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Body temperature and cardiovascular control during exercise in the heat: implications for special populations and athletic performance

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BODY TEMPERATURE AND CARDIOVASCULAR CONTROL DURING EXERCISE IN THE HEAT: IMPLICATIONS FOR SPECIAL POPULATIONS AND ATHLETIC PERFORMANCE

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ABSTRACT

Exercise in the heat increases the risk of dehydration and hyperthermia, subsequently impaired cardiovascular function and increased thermal stress. The purpose of this dissertation is to determine the fluid balance, cardiovascular function, thermoregulation, and cooling intervention during exercise in the heat. Four novel studies were conducted in this dissertation.

The first and second studies investigated the fluid balance during exercise in the heat across different sports with their special concerns. The first study conducted in female soccer, suggesting most players were in a hypohydration state after practice, and various fluid needs were exhibited by different positions, possibly associated with on-field physical exertion characteristics. The second study was a longitudinal study examining the physiological and hematological responses of football players with sickle cell trait (SCT). SCT exhibited ~37% of hemoglobin S and had a greater serum uric acid concentration and red blood cell distribution width. Furthermore, SCT had 14% less distance ran on the field across the same intensity compared to position-matched controls. These two hydration studies provided the applicable information for teams to promote hydration guidelines and better monitor biomarkers in SCT during exercise in the heat.

The third and fourth studies examined the effects of cooling interventions on lowering body temperature and thermal stress. The third study was a pilot study to examine the effects of leg cooling on soccer-simulated intermittent exercise performance. The results suggested leg cooling lowered thigh skin temperature by 4.3°C and was effective to decrease auditory canal temperature, core temperature, and thermal sensation. Tissue saturation index was not changed, suggesting muscle blood flow was not affected in this cooling treatment. The last study

determined the effects of t-shirt fabric materials on upper-body heat dissipation during exercise in the heat with or without simulated wind. The results suggested the novel shirt with the cooling fan exhibited a superior upper-body heat dissipation during exercise in the heat, mainly decrease averaged skin temperature, ratings of perceived exertion, and promote subjective overall feeling. Given these two cooling studies above, we provided the noticeable applications in external cooling method and clothing material factor to decrease body thermal stress.

CHAPTER 1. INTRODUCTION

Exercise in hot and humid environment conditions brings extra stress in human body inducing hyperthermia and involuntary dehydration, subsequently impairing cardiovascular function and exercise performance reduction.[1,2] Exercise performance impaired by hyperthermia has been well documented in previous sports studies, such as American football,[3] soccer,[4] and marathon runners.[5] Ely et al. (2010) found the running performance decreased by 2~3% in elite marathon runners when the ambient temperature increased from 8°C to 22°C.[5] Moreover, involuntary dehydration is another critical consideration and commonly occurs when exercises in the heat due to the loss of body fluid and electrolyte through sweat. Hypohydration as little as 2% is commonly found in outdoor sports,[6] and exercise performance starts to decline as fluid balance deficit over 2%.[7] In addition, studies showed that 2% hypohydration resulted in a resistance performance reduction by 3.9%,[8] and intermittent running performance decreased by 4%.[9]

Exercise performance can be further exacerbated when hyperthermia combines with hypohydration compared any of these conditions alone. Hyperthermia is defined as skin temperature over 37°C and core temperature over 40°C. Hyperthermia can significantly alter cardiovascular function by increasing heart rates (HR), decreasing stroke volume (SV), and even cardiac output.[2] Nybo et al. (1985) found the VO_2 consumption decreased by 5.7% in 4% of hypohydration status, and by 16.5% in combination of both hypohydration and hyperthermia (38.5°C).[10] In addition, previous study showed high skin temperature caused a decline in VO_{2max} because the amount of blood flow perfused to the peripheral leading to a reduced cardiac filling and cardiac output.[11-13] High core temperature also reduces cardiac

filling by tachycardia as increasing sympathetic activity.[14,15]

One of the strategies to prevent exercise performance reduction in the heat is rehydration with recommended amount fluid consumption to compensate fluid loss. American College of Sports and Medicine (ACSM) guidelines recommends fluid replacement regimen during exercise is 3-8 ounces (85-227 g) every 15-20 minutes in over 60 min exercise.[16] Previous studies also showed the beneficial ingestion of carbohydrate-electrolyte solutions (CES) and sodium beverages to promote fluid retention and electrolyte replenishment.[17-19] However, the inter-individual variability of fluid loss and sweat sodium loss need to be considered in the recommendations due to the differences of sex, drinking behaviors, fitness level, and exertion level.[20] Besides rehydration, various cooling interventions have been developed to prevent hyperthermia by convection and conduction techniques, such as misting fans, cold-water immersion, and circulation suit. Previous studies examined the efficacy of the cooling interventions on decreasing body temperature and increasing exercise performance by applying cold water immersion,[21] cooling vest,[22] and neck cooling.[23] They suggested the cooling interventions can be an efficient strategy to prevent hyperthermia and maintain exercise performance.

The purpose of this dissertation was to investigate the effects of hot and humid environmental conditions on exercise, preventing heat-related illness and exercise performance decrement in the heat. Chapter 2 is an extensive literature review related to the background knowledge of human thermoregulation, fluid balance, hyperthermia, cardiovascular function, and cooling interventions. Chapters (3-6) are the novel studies to examine the following questions: 1) fluid balance assessments in field-based study design, 2) physiological and

hematological responses in football players with sickle cell trait, and 3) the effects of different cooling interventions on human thermoregulations. Finally, Chapter 7 is a conclusion to discuss the main findings in this dissertation and the future research directions.

The first study (Chapter 3) examined the fluid balance and positional differences in collegiate female soccer players. Fluid replacement guidelines are developed for athletes but are neither sport-specific nor gender-specific. The lack of fluid balance research in female athletes cause inappropriate hydration strategies, thus enhancing the risk of dehydration. To understand fluid balance and develop rehydration guidelines for female soccer players, a field-based observational study was conducted to determine hydration status, fluid intake, and sweat electrolyte losses. In addition, this study examined the differential effects on the aforementioned outcomes across on-field positions due to different exertion characteristics by positions.

The second study (Chapter 4) investigated the physiological and hematological responses in America football players with sickle cell trait. Sickle cell trait is characterized by the presence of hemoglobin S, which has less oxygen affinity compared to the normal hemoglobin. Reduced oxygen affinity impairs oxygen availability to the muscle and organs causing tissue hypoxia. Even though sickle cell trait is a benign condition, it is associated with greater risk of exertional death in sport events. Recently, the main strategy to prevent exertional sickling illness is to educate players to avoid dehydration and heat stress due to lack of research. Therefore, this study provided important findings and precautions for athletic trainers and coach staff to avoid sickling injuries.

The third study (Chapter 5) investigated the different cooling interventions on a

soccