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Observing Renal Responses to Endurance Cycling in the Heat

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Observing Renal Responses to Endurance Cycling in the Heat

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Kinesiology

by

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Abstract

AIM: To observe the effects of endurance cycling in the heat on renal function, and determine if the extent of impairment is related to hydration. **METHODS:** 40 cyclists (34 male, 6 female, 52 ± 9 y, 21.7 ± 6.5 % body fat) completed an endurance cycling event (5.7 ± 1.2 hours) in the heat ($33.2 \pm 5.0^\circ\text{C}$, $38.4 \pm 10.7\%$ RH). Body mass was assessed to determine net fluid loss, muscle damage in the legs was assessed with a pain scale, a urine sample was collected to analyze hydration status, and a blood sample was drawn. All measurements were taken pre- and post-event. Serum creatinine was measured by colorimetric assay and analyzed via the Jaffe reaction, and creatinine clearance was estimated. An ELISA kit was used to measure serum neutrophil gelatinase-associated lipocalin. **RESULTS:** A net fluid loss of 1.3 ± 1.1 kg was observed. Urine specific gravity ($P < 0.001$) and leg muscle pain ($P < 0.001$) increased post-event. Serum creatinine increased from pre- (0.52 ± 0.14 mg/dL) to post-event (0.88 ± 0.21 mg/dL; $P < 0.001$). Creatinine clearance decreased from pre- (160.9 ± 48.9 mL/min) to post-event (95.2 ± 28.1 mL/min; $P < 0.001$). Serum neutrophil gelatinase-associated lipocalin increased from pre- (68.51 ± 17.54) to post-event (139.12 ± 36.52 ; $P < 0.001$) **CONCLUSION:** Changes observed in renal measures suggest acute kidney injury and reduced kidney function after endurance cycling in the heat.

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I. Introduction

Strenuous running (1-3) and cycling (4) has been reported to compromise kidney function. A decrease in estimated creatinine clearance (CCr) from serum to urine as a result of reduced renal blood flow during exercise suggests this finding (4, 5). The kidneys play a vital role in maintaining fluid and electrolyte balance by filtering and excreting metabolic waste products from blood into urine, while also reabsorbing essential molecules such as water, glucose and amino acids. In a homeostatic state, the kidneys are supplied via the renal arteries with 20% of cardiac output. Each kidney has many nephrons that together produce 180 liters of filtrate each day, of which an estimated 2 liters are excreted in urine.

Upon reaching the kidney, blood flows to the nephron through the afferent arteriole and subsequently into the glomerular capillaries. Molecules are then filtered from the blood through the capillaries into the renal corpuscle, and the filtrate is either reabsorbed into the peritubular capillaries or excreted in urine. The rate at which molecules are filtered through the kidneys is known as the glomerular filtration rate (GFR). Creatinine (Cr) is a muscle metabolic waste product that can be measured in serum and in urine to estimate GFR and subsequently manifest renal function.

Cr is produced by the breakdown of creatine phosphate by creatine kinase, and is released into the blood stream where it is eventually filtered through the kidneys and excreted in urine. In a normal functioning kidney, the CCr in the blood is equal to the concentration of creatinine that is filtered and excreted in urine (6). Two determinants that contribute to an increase in serum creatinine (sCr) are hydration status (7) and skeletal muscle damage (8). Hydration status is more important when exercising in the heat due to the loss of water via increased sweat rate and

respiration (9). Skeletal muscle damage is less common during exercise cycling versus running due to the lack of eccentric contractions of working skeletal muscle (4, 10).

Other studies involving endurance events have examined the kidney biomarker neutrophil gelatinase-associated lipocalin (NGAL) as an early indicator of acute kidney injury (AKI) during exercise (7, 11, 12). AKI is a result of renal tissue damage that can occur during strenuous endurance exercise as a result of renal ischemia. NGAL is a glycoprotein that is synthesized as a result of inflamed epithelial cells in several human tissues such as uterus, prostate, lung, stomach, and kidney (13). Dramatic upregulation of NGAL is found in proximal tubules of mice within 3 hours of ischemic renal injury, leading to its suggested use of determining AKI in humans (14). Although NGAL has been thoroughly studied in clinical settings there is a need for further research to observe instances and determine causes of AKI in humans after strenuous exercise.

II. Review of Literature

During exercise, renal blood flow is reduced to as low as 25% of resting values as a result of renal blood vessel vasoconstriction (15). Reduction in blood flow to the kidneys is exacerbated by the effects of dehydration, particularly while exercising in the heat (9). A common assessment of dehydration involves participants to void their bladder before exercise, drink water to replenish fluid loss during exercise, and examine the difference in body weight post exercise. A greater loss of body weight during exercise suggests greater dehydration. Neviackas and Bauer reported 3.0 to 4.0kg were lost during warm weather marathon running versus just 2.0 to 2.5kg lost during cold weather marathon running (16). This finding suggests that exercising in the heat can lead to a greater reduction of renal blood flow than exercising in the cold.

Fluid replacement while running can be difficult to achieve due to the swinging motion of the arms to maintain balance and the unstable effect the movement of the legs have on the body. Therefore, replacing the amount of fluid necessary for maintaining hydration can be difficult during a marathon run in which great amounts of fluid are lost over a long period of exercise. Cycling, however, allows free use of the arm and a steady posture of the body, which can facilitate drinking during exercise. Neumayr et al. reported a moderate weight loss of 1.7kg, as well as no significant difference in urine osmolarity from pre to post event while marathon cycling in a cold environment (4). These findings, along with the findings from Neviackas et al., suggest a need to examine the effects of hydration status on the kidneys while endurance cycling in the heat.

Dehydration decreases blood volume, reducing renal blood flow. Reductions in renal blood flow, and subsequent reductions in GFR, are increased when dehydration is associated with exercise in a hot environment (17). Otani et al. found that when full fluid loss is replaced during heavy exercise in a hot environment, dehydration is attenuated and GFR is unchanged (18). Conversely, when fluid is ingested voluntarily dehydration is augmented and GFR is reduced (18). Reduced GFR results in increased concentration of waste products in the blood, which leads to the method by which GFR can be estimated – creatinine clearance.

As a muscle metabolic waste product, creatinine is filtered from the blood and excreted in urine. Creatinine clearance is commonly assessed by observing the increase in SCr before and after insult. When renal function is impaired the filtration of waste products is reduced as evidenced by an increase in SCr. Therefore, decreases in creatinine clearance suggest detriments in renal function. The Acute Kidney Injury Network (AKIN) defines AKI as a 50% increase or an absolute increase of 0.3 mg/dl in SCr (19). Endurance running requires eccentric muscle

contractions resulting in muscle damage (10) leading to increases in creatinine in the blood immediately following exercise (1). Kao et al. found associations between increased plasma creatinine and reduced kidney function in ultramarathon runners (20). These findings present the question of whether muscle damage during endurance running overloads the kidney filtration system with creatinine or reductions in renal blood flow is the main contributor to increases in SCr. Using creatinine as a marker to assess kidney injury is questionable since increases in SCr could be only a reflection of the renal filtration system. Therefore, creatinine may be better described as a marker of kidney function instead of injury.

Reductions in creatinine clearance are directly related to the intensity of exercise (15). Although many studies observing renal function have incorporated endurance running, endurance cycling has been examined as well (4, 21). Differences in mechanics while running and cycling reduce the likelihood to eccentrically contract leg muscles while cycling, resulting in reduced muscle damage (10). Neumayr et al. found that renal function is minimally altered during strenuous endurance cycling (4, 21). They hypothesized that exercise induced reduction of renal blood flow is likely the cause of decreases found in creatinine clearance since muscle damage was not identified (4). Further research of endurance cycling in a hot environment is necessary to verify this hypothesis as hypohydration could reduce renal blood flow even greater than their results.

Cr has historically been used to diagnose AKI, but recent research has recognized NGAL as a more accurate and earlier marker of renal injury. Clinical research of NGAL has primarily investigated the biomarker's synthesis in rodents and human patients with induced or confirmed renal injury, respectively. Mishra et al. found NGAL to be a sensitive urinary biomarker for early detection of ischemic renal injury in mice (22). Interestingly, sCr levels were normal in this study despite kidney injury, further suggesting Cr as a poor marker for AKI. In a study of 635

patients, single measurements of NGAL in urine helped distinguish AKI from CKD, azotemia and normal function (23). Makris et al. reported urinary NGAL as an early detector of AKI in severe trauma patients (24). While NGAL has been identified as a reliable marker of AKI in clinical settings, its application in exercise settings requires more research.

In a study by McCullough et al., plasma NGAL (pNGAL) was measured in runners before and after a marathon in the cold, and then 24 hours after finishing the run. Results of this study showed a dramatic rise in pNGAL immediately after the race, but the levels returned to near baseline after 24 hours (12). Junglee et al. also found a significant increase in pNGAL after a heat stressed running protocol following exercise induced muscle damage (7). Furthermore, the increase in pNGAL was suggested to be a result of inflammation from the muscle damaging exercise (7). Lippi et al. observed NGAL variations in athletes after an ultramarathon run in cold weather and found an almost two fold increase in sNGAL (11). Based on these three studies involving running protocols strenuous running exercise could lead to AKI. However, to the best of our knowledge, there are no cycling protocols in which sCr and sNGAL have been measured to observe renal function and assess potential injury.

Electrolyte concentration has been reported to change post marathon running (1, 25). Fallon et al. found increased serum sodium (sNa) and chloride (sCl) levels post event, while Kratz et al. reported no change in sNa or serum potassium (sK) but decreased sCl levels post event (1, 25). Neumayr et al. reported increased sK and unchanged sNa and sCl post marathon cycling (4). Kratz et al. hypothesized that differences in fluid intake may be the cause of differences in post exercise serum electrolyte concentrations (25). Neumayr et al. found that urine was more dilute post-race, surmising that fluid replacement while cycling is more sufficient than that of runners who observe greater urine concentration post event (4).

Fluid replacement can become dangerous when fluid is replaced at a higher rate than necessary. Although insufficient replacement of fluid causes dehydration, over sufficient replacement of fluid could result in hyponatremia (26). Hyponatremia is characterized by an unsafe decrease in Na in the blood and could result in exhaustion, unconsciousness, and even death. The normal reference range of sNa is 135-145 mmol/L (25). Therefore, sNa levels below 135 mmol/L are classified as hyponatremic.

Urine sodium (uNa) levels are important to monitor as well. When filtered through healthy kidneys, Na is mostly reabsorbed before urine is excreted and a relatively low concentration of Na remains in urine. Irving et al. found that serum electrolytes were increased and uNa was decreased after ultramarathon running (3). Neumayr et al. also reported lower uNa post marathon cycling (4). Neither Irving et al. nor Neumayr et al. found differences in hydration, suggesting that proper fluid replacement during exercise may reduce sNa imbalance. Fractional excretion of sodium (FENa) can be measured to determine how well the renal tubules are functioning to reabsorb molecules into the blood stream. Junglee et al. reported that a significant reduction in FENa after exercising in the heat suggests kidney stress due to higher energy demands from increased Na-K-ATPase activity in the renal tubules (7).

The limited research available observing the effects of endurance cycling on renal function has been performed in environments lacking heat stress, and no research has measured preferred kidney injury markers. With knowledge of the effects of heat on fluid balance during exercise, an observational study of the effects of endurance cycling in a hot environment on the kidneys should be conducted. Based on previous research, we hypothesize: 1) that renal function will be minimally impaired due to normalized CCr as a result of attenuated muscle damage, 2) that hypohydration and alterations in electrolyte concentrations will be observed due to a lack of

sufficient fluid replacement, and 3) that sNGAL values will increase dramatically, possibly due to renal ischemia as a result of hypohydration and heat effects.

III. Methodology

Participants in either the 100 mile or 100 kilometer 2015 Hotter'n Hell Hundred event (HHH) ($33.2 \pm 5.0^{\circ}\text{C}$, $38.4 \pm 10.7\%$ RH) were recruited to participate in this study. During the two days leading up to the event a station was set up near the HHH expo with information about the study. Researchers actively sought and requested the participation of cyclists who were visiting the expo. Cyclists interested in participating were briefed of the details of the study, as described below, and what was required of them throughout the next couple of days.

Upon being recruited and verbally consenting to participation, participants completed a medical history questionnaire. Participants were asked to comply with exclusion criteria including: medical condition or medication that alters body fluid balance, dietary manipulation that omits one nutrient or class of nutrients, present musculoskeletal injury, and use of tobacco products. An investigator then took 3-site skin fold measures to assess body composition using the Brozek equation (26). Those who were able and willing to participate in the study signed an informed consent document.

The morning of the event, before starting HHH, participants arrived to our station where body mass was measured using a standard scale (Health-o-med 349 KLX, McCook, IL), a urine sample was collected, and a blood sample (50mL) was drawn via venipuncture of a cubital vein into a vacutainer serum separating tube where it remained in a vertical position until the blood clotted. After clotting, the sample was spun in a centrifuge at 1500 g for 10 minutes to separate serum from whole blood. Serum was then be aliquoted into a microcentrifuge tube and frozen

until analysis. Urine specific gravity (USG) and urine color (hydrationcheck.com) was assessed immediately to determine hydration status. Once analyzed for hydration, urine samples were aliquoted into microcentrifuge tubes and frozen. Serum and urine samples were stored in the tubes and frozen in coolers with dry ice until transported back to the laboratory.

Immediately after HHH was completed participants returned to our station where body mass was assessed, a urine sample was collected, and a blood sample (50mL) was drawn. Urine was assessed immediately to determine hydration status. Serum and urine samples were stored in microcentrifuge tubes and frozen for transport as described before. Once transported to the laboratory, samples were frozen at -80°C until analysis.

Samples were removed from the freezer at least one hour before analysis to ensure thawing. Pre- and post-urine and serum sodium, potassium and chloride concentrations were measured and recorded in duplicate via ion-sensitive electrodes. Pre- and post-event sCr and uCr was measured in duplicate by colorimetric assay and analyzed via the Jaffe reaction. CCr was calculated via the Cockcroft and Gault formula (27). sNGAL was measured via commercially based ELISA kit as per instructions (R&D Systems, Minneapolis, MN). FENa was calculated using the equation $[(uNa \times sCr) \div (sNa \times uCr) \times 100]$.

Pre- and post-event body mass difference was used to determine net fluid loss. Paired samples T-tests were used to determine whether sNGAL or sCr concentrations were statistically different pre- and post-event, and whether any electrolyte measures were statistically different post-event compared to pre-event. Correlation tests were used to determine the cause of any differences in NGAL and Cr. An alpha level of 0.05 was set *a priori*.

IV. Manuscript

Introduction

Strenuous running (1-3) and cycling (4) has been reported to compromise kidney function. A decrease in estimated creatinine clearance (CCr) from the blood to urine as a result of reduced renal blood flow during exercise suggests this finding (4, 5). The kidneys play a vital role in maintaining fluid and electrolyte balance by filtering and excreting metabolic waste products from the blood into urine, while also reabsorbing essential molecules such as water, glucose and amino acids. The rate at which molecules are filtered through the kidneys is known as the glomerular filtration rate (GFR). Creatinine (Cr) is a muscle metabolic waste product that is filtered from the blood to urine. Cr can be measured in serum and in urine to estimate GFR and subsequently manifest renal function. Therefore, an increase in sCr and a subsequent decrease in CCr suggests detriments in renal function.

In a normal functioning kidney, the concentration of Cr in the blood is equal to the concentration of Cr that is filtered and excreted in urine (6). Therefore, observing an increase in serum creatinine (sCr) would reveal a decrease in CCr. Two determinants that contribute to an increase in sCr are hydration status (7) and skeletal muscle damage (8). Hydration status is important when exercising in the heat due to the loss of water via increased sweat rate and respiration (9). Skeletal muscle damage is less common during exercise cycling versus running due to the lack of eccentric contractions of working skeletal muscle (4, 10).

Reductions in CCr are directly related to the intensity of exercise (11). Although studies observing renal function during exercise have mostly incorporated endurance running, endurance cycling has been examined as well (4, 12). Differences in mechanics while running and cycling reduce the likelihood to eccentrically contract leg muscles while cycling, resulting in reduced muscle damage (10). Neumayr et al. found that renal function is minimally altered during

strenuous endurance cycling (4, 12). They hypothesized that exercise induced reduction of renal blood flow is likely the cause of decreases found in CCr since muscle damage was not identified (4). Further research of endurance cycling in a hot environment is necessary to verify this hypothesis as hypohydration could reduce renal blood flow even greater than their results.

During exercise, renal blood flow is reduced to as low as 25% of resting values as a result of renal blood vessel vasoconstriction (11). A reduction in blood flow to the kidneys is exacerbated by the effects of dehydration, particularly while exercising in the heat (9). Dehydration decreases blood volume, reducing renal blood flow. Replenishing fluid losses during exercise can augment blood volume, increase the amount of blood available to flow to the kidneys, and subsequently diminish the effects of dehydration. A greater loss of body weight during exercise suggests a lack of fluid replacement and greater dehydration. Neviackas and Bauer reported 3.0 to 4.0 kg were lost during warm weather marathon running versus just 2.0 to 2.5 kg lost during cold weather marathon running (13). This finding suggests that exercising in the heat can lead to a greater reduction of renal blood flow than exercising in the cold.

Reductions in renal blood flow, and subsequent reductions in GFR, are increased when dehydration is associated with exercise in a hot environment due to greater fluid loss through increased sweating (14). Otani et al. found that when full fluid loss is replaced during heavy exercise in a hot environment, dehydration is attenuated and GFR is unchanged (15). Conversely, when fluid is ingested voluntarily dehydration is augmented and GFR is reduced (15). Fluid replacement is simpler while cycling versus running due to the differences in stability and the use of the hands. Neumayr et al. reported a moderate weight loss of 1.7 kg, as well as no significant difference in urine osmolarity from pre- to post-event while marathon cycling in a cold environment (4). These findings, along with the findings from Neviackas et al., suggest a

need to examine the effects of hydration status on the kidneys while endurance cycling in the heat.

Neumayr et al. found that urine was more dilute post-race, surmising that fluid replacement while cycling is more sufficient than that of runners who observe greater urine concentration post event (4). Fractional excretion of sodium (FENa) determines how well the renal tubules are functioning to reabsorb molecules into the blood stream. Junglee et al. reported that a significant reduction in FENa after exercising in the heat suggests kidney stress due to higher energy demands from increased Na-K-ATPase activity in the renal tubules (7).

Studies involving endurance exercise have examined the biomarker neutrophil gelatinase-associated lipocalin (NGAL) as an early indicator of acute kidney injury (AKI) during exercise (7, 16, 17). AKI is a result of renal tissue damage that can occur during strenuous endurance exercise as a result of renal ischemia. NGAL is a glycoprotein that is synthesized as a result of inflamed epithelial cells in several human tissues such as uterus, prostate, lung, stomach, and kidney (18). Dramatic upregulation of NGAL is found in the proximal tubules of mice within 3 hours of ischemic renal injury, leading to its suggested use of determining AKI in humans (19). Though NGAL has been thoroughly studied in clinical settings (19-21), there is a need for further research to observe instances of AKI in humans during endurance exercise.

