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Dehydration, Muscle Damage, and Exercise in the Heat:
Impacts on Renal Stress, Thermoregulation, and Muscular Damage Recovery

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Kinesiology

by

Cory L. Butts
Colorado State University – Pueblo
Bachelor of Science in Exercise Science, 2011
University of Texas at Arlington
Master of Science in Exercise Physiology, 2013

August 2018
University of Arkansas

This dissertation is approved for recommendation to the Graduate Council.

_____
Brendon P. McDermott, Ph.D.
Dissertation Director

_____
Matthew S. Ganio, Ph.D.
Committee Member

_____
Stavros A. Kavouras, Ph.D.
Committee Member

_____
Nicholas P. Greene, Ph.D.
Committee Member

_____
Ronna C. Turner, Ph.D.
Committee Member

Abstract

Purpose: The purpose was to identify the combined influence of dehydration, muscle damage, and exertional hyperthermia on biological markers of acute kidney injury and renal function. We also investigated the effects of performing muscle damaging exercise during mild hypohydration on muscle damage biomarkers and muscular strength recovery. **Methods:** Eighteen recreationally-active males (age 24 ± 5 y, body fat 17.3 ± 6.2%) completed a familiarization visit and two experimental trials separated by ≥28 days. The two experimental conditions consisted of either euhydration (EU; maintaining hydration, -1.2 ± 0.8%) or hypohydration (HY; restricting fluid consumption for 24 hours prior to and during the trial, -4.4 ± 1.9%). Participants completed a unilateral eccentric knee flexion muscle damaging protocol, 60-minute treadmill exercise in the heat, 30-minute passive recovery, and a rehydrated 24-h follow-up visit, respectively. **Results:** Strength was reduced across time independent of trial for isometric strength at 70° (P<0.001), isometric strength at 90° (P=0.001), and isokinetic strength at 60°·sec^{-1} (P=0.001). Serum creatine kinase increased regardless of trial (P<0.001), with the 24-h follow-up greater (grand mean; 58.7 ± 25.1 U/L) than at baseline (grand mean; 35.7 ± 23.1 U/L, P<0.001) and post-exercise (grand mean; 51.6 ± 23.2 U/L, P=0.009). Percent change in plasma neutrophil gelatinous associated lipocalin was greater in the HY trial post-exercise (EU 28.0 ± 15.2%, HY 41.8 ± 17.5%, P<0.001), but not at 24-h follow-up (P=0.39). Serum creatinine was increased in the HY trial regardless of time (EU 0.97 ± 0.14, HY 1.04 ± 0.15, mg/dL, P=0.025). Urine NGAL and urine creatinine were also elevated in the HY trial pre-exercise and post-exercise (all, P<0.05) but were returned to EU levels by 24-h follow-up (all, P>0.05). **Conclusions:** We demonstrated no significant impact of hydration status when performing muscle damaging exercise, followed by exercise in the heat, on indices of muscle damage recovery. Exercise in

the heat with muscle damage increased physiological and renal strain when HY, but the rehydration protocol ameliorated differences between trials by the 24-h follow-up. These findings highlight the importance of proper fluid intake following exercise to mitigate renal stress.

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I. Introduction

Exposure to heat stress yields a spectrum of responses ranging from positive cardiovascular adaptations (e.g. plasma volume expansion, improved vascular function) to the potential life-threatening risk of heat illness (e.g. exertional heat stroke) (Casa et al., 2015a; Epstein & Roberts, 2011; Laukkanen, Khan, Zaccardi, & Laukkanen, 2015; Lorenzo, Halliwill, Sawka, & Minson, 2010; Lorenzo & Minson, 2010; Nadel, Pandolf, Roberts, & Stolwijk, 1974; Patterson, Stocks, & Taylor, 2004; Xiang, Hansen, Pisaniello, & Bi, 2015). When exposed to heat stress, increases in skin blood flow and sweating occur to maintain thermoregulatory homeostasis, leading to reductions in splanchnic and renal blood flow (Hohimer, Hales, Rowell, & Smith, 1983; Rowell, 1974; Sawka, Leon, Montain, & Sonna, 2011). The reductions in blood flow to vital organs at rest and during exercise in the heat are often transient attenuations in perfusion, resulting in minor functional alterations as evidenced by a return to baseline function shortly after exposure (e.g. within hours) (Junglee et al., 2013; Melin et al., 1997; Radigan & Robinson, 1949). However, heat stress is often experienced in combination with other physiological challenges, such as dehydration and muscle damage. Hypohydration, muscle damage, and environmental heat stress are commonly experienced in athletic, military, and occupational settings (Armstrong et al., 2010; Godek, Bartolozzi, Burkholder, Sugarman, & Dorshimer, 2006; Johnson et al., 2016; Knochel, Dotin, & Hamburger, 1974; Meade, Lauzon, Poirier, Flouris, & Kenny, 2015; Poirier et al., 2015; Schlader et al., 2017; Schrier et al., 1970; Smoot, Cavanaugh, Amendola, West, & Herwaldt, 2014; Yeargin et al., 2010). The combined effects of these stressors may compromise renal function and increase acute kidney injury risk, however, long term consequences remain unknown (Johnson et al., 2016; Junglee et al., 2013; Schrier et al., 1970; Smith, Robinson, & Pearcy, 1952).

Adequate perfusion of the renal vasculature is vital for maintaining optimal kidney function (e.g. fluid homeostasis and filtration of waste products from the blood). Reductions in renal blood flow may occur in response to a variety of physiological and thermoregulatory challenges, such as exercise (Tidgren, Hjemdahl, Theodorsson, & Nussberger, 1991), heat stress (Minson, Wladkowski, Cardell, Pawelczyk, & Kenney, 1998), or hypohydration (Melin et al., 1997). Schlader et al. (2017) recently found greater increases in biomarkers of renal stress with longer duration treadmill exercise in the heat. Preliminary field work from our laboratory (unpublished) has investigated the renal responses to a 100 mile or 100 km cycling event in the heat (22 – 34°C) in recreational riders (age 52 ± 9 y) completing the race in ~5.7 hours. We demonstrated significant elevations in the acute kidney injury biomarker neutrophil gelatinase associated lipocalin (NGAL), as well as increases in the renal function biomarker, serum creatinine, immediately following the ride. Bongers et al. (2017) also showed elevations in urinary markers of renal stress after one and three days of long distance walking, however, these increases were relatively low and likely not indicative of serious complications. The exercise in this study was walking, therefore the intensity (average heart rate = 112 bpm) may not have been severe enough to induce reductions in renal perfusion and subsequent kidney stress. Studies of marathon (McCullough et al., 2011) and ultramarathon runners (Hoffman & Weiss, 2016; Lippi et al., 2012; Skenderi, Kavouras, Anastasiou, Yiannakouris, & Matalas, 2006) have also revealed significant elevations in biomarkers of muscle damage and acute kidney injury immediately post-race. Interestingly, McCullough et al. (2011) demonstrated that 24 hours post-race, NGAL and creatinine had returned to near-baseline levels. Hoffman and Weiss (2016) reported similar renal stress responses during a subsequent ultramarathon, importantly noting that these individuals did not appear to experience lasting effects from the first ultramarathon. Thus, stress induced during

the marathon may only transiently alter renal function. These findings suggest mild acute kidney injury and reduced renal function may also be related to the duration of the exposure to exertional hyperthermia.

Melin et al. (1997) demonstrated significant reductions in creatinine clearance (a marker of renal function) with dehydration compared to a euhydrated control during treadmill walking in the heat. Reductions in plasma volume with hypohydration lead to blood volume attenuations, which can increase cardiovascular strain and compromise thermoregulation (González-Alonso, Mora-Rodríguez, & Coyle, 2000). Hypohydration with concomitant exercise in the heat challenges thermoregulation and performance due to the competition for blood flow between active skeletal muscle and the skin for heat dissipation (Casa et al., 2010; González-Alonso, Calbet, & Nielsen, 1998; González-Alonso et al., 1999). Thus, to compensate for increased demand for blood flow in the cutaneous vasculature, blood flow is further reduced to inactive tissues (i.e. splanchnic and renal vasculature), potentially compromising function in these areas.

Heat stress, hypohydration, and muscle damage are factors commonly found in preseason athletic practices, such as in American football (Yeargin et al., 2010). Smoot et al. (Smoot et al., 2014) demonstrated elevated serum creatine kinase, a marker of muscle damage, throughout preseason football practices in NCAA Division I football players. These findings have been further confirmed in several observational studies of offseason, pre-season, and in-season play as well (Ehlers, Ball, & Liston, 2002; J. R. Hoffman, Kang, Ratamess, & Faigenbaum, 2005; Kraemer et al., 2013; Kraemer et al., 2009; Smoot et al., 2013). Severe skeletal muscle damage (i.e. exertional rhabdomyolysis) may lead to acute kidney injury due to the nephrotoxic effects of intracellular contents (i.e. myoglobin) entering the circulation from skeletal muscle cell breakdown. However, in settings of optimal hydration and thermoneutral environmental

temperatures, muscle damage does not appear to alter renal function. Therefore, the implications for sustained mild muscle damage throughout preseason practices are not yet known.

Athletes also often report to activities in a water conserving state (i.e. sub-optimally hydrated) as evidenced by urinary markers (Godek, Godek, & Bartolozzi, 2005; Phillips, Sykes, & Gibson, 2014; Yeargin et al., 2010), potentially increasing physiological strain and decreasing performance during exercise (Bardis, Kavouras, Arnaoutis, Panagiotakos, & Sidossis, 2013; Casa et al., 2010). Furthermore, football players were found to progressively dehydrate throughout preseason practices (Godek et al., 2006; Godek et al., 2005; Stover, Zachwieja, Stofan, Murray, & Horswill, 2006; Yeargin et al., 2010). Concomitant muscle damage and dehydration may only be exacerbated by the high ambient temperatures often experienced during preseason American football practices. Junglee et al. (2013) revealed elevations in biomarkers of acute kidney injury with muscle damage during exercise in the heat. Another study (Fortes et al., 2013), also demonstrated muscle damage to elicit elevations in thermal strain during subsequent exercise in the heat. However, both studies maintained hydration state to a similar degree in all trials, thus the impact of hypohydration compared to euhydration is unknown. Furthermore, the renal responses among many other sporting activities (e.g. American football, soccer, rugby) have received little investigation. Thus, sports that require individuals to exercise regularly (i.e. several times per week) in high ambient temperatures and humidity, when muscle damage and dehydration are present, may provide a unique stress to renal function, of which the consequences remain unknown. Elucidating the role of adequate hydration may be pivotal to improving the overall safety of athletics, especially since factors such as muscle damage (often induced by strength training) and heat stress are not easily avoidable and inherent in typical conditioning regimens.

In working populations, it has been suggested that the regular exposure to heat stress with concomitant dehydration and mild muscle damage may increase the risk of chronic kidney disease (García-Trabanino et al., 2015; Johnson et al., 2016; Moyce, Joseph, Tancredi, Mitchell, & Schenker, 2016; Roncal-Jimenez et al., 2016). The recent rise in chronic kidney disease in Mesoamerican sugar cane workers has been related to physiological responses to environmental working conditions (Bodin et al., 2016; Crowe, Nilsson, Kjellstrom, & Wesseling, 2015; García-Trabanino et al., 2015; Laws et al., 2015, 2016; Roncal-Jimenez et al., 2016). As glomerular filtration rate, a marker of kidney function, has been shown to decrease throughout the work day, the increased prevalence of chronic kidney disease may be due to the additive effects of repetitive kidney stress from the concomitant environmental and physiological strain experienced by these workers (Bodin et al., 2016; Crowe et al., 2015; García-Trabanino et al., 2015; Laws et al., 2015, 2016; Roncal-Jimenez et al., 2016). Furthermore, these workers also experience progressive dehydration throughout the workday through elevated urine specific gravity and decreased glomerular filtration rates (García-Trabanino et al., 2015; Roncal-Jimenez et al., 2016; Wesseling et al., 2016). The combination of heat stress with strenuous exercise and gradual dehydration throughout the workday places a high demand on the kidneys to retain fluid while clearing excess waste from potential muscle damage. These characteristics may apply to many other occupations as well, such as firefighters, military, agricultural and industrial settings.

Specific Aims

Aim #1: Identify the combined influence of dehydration, exercise in the heat, and muscle damage on biomarkers of acute kidney injury and renal function.

Research Hypothesis #1: The thermoregulatory strain associated with dehydration during exercise in the heat would augment renal biomarker elevations immediately post-exercise as

compared to the euhydrated trial, however, these differences would be transient with returns to baseline 24 hours post.

Aim #2: Identify the effects of performing muscle damaging exercise during mild hypohydration on muscle damage biomarkers (creatine kinase) and muscular strength recovery indices (i.e. isometric and isokinetic strength).

Research Hypothesis #2: There would be no differences in muscle damage biomarkers with hypohydration, however, muscle strength recovery would be modestly impaired as compared to a euhydrated state.

II. Literature Review

Renal Function & Biomarkers

The homeostatic role of the renal system in maintaining fluid balance, waste elimination, acid-base balance, and blood pressure, is vital to the preservation of normal health and function (Poortmans & Vanderstraeten, 1994). As such, physiological and environmental challenges may provide stress to the kidneys to maintain optimal function. Reports of renal compromise in athletics primarily focus on renal trauma, unless the individual has experienced exertional rhabdomyolysis or heat illness (Bosch, Poch, & Grau, 2009; Brophy et al., 2008; Gerstenbluth, Spirnak, & Elder, 2002; Grinsell, Butz, Gurka, Gurka, & Norwood, 2012). It was found that of the 52 kidney injuries reported in the National Football League from 1984 to 2004, only two were caused by dysfunction, with trauma (contusion or laceration) being most common (Brophy et al., 2008). In contrast, 30-80% of ultra-marathon runners are suspected to develop transient acute kidney injury (AKI) following a race (M. D. Hoffman & Weiss, 2016). Renal function and acute renal failure have received much investigation in clinical populations, however, the renal responses during and following exercise with environmental stress and muscle damage has received considerably less attention.

The role of the kidneys in fluid balance is necessary for maintaining optimal hydration, both during resting and exercise conditions. Losses in total body water (i.e. dehydration) can be detrimental to physiological and psychological performance (Bardis et al., 2013; Casa et al., 2015a; Casa et al., 2010; Cheuvront & Kenefick, 2014; Cheuvront, Kenefick, Montain, & Sawka, 2010; Distefano et al., 2013; Judelson et al., 2007; Lopez et al., 2011; McDermott, Casa, Lee, Yamamoto, Beasley, Emmanuel, Pescatello, et al., 2013; Yamamoto et al., 2008). Further, operating in a state of low body water (i.e. hypohydration) for chronic periods of time has been linked to several negative health consequences (Clark et al., 2016; Clark et al., 2014; García-

Trabanino et al., 2015; Glaser et al., 2016; Johnson et al., 2016; Rosinger, Lawman, Akinbami, & Ogden, 2016).

Fluid homeostasis is maintained through an intricate balance between behavioral (e.g. water-seeking, removal from challenging thermal environments) and hormonal mechanisms. When fluids are inadequately consumed (i.e. drinking or food intake), water retention is dependent on hormonal release. A hormone vital to the process of fluid maintenance is arginine vasopressin (AVP), also known as antidiuretic hormone. AVP is produced in the paraventricular nucleus and supraoptic nucleus of the hypothalamus and secreted by the posterior pituitary gland. The primary drivers of AVP release are osmoreceptor and baroreceptor feedback in response to osmolality and blood pressure changes, respectively (Bankir, 2013; Baylis & Robertson, 1980; Koshimizu et al., 2012; Robertson, 1984; Robertson & Athar, 1976; Robertson, Shelton, & Athar, 1976; Share, 1996). AVP release typically occurs at a plasma osmolality of ~280 mOsm/kg (Robertson, 1984; Robertson et al., 1976). Bayliss and Robertson (1980) also demonstrated a similar release threshold and further showed that every 1% increase in plasma osmolality induced a 1.8 pg/mL increase in AVP. Therefore, when fluid losses are greater than gains (i.e. dehydration), plasma osmolality increases and subsequently AVP is released. Similarly, blood volume decreases, causing reductions in blood pressure, also stimulates AVP release. However, greater blood volume reductions (10-20%) are typically necessary for the stimulation of AVP as compared to osmotic regulation (Share, 1996). The action of AVP is widespread, however, arguably the most important is water conservation at the kidney. AVP acts on V2 receptors in the renal tubules and collecting duct, which, stimulates the action of aquaporin channels to reabsorb water into the vasculature, producing a concentrated urine (Johnson et al., 2016; Koshimizu et al., 2012). The action of AVP on V1a receptors located in

the walls of the vasculature also causing increases in blood pressure, subsequently increasing cardiovascular stability (Koshimizu et al., 2012). AVP also stimulates water-seeking behaviors through thirst, therefore, once blood volume and osmolality are increased, individuals will drink fluids causing a decrease in osmolality and AVP secretion. AVP has many other non-fluid regulatory actions (e.g. stimulating release of ACTH through V1b receptor in anterior pituitary), thus it has also been termed a survival hormone (Johnson et al., 2016; Koshimizu et al., 2012). As such, chronically elevated levels of AVP due to improper hydration strategies have been suggested to have significant health consequences (Bankir, 2013; Bouby, Bachmann, Bichet, & Bankir, 1990; Bouby, Hassler, & Bankir, 1999; Clark et al., 2016; Clark et al., 2014; Johnson et al., 2016; Kuwabara et al., 2017; Roussel et al., 2014; Share, 1996).

Others hormones are also responsible for fluid balance as urine concentration and fluid conservation has been shown to occur in the absence of AVP (Gellai, Edwards, & Valtin, 1979). As renal perfusion is reduced, the juxtaglomerular apparatus detects these changes, and releases renin (Sparks, Crowley, Gurley, Mirotsou, & Coffman, 2014). Renin then acts to convert angiotensinogen to angiotensin I, a biologically inert hormone (Sparks et al., 2014). Angiotensin I is converted to angiotensin II through angiotensin converting enzyme, which directly induces blood pressure increases through actions on the smooth muscle of the vasculature (Sparks et al., 2014). Angiotensin II also stimulates the release of aldosterone from the adrenal glomerulosa and thirst centers in the brain (Sparks et al., 2014; Thornton, 2010). Aldosterone increases blood pressure through actions in the vasculature, however, is also known for stimulating the reabsorption of sodium from the kidney. This is an important mechanism to aid in fluid preservation, as the increased sodium retention allows for greater water movement into the vasculature as a result of increased osmolality (Thornton, 2010). Further, the actions of the

renin-angiotensin-aldosterone system play a vital role in the maintenance of fluid balance through thirst stimulation to increase water seeking behaviors, which are essential to proper hydration (Thornton, 2010). Evidence is continually increasing to support the role of proper water consumption to mitigate reliance on hormonal fluid regulation and prevent renal dysfunction and disease (Clark et al., 2016; Wang, Grantham, & Wetmore, 2013).

Identifying renal dysfunction may be vital to improving safety in athletic, military, and working populations alike. Furthermore, elucidating the effects of exertion, with or without environmental and physiological stressors (i.e. heat stress, dehydration, and muscle damage), may also provide implications for the development of acute kidney injury (AKI) or chronic kidney disease (CKD). The classic clinical definition for AKI involves the decrease in glomerular filtration rate (GFR) over a period of hours to days leading to the buildup of creatinine and blood urea nitrogen (Basile, Anderson, & Sutton, 2012). However, the mechanism eliciting these elevations may drastically alter clinical treatment and the definition of this injury. Prerenal AKI occurs as a consequence of renal perfusion alterations, leading to changes in filtration through the glomeruli (Basile et al., 2012). In contrast, impedances to normal urinary tract flow may induce postrenal AKI. Lastly, renal AKI encompasses etiologies that compromise tissue structure, such as tubular or glomerular damage (Basile et al., 2012). Renal compromise by any mechanism is of serious clinical concern as the development of AKI is associated with morbidity and mortality rates of 40-60% (Schiffl & Lang, 2012).

A primary focus in the literature regarding AKI is patients in hospital settings with serious illness or injury. Therefore, it should be noted that the term AKI discussed in this review with respect to exercise, may be misleading given the severity and duration of the renal compromise. In certain clinical situations associated with exercise (e.g. exertional

rhabdomyolysis), the risk of AKI may place the individual at an increased potential for negative outcomes. Accordingly, hospitalizations due to rhabdomyolysis have been reported to develop AKI in 13 to 50% of cases (Bosch et al., 2009). However, the use of AKI to describe renal responses to exercise and environmental stress in otherwise healthy individuals may be inappropriate. This is not to infer that the renal function alterations described in this review do not provide the potential for development of CKD, but rather the epidemiological data is absent and therefore cannot be exclusively stated at this time. Regardless, the depth of literature on this topic is relatively lacking, thus comparison to clinical standards for AKI are commonly used throughout the exercise renal physiology literature and will be used in this review.

Assessment of short and long-term detriments to renal function and health are essential in identifying AKI and CKD. The risk for CKD increases with AKI occurrences in clinical settings, however, the risk of CKD following elevations in AKI markers induced by exercise or environmental stress remains relatively unstudied. Mesoamerican nephropathy may perhaps be the closest human model to athletics to represent the impact of recurrent AKI induced via physiological and environmental stress, however, this is still limited due to several confounding variables not often present in organized sport. Nonetheless, this population is experiencing CKD at alarming rates, hypothesized to be driven by recurrent dehydration with concomitant subclinical rhabdomyolysis and heat stress (García-Trabanino et al., 2015; Johnson et al., 2016; Roncal-Jimenez et al., 2016). The mechanisms for acute renal stress leading to CKD in this population will be further detailed later in this literature review. While the long-term health complications associated with acute renal stress remain unknown, it is well demonstrated that acute renal failure occurs on a spectrum, and if improperly managed, may result in sequela and potential fatality.

Distinguishing between appropriate biological markers in both urine and blood samples is essential to proper diagnosis of AKI and establishing practical treatment or prevention strategies. The use of different biomarkers also allows for the specific identification of renal injury or dysfunction location, as well as provide clarity for the functional significance in these elevations. While all biomarkers have pitfalls and benefits, understanding the mechanism of action for each is pertinent for identifying details regarding the location of renal dysfunction (Vanmassenhove, Vanholder, Nagler, & Van Biesen, 2013). This is of importance in cases of sub-clinical AKI often shown with exercise, as the impact of transient renal dysfunction in this instance is not well understood.

The assessment of kidney function via glomerular filtration rate (GFR) is a primary assessment in renal health, as failure to properly filter the plasma through the glomerulus or reabsorb molecules in the tubules will alter the excretion of substances. Glomerular filtration has been assessed with a variety of markers, both exogenous and endogenous (Beierwaltes, Harrison-Bernard, Sullivan, & Mattson, 2013). The gold standard assessment of GFR is performed through inulin injection into the circulation combined with collection in the urine. Because inulin is readily filtered by the glomerulus, with no reabsorption, anything collected in the urine can be compared to what is left in the plasma to identify excretion rates (Beierwaltes et al., 2013). The use of inulin however, requires time and expenses that may not be available in clinical or field settings. Hence, the use of an endogenously produced marker may be favored in clinical practice.

Creatinine is commonly used to assess GFR as it is endogenously produced and can be measured in the blood and urine (Beierwaltes et al., 2013). Further, creatinine is freely filtered by the glomerulus, and when excretion rates are high, minimal reabsorption occurs with only

slight secretion by the proximal tubule (Beierwaltes et al., 2013). Similar to inulin, the assessment of GFR with creatinine uses collection in the blood and urine over a period of time to identify excretion rates. Typical values for blood creatinine range from (0.8-1.4 ml/dL) while urine values provide a much greater range (Beierwaltes et al., 2013). Because creatinine is produced as a byproduct of the reaction between phosphocreatine and ADP, there is a large release by skeletal muscle and can be dependent on muscle mass (Beierwaltes et al., 2013). This also creates an issue regarding the steady state values of creatinine in the blood. If rises in creatinine are found in the blood, it is difficult to ascertain whether the increases occurred due to decreases in GFR or increased production by other tissues.

Serum creatinine (SCr) can also be utilized to estimate GFR, independent of the urinary collection (Beierwaltes et al., 2013; Poortmans, Gulbis, De Bruyn, Baudry, & Carpentier, 2013). Poortmans et al. (2013) demonstrated a lower estimated GFR from SCr alone compared to GFR measured using both urine and serum creatinine. Further, when creatinine clearance via urine and serum samples returned to baseline values, the estimated GFR via SCr was still reduced below baseline by ~10% (Poortmans et al., 2013). The assumption that GFR is altered when it has returned to normal may impact clinical decision-making, however, the use of this marker in research may still be implicated in instances when urine creatinine assessment is unavailable.

SCr can also be used to classify levels of AKI. Many foundations have guidelines regarding stages of AKI and CKD, however two commonly used in clinical and exercise settings are the RIFLE criteria (Risk, Injury, Failure, Loss, End-stage kidney disease) and AKIN (acute kidney injury network) (Mehta et al., 2007; Vaidya, Ferguson, & Bonventre, 2008). According to the AKIN classifications, there are three stages of AKI, including stage one, which occurs with an increase in SCr $\geq$0.3 mg/dl or 150-200% increase from baseline. Stage two requires a

200-300% increase from baseline and stage three necessitates >300% increase from baseline or >4.0 mg/dl with an acute 0.5 mg/dl increase (Mehta et al., 2007; Vaidya et al., 2008). These stages can also use urine output of <0.5 ml/kg per six hours, <0.5ml/kg per 12 hours, and <0.3ml/kg per 24 hours or anuria for 12 hours, for stages one, two and three respectively (Mehta et al., 2007; Vaidya et al., 2008). Stages one through three also correspond to the first three stages according to the RIFLE criteria (i.e. risk, injury, failure). The RIFLE criteria also includes a Loss stage (stage four) which indicates a complete loss of function greater than four weeks and an end stage renal disease stage (stage five), which is a greater than three month loss of kidney function (Vaidya et al., 2008). The RIFLE criteria also includes reductions in GFR, allowing for use with different biomarkers (Mehta et al., 2007; Ricci, Cruz, & Ronco, 2011). A concern with the AKIN and RIFLE criteria, however, is the mandate for a baseline sample of SCr, which, clinically, may be very challenging. Further, reductions in renal perfusion with exercise induce elevations in SCr that may be misinterpreted as AKI, when they are instead transient alterations in GFR, potentially with minimal negative outcomes.

Interestingly, certain disease states and illnesses also induce hyperfiltration (i.e. increased GFR), which may result in renal injury and GFR impairments long term (Palatini, 2012). It has been suggested that glomerular hyperfiltration may even be a risk factor in pre-diabetes or pre-hypertension, due to the potential development of microalbuminuria (Palatini, 2012). Oxidative stress and inflammation are also suggested with hyperfiltration, leading to potential nephropathy (Palatini, 2012). These conditions are often associated with underlying etiologies, but are thought to arise from endothelial dysfunction or altered tubuloglomerular feedback, causing vasodilation of the afferent arteriole and increased permeability through the glomerulus. Thus,

high GFR in patients is also detrimental to renal health, and as such, requires immediate medical attention.

The use of creatinine to assess renal function has received much criticism due to the delayed response time and lack of sensitivity, thus the use of novel biomarkers has received much attention (Ferguson, Vaidya, & Bonventre, 2008). Cystatin C (CyC) is a 13 kD protein that has been suggested for use instead of creatinine for assessment of GFR, due to a greater ability to detect acute renal failure (Charlton, Portilla, & Okusa, 2014; Colombini et al., 2012; Herget-Rosenthal, Metzger, Albalat, Bitsika, & Mischak, 2012). CyC is produced by all nucleated cells, and similar to creatinine, is freely filtered by the glomerulus (Charlton et al., 2014). However, CyC is reabsorbed at the proximal tubule, therefore, excretion of CyC in the urine is indicative of tubular damage (Charlton et al., 2014). It is because of this mechanism that CyC is suggested to be a better marker in the detection of AKI than creatinine. Also, in contrast to creatinine, CyC estimates of GFR are affected by obesity, whereas creatinine is affected by muscle mass (Chew-Harris, Florkowski, George, Elmslie, & Endre, 2013).

Interestingly, the use of serum CyC demonstrated underestimation of GFR compared to creatinine clearance measured in the urine and serum (Poortmans et al., 2013). Further, CyC was also ~10% below baseline values when GFR returned to baseline (Poortmans et al., 2013). Mingels et al. (2009) found CyC to produce lower elevations compared to SCr immediately following a marathon and returned to baseline values by one day post-race, which SCr did not. These findings suggest that CyC may be better for the evaluation of GFR as it is less affected by confounding factors, such as muscle mass or breakdown. These findings have also been mirrored in rugby populations, where muscle mass may considerably impact creatinine assessment by underestimating GFR as compared to actual creatinine clearance (Banfi, Del

Fabbro, d'Eril, & Melegati, 2009; Banfi et al., 2012). Further, CyC estimated GFR was less correlated with creatine kinase (a marker of muscle breakdown) during a 3-week endurance cycling event than GFR estimated by creatinine (Colombini et al., 2012). This also lends to the argument that creatinine may be altered by muscle mass.

GFR only provides assessment of the functional status of the glomerulus and is largely altered by differences in renal perfusion. However, renal health assessment should also analyze the renal tissue, which includes cells of the renal tubules where reabsorption and excretion are regulated. Stress to the tubular cells provides an alternative view of the effects of different stresses and recovery status. With this, several different biological markers (i.e. biomarkers) have been assessed for validity and usefulness in the evaluation of the state of the tubules. While there have been many biomarkers suggested for use in clinical settings of AKI (e.g. IL-18, FABP, NAG), this review will focus on those also being utilized in the renal responses to exercise literature.

Neutrophil-gelatinase associated lipocalin (NGAL), a 25 kD protein measured in both urine and plasma has been found to be a reliable and accurate predictor of AKI in clinical settings (Alge & Arthur, 2015; Charlton et al., 2014; Ferguson et al., 2008; Mårtensson, Martling, & Bell, 2012). NGAL is produced in many tissues (e.g. bone marrow, epithelial cells) throughout the body in response to inflammation, however, it is also readily expressed in the proximal tubule cells (Ferguson et al., 2008; Mishra et al., 2003; Mårtensson et al., 2012). NGAL secretion is increased following ischemia or nephrotoxic injury, with urine value increases in as little as three hours post-insult (Alge & Arthur, 2015; Mishra et al., 2003). In a study of intensive care unit patients, NGAL diagnosed AKI in less than six hours with an area under the curve of 0.82 in patients with estimated GFR values of 90-120 ml/min (i.e. normal)

(Endre et al., 2011). However, when patients had low estimated GFR (<60 ml/min), NGAL only predicted AKI in less than six hours with an area under the curve of 0.45 (Endre et al., 2011). As ischemia is a driver for NGAL production, the use of this biomarker to evaluate the renal response to exercise may be beneficial due to the reductions in renal blood flow that are commonly associated with exertion. ICU patients with prerenal etiologies of AKI (e.g. perfusion) demonstrated elevations in urinary NGAL (Nejat et al., 2012), which may provide some extrapolation to exercise due to reduced blood flow as a potential prerenal cause. As such, many investigations have evaluated urinary and blood NGAL responses following exercise (Junglee et al., 2013; Junglee et al., 2012; Lippi et al., 2012; Mansour et al., 2017; McCullough et al., 2011; Schlader et al., 2017). Further, NGAL has been shown to have a relationship to the development of acute mountain sickness and the negative response to altitude (Mellor et al., 2013). NGAL is also involved in the repair process from renal injuries such as ischemia-reperfusion (Alge & Arthur, 2015). The differentiation of progenitor cells in the renal tubules is thought to be caused by NGAL (Mårtensson et al., 2012). Therefore, the elevation of NGAL following ischemic injury may indicate a repair mechanism rather than continued damage. Using NGAL elevations post-insult may be beneficial in understanding long-term renal tissue responses to potential ischemic activities such as exercise.

Kidney Injury Molecule 1 (KIM-1) is another marker that has shown promise in clinical and exercise settings to evaluate AKI (Alge & Arthur, 2015; Nejat et al., 2012). Expressed in the epithelial cells of the proximal tubules of the kidney in response to ischemic injury, KIM-1 is a 38.7 kD protein that provides implications for injury when measured in the urine (Alge & Arthur, 2015; Charlton et al., 2014). KIM-1 has been found to elicit phagocytic activities to aid in the removal of cellular debris following AKI. KIM-1 is primarily used as a urinary target with

peak values usually occurring at ~48 hours post injury (Alge & Arthur, 2015; Nejat et al., 2012). KIM-1 identified AKI with an area under the curve of 0.85 in six to 12 hours in intensive care unit patients with normal estimated GFR (90-120 ml/min) (Endre et al., 2011). Urinary KIM-1 also increased in ICU patients with pre-renal causes of AKI. While use of KIM-1 in clinical practice is somewhat controversial, it may have benefit in the recognition of kidney stress with exertion or thermal challenges (Ferguson et al., 2008; McCullough et al., 2011; Vaidya et al., 2008; Vaidya et al., 2010). As with NGAL, KIM-1 provides information regarding the recovery state of the renal tissue, which following exertion driven renal ischemia, may alert clinicians to potential negative health outcomes.

Renal Function During Exercise

Exercise poses a transient challenge to renal function (e.g. GFR), driven by renal perfusion decreases during exertion. However, upon cessation of activity, kidney blood flow and subsequent function returns to normal. Therefore, renal blood flow is a pivotal driver in mediating functional response with exercise.

At the onset of exercise, the increase in sympathetic nervous system activity mandates a redirection of blood flow to the active tissue (Hohimer & Smith, 1979). Vasoconstriction of the renal and splanchnic vasculature greatly reduce blood flow to these organs in direct relation to exercise intensity (Grimby, 1965; Rowell, 1974). Grimby (1965) assessed renal clearance of inulin and para-aminohippuric acid during exercise intensities from 150 to 900 kpm/min, noting greater reductions in clearance at higher workloads. It was further determined that the fraction of cardiac output directed toward the renal vasculature was reduced from ~17% at rest to <5% at oxygen uptakes of 2.0 to 2.5 L/min. Baboons conducting dynamic leg exercise also demonstrated decreases in renal blood flow of ~19% (Hohimer & Smith, 1979). Further, one

kidney in the baboons was denervated, which exhibited increased blood flow during exercise, confirming that the vasoconstriction of the renal vasculature with exercise is neurally mediated (Hohimer & Smith, 1979). Renal vascular conductance also decreases during moderate intensity dynamic exercise (Pricher, Holowatz, Williams, Lockwood, & Halliwill, 2004). Exercise induced renal blood flow reductions are suggested to be attenuated following endurance training (McAllister, 1998). The mechanism for this is not well described, however, it is likely the result of alterations in sympathetically mediated vasoconstriction (McAllister, 1998).