The Acute Kidney Injury Network (AKIN) defines AKI as a 50% increase or an absolute increase of 0.3 mg/dl in sCr (22). Endurance running requires eccentric muscle contractions resulting in muscle damage (10) leading to increases in creatinine in the blood immediately following exercise (1). Kao et al. found associations between increased plasma creatinine and reduced kidney function in ultramarathon runners (23). These findings present the question of whether muscle-damaging exercise overloads the kidney filtration system with creatinine, or if a

reduction in renal blood flow is the main contributor to increases in sCr. The use of creatinine as a marker to assess kidney injury is questionable as increases in sCr could only be a reflection of the renal filtration system. Therefore, creatinine may be best utilized as a marker of kidney function instead of injury.

In a study by McCullough et al., plasma NGAL (pNGAL) was measured in runners before and after a marathon in the cold, and then 24 hours after finishing the run. They found a dramatic rise in pNGAL immediately after the race, but the levels returned to near baseline after 24 hours (17). Junglee et al. found a two-fold increase in pNGAL, which exceeded their reported normal range (37-106 ng/mL), after a heat stressed running protocol following exercise induced muscle damage (7). Furthermore, the increase in pNGAL was suggested to be a result of inflammation from the muscle damaging exercise (7). Lippi et al. observed NGAL variations in athletes after an ultramarathon run in cold weather and found an almost two fold increase in sNGAL (16). Based on these three studies involving running protocols, endurance running could lead to AKI. However, to the best of our knowledge, there are no cycling protocols in the heat that have measured sCr and sNGAL to observe renal function and assess potential injury.

The limited research available observing the effects of endurance cycling on renal function has been performed in environments lacking heat stress, and no research has measured preferred kidney injury markers. Therefore, the purpose of this study is to observe renal function and potential instances of AKI during endurance cycling in the heat.

Methods

The present study was designed to observe kidney function and instances of AKI in cyclists participating in an endurance event in the heat. The event was completed the same day it was

started and all measurements were taken the days leading up to and the day of the event. No variables were controlled, but this study was in collaboration with another study investigating NSAID use. Therefore, some of the participants took ibuprofen before the event. This study was approved by the University of Arkansas Institutional Review Board.

Participants in either the 100 mi or 100 km 2015 Hotter'n Hell Hundred event (HHH) ($33.2 \pm 5.0^{\circ}\text{C}$, $38.4 \pm 10.7\% \text{RH}$) were recruited to participate in this study. During the two days leading up to the event a station was set up near the HHH expo with information about the study. Researchers actively sought and requested the participation of cyclists who were visiting the expo. Cyclists interested in participating were briefed of the details of the study, as described below, and what was required of them throughout the next couple of days.

Upon being recruited and consenting to participation, participants completed a medical history questionnaire. Exclusion criteria for this study included: medical condition or medication that alters body fluid balance, dietary manipulation that omits one nutrient or class of nutrients, present musculoskeletal injury, and use of tobacco products. Those who were able and willing to participate in the study signed an informed consent document. An investigator then measured body mass and took 3-site skin fold measures to assess body composition using the Brozek equation (24).

The morning of the event, before starting HHH, participants arrived to our station where body mass was measured using a standard scale (Health-o-med 349 KLX, McCook, IL), a urine sample was collected, and a blood sample (50mL) was drawn via venipuncture of a cubital vein into a vacutainer serum separating tube where it remained in a vertical position until the blood clotted. After clotting, the sample was spun in a centrifuge at 1500 g for 10 minutes to separate serum from whole blood. Serum was then be aliquoted into a microcentrifuge tube and frozen

until analysis. Urine specific gravity (USG) and urine color (hydrationcheck.com) was assessed immediately to determine hydration status. Once analyzed for hydration, urine samples were aliquoted into microcentrifuge tubes and frozen. Serum and urine samples were stored in the tubes and frozen in coolers with dry ice until transported back to the laboratory. Participants were asked about their perceived pain in the legs and overall pain from a 0-10 muscle pain scale (25) pre- and post-event.

Immediately after HHH was completed participants returned to our station where body mass was assessed, a urine sample was collected, and a blood sample (50mL) was drawn. Urine was assessed immediately to determine hydration status. Serum and urine samples were stored in microcentrifuge tubes and frozen for transport as described before. Once transported to the laboratory, samples were frozen at -80°C until analysis.

Samples were removed from the freezer at least one hour before analysis to ensure thawing. Pre- and post-urine and serum sodium, potassium and chloride concentrations were measured and recorded in duplicate via ion-sensitive electrodes (Medica EasyElectrolytes, Bedford, MA). Pre- and post-event sCr and uCr was measured in duplicate by colorimetric assay and analyzed via the Jaffe reaction. CCr was calculated via the Cockcroft and Gault formula (26). A commercially based ELISA kit was used to measure sNGAL as per instructions (R&D Systems, Minneapolis, MN). FENa was calculated using the equation $[(uNa \times sCr) \div (sNa \times uCr) \times 100]$.

Pre- and post-event body mass difference was used to determine net fluid loss. Paired samples t-tests were used to determine any pre- versus post-event differences. Spearman's rho correlation tests were used to determine any relationships between select variables. An alpha level of 0.05 was set *a priori*.

Results

Forty cyclists (34 male, 6 female) who completed the event (100 mi = 31, 100 km = 9) participated in this study. Average race time was 5.7 ± 1.2 hours and average body mass difference was -1.3 ± 1.1 kg (-1.6 ± 1.4 %, $P < 0.001$). Table 1 displays the demographic information of the participants included in our analysis. Pre- and post-event sCr, sNGAL, CCr, muscle pain, and FENa results are displayed in Table 2 and hydration status results are displayed in Table 3. sNGAL increased 70.61 ± 34.25 ng/mL from pre- to post-event, approximately a two-fold increase ($P < 0.001$) as displayed in Figure 1. sCr increased 0.32 ± 0.22 mg/dL from pre- to post-event, a 62% increase ($P < 0.001$), as displayed in Figure 2. Figure 3 shows a 60.9 ± 42.4 mL/min decrease ($P < 0.001$) in CCr from pre- to post-event. Table 4 is a correlation matrix displaying post-event relationships of sNGAL, sCr and CCr with hydration status, muscle pain, FENa, and finish time. Another correlation matrix of the relationships between changes in pre- and post-event sNGAL, sCr and CCr with hydration status, muscle pain, FENa, and finish time is shown in Table 5. Table 6 displays all electrolyte results.