Renal function during exercise may also exhibit a mode dependent effect. Many of the aforementioned studies have consisted of dynamic, aerobic endurance exercise, however, static exercise also mediates kidney function. Both passive stretch and static contraction of the triceps surae in rats induced renal sympathetic nervous system increases, subsequently reducing renal cortical vascular conductance and renal cortical blood flow (Koba, Yoshida, & Hayashi, 2006). The control of renal blood flow due to electrically stimulated contractions suggests that the exercise pressor reflex may mediate the renal response to an exercise stimulus (Koba et al., 2006). Static handgrip exercise performed by healthy controls and kidney transplant patients, elicited much greater reductions in renal blood flow velocity assessed by Doppler ultrasound in the healthy controls compared to the renal transplant group (Momen et al., 2005). These support the vital role of sympathetic neural mediated mechanisms in altering renal blood flow during exercise rather than autoregulatory mechanisms (Momen et al., 2005). Interestingly, neither gender nor muscle mass engaged (i.e. leg vs arm) impacted the renal vascular resistance increases or renal blood flow reductions during static exercise (Momen, Handly, Kunselman, Leuenberger, & Sinoway, 2006). Further, baroreceptor unloading via orthostatic stress did not significantly alter the renal vascular response to handgrip exercise, again supporting that the

primary regulation of renal vasoconstrictor tone with exercise occurs via central command and the exercise pressor reflex (Momen, Thomas, et al., 2006). It should be noted that orthostatic stress induced using lower body negative pressure increased renal vascular resistance in the absence of exercise (Momen, Thomas, et al., 2006).

Renal blood flow and renal vascular conductance following moderate intensity dynamic exercise has been shown to return to baseline levels within 20 minutes of exercise completion (Pricher et al., 2004). This is particularly interesting given the exercise induced systemic hypotension that can last at least two hours following exercise (Pricher et al., 2004). As muscle blood flow is still elevated due to a reduced sympathetic activity post exercise, it would be expected that vasoconstriction of the splanchnic and renal vasculature would occur to prevent marked reductions in mean arterial pressure (Pricher et al., 2004). However, because there is a lack in sympathetic activity to induce vasoconstriction, the renal vascular conductance returns to resting levels (Pricher et al., 2004).

In contrast, renal function remains reduced immediately following exhausting exercise. Suzuki et al. (Suzuki et al., 1996) utilized a radioactive tracer (technetium 99m phytate) to identify changes in renal blood flow up to 60 minutes after a graded maximal cycling test. Renal blood flow immediately post-exercise was determined to be 53% reduced compared to a resting baseline (Suzuki et al., 1996). Further, at 30 and 60 minutes, renal blood flow was still reduced 17.5% and 21.1%, respectively. The reductions in renal blood flow were mirrored by reductions in creatinine clearance of similar magnitudes from immediately after exercise through 60 minutes (Suzuki et al., 1996). Given that the exercise performed only lasted an average of 11.4 minutes in this protocol, the delayed return to normal clearance may be of impact when

exhaustive exercise lasts longer or additional stressors are present (e.g. heat stress, dehydration, muscle damage).

Dr. Poortmans and colleagues have conducted a multitude of studies investigating the effects of exercise on renal function, particularly the consequences of protein in the urine (Poortmans, 1977, 1984, 1985, 1995; Poortmans, Auquier, et al., 1997; Poortmans, Blommaert, Baptista, De Broe, & Nouwen, 1997; Poortmans et al., 1988; Poortmans et al., 2013; Poortmans & Haralambie, 1979; Poortmans, Jeannaud, Baudry, & Carpentier, 2015; Poortmans & Labilloy, 1988; Poortmans, Mathieu, & De Plaen, 1996; Poortmans, Rampaer, & Wolfs, 1989; Poortmans & Vancalck, 1978; Poortmans & Vanderstraeten, 1994). Protein found in the urine (i.e. proteinuria) has been well documented following exercise and is implicated as a marker of renal function alterations (Junglee et al., 2012; Poortmans, 1984, 1985; Poortmans, Blommaert, et al., 1997; Poortmans et al., 1988; Poortmans & Haralambie, 1979; Poortmans et al., 2015; Poortmans & Labilloy, 1988; Poortmans et al., 1989; Poortmans & Vancalck, 1978; Poortmans & Vanderstraeten, 1994; Schrier et al., 1970). The presence of proteinuria can indicate increased glomerular permeability, tubular dysfunction, or both. Recently, proteinuria has been linked to mTOR-mediated autophagy impairments in the proximal tubule of mice, potentially leading to tubular injury and the progression of disease (Nolin et al., 2016). However, this model did not involve exercise, limiting the extrapolation to exercising humans.

Male participants running distances from 100 meters to 3000 meters at maximal effort displayed increases in total protein excreted for all events, however, the greatest increases were found with 400 and 800 meter events (Poortmans et al., 1996). This pattern was also shown with individual proteins assessed (e.g. albumin, β_2-microglobulin, retinol-binding protein) and plasma lactate values. Furthermore, there was a direct relationship ($R^2 = 0.996$) between protein

excretion and plasma lactate. These findings demonstrate that supramaximal intensity races (400 and 800 meter) produce the greatest protein clearance, indicating increased glomerular permeability, as well as tubular reabsorption limitations with increases in exercise intensity. The increased excretion of protein may have also contributed to the greater reductions in plasma volume with these events due to reductions in oncotic forces. Interestingly, excretion of creatinine was not altered with shorter and middle distance events, but the 1500 and 3000 meter runs both demonstrated reductions in urine creatinine, in a dose-dependent manner (Poortmans et al., 1996). These races also exhibited the greatest increases in plasma creatinine. It is likely that the reductions in creatinine clearance (i.e. glomerular filtration) occurred in the longer duration activities due to the length of time reductions in renal perfusion were present. While there were likely marked renal blood flow reductions with the 400 and 800-meter events, these races were short enough in duration that the glomerular filtration rate was not affected, but rather permeability increases (as evidenced by greater protein excretion) were possibly driven by higher blood pressures with these events (not measured). The findings of increased protein excretion without creatinine clearance alterations have also been demonstrated in women conducting one minute interval sprints (Poortmans & Vancalck, 1978). Regardless, increased protein excretion at higher intensities merely indicates the tubular cells of the kidneys were not able to meet the demands for reabsorption. However, the findings from 1500 and 3000 meter races provide greater evaluation of changes in renal function, as glomerular filtration was decreased with concomitant permeability increases and tubular reabsorption saturation for protein (Poortmans et al., 1996). These events only lasted between 5 and 12 minutes as well, which could provide greater challenges with longer events.

In addition to protein excretion and creatinine clearance, other markers of renal tissue stress have been investigated during exercise. During 400 and 3000-meter maximal effort running exercise, N-acetyl-β-D-glucosaminidase (NAG) and tissue-nonspecific alkaline phosphatase (TNAP) were significantly elevated above resting levels, with greater increases in the 400-meter run. Increases in these markers indicate changes to the proximal tubule cells, however, the extent the alteration in these related to kidney function is not well understood (Poortmans, Blommaert, et al., 1997). When evaluating increased expression of renal tubular enzymes, glomerular permeability should also be considered. Augmented glomerular permeability evidenced by increased total protein excretion also challenges the tubular reabsorption. Plasma proteins of high molecular weight, such as albumin, may saturate the renal tubular ability for reabsorption (Poortmans, Blommaert, et al., 1997). This may subsequently stress the cells of the proximal tubule, therefore eliciting the release of these tubular markers.

Junglee and colleagues (2012) also evaluated proteinuria inducing exercise (800-meter run) effects on NGAL production, demonstrating transient elevations in urinary NGAL, peaking at 25 minutes post-exercise and returning to baseline by two hours post-exercise. Interestingly, plasma NGAL levels slightly decreased following exercise, providing conflicting evidence regarding the expression of NGAL in response to a high intensity bout of exercise. However, all participants were well hydrated and performed the exercise bout only one time (Junglee et al., 2012). Therefore, it is difficult to ascertain whether elevations in urinary NGAL immediately post-exercise occurred due to plasma NGAL reductions (i.e. increased filtration and excretion of plasma NGAL) or increased expression of NGAL in the proximal tubule.

Urea and uric acid clearance also decrease significantly during exercise (Poortmans, 1984; Poortmans & Vanderstraeten, 1994). This results in a greater reabsorption for urea and

uric acid in the tubule, causing plasma elevations (Poortmans & Vanderstraeten, 1994). While likely unsubstantial, low urea may be linked to the formation of casts in renal tissue (Poortmans, 1984). Uric acid has also been implicated in the development of CKD with certain working populations (Johnson et al., 2016; Roncal-Jimenez et al., 2016).

Marathon, ultramarathon, and triathlon races also provide a unique model for evaluation of renal function due to the long duration of exercise. Protein excretion in the urine during marathon running has been shown to be elevated relative to pre-race values, however, total serum protein remained unchanged relative to pre-race values (Poortmans & Haralambie, 1979). The day following the race also revealed a decreased total serum protein compared to pre-race and race values, yet urinary protein was only slightly elevated above baseline (57 vs 50 µg/min). These findings indicate that marathon running only slightly increases glomerular permeability, however, filtration was not assessed. Poortmans et al. (2015) also evaluated the renal response after each event of a half triathlon (swim, cycle, run). Interestingly, the total protein excretion was the greatest (~10-fold increase above baseline) after the first event (i.e. swimming), with ~2-3 fold elevations above baseline during the two subsequent events. In line with findings from previous work (Poortmans et al., 1996), the plasma lactate levels were also the greatest following the swim, indicating that the greatest excretion of protein occurred with the highest intensity activity. Urine creatinine was decreased continuously throughout the triathlon with greatest reductions in the last event, however, there were no changes in plasma creatinine throughout the event (Poortmans et al., 2015). Thus, the glomerular filtration may have been maintained, despite likely perfusion reductions. It should be noted that the environmental conditions were cool ~16°C, therefore thermal stress and dehydration may have been minimal.

Findings from 2001 Boston Marathon runners demonstrated only minimal increases in SCr from prerace values (4 hours post-, 1.3 vs pre-race 1.0 mg/dL) (Kratz et al., 2002). Further, total protein only increased by 0.3 g/dL four hours post-marathon and returned to baseline values within 24 hours (Kratz et al., 2002). Therefore, renal function was stressed, yet only transiently by marathon running in cool weather conditions. Clarkson (2007) suggested that acute renal failure in marathon runners generally requires a cumulative effect of several physiological and environmental factors (i.e. rhabdomyolysis, heat stress, dehydration) concomitant with prior illness or medication use (e.g. viral infection or non-steroidal anti-inflammatory drugs).

In contrast, Mansour and colleagues (Mansour et al., 2017) recently assessed biomarkers of renal function and cellular stress immediately and 24-hours following the Hartford marathon (race temperature ~16°C). Per AKIN criteria, 82% of runners developed stage one AKI, with one runner developing stage two AKI. SCr, urinary albumin, and biomarkers of renal stress and inflammation (NGAL, IL-18, IL-6, TNF-α) were all significantly elevated immediately following the race, however, by 24-hours post-race, these markers had returned to baseline or near-baseline levels (Mansour et al., 2017). Interestingly, KIM-1 remained significantly elevated 24-hours post-race, potentially indicating a supporting role of this biomarker in cellular repair. It should be noted that the elevations in KIM-1, even immediately post-race, were minor compared to reference ranges for AKI, thus the extent of the damage or stress expressed by this marker should be interpreted with caution.

McCullough et al. (2011) mirrored the findings of transient elevations in biomarkers of AKI and renal function (i.e. NGAL, KIM-1, SCr, CyC) following a marathon in a cool climate (~1°C). Approximately 40% of runners also met the criteria for stage one AKI per AKIN, however, no runners were identified for stages two or three (McCullough et al., 2011). The

cooler climate in this investigation may explain the reduction in AKI occurrence compared to Mansour et al. (1°C vs 16°C). In a slightly warmer (24-28°C) 100 km ultramarathon, 22 of the 26 study participants demonstrated at least stage one AKI, with significant increases in SCr and NGAL. However, by one day post-race, the SCr values had already returned to near baseline (Kao et al., 2015). Lippi et al. (2012) also demonstrated acute elevations in NGAL and creatinine following an ultramarathon race completed in high humidity (54-87%) albeit cooler temperatures (6-8°C).

The long term consequences of marathon and ultramarathon running are relatively unknown, though it has been suggested that completing ultramarathon running does not impact future renal responses (Hoffman & Weiss, 2016). Hoffman et al. (2016) found renal responses following an ultramarathon race did not differ from those in subsequent races. Interestingly, individuals who experienced marked elevations in SCr in the first race also experienced similar magnitude increases in the race the following year (Hoffman & Weiss, 2016). A key finding, however, is that participation in ultramarathon running did not cause more severe renal responses during subsequent races. Therefore, it is possible that the transient elevations in renal biomarkers merely reveal a stressed kidney, and, as such, indicate a natural recovery process. These findings are limited to ultramarathon runners as the physiological and cardiovascular fitness is much different than in other sports (e.g. soccer, American football). Bongers et al. (Bongers et al., 2017) also showed elevations in urinary markers of renal stress after one and three days of long distance walking at a light intensity (average heart rate = 112 bpm). Further, the intensity may have been light enough to minimize reductions in renal perfusion and subsequent kidney stress, as the biomarker increases were relatively low and likely not indicative of serious complications.

Resistance exercise also impacts renal function, with SCr increases and estimated GFR decreases demonstrated up to 72 hours post-exercise (Machado et al., 2012). A strong correlation (-0.92) was found between changes in estimated GFR and changes in serum creatine kinase (Machado et al., 2012). This is in contrast to other work (Clarkson, Kearns, Rouzier, Rubin, & Thompson, 2006), demonstrating no relationship ($r = 0.23$) between SCr and creatine kinase following exercise induced muscle damage. It should be noted that Clarkson et al. (2006) conducted elbow flexor exercises, whereas Machado et al. (2012) conducted resistance exercises typically performed by athletes. The difference in muscle mass engaged, as well as exercise duration and intensity, may explain variations in these findings. Additionally, the practical implications for the Clarkson et al. findings that SCr is not directly impacted by creatine kinase elevations are limited, as most athletes are not conducting exercise on a unilateral single muscle group (i.e. elbow flexors). The participants in this study were also well hydrated with no environmental stress or prior exercise, not commonly experienced in athletics.

Regular resistance training may also impact renal health. A murine model assessing renal outcomes after 12 weeks of high intensity training compared with no training found lower plasma creatinine levels with high intensity exercise (Aparicio et al., 2014). Interestingly, negative morphological renal effects were found in the high intensity exercise intervention, which the authors suggest could lead to long-term kidney disease (Aparicio et al., 2014). Unfortunately, there is little epidemiological evidence to support the negative aspects of this hypothesis. Apoptosis of renal tubular cells has been shown following exercise to exhaustion in rats, however, regular endurance training reduced the number of apoptotic cells compared to a sedentary group following exhaustive exercise (Podhorska-Okolow et al., 2007). These results suggest that the type of exercise training program may affect the outcomes of renal health, yet

further evaluation is necessary to ensure individuals completing long-term high intensity exercise are not increasing risk for renal disease.

Renal Function with Passive and Active Heat Stress

Increases in global temperatures have resulted in heat waves, subsequently increasing the thermoregulatory strain in populations across the world (Glaser et al., 2016; Kjellstrom, Butler, Lucas, & Bonita, 2010). In Florida from 2005 to 2012, there were nearly 24,000 heat related illnesses not related to work treated in the emergency department (Harduar Morano, Watkins, & Kintziger, 2016). This is a rate of 33.11 visits per 100,000 person-years (Harduar Morano et al., 2016). Analysis from 12 years of hospital admissions in South Australia, revealed increases in hospital admissions during heat waves for renal disease and acute renal failure (IRR; 1.13 and 1.25, respectively) (Hansen et al., 2008). Further, prolonged occupational exposure to heat stress in Thailand led to CKD at odds 2.22 times greater than men without exposure (Tawatsupa et al., 2012). High skin temperature induced by heat stress causes significant impact on renal perfusion (Wilson, 2017). Similar to exercise, heat stress causes redistribution of blood flow away from vital organs such as the splanchnic and renal vasculatures (Radigan & Robinson, 1949; Rowell, Brengelmann, Blackmon, & Murray, 1970; Wilson, 2017). Rowell and colleagues demonstrated progressive decreases in renal blood flow as skin temperature and rectal temperature increased via passive heating (Rowell et al., 1970). Hales et al. (1979) confirmed these findings with reduced renal blood flow by ~27% during passive heat stress in baboons.

Hyperthermia has also been found to induce heat shock protein 72 upregulation in renal cells (Borkan, Emami, & Schwartz, 1993; Emami, Schwartz, & Borkan, 1991). This is thought to occur as a mechanism to induce thermal protection for subsequent bouts of heat stress (Borkan et al., 1993; Emami et al., 1991). Further, this may be beneficial to prevent mitochondrial

function impairments that can occur with extreme levels of heat stress (Borkan et al., 1993). The upregulation of heat shock proteins has also shown to be promising in reducing the negative effects of ischemia/reperfusion injuries (Harrison et al., 2008).

The addition of heat stress to exercise provides a further challenge to maintain cardiac output in the face of cutaneous vasodilation and increased perfusion to active skeletal muscle. As such, blood flow to the splanchnic and renal vasculature may be further attenuated (Radigan & Robinson, 1949; Rowell, 1974). Radigan and Robinson reported renal plasma flow and GFR decreases of 38% and 25% from resting values, respectively, during treadmill walking in a hot environment (50°C) (Radigan & Robinson, 1949). Renal function following a soccer match played in 27°C was also found to be compromised with significant elevations in SCr and substantial reductions in estimated GFR (Colombini, Machado, Lombardi, Lanteri, & Banfi, 2014). Schlader et al. (2017) recently evaluated different durations of treadmill walking (two 20 minute bouts vs three 20 minute bouts) in the heat on biomarkers of renal function. The authors revealed greater changes in plasma NGAL and augmented creatinine responses during the long protocol (Schlader et al., 2017). In congruence with previous literature from marathon and ultramarathons, the NGAL and creatinine values returned to baseline by 24 hours post exercise (Schlader et al., 2017). The authors also demonstrated a weak, but significant relationship ($r = 0.32$), between core temperature and plasma NGAL (Schlader et al., 2017). This could suggest that higher body temperatures, which in this protocol, increased with protocol duration, may be a contributor to the extent of NGAL expression. This hypothesis also fits with data from our own laboratory regarding a relationship between NGAL and exercise finishing time at an endurance-cycling event (100 km or 100 mile) in the heat. As exercise in the heat may induce an ischemic-like event in the kidneys, the duration of exercise may elicit greater stress on the tubules.

A common risk with exercise in the heat is the development of exertional heat illness, with exertional heat stroke representing the most severe condition (Casa et al., 2015b; Leon & Bouchama, 2015). If treated improperly, heat stroke can result in multiple organ failure, renal compromise, and potential fatality (Leon & Bouchama, 2015; Leon & Helwig, 2010; Sawka et al., 2011). Furthermore, heat stroke is commonly associated with a systemic inflammatory response, thought to potentially leading to the development of multiple organ dysfunction or failure (Leon & Bouchama, 2015; Leon & Helwig, 2010). One factor thought to induce this inflammatory response is the release of endotoxins (e.g. lipopolysaccharide, LPS) into the blood from the intestinal tract caused by severe reductions in splanchnic blood flow (Leon & Helwig, 2010). The impact of LPS on renal function has also received investigation in the absence of heat stroke. In rats injected with LPS, SCr was significantly elevated from three to 12 hours post-injection, meeting guidelines for AKI (Han, Li, Liu, & Cong, 2012). Further, at three hours and six hours post-administration, plasma NGAL and urinary NGAL reached peak values, indicating stress in the proximal tubule (Han et al., 2012). Interestingly, TNF-α mRNA were strongly correlated with NGAL mRNA ($r = 0.99$), suggesting that the upregulation of NGAL following sepsis may be regulated by a TNF-α cytokine response (Han et al., 2012). However, NGAL was not related to IL-6 expression, which has been shown in other models of AKI (Han et al., 2012; Junglee et al., 2013).

In addition to dynamic exercise, the effects of forearm heating on renal vascular responses to static handgrip exercise have also been evaluated (Kuipers, Sauder, Kearney, & Ray, 2007). The exercise-induced reductions in renal blood flow velocity were augmented with forearm heating, potentially indicating a greater activation of the exercise pressor reflex with heat stress. The authors suggested the increased renal vasoconstriction with heating likely

occurred due to enhanced mechanoreceptor sensitivity, as post-exercise ischemia (i.e. metaboreceptor stimulation) did not increase renal vasoconstriction (Kuipers et al., 2007). In contrast, cooling the forearm reduced vasoconstriction in the renal vasculature via a dampened metaboreflex response (Kuipers et al., 2007).

Influence of Hydration on Renal Function

Hydration also affects renal structure and function both acutely and chronically. Dehydration is defined as the process of losing total body water and can be divided into extracellular (e.g. diuretics, diarrhea) and intracellular (e.g. thermoregulatory sweating) deficits (Cheuvront & Kenefick, 2014). Common measures of dehydration include blood (serum or plasma), urine, and body mass, however, the proper assessment depends largely on the mechanism of fluid loss (Cheuvront & Kenefick, 2014). Regardless, deficits in total body water, acute and chronically, necessitate return to homeostasis through fluid retention strategies via renal mechanisms. As such, proper renal function with suboptimal hydration is of serious concern to prevent negative consequences related to physiological, performance, or health outcomes.

Acutely changing an individual's drinking pattern to high fluid volumes has been shown to reduce the kidney's ability to concentrate urine following subsequent fluid deprivation (DE WARDENER & HERXHEIMER, 1957). Short-term dehydration (60 hours) in rats has been shown to upregulate aquaporin-2 mRNA expression, sodium chloride creatine transporter mRNA expression, and creatine uptake compared to a water loaded animal (Garcia-Miranda, Peral, & Ilundain, 2010). In clinical settings, the use of early hydration has been found to reduce the incidence of contrast-induced AKI (Rihal & Kashani, 2011).

Heat stress following 48 hours of water deprivation in rats was shown to induce substantial reductions in renal and mesenteric blood flow (Massett, Johnson, & Kregel, 1996). Interestingly, the change in renal blood flow during heating in the euhydrated rats were greater than those in the 48-hour water deprivation trials. The altered pressor response following water deprivation may have been due to adrenergic receptor sensitivity or lower cardiac output (Massett et al., 1996).

Smith et al. (1952) conducted early work evaluating the influence of dehydration on renal function during treadmill walking in the heat. High ambient temperatures induced marked reductions in GFR and renal plasma flow, with greater attenuations found when work was conducted in the heat while dehydrated (Smith et al., 1952). This work was pivotal in demonstrating that dehydration during exercise in the heat induces substantial GFR and renal blood flow reductions, with some greater than 50% of those during exercise in a cool environment (Smith et al., 1952). These interruptions in renal plasma flow may have a substantial impact on the ischemic response to exercise. The response noted by Smith and colleagues were during light activity as well, therefore it would be expected that a graded decrease in renal blood flow during dehydration would occur as exercise intensity increases. Melin et al. (1997) also evaluated the influence of dehydration on renal responses during one hour of treadmill walking in the heat. Compared with a euhydrated control trial, dehydration reduced creatinine clearance, urine volume, and free water clearance.

Method of dehydration also impacts renal and hormonal responses (Melin et al., 2001). Melin et al. (2001) evaluated similar levels of dehydration induced by passive heating or exertional hyperthermia, demonstrating greater plasma levels of renin and aldosterone as well as larger reductions in creatinine clearance with exercise induced dehydration. The reduction in

creatinine clearance likely contributed to the reduced osmolar clearance and free water clearance with exercise (Melin et al., 2001). Although not directly measured, the renal hormonal and filtration responses suggest a greater reduction in renal blood flow during the exercise trial compared to passive heating.

Renal GFR during recovery from dehydrating exercise is also significantly reduced, however by 240 minutes post-exercise GFR had returned to baseline values (Stachenfeld, Gleim, Zabetakis, & Nicholas, 1996). Subsequently, urine volume was also reduced from the end of exercise through 240 minutes of recovery, even though the participants had consumed to within 1% of their baseline mass (Stachenfeld et al., 1996). Interestingly, a gender comparison revealed males to have slower recovery of aldosterone at one and two hours post exercise. Yet, males also had greater osmolar clearance than females at two hours post exercise (Stachenfeld et al., 1996). These findings suggest an influence of gender (potentially oestrogen driven) on the hormonal regulation of fluid post dehydrating exercise (Stachenfeld et al., 1996).

Beverage choice during rehydration following exercise induced dehydration also impacts the renal functionality. Kamijo et al. (Kamijo et al., 2012) provided participants with high carbohydrate, low carbohydrate, or control following mild dehydration induced by exercise in the heat. The consumption of a high carbohydrate beverage, increased sodium reabsorption in the kidney and decreased urine volume. Interestingly, GFR (assessed by inulin) was greater in the high carbohydrate trial during and immediately after drinking. The authors suggest that this increase was due to greater insulin with the high glucose concentration, inducing vasodilation and thus increased blood flow through the glomerulus (Kamijo et al., 2012). Further, the increased sodium reabsorption was suggested to occur via insulin stimulation in the proximal tubule (Kamijo et al., 2012).

Acute intense exercise also enhances proximal tubular sodium reabsorption during the following day (Nagashima, Wu, Kavouras, & Mack, 2001). The reabsorption of sodium then aids to enhance plasma volume expansion. This is potentially due to reduced renal blood flow driving a decreased hydrostatic pressure in the peritubular capillaries (Nagashima et al., 2001). These findings provide a mechanism for improvements in plasma volume expansion with endurance exercise training.

Hydration is also suggested to play a role in the development and progression of CKD (Clark et al., 2016; Kuwabara et al., 2017). A recent retrospective analysis of over 12,000 subjects over a 5 year period evaluated the effect of elevated serum sodium, a potential indicator of inadequate hydration, on CKD development (Kuwabara et al., 2017). Regression analysis revealed every 5 mmol/L increase in serum sodium was associated with an 18% increase in risk of CKD (Kuwabara et al., 2017). Roussel et al. (2014) evaluated the relationship between copeptin or vasopressin and CKD in a sample of ~2300 participants. The authors determined a strong relationship between AVP and copeptin, supporting the use of copeptin as a surrogate of AVP (Roussel et al., 2014). Elevated copeptin levels have also been related to greater prevalence of renal cyst formation and number of cysts (Ponte et al., 2015). Using water restriction to induce recurrent dehydration in spontaneously hypertensive rats has recently been found to increase renal fibrosis, pro-inflammatory cytokine release and urinary NGAL levels (Hilliard et al., 2016). These findings support the work from Bouby et al. (Bouby et al., 1990), augmenting the progression of CKD as well as hypertension with poor drinking habits (Hilliard et al., 2016).

Modifiers of Exercise Induced Muscle Damage

Exercise induced muscle damage may range from asymptomatic increases in damage biomarkers to exertional rhabdomyolysis requiring medical attention. Subclinical rhabdomyolysis can easily be treated with rest and hydration (Tietze & Borchers, 2014). Rhabdomyolysis is characterized as the breakdown of skeletal muscle, resulting in the release of intracellular components such as creatine kinase and myoglobin in the circulation (Tietze & Borchers, 2014). Exertional rhabdomyolysis is characterized as an overuse injury, often due to unaccustomed exercise. Exertional rhabdomyolysis is typically defined as a creatine kinase response greater than 5 to 10 times the upper limit or values >1000 U/L (Tietze & Borchers, 2014).

While creatine kinase may not be an ideal marker of muscle damage, it does provide indication that there is a disturbance to the skeletal muscle. This information is important given the presence of creatine kinase elevations and possible hospitalizations for rhabdomyolysis in preseason and offseason athletes, military settings, and working populations, both with and without exertional rhabdomyolysis (Aizawa, Morita, Minami, Sasaki, & Tobise, 1995; Bhalla & Dick-Perez, 2014; M. A. Cleary, Sadowski, Lee, Miller, & Nichols, 2011; Ehlers et al., 2002; Galvez, Stacy, & Howley, 2008; Hummel, Gregory, Desai, & Diamond, 2016; Kahanov, Eberman, Wasik, & Alvey, 2012; Smoot et al., 2013; Smoot et al., 2014; "Update: Exertional rhabdomyolysis, active component, U.S. Armed Forces, 2011," 2012). Creatine kinase responses are criticized in clinical diagnosis of exertional rhabdomyolysis as the values exhibit large inter-individual variabilities, even when individuals perform similar exercises (Lin, Chie, & Lien, 2006). Individuals completing a 246 km road race demonstrated dramatic increases in

creatine kinase (>43000 U/L), yet were essentially asymptomatic for exertional rhabdomyolysis and did not require clinical treatment (Skenderi et al., 2006).

Castellani et al. (2016) conducted eccentric elbow flexor exercise with and without heat stress, demonstrating that muscle temperature >40°C does impact injury biomarkers or skeletal muscle strength assessment. However, these findings are limited as only skeletal muscle temperature was increased (via short-wave diathermy), which would not occur in exercise settings without core body temperature increases as well.

Hydration impacts resistance exercise performance, however has no impact on circulating markers of muscle damage (i.e. creatine kinase and myoglobin) with exercise (Yamamoto et al., 2008). Moderate hypohydration (5%) was successful at inducing slight increases in myoglobin one and two hours post-exercise, however, total work was not affected compared to an euhydrated condition. In contrast, work in wrestlers found the greatest creatine kinase levels in those who reported the greatest body mass change to compete in a certain weight class (Ozkan & Ibrahim, 2016). These data were purely observational, and body mass changes were reported by the athletes. The impact of dehydration on anaerobic exercise performance remains somewhat controversial. An excellent review of the literature on this topic, concluded that the overall effect of dehydration on muscular strength and power was negative (Judelson et al., 2007).

Finally, dehydration combined with hyperthermia may impact recovery from muscle damaging exercise (M. A. Cleary, Sweeney, Kendrick, & Sitler, 2005). Cleary et al. (2005) investigated the effects of fluid restriction on delayed onset muscle soreness (DOMS) recovery from downhill running in the heat. Perceptions of muscle pain were found to be significantly elevated 24- and 48 hours post exercise, with nonsignificant elevations through 96 hours post exercise (M. A. Cleary et al., 2005). Overall perceptions of pain were elevated throughout the 96

hour recovery (M. A. Cleary et al., 2005). However, there were no differences between trials in strength decreases, indicating no impacts on the severity of muscle damage. Interestingly, when the downhill running was performed in a thermoneutral environment, the effects of dehydration on DOMS were ameliorated (M. A. Cleary, Sitler, & Kendrick, 2006). Therefore, the addition of hyperthermia with concomitant dehydration may impact perceptions of skeletal muscle recovery, yet, the authors did not evaluate biomarkers of muscle damage (creatine kinase levels) at any time point. In organized sport, when compared with a normothermic soccer match, there was no impact of heat stress on markers of muscle damage recovery, however perceptions were not assessed (Nybo et al., 2013). Furthermore, this was post-soccer match therefore, the hydration and damage responses may have differed significantly between individuals. Regardless, the impact of muscle damaging exercise with concomitant dehydration may exacerbate symptoms of DOMS, potentially due to delayed recovery induced via hyperthermia and dehydration.

Renal Function with Exercise-Induced Muscle Damage

Exertional rhabdomyolysis is the breakdown of skeletal muscle, causing the release of cellular contents, such as creatine kinase and myoglobin (a nephrotoxic substance), into circulation (Huerta-Alardín, Varon, & Marik, 2005). Acute renal failure with exertional rhabdomyolysis has been well documented. Athletes, soldiers, or workers completing unaccustomed strenuous activity induce significant muscle damage which then overwhelms the kidneys, potentially inducing AKI (Bach & Clement, 1980). Furthermore, the presence of heat stress and dehydration often create the "perfect storm" to accentuate the severity of the muscle damage and prompt AKI (Clarkson, 2007; M. Cleary, Ruiz, Eberman, Mitchell, & Binkley, 2007; Kodama et al., 1985). This is often transient with aggressive fluid resuscitation and rest resulting in full recovery of function after the event (M. Cleary et al., 2007). In certain cases,

however, rhabdomyolysis severity may induce significant AKI resulting in renal replacement therapy to return renal function. The presence of genetic predispositions, such as sickle cell trait, also facilitate the risk of rhabdomyolysis and AKI, therefore, precautions are necessary to prevent negative health outcomes in challenging physiological and environmental conditions (Anzalone et al., 2010; O'Connor et al., 2012)

The risk of exertional rhabdomyolysis is of concern for athletes, particularly those completing unaccustomed exercise. However, the impact of subclinical rhabdomyolysis (i.e. low-grade muscle injury) on renal function and structure has received considerably less attention as this event does not require medical attention and likely goes unnoticed by medical professionals. After exercise, creatine kinase levels in the blood can rise considerably without any clinical symptoms, making this marker rather unreliable in the assessment of risk for immediate negative renal outcomes (Clarkson & Eichner, 2006). Furthermore, this suggests that, for the development of AKI, other stressors (i.e. environmental heat, dehydration) are necessary to compromise renal function (Clarkson & Eichner, 2006). Renal stress induced by subclinical rhabdomyolysis with concomitant heat stress and dehydration may have substantial impact on AKI and the development of CKD.

Knochel and colleagues evaluated military recruits undergoing physical training at Fort Sam Houston in San Antonio, Texas during hot, warm, and cool weather (Knochel et al., 1974). Uric acid excretion continuously increased through study day 11 in recruits while creatinine clearance fell during the initial days of observation, before returning to basal levels during training in the hot weather. Further, urine volume in hot weather was nearly half the production in cool weather, while urine creatinine initially decreased through day four, but then steadily increased through day 25 of training (Knochel et al., 1974).

The assessment of AKI in military recruits was also assessed by another study (Schrier et al., 1970). The findings demonstrated similar creatinine elevations to severe exercise at 10 days and three weeks of training. The authors showed similar increases in uric acid at these time points (Schrier et al., 1970). Further, the authors provided a schematic suggesting contributing mechanisms leading to acute renal failure following exercise in the heat. Interestingly, this model was proposed in 1970, yet the recent rise in CKD associated with Mesoamerican sugarcane workers has revealed a model that provides nearly identical mechanisms (dehydration, heat stress, physical exertion, subclinical rhabdomyolysis) for the development of AKI and CKD in this population (Johnson et al., 2016; Roncal-Jimenez et al., 2016; Schrier et al., 1970).

To date, there have been few controlled laboratory investigations of the cumulative effects of dehydration, heat stress, and muscle damage on renal function during and following exercise. Junglee and colleagues investigated the renal responses to exertional hyperthermia following 40 minutes of moderate intensity treadmill running in the heat with muscle damage previously induced via downhill running (Junglee et al., 2013). Compared with the no muscle damage trials, exercise in the heat following muscle damage elicited elevations in urinary NGAL, plasma NGAL, and SCr. The hydration status of individuals was not well controlled, likely resulting in minor dehydration (1-2%) in these individuals. Additionally, no follow-up measures were collected, therefore it is difficult to ascertain whether these responses followed a similar transient path shown in marathon running (Mansour et al., 2017; McCullough et al., 2011). Thus, the impact of dehydration with muscle damage during exercise in the heat remains a heretofore unstudied phenomenon.

Unfortunately, the long-term impact of low-grade muscle injury on the development of CKD is relatively unknown in athletic or working populations in the United States. Agricultural

workers in central California demonstrated renal dysfunction after a single shift (Moyce et al., 2016). Using SCr measurements before and after a work shift, 11.8% of the 295 workers assessed met the criteria for stage one AKI (Moyce et al., 2016). Mesoamerican sugarcane workers represent one such population that regularly experiences subclinical rhabdomyolysis with extreme heat stress and dehydration (García-Trabanino et al., 2015; Glaser et al., 2016; Paula Santos, Zanetta, Terra-Filho, & Burdmann, 2015; Peraza et al., 2012; Roncal-Jimenez et al., 2016). The recent rise in CKD in Mesoamerican sugar cane workers has been largely related to physiological responses to environmental working conditions (Bodin et al., 2016; Crowe et al., 2015; García-Trabanino et al., 2015; Laws et al., 2015, 2016; Roncal-Jimenez et al., 2016). These workers experience progressive dehydration throughout the work day through elevated urine specific gravity and decreased glomerular filtration rates (García-Trabanino et al., 2015; Roncal-Jimenez et al., 2016; Wesseling et al., 2016). The combination of heat stress with strenuous exercise and gradual dehydration throughout the work day places a high demand on the kidneys to retain fluid while clearing excess waste from potential muscle damage. As GFR has been shown to decrease throughout the work day, the increased prevalence of CKD may be due to the additive effects of repetitive kidney stress from the concomitant environmental and physiological strain experienced by these workers (Bodin et al., 2016; Crowe et al., 2015; García-Trabanino et al., 2015; Laws et al., 2015, 2016; Peraza et al., 2012; Roncal-Jimenez et al., 2016). Cutters regularly experience uncompensable heat stress with wet bulb globe temperatures >30°C by 9:00 AM in coastland working areas (Bodin et al., 2016). Interestingly, coastland areas tend to have greater elevations in creatinine as well, suggesting a link to environmental heat stress (Peraza et al., 2012). Furthermore, metabolic loads in the Costa Rican sugarcane cutters have been estimated to be 261 W/m^2, which, using recommendations from the National

Institute of Occupational Safety and Health in Spain, is suggested to be conducted at a maximum wet bulb globe temperature of 26°C (Crowe et al., 2013). The characteristics of the heat stress experienced may apply to many other occupations as well, such as firefighters, military, agricultural and industrial settings.

Interestingly, implementation of a simple water (CamelBaks), shade, and rest break intervention by Bodin and colleagues in sugar cane workers in El Salvador, not only increased water consumption, but also improved worker productivity and reported symptoms of heat stress and dehydration (Bodin et al., 2016). However, renal function in these individuals was not evaluated therefore it is difficult to ascertain whether the increased fluid consumption or rest breaks resulted in substantial improvements in the development of CKD.

Beneficial Effects of Exercise on Renal Function

It should be clarified that the aim of this review is not to indemnify the beneficial effects of exercise on long term renal functionality. Following 16 weeks of treadmill running, blood pressure increases and renal function impairments were prevented in spontaneously hypertensive rats compared to their sedentary counterparts (Agarwal et al., 2012). Furthermore, the exercise training program prevented elevations in inflammatory markers such as TNF-α or NF-κB (Agarwal et al., 2012). Twelve weeks of resistance training in patients receiving hemodialysis was found to have no impact on circulating pro-inflammatory markers (e.g. TNF-α, IL-8) with a slight positive impact on anti-inflammatory markers (IL-6) associated with muscular adaptations (Cheema et al., 2011). Skeletal muscle growth induced in Akt-1 transgenic mice has also shown mitigation of renal damage, inflammation, and fibrosis following surgery (Hanatani et al., 2014). These findings are substantial as the benefit of skeletal muscle growth occurred independent of exercise. While clinically less applicable than an exercise model, the improvement in skeletal

muscle mass inducing renal protective effects, further supports the need for exercise and other benefits associated with exertion. Renal resistance artery sensitivity to vasoconstrictor stimuli was increased following an exercise training protocol in rats (Koçer et al., 2011).

A concern with elevations in kidney injury biomarkers or changes in renal function is the development of chronic kidney disease. Hiraki and colleagues utilized chronic kidney disease patients to evaluate the effects of an acute bout of moderate intensity treadmill walking on renal biomarkers (Hiraki et al., 2013). The authors demonstrated slight, but non-significant, increases in L-type fatty acid binding protein (L-FABP) with no changes in NAG or estimated GFR (Hiraki et al., 2013). Thus, moderate intensity treadmill walking in this population provides a safe modality for physical activity. These findings are also confirmed in healthy populations, with lower intensity exercise producing no changes in GFR (Poortmans & Vanderstraeten, 1994). The aforementioned return of renal blood flow shortly after ceasing moderate intensity exercise (Pricher et al., 2004) likely plays an important role in the ability for individuals to tolerate or recover renal function from exercise.

Cardiovascular and Thermoregulatory Responses to Exercise in the Heat

The challenge of exercising in the heat requires marked cardiovascular and thermoregulatory adjustments to maintain both core body temperature and mean arterial pressure (González-Alonso, Mora-Rodríguez, Below, & Coyle, 1997; González-Alonso et al., 2000; González-Alonso et al., 1999; Montain & Coyle, 1992; Montain, Latzka, & Sawka, 1995; Montain, Sawka, Latzka, & Valeri, 1998; Rowell, 1974; Sawka, Cheuvront, & Kenefick, 2012; Sawka et al., 2011). During the initial transition to exercise, feed-forward signals from central command and feedback from the exercise pressor reflex (i.e. mechanoreflex and metaboreflex) dictate an increase in heart rate and blood pressure to maintain perfusion of cerebral vasculature

and exercising tissue. Further, the cutaneous vasculature experiences marked vasoconstriction to aid in the maintenance of central venous pressure (i.e. cardiac filling). As exercise continues, especially in the heat, cutaneous blood flow and sweating increase as core temperature and skin temperature rise (Nadel, 1979; Nadel, Bullard, & Stolwijk, 1971; Rowell, 1974; Sawka et al., 2011). The redistribution of blood flow to the cutaneous vasculature causes a subsequent reduction in central blood volume. To maintain cardiac output, heart rate and cardiac contractility are increased, and blood flow to the splanchnic and renal vasculature is decreased (Rowell, 1974). As intensity increases during exercise in the heat, however, the competition for blood flow between the cutaneous and skeletal muscle vasculature induces greater cardiovascular strain. As a result, performance is attenuated, particularly during endurance events (Ely, Cheuvront, Roberts, & Montain, 2007; Sawka et al., 2011).

When dehydration is combined with exercise in the heat, there is substantial impairment in stroke volume due to blood volume reductions (González-Alonso et al., 2000; González-Alonso et al., 1999). Cardiac output is therefore reduced compared to a euhydrated state (González-Alonso et al., 1997). Further, there is a reduction in muscle blood flow which leads to sacrificed performance (Casa et al., 2010; González-Alonso et al., 1998). Dehydration has also been found to impair skin blood flow and sweating (González-Alonso et al., 2000). Therefore, there is a decreased ability to dissipate heat, resulting in elevated core temperatures during exercise as compared to a euhydrated state (Casa et al., 2010; Cheuvront & Kenefick, 2014; González-Alonso, Mora-Rodríguez, Below, & Coyle, 1995; González-Alonso et al., 1997).

Thermal strain is also increased with prior muscle damage (Fortes et al., 2013). Fortes et al. (2013) induced muscle damage via a downhill running protocol, followed by exercise in the heat either 30 minutes or 24 hours post-muscle damage. Interestingly, rectal temperature during

exercise in the heat was elevated above the non-muscle damage trial when exercise was completed 30 minutes post damage. There were no differences in sweat rate in any of the trials, however IL-6, an inflammatory cytokine, was significantly greater in the muscle damage trial versus no damage. Interestingly, when the exercise was performed 24 hours post muscle damage there were no differences as compared to no damage. Hence, the acute inflammatory immediately post-muscle damage may have led to the decreases in thermoregulation (Fortes et al., 2013). However, the authors did not assess skin blood flow, thus potential differences in heat losses via convective mechanisms may have contributed. There were also no differences in skin temperature, so it is likely that the cutaneous blood flow responses were similar between trials (Fortes et al., 2013). Additionally, the authors provided fluids during the muscle damaging exercise but not during exercise in the heat, allowing for potential hydration changes. Therefore, the impact of dehydration in thermal strain during exercise in the heat following exercise induced muscle damage has not been fully investigated.

Cardiovascular and Thermoregulatory Responses Following Exercise in the Heat

Upon completion of exercise, there is a marked decrease in sympathetic nervous system activity. This may induce post-exercise hypotension due to the continued vasodilation of skeletal muscle vasculature in combination with increases in blood flow to splanchnic and renal systems (Charkoudian, Halliwill, Morgan, Eisenach, & Joyner, 2003; Halliwill, 2001; Pricher et al., 2004). Interestingly, skin blood flow and sweating also decrease following exercise completion, despite continued elevations in core body temperature (Kenny et al., 2008; Kenny, Jay, & Journeay, 2007; Kenny et al., 2006). It has been suggested that these decreases in thermoregulatory mechanisms are largely baroreflex driven as adjusting body posture to the supine position (i.e. baroreflex loading) improves sweating and skin blood flow (Jay et al., 2008;

Kenny et al., 2008; Kenny et al., 2007; Kenny et al., 2006). As dehydration impairs thermoregulatory mechanisms, cardiovascular stability, and blood flow to the muscle during exercise, it has been shown that some of these mechanisms continue to be altered during immediate recovery as well (Charkoudian et al., 2003; Gagnon, Lynn, Binder, Boushel, & Kenny, 2012; González-Alonso et al., 1998; González-Alonso et al., 1997; González-Alonso et al., 2000; González-Alonso et al., 1999; McDermott, Casa, Lee, Yamamoto, Beasley, Emmanuel, Anderson, et al., 2013; McDermott, Casa, Lee, Yamamoto, Beasley, Emmanuel, Pescatello, et al., 2013). Lower mean arterial pressures were demonstrated when dehydrated following exercise, with greater effects reductions in trained individuals (Gagnon et al., 2012). Further, esophageal temperature was elevated by ~ 0.8°C in untrained and trained individuals with dehydration after 210 minutes of recovery from exercise (Gagnon et al., 2012). However, esophageal temperature at the end of exercise was greater with dehydration than the euhydrated condition. Consequently, the rate of passive cooling may have been similar. Our laboratory has demonstrated small but significant reductions in cooling rates during cold water immersion with mild dehydration (Butts et al., 2016). McDermott et al. also revealed a slowed recovery rate of rectal temperature when dehydrated compared to conditions of post-exercise rehydration (McDermott, Casa, Lee, Yamamoto, Beasley, Emmanuel, Anderson, et al., 2013). Interestingly, skin temperature was similar between dehydrated and rehydrated individuals, suggesting a possible mechanism that would lead to similar skin blood flow responses.

Lynn and colleagues found a slower core temperature recovery with elevated skin blood following exercise in the heat without fluid replacement (Lynn, Minson, & Halliwill, 2009). As hyperosmolality associated with dehydration is shown to induce alterations in skin blood flow and sweating, it is possible that this mechanism would continue to alter thermoregulatory

responses post-exercise (Charkoudian, 2010; Charkoudian et al., 2003; Fortney, Wenger, Bove, & Nadel, 1984; Shibasaki, Aoki, Morimoto, Johnson, & Takamata, 2009). These responses were only compared to compared to fluid and no fluid replacement exercise bouts in thermoneutral environments, thus the impact of elevated skin and core temperature on these responses cannot be stated (Lynn et al., 2009). Paull et al. (2016) recently assessed the effects of plasma osmolality on heat loss mechanisms post-exercise (i.e. skin blood flow and sweating) using hypertonic and isotonic saline infusion during exercise. Interestingly, hypertonic saline during exercise resulted in a slower recovery of skin temperatures, with elevations through 60 minutes. However, there were no differences in skin blood flow, esophageal temperature, or local sweat rate between isotonic and hypertonic saline infusion. Conversely, hypertonic saline resulted in lower sweat rates on the upper back and chest. Thus, the effects of hyperosmolality appear to moderately impact heat loss mechanisms post-exercise. However, the post-exercise responses of skin blood flow and sweating when fluid replacement during exercise was provided by oral drinking compared with no drinking has not been exclusively investigated.

Summary

There is overwhelming evidence to suggest a negative role of dehydration in renal function, thermoregulation, performance, and cardiovascular stability. The concomitant exposure to physiological (i.e. exercise, muscle damage) and environmental (high ambient temperature and humidity) stressors commonly experienced by athletes, military, and occupational populations may augment the deleterious responses to dehydration. The recent use of novel biological markers (e.g. NGAL, KIM-1) to evaluate renal function during and following exercise has also provided the ability to evaluate stress placed on renal components outside of the glomerulus, further detailing the response of renal health with exertion. However, the role of

optimal hydration during exercise on renal function and the expression of AKI biomarkers, has not been exclusively investigated. Elucidating the acute effects of dehydration with concomitant exercise, muscle damage, and heat exposure may provide insight for long-term consequences in populations regularly exposed to these combined stressors.

III. Methods

Eighteen healthy, recreationally active males (age 24 ± 5 y, wt 75.9 ± 10.0 kg, ht 1.79 ± 0.05m, body fat 17.3 ± 6.2%, VO_2max, 51.0 ± 6.0 ml/kg/min) were recruited from the University and surrounding areas to participate in this randomized, crossover, counterbalanced design study. All procedures were approved by the University Institutional Review Board and written informed consent was acquired from all individuals prior to participation. Participants completed five total visits including one familiarization day and two experimental days (one hypohydrated, HY, and one euhydrated, EU) each with 24-h follow-up visits. Experimental visits were separated by ≥28 days (average; 41 ± 16 days) to allow for muscle damage recovery and prevent acclimation to the heat. Exclusionary criteria included previous heat exhaustion or heat stroke within the past 3 years, current musculoskeletal injury, hypertension where vigorous exercise is contraindicated, diagnosed sickle cell trait, use of medications that may alter thermoregulation or kidney function, current use of creatine supplementation, and a history of kidney disease. All participants were asked to refrain from alcohol use for 24-h, caffeine use for 12-h, resistance training for 5-days and exercise for 24-h prior to each trial. Body composition was assessed via dual energy x-ray absorptiometry (DXA, Lunar Prodigy, General Electric, Madison, WI, USA).

Familiarization Day:

During the initial familiarization visit, participants signed an informed consent form and completed a medical history questionnaire. Upon approval, baseline demographic information was collected and body composition assessed via DXA. Participants then completed a five-minute warm-up on a cycle ergometer (~50W) and were fitted to the isokinetic dynamometer (Biodex System 3, Biodex Medical Systems, New York) with seat and leg positions recorded for

future testing. Baseline isometric strength at 70° and 90°, and isokinetic strength at 60°·sec-1 were competed in triplicate. Because these were used as a familiarization, these measures were not included in analysis. Participants then underwent a graded maximal exercise test on a treadmill to assess maximal oxygen uptake (VO_2max) to establish exercise intensities for subsequent trials. The test consisted of a constant jogging speed (selected by the participant) with grade increasing by 2% every two minutes until volitional exhaustion.

Experimental Days:

Prior to arrival, participants completed three-day euhydrated baseline body weights with a provided scale (BalanceFrom High Accuracy Bathroom Scale, BalanceFrom LLC, China) for both trials, as well as a diet record on a standard log for 24-h prior to each trial. Additionally, participants collected all urinations for the 24-h prior to the start of trial, which was subsequently analyzed for 24-hr urine osmolality (freezing point depression, Model 3250, Advanced Instruments Inc., Norwood, MA). For the HY trial, the dehydration protocol consisted of 24-h fluid restriction in addition to fluid restriction during the protocol. Prior to the euhydrated protocol, participants were instructed to consume fluids prior to arrival, while water was provided during the trial to ensure less than 2% body mass loss.

Upon arrival, participants completed a 24-h history, provided a spot urine sample, and completed a nude body mass. The 24-h urine collection and spot urine were analyzed for urine specific gravity (refractometer, model Master-SUR,NM, Atago Co Ltd, Tokyo, Japan) and osmolality (freezing point depression, Model 3250, Advanced Instruments Inc., Norwood, MA) to confirm hydration status. Spot urine was also stored for later assessment of the acute kidney injury biomarker, (UNGAL) and creatinine (UCr) via their respective assays. Participants were provided a rectal thermometer (RET-1, Physitemp Instruments Inc, Clifton, NJ, USA) to insert

~15 cm past the anal sphincter to assess rectal temperature (T_{re}). Participants were also instrumented with a Polar heart rate monitor (FT1/T31, Polar Inc, Lake Success, NY, USA), automated blood pressure cuff (Tango+, Suntech, Medical Inc., Morrisville, NC, USA) and skin temperature thermochrons (iButton, Maxim Integrated, San Jose, CA, USA) to assess four-site mean weighted skin temperature (T_{sk}) (Ramanathan, 1964). Participant attire consisted of running shorts, socks, and shoes. prior to arrival will also be analyzed.

Participants completed a 20-minute semi-recumbent baseline rest in a thermoneutral environment (~20°C). During this time, participants were informed on the perceptual scales for rating of perceived exertion (RPE) (Borg, 1970), thermal sensation (Toner, Drolet, & Pandolf, 1986), and perceived thirst (Engell et al., 1987), as well as visual analog scales to identify overall and leg-specific muscle pain. Following the 20 minutes of rest, baseline physiological and perceptual measures were recorded and a baseline blood draw via venipuncture was conducted to collect serum and sodium heparin plasma vacutainers (BD, Ontario, Canada

Participants moved to a cycle ergometer (Monark 828E, Monark Exercise AB, Sweden) to complete a 5-minute warm-up at 50W before completing the muscle damaging protocol on an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems, New York) (Xin, Hyldahl, Chipkin, & Clarkson, 2014). The muscle damaging procedure involved unilateral eccentric knee extension exercise, with the contralateral leg utilized during the second trial to minimize the potential repeated bout effects. The leg used during the first trial was randomized and counterbalanced for hydration and dominance between participants. Prior to, and immediately following the eccentric protocol, isometric strength (i.e. peak torque) measures were performed via three 5-second maximal voluntary isometric knee extensor contractions at 70° and 90° knee flexion with one minute of rest between repetitions. Isokinetic knee extensor strength (i.e. peak

torque) was also completed in triplicate at 60°·sec^{-1}. For each trial, participants completed 10 sets of 10 eccentric maximal effort knee flexion repetitions at a speed of 30°/s with sets separated by one-minute (Xin et al., 2014). Perceptions of muscle pain and RPE were recorded following the exercise induced muscle damage. Decreases in strength, as well as elevations in SCK and muscle pain, served as indices of muscle damage (Damas, Nosaka, Libardi, Chen, & Ugrinowitsch, 2016). While it is recognized that there are contralateral adaptations following single leg eccentric exercise, the 4 weeks trial separation should minimize many of the responses. Xin et al. (2014) demonstrated no differences in muscle soreness or creatine kinase following contralateral eccentric exercise separated by 4 weeks, while isometric strength was lower in the second bout from 24 to 96 hours post exercise. Further, the purpose of the eccentric exercise protocol was to induce mild muscle damage similar to athletic practices or labor-intensive occupational settings, thus physiological adaptations were not expected to alter the findings given our counterbalanced, crossover study design. Also, the participants were directed to avoid changes in their exercise regimen between trials to minimize changes in fitness.

Next, the participants transitioned to an environmental chamber (33.0 ± 0.3°C, 54 ± 2% relative humidity) and complete a 10-minute up-right seated acclimation period. During this period sweat patches (Tegaderm+Pad, 3M, St. Paul, MN, USA) were applied to the dorsal or ventral forearm (depending on the forearm hair) and superior scapula (back) (Baker, Stofan, Hamilton, & Horswill, 2009). Physiological and perceptual measures were assessed at the end of the ten minutes, at which time a body mass was obtained and the participant moved to a treadmill to begin a 5-minute walking warm-up (1.3 m/s) followed by running at 60% VO_2max (no difference between trials; EU 61.8 ± 3.9%, HY 62.5 ± 3.85%; P = 0.18) (1% grade) for 60 minutes with a 5- minute walking (1.3 m/s) break at 30 minutes and 5-minute cool down (1.3

m/s). Heart rate, T_{re}, T_{sk}, and perceptual measures were recorded every 10 minutes. Exercise was paused every 10 minutes to complete a body mass, which was subsequently used to provide water (warmed to 38°C) to replace sweat losses within 2% body mass loss in the EU trial (average intake; 0.96 ± 0.63 L). In the HY trial, small amounts of water were provided every 10-20 minutes to prevent excessive dehydration and improve participant comfort (average intake; 0.19 ± 0.12 L). Exercise was terminated early in 3 individuals due to rectal temperature equal to 40°C in the HY trial and in one individual in the EU trial due to trial time.

Immediately following exercise, a body mass was obtained and participants remained in the chamber to complete a 30-minute semi-recumbent recovery period with physiological (heart rate, T_{re}, T_{sk}) and perceptual measures (thirst, thermal sensation, muscle pain) recorded every 10 minutes. A blood draw was also collected at 20 minutes of recovery. The participants then exited the chamber, removed instrumentation, and provided a final nude body mass and urine sample.

Participants were provided with a 24-hour urine collection container, diet log, and rehydration instructions such that 100% of fluid losses were replaced within four hours of completion and an additional 2.5 L were consumed before arriving for the follow-up visit ~24-h later (actual time; 21.5 ± 0.9 h). Compliance was verbally confirmed upon arrival at the 24-h follow-up visit.

At the 24-h follow-up visit, participants provided a nude body mass and spot urine sample, followed by a 20-minute semi-recumbent rest. At the end of the rest, physiological and perceptual measures were recorded and a blood draw was collected. Participants then completed the five-minute warm-up on a cycle ergometer at 50W before moving to the isokinetic dynamometer. Knee extension strength was once again recorded for isometric contractions at

70° and 90° of knee flexion as well as isokinetic knee extension contractions at 60°·sec^{-1}. Participants were then instructed to resume normal exercise routines and second visits were scheduled, if applicable.

Blood analysis

Serum collected at each time point clotted at room temperature followed by centrifugation at 1000g and 4°C for 15 minutes. Serum was then used to assess osmolality via freezing point depression, sodium (ion-selective electrode, EasyElectrolyte, Medica Corporation, Bedford, MA, USA), and protein (refractometer, model Master-SUR,NM, Atago Co Ltd, Tokyo, Japan) in duplicate. Serum was also aliquoted and stored at -80°C for subsequent analysis of creatine kinase (SCK) and creatinine (SCr) performed per manufacturer's instructions via their respective commercially available colorimetric assays (BioAssay Systems, Hayward, CA, USA). Plasma collected at each time point was used to assess hemoglobin (Hb) in duplicate (HemoCueHb 201+, HemoCue, Angelholm, Sweden) and hematocrit read in triplicate using microcapillary tubes with a Micro-Capillary Reader (International Equipment Company, Needham Heights, MA). Plasma was also aliquoted and stored at -80°C for subsequent analysis of NGAL (PNGAL) performed per manufacturer's instructions via a commercially available enzyme linked immunosorbent assay kit (R&D Systems Inc, Minneapolis, MN, USA). UNGAL assessment was also completed per manufacturer's instructions via a commercially available enzyme linked immunosorbent assay kit (R&D Systems Inc, Minneapolis, MN, USA). UCr was assessd performed per manufacturer's instructions via a commercially available colorimetric assay (BioAssay Systems, Hayward, CA, USA). The coefficients of variation for SCK, SCr, UCr, PNGAL, and UNGAL were 2.1%, 2.8%, 4.9%, 3.2%, and 3.8% respectively.

Statistical Analysis

All statistical analyses were completed using SPSS version 24 (IBM Corporation, Somers, NY). Normality was assessed via Shapiro-Wilks test and histogram analysis and outliers were identified as three x interquartile range. Statistical analyses were initially completed with outliers removed. Outliers were then returned to the data to identify the impacts on statistical outcomes. Results were reported with outliers maintained if they did not impact statistical conclusions. Statistical findings that required outlier removal (i.e. decreasing sample size) are noted in the results. All partial eta squared (η_p^2) and Hedge's g values were calculated using a spreadsheet from Lakens (2013). Based on a .80 power calculation using the primary outcome variable NGAL (Junglee et al., 2013; Melin et al., 1997) with a correlation between time points of 0.42, a 2-standard deviation effect size, β of 0.20, and α of 0.05, it was determined 17 participants would be sufficient to complete this study. An increased experiment-wise type I error rate is acknowledged due to the multiple multivariate and univariate analyses conducted. Because the experimental protocol is time, resource, and cost intensive, power estimates were calculated based on singular analyses to provide initial experimental outcome indicators and guide future research.

For paper number one, repeated measures multivariate analysis of variance were used to assess blood NGAL and creatinine differences with a 3-way repeated measures analysis. Repeated measures multivariate analysis of variance were also used to assess urine NGAL and creatinine differences with a 3-way repeated measures analysis. All thermoregulatory, cardiovascular, hydration, and muscle damage variables (i.e. T_{re}, T_{sk}, thermal sensation, RPE, thirst, heart rate, blood pressure, body mass, urine specific gravity, urine osmolality, and isometric strength changes) were analyzed using two-way (time x hydration) repeated measures analysis of variance. When sphericity was violated, Greenhouse-Geisser adjustments were used

in the omnibus test. Post-hoc analyses involved pairwise comparisons with an appropriate Bonferroni corrected alpha to identify significant time point differences. Data that failed normality tests (thirst sensation and muscle pain) were analyzed with a Friedman test across time and between hydration states. Follow-up pairwise analysis were conducted using a Wilcoxon signed rank test for individual time point differences. Alpha of 0.05 was set *a priori* to determine significance at the omnibus level for each analysis.

For paper number two, repeated measures multivariate analysis of variance was used to assess absolute peak torque (i.e. strength) across time (pre-muscle damage, post-muscle damage, and the 24-h follow-up) and between hydration (EU and HY) for isometric peak torque at 70° and 90° knee flexion and $60°\cdot sec^{-1}$ isokinetic peak torque with a 3-way repeated measures analysis. Additional variables (body mass, creatine kinase, and muscle pain) were analyzed using two-way (time x hydration) repeated measures analysis of variance. When sphericity was violated, Greenhouse-Geisser adjustments were used in the omnibus test. Post-hoc analyses involved pairwise comparisons with an appropriate Bonferroni corrected alpha to identify significant time point differences. Dependent t-tests were used to assess differences in 24-h urine osmolality, total eccentric work, average eccentric peak torque and ratings of perceived exertion between trials. Data that failed normality tests (muscle pain) were analyzed with a Friedman test across time and between hydration states. Follow-up pairwise analysis were conducted using a Wilcoxon signed rank test for individual time point differences. Alpha of 0.05 was set *a priori* to determine significance at the omnibus level for each analysis.

IV. Manuscript #1: Combined Effects of Hypohydration, Muscle Damage, and Exertional Hyperthermia on Biomarkers of Acute Kidney Injury

Cory L. Butts, Aaron R. Caldwell, Samantha A. Mohler, Matthew S. Ganio, Nicholas P. Greene, Stavros A. Kavouras, Ronna C. Turner, Brendon P. McDermott.

Exercise Science Research Center, University of Arkansas, Fayetteville, Arkansas, USA

Abstract

Purpose: We investigated the combination of dehydration, muscle damage, and exercise in the heat on biomarkers of renal stress. **Methods**: Eighteen male participants (age 24±5 y, mass 75.9±10.0 kg, body fat 17.3±6.2%, VO_2peak 51.0 ± 6.0 ml/kg/min) completed two trials, one euhydrated (EU; fluid replacement ≤2% body mass loss; actual loss -1.2 ± 0.8%) and one hypohydrated (HY; fluid restriction 24-h prior to and throughout exercise; total loss -4.4 ± 1.9%), separated by ≥28 days. Trials consisted of muscle damaging unilateral eccentric knee flexion, 60 minutes of treadmill running (~60% VO_2peak) in the heat (33.0 ± 0.3°C, 54 ± 2% RH), and 30 minutes of passive recovery. Participants were provided a rehydration protocol in both trials and returned 24-h later for a follow-up visit. **Results**: The HY trial caused greater changes in rectal temperature during exercise (1.8 ± 0.5°C) compared to the EU trial (1.5 ± 0.4°C, $P = 0.04$). Percent change in plasma neutrophil gelatinous associated lipocalin (NGAL, a biomarker of acute kidney injury) was greater in the HY trial post-exercise (EU 28.0 ± 15.2%, HY 41.8 ± 17.5%, $P < 0.001$), but not at 24-h follow-up ($P = 0.39$). Serum creatinine also exhibited a main effect of trial (EU 0.97 ± 0.14, HY 1.04 ± 0.15, mg/dL, $P = 0.025$). Urine NGAL and urine creatinine were also elevated in the HY trial pre-exercise and post exercise (all, $P<0.05$) but were returned to EU levels by 24-h follow-up (all, $P > 0.05$). **Conclusion**: These findings suggest that improper fluid consumption prior to and during exercise may augment renal stress, yet the long-term consequences of these detriments require further investigation.

Key Words: acute kidney injury, hyperthermia, dehydration

Introduction

Heat stress and humidity can place high levels of physiological and perceptual strain on individuals conducting physical labor and exercise (1-5). Poor hydration practices in these environments, whether due to unavailability of fluids or voluntary under-consumption, may result in progressive dehydration (2, 3, 5). If individuals are not able to replace fluids adequately, this may lead to a cycle in which people report for subsequent bouts of work or exercise sub-optimally hydrated (5). As such, populations including agricultural workers, military members, and athletes may be at risk for negative health outcomes in these settings. For example, Mesoamerican nephropathy, also known as chronic interstitial nephritis of agricultural communities, occurring in Central American agricultural workers has been suggested to occur partially due to recurrent dehydration, exertional hyperthermia, and subclinical rhabdomyolysis (3, 6-8). From 1997 to 2013, an estimated 47,885 deaths occurred in Costa Rica, El Salvador, Nicaragua and Panamá because of chronic kidney disease, with ~40% of deaths occurring in persons aged 60 years or younger (6). While certain aspects of these occupations are unavoidable (i.e. muscle damaging labor, heat stress, pesticides), understanding the role of sub-optimal hydration in the elevation of biomarkers of acute kidney injury may allow for proper recommendations in fluid intake that would mitigate long term health consequences.

During exercise and exposure to heat stress, blood flow to the splanchnic and renal regions decrease, allowing for increased perfusion of the skin for thermoregulation (9, 10). This often results in elevated blood markers of renal function (i.e. creatinine), however, these alterations are transient and a consequence of reduced glomerular filtration associated with the lower renal perfusion (11, 12). Due to the energetic demands of the renal tubules, this reduction in blood flow during exercise in the heat may cause an ischemic atmosphere and subsequent

oxidative stress. Ischemic damage and nephrotoxicity in the renal tubules lead to the release of biological markers in the urine and blood (13). Novel biomarkers, such as neutrophil gelatinase associated lipocalin (NGAL), have been implicated as an alternative to traditional creatinine measurement due the expression of these markers in the renal tubules (13, 14). Schlader et al. (15) showed augmented levels of NGAL by extending the duration of exercise in the heat. Further, NGAL elevations have been shown immediately following distance running events (12, 16). However, these studies did not evaluate the influence of hydration on NGAL responses with exercise.

The addition of other physiological stressors such as muscle damage has been shown to cause further strain during exercise in the heat (17, 18). Muscle damage can be a consequence of exercise, particularly when individuals are unaccustomed to the activity or an eccentric component is present in the movements. Biological markers of muscle damage (e.g. serum creatine kinase) can be elevated in military training (19), agriculture workers (20), and athletes during preseason American football practices (21). Intramuscular contents leaked following muscle damage (e.g. myoglobin) may have nephrotoxic effects therefore, managing breakdown is necessary to mitigate renal stress (22). Junglee et al. (18) demonstrated significantly greater increases in NGAL and creatinine when muscle damaging exercise was performed before a bout of exertional hyperthermia compared with no damage. Thus, the presence of heat stress and exercise increase the nephrotoxic effects associated with muscle damage (18). However, hydration status of these individuals was maintained similarly between trials, therefore the potential protective effect of proper fluid intake when completing exercise in the heat with concomitant muscle damage is unclear.

Dehydration leads to significant reductions in creatinine clearance (i.e. glomerular impairment), however, the impact of dehydration on biomarkers of acute kidney injury (i.e. tubular damage) has received little investigation in humans (11). Combining heat stress with dehydration and exercise causes to further decreases in renal blood flow and subsequently greater increases in creatinine (11, 23). As dehydration combined with muscle damage, exercise, and heat stress are commonly experienced by a variety of occupations, it is necessary to understand the role of adequate fluid intake in the mitigation of renal stress (5, 19, 20). As such, the aim of this study was to identify the combined influence of dehydration, exercise in the heat, and muscle damage on biomarkers of acute kidney injury and renal function. It was hypothesized that the thermoregulatory strain associated with dehydration during exercise in the heat would augment renal biomarker elevations immediately post-exercise as compared to the euhydrated trial, however, these differences would be transient with returns to baseline 24-h post-exercise.