Discussion

Our main findings were that endurance cycling in the heat resulted in renal function impairment and AKI based on decreases in CCr and increases in sNGAL (7, 17). The absolute increase in sCr was consistent with the AKIN definition of AKI, but as mentioned earlier sCr may not be the best variable to determine AKI (22). Interestingly, the identified rise in sNGAL was independent of hydration status and muscle pain, suggesting for the first time that AKI could result from heat exposure in an endurance exercise event.

An increase in sCr suggests that renal function was reduced. Although hydration status significantly decreased, which can contribute to increases in sCr (7), there was no relationship

found between the two in the present study. There was a significant decrease in body mass, however it was moderate in comparison to the study by Neumayr et al. which was performed in a cool environment and it was also not related to sCr (4). Therefore, our findings suggest that any potential renal blood flow impairment would be a result of the demands of blood elsewhere in the body during endurance exercise in the heat. Environmental conditions likely reduced renal blood flow and could have induced kidney stress. Endurance exercise combined with heat stress increases sweating which requires an increase in blood flow to the skin (14). As mentioned earlier, renal blood flow is reduced to as low as 25% during exercise and has even greater consequences when blood volume is reduced by dehydration (11). FENa was significantly reduced and in agreement with the results of Juglee et al. which further suggests heat stress contributes to kidney stress during exercise (7).

CCr was reduced post-event which suggests renal function impairment during exercise in the heat. However, there were no correlations between CCr and any of the variables that would suggest that the reduction in CCr is related to hydration status, muscle damage, FENa, or heat exposure. Our findings are consistent with those of a cycling protocol by Neumayr et al. who suggested that exercise-induced renal blood flow reduction leads to a decrease in CCr (4).

However, the difference between our study and that of Neumayr et al. is not only environmental conditions, but length of cycling as well. Cyclists in the present study rode for almost half the time of those in the study by Neumayr et al. Therefore, the duration of cycling exercise and the subsequent amount of time in which renal blood flow is reduced may play a role in the severity of kidney stress. Further research involving a shorter duration of cycling exercise should be conducted in order to determine the onset of renal function reduction.

Unlike the results of a similar cycling study by Neumayr and colleagues, we found an increase in muscle pain in the legs which would lead to the conclusion that there was muscle damage during the event (4). Furthermore, we found that muscle pain the legs post-event was related to the increase in sCr. Our differing results could be due to the variances in terrain cycled between the two studies. Neumayr et al. performed their protocol in the mountains in Austria while the present study was conducted in a mostly flat area of Texas. With changes in elevation there are more opportunities to not need to pedal and thus not use the leg muscles, while on a flat course there would be a need for pedaling almost throughout the event.

sNGAL more than doubled from pre- to post-event and reached a level consistent with previous studies involving a running protocol which observed AKI in exercise protocols (7, 17). Results from clinical studies suggests that an upregulation in serum is due to ischemic renal injury (19, 20). However, like the results of Cr, sNGAL results were not linked to hydration status or muscle pain. Therefore, it could be suggested that dehydration combined with endurance exercise, heat stress, or both may bring about AKI during endurance exercise. Unlike Junglee et al., who's protocol was designed to induce muscle damage, we did not find that the increase in sNGAL was related to muscle pain (7). Our results show sNGAL was increased to a level much greater than both McCullough et al. and Junglee et al. found, but was closest to the results of Junglee et al. (7, 17).

Finish time was equated as the time from when the participants started the event until the time they finished, and tells how long they were exposed to the heat. Post-event sNGAL had a tendency to relate to finish time, suggesting for the first time that AKI could be due to heat exposure during exercise. This could be caused by several mechanistic physiological responses to the heat such as renal ischemia due to increased blood flow to the skin or decreased blood

volume. Participants were exposed to the heat for almost 6 hours during the present study compared to just 40 minutes in the study by Junglee et al., which may explain the greater level of sNGAL post-event in the present study (7). Because both studies induced increases in NGAL consistent with AKI, the differing suggested causes may not be mutually exclusive. Muscle damaging exercise as well as long periods of heat exposure while exercising may have a similar effect on the kidneys.

In conclusion, endurance cycling in the heat stresses the kidneys and could result in AKI. While hydration and muscle pain do not have a direct relationship with these findings, the combination of them with the duration of heat exposure while exercising may increase the likelihood of renal stress and injury.

Limitations

We were unable to collect data the day following the event and therefore could not determine if the participants' biomarkers recovered to baseline levels. It is also unknown if instances of AKI accumulated over time leads to CKD. Future research should investigate the role of AKI during exercise in CKD.

V. Discussion

Our main findings were that endurance cycling in the heat resulted in renal function impairment and AKI based on decreases in CCr and increases in sNGAL (7, 17). The absolute increase in sCr was consistent with the AKIN definition of AKI, but as mentioned earlier sCr may not be the best variable to determine AKI (22). Interestingly, the identified rise in sNGAL was independent of hydration status and muscle pain, suggesting for the first time that AKI could result from heat exposure in an endurance exercise event.

An increase in sCr suggests that renal function was reduced. Although hydration status significantly decreased, which can contribute to increases in sCr (7), there was no relationship found between the two in the present study. There was a significant decrease in body mass, however it was moderate in comparison to the study by Neumayr et al. which was performed in a cool environment and it was also not related to sCr (4). Therefore, our findings suggest that any potential renal blood flow impairment would be a result of the demands of blood elsewhere in the body during endurance exercise in the heat. Environmental conditions likely reduced renal blood flow and could have induced kidney stress. Endurance exercise combined with heat stress increases sweating which requires an increase in blood flow to the skin (14). As mentioned earlier, renal blood flow is reduced to as low as 25% during exercise and has even greater consequences when blood volume is reduced by dehydration (11). FENa was significantly reduced and in agreement with the results of Juglee et al. which further suggests heat stress contributes to kidney stress during exercise (7).

CCr was reduced post-event which suggests renal function impairment during exercise in the heat. However, there were no correlations between CCr and any of the variables that would suggest that the reduction in CCr is related to hydration status, muscle damage, FENa, or heat

exposure. Our findings are consistent with those of a cycling protocol by Neumayr et al. who suggested that exercise-induced renal blood flow reduction leads to a decrease in CCr (4).