Methods

Participants

Eighteen healthy, recreationally active males (age 24 ± 5 y, wt 75.9 ± 10.0 kg, ht 1.79 ± 0.05m, body fat 17.3 ± 6.2%, VO_2peak, 51.0 ± 6.0 ml/kg/min) were recruited from the University and surrounding areas to participate in this randomized crossover counterbalanced design study. All procedures were approved by the University Institutional Review Board and written informed consent was acquired from all individuals prior to participation. Participants completed five total visits including one familiarization day and two experimental days (one hypohydrated, HY, and one euhydrated, EU) each with 24-h follow-up visits. Experimental visits were separated by ≥28 days (average; 41 ± 16 days) to allow for muscle damage recovery

and prevent acclimation to the heat. Exclusionary criteria included previous heat exhaustion or heat stroke within the past 3 years, current musculoskeletal injury, hypertension where vigorous exercise is contraindicated, diagnosed sickle cell trait, use of medications that may alter thermoregulation or kidney function, current use of creatine supplementation, and a history of kidney disease. All participants were asked to refrain from alcohol use for 24-h, caffeine use for 12-h, resistance training for 5-days and exercise for 24-h prior to each trial. Body composition was assessed via dual energy x-ray absorptiometry (DXA, Lunar Prodigy, General Electric, Madison, WI, USA).

Familiarization Visit

Participants completed baseline knee extensor isometric strength at 70° and 90° of knee flexion as well as isokinetic strength at $60°\cdot sec^{-1}$, following a 5-minute warm-up on a cycle ergometer (Monark 828E, Monark Exercise AB, Sweden) at ~50W (~50 rpm at 1 kilopond). Participants then underwent a graded maximal exercise test on a treadmill to assess maximal oxygen uptake (VO_2peak) to establish exercise intensity for subsequent trials. The test consisted of a constant jogging speed (selected by the participant) with grade increasing by 2% every two minutes until volitional exhaustion. VO_2peak was verified by a plateau in VO_2, respiratory exchange ratio ≥1.1, heart rate within 10 beats of age predicted maximum heart rate, or rating of perceived exertion ≥ 17.

Experimental Visits

Prior to arrival, participants completed three-day euhydrated baseline body weights with a provided scale (BalanceFrom High Accuracy Bathroom Scale, BalanceFrom LLC, China) for both trials, as well as a diet record on a standard log for 24-h prior to each trial. For the HY trial, the dehydration protocol consisted of 24-h fluid restriction in addition to fluid restriction during

the protocol. Prior to the euhydrated protocol, participants were instructed to consume fluids prior to arrival, while water was provided during the trial to ensure less than 2% body mass loss.

Upon arrival, participants completed a 24-h history, provide a spot urine sample, and completed a nude body mass. Spot urine was analyzed for urine specific gravity (refractometer, model Master-SUR,NM, Atago Co Ltd, Tokyo, Japan) and osmolality (freezing point depression, Model 3250, Advanced Instruments Inc., Norwood, MA) to confirm hydration status. Urine was also stored for later assessment of acute kidney injury biomarkers (uNGAL) and creatinine (uCr) via their respective assays. Participants were provided a rectal thermometer (RET-1, Physitemp Instruments Inc, Clifton, NJ, USA) to insert ~15 cm past the anal sphincter to assess rectal temperature (T_{re}). Participants were also instrumented with a Polar heart rate monitor (FT1/T31, Polar Inc, Lake Success, NY, USA), automated blood pressure cuff (Tango+, Suntech, Medical Inc., Morrisville, NC, USA) and skin temperature thermochrons (iButton, Maxim Integrated, San Jose, CA, USA) to assess four-site mean weighted skin temperature (T_{sk}) (24). Mean arterial blood pressure was calculated as (systolic blood pressure – diastolic blood pressure) *1/3 + diastolic blood pressure). Participant attire consisted of running shorts, socks, and shoes.

Participants completed a 20-minute semi-recumbent baseline rest in a thermoneutral environment (~20°C). During this time, participants were informed on the perceptual scales for rating of perceived exertion (RPE) (25), thermal sensation (26), and perceived thirst (27), as well as visual analog scales to identify overall and leg-specific muscle pain. Following the 20 minutes of rest, baseline physiological and perceptual measures were recorded. A baseline blood draw was also conducted via venipuncture to obtain serum and plasma (sodium heparin) samples (BD, Ontario, Canada). The participants then moved to a cycle ergomenter to complete a 5-minute warm-up at ~50W. Eccentric muscle damaging exercise was then performed on an

isokinetic dynamometer (Biodex System 3, Biodex Medical Systems, New York) (28). The procedure consisted of 10 sets of 10 repetitions of maximal unilateral eccentric knee flexion exercise at a speed of 30°/s with one minute of rest between sets (28). During the second trial, the contralateral leg was utilized to minimize the repeated bout effect and the leg used during the first trial was randomized and counterbalanced between participants for hydration and dominance. Prior to, and immediately following the eccentric protocol, isometric strength measures were performed via three 5-second maximal isometric knee extensor voluntary contractions at 70° and 90° knee flexion with one minute of rest between trials. Isokinetic knee extensor strength was also completed in triplicate at 60°/sec. Decreases in strength, as well as elevations in muscle pain, served as indices of muscle damage. While it is recognized that there are contralateral adaptations following single leg eccentric exercise, the 4 weeks trial separation should have minimized many of the responses. Xin et al (28) demonstrated no differences in muscle soreness or creatine kinase following contralateral eccentric exercise separated by 4 weeks, while isometric strength was lower in the second bout from 24 to 96 hours post exercise. Further, the purpose of the eccentric exercise protocol was to induce mild muscle damage similar to athletic practices or labor-intensive occupational settings, thus physiological adaptations did not alter the findings given the counterbalanced, crossover study design. Also, the participants were directed to avoid changes in their exercise regimen between trials to minimize changes in fitness.

Next, the participants transitioned to an environmental chamber (33.0 ± 0.3°C, 54 ± 2% relative humidity) and completed a 10-minute up-right seated acclimation period. During this period, sweat patches (Tegaderm+Pad, 3M, St. Paul, MN, USA) were applied to the dorsal or ventral forearm (depending on the forearm hair) and superior scapula (back) (29). Physiological

and perceptual measures were assessed at the end of the 10 minutes, at which time a body mass was obtained and the participant moved to a treadmill to begin a 5-minute walking warm-up (1.3 m/s) followed by running at 62.4 ± 4.4% VO_2peak (1% grade) for 60 minutes with a 5-minute walking (1.3 m/s) break at 30 minutes and 5-minute cool down (1.3 m/s). Exercise was paused every 10 minutes to complete a body mass, which was subsequently used to provide water (warmed to 38°C) to replace sweat losses within 2% body mass loss in the EU trial (average intake; 0.96 ± 0.63 L). In the HY trial, small amounts of water (25-50 mL) were provided every 10-20 minutes to prevent excessive dehydration and improve participant comfort (average intake; 0.19 ± 0.12 L). Physiological and perceptual measures were recorded at the beginning, 30 minutes, and end of exercise. Exercise was terminated early for 3 individuals due to rectal temperature equal to 40°C in the HY trial and in one individual in the EU trial due to non-trial related reasons.

Immediately following exercise, a body mass was obtained and participants remained in the environmental chamber to complete a 30-minute semi-recumbent recovery period with measures recorded every 10 minutes. A blood draw was also collected at 20 minutes of recovery. The participants then exited the chamber, removed instrumentation, and provided a final nude body mass and urine sample.

Participants were provided with a 24-hour urine collection container, diet log, and rehydration instructions such that 100% of fluid losses from HY and/or exercise heat stress were replaced within four hours of completion for both trials and an additional 2.5 L were consumed before arriving for the follow-up visit ~24-h later (actual time; 21.5 ± 0.9 h). Compliance with this protocol was verbally confirmed upon arrival at the 24-h follow-up visit and diet logs were compared between trials.

At the 24-h follow-up visit, participants provided a nude body mass and spot urine sample, followed by a 20-minute semi-recumbent rest. At the end of the rest, physiological and perceptual measures were recorded and a blood draw was collected. A five-minute warm-up on a cycle ergometer at ~50W was then conducted, followed by concentric knee extensor isometric and isokinetic strength assessment, respectively.

Blood analysis

Serum collected at each time point clotted at room temperature followed by centrifugation at 1000g and 4°C for 15 minutes. Serum was then used to assess osmolality via freezing point depression, sodium (ion-selective electrode, EasyElectrolyte, Medica Corporation, Bedford, MA, USA), and protein (refractometer, model Master-SUR, NM, Atago Co Ltd, Tokyo, Japan) were measured in duplicate. Serum was also aliquoted and stored at -80°C for subsequent analysis of creatinine (sCr) performed per manufacturer's instructions via a commercially available colorimetric assay (BioAssay Systems, Hayward, CA, USA). Whole blood collected at each time point was used to assess hemoglobin (Hb) in duplicate (HemoCueHb 201+, HemoCue, Angelholm, Sweden) and hematocrit was read in triplicate using microcapillary tubes with a Micro-Capillary Reader (International Equipment Company, Needham Heights, MA). Plasma was also aliquoted and stored at -80°C for subsequent analysis of NGAL (pNGAL) performed per manufacturer's instructions via a commercially available enzyme linked immunosorbent assay kit (R&D Systems Inc, Minneapolis, MN, USA). uNGAL assessment was also completed per manufacturer's instructions via a commercially available enzyme linked immunosorbent assay kit (R&D Systems Inc, Minneapolis, MN, USA). uCr analysis was performed per manufacturer's instructions via a commercially available colorimetric assay (BioAssay Systems, Hayward, CA, USA). uNGAL was also corrected for U_{osm} and uCr to remove the effects of urine

concentration on outcomes (30). The coefficients of variation for sCr, uCr, pNGAL, and uNGAL were 2.8%, 4.9%, 3.2%, and 3.8% respectively.

Statistical Analysis

All statistical analyses were completed using SPSS version 24 (IBM Corporation, Somers, NY). Normality was assessed via Shapiro-Wilks test and histogram analysis and outliers were identified as three x interquartile range and removed. Statistical analyses were initially completed with outliers removed. Outliers were then returned to the data to identify the impacts on statistical outcomes. Results were reported with outliers maintained if they did not impact statistical conclusions. Statistical findings that required outlier removal (i.e. decreasing sample size) are noted in the results. All partial eta squared (η_p^2) and Hedge's g values were calculated using a spreadsheet from Lakens (31). Repeated measures multivariate analysis of variance was used to assess pNGAL and sCr differences with a 3-way repeated measures analysis. Repeated measures multivariate analysis of variance was also used to assess uNGAL and uCr differences with a 3-way repeated measures analysis. All thermoregulatory, cardiovascular, hydration, and muscle damage variables (i.e. T_{re}, T_{sk}, thermal sensation, RPE, heart rate, blood pressure, body mass, urine specific gravity, urine osmolality, and isometric strength changes) were analyzed using two-way (time x hydration) repeated measures analysis of variance. When sphericity was violated, Greenhouse-Geisser adjustments were used. Post-hoc analyses involved pairwise comparisons with an appropriate Bonferroni corrected alpha to identify significant time point differences. Data that failed normality tests (thirst sensation and muscle pain) were analyzed with a Friedman test across time and between hydration states. Follow-up pairwise analyses were conducted using a Wilcoxon signed rank test for individual time point differences. Alpha of 0.05 was set *a priori* to determine significance at the omnibus

level for each analysis. Based on a .80 power calculation using the primary outcome variable pNGAL (11, 18) with a correlation between time points of 0.42, a 2-standard deviation effect size, β of 0.20, and α of 0.05, it was determined 17 participants would be sufficient for adequate power. An increased experiment-wise type I error rate is acknowledged due to the multiple multivariate and univariate analyses conducted. Because the experimental protocol was time, resource, and cost intensive, power estimates were calculated based on singular analyses to provide initial experimental outcome indicators to guide future research.

Results

Hydration Measures

Body mass was influenced by the interaction effect of time and trial ($F_{1.475,23.598}$ = 39.64, P < 0.001, η_p^2 = 0.71, Table 1). Pairwise comparisons revealed no differences between trials for 3-day baseline masses (P = 0.87), but lower masses for the HY treatment at baseline (P < 0.001) and at end of trial (P < 0.001). There were no differences in masses at the 24-h follow-up (P = 0.29). The dehydration protocol in the HY trial resulted in significantly greater reduction in body mass compared to the hydration protocol (t_{17} = 5.79, Hedge's g = 2.60, P < 0.001, Table 1). There was an interaction effect of hydration and time for urine osmolality ($F_{3,42}$ = 13.44, η_p^2 = 0.49, P < 0.001, Table 1). By design, U_{osm} was more concentrated in the HY pre-trial spot sample than the EU sample (P < 0.001) with similar differences in post-trial spot samples (P < 0.001). There was no difference in U_{osm} between the 24-h follow-up urine spot samples (P = 0.96, Table 1) or the 24-h urine collected between the end of the trial and the follow-up visit (EU 367 ± 197, HY 533 ± 316, P = 0.08). Urine specific gravity was also influenced by hydration and time ($F_{3, 39}$ = 7.13, η_p^2 = 0.35, P < 0.001, Table 1) with greater values in the pre-trial spot sample (P < 0.001) and post-trial spot sample (P < 0.001) of the HY group using a pairwise

alpha of 0.0125. There were no differences between trials in the 24-h follow-up spot sample (P = 0.697) or 24-h urine collected between the trial and follow-up visit (EU 1.011 ± 0.006, HY 1.016 ± 0.008, P = 0.048).

There was an interaction of trial and time for serum osmolality ($F_{2,34}$ = 29.22, η_p^2 = 0.63 P < 0.001, Table 1). Serum osmolality was greater for the HY trial compared to the EU trial pre (P < 0.001) and post-trial (P < 0.001), but not at the 24-h follow-up (P = 0.13). Serum Na^+ exhibited an interaction effect for trial and time ($F_{2,32}$ = 45.68, η_p^2 = 0.74, P <0.001, Table 1), where there were elevations for the HY trial at baseline (P < 0.001), and post-trial (P < 0.001), but no differences at 24-h follow-up (P = 0.54). There were no differences between trials for sweat sodium on the back (EU 84.7 ± 27.1, HY 86.5 ± 17.3 mEq/L, t_{17} = -0.46, Hedge's g = -0.21, P = 0.66) and arm (EU 64.7 ± 24.8, HY 67.4 ± 23.0 mEq/L, t_{16} = -0.62, Hedge's g = -0.29, P = 0.55). Hb content was influenced by time and trial (i.e. interaction effect) ($F_{2,34}$ = 14.60, η_p^2 = 0.46, P <0.001, Table 1). Hb tended to be elevated for the HY group at baseline (P = 0.018; pairwise alpha = 0.0167) and was significantly higher post-trial (P < 0.001), but was not different at the 24-h follow-up (P = 0.84). Hct was greater in the HY trial independent of time ($F_{1,17}$ = 5.429, η_p^2 = 0.24, P = 0.03, Table 1), and was decreased at the 24-h follow-up compared to baseline and post-exercise independent of trial ($F_{2,34}$ = 15.14, η_p^2 = 0.47, P < 0.001), but there was not a significant interaction effect ($F_{2,34}$ = 3.22, η_p^2 = 0.16, P = 0.053). There was a significant interaction for serum protein ($F_{2,28}$ = 4.93, η_p^2 = 0.26, P = 0.02, Table 1), with levels only different at baseline (P = 0.002), and no differences at post exercise (P = 0.07) or 24-h follow-up (P = 0.68).

Exercise Measures

Muscle damage was confirmed by evaluating percent changes in muscular strength from baseline using a multivariate analysis including isometric peak torque measured at 70° and 90° as well as peak isokinetic torque assessed at 60°·sec^{-1}. At the multivariate level, there was not a significant interaction of time and hydration (Wilks $\Lambda = 0.62$, $F_{6,11} = 1.13$, $P = 0.41$) nor a main effect of hydration (Wilks $\Lambda = 0.70$, $F_{3,14} = 2.05$, $P = 0.15$), but there was a main effect of time (Wilks $\Lambda = 0.27$, $F_{6,11} = 4.91$, $P = 0.01$). The analysis of time was then conducted using an alpha of 0.017. Strength was reduced independent of trial (i.e. main effect of time) for isometric strength at 70° ($F_{2,32} = 15.19$, $\eta_p^2 = 0.49$, $P < 0.001$), isometric strength at 90° ($F_{2,32} = 8.03$, $\eta_p^2 = 0.33$, $P = 0.003$), and isokinetic strength at 60°·sec^{-1} ($F_{2,32} = 6.16$, $\eta_p^2 = 0.28$, $P = 0.005$). Pairwise comparisons for each of the strength measures were then completed using an alpha of 0.006. Isometric strength at 70° decreased immediately post-damage (grand mean; -16.9 ± 12.7%, $P < 0.001$) and remained reduced at the 24-h follow-up (grand mean; -9.5 ± 11.3%, $P = 0.003$). Isometric strength at 90° was also reduced from immediate-post damage (grand mean; -15.7 ± 14.3%, $P < 0.001$) and tended to be reduced at the 24-h follow-up (grand mean; -10.6 ± 14.9%, $P = 0.010$).

Analysis of rectal temperature revealed an interaction effect of hydration and time ($F_{2,34} = 4.28$, $P = 0.02$, $\eta_p^2 = 0.20$, Figure 1C). Pairwise comparisons using a corrected alpha of 0.0167 revealed no differences between trials at the beginning ($P = 0.05$) or 30 minutes of exercise in the heat ($P = 0.06$), however, end of exercise T_{re} were greater in the HY trial compared to the EU trial ($P < 0.001$). Pairwise analysis of time also revealed significant elevations from baseline through end of exercise in both the EU (all $P < 0.001$) and HY trials (all $P < 0.001$). Overall change in T_{re} during exercise was greater in the HY (1.8 ± 0.5°C) compared to the EU trial (1.5 ± 0.4°C; $t_{17} = -2.26$, Hedge's g = -1.01, $P = 0.04$).

There was a significant interaction of hydration and time for skin temperature ($F_{2,34}$ = 5.49, η_p^2 = 0.24, P = 0.01, Figure 1B), however, pairwise analysis (adjusted alpha = 0.008) revealed no differences between trials at any time point (all $P > 0.05$). In the EU trial, skin temperature was lower at baseline compared to 30-minutes of exercise ($P < 0.001$) and the end of exercise ($P < 0.001$), however, in the HY trial, baseline was only lower than the 30-minutes of exercise time point ($P < 0.001$), with a trend to be lower than the end of exercise ($P = 0.01$).

There was an interaction of time and hydration for heart rate ($F_{2.09, 35.50}$ = 10.61, η_p^2 = 0.38, $P < 0.001$, Figure 1A), with no differences between conditions at the beginning of trials (P = 0.73) or pre-exercise ($P = 0.43$). Heart rate was greater in the HY trial at mid-exercise ($P <$ 0.001) and end of exercise ($P < 0.001$). Pairwise analysis of time also revealed significant elevations from baseline through end of exercise in both the EU (all $P < 0.001$) and HY trials (all $P < 0.001$).

Perceptual Measures

Ratings of perceived exertion exhibited an interaction effect for hydration and time ($F_{1,16}$ = 10.32, P = 0.005, η_p^2 = 0.39), with greater levels of perceived exertion in the HY trial at the mid-exercise (EU 14 ± 3, HY 15 ± 3, $P < 0.001$) and end of exercise (EU 14 ± 2, HY 17 ± 2, $P <$ 0.001) time periods. Thermal sensation was not affected by hydration status ($F_{1,15}$ = 1.29, P = 0.27, η_p^2 = 0.08), but increased independent of trial ($F_{2,30}$ = 57.01, $P < 0.001$, η_p^2 = 0.79) from pre-exercise (5.1 ± 0.6) to the end of exercise (6.6 ± 0.5, $P < 0.001$), with no interaction ($F_{2,30}$ = 1.69, P = 0.20, η_p^2 = 0.10). Thirst sensation was influenced by hydration (χ^2_1 = 16.00, P <0.001) and time (χ^2_5 = 53.88, P <0.001). Pairwise comparisons completed using an adjusted alpha of 0.008, revealed significantly greater thirst from baseline (EU 2.2 ± 1.4, HY 6.1 ± 1.5, $P < 0.001$) through pre-exercise (EU 3.2 ± 1.9, HY 6.8 ± 1.2, $P < 0.001$) to the end of recovery (EU 2.0 ±

0.9, HY 8.2 ± 1.2, $P < 0.001$). There were no differences in perceived thirst at the 24-h follow-up time point (EU 1.7 ± 1.0, HY 2.3 ± 1.3, $P = 0.02$). There was no effect of hydration ($\chi^2_1 = 1.00$, $P = 0.32$) for muscle pain, however, there was an effect of time ($\chi^2_3 = 29.60$, $P < 0.001$). Using a pairwise alpha of 0.008, muscle pain was increased in both trials from baseline (EU 1.3 ± 1.6, HY 2.2 ± 3.1 mm) to the end of exercise (EU 20.9 ± 22.0, HY 29.7 ± 22.9 mm, both $P < 0.001$), and at the 24-h follow-up (EU 12.2 ± 12.4, HY 10.7 ± 12.2 mm, both $P < 0.008$). Overall pain was influenced by hydration ($\chi^2_1 = 4.00$, $P = 0.046$) and time ($\chi^2_3 = 35.76$, $P <0.001$), with significantly greater pain at baseline in the HY trial (2.5 ± 2.6 mm) compared to the EU trial (0.9 ± 1.3 mm, $P = 0.007$), but no difference from any other point (all $P > 0.008$).

Recovery Measures

Analysis of rectal temperature responses during recovery revealed no interaction effects ($F_{3,51} = 0.21$, $P = 0.89$, $\eta_p^2 = 0.012$, Figure 2C) with greater temperatures in the HY trial compared to the EU trial independent of time ($F_{1,17} = 18.40$, $P < 0.001$, $\eta_p^2 = 0.52$). Rectal temperature was also reduced in recovery regardless of trial ($F_{1.32, 22.49} = 186.98$, $P < 0.001$, $\eta_p^2 =0.92$). Pairwise analysis revealed differences at every time point ($P <0.001$) with decreases from the beginning of recovery through 30 minutes in both trials. Skin temperature in recovery exhibited an interaction effect ($F_{1.53,24.53} = 4.09$, $P = 0.04$, $\eta_p^2 = 0.20$), with no differences between trials at any time point (all pairwise $P > 0.05$), however, temperatures were reduced in the EU trial from the beginning of recovery to the end of recovery with differences between all-time points (all $P < 0.001$) except 20 and 30 minutes. In the HY trial, temperatures were reduced from the beginning through 20 min of recovery ($P = 0.002$). Heart rate in recovery was greater in the HY trial compared to the EU trial independent of time ($F_{1,17} = 91.45$, $P < 0.001$, $\eta_p^2 = 0.84$, Figure 2A). Heart rate was also reduced regardless of trial ($F_{3,51} = 73.70$, $P < 0.001$, $\eta_p^2 = 0.81$),

from the beginning of recovery to the end of recovery ($P < 0.001$) with no differences between 20 and 30 minutes ($P = 0.63$), and no interaction effects ($F_{3,51} = 2.02$, $P = 0.12$, $\eta_p^2 = 0.11$). Mean arterial pressure during recovery was not different between trials (grand means; EU 80.7 ± 5.7, HY 82.4 ± 8.0 mmHg; $F_{1,17} = 1.83$, $P = 0.19$, $\eta_p^2 = 0.10$), and did not change significantly across time ($F_{5,51} = 1.07$, $P = 0.37$, $\eta_p^2 = 0.10$), with no interaction of time and hydration ($F_{2.06,35.10} = 2.84$, $P = 0.07$, $\eta_p^2 = 0.14$). Mean arterial pressure at the 24-h follow-up was not different between the EU trial (85.3 ± 8.1 mmHg) and HY trial (85.7 ± 6.2 mmHg; $t_{17} = -0.21$, $P = 0.84$, Hedge's g = -0.09).

Renal Biomarkers

Analyses of blood markers of acute kidney injury and kidney function (pNGAL and sCr, respectively) were conducted using multivariate repeated measures analysis of variance. Because of outliers causing violations of normality, the data for three participants were set aside, leaving 15 participants for the analysis. The initial multivariate analysis revealed a significant interaction of time by hydration by outcome (Wilks $\Lambda = 0.45$, $F_{4,11} = 3.37$, $P = 0.05$), however, follow-up analysis revealed no time by hydration interaction (Wilks $\Lambda = 0.77$, $F_{4,54} = 1.87$, $P = 0.13$). Rather, there were significant main effects of hydration (Wilks $\Lambda = 0.61$, $F_{2,13} = 4.08$, $P = 0.04$), as well as time (Wilks $\Lambda = 0.14$, $F_{4,54} = 22.64$, $P < 0.01$). Univariate analysis of main effects were then conducted using an adjusted alpha of 0.025. At the univariate level, there was no main effect of hydration for pNGAL ($F_{1, 14} = 1.40$, $P = 0.26$, $\eta_p^2 = 0.09$, Figure 3C), however there was a main effect of time ($F_{2,28} = 75.93$, $P < 0.001$, $\eta_p^2 = 0.84$). Pairwise comparisons were then completed using an adjusted alpha of 0.0083, with post-exercise combined values significantly elevated above pre-exercise ($P < 0.001$) and the 24-h follow-up ($P < 0.001$), however, there were no differences between baseline and the 24-h follow-up ($P = 0.82$). At the

univariate level, sCr was different between hydration trials regardless of time ($F_{1,14}$ = 6.27, P = 0.025, η_p^2 = 0.31), and changed across time independent of trial ($F_{2,28}$ = 11.85, P < 0.001, η_p^2 = 0.46). Pairwise comparisons were then completed using an adjusted alpha of 0.0083, with post exercise elevated significantly above pre-exercise (P = 0.004) and the 24-h follow-up (P < 0.001), however, there were no differences between baseline and the 24-h follow-up (P = 0.25).

A separate repeated measures analysis of variance was also conducted to assess percent change from baseline for pNGAL. This analysis revealed an interaction of hydration and time ($F_{1,17}$ = 4.49, P = 0.05, η_p^2 = 0.21). Pairwise comparisons revealed a greater change in the HY trial post-exercise compared to the EU trial (P < 0.001, Hedge's g = 0.80), while there were no differences at the 24-h follow-up (P = 0.39).

Multivariate analysis was performed using urinary markers of acute kidney injury and kidney function (uNGAL and uCr, repectively) with data from 16 participants, exhibiting a significant time by hydration trial by outcome interaction (Wilks Λ = 0.41, $F_{4,12}$ = 4.33, P = 0.02). There was also a significant time by trial interaction (Wilks Λ = 0.52, $F_{4,58}$ = 5.57, P < 0.001). Univariate analysis was then conducted using an adjusted alpha of 0.025. There was a significant time and hydration trial interaction for uNGAL ($F_{1.43,\ 21.45}$ = 7.11, P = 0.008, η_p^2 = 0.32, Figure 3A) and uCr ($F_{2,30}$ = 10.10, P < 0.001, η_p^2 = 0.40, Figure 3B). Pairwise comparisons were conducted for each variable using an adjusted alpha of 0.005. uNGAL was greater in the HY trial compared to the EU trial at baseline (P < 0.001) and post-exercise (P < 0.001), however, there were no differences at 24-h post (P = 0.91). The uCr concentrations were also lower in the EU trial compared to the HY trial at baseline (P < 0.001) and post-exercise (P < 0.001), but not at 24-h follow-up (P = 0.47).

Following the correction of uNGAL for U_{osm}, there were no effects of trial (grand means; EU 11.2 ± 10.8, HY 11.3 ± 6.6 pg/mOsm; $F_{1,14} = 0.01$, $P = 0.94$, $\eta_p^2 = 0.0007$), time ($F_{2,28} = 0.51$, $P = 0.60$, $\eta_p^2 = 0.04$), or interaction ($F_{2,28} = 1.31$, $P = 0.29$, $\eta_p^2 = 0.09$). uNGAL was also corrected for uCr (pg/mg) with similar results of no differences in hydration ($F_{1,14} = 1.17$, $P = 0.30$, $\eta_p^2 = 0.08$), time, ($F_{1.13,15.85} = 1.04$, $P = 0.33$, $\eta_p^2 = 0.07$), or interaction ($F_{1.16,16.22} = 1.13$, $P = 0.32$, $\eta_p^2 = 0.07$). uCr was also corrected for urine osmolality, demonstrating elevated responses in the HY trial independent of time (grand means; EU 0.217 ± 0.068, HY 0.248 ± 0.064 mg/mOsm, $F_{1,17} = 5.55$, $P = 0.03$, $\eta_p^2 = 0.25$). There was also a main effect of time ($F_{2,34} = 41.51$, $P < 0.001$, $\eta_p^2 = 0.71$), with post-exercise (grand mean; 0.279 ± 0.068 mg/mOsm) elevated above baseline (0.210 ± 0.064 mg/mOsm, $P < 0.001$) and the 24-h follow-up (0.211 ± 0.055, mg/mOsm, $P < 0.001$), however, no interaction effects for time and hydration ($F_{2,34} = 0.12$, $P = 0.89$, $\eta_p^2 = 0.007$).

Discussion

The purpose of this investigation was to evaluate the combined influence of hypohydration, muscle damage, and exercise in the heat on biological markers of renal stress. Further, we sought to evaluate the impact of proper rehydration on these responses to isolate the impact of hypohydration during exercise. As hypothesized, pNGAL and sCr showed significantly greater changes post-exercise when HY, but elevations were transient and returned to baseline by 24-h follow-up. We also demonstrated significant elevations in uNGAL and uCr at baseline and post-exercise, but there were no differences following rehydration by the 24-h follow-up. Interestingly, when uNGAL but not uCr values were corrected for the corresponding urine osmolality, these differences were ameliorated, suggesting the elevations may have been due to concentration of the sample rather than increased production. Overall, we demonstrated

that HY caused greater thermoregulatory, physiological, and renal stress following exercise in the heat, however, our rehydration protocol successfully mitigated these elevations by the 24-h follow-up.

Animal models have shown the negative impacts of recurrent exposure to heat stress and dehydration on renal health through glomerular and tubulointerstitial changes (32-36). Repeated exposure to heat stress and dehydration causes elevated osmolality and activates the aldose reductase pathway, which leads to greater fructose metabolism in the proximal tubule (34). Because this is an energetically demanding process, these elevations in fructose metabolism may lead to ATP depletion and subsequent oxidative and inflammatory stress (34). In addition, chronic vasopressin elevations may lead to hyperfiltration in the glomerulus as well as increased permeability and albuminuria (35). This results in augmented formation of fibrosis, inflammatory responses, and overall renal injury (35). When rehydration is delayed or replaced with sugar-sweetened beverages, the resultant elevations in biomarkers of acute kidney injury (e.g. uNGAL), renal dysfunction (e.g. creatinine), and histological changes (i.e. brush border reduction) show damage to the tubules as well as glomerulus (32-34). This worsening of renal injury highlights the importance of proper rehydration as well as beverage choice.

In agricultural workers, mechanisms previously shown in animal models are suggested to contribute to early onset chronic kidney disease in central American countries due to daily exposure to dehydration, subclinical rhabdomyolysis, heat stress, and exertion (7, 37). Increases in biomarkers of renal injury (e.g. NGAL, creatinine) occur across singular shifts as well as throughout harvesting seasons (2, 3, 20). These findings are substantial given the heat stress, muscle damage, and dehydration experienced by athletes, military personnel, and a variety of employees in occupations on a regular basis (1, 2, 5, 19, 21).

Laboratory investigations of heat stress, muscle damage, and exercise on markers of renal function and injury tend to confirm the findings in animal and field studies. Schlader et al. (15) revealed greater elevations in NGAL and creatinine by extending exercise duration in the heat, however, these responses returned to baseline by 24-h post. When completing exercise in the heat, the addition of muscle damage was also shown to increase NGAL and creatinine responses (18). However, these studies either focused on singular aspects (i.e. exertional hyperthermia), did not evaluate the role of hydration, or focused on traditional markers of renal function that may be limited in exercise settings (38). Regardless, we showed similar increases in NGAL (Figure 3C and 4) and creatinine (Figure 3D) with exercise in the heat. Further, we induced similar thermal (i.e. T_{re} changes) and physiological (i.e. heart rate) stress during exercise compared to previous studies (15, 17, 18). However, our HY trial caused greater increases during exercise and in recovery, as were expected with dehydration and exercise in the heat (4, 39). Additionally, our responses returned to baseline by the 24-h follow-up visit. Because these stressors (heat, exertion, muscle damage) often occur concomitantly, it is important to understand the combined influence on markers of renal function in humans.

According to our absolute values, we showed greater elevations in sCr but not pNGAL post exercise when HY. When controlling for the variability in baseline values, pNGAL demonstrated greater increases post-exercise (Figure 4). These support previous findings (11), demonstrating significant reductions in creatinine clearance when performing treadmill exercise in the heat while dehydrated. Further, the present study HY trial resulted in significantly greater pre-exercise and post-exercise values for uNGAL and uCr. Our dehydration protocol was sufficient to induce marked elevations in serum osmolality, hemoconcentration, as well as urine concentration. Therefore, given the relationship between serum osmolality and vasopressin, it is

likely that there was marked elevations throughout the HY trial in vasopressin that may have impacted renal function (i.e. hyperfiltration) and contributed to increased renal injury (33, 35, 40). These differences were ameliorated via our rehydration protocol as all values (blood and urine) were similar between trials at the 24-h follow-up. Field studies of marathon runners where exertion, hyperthermia, dehydration, and muscle damage tend to occur, have also shown significant elevations in biomarkers of renal injury, albeit these responses were transient, resolving by 24-h post (12, 16). Therefore, these studies suggest that singular bouts of exertional hyperthermia, dehydration and muscle damage are sufficient to elevate novel (NGAL) and traditional (creatinine) biomarkers of renal injury, however, these can be resolved by 24-h post with rest and adequate rehydration.

These findings highlight the protective role of adequate hydration during and following exercise in the heat. There is overwhelming evidence to support a negative impact of dehydration in renal health, thermoregulation, performance, and cardiovascular stability (4, 11, 33, 37, 39, 41). As heat stress, exertion, and muscle damage are often unavoidable, providing proper recommendations to rehydrate individuals may mitigate these stressors. We utilized recommendations provided by the National Athletic Training Association and American College of Sports Medicine to rapidly rehydrate participants within four hours (self-reported) (42, 43). These protocols were successful in returning renal, perceptual, and physiological responses to baseline EU conditions. In a field setting, Bodin et al. (44) successfully introduced a water-rest-shade intervention in sugar cane cutters, showing improved fluid intake behaviors (self-reported), symptoms associated with heat stress, and overall productivity across a harvest season. Wegman et al. (45) used the same intervention, however, there were still decreases in estimated glomerular filtration rate across the harvest and dehydration across work shifts. Reductions in

glomerular filtration rate indicate impairments in renal function at the level of the glomerulus, potentially due to attenuations in renal blood flow associated with exertion in hot environments. However, these findings are also limited as sCr is used to estimate filtration rate and can be affected by body mass and exertion. If these detriments are continuous, however, over time this may indicate the underlying development of chronic kidney disease. As the authors did not use a control group (i.e. no intervention), it is difficult to assess the efficacy of the intervention used in preventing acute kidney injury. Regardless, future research should focus on rehydration strategies post-work as well as throughout the work-day to identify the longitudinal effects of proper hydration on renal health.

Our results involving corrections for concentration and creatinine provided interesting commentary on the meaningfulness of biomarker elevations. We showed elevations in urine markers of acute kidney injury with HY, however, when corrected for concentration (i.e. osmolality) and creatinine, these elevations were ameliorated. Therefore, the elevation of these biomarkers may have been the product of concentrated sample collection rather than increased expression. However, it is also possible that this correction may be masking an increase in renal stress due to the relationship between concentration and expression of injury biomarkers. Further, the correction of samples to creatinine must be interpreted with caution, as this assumes a steady production of creatinine – often not the case with strenuous exercise. It is well known that muscle mass and exercise can influence creatinine release, making it a flawed variable for use in exercise settings (38). Regardless, the exclusive use of biomarkers, as in the current study cannot absolutely confirm the presence of renal damage without histological examination of renal tissue. Rather, these findings suggest renal stress may have been present with these

biomarkers, but correcting for concentration indicates that this conclusion must be interpreted with caution.

This study utilized a singular bout of exercise in the heat (total ~75 minutes) to induce changes in renal biomarkers. Agricultural workers experience these stresses for entire work shifts (i.e. multiple hours), therefore these findings from a relatively short duration have limited applicability to a whole work day. However, the detrimental relationships we highlighted in physiological and renal function with a relatively short bout of exercise are likely to be exacerbated with increased duration, and should raise concern for individuals experiencing prolonged exposure to these environmental stressors. Further, it is difficult to ascertain the effects of repetitive bouts (i.e. daily) on these indices of renal health. Additionally, we rehydrated individuals adequately, which may have limited the ability to find prolonged recovery of renal biomarkers. As such, further investigation is warranted to identify the impacts of limited or delayed rehydration on biomarker elevations. Another limitation of the current study pertains to the muscle damage protocol utilizing only a single leg to induce damage, limiting application to whole body exercise. However, the design of this protocol was to induce mild muscle damage similar to many athletic and occupational settings. Further, the conditions of the current study replicate those commonly association with clinically significant rhabdomyolysis (i.e. heat stress and dehydration), therefore we chose a protocol that ensured the safety of participants. Because the HY trial induced greater physiological stress (i.e. T_{re} and cardiovascular strain) we cannot delineate between the effects of dehydration and stress on the biomarker elevations of acute kidney injury. Increased thermal and physiological stresses are commonly associated with hypohydration and exercise in the heat (4, 39, 41), therefore, the authors chose to match workload rather than heat stress as this increases real world applicability.

Conclusions

The combination of heat stress with strenuous exercise and gradual dehydration throughout the work day or athletic practice places a high demand on the kidneys to retain fluid while clearing excess waste from inherent muscle damage. The concomitant exposure to physiological (i.e. exercise, muscle damage) and environmental (high ambient temperature and humidity) stressors commonly experienced by athletes, military, and occupational populations may augment the deleterious responses to dehydration. Our results confirm previously reported increases in physiological and perceptual stress associated with hypohydration during exercise in the heat. We demonstrated elevations in novel renal biomarkers of acute kidney injury (NGAL) as well as traditional markers of renal function (creatinine). Although, correcting for concentration ameliorated these elevations, thus these findings must be interpreted caution. Regardless, the rehydration protocol used during recovery in this study highlighted the importance of proper fluid intake post-exertion by returning function biomarkers to baseline by 24-h follow-up. Therefore, these findings support the need for proper hydration strategies before and after dehydrating exercise in the heat to mitigate stresses and reduce negative health outcomes.

References

1. Meade RD, Lauzon M, Poirier MP, Flouris AD, Kenny GP. An Evaluation of the Physiological Strain Experienced by Electrical Utility Workers in North America. J Occup Environ Hyg. 2015;12(10):708-20. doi: 10.1080/15459624.2015.1043054. PubMed PMID: 26011148.

2. Moyce S, Mitchell D, Armitage T, Tancredi D, Joseph J, Schenker M. Heat strain, volume depletion and kidney function in California agricultural workers. Occup Environ Med. 2017;74(6):402-9. Epub 2017/01/16. doi: 10.1136/oemed-2016-103848. PubMed PMID: 28093502; PubMed Central PMCID: PMCPMC5771643.

3. García-Trabanino R, Jarquín E, Wesseling C, Johnson RJ, González-Quiroz M, Weiss I, et al. Heat stress, dehydration, and kidney function in sugarcane cutters in El Salvador--A cross-

shift study of workers at risk of Mesoamerican nephropathy. Environ Res. 2015;142:746-55. doi: 10.1016/j.envres.2015.07.007. PubMed PMID: 26209462.

4. González-Alonso J, Teller C, Andersen SL, Jensen FB, Hyldig T, Nielsen B. Influence of body temperature on the development of fatigue during prolonged exercise in the heat. J Appl Physiol (1985). 1999;86(3):1032-9. PubMed PMID: 10066720.

5. Yeargin SW, Casa DJ, Judelson DA, McDermott BP, Ganio MS, Lee EC, et al. Thermoregulatory responses and hydration practices in heat-acclimatized adolescents during preseason high school football. J Athl Train. 2010;45(2):136-46. doi: 10.4085/1062-6050-45.2.136. PubMed PMID: 20210617; PubMed Central PMCID: PMCPMC2838465.

6. Ordunez P, Nieto FJ, Martinez R, Soliz P, Giraldo GP, Mott SA, et al. Chronic kidney disease mortality trends in selected Central America countries, 1997-2013: clues to an epidemic of chronic interstitial nephritis of agricultural communities. J Epidemiol Community Health. 2018;72(4):280-6. Epub 2018/02/02. doi: 10.1136/jech-2017-210023. PubMed PMID: 29437864.

7. Roncal-Jimenez C, García-Trabanino R, Barregard L, Lanaspa MA, Wesseling C, Harra T, et al. Heat Stress Nephropathy From Exercise-Induced Uric Acid Crystalluria: A Perspective on Mesoamerican Nephropathy. Am J Kidney Dis. 2016;67(1):20-30. doi: 10.1053/j.ajkd.2015.08.021. PubMed PMID: 26455995.

8. Wesseling C, Aragón A, González M, Weiss I, Glaser J, Bobadilla NA, et al. Kidney function in sugarcane cutters in Nicaragua - A longitudinal study of workers at risk of Mesoamerican nephropathy. Environ Res. 2016;147:125-32. doi: 10.1016/j.envres.2016.02.002. PubMed PMID: 26866450.

9. Hales JR, Rowell LB, King RB. Regional distribution of blood flow in awake heat-stressed baboons. Am J Physiol. 1979;237(6):H705-12. PubMed PMID: 117718.

10. Hohimer AR, Smith OA. Decreased renal blood flow in the baboon during mild dynamic leg exercise. Am J Physiol. 1979;236(1):H141-50. PubMed PMID: 107812.

11. Melin B, Jimenez C, Savourey G, Bittel J, Cottet-Emard JM, Pequignot JM, et al. Effects of hydration state on hormonal and renal responses during moderate exercise in the heat. Eur J Appl Physiol Occup Physiol. 1997;76(4):320-7. doi: 10.1007/s004210050255. PubMed PMID: 9349646.

12. Mansour SG, Verma G, Pata RW, Martin TG, Perazella MA, Parikh CR. Kidney Injury and Repair Biomarkers in Marathon Runners. Am J Kidney Dis. 2017. Epub 2017/03/24. doi: 10.1053/j.ajkd.2017.01.045. PubMed PMID: 28363731.

13. Mishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, et al. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. J Am Soc Nephrol. 2003;14(10):2534-43. PubMed PMID: 14514731.

14. Nejat M, Pickering JW, Devarajan P, Bonventre JV, Edelstein CL, Walker RJ, et al. Some biomarkers of acute kidney injury are increased in pre-renal acute injury. Kidney Int. 2012;81(12):1254-62. Epub 2012/03/14. doi: 10.1038/ki.2012.23. PubMed PMID: 22418979; PubMed Central PMCID: PMCPMC3365288.

15. Schlader ZJ, Chapman CL, Sarker S, Russo L, Rideout TC, Parker MD, et al. Firefighter Work Duration Influences the Extent of Acute Kidney Injury. Med Sci Sports Exerc. 2017. Epub 2017/03/07. doi: 10.1249/MSS.0000000000001254. PubMed PMID: 28272268.

16. McCullough PA, Chinnaiyan KM, Gallagher MJ, Colar JM, Geddes T, Gold JM, et al. Changes in renal markers and acute kidney injury after marathon running. Nephrology (Carlton). 2011;16(2):194-9. doi: 10.1111/j.1440-1797.2010.01354.x. PubMed PMID: 21272132.

17. Fortes MB, Di Felice U, Dolci A, Junglee NA, Crockford MJ, West L, et al. Muscle-damaging exercise increases heat strain during subsequent exercise heat stress. Med Sci Sports Exerc. 2013;45(10):1915-24. doi: 10.1249/MSS.0b013e318294b0f8. PubMed PMID: 23559121.

18. Junglee NA, Di Felice U, Dolci A, Fortes MB, Jibani MM, Lemmey AB, et al. Exercising in a hot environment with muscle damage: effects on acute kidney injury biomarkers and kidney function. Am J Physiol Renal Physiol. 2013;305(6):F813-20. doi: 10.1152/ajprenal.00091.2013. PubMed PMID: 23825078.

19. Knochel JP, Dotin LN, Hamburger RJ. Heat stress, exercise, and muscle injury: effects on urate metabolism and renal function. Ann Intern Med. 1974;81(3):321-8. PubMed PMID: 4852235.

20. Paula Santos U, Zanetta DM, Terra-Filho M, Burdmann EA. Burnt sugarcane harvesting is associated with acute renal dysfunction. Kidney Int. 2015;87(4):792-9. Epub 2014/09/17. doi: 10.1038/ki.2014.306. PubMed PMID: 25229334.

21. Smoot MK, Cavanaugh JE, Amendola A, West DR, Herwaldt LA. Creatine kinase levels during preseason camp in National Collegiate Athletic Association Division I football athletes. Clin J Sport Med. 2014;24(5):438-40. doi: 10.1097/JSM.0000000000000057. PubMed PMID: 24346738.

22. Bosch X, Poch E, Grau JM. Rhabdomyolysis and acute kidney injury. N Engl J Med. 2009;361(1):62-72. doi: 10.1056/NEJMra0801327. PubMed PMID: 19571284.

23. Smith Jh, Robinson S, Pearcy M. Renal responses to exercise, heat and dehydration. J Appl Physiol. 1952;4(8):659-65. PubMed PMID: 14907583.

24. Ramanathan Nl. A New Weighting System For Mean Surface Temperature Of The Human Body. J Appl Physiol. 1964;19:531-3. PubMed PMID: 14173555.

25. Borg G. Perceived exertion as an indicator of somatic stress. Scand J Rehabil Med. 1970;2(2):92-8. PubMed PMID: 5523831.

26. Toner MM, Drolet LL, Pandolf KB. Perceptual and physiological responses during exercise in cool and cold water. Percept Mot Skills. 1986;62(1):211-20. doi: 10.2466/pms.1986.62.1.211. PubMed PMID: 3960662.

27. Engell DB, Maller O, Sawka MN, Francesconi RN, Drolet L, Young AJ. Thirst and fluid intake following graded hypohydration levels in humans. Physiol Behav. 1987;40(2):229-36. PubMed PMID: 3306730.

28. Xin L, Hyldahl RD, Chipkin SR, Clarkson PM. A contralateral repeated bout effect attenuates induction of NF-κB DNA binding following eccentric exercise. J Appl Physiol (1985). 2014;116(11):1473-80. doi: 10.1152/japplphysiol.00133.2013. PubMed PMID: 23950163.

29. Baker LB, Stofan JR, Hamilton AA, Horswill CA. Comparison of regional patch collection vs. whole body washdown for measuring sweat sodium and potassium loss during exercise. J Appl Physiol (1985). 2009;107(3):887-95. Epub 2009/06/18. doi: 10.1152/japplphysiol.00197.2009. PubMed PMID: 19541738.

30. Bongers CCWG, Alsady M, Nijenhuis T, Hartman YAW, Eijsvogels TMH, Deen PMT, et al. Impact of acute versus repetitive moderate intensity endurance exercise on kidney injury markers. Physiol Rep. 2017;5(24). doi: 10.14814/phy2.13544. PubMed PMID: 29263119; PubMed Central PMCID: PMCPMC5742704.

31. Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. Front Psychol. 2013;4:863. Epub 2013/11/26. doi: 10.3389/fpsyg.2013.00863. PubMed PMID: 24324449; PubMed Central PMCID: PMCPMC3840331.

32. García-Arroyo FE, Cristóbal M, Arellano-Buendía AS, Osorio H, Tapia E, Soto V, et al. Rehydration with soft drink-like beverages exacerbates dehydration and worsens dehydration-associated renal injury. Am J Physiol Regul Integr Comp Physiol. 2016;311(1):R57-65. Epub 2016/04/06. doi: 10.1152/ajpregu.00354.2015. PubMed PMID: 27053647.

33. García-Arroyo FE, Tapia E, Blas-Marron MG, Gonzaga G, Silverio O, Cristóbal M, et al. Vasopressin Mediates the Renal Damage Induced by Limited Fructose Rehydration in Recurrently Dehydrated Rats. Int J Biol Sci. 2017;13(8):961-75. Epub 2017/07/18. doi: 10.7150/ijbs.20074. PubMed PMID: 28924378; PubMed Central PMCID: PMCPMC5599902.

34. Roncal Jimenez CA, Ishimoto T, Lanaspa MA, Rivard CJ, Nakagawa T, Ejaz AA, et al. Fructokinase activity mediates dehydration-induced renal injury. Kidney Int. 2014;86(2):294-302. Epub 2013/12/11. doi: 10.1038/ki.2013.492. PubMed PMID: 24336030; PubMed Central PMCID: PMCPMC4120672.

35. Roncal-Jimenez CA, Milagres T, Andres-Hernando A, Kuwabara M, Jensen T, Song Z, et al. Effects of exogenous desmopressin on a model of heat stress nephropathy in mice. Am J Physiol Renal Physiol. 2017;312(3):F418-F26. Epub 2016/12/21. doi: 10.1152/ajprenal.00495.2016. PubMed PMID: 28003190; PubMed Central PMCID: PMCPMC5374310.

36. Song 宋志林 Z, Roncal-Jimenez CA, Lanaspa-Garcia MA, Oppelt SA, Kuwabara M, Jensen T, et al. Role of fructose and fructokinase in acute dehydration-induced vasopressin gene expression and secretion in mice. J Neurophysiol. 2017;117(2):646-54. Epub 2016/11/16. doi: 10.1152/jn.00781.2016. PubMed PMID: 27852737; PubMed Central PMCID: PMCPMC5288484.

37. Roncal-Jimenez C, Lanaspa MA, Jensen T, Sanchez-Lozada LG, Johnson RJ. Mechanisms by Which Dehydration May Lead to Chronic Kidney Disease. Ann Nutr Metab. 2015;66 Suppl 3:10-3. Epub 2015/06/18. doi: 10.1159/000381239. PubMed PMID: 26088040.

38. Poortmans JR, Gulbis B, De Bruyn E, Baudry S, Carpentier A. Limitations of serum values to estimate glomerular filtration rate during exercise. Br J Sports Med. 2013;47(18):1166-70. Epub 2012/07/07. doi: 10.1136/bjsports-2012-090976. PubMed PMID: 22773320.

39. González-Alonso J, Mora-Rodríguez R, Below PR, Coyle EF. Dehydration markedly impairs cardiovascular function in hyperthermic endurance athletes during exercise. J Appl Physiol (1985). 1997;82(4):1229-36. PubMed PMID: 9104860.

40. Baylis PH, Robertson GL. Plasma vasopressin response to hypertonic saline infusion to assess posterior pituitary function. J R Soc Med. 1980;73(4):255-60. PubMed PMID: 7241442; PubMed Central PMCID: PMCPMC1437414.

41. Cheuvront SN, Kenefick RW. Dehydration: physiology, assessment, and performance effects. Compr Physiol. 2014;4(1):257-85. doi: 10.1002/cphy.c130017. PubMed PMID: 24692140.

42. McDermott BP, Anderson SA, Armstrong LE, Casa DJ, Cheuvront SN, Cooper L, et al. National Athletic Trainers' Association Position Statement: Fluid Replacement for the Physically Active. J Athl Train. 2017;52(9):877-95. doi: 10.4085/1062-6050-52.9.02. PubMed PMID: 28985128; PubMed Central PMCID: PMCPMC5634236.

43. Sawka MN, Burke LM, Eichner ER, Maughan RJ, Montain SJ, Stachenfeld NS, et al. American College of Sports Medicine position stand. Exercise and fluid replacement. Med Sci Sports Exerc. 2007;39(2):377-90. doi: 10.1249/mss.0b013e31802ca597. PubMed PMID: 17277604.

44. Bodin T, García-Trabanino R, Weiss I, Jarquín E, Glaser J, Jakobsson K, et al. Intervention to reduce heat stress and improve efficiency among sugarcane workers in El Salvador: Phase 1. Occup Environ Med. 2016. doi: 10.1136/oemed-2016-103555. PubMed PMID: 27073211.

45. Wegman DH, Apelqvist J, Bottai M, Ekström U, García-Trabanino R, Glaser J, et al. Intervention to diminish dehydration and kidney damage among sugarcane workers. Scand J Work Environ Health. 2018;44(1):16-24. Epub 2017/07/07. doi: 10.5271/sjweh.3659. PubMed PMID: 28691728.

Figure Legends

Figure 1. A) Heart rate **B)** Skin Temperature (Tsk) and **C)** Rectal Temperature (T_{re}) assessed at pre-exercise, 30 minutes of exercise, and end of exercise in the heat. [a]Indicates different from pre-exercise in EU trial ($P < 0.05$). [b]Indicates different from pre-exercise in HY trial ($P < 0.05$). [c]Indicates difference between hydration at time point ($P < 0.05$). [d]Indicates different from 30-minutes exercise in EU trial ($P < 0.05$). [e]Indicates different from 30-minutes exercise in HY trial ($P < 0.05$). [f]Indicates difference from baseline in EU trial ($P <0.05$). [g]Indicates difference from baseline in HY trial ($P <0.05$).

Figure 2: **A)** Heart rate **B)** Skin Temperature (Tsk) and **C)** Rectal Temperature (T_{re}) assessed during 30 minutes of recovery from exercise in the heat. [a]Indicates difference between trial independent of time ($P < 0.05$). [b]Indicates different from baseline independent of trial ($P < 0.05$). [c]Indicates different from 10 minutes independent of trial ($P < 0.05$). [d]Indicates different from 20 minutes independent of trial ($P < 0.05$). [e]Indicates different from the onset of recovery in EU trial ($P < 0.05$). [f]Indicates difference onset of recovery in HY trial ($P < 0.05$). [g]Indicates different from 10 minutes of recovery in EU trial ($P < 0.05$).

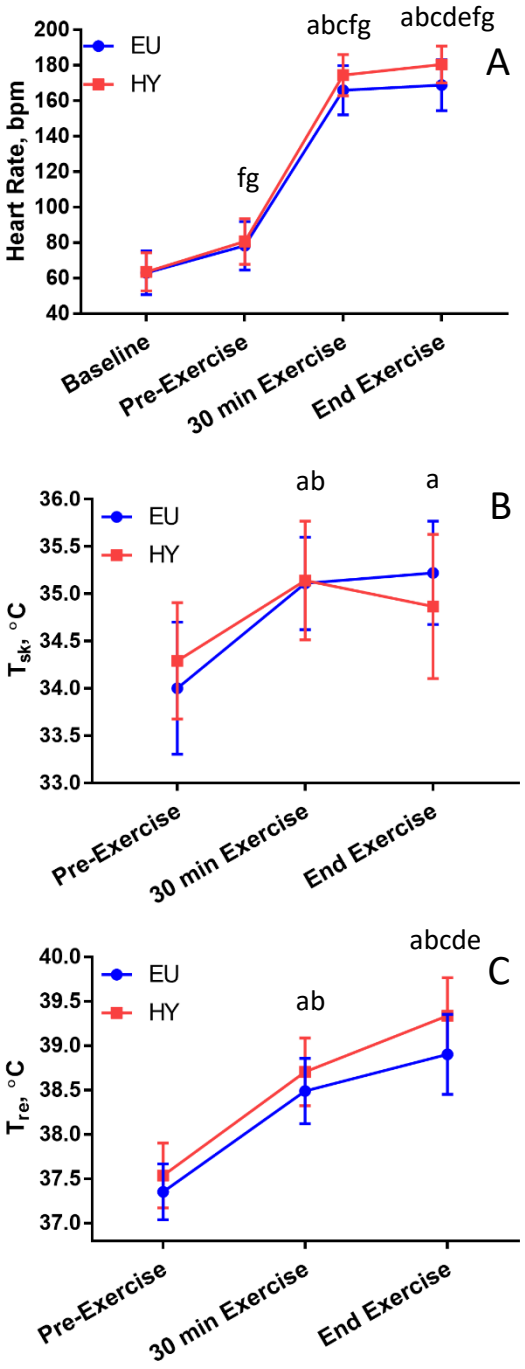
Figure 3: A) Urine NGAL **B)** Urine Creatinine **C)** Plasma NGAL and **D)** Serum Creatinine assessed at pre-exercise, post-exercise, and the 24-h follow-up visit. [a]Indicates different from baseline independent in hydration ($P < 0.05$). [b]Indicates different from post-exercise independent in hydration ($P < 0.05$). [c]Indicates different between hydration trials independent of time ($P < 0.05$). [d]Indicates different from EU trial at time point ($P < 0.05$).

Figure 4: Percent changes from baseline in Plasma NGAL at post-exercise and the 24-h follow-up visit. [a]Indicates different from EU trial at designated time point ($P < 0.05$).

Table 1. *Blood and Urine Markers of Hydration Assessed Pre-Exercise, Post-Exercise, and at the 24-H Follow-Up Visit.*

	Euhydrated Trial			Hypohydrated Trial		
Measure	Pre-Exercise	Post-Exercise	24-h Follow-up	Pre-Exercise	Post-Exercise	24-h Follow-up
Body Mass, kg	74.1 ± 8.5	73.1 ± 8.3	74.0 ± 8.4	72.1 ± 8.8[a]	70.5 ± 8.5[a]	73.6 ± 9.0
Body Mass Δ, %	-0.2 ± 1.1	-1.2 ± 0.8	-0.2 ± 0.9	-2.6 ± 1.5[a]	-4.4 ± 1.9[a]	-0.6 ± 1.5
Uosm, mOsm·kg^{-1}	607 ± 232	503 ± 252	554 ± 295	1012 ± 130[a]	977 ± 112[a]	559 ± 392
Usg	1.017 ± 0.007	1.015 ± 0.009	1.015 ±0.009	1.026 ± 0.005[a]	1.028 ± 0.004[a]	1.016 ± 0.010
Sosm, mOsm·kg^{-1}	291 ± 4	288 ± 5	292 ± 4	299 ± 6[a]	302 ± 8[a]	293 ± 5
Serum Na^+, mEq·L^{-1}	137.2 ± 1.6	135.9 ± 1.6	137.3 ± 1.3	140.3 ± 2.4[a]	141.5 ± 2.7[a]	137.6 ± 1.8
Serum Protein	6.7 ± 0.4	7.5 ± 0.7	6.9 ± 0.4	7.3 ± 0.4	7.9 ± 0.4	6.8 ± 0.4
Hb, g·dL^{-1}	14.9 ± 1.0	15.2 ± 0.9	14.6 ± 0.9	15.4 ± 1.0	16 ± 1.2	14.6 ± 1.1
Hct, %[b]	44.6 ± 2.9	44.6 ± 2.8	43.6 ± 2.3[cd]	45.1 ± 2.2	46.1 ± 2.3	44.1 ± 2.3[cd]

Note: Δ=change; Uosm=urine osmolality; Usg=urine specific gravity; Sosm=serum osmolality; Serum Na^+=serum sodium; Hb=hemoglobin; Hct=hematocrit. [a] *Indicates difference from EU trial at respective time point.* [b] *Indicates main effect of trial.* [c] *Indicates different from pre-exercise.* [d] *Indicates different from post-exercise.*


A
EU
HY
Heart Rate, bpm
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140
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abcfg
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Baseline
Pre-Exercise
30 min Exercise
End Exercise
B
EU
HY
T_{sk}, °C
36.0
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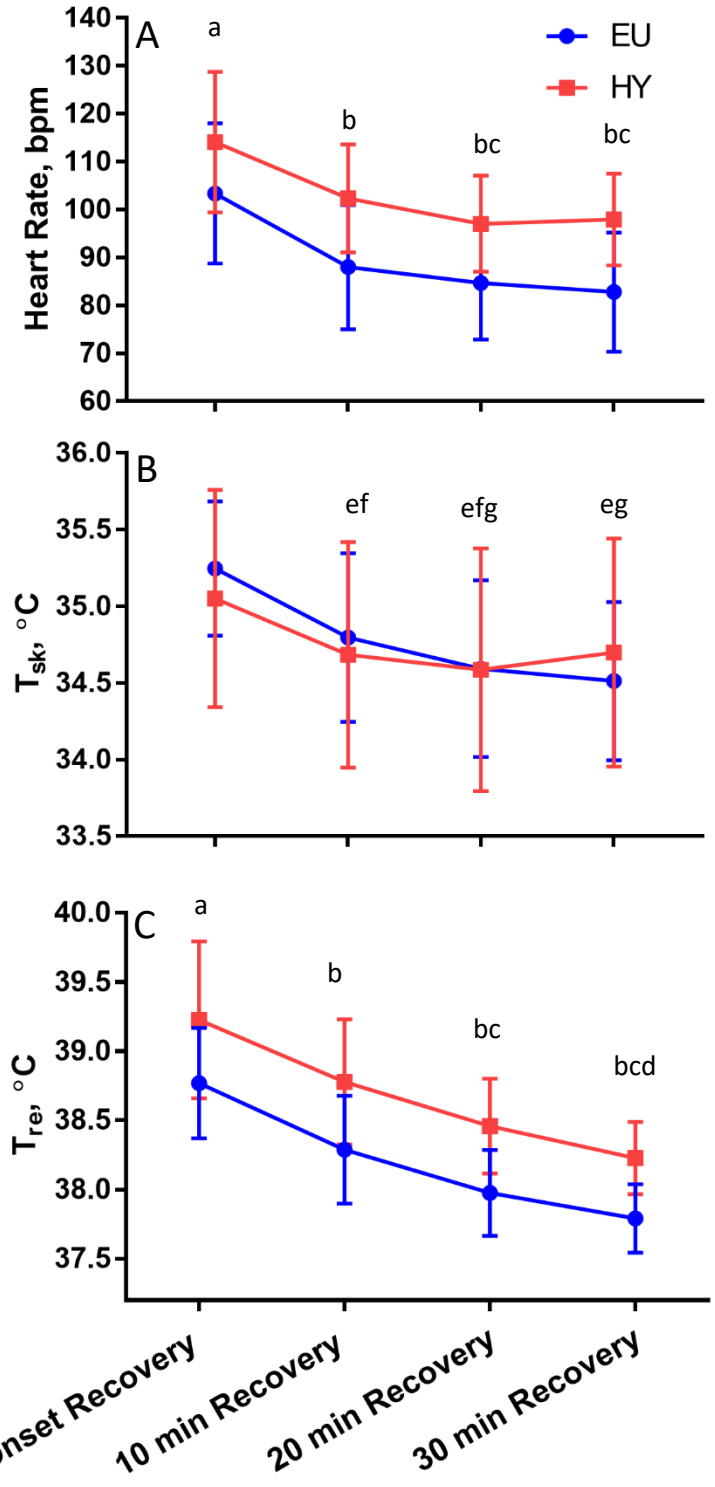
Figure 1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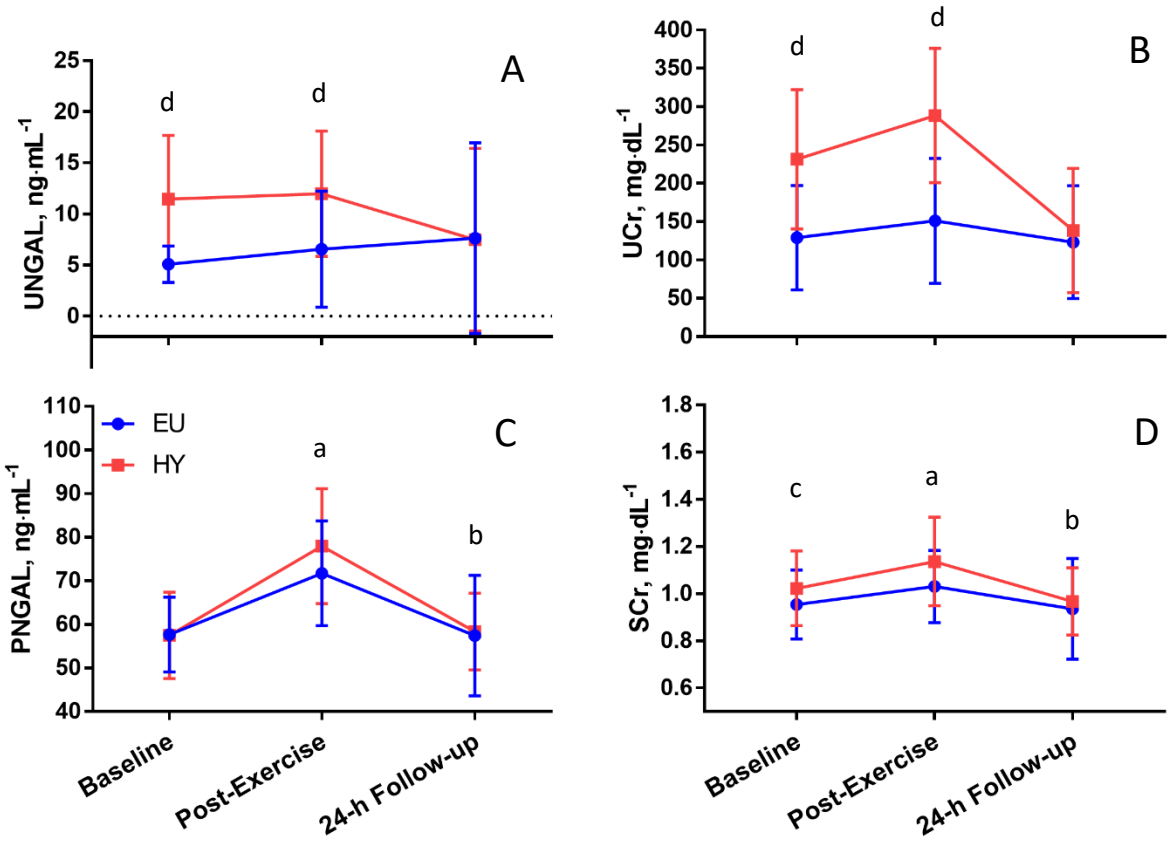
Onset Recovery
10 min Recovery
20 min Recovery
30 min Recovery

Figure 2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UNGAL, ng·mL-1
UCr, mg·dL-1
PNGAL, ng·mL-1
SCr, mg·dL-1
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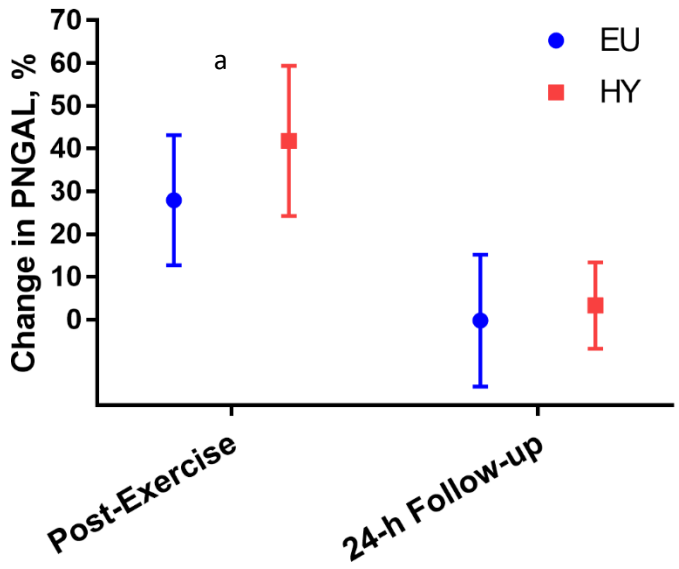
Baseline
Post-Exercise
24-h Follow-up
Baseline
Post-Exercise
24-h Follow-up

Figure 3


70
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Change in PNGAL, %
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Post-Exercise
24-h Follow-up

Figure 4

V. Manuscript #2: Influences of Hypohydration During Exercise-Induced Muscle Damage on Recovery

Cory L. Butts, Aaron R. Caldwell, Matthew S. Ganio, Nicholas P. Greene, Stavros A. Kavouras, Ronna C. Turner, Brendon P. McDermott

Exercise Science Research Center, University of Arkansas, Fayetteville, Arkansas, USA

Abstract

Purpose: Identify the effects of mild hypohydration on muscular strength recovery indices as well as muscle damage biomarkers from eccentric knee flexion followed by exercise in the heat. **Methods:** Recreationally active males (n = 18, age 24±5 y, mass 75.9±10.0 kg, bf 17.3±6.2%) completed two experimental conditions consisting of either euhydration (EU; maintaining hydration) or hypohydration (HY; restricting fluid consumption for 24 hours prior to and during the trial) separated by ≥28 days. Participants completed a baseline 20-minute rest, muscle damaging protocol, treadmill exercise in the heat, passive recovery, and a rehydrated 24-h follow-up visit, respectively. The muscle damage was induced through contralateral (opposite leg for each trial) eccentric knee flexion exercises (30°/sec) on an isokinetic dynamometer. Isometric (70° and 90°) and isokinetic (60°/sec) strength was performed immediately before and after damage as well as during the 24-h follow-up. **Results:** Fluid restriction induced -2.6±1.5% reduction in body mass at the beginning of the trial, while body mass was maintained in the EU trial (-0.2±1.1%, $P < 0.001$). Strength was reduced independent of trial for isometric strength at 70° ($F_{2,32} = 12.54$, $P < 0.001$), isometric strength at 90° ($F_{2,32} = 8.96$, $P = 0.001$), and isokinetic strength at 60°·sec^{-1} ($F_{2,32} = 8.11$, $P = 0.001$). Serum creatine kinase increased regardless of trial ($F_{1.32,18.4} = 24.42$, $P < 0.001$), with the 24-h follow-up greater (grand mean; 58.7±25.1 U/L) than at baseline (grand mean; 35.7±23.1, $P < 0.001$) and post exercise (grand mean; 51.6±23.2 U/L, P=0.009). **Conclusions:** We demonstrated no significant impact of hydration status when performing muscle damaging exercise, followed by exercise in the heat, on indices of muscle damage recovery. Further, the rehydration protocol successfully returned participants to a EU state by the 24-h follow-up, which may have impacted the recovery from muscle damaging protocol.