However, the difference between our study and that of Neumayr et al. is not only environmental conditions, but length of cycling as well. Cyclists in the present study rode for almost half the time of those in the study by Neumayr et al. Therefore, the duration of cycling exercise and the subsequent amount of time in which renal blood flow is reduced may play a role in the severity of kidney stress. Further research involving a shorter duration of cycling exercise should be conducted in order to determine the onset of renal function reduction.

Unlike the results of a similar cycling study by Neumayr and colleagues, we found an increase in muscle pain in the legs which would lead to the conclusion that there was muscle damage during the event (4). Furthermore, we found that muscle pain the legs post-event was related to the increase in sCr. Our differing results could be due to the variances in terrain cycled between the two studies. Neumayr et al. performed their protocol in the mountains in Austria while the present study was conducted in a mostly flat area of Texas. With changes in elevation there are more opportunities to not need to pedal and thus not use the leg muscles, while on a flat course there would be a need for pedaling almost throughout the event.

sNGAL more than doubled from pre- to post-event and reached a level consistent with previous studies involving a running protocol which observed AKI in exercise protocols (7, 17). Results from clinical studies suggests that an upregulation in serum is due to ischemic renal injury (19, 20). However, like the results of Cr, sNGAL results were not linked to hydration status or muscle pain. Therefore, it could be suggested that dehydration combined with endurance exercise, heat stress, or both may bring about AKI during endurance exercise. Unlike Junglee et al., who's protocol was designed to induce muscle damage, we did not find that the increase in sNGAL was

related to muscle pain (7). Our results show sNGAL was increased to a level much greater than both McCullough et al. and Junglee et al. found, but was closest to the results of Junglee et al. (7, 17).

Finish time was equated as the time from when the participants started the event until the time they finished, and tells how long they were exposed to the heat. Post-event sNGAL had a tendency to relate to finish time, suggesting for the first time that AKI could be due to heat exposure during exercise. This could be caused by several mechanistic physiological responses to the heat such as renal ischemia due to increased blood flow to the skin or decreased blood volume. Participants were exposed to the heat for almost 6 hours during the present study compared to just 40 minutes in the study by Junglee et al., which may explain the greater level of sNGAL post-event in the present study (7). Because both studies induced increases in NGAL consistent with AKI, the differing suggested causes may not be mutually exclusive. Muscle damaging exercise as well as long periods of heat exposure while exercising may have a similar effect on the kidneys.

In conclusion, endurance cycling in the heat stresses the kidneys and could result in AKI. While hydration and muscle pain do not have a direct relationship with these findings, the combination of them with the duration of heat exposure while exercising may increase the likelihood of renal stress and injury.

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Tables and Figures

Table 1. Demographic information ($n = 40$) expressed as mean \pm standard deviation.

Age (years)	52 \pm 9
Height (cm)	173.6 \pm 18.2
Body Mass (kg)	84.5 \pm 16.1
Body Fat (%)	21.7 \pm 6.5

Table 2. Pre- and post-event measurements expressed as mean \pm standard deviation when appropriate.

	Pre-event	Post-event	<i>P</i> -value
sNGAL (ng/mL)	68.51 \pm 17.54	139.12 \pm 36.52	<0.001
sCr (mg/dL)	0.53 \pm 0.14	0.85 \pm 0.19	<0.001
CCr (mL/min)	158.0 \pm 44.5	97.1 \pm 29.1	<0.001
Muscle Pain (legs)	0 \pm 1	1 \pm 2	<0.001
Muscle Pain (overall)	0 \pm 1	1 \pm 1	0.001
Fractional Excretion Na (%)	0.52 \pm 0.24	0.27 \pm 0.18	<0.001

Table 3. Hydration status measurements pre- and post- event expressed as mean \pm standard deviation.

	Pre-event	Post-event	<i>P</i> -value
Urine Specific Gravity	1.015 \pm 0.007	1.020 \pm 0.007	0.002
Color	2 \pm 1	3 \pm 1	<0.001
Body Mass (kg)	84.5 \pm 16.0	82.9 \pm 15.9	<0.001

Table 4. Correlation matrix of post-event measurements expressed as Spearman's rho and *P*-value.

		sNGAL	sCr	CCr
USG	Correlation Coefficient	0.070	-0.188	-0.031
	Sig. (2-tailed)	0.71	0.32	0.87
	N	29	29	29
Body Mass Loss	Correlation Coefficient	0.161	0.094	0.097
	Sig. (2-tailed)	0.32	0.56	0.55
	N	39	39	39
Leg Pain	Correlation Coefficient	0.172	0.531*	-0.208
	Sig. (2-tailed)	0.38	0.004	0.30
	N	28	28	26
Overall Pain	Correlation Coefficient	0.303	0.273	-0.205
	Sig. (2-tailed)	0.11	0.16	0.30
	N	28	28	27
FENa	Correlation Coefficient	-0.131	0.016	0.311
	Sig. (2-tailed)	0.48	0.93	0.094
	N	31	31	30
Finish Time	Correlation Coefficient	0.363	-0.295	0.195
	Sig. (2-tailed)	0.058	0.128	0.31
	N	28	28	28

* Correlation is significant at the 0.01 level.

Table 5. Correlation matrix of the changes in pre- and post-event measurements expressed as Spearman's rho correlation coefficient and *P*-value.

		Δ sNGAL	Δ sCr	Δ CCr
Δ USG	Correlation Coefficient	0.065	0.102	0.033
	Sig. (2-tailed)	0.73	0.59	0.86
	N	29	29	29
Body Mass Loss	Correlation Coefficient	0.120	0.154	0.213
	Sig. (2-tailed)	0.46	0.34	0.19
	N	39	39	39
Δ Leg Pain	Correlation Coefficient	-0.020	0.336	-0.058
	Sig. (2-tailed)	0.91	0.075	0.77
	N	29	29	26
Δ Overall Pain	Correlation Coefficient	0.152	0.234	-0.054
	Sig. (2-tailed)	0.43	0.22	0.78
	N	29	29	28
Δ FENa	Correlation Coefficient	0.019	0.081	-0.240
	Sig. (2-tailed)	0.91	0.66	0.20
	N	31	31	30
Finish Time	Correlation Coefficient	0.360	-0.263	0.120
	Sig. (2-tailed)	0.060	0.17	0.54
	N	28	28	28

Table 6. Electrolyte measurements pre- and post- event expressed as mean \pm standard deviation.