Introduction

A potential consequence of resistance exercise involves damaging muscle tissue, particularly if the movements involve an eccentric component (1-3). Exercise induced muscle damage may range from asymptomatic increases in biomarkers to exertional rhabdomyolysis requiring medical attention. Subclinical rhabdomyolysis not requiring medical treatment can easily be treated with rest and hydration (1). Muscle damaging exercise decreases the ability to generate force and may have implications for subsequent performance (4-10). As such, controlling factors that may affect muscle damage and recovery is essential for athletes to enable expeditious returns in performance.

Athletes commonly arrive to exercise bouts, athletic events, and practices in a fluid conservation state when evaluated by urinary indices (e.g. specific gravity, color, osmolality) (11-14). Further, reductions in body weight have been shown across pre-season practices in addition to concentrated urine production, suggesting potential losses in total body water (12). As such, poor hydration practices during and following practices may lead to dehydration, potentially impacting performance and altering recovery (2, 15, 16). This particularly applies in settings where there is a reduced time between practices (i.e. two-a-days) or preseason where individuals are undergoing rigorous workouts day after day (i.e. muscle damaging exercise).

The impact of suboptimal hydration on muscle damaging exercise has received relatively little investigation. While hypohydration is consistently found to reduce endurance performance, impacts on muscular strength or power are more controversial (2, 15). In a review by Judelson et al. (2), authors concluded that the overall effect of dehydration on muscular strength and power was negative. However, study limitations often prevent the clear interpretation of findings, making it difficult to ascertain the effects of hypohydration on resistance and power performance

(2, 15). Regardless, increases in physiological and perceptual strain associated with hypohydration merit the support for athletes to perform these exercises in a well hydrated state.

Hydration impacts resistance exercise performance, however, it has been shown to have no impact on circulating markers of muscle damage (i.e. creatine kinase and myoglobin) often seen following exercise (3). Moderate hypohydration (5%) was successful at inducing slight increases in myoglobin one and two hours post-exercise, however, total work was not affected compared to an euhydrated condition. Dehydration combined with hyperthermia may also impact recovery from muscle damaging exercise (6). Cleary et al. (6) investigated the effects exercise induced dehydration on delayed onset muscle soreness (DOMS) recovery from downhill running in the heat. Perceptions of lower extremity pain were significantly elevated in the dehydrated trial, albeit with no differences in muscular strength (6). Interestingly, when downhill running was performed in a thermoneutral environment, the effects of dehydration on DOMS were ameliorated (7). Therefore, the addition of hyperthermia with concomitant dehydration may impact skeletal muscle recovery. In organized sport, when compared with a normothermic soccer match, there was no impact of heat stress on markers of muscle damage recovery, however perceptions were not assessed (17). Furthermore, these data were collected post-soccer match therefore, the hydration and damage responses may have differed significantly between individuals. Regardless, the impact of muscle damaging exercise with concomitant dehydration may exacerbate symptoms of DOMS, potentially due to delayed recovery induced via hyperthermia and dehydration.

Recovery from muscle damaging exercise performed while in a fluid conserving state has not been extensively investigated. Further, muscle damaging exercise is likely associated with a strength and conditioning session that would be completed in a normothermic environment

followed by exercise in the heat, rather than all in a heated environment. Therefore, the purpose of this study was to identify the effects of performing muscle damaging exercise followed by exercise in the heat while hypohydrated, on muscular strength indices (i.e. isometric and isokinetic strength) as well as muscle damage biomarkers (creatine kinase), when full rehydration was conducted in recovery. Based on previous investigations, it was hypothesized that there would be no differences in muscle damage biomarkers with hypohydration, however, muscle strength or recovery would be modestly impaired (i.e. slightly greater reductions) when compared to a euhydrated state.

Methods

Eighteen healthy, recreationally active males (age 24 ± 5 y, wt 75.9 ± 10.0 kg, ht 1.79 ± 0.05m, bf 17.3 ± 6.2%) were recruited from the University and surrounding areas to participate in this randomized, counterbalanced, crossover design study. All procedures were approved by the University Institutional Review Board and written informed consent was acquired from all individuals prior to participation. The study consisted of five visits including one familiarization day and two experimental days (one euhydrated; EU and one hypohydrated; HY) each with 24-h follow-ups. Experimental days were separated by ≥28 days (average; 41 ± 16 days), and muscle damaging exercise was completed on contralateral legs to mitigate the repeated bout effect (18).

Familiarization Day:

During the initial familiarization visit, participants completed a medical history questionnaire and signed an informed consent form. Exclusionary criteria included previous heat exhaustion or heat stroke within the past 3 years, current musculoskeletal injury, hypertension where vigorous exercise is contraindicated, diagnosed sickle cell trait, use of medications that may alter thermoregulation or kidney function, current use of creatine supplementation, and a

history of kidney disease. Upon approval, baseline demographic information was collected and body composition assessed via dual energy x-ray absorptiometry (DXA). Participants then completed a five-minute warm-up on a cycle ergometer (~50W) and were fitted to the isokinetic dynamometer (Biodex System 3, Biodex Medical Systems, New York) with seat and leg positions recorded for future testing. Baseline isometric strength at 70° and 90°, and isokinetic strength at $60°\cdot sec^{-1}$ were competed in triplicate and future eccentric procedures were explained. Because these were used as a familiarization, these measures were not included in analysis.

Experimental Days:

All participants were asked to refrain from alcohol use for 24-h, caffeine use for 12-h, resistance training for 5-days and exercise for 24-h prior to each trial. For the HY trial, the dehydration protocol consisted of 24-h fluid restriction with minimal fluid provided throughout the trial. Prior to the EU trial, participants were instructed to consume fluids prior to arrival, while water was provided during the trial to ensure less than 2% body mass loss.

Prior to arrival for both trials, participants recorded three-day baseline body masses on a lab provided scale (BalanceFrom High Accuracy Bathroom Scale, BalanceFrom LLC, China) on the days leading up to the trials. For the 24-h prior to arrival, participants recorded their diet using a standard diet log and were asked to repeat a similar diet for the second trial. Additionally, participants collected all urinations for the 24-h prior to the start of trial, which was subsequently analyzed for 24-hr urine osmolality (freezing point depression, Model 3250, Advanced Instruments Inc., Norwood, MA).

Upon arrival to the laboratory, participants completed a nude body mass and dressed in shorts and a t-shirt, followed by a 20-minute semi-recumbent baseline rest in a thermoneutral environment (~20°C). During this time, participants were informed of perceptual scales for

rating of perceived exertion (RPE) and muscle pain and baseline values were recorded. This was followed by a blood draw via venipuncture to assess serum creatine kinase (SCK).

Participants moved to a cycle ergometer (Monark 828E, Monark Exercise AB, Sweden) to complete a 5-minute warm-up at 50W before completing the muscle damaging protocol on an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems, New York) (18). The muscle damaging procedure involved maximal effort unilateral eccentric knee extension exercise, with the contralateral leg utilized during the second trial to minimize potential repeated bout effects. The leg used during the first trial was randomized and counterbalanced for hydration and dominance between participants. For each trial, participants completed 10 sets of 10 maximal effort eccentric knee flexion repetitions at a speed of 30°/s with sets separated by one-minute (18). Prior to, and immediately following the eccentric protocol, isometric strength (i.e. peak torque) measures were performed via three 5-second maximal voluntary concentric knee extensor contractions at 70° and 90° knee flexion with one minutes of rest between repetitions. Isokinetic concentric knee extensor strength (i.e. peak torque) was also completed in triplicate at $60° \cdot sec^{-1}$. Perceptions of muscle pain and RPE were recorded following the exercise induced muscle damage. Decreases in strength, as well as elevations in SCK and muscle pain, served as indices of muscle damage (5). As this was part of a larger study on renal biomarkers, the purpose of the eccentric exercise protocol was to induce mild muscle damage similar to athletic practices or labor-intensive occupational settings, thus physiological adaptations were not expected to alter the findings given our counterbalanced, crossover study design. Also, the participants were directed to avoid changes in their exercise regimen between trials to minimize changes in fitness.

Upon completion of the exercise induced muscle damage, participants moved to an environmental chamber set to a hot, humid environment (33°C, 50% relative humidity) to complete treadmill running at 60% VO_2max (1% grade) for 60 minutes with a five-minute walking (3 mph) warm-up, break at 30 minutes, and cool-down at 60 minutes. This protocol was designed to increase thermal stress and further dehydrate participants during the HY trial and prevent >2% body mass loss in the EU trial. Immediately following exercise, participants remained in the chamber to commence a 30-minute semi-recumbent recovery with a post-exercise blood draw taken at 20 minutes.

After the recovery period, participants provided another body mass and urine sample, and were provided with a rehydration protocol in both trials, such that the participant would replace 100% of losses in the initial four hours following the trial, ensuring to consume food to prevent over-hydration. Additionally, participants were then encouraged to consume at least an additional 2.5 liters to aid in the production of dilute urine samples.

Participants returned to the laboratory for a 24-h follow-up visit. Visits involved a nude body mass followed by 20-minute semi-recumbent rest period, during which perceptual measures, and a blood draw were collected. Participants then completed the five-minute warm-up on a cycle ergometer at 50W before moving to the isokinetic dynamometer. Concentric knee extension strength was once again recorded for isometric contractions at 70° and 90° of knee flexion as well as isokinetic contractions at $60° \cdot sec^{-1}$ knee extension. Participants were then instructed to resume normal exercise routines and second visits were scheduled, if applicable.

Blood Analysis

After clotting, serum samples were centrifuged for 15 minutes at 1000 g and 4°C. Serum was aliquoted and stored at -80°C for further analysis. Serum creatine kinase (SCK) was

assessed using a commercially available colorimetric assay (Bioassay Systems Inc, Hayward, CA) with an average coefficient of variation of 2.1%.

Statistical Analysis

All statistical analyses were completed using SPSS version 24 (IBM Corporation, Somers, NY). A repeated measures multivariate analysis of variance was used to assess absolute peak torque (i.e. strength) across time (pre-muscle damage, post-muscle damage, and the 24-h follow-up) and between hydration (EU and HY) for isometric peak torque at 70° and 90° knee flexion and $60°\cdot sec^{-1}$ isokinetic peak torque. Additional variables (body mass, creatine kinase) were analyzed using two-way (time x hydration) repeated measures analysis of variance. When sphericity was violated, Greenhouse-Geisser adjustments were used. Post-hoc analyses involved pairwise comparisons with an appropriate Bonferroni corrected alpha to identify significant time point differences. Dependent t-tests were used to assess differences in 24-h urine osmolality, total eccentric work, average eccentric peak torque and ratings of perceived exertion between trials. Data that failed normality tests (muscle pain) were analyzed with a Friedman test across time and between hydration states. Follow-up pairwise analysis were conducted using a Wilcoxon signed rank test for individual time point differences. All partial eta squared (η_p^2) and Hedge's g values were calculated using a spreadsheet from Lakens (19). Alpha of 0.05 was set *a priori* to determine significance at the omnibus level. Based on a .80 power calculation using the primary outcome variable NGAL (20, 21) with a correlation between time points of 0.42, a 2-standard deviation effect size, β of 0.20, and α of 0.05, it was determined 17 participants would be sufficient to complete this study. An increased experiment-wise type I error rate is acknowledged due to the multiple multivariate and univariate analyses conducted. Because the experimental protocol is time, resource, and cost intensive, power estimates were calculated

based on singular analyses to provide initial experimental outcome indicators and guide future research.

Results

Hydration

There was a significant interaction of body mass for time and trial ($F_{2,32} = 24.51$, $P < 0.001$, $\eta_p^2 = 0.61$), as three-day baseline body masses were not different between trials (EU 73.7 ± 8.6, HY 73.8 ± 9.4 kg, $P = 0.87$), while body mass at the beginning of the trial was reduced following fluid restriction (HY 72.1 ± 8.8kg, EU 74.1 ± 8.5kg, $P < 0.001$). By design, the body masses were not different at the 24-h follow-up visit (EU 74.0 ± 8.4, HY 73.6 ± 9.0 kg, $P = 0.29$). There was also an interaction of time and trial ($F_{1,15} = 103.50$, $P < 0.001$, $\eta_p^2 = 0.87$) for percent body mass change, with the fluid restriction inducing a -2.6±1.5% reduction in body mass from the three-day baseline body mass at the beginning of the trial, while body mass was maintained in the EU trial (-0.2±1.1%, $P < 0.001$). There were no differences between trials at the 24-h follow-up for percent change from the three-day baseline (EU -0.2±0.9, HY -0.6±1.5%, $P=0.26$). Urine collection for 24 h prior to the trial days showed greater urine osmolality following fluid restriction (HY 775±180, EU 427±188 mOsm/kg, $t_{17} = -6.71$, $P < 0.001$, Hedge's g = -3.01).

Muscular Strength & Muscle Damage Markers

Total work completed during eccentric exercise was not different between trials (EU 8663 ± 2651, HY 8280 ± 2102 J, $t_{17} = 0.90$, $P = 0.38$, Hedge's g = 0.40). Average peak eccentric torque was also not different between trials (EU 196.1±62.5, HY 186.3±57.1 N·m, $t_{17} = 1.22$, $P = 0.24$, Hedge's g = 0.55).

Muscular strength was assessed using a multivariate analysis including isometric peak torque measured at 70° and 90° as well as peak isokinetic torque assessed at 60°·sec^{-1}. At the multivariate level, there was a main effect of time (Wilks $\Lambda = 0.35$, $F_{6,11} = 3.49$, $P = 0.04$), however, there was no effect of hydration (Wilks $\Lambda = 0.80$, $F_{3,14} = 1.14$, $P = 0.37$) or interaction of time and hydration (Wilks $\Lambda = 0.51$, $F_{6,11} = 1.76$, $P = 0.20$).

Univariate analysis of time was then conducted using an alpha of 0.017. Strength was reduced independent of trial (i.e. main effect of time) for isometric strength at 70° ($F_{2,32} = 12.54$, $P < 0.001$, $\eta_p^2 = 0.44$, Figure 1C), isometric strength at 90° ($F_{2,32} = 8.96$, $P = 0.001$, $\eta_p^2 = 0.36$, Figure 1B), and isokinetic strength at 60°·sec^{-1} ($F_{2,32} = 8.11$, $P = 0.001$, $\eta_p^2 = 0.34$, Figure 1A). Pairwise comparisons for each of the strength measures were then completed using an alpha of 0.006. Isometric strength at 70° decreased from baseline (grand mean; 203.2 ± 56.8 N·m) to immediate-post damage (grand mean; 166.0±45.2 N·m, $P = 0.001$) and remained reduced at the 24 h follow-up (grand mean; 182.9 ± 50.8 N·m, $P = 0.006$). Isometric at 90° was also reduced from baseline (grand mean; 227.2 ± 65.7 N·m) to immediate-post damage (grand mean; 188.9 ± 55.1 N·m, $P < 0.001$) and tended to be reduced at the 24 h follow-up (grand mean; 200.2 ± 58.6 N·m, $P = 0.007$). Isokinetic strength at 60°·sec^{-1} was reduced immediately post-damage (grand mean; 143.2 ± 42.0 N·m) compared to baseline (grand mean; 168.0 ± 53.7 N·m, P =0.003), however, there was no difference between baseline and the 24-h follow-up (grand mean; 156.5 ± 38.0 N·m, $P = 0.10$).

SCK analysis revealed increases regardless of hydration ($F_{1.32,18.4} = 24.42$, $P < 0.001$, $\eta_p^2 = 0.64$) with no differences between trials ($F_{1,14} = 0.36$, $P = 0.56$, $\eta_p^2 = 0.04$) and no interaction effects ($F_{1.2,16.5} = 1.002$, $P = 0.35$, $\eta_p^2 = 0.07$, Figure 2). The 24-h follow-up SCK was greater (grand mean; 58.7 ± 25.1 U/L) than baseline (grand mean; 35.7 ± 23.1, $P < 0.001$) and post-

exercise (grand mean; 51.6 ± 23.2 U/L, P=0.009). In addition, the post-exercise SCK was greater than at baseline ($P < 0.001$).

Perceptual Measures

Muscle pain was affected by time ($\chi^2_2 = 30.10$, $P < 0.001$) and trial ($\chi^2_1 = 4.57$, $P = 0.03$), however, there were no differences between trials for muscle pain at any time (all $P > 0.017$). Rather, pain was elevated above baseline (EU 0.1 ± 0.2, HY 0.1 ± 0.2) immediately post damage (EU 2.7 ± 1.7, HY 3.5 ± 1.6, both $P < 0.001$) and at the 24-h follow-up (EU 1.3 ± 1.4, HY 1.4 ± 1.6, both $P < 0.001$). Ratings of Perceived Exertion at the end of the muscle damaging exercise were lower in the EU trial (EU 15.8 ± 2.0, HY 16.6 ± 1.8, $t_{16} = -2.75$, $P = 0.01$, Hedge's g = -1.27).

Discussion

The purpose of this investigation was to evaluate the influence of performing muscle damaging exercise while fluid restricted on indices of muscle damage recovery when muscle damaging exercise was followed by exertional hyperthermia. Further, we removed the impact of hypohydration in recovery by prescribing fluid replacement following both EU and HY trials, thus isolating the impact of hypohydration during muscle damaging exercise. Contrary to our hypothesis, our participants demonstrated no differences between trials in strength decreases, a marker of muscle damage. As expected, however, there was no difference between trials for SCK responses at any time point.

Maintaining a positive fluid balance with exercise can be difficult, particularly when completing repeated bouts in hot, humid conditions (e.g. pre-season practice). This is demonstrated by athletes regularly reporting to activities in a state of water conservation (i.e. producing concentrated urine samples) (11, 12, 14, 22). This suboptimal hydration has many

implications on athlete safety and performance. For example, many sources have found detriments in resistance exercise performance when dehydrated (2, 15). Poor hydration is also implicated to alter physiological and perceptual recovery following exercise in the heat (23-26). Therefore, proper rehydration strategies such as those recommended in consensus statements (27, 28) are important to prevent delayed recovery and enhance the preparation for subsequent bouts (24, 26).

The impacts of exercise induced dehydration on symptoms of DOMS have been conducted in both hyperthermic (6) and normothermic males (7). Similar to the current study, Cleary et al. (7) found no impact of hypohydration on measures of isometric strength or muscle pain following muscle damaging exercise when individuals performed the exercise in a normothermic state. The authors utilized 45 minutes of downhill running to induce muscle damage presenting isometric strength decreases similar to the current study. In contrast, dehydration was conducted using walking in a hot, humid environment while we utilized 24-h fluid restriction to initiate dehydration. Regardless, the work by Cleary et al. (7) suggests that dehydration alone does not impact the extent of muscle damage, perceptions, or recovery.

In a separate study, Cleary et al. found that performing muscle damaging exercise with concomitant dehydration and hyperthermia led to increased perceived pain compared to the euhydrated hyperthermic trial (6). The current study utilized muscle damaging exercise while in a normothermic state, then participants experienced marked exertional hyperthermia, without showing any differences between hydration states. Therefore, the presence of increased muscle temperature during muscle damaging exercise may be required for hypohydration to have an impact on perceived muscular soreness (6). Cleary et al. (6) suggested that the presence of hypohydration augmented the intramuscular temperature due to thermoregulatory

compensations, which compromised the structural integrity of the tissue and led to increased damage. However, the authors did not report any differences between hydration trials for isometric quadriceps strength measures, therefore the extent of damage may have been related purely to perceptual measures (6). Further, another study confirmed these strength findings by elevating intramuscular temperature and completing muscle damaging exercise of the biceps (29). The authors found no impact of higher muscle temperature on indices of muscle damage compared to exercise performed in a normothermic state (29). This would suggest that the presence of hypohydration is necessary to influence changes in perception when muscle damage is performed in a hyperthermic condition and that these moderations are not purely temperature driven. Rather, hypohydration may alter the inflammatory response to increase sensitivity and subsequently drive pain (9). Fielding et al. showed that a 2% dehydration exercise protocol followed by exercise induced muscle damage caused greater circulating neutrophil release compared to the rest trial (30). Further, there were no differences in z-band damage nor SCK between the trials (30). The lack of differences in muscle damage indices in the current study further support that hyperthermia with concomitant hypohydration is necessary during the damaging process to alter recovery.

Hypohydration has also been shown to have minimal impact on biomarkers of muscle damage (3). Yamamoto et al. (3) evaluated the impact of hypohydration at 2.5% and 5% body mass loss on muscle damage markers following an intense resistance exercise protocol. The authors found no differences in SCK, but there were minor elevations in myoglobin with dehydration (3). Although the present study did not investigate myoglobin, we found no differences between hydration on the elevations in SCK, however, our damaging protocol resulted in >1.5-fold elevations in SCK at 24-h. Peak SCK values are generally reported at ~3-4

days post damage, therefore it is likely there were greater elevations in these values (9). Additionally, it is recognized that the values reported for the current study are within normal range for healthy individuals (<175 U/L) (8). However, SCK as a biomarker of muscle damage does not necessarily reflect the extent of disruption in the tissue with a great amount of inter-individual variability (5, 8, 30). Regardless, the low SCK response may have been a function of the methodology used for inducing muscle damage (i.e. unilateral knee extension) resulting in low concentrations or possibly the colorimetric assay used to assess CK activity rather than true concentration. Regardless, the SCK response was one that increased, thus confirming the presence of muscle damage.

As this study was part of a larger investigation of biomarkers of acute kidney injury, there were limitations in the methodology. We provided instructions to participants to rehydrate within four hours of finishing the trial and continue recommended fluid consumption until the 24-h follow-up. This rehydration was performed by the participant outside the laboratory, therefore we relied on participants to complete the procedure and verbally confirmed compliance during the 24-h follow-up. Additionally, we cannot comment on the impacts of poor hydration following muscle damaging exercise on recovery indices. However, restricting fluids following a muscle damaging exercise bout with exercise in the heat may place substantial strain on the renal system and increase risk for exertional rhabdomyolysis. The presence of hypohydration, heat stress, and unaccustomed exercise are thought to create the "perfect storm" and have been reported in case studies to contribute to clinically significant cases of exertional rhabdomyolysis (31, 32). As such, the goal of this study was to evaluate the implications for reporting to an exercise session following poor fluid intake practices but finishing trials with proper rehydration to evaluate the impacts on muscle damage recovery. To further ensure safety, we used a

unilateral knee extensor protocol for inducing muscle damage to mitigate the risk of rhabdomyolysis in participants. As such, future studies may be necessary to evaluate the impacts of delayed rehydration or partial rehydration on muscle damage severity and recovery.

In conclusion, the findings of this study apply particularly well in settings where teams may undergo a strength training protocol (i.e. muscle damaging) followed by practice or conditioning session in a hot, humid environment. We demonstrated no significant impact of hydration status when performing muscle damaging exercise, followed by exercise in the heat, on severity of muscle damage and pain, or indices of recovery. Further, the rehydration protocol successfully returned participants to a euhydrated state by the 24-h follow-up, which may have facilitated the recovery from our muscle damaging protocol. Therefore, when athletes report to activities in a state of water conservation, it is unlikely that recovery form any muscle damaging exercise will be impacted as long as the athlete rehydrates according to the National Athletic Training Association and American College of Sport Medicine guidelines (27, 28). However, hypohydration, heat stress and unaccustomed exercise may increase risk for exertional rhabdomyolysis. As such, to improve overall safety, coaches, strength staff, and clinicians should encourage athletes to report to practices to well prepared with proper hydration and diet.

References

1. Tietze DC, Borchers J. Exertional rhabdomyolysis in the athlete: a clinical review. Sports Health. 2014;6(4):336-9. doi: 10.1177/1941738114523544. PubMed PMID: 24982707; PubMed Central PMCID: PMCPMC4065559.

2. Judelson DA, Maresh CM, Anderson JM, Armstrong LE, Casa DJ, Kraemer WJ, et al. Hydration and muscular performance: does fluid balance affect strength, power and high-intensity endurance? Sports Med. 2007;37(10):907-21. PubMed PMID: 17887814.

3. Yamamoto LM, Judelson DA, Farrell MJ, Lee EC, Armstrong LE, Casa DJ, et al. Effects of hydration state and resistance exercise on markers of muscle damage. J Strength Cond Res. 2008;22(5):1387-93. doi: 10.1519/JSC.0b013e3181739403. PubMed PMID: 18714253.

4. Doma K, Leicht A, Sinclair W, Schumann M, Damas F, Burt D, et al. The impact of exercise-induced muscle damage on performance test outcomes in elite female basketball players. J Strength Cond Res. 2017. Epub 2017/09/11. doi: 10.1519/JSC.0000000000002244. PubMed PMID: 28930877.

5. Damas F, Nosaka K, Libardi CA, Chen TC, Ugrinowitsch C. Susceptibility to Exercise-Induced Muscle Damage: a Cluster Analysis with a Large Sample. Int J Sports Med. 2016;37(8):633-40. Epub 2016/04/26. doi: 10.1055/s-0042-100281. PubMed PMID: 27116346.

6. Cleary MA, Sweeney LA, Kendrick ZV, Sitler MR. Dehydration and symptoms of delayed-onset muscle soreness in hyperthermic males. J Athl Train. 2005;40(4):288-97. PubMed PMID: 16404450; PubMed Central PMCID: PMCPMC1323290.

7. Cleary MA, Sitler MR, Kendrick ZV. Dehydration and symptoms of delayed-onset muscle soreness in normothermic men. J Athl Train. 2006;41(1):36-45. PubMed PMID: 16619093; PubMed Central PMCID: PMCPMC1421497.

8. Baird MF, Graham SM, Baker JS, Bickerstaff GF. Creatine-kinase- and exercise-related muscle damage implications for muscle performance and recovery. J Nutr Metab. 2012;2012:960363. Epub 2012/01/11. doi: 10.1155/2012/960363. PubMed PMID: 22288008; PubMed Central PMCID: PMCPMC3263635.

9. Peake JM, Neubauer O, Della Gatta PA, Nosaka K. Muscle damage and inflammation during recovery from exercise. J Appl Physiol (1985). 2017;122(3):559-70. Epub 2016/12/29. doi: 10.1152/japplphysiol.00971.2016. PubMed PMID: 28035017.

10. Hubal MJ, Rubinstein SR, Clarkson PM. Mechanisms of variability in strength loss after muscle-lengthening actions. Med Sci Sports Exerc. 2007;39(3):461-8. doi: 10.1249/01.mss.0000247007.19127.da. PubMed PMID: 17473772.

11. Osterberg KL, Horswill CA, Baker LB. Pregame urine specific gravity and fluid intake by National Basketball Association players during competition. J Athl Train. 2009;44(1):53-7. doi: 10.4085/1062-6050-44.1.53. PubMed PMID: 19180219; PubMed Central PMCID: PMCPMC2629040.

12. Godek SF, Godek JJ, Bartolozzi AR. Hydration status in college football players during consecutive days of twice-a-day preseason practices. Am J Sports Med. 2005;33(6):843-51. doi: 10.1177/0363546504270999. PubMed PMID: 15827364.

13. Yeargin SW, Casa DJ, Armstrong LE, Watson G, Judelson DA, Psathas E, et al. Heat acclimatization and hydration status of American football players during initial summer workouts. J Strength Cond Res. 2006;20(3):463-70. doi: 10.1519/20596.1. PubMed PMID: 16937956.

14. Yeargin SW, Casa DJ, Judelson DA, McDermott BP, Ganio MS, Lee EC, et al. Thermoregulatory responses and hydration practices in heat-acclimatized adolescents during preseason high school football. J Athl Train. 2010;45(2):136-46. doi: 10.4085/1062-6050-45.2.136. PubMed PMID: 20210617; PubMed Central PMCID: PMCPMC2838465.

15. Cheuvront SN, Kenefick RW. Dehydration: physiology, assessment, and performance effects. Compr Physiol. 2014;4(1):257-85. doi: 10.1002/cphy.c130017. PubMed PMID: 24692140.

16. Judelson DA, Maresh CM, Farrell MJ, Yamamoto LM, Armstrong LE, Kraemer WJ, et al. Effect of hydration state on strength, power, and resistance exercise performance. Med Sci Sports Exerc. 2007;39(10):1817-24. doi: 10.1249/mss.0b013e3180de5f22. PubMed PMID: 17909410.

17. Nybo L, Girard O, Mohr M, Knez W, Voss S, Racinais S. Markers of muscle damage and performance recovery after exercise in the heat. Med Sci Sports Exerc. 2013;45(5):860-8. doi: 10.1249/MSS.0b013e31827ded04. PubMed PMID: 23247710.

18. Xin L, Hyldahl RD, Chipkin SR, Clarkson PM. A contralateral repeated bout effect attenuates induction of NF-κB DNA binding following eccentric exercise. J Appl Physiol (1985). 2014;116(11):1473-80. doi: 10.1152/japplphysiol.00133.2013. PubMed PMID: 23950163.

19. Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. Front Psychol. 2013;4:863. Epub 2013/11/26. doi: 10.3389/fpsyg.2013.00863. PubMed PMID: 24324449; PubMed Central PMCID: PMCPMC3840331.

20. Junglee NA, Di Felice U, Dolci A, Fortes MB, Jibani MM, Lemmey AB, et al. Exercising in a hot environment with muscle damage: effects on acute kidney injury biomarkers and kidney function. Am J Physiol Renal Physiol. 2013;305(6):F813-20. doi: 10.1152/ajprenal.00091.2013. PubMed PMID: 23825078.

21. Melin B, Jimenez C, Savourey G, Bittel J, Cottet-Emard JM, Pequignot JM, et al. Effects of hydration state on hormonal and renal responses during moderate exercise in the heat. Eur J Appl Physiol Occup Physiol. 1997;76(4):320-7. doi: 10.1007/s004210050255. PubMed PMID: 9349646.

22. Godek SF, Bartolozzi AR, Burkholder R, Sugarman E, Dorshimer G. Core temperature and percentage of dehydration in professional football linemen and backs during preseason practices. J Athl Train. 2006;41(1):8-14; discussion -7. PubMed PMID: 16619090; PubMed Central PMCID: PMCPMC1421487.

23. Gagnon D, Lynn AG, Binder K, Boushel RC, Kenny GP. Mean arterial pressure following prolonged exercise in the heat: influence of training status and fluid replacement. Scand J Med Sci Sports. 2012;22(5):e99-e107. doi: 10.1111/j.1600-0838.2012.01506.x. PubMed PMID: 22830505.

24. McDermott BP, Casa DJ, Lee E, Yamamoto L, Beasley K, Emmanuel H, et al. Thermoregulation and stress hormone recovery after exercise dehydration: comparison of rehydration methods. J Athl Train. 2013;48(6):725-33. Epub 2013/10/23. doi: 10.4085/1062-6050-48.6.01. PubMed PMID: 24143900; PubMed Central PMCID: PMCPMC3867082.

25. Distefano LJ, Casa DJ, Vansumeren MM, Karslo RM, Huggins RA, Demartini JK, et al. Hypohydration and hyperthermia impair neuromuscular control after exercise. Med Sci Sports Exerc. 2013;45(6):1166-73. doi: 10.1249/MSS.0b013e3182805b83. PubMed PMID: 23274594.

26. McDermott BP, Casa DJ, Lee EC, Yamamoto LM, Beasley KN, Emmanuel H, et al. The influence of rehydration mode after exercise dehydration on cardiovascular function. J Strength Cond Res. 2013;27(8):2086-95. doi: 10.1519/JSC.0b013e318295d548. PubMed PMID: 23615479.

27. Sawka MN, Burke LM, Eichner ER, Maughan RJ, Montain SJ, Stachenfeld NS, et al. American College of Sports Medicine position stand. Exercise and fluid replacement. Med Sci Sports Exerc. 2007;39(2):377-90. doi: 10.1249/mss.0b013e31802ca597. PubMed PMID: 17277604.

28. McDermott BP, Anderson SA, Armstrong LE, Casa DJ, Cheuvront SN, Cooper L, et al. National Athletic Trainers' Association Position Statement: Fluid Replacement for the Physically Active. J Athl Train. 2017;52(9):877-95. doi: 10.4085/1062-6050-52.9.02. PubMed PMID: 28985128; PubMed Central PMCID: PMCPMC5634236.

29. Castellani JW, Zambraski EJ, Sawka MN, Urso ML. Does high muscle temperature accentuate skeletal muscle injury from eccentric exercise? Physiol Rep. 2016;4(9). Epub 2016/05/15. doi: 10.14814/phy2.12777. PubMed PMID: 27185904; PubMed Central PMCID: PMCPMC4873630.

30. Fielding RA, Violan MA, Svetkey L, Abad LW, Manfredi TJ, Cosmas A, et al. Effects of prior exercise on eccentric exercise-induced neutrophilia and enzyme release. Med Sci Sports Exerc. 2000;32(2):359-64. PubMed PMID: 10694117.

31. Cleary M, Ruiz D, Eberman L, Mitchell I, Binkley H. Dehydration, cramping, and exertional rhabdomyolysis: a case report with suggestions for recovery. J Sport Rehabil. 2007;16(3):244-59. PubMed PMID: 17923731.

32. Cleary MA, Sadowski KA, Lee SY, Miller GL, Nichols AW. Exertional rhabdomyolysis in an adolescent athlete during preseason conditioning: a perfect storm. J Strength Cond Res. 2011;25(12):3506-13. doi: 10.1519/JSC.0b013e318216302f. PubMed PMID: 22080315.

Figure Legends

Figure 1. A) Isokinetic peak torque at 60°/sec and isometric peak torque at **B)** 90° of knee flexion and **C)** 70° of knee flexion assessed immediately prior to and after muscle damaging exercise and during 24-h follow-up. [a]Indicates different from baseline independent of hydration ($P < 0.05$).

Figure 2. Serum creatine kinase activity measured immediately prior to and after muscle damaging exercise and during the 24-h follow-up. [a]Indicates different from baseline independent of hydration ($P < 0.05$). [b]Indicates different from post-damage independent of hydration ($P < 0.05$).

Figure 3. Perceived muscle pain recorded immediately prior to and after muscle damaging exercise and during 24-h follow-up. [a]Indicates different from baseline in both hydration states ($P < 0.05$).

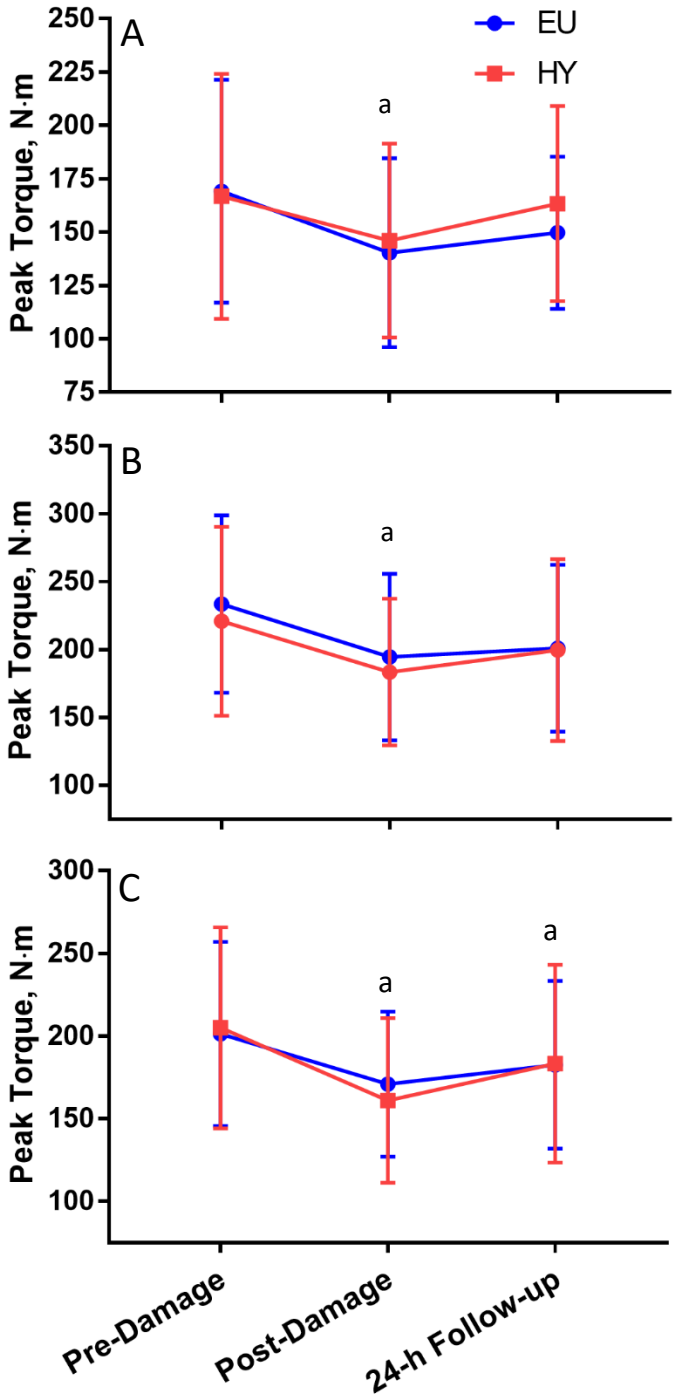
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Figure 1

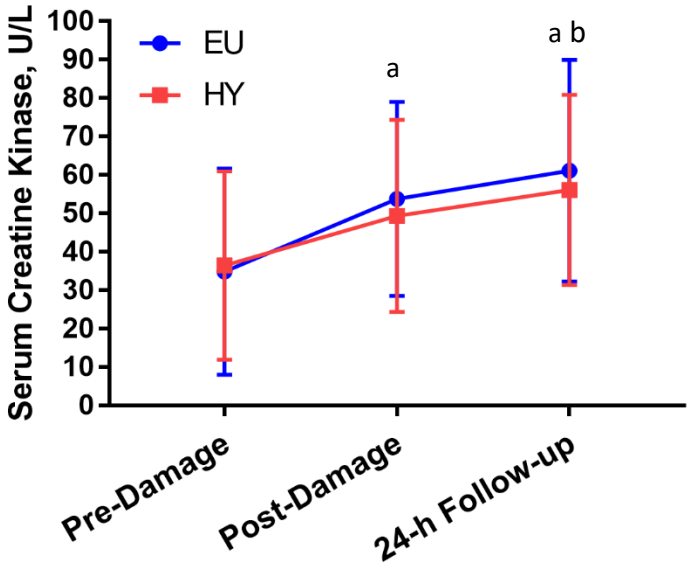
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Figure 2.

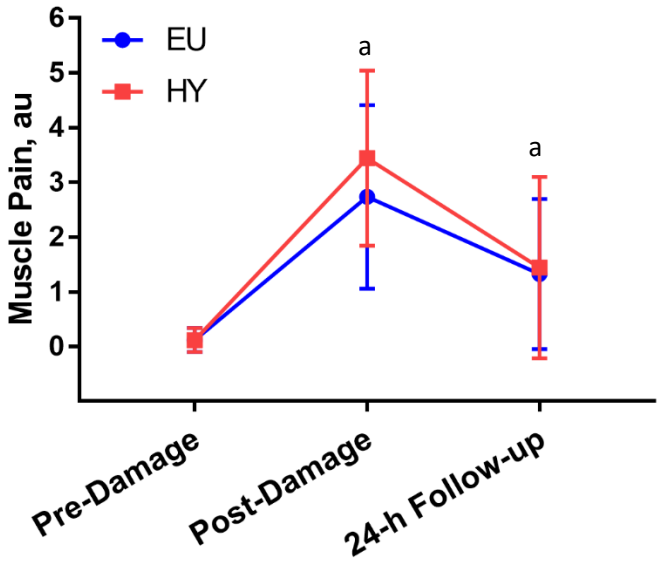
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Figure 3.

IV. Conclusions

These investigations were conducted to evaluate the influence of poor fluid intake practices on renal stress and recovery from muscle damaging exercise. By using fluid restriction prior to exercise, we mimicked conditions found in athletic, military, and occupational populations when reporting for activity. Further, providing minimal fluid during exercise further dehydrated individuals leading to greater physiological and perceptual strain. Therefore, our dehydration protocol was effective in creating a state of hypohydration prior to and throughout exercise. We followed this by providing fluid intake recommendations based on recent position stands for rapid rehydration. As such, the rehydration protocol was successful at returning individuals to a euhydrated state. By using these methodologies, we were able to evaluate the effects of performing exercise while suboptimally hydrated on renal stress and muscle damage recovery.

In Study 1, we demonstrated significantly greater increases in biomarkers of AKI when participants were hypohydrated. However, the fluid replacement protocol in this study returned biomarkers to baseline levels, indicating no lasting impairments in renal function. The dehydration protocol also augmented cardiovascular, thermal strain, and perceptual strain during exercise in the heat.

In Study 2, we demonstrated no impact of hydration status when performing muscle damaging exercise, followed by exercise in the heat, on muscle damage and pain recovery. We also demonstrated no influence of hydration on serum creatine kinase, a common biomarker of muscle damage or on isokinetic or isometric muscular strength. The rehydration protocol used may have aided in recovery to mitigate any detriments in muscular strength between hydration trials.

There is overwhelming evidence to suggest a negative role of dehydration in renal function, thermoregulation, performance, and cardiovascular stability. The concomitant exposure to physiological (i.e. exercise, muscle damage) and environmental (high ambient temperature and humidity) stressors commonly experienced by athletes, military, and occupational populations augment the deleterious responses to dehydration. We demonstrated that poor fluid intake practices leading into, and throughout, activity negatively impact renal, physiological, and perceptual measures. However, proper fluid intake following this stressful environment ameliorated the negative impacts of the previous hypohydration. Together, these studies provide support for proper rehydration following dehydrating activity to prevent deleterious impacts on renal and muscular recovery.

References

Agarwal, D., Elks, C. M., Reed, S. D., Mariappan, N., Majid, D. S., & Francis, J. (2012). Chronic exercise preserves renal structure and hemodynamics in spontaneously hypertensive rats. *Antioxid Redox Signal, 16*(2), 139-152. doi:10.1089/ars.2011.3967

Aizawa, H., Morita, K., Minami, H., Sasaki, N., & Tobise, K. (1995). Exertional rhabdomyolysis as a result of strenuous military training. *J Neurol Sci, 132*(2), 239-240.

Alge, J. L., & Arthur, J. M. (2015). Biomarkers of AKI: a review of mechanistic relevance and potential therapeutic implications. *Clin J Am Soc Nephrol, 10*(1), 147-155. doi:10.2215/CJN.12191213

Anzalone, M. L., Green, V. S., Buja, M., Sanchez, L. A., Harrykissoon, R. I., & Eichner, E. R. (2010). Sickle cell trait and fatal rhabdomyolysis in football training: a case study. *Med Sci Sports Exerc, 42*(1), 3-7. doi:10.1249/MSS.0b013e3181ae0700

Aparicio, V. A., Tassi, M., Nebot, E., Camiletti-Moirón, D., Ortega, E., Porres, J. M., & Aranda, P. (2014). High-intensity exercise may compromise renal morphology in rats. *Int J Sports Med, 35*(8), 639-644. doi:10.1055/s-0033-1354383

Armstrong, L. E., Johnson, E. C., Casa, D. J., Ganio, M. S., McDermott, B. P., Yamamoto, L. M., . . . Emmanuel, H. (2010). The American football uniform: uncompensable heat stress and hyperthermic exhaustion. *J Athl Train, 45*(2), 117-127. doi:10.4085/1062-6050-45.2.117

Bach, T. M., & Clement, D. B. (1980). Exercise induced acute renal failure in an athlete. *Can Fam Physician, 26*, 591-595.

Baker, L. B., Stofan, J. R., Hamilton, A. A., & Horswill, C. A. (2009). Comparison of regional patch collection vs. whole body washdown for measuring sweat sodium and potassium loss during exercise. *J Appl Physiol (1985), 107*(3), 887-895. doi:10.1152/japplphysiol.00197.2009

Banfi, G., Del Fabbro, M., d'Eril, G. M., & Melegati, G. (2009). Reliability of cystatin C in estimating renal function in rugby players. *Ann Clin Biochem, 46*(Pt 5), 428. doi:10.1258/acb.2009.009135

Banfi, G., Sloand, J., Shelly, M., Del Fabbro, M., Barassi, A., & Melzi d'Eril, G. V. (2012). Limitations of Cockcroft-Gault and MDRD formulas in estimating GFR among top-level rugby players. *J Nephrol, 25*(6), 1047-1053. doi:10.5301/jn.5000094

Bankir, L. (2013). Water and Kidney Physiology. In (pp. S13-S17). Nutrition Today

Bardis, C. N., Kavouras, S. A., Arnaoutis, G., Panagiotakos, D. B., & Sidossis, L. S. (2013). Mild dehydration and cycling performance during 5-kilometer hill climbing. *J Athl Train, 48*(6), 741-747. doi:10.4085/1062-6050-48.5.01

Basile, D. P., Anderson, M. D., & Sutton, T. A. (2012). Pathophysiology of acute kidney injury. *Compr Physiol, 2*(2), 1303-1353. doi:10.1002/cphy.c110041

Baylis, P. H., & Robertson, G. L. (1980). Plasma vasopressin response to hypertonic saline infusion to assess posterior pituitary function. *J R Soc Med, 73*(4), 255-260.

Beierwaltes, W. H., Harrison-Bernard, L. M., Sullivan, J. C., & Mattson, D. L. (2013). Assessment of renal function; clearance, the renal microcirculation, renal blood flow, and metabolic balance. *Compr Physiol, 3*(1), 165-200. doi:10.1002/cphy.c120008

Bhalla, M. C., & Dick-Perez, R. (2014). Exercise induced rhabdomyolysis with compartment syndrome and renal failure. *Case Rep Emerg Med, 2014*, 735820. doi:10.1155/2014/735820

Bodin, T., García-Trabanino, R., Weiss, I., Jarquín, E., Glaser, J., Jakobsson, K., . . . Group, W. P. W. (2016). Intervention to reduce heat stress and improve efficiency among sugarcane workers in El Salvador: Phase 1. *Occup Environ Med*. doi:10.1136/oemed-2016-103555

Bongers, C. C. W. G., Alsady, M., Nijenhuis, T., Hartman, Y. A. W., Eijsvogels, T. M. H., Deen, P. M. T., & Hopman, M. T. E. (2017). Impact of acute versus repetitive moderate intensity endurance exercise on kidney injury markers. *Physiol Rep, 5*(24). doi:10.14814/phy2.13544

Borg, G. (1970). Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med, 2*(2), 92-98.

Borkan, S. C., Emami, A., & Schwartz, J. H. (1993). Heat stress protein-associated cytoprotection of inner medullary collecting duct cells from rat kidney. *Am J Physiol, 265*(3 Pt 2), F333-341.

Bosch, X., Poch, E., & Grau, J. M. (2009). Rhabdomyolysis and acute kidney injury. *N Engl J Med, 361*(1), 62-72. doi:10.1056/NEJMra0801327

Bouby, N., Bachmann, S., Bichet, D., & Bankir, L. (1990). Effect of water intake on the progression of chronic renal failure in the 5/6 nephrectomized rat. *Am J Physiol, 258*(4 Pt 2), F973-979.

Bouby, N., Hassler, C., & Bankir, L. (1999). Contribution of vasopressin to progression of chronic renal failure: study in Brattleboro rats. *Life Sci, 65*(10), 991-1004.

Brophy, R. H., Gamradt, S. C., Barnes, R. P., Powell, J. W., DelPizzo, J. J., Rodeo, S. A., & Warren, R. F. (2008). Kidney injuries in professional American football: implications for management of an athlete with 1 functioning kidney. *Am J Sports Med, 36*(1), 85-90. doi:10.1177/0363546507308940

Butts, C. L., Luhring, K. E., Smith, C. R., Tucker, M. A., Moyen, N. E., Ganio, M. S., & McDermott, B. P. (2016). Effects of mild hypohydration on cooling during cold-water

immersion following exertional hyperthermia. *Eur J Appl Physiol, 116*(4), 687-695. doi:10.1007/s00421-016-3329-7

Casa, D. J., DeMartini, J. K., Bergeron, M. F., Csillan, D., Eichner, E. R., Lopez, R. M., . . . Yeargin, S. W. (2015a). National Athletic Trainers' Association Position Statement: Exertional Heat Illnesses. *J Athl Train, 50*(9), 986-1000. doi:10.4085/1062-6050-50.9.07

Casa, D. J., DeMartini, J. K., Bergeron, M. F., Csillan, D., Eichner, E. R., Lopez, R. M., . . . Yeargin, S. W. (2015b). National Athletic Trainers' Association Position Statement: Exertional Heat Illnesses. *J Athl Train*. doi:10.4085/1062-6050-50-9-07

Casa, D. J., Stearns, R. L., Lopez, R. M., Ganio, M. S., McDermott, B. P., Walker Yeargin, S., . . . Maresh, C. M. (2010). Influence of hydration on physiological function and performance during trail running in the heat. *J Athl Train, 45*(2), 147-156. doi:10.4085/1062-6050-45.2.147

Castellani, J. W., Zambraski, E. J., Sawka, M. N., & Urso, M. L. (2016). Does high muscle temperature accentuate skeletal muscle injury from eccentric exercise? *Physiol Rep, 4*(9). doi:10.14814/phy2.12777

Charkoudian, N. (2010). Mechanisms and modifiers of reflex induced cutaneous vasodilation and vasoconstriction in humans. *J Appl Physiol (1985), 109*(4), 1221-1228. doi:10.1152/japplphysiol.00298.2010

Charkoudian, N., Halliwill, J. R., Morgan, B. J., Eisenach, J. H., & Joyner, M. J. (2003). Influences of hydration on post-exercise cardiovascular control in humans. *J Physiol, 552*(Pt 2), 635-644. doi:10.1113/jphysiol.2003.048629

Charlton, J. R., Portilla, D., & Okusa, M. D. (2014). A basic science view of acute kidney injury biomarkers. *Nephrol Dial Transplant, 29*(7), 1301-1311. doi:10.1093/ndt/gft510

Cheema, B. S., Abas, H., Smith, B. C., O'Sullivan, A. J., Chan, M., Patwardhan, A., . . . Fiatarone Singh, M. A. (2011). Effect of resistance training during hemodialysis on circulating cytokines: a randomized controlled trial. *Eur J Appl Physiol, 111*(7), 1437-1445. doi:10.1007/s00421-010-1763-5

Cheuvront, S. N., & Kenefick, R. W. (2014). Dehydration: physiology, assessment, and performance effects. *Compr Physiol, 4*(1), 257-285. doi:10.1002/cphy.c130017

Cheuvront, S. N., Kenefick, R. W., Montain, S. J., & Sawka, M. N. (2010). Mechanisms of aerobic performance impairment with heat stress and dehydration. *J Appl Physiol (1985), 109*(6), 1989-1995. doi:10.1152/japplphysiol.00367.2010

Chew-Harris, J. S., Florkowski, C. M., George, P. M., Elmslie, J. L., & Endre, Z. H. (2013). The relative effects of fat versus muscle mass on cystatin C and estimates of renal function in healthy young men. *Ann Clin Biochem, 50*(Pt 1), 39-46. doi:10.1258/acb.2012.011241

Clark, W. F., Sontrop, J. M., Huang, S. H., Moist, L., Bouby, N., & Bankir, L. (2016). Hydration and Chronic Kidney Disease Progression: A Critical Review of the Evidence. *Am J Nephrol, 43*(4), 281-292. doi:10.1159/000445959

Clark, W. F., Susan Huang, S. H., Garg, A. X., House, A., Moist, L. M., Weir, M., & Sontrop, J. M. (2014). "Drink at least 8 glasses of water a day to be healthy???". *Home Healthc Nurse, 32*(4), 237-242. doi:10.1097/NHH.0000000000000050

Clarkson, P. M. (2007). Exertional rhabdomyolysis and acute renal failure in marathon runners. *Sports Med, 37*(4-5), 361-363.

Clarkson, P. M., & Eichner, E. R. (2006). Exertional rhabdomyolysis: does elevated blood creatine kinase foretell renal failure? *Curr Sports Med Rep, 5*(2), 57-60.

Clarkson, P. M., Kearns, A. K., Rouzier, P., Rubin, R., & Thompson, P. D. (2006). Serum creatine kinase levels and renal function measures in exertional muscle damage. *Med Sci Sports Exerc, 38*(4), 623-627. doi:10.1249/01.mss.0000210192.49210.fc

Cleary, M., Ruiz, D., Eberman, L., Mitchell, I., & Binkley, H. (2007). Dehydration, cramping, and exertional rhabdomyolysis: a case report with suggestions for recovery. *J Sport Rehabil, 16*(3), 244-259.

Cleary, M. A., Sadowski, K. A., Lee, S. Y., Miller, G. L., & Nichols, A. W. (2011). Exertional rhabdomyolysis in an adolescent athlete during preseason conditioning: a perfect storm. *J Strength Cond Res, 25*(12), 3506-3513. doi:10.1519/JSC.0b013e318216302f

Cleary, M. A., Sitler, M. R., & Kendrick, Z. V. (2006). Dehydration and symptoms of delayed-onset muscle soreness in normothermic men. *J Athl Train, 41*(1), 36-45.

Cleary, M. A., Sweeney, L. A., Kendrick, Z. V., & Sitler, M. R. (2005). Dehydration and symptoms of delayed-onset muscle soreness in hyperthermic males. *J Athl Train, 40*(4), 288-297.

Colombini, A., Corsetti, R., Machado, M., Marco, M., Graziani, R., Lombardi, G., . . . Banfi, G. (2012). Serum creatine kinase activity and its relationship with renal function indices in professional cyclists during the Giro d'Italia 3-week stage race. *Clin J Sport Med, 22*(5), 408-413. doi:10.1097/JSM.0b013e31825e66cc

Colombini, A., Machado, M., Lombardi, G., Lanteri, P., & Banfi, G. (2014). Modifications of biochemical parameters related to protein metabolism and renal function in male soccer players after a match. *J Sports Med Phys Fitness, 54*(5), 658-664.

Crowe, J., Nilsson, M., Kjellstrom, T., & Wesseling, C. (2015). Heat-related symptoms in sugarcane harvesters. *Am J Ind Med, 58*(5), 541-548. doi:10.1002/ajim.22450

Crowe, J., Wesseling, C., Solano, B. R., Umaña, M. P., Ramírez, A. R., Kjellstrom, T., . . . Nilsson, M. (2013). Heat exposure in sugarcane harvesters in Costa Rica. *Am J Ind Med, 56*(10), 1157-1164. doi:10.1002/ajim.22204

Damas, F., Nosaka, K., Libardi, C. A., Chen, T. C., & Ugrinowitsch, C. (2016). Susceptibility to Exercise-Induced Muscle Damage: a Cluster Analysis with a Large Sample. *Int J Sports Med, 37*(8), 633-640. doi:10.1055/s-0042-100281

DE WARDENER, H. E., & HERXHEIMER, A. (1957). The effect of a high water intake on the kidney's ability to concentrate the urine in man. *J Physiol, 139*(1), 42-52.

Distefano, L. J., Casa, D. J., Vansumeren, M. M., Karslo, R. M., Huggins, R. A., Demartini, J. K., . . . Maresh, C. M. (2013). Hypohydration and hyperthermia impair neuromuscular control after exercise. *Med Sci Sports Exerc, 45*(6), 1166-1173. doi:10.1249/MSS.0b013e3182805b83

Ehlers, G. G., Ball, T. E., & Liston, L. (2002). Creatine Kinase Levels are Elevated During 2-A-Day Practices in Collegiate Football Players. *J Athl Train, 37*(2), 151-156.

Ely, M. R., Cheuvront, S. N., Roberts, W. O., & Montain, S. J. (2007). Impact of weather on marathon-running performance. *Med Sci Sports Exerc, 39*(3), 487-493. doi:10.1249/mss.0b013e31802d3aba

Emami, A., Schwartz, J. H., & Borkan, S. C. (1991). Transient ischemia or heat stress induces a cytoprotectant protein in rat kidney. *Am J Physiol, 260*(4 Pt 2), F479-485.

Endre, Z. H., Pickering, J. W., Walker, R. J., Devarajan, P., Edelstein, C. L., Bonventre, J. V., . . . George, P. M. (2011). Improved performance of urinary biomarkers of acute kidney injury in the critically ill by stratification for injury duration and baseline renal function. *Kidney Int, 79*(10), 1119-1130. doi:10.1038/ki.2010.555

Engell, D. B., Maller, O., Sawka, M. N., Francesconi, R. N., Drolet, L., & Young, A. J. (1987). Thirst and fluid intake following graded hypohydration levels in humans. *Physiol Behav, 40*(2), 229-236.

Epstein, Y., & Roberts, W. O. (2011). The pathopysiology of heat stroke: an integrative view of the final common pathway. *Scand J Med Sci Sports, 21*(6), 742-748. doi:10.1111/j.1600-0838.2011.01333.x

Ferguson, M. A., Vaidya, V. S., & Bonventre, J. V. (2008). Biomarkers of nephrotoxic acute kidney injury. *Toxicology, 245*(3), 182-193. doi:10.1016/j.tox.2007.12.024

Fortes, M. B., Di Felice, U., Dolci, A., Junglee, N. A., Crockford, M. J., West, L., . . . Walsh, N. P. (2013). Muscle-damaging exercise increases heat strain during subsequent exercise heat stress. *Med Sci Sports Exerc, 45*(10), 1915-1924. doi:10.1249/MSS.0b013e318294b0f8

Fortney, S. M., Wenger, C. B., Bove, J. R., & Nadel, E. R. (1984). Effect of hyperosmolality on control of blood flow and sweating. *J Appl Physiol Respir Environ Exerc Physiol, 57*(6), 1688-1695.

Gagnon, D., Lynn, A. G., Binder, K., Boushel, R. C., & Kenny, G. P. (2012). Mean arterial pressure following prolonged exercise in the heat: influence of training status and fluid replacement. *Scand J Med Sci Sports, 22*(5), e99-e107. doi:10.1111/j.1600-0838.2012.01506.x

Galvez, R., Stacy, J., & Howley, A. (2008). Exertional rhabdomyolysis in seven division-1 swimming athletes. *Clin J Sport Med, 18*(4), 366-368. doi:10.1097/JSM.0b013e31817dd506

Garcia-Miranda, P., Peral, M. J., & Ilundain, A. A. (2010). Effect of antidiuresis on renal creatine metabolism. *J Physiol Pharmacol, 61*(1), 83-88.

García-Trabanino, R., Jarquín, E., Wesseling, C., Johnson, R. J., González-Quiroz, M., Weiss, I., . . . Barregard, L. (2015). Heat stress, dehydration, and kidney function in sugarcane cutters in El Salvador--A cross-shift study of workers at risk of Mesoamerican nephropathy. *Environ Res, 142*, 746-755. doi:10.1016/j.envres.2015.07.007

Gellai, M., Edwards, B. R., & Valtin, H. (1979). Urinary concentrating ability during dehydration in the absence of vasopressin. *Am J Physiol, 237*(2), F100-104.

Gerstenbluth, R. E., Spirnak, J. P., & Elder, J. S. (2002). Sports participation and high grade renal injuries in children. *J Urol, 168*(6), 2575-2578. doi:10.1097/01.ju.0000037535.88844.cc

Glaser, J., Lemery, J., Rajagopalan, B., Diaz, H. F., García-Trabanino, R., Taduri, G., . . . Johnson, R. J. (2016). Climate Change and the Emergent Epidemic of CKD from Heat Stress in Rural Communities: The Case for Heat Stress Nephropathy. *Clin J Am Soc Nephrol, 11*(8), 1472-1483. doi:10.2215/CJN.13841215

Godek, S. F., Bartolozzi, A. R., Burkholder, R., Sugarman, E., & Dorshimer, G. (2006). Core temperature and percentage of dehydration in professional football linemen and backs during preseason practices. *J Athl Train, 41*(1), 8-14; discussion 14-17.

Godek, S. F., Godek, J. J., & Bartolozzi, A. R. (2005). Hydration status in college football players during consecutive days of twice-a-day preseason practices. *Am J Sports Med, 33*(6), 843-851. doi:10.1177/0363546504270999

González-Alonso, J., Calbet, J. A., & Nielsen, B. (1998). Muscle blood flow is reduced with dehydration during prolonged exercise in humans. *J Physiol, 513 (Pt 3)*, 895-905.

González-Alonso, J., Mora-Rodríguez, R., Below, P. R., & Coyle, E. F. (1995). Dehydration reduces cardiac output and increases systemic and cutaneous vascular resistance during exercise. *J Appl Physiol (1985), 79*(5), 1487-1496.

González-Alonso, J., Mora-Rodríguez, R., Below, P. R., & Coyle, E. F. (1997). Dehydration markedly impairs cardiovascular function in hyperthermic endurance athletes during exercise. *J Appl Physiol (1985), 82*(4), 1229-1236.

González-Alonso, J., Mora-Rodríguez, R., & Coyle, E. F. (2000). Stroke volume during exercise: interaction of environment and hydration. *Am J Physiol Heart Circ Physiol, 278*(2), H321-330.

González-Alonso, J., Teller, C., Andersen, S. L., Jensen, F. B., Hyldig, T., & Nielsen, B. (1999). Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *J Appl Physiol (1985), 86*(3), 1032-1039.

Grimby, G. (1965). Renal clearances during prolonged supine exercise at different loads. In (Vol. 20, pp. 1294-1298). J Appl Physiol.

Grinsell, M. M., Butz, K., Gurka, M. J., Gurka, K. K., & Norwood, V. (2012). Sport-related kidney injury among high school athletes. *Pediatrics, 130*(1), e40-45. doi:10.1542/peds.2011-2082

Hales, J. R., Rowell, L. B., & King, R. B. (1979). Regional distribution of blood flow in awake heat-stressed baboons. *Am J Physiol, 237*(6), H705-712.

Halliwill, J. R. (2001). Mechanisms and clinical implications of post-exercise hypotension in humans. *Exerc Sport Sci Rev, 29*(2), 65-70.

Han, M., Li, Y., Liu, M., & Cong, B. (2012). Renal neutrophil gelatinase associated lipocalin expression in lipopolysaccharide-induced acute kidney injury in the rat. *BMC Nephrol, 13*, 25. doi:10.1186/1471-2369-13-25

Hanatani, S., Izumiya, Y., Araki, S., Rokutanda, T., Kimura, Y., Walsh, K., & Ogawa, H. (2014). Akt1-mediated fast/glycolytic skeletal muscle growth attenuates renal damage in experimental kidney disease. *J Am Soc Nephrol, 25*(12), 2800-2811. doi:10.1681/ASN.2013091025

Hansen, A. L., Bi, P., Ryan, P., Nitschke, M., Pisaniello, D., & Tucker, G. (2008). The effect of heat waves on hospital admissions for renal disease in a temperate city of Australia. *Int J Epidemiol, 37*(6), 1359-1365. doi:10.1093/ije/dyn165

Harduar Morano, L., Watkins, S., & Kintziger, K. (2016). A Comprehensive Evaluation of the Burden of Heat-Related Illness and Death within the Florida Population. *Int J Environ Res Public Health, 13*(6). doi:10.3390/ijerph13060551

Harrison, E. M., Sharpe, E., Bellamy, C. O., McNally, S. J., Devey, L., Garden, O. J., . . . Wigmore, S. J. (2008). Heat shock protein 90-binding agents protect renal cells from oxidative stress and reduce kidney ischemia-reperfusion injury. *Am J Physiol Renal Physiol, 295*(2), F397-405. doi:10.1152/ajprenal.00361.2007

Herget-Rosenthal, S., Metzger, J., Albalat, A., Bitsika, V., & Mischak, H. (2012). Proteomic biomarkers for the early detection of acute kidney injury. *Prilozi, 33*(1), 27-48.

Hilliard, L. M., Colafella, K. M., Bulmer, L. L., Puelles, V. G., Singh, R. R., Ow, C. P., . . . Denton, K. M. (2016). Chronic recurrent dehydration associated with periodic water

intake exacerbates hypertension and promotes renal damage in male spontaneously hypertensive rats. *Sci Rep, 6*, 33855. doi:10.1038/srep33855

Hiraki, K., Kamijo-Ikemori, A., Yasuda, T., Hotta, C., Izawa, K. P., Watanabe, S., . . . Kimura, K. (2013). Moderate-intensity single exercise session does not induce renal damage. *J Clin Lab Anal, 27*(3), 177-180. doi:10.1002/jcla.21579

Hoffman, J. R., Kang, J., Ratamess, N. A., & Faigenbaum, A. D. (2005). Biochemical and hormonal responses during an intercollegiate football season. *Med Sci Sports Exerc, 37*(7), 1237-1241.

Hoffman, M. D., & Weiss, R. H. (2016). Does Acute Kidney Injury From an Ultramarathon Increase the Risk for Greater Subsequent Injury? *Clin J Sport Med, 26*(5), 417-422. doi:10.1097/JSM.0000000000000277

Hohimer, A. R., Hales, J. R., Rowell, L. B., & Smith, O. A. (1983). Regional distribution of blood flow during mild dynamic leg exercise in the baboon. *J Appl Physiol Respir Environ Exerc Physiol, 55*(4), 1173-1177.

Hohimer, A. R., & Smith, O. A. (1979). Decreased renal blood flow in the baboon during mild dynamic leg exercise. *Am J Physiol, 236*(1), H141-150.

Huerta-Alardín, A. L., Varon, J., & Marik, P. E. (2005). Bench-to-bedside review: Rhabdomyolysis -- an overview for clinicians. *Crit Care, 9*(2), 158-169. doi:10.1186/cc2978

Hummel, K., Gregory, A., Desai, N., & Diamond, A. (2016). Rhabdomyolysis in adolescent athletes: review of cases. *Phys Sportsmed, 44*(2), 195-199. doi:10.1080/00913847.2016.1170582

Jay, O., Gagnon, D., DuCharme, M. B., Webb, P., Reardon, F. D., & Kenny, G. P. (2008). Human heat balance during postexercise recovery: separating metabolic and nonthermal effects. *Am J Physiol Regul Integr Comp Physiol, 294*(5), R1586-1592. doi:10.1152/ajpregu.00717.2007

Johnson, R. J., Stenvinkel, P., Jensen, T., Lanaspa, M. A., Roncal, C., Song, Z., . . . Sánchez-Lozada, L. G. (2016). Metabolic and Kidney Diseases in the Setting of Climate Change, Water Shortage, and Survival Factors. *J Am Soc Nephrol, 27*(8), 2247-2256. doi:10.1681/ASN.2015121314

Judelson, D. A., Maresh, C. M., Farrell, M. J., Yamamoto, L. M., Armstrong, L. E., Kraemer, W. J., . . . Anderson, J. M. (2007). Effect of hydration state on strength, power, and resistance exercise performance. *Med Sci Sports Exerc, 39*(10), 1817-1824. doi:10.1249/mss.0b013e3180de5f22

Junglee, N. A., Di Felice, U., Dolci, A., Fortes, M. B., Jibani, M. M., Lemmey, A. B., . . . Macdonald, J. H. (2013). Exercising in a hot environment with muscle damage: effects

on acute kidney injury biomarkers and kidney function. *Am J Physiol Renal Physiol, 305*(6), F813-820. doi:10.1152/ajprenal.00091.2013

Junglee, N. A., Lemmey, A. B., Burton, M., Searell, C., Jones, D., Lawley, J. S., . . . Macdonald, J. H. (2012). Does proteinuria-inducing physical activity increase biomarkers of acute kidney injury? *Kidney Blood Press Res, 36*(1), 278-289. doi:10.1159/000343417

Kahanov, L., Eberman, L. E., Wasik, M., & Alvey, T. (2012). Exertional rhabdomyolysis in a collegiate american football player after preventive cold-water immersion: a case report. *J Athl Train, 47*(2), 228-232.