	Pre-event	Post-event	<i>P</i> -value
sNa (mmol/L)	141.2 \pm 2.4	143.0 \pm 3.0	0.002
sK (mmol/L)	4.4 \pm 0.5	4.7 \pm 0.6	0.005
sCl (mmol/L)	102.9 \pm 2.4	103.4 \pm 3.6	0.35
uNa (mmol/L)	95.8 \pm 51.2	52.4 \pm 33.9	<0.001
uK (mmol/L)	34.6 \pm 21.3	90.2 \pm 38.4	<0.001
uCl (mmol/L)	117.2 \pm 51.1	126.6 \pm 49.8	0.27

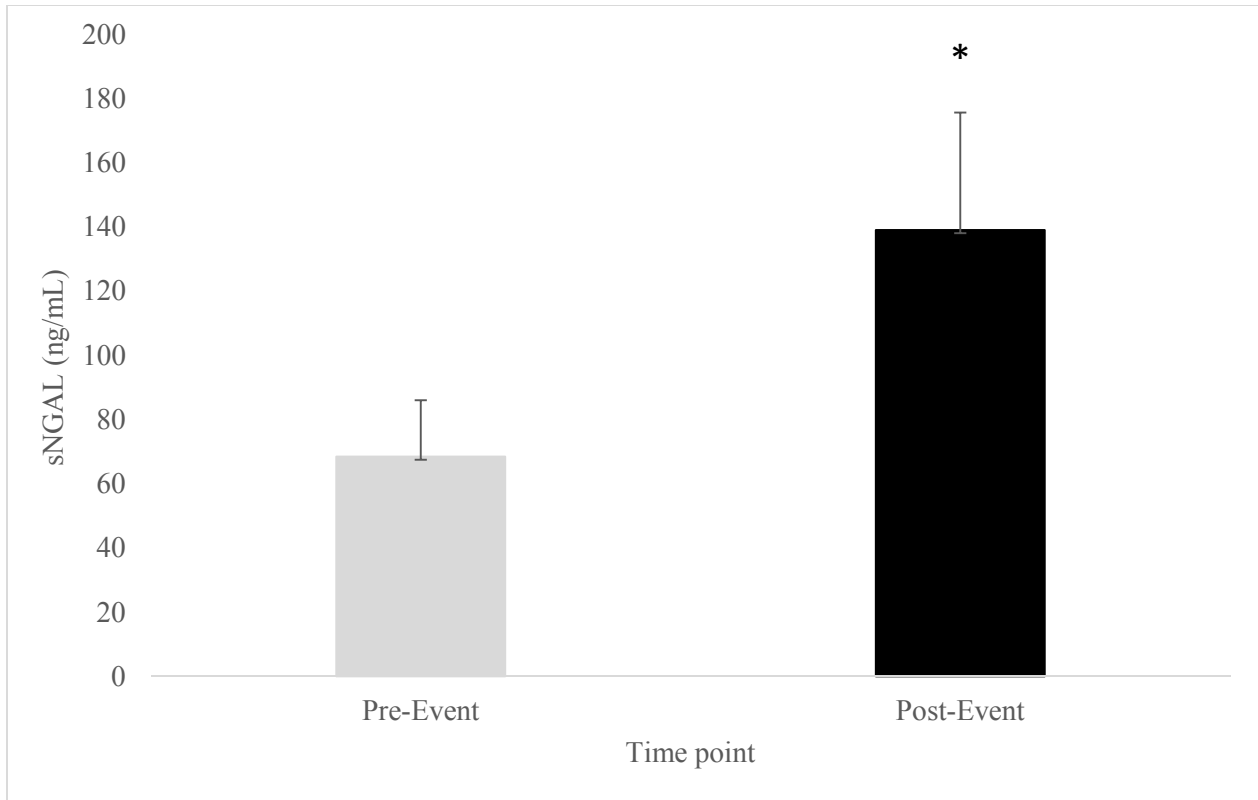


Figure 1. sNGAL measurements pre- and post-event expressed as means (SD).
* Indicates significantly different than pre-event ($P < 0.001$).

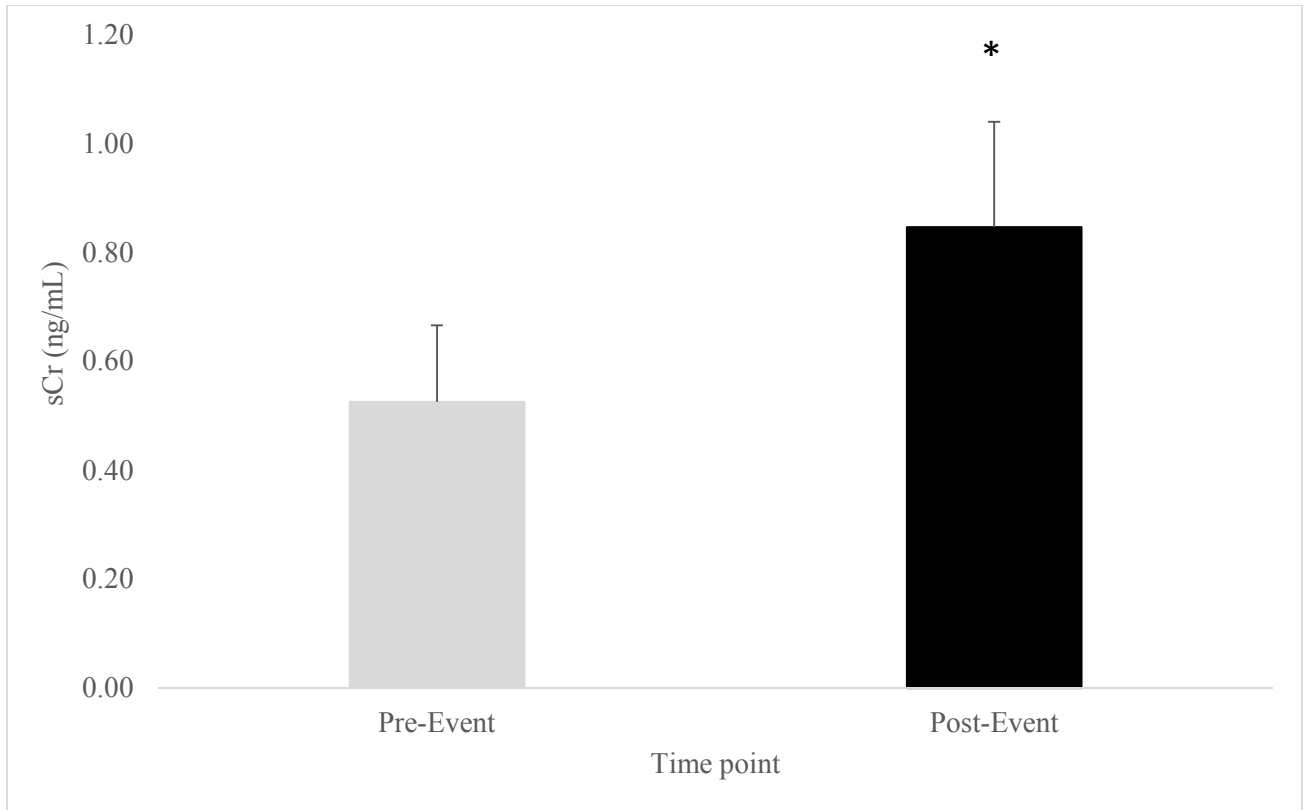


Figure 2. sCr measurements pre- and post-event expressed as means (SD).

* Indicates significantly different than pre-event ($P < 0.001$).

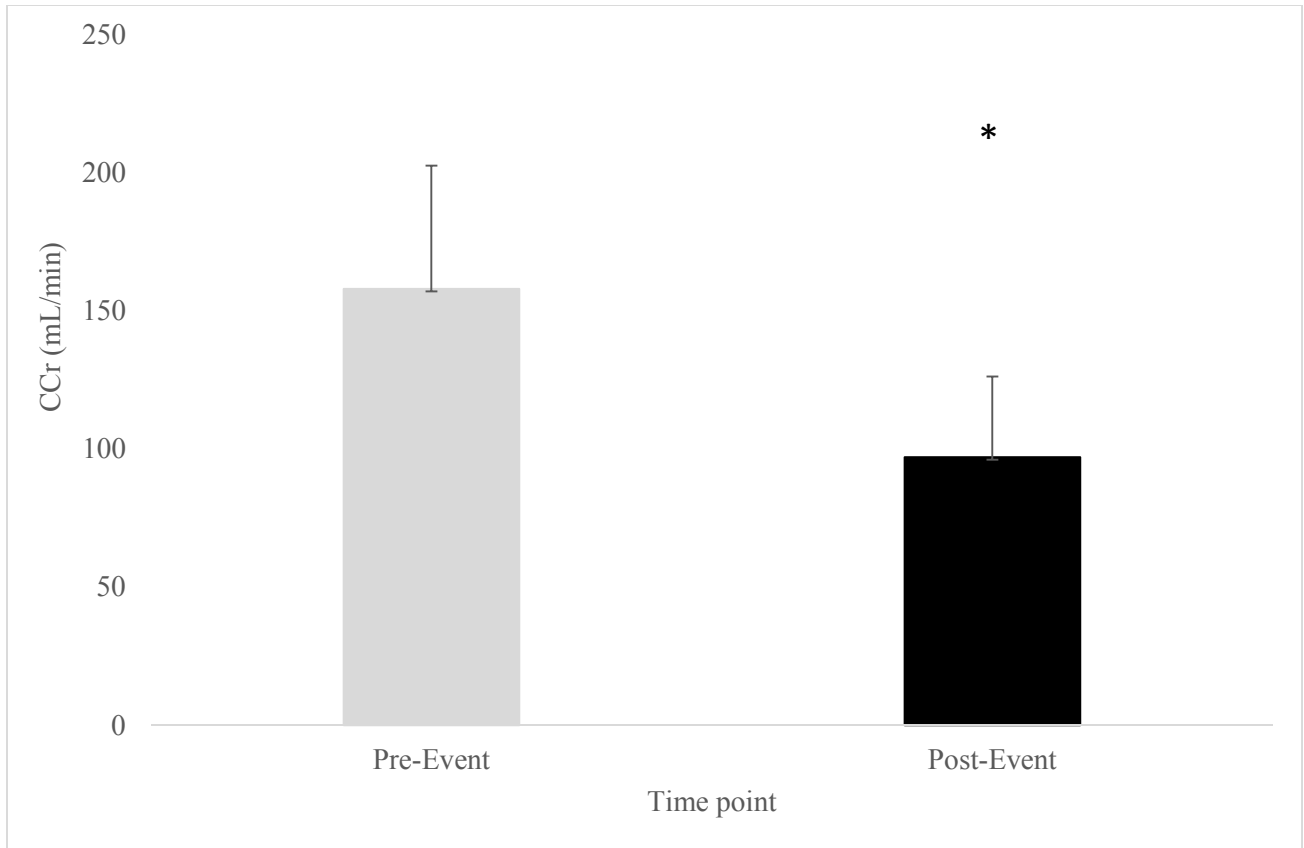


Figure 3. CCr measurements pre- and post-event expressed as means (SD).

* Indicates significantly different than pre-event ($P < 0.001$).

Appendix

IRB Approval Letter

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RESEARCH COMPLIANCE

Institutional Review Board (IRB) Authorization Agreement

Name of Institution or Organization Providing IRB Review (Institution/Organization A):

University of North Texas

IRB Registration #: 00000704 Federalwide Assurance (FWA) #: 00007479

Name of Institution Relying on the Designated IRB (Institution B):

University of Arkansas IRB #1

IRB Registration: 00001843 FWA #: 00001952

The Officials signing below agree that the University of Arkansas IRB#1, may rely on the designated IRB for review and continuing oversight of its human subjects research described below: (*check one*)

This agreement applies to all human subjects research covered by Institution B's FWA.

This agreement is limited to the following specific protocol(s):

Name of Research Project: Cycling in the Heat
Protocol #: UNT #15231, UA #15-06-790
Name of Principal Investigator: Jakob Vingren
University of Arkansas Researcher: Matthew Ganio
Sponsor or Funding Agency:
Award Number, if any: N/A

The review performed by the designated IRB will meet the human subject protection requirements of Institution B's OHRP-approved FWA. The IRB at Institution/Organization A will follow written procedures for reporting its findings and actions to appropriate officials at Institution B. Relevant minutes of IRB meetings will be made available to Institution B upon request. Institution B remains responsible for ensuring compliance with the IRB's determinations and with the Terms of its OHRP-approved FWA. This document must be kept on file by both parties and provided to OHRP upon request.

Signature of Signatory Official (Institution/Organization A):



Date: 7/22/15

Print Full Name:
Institutional Title:

Signature of Signatory Official (Institution B):



Date: 07/17/2015

Print Full Name: Rosemary Ruff
Institutional Title: Director, Research Compliance