Kamijo, Y., Ikegawa, S., Okada, Y., Masuki, S., Okazaki, K., Uchida, K., . . . Nose, H. (2012). Enhanced renal Na+ reabsorption by carbohydrate in beverages during restitution from thermal and exercise-induced dehydration in men. *Am J Physiol Regul Integr Comp Physiol, 303*(8), R824-833. doi:10.1152/ajpregu.00588.2011

Kao, W. F., Hou, S. K., Chiu, Y. H., Chou, S. L., Kuo, F. C., Wang, S. H., & Chen, J. J. (2015). Effects of 100-km ultramarathon on acute kidney injury. *Clin J Sport Med, 25*(1), 49-54. doi:10.1097/JSM.0000000000000116

Kenny, G. P., Gagnon, D., Jay, O., McInnis, N. H., Journeay, W. S., & Reardon, F. D. (2008). Can supine recovery mitigate the exercise intensity dependent attenuation of post-exercise heat loss responses? *Appl Physiol Nutr Metab, 33*(4), 682-689. doi:10.1139/H08-053

Kenny, G. P., Jay, O., & Journeay, W. S. (2007). Disturbance of thermal homeostasis following dynamic exercise. *Appl Physiol Nutr Metab, 32*(4), 818-831. doi:10.1139/H07-044

Kenny, G. P., Jay, O., Zaleski, W. M., Reardon, M. L., Sigal, R. J., Journeay, W. S., & Reardon, F. D. (2006). Postexercise hypotension causes a prolonged perturbation in esophageal and active muscle temperature recovery. *Am J Physiol Regul Integr Comp Physiol, 291*(3), R580-588. doi:10.1152/ajpregu.00918.2005

Kjellstrom, T., Butler, A. J., Lucas, R. M., & Bonita, R. (2010). Public health impact of global heating due to climate change: potential effects on chronic non-communicable diseases. *Int J Public Health, 55*(2), 97-103. doi:10.1007/s00038-009-0090-2

Knochel, J. P., Dotin, L. N., & Hamburger, R. J. (1974). Heat stress, exercise, and muscle injury: effects on urate metabolism and renal function. *Ann Intern Med, 81*(3), 321-328.

Koba, S., Yoshida, T., & Hayashi, N. (2006). Renal sympathetic and circulatory responses to activation of the exercise pressor reflex in rats. *Exp Physiol, 91*(1), 111-119. doi:10.1113/expphysiol.2005.031666

Kodama, K., Ikeda, K., Kawamura, S., Oyama, T., Fujita, S., & Kobayashi, Y. (1985). A case of severe dehydration with marked rhabdomyolysis. *Jpn J Med, 24*(2), 150-154.

Koshimizu, T. A., Nakamura, K., Egashira, N., Hiroyama, M., Nonoguchi, H., & Tanoue, A. (2012). Vasopressin V1a and V1b receptors: from molecules to physiological systems. *Physiol Rev, 92*(4), 1813-1864. doi:10.1152/physrev.00035.2011

Koçer, G., Kuru, O., Gündüz, F., Bayram, Z., Ozdem, S., Aksoy, D., & Sentürk, U. K. (2011). The effect of exercise training on the responsiveness of renal resistance arteries in rats. *Ren Fail, 33*(6), 587-592. doi:10.3109/0886022X.2011.585000

Kraemer, W. J., Looney, D. P., Martin, G. J., Ratamess, N. A., Vingren, J. L., French, D. N., . . . Fleck, S. J. (2013). Changes in creatine kinase and cortisol in National Collegiate Athletic Association Division I American football players during a season. *J Strength Cond Res, 27*(2), 434-441. doi:10.1519/JSC.0b013e318281d1b0

Kraemer, W. J., Spiering, B. A., Volek, J. S., Martin, G. J., Howard, R. L., Ratamess, N. A., . . . Maresh, C. M. (2009). Recovery from a national collegiate athletic association division I football game: muscle damage and hormonal status. *J Strength Cond Res, 23*(1), 2-10. doi:10.1519/JSC.0b013e31819306f2

Kratz, A., Lewandrowski, K. B., Siegel, A. J., Chun, K. Y., Flood, J. G., Van Cott, E. M., & Lee-Lewandrowski, E. (2002). Effect of marathon running on hematologic and biochemical laboratory parameters, including cardiac markers. *Am J Clin Pathol, 118*(6), 856-863. doi:10.1309/14TY-2TDJ-1X0Y-1V6V

Kuipers, N. T., Sauder, C. L., Kearney, M. L., & Ray, C. A. (2007). Changes in forearm muscle temperature alter renal vascular responses to isometric handgrip. *Am J Physiol Heart Circ Physiol, 293*(6), H3432-3439. doi:10.1152/ajpheart.00822.2007

Kuwabara, M., Hisatome, I., Roncal-Jimenez, C. A., Niwa, K., Andres-Hernando, A., Jensen, T., . . . Johnson, R. J. (2017). Increased Serum Sodium and Serum Osmolarity Are Independent Risk Factors for Developing Chronic Kidney Disease; 5 Year Cohort Study. *PLoS One, 12*(1), e0169137. doi:10.1371/journal.pone.0169137

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Front Psychol, 4*, 863. doi:10.3389/fpsyg.2013.00863

Laukkanen, T., Khan, H., Zaccardi, F., & Laukkanen, J. A. (2015). Association between sauna bathing and fatal cardiovascular and all-cause mortality events. *JAMA Intern Med, 175*(4), 542-548. doi:10.1001/jamainternmed.2014.8187

Laws, R. L., Brooks, D. R., Amador, J. J., Weiner, D. E., Kaufman, J. S., Ramírez-Rubio, O., . . . McClean, M. D. (2015). Changes in kidney function among Nicaraguan sugarcane workers. *Int J Occup Environ Health, 21*(3), 241-250. doi:10.1179/2049396714Y.0000000102

Laws, R. L., Brooks, D. R., Amador, J. J., Weiner, D. E., Kaufman, J. S., Ramírez-Rubio, O., . . . McClean, M. D. (2016). Biomarkers of Kidney Injury Among Nicaraguan Sugarcane Workers. *Am J Kidney Dis, 67*(2), 209-217. doi:10.1053/j.ajkd.2015.08.022

Leon, L. R., & Bouchama, A. (2015). Heat stroke. *Compr Physiol, 5*(2), 611-647. doi:10.1002/cphy.c140017

Leon, L. R., & Helwig, B. G. (2010). Heat stroke: role of the systemic inflammatory response. *J Appl Physiol (1985), 109*(6), 1980-1988. doi:10.1152/japplphysiol.00301.2010

Lin, H., Chie, W., & Lien, H. (2006). Epidemiological analysis of factors influencing an episode of exertional rhabdomyolysis in high school students. *Am J Sports Med, 34*(3), 481-486. doi:10.1177/0363546505281243

Lippi, G., Sanchis-Gomar, F., Salvagno, G. L., Aloe, R., Schena, F., & Guidi, G. C. (2012). Variation of serum and urinary neutrophil gelatinase associated lipocalin (NGAL) after strenuous physical exercise. *Clin Chem Lab Med, 50*(9), 1585-1589. doi:10.1515/cclm-2011-0954

Lopez, R. M., Casa, D. J., Jensen, K. A., DeMartini, J. K., Pagnotta, K. D., Ruiz, R. C., . . . Maresh, C. M. (2011). Examining the influence of hydration status on physiological responses and running speed during trail running in the heat with controlled exercise intensity. *J Strength Cond Res, 25*(11), 2944-2954. doi:10.1519/JSC.0b013e318231a6c8

Lorenzo, S., Halliwill, J. R., Sawka, M. N., & Minson, C. T. (2010). Heat acclimation improves exercise performance. *J Appl Physiol (1985), 109*(4), 1140-1147. doi:10.1152/japplphysiol.00495.2010

Lorenzo, S., & Minson, C. T. (2010). Heat acclimation improves cutaneous vascular function and sweating in trained cyclists. *J Appl Physiol (1985), 109*(6), 1736-1743. doi:10.1152/japplphysiol.00725.2010

Lynn, B. M., Minson, C. T., & Halliwill, J. R. (2009). Fluid replacement and heat stress during exercise alter post-exercise cardiac haemodynamics in endurance exercise-trained men. *J Physiol, 587*(Pt 14), 3605-3617. doi:10.1113/jphysiol.2009.171199

Machado, M., Zini, E. N., Valadão, S. D., Amorim, M. Z., Barroso, T. Z., & de Oliveira, W. (2012). Relationship of glomerular filtration rate and serum CK activity after resistance exercise in women. *Int Urol Nephrol, 44*(2), 515-521. doi:10.1007/s11255-011-9963-4

Mansour, S. G., Verma, G., Pata, R. W., Martin, T. G., Perazella, M. A., & Parikh, C. R. (2017). Kidney Injury and Repair Biomarkers in Marathon Runners. *Am J Kidney Dis*. doi:10.1053/j.ajkd.2017.01.045

Massett, M. P., Johnson, D. G., & Kregel, K. C. (1996). Cardiovascular and sympathoadrenal responses to heat stress following water deprivation in rats. *Am J Physiol, 270*(3 Pt 2), R652-659.

McAllister, R. M. (1998). Adaptations in control of blood flow with training: splanchnic and renal blood flows. *Med Sci Sports Exerc, 30*(3), 375-381.

McCullough, P. A., Chinnaiyan, K. M., Gallagher, M. J., Colar, J. M., Geddes, T., Gold, J. M., & Trivax, J. E. (2011). Changes in renal markers and acute kidney injury after marathon running. *Nephrology (Carlton), 16*(2), 194-199. doi:10.1111/j.1440-1797.2010.01354.x

McDermott, B. P., Casa, D. J., Lee, E., Yamamoto, L., Beasley, K., Emmanuel, H., . . . Maresh, C. (2013). Thermoregulation and stress hormone recovery after exercise dehydration: comparison of rehydration methods. *J Athl Train, 48*(6), 725-733. doi:10.4085/1062-6050-48.6.01

McDermott, B. P., Casa, D. J., Lee, E. C., Yamamoto, L. M., Beasley, K. N., Emmanuel, H., . . . Maresh, C. M. (2013). The influence of rehydration mode after exercise dehydration on cardiovascular function. *J Strength Cond Res, 27*(8), 2086-2095. doi:10.1519/JSC.0b013e318295d548

Meade, R. D., Lauzon, M., Poirier, M. P., Flouris, A. D., & Kenny, G. P. (2015). An Evaluation of the Physiological Strain Experienced by Electrical Utility Workers in North America. *J Occup Environ Hyg, 12*(10), 708-720. doi:10.1080/15459624.2015.1043054

Mehta, R. L., Kellum, J. A., Shah, S. V., Molitoris, B. A., Ronco, C., Warnock, D. G., . . . Network, A. K. I. (2007). Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care, 11*(2), R31. doi:10.1186/cc5713

Melin, B., Jimenez, C., Savourey, G., Bittel, J., Cottet-Emard, J. M., Pequignot, J. M., . . . Gharib, C. (1997). Effects of hydration state on hormonal and renal responses during moderate exercise in the heat. *Eur J Appl Physiol Occup Physiol, 76*(4), 320-327. doi:10.1007/s004210050255

Melin, B., Koulmann, N., Jimenez, C., Savourey, G., Launay, J. C., Cottet-Emard, J. M., . . . Gharib, C. (2001). Comparison of passive heat or exercise-induced dehydration on renal water and electrolyte excretion: the hormonal involvement. *Eur J Appl Physiol, 85*(3-4), 250-258.

Mellor, A., Boos, C., Stacey, M., Hooper, T., Smith, C., Begley, J., . . . Woods, D. R. (2013). Neutrophil gelatinase-associated lipocalin: its response to hypoxia and association with acute mountain sickness. *Dis Markers, 35*(5), 537-542. doi:10.1155/2013/601214

Mingels, A., Jacobs, L., Kleijnen, V., Wodzig, W., & Dieijen-Visser, M. (2009). Cystatin C a marker for renal function after exercise. *Int J Sports Med, 30*(9), 668-671. doi:10.1055/s-0029-1220733

Minson, C. T., Wladkowski, S. L., Cardell, A. F., Pawelczyk, J. A., & Kenney, W. L. (1998). Age alters the cardiovascular response to direct passive heating. *J Appl Physiol (1985), 84*(4), 1323-1332.

Mishra, J., Ma, Q., Prada, A., Mitsnefes, M., Zahedi, K., Yang, J., . . . Devarajan, P. (2003). Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. *J Am Soc Nephrol, 14*(10), 2534-2543.

Momen, A., Bower, D., Leuenberger, U. A., Boehmer, J., Lerner, S., Alfrey, E. J., . . . Sinoway, L. I. (2005). Renal vascular response to static handgrip exercise: sympathetic vs. autoregulatory control. *Am J Physiol Heart Circ Physiol, 289*(4), H1770-1776. doi:10.1152/ajpheart.01213.2004

Momen, A., Handly, B., Kunselman, A., Leuenberger, U. A., & Sinoway, L. I. (2006). Influence of sex and active muscle mass on renal vascular responses during static exercise. *Am J Physiol Heart Circ Physiol, 291*(1), H121-126. doi:10.1152/ajpheart.00931.2005

Momen, A., Thomas, K., Blaha, C., Gahremanpour, A., Mansoor, A., Leuenberger, U. A., & Sinoway, L. I. (2006). Renal vasoconstrictor responses to static exercise during orthostatic stress in humans: effects of the muscle mechano- and the baroreflexes. *J Physiol, 573*(Pt 3), 819-825. doi:10.1113/jphysiol.2005.104612

Montain, S. J., & Coyle, E. F. (1992). Influence of graded dehydration on hyperthermia and cardiovascular drift during exercise. *J Appl Physiol (1985), 73*(4), 1340-1350.

Montain, S. J., Latzka, W. A., & Sawka, M. N. (1995). Control of thermoregulatory sweating is altered by hydration level and exercise intensity. *J Appl Physiol (1985), 79*(5), 1434-1439.

Montain, S. J., Sawka, M. N., Latzka, W. A., & Valeri, C. R. (1998). Thermal and cardiovascular strain from hypohydration: influence of exercise intensity. *Int J Sports Med, 19*(2), 87-91. doi:10.1055/s-2007-971887

Moyce, S., Joseph, J., Tancredi, D., Mitchell, D., & Schenker, M. (2016). Cumulative Incidence of Acute Kidney Injury in California's Agricultural Workers. *J Occup Environ Med, 58*(4), 391-397. doi:10.1097/JOM.0000000000000668

Mårtensson, J., Martling, C. R., & Bell, M. (2012). Novel biomarkers of acute kidney injury and failure: clinical applicability. *Br J Anaesth, 109*(6), 843-850. doi:10.1093/bja/aes357

Nadel, E. R. (1979). Control of sweating rate while exercising in the heat. *Med Sci Sports, 11*(1), 31-35.

Nadel, E. R., Bullard, R. W., & Stolwijk, J. A. (1971). Importance of skin temperature in the regulation of sweating. *J Appl Physiol, 31*(1), 80-87.

Nadel, E. R., Pandolf, K. B., Roberts, M. F., & Stolwijk, J. A. (1974). Mechanisms of thermal acclimation to exercise and heat. *J Appl Physiol, 37*(4), 515-520.

Nagashima, K., Wu, J., Kavouras, S. A., & Mack, G. W. (2001). Increased renal tubular sodium reabsorption during exercise-induced hypervolemia in humans. *J Appl Physiol (1985), 91*(3), 1229-1236.

Nejat, M., Pickering, J. W., Devarajan, P., Bonventre, J. V., Edelstein, C. L., Walker, R. J., & Endre, Z. H. (2012). Some biomarkers of acute kidney injury are increased in pre-renal acute injury. *Kidney Int, 81*(12), 1254-1262. doi:10.1038/ki.2012.23

Nolin, A. C., Mulhern, R. M., Panchenko, M. V., Pisarek-Horowitz, A., Wang, Z., Shirihai, O., . . . Havasi, A. (2016). Proteinuria causes dysfunctional autophagy in the proximal tubule. *Am J Physiol Renal Physiol, 311*(6), F1271-F1279. doi:10.1152/ajprenal.00125.2016

Nybo, L., Girard, O., Mohr, M., Knez, W., Voss, S., & Racinais, S. (2013). Markers of muscle damage and performance recovery after exercise in the heat. *Med Sci Sports Exerc, 45*(5), 860-868. doi:10.1249/MSS.0b013e31827ded04

O'Connor, F. G., Bergeron, M. F., Cantrell, J., Connes, P., Harmon, K. G., Ivy, E., . . . Deuster, P. A. (2012). ACSM and CHAMP summit on sickle cell trait: mitigating risks for warfighters and athletes. *Med Sci Sports Exerc, 44*(11), 2045-2056. doi:10.1249/MSS.0b013e31826851c2

Ozkan, I., & Ibrahim, C. H. (2016). Dehydration, skeletal muscle damage and inflammation before the competitions among the elite wrestlers. *J Phys Ther Sci, 28*(1), 162-168. doi:10.1589/jpts.28.162

Palatini, P. (2012). Glomerular hyperfiltration: a marker of early renal damage in pre-diabetes and pre-hypertension. *Nephrol Dial Transplant, 27*(5), 1708-1714. doi:10.1093/ndt/gfs037

Patterson, M. J., Stocks, J. M., & Taylor, N. A. (2004). Sustained and generalized extracellular fluid expansion following heat acclimation. *J Physiol, 559*(Pt 1), 327-334. doi:10.1113/jphysiol.2004.063289

Paula Santos, U., Zanetta, D. M., Terra-Filho, M., & Burdmann, E. A. (2015). Burnt sugarcane harvesting is associated with acute renal dysfunction. *Kidney Int, 87*(4), 792-799. doi:10.1038/ki.2014.306

Paull, G., Dervis, S., Barrera-Ramirez, J., McGinn, R., Haqani, B., Flouris, A. D., & Kenny, G. P. (2016). The effect of plasma osmolality and baroreceptor loading status on postexercise heat loss responses. *Am J Physiol Regul Integr Comp Physiol, 310*(6), R522-531. doi:10.1152/ajpregu.00435.2015

Peraza, S., Wesseling, C., Aragon, A., Leiva, R., García-Trabanino, R. A., Torres, C., . . . Hogstedt, C. (2012). Decreased kidney function among agricultural workers in El Salvador. *Am J Kidney Dis, 59*(4), 531-540. doi:10.1053/j.ajkd.2011.11.039

Phillips, S. M., Sykes, D., & Gibson, N. (2014). Hydration Status and Fluid Balance of Elite European Youth Soccer Players during Consecutive Training Sessions. *J Sports Sci Med, 13*(4), 817-822.

Podhorska-Okolow, M., Dziegiel, P., Murawska-Cialowicz, E., Saczko, J., Kulbacka, J., Gomulkiewicz, A., . . . Zabel, M. (2007). Effects of adaptive exercise on apoptosis in cells of rat renal tubuli. *Eur J Appl Physiol, 99*(3), 217-226. doi:10.1007/s00421-006-0335-1

Poirier, M. P., Meade, R. D., McGinn, R., Friesen, B. J., Hardcastle, S. G., Flouris, A. D., & Kenny, G. P. (2015). The Influence of Arc-Flash and Fire-Resistant Clothing on Thermoregulation during Exercise in the Heat. *J Occup Environ Hyg, 12*(9), 654-667. doi:10.1080/15459624.2015.1029615

Ponte, B., Pruijm, M., Ackermann, D., Vuistiner, P., Guessous, I., Ehret, G., . . . Bochud, M. (2015). Copeptin is associated with kidney length, renal function, and prevalence of simple cysts in a population-based study. *J Am Soc Nephrol, 26*(6), 1415-1425. doi:10.1681/ASN.2014030260

Poortmans, J. R. (1977). Exercise and renal function. *Exerc Sport Sci Rev, 5*, 255-294.

Poortmans, J. R. (1984). Exercise and renal function. *Sports Med, 1*(2), 125-153.

Poortmans, J. R. (1985). Postexercise proteinuria in humans. Facts and mechanisms. *JAMA, 253*(2), 236-240.

Poortmans, J. R. (1995). [Renal response to exercise in healthy and diseased patients]. *Nephrologie, 16*(4), 317-324.

Poortmans, J. R., Auquier, H., Renaut, V., Durussel, A., Saugy, M., & Brisson, G. R. (1997). Effect of short-term creatine supplementation on renal responses in men. *Eur J Appl Physiol Occup Physiol, 76*(6), 566-567. doi:10.1007/s004210050291

Poortmans, J. R., Blommaert, E., Baptista, M., De Broe, M. E., & Nouwen, E. J. (1997). Evidence of differential renal dysfunctions during exercise in men. *Eur J Appl Physiol Occup Physiol, 76*(1), 88-91.

Poortmans, J. R., Brauman, H., Staroukine, M., Verniory, A., Decaestecker, C., & Leclercq, R. (1988). Indirect evidence of glomerular/tubular mixed-type postexercise proteinuria in healthy humans. *Am J Physiol, 254*(2 Pt 2), F277-283.

Poortmans, J. R., Gulbis, B., De Bruyn, E., Baudry, S., & Carpentier, A. (2013). Limitations of serum values to estimate glomerular filtration rate during exercise. *Br J Sports Med, 47*(18), 1166-1170. doi:10.1136/bjsports-2012-090976

Poortmans, J. R., & Haralambie, G. (1979). Biochemical changes in a 100 km run: proteins in serum and urine. *Eur J Appl Physiol Occup Physiol, 40*(4), 245-254.

Poortmans, J. R., Jeannaud, F., Baudry, S., & Carpentier, A. (2015). Changes in Kidney Functions during Middle-distance Triathlon in Male Athletes. *Int J Sports Med, 36*(12), 979-983. doi:10.1055/s-0034-1398681

Poortmans, J. R., & Labilloy, D. (1988). The influence of work intensity on postexercise proteinuria. *Eur J Appl Physiol Occup Physiol, 57*(2), 260-263.

Poortmans, J. R., Mathieu, N., & De Plaen, P. (1996). Influence of running different distances on renal glomerular and tubular impairment in humans. *Eur J Appl Physiol Occup Physiol, 72*(5-6), 522-527.

Poortmans, J. R., Rampaer, L., & Wolfs, J. C. (1989). Renal protein excretion after exercise in man. *Eur J Appl Physiol Occup Physiol, 58*(5), 476-480.

Poortmans, J. R., & Vancalck, B. (1978). Renal glomerular and tubular impairment during strenuous exercise in young women. *Eur J Clin Invest, 8*(3), 175-178.

Poortmans, J. R., & Vanderstraeten, J. (1994). Kidney function during exercise in healthy and diseased humans. An update. *Sports Med, 18*(6), 419-437.

Pricher, M. P., Holowatz, L. A., Williams, J. T., Lockwood, J. M., & Halliwill, J. R. (2004). Regional hemodynamics during postexercise hypotension. I. Splanchnic and renal circulations. *J Appl Physiol (1985), 97*(6), 2065-2070. doi:10.1152/japplphysiol.00465.2004

Radigan, L. R., & Robinson, S. (1949). Effects of environmental heat stress and exercise on renal blood flow and filtration rate. *J Appl Physiol, 2*(4), 185-191.

Ramanathan, N. L. (1964). A New Weighting System For Mean Surface Temperature Of The Human Body. *J Appl Physiol, 19*, 531-533.

Ricci, Z., Cruz, D. N., & Ronco, C. (2011). Classification and staging of acute kidney injury: beyond the RIFLE and AKIN criteria. *Nat Rev Nephrol, 7*(4), 201-208. doi:10.1038/nrneph.2011.14

Rihal, C. S., & Kashani, K. B. (2011). Intravascular volume expansion before primary angioplasty for prevention of acute kidney injury: hydration or dilution? *Circ Cardiovasc Interv, 4*(5), 405-406. doi:10.1161/CIRCINTERVENTIONS.111.964304

Robertson, G. L. (1984). Abnormalities of thirst regulation. *Kidney Int, 25*(2), 460-469.

Robertson, G. L., & Athar, S. (1976). The interaction of blood osmolality and blood volume in regulating plasma vasopressin in man. *J Clin Endocrinol Metab, 42*(4), 613-620. doi:10.1210/jcem-42-4-613

Robertson, G. L., Shelton, R. L., & Athar, S. (1976). The osmoregulation of vasopressin. *Kidney Int, 10*(1), 25-37.

Roncal-Jimenez, C., García-Trabanino, R., Barregard, L., Lanaspa, M. A., Wesseling, C., Harra, T., . . . Johnson, R. J. (2016). Heat Stress Nephropathy From Exercise-Induced Uric Acid Crystalluria: A Perspective on Mesoamerican Nephropathy. *Am J Kidney Dis, 67*(1), 20-30. doi:10.1053/j.ajkd.2015.08.021

Rosinger, A. Y., Lawman, H. G., Akinbami, L. J., & Ogden, C. L. (2016). The role of obesity in the relation between total water intake and urine osmolality in US adults, 2009-2012. *Am J Clin Nutr, 104*(6), 1554-1561. doi:10.3945/ajcn.116.137414

Roussel, R., Fezeu, L., Marre, M., Velho, G., Fumeron, F., Jungers, P., . . . Bichet, D. G. (2014). Comparison between copeptin and vasopressin in a population from the community and in people with chronic kidney disease. *J Clin Endocrinol Metab, 99*(12), 4656-4663. doi:10.1210/jc.2014-2295

Rowell, L. B. (1974). Human cardiovascular adjustments to exercise and thermal stress. *Physiol Rev, 54*(1), 75-159.

Rowell, L. B., Brengelmann, G. L., Blackmon, J. R., & Murray, J. A. (1970). Redistribution of blood flow during sustained high skin temperature in resting man. *J Appl Physiol, 28*(4), 415-420.

Sawka, M. N., Cheuvront, S. N., & Kenefick, R. W. (2012). High skin temperature and hypohydration impair aerobic performance. *Exp Physiol, 97*(3), 327-332. doi:10.1113/expphysiol.2011.061002

Sawka, M. N., Leon, L. R., Montain, S. J., & Sonna, L. A. (2011). Integrated physiological mechanisms of exercise performance, adaptation, and maladaptation to heat stress. *Compr Physiol, 1*(4), 1883-1928. doi:10.1002/cphy.c100082

Schiffl, H., & Lang, S. M. (2012). Update on biomarkers of acute kidney injury: moving closer to clinical impact? *Mol Diagn Ther, 16*(4), 199-207. doi:10.2165/11634310-000000000-00000

Schlader, Z. J., Chapman, C. L., Sarker, S., Russo, L., Rideout, T. C., Parker, M. D., . . . Hostler, D. (2017). Firefighter Work Duration Influences the Extent of Acute Kidney Injury. *Med Sci Sports Exerc*. doi:10.1249/MSS.0000000000001254

Schrier, R. W., Hano, J., Keller, H. I., Finkel, R. M., Gilliland, P. F., Cirksena, W. J., & Teschan, P. E. (1970). Renal, metabolic, and circulatory responses to heat and exercise. Studies in military recruits during summer training, with implications for acute renal failure. *Ann Intern Med, 73*(2), 213-223.

Share, L. (1996). Control of Vasopressin Release: An Old But Continuing Story In (Vol. 11, pp. 7-13). Physiology

Shibasaki, M., Aoki, K., Morimoto, K., Johnson, J. M., & Takamata, A. (2009). Plasma hyperosmolality elevates the internal temperature threshold for active thermoregulatory vasodilation during heat stress in humans. *Am J Physiol Regul Integr Comp Physiol, 297*(6), R1706-1712. doi:10.1152/ajpregu.00242.2009

Skenderi, K. P., Kavouras, S. A., Anastasiou, C. A., Yiannakouris, N., & Matalas, A. L. (2006). Exertional Rhabdomyolysis during a 246-km continuous running race. *Med Sci Sports Exerc, 38*(6), 1054-1057. doi:10.1249/01.mss.0000222831.35897.5f

Smith, J. H., Robinson, S., & Pearcy, M. (1952). Renal responses to exercise, heat and dehydration. *J Appl Physiol, 4*(8), 659-665.

Smoot, M. K., Amendola, A., Cramer, E., Doyle, C., Kregel, K. C., Chiang, H. Y., . . . Herwaldt, L. A. (2013). A cluster of exertional rhabdomyolysis affecting a Division I Football team. *Clin J Sport Med, 23*(5), 365-372. doi:10.1097/JSM.0b013e3182914fe2

Smoot, M. K., Cavanaugh, J. E., Amendola, A., West, D. R., & Herwaldt, L. A. (2014). Creatine kinase levels during preseason camp in National Collegiate Athletic Association Division I football athletes. *Clin J Sport Med, 24*(5), 438-440. doi:10.1097/JSM.0000000000000057

Sparks, M. A., Crowley, S. D., Gurley, S. B., Mirotsou, M., & Coffman, T. M. (2014). Classical Renin-Angiotensin system in kidney physiology. *Compr Physiol, 4*(3), 1201-1228. doi:10.1002/cphy.c130040

Stachenfeld, N. S., Gleim, G. W., Zabetakis, P. M., & Nicholas, J. A. (1996). Fluid balance and renal response following dehydrating exercise in well-trained men and women. *Eur J Appl Physiol Occup Physiol, 72*(5-6), 468-477.

Stover, E. A., Zachwieja, J., Stofan, J., Murray, R., & Horswill, C. A. (2006). Consistently high urine specific gravity in adolescent American football players and the impact of an acute drinking strategy. *Int J Sports Med, 27*(4), 330-335. doi:10.1055/s-2005-865667

Suzuki, M., Sudoh, M., Matsubara, S., Kawakami, K., Shiota, M., & Ikawa, S. (1996). Changes in renal blood flow measured by radionuclide angiography following exhausting exercise in humans. *Eur J Appl Physiol Occup Physiol, 74*(1-2), 1-7.

Tawatsupa, B., Lim, L. L., Kjellstrom, T., Seubsman, S. A., Sleigh, A., & Team, T. C. S. (2012). Association between occupational heat stress and kidney disease among 37,816 workers in the Thai Cohort Study (TCS). *J Epidemiol, 22*(3), 251-260.

Thornton, S. N. (2010). Thirst and hydration: physiology and consequences of dysfunction. *Physiol Behav, 100*(1), 15-21. doi:10.1016/j.physbeh.2010.02.026

Tidgren, B., Hjemdahl, P., Theodorsson, E., & Nussberger, J. (1991). Renal neurohormonal and vascular responses to dynamic exercise in humans. *J Appl Physiol (1985), 70*(5), 2279-2286.

Tietze, D. C., & Borchers, J. (2014). Exertional rhabdomyolysis in the athlete: a clinical review. *Sports Health, 6*(4), 336-339. doi:10.1177/1941738114523544

Toner, M. M., Drolet, L. L., & Pandolf, K. B. (1986). Perceptual and physiological responses during exercise in cool and cold water. *Percept Mot Skills, 62*(1), 211-220. doi:10.2466/pms.1986.62.1.211

Update: Exertional rhabdomyolysis, active component, U.S. Armed Forces, 2011. (2012). *MSMR, 19*(3), 17-19.

Vaidya, V. S., Ferguson, M. A., & Bonventre, J. V. (2008). Biomarkers of acute kidney injury. *Annu Rev Pharmacol Toxicol, 48*, 463-493. doi:10.1146/annurev.pharmtox.48.113006.094615

Vaidya, V. S., Ozer, J. S., Dieterle, F., Collings, F. B., Ramirez, V., Troth, S., . . . Bonventre, J. V. (2010). Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies. *Nat Biotechnol, 28*(5), 478-485. doi:10.1038/nbt.1623

Vanmassenhove, J., Vanholder, R., Nagler, E., & Van Biesen, W. (2013). Urinary and serum biomarkers for the diagnosis of acute kidney injury: an in-depth review of the literature. *Nephrol Dial Transplant, 28*(2), 254-273. doi:10.1093/ndt/gfs380

Wang, C. J., Grantham, J. J., & Wetmore, J. B. (2013). The medicinal use of water in renal disease. *Kidney Int, 84*(1), 45-53. doi:10.1038/ki.2013.23

Wesseling, C., Aragón, A., González, M., Weiss, I., Glaser, J., Bobadilla, N. A., . . . Barregard, L. (2016). Kidney function in sugarcane cutters in Nicaragua - A longitudinal study of workers at risk of Mesoamerican nephropathy. *Environ Res, 147*, 125-132. doi:10.1016/j.envres.2016.02.002

Wilson, T. E. (2017). Renal sympathetic nerve, blood flow, and epithelial transport responses to thermal stress. *Auton Neurosci, 204*, 25-34. doi:10.1016/j.autneu.2016.12.007

Xiang, J., Hansen, A., Pisaniello, D., & Bi, P. (2015). Extreme heat and occupational heat illnesses in South Australia, 2001-2010. *Occup Environ Med, 72*(8), 580-586. doi:10.1136/oemed-2014-102706

Xin, L., Hyldahl, R. D., Chipkin, S. R., & Clarkson, P. M. (2014). A contralateral repeated bout effect attenuates induction of NF-κB DNA binding following eccentric exercise. *J Appl Physiol (1985), 116*(11), 1473-1480. doi:10.1152/japplphysiol.00133.2013

Yamamoto, L. M., Judelson, D. A., Farrell, M. J., Lee, E. C., Armstrong, L. E., Casa, D. J., . . . Maresh, C. M. (2008). Effects of hydration state and resistance exercise on markers of muscle damage. *J Strength Cond Res, 22*(5), 1387-1393. doi:10.1519/JSC.0b013e3181739403

Yeargin, S. W., Casa, D. J., Judelson, D. A., McDermott, B. P., Ganio, M. S., Lee, E. C., . . . Maresh, C. M. (2010). Thermoregulatory responses and hydration practices in heat-acclimatized adolescents during preseason high school football. *J Athl Train, 45*(2), 136-146. doi:10.4085/1062-6050-45.2.136

Appendix

UNIVERSITY OF ARKANSAS

Office of Research Compliance
Institutional Review Board

May 15, 2017

MEMORANDUM

TO:	Brendon McDermott	Cory Butts
	Kathleen Paulsen	Eric Schwartz
	Aaron Caldwell	Matthew Ganio
	Stavros Kavouras	Lisa Jansen
	Hyungyu Suh	Adam Seal
FROM:	Ro Windwalker IRB Coordinator	
RE:	New Protocol Approval	
IRB Protocol #:	17-05-685	
Protocol Title:	*Dehydration, Muscle Damage, and Exercise in the Heat: Impacts on Renal Stress, Thermoregulation, and Muscular Damage Recovery*	
Review Type:	☐ EXEMPT ☐ EXPEDITED ☒ FULL IRB	
Approved Project Period:	Start Date: 05/15/2017 Expiration Date: 05/14/2018	

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form *Continuing Review for IRB Approved Projects*, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (https://vpred.uark.edu/units/rscp/index.php). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in Termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

This protocol has been approved for 70 participants. If you wish to make *any* modifications in the approved protocol, including enrolling more than this number, you must seek approval *prior to* implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 109 MLKG Building, 5-2208, or irb@uark.edu.

109 MLKG • 1 University of Arkansas • Fayetteville, AR 72701-1201 • (479) 575-2208 • Fax (479) 575-6527 • Email irb@uark.edu
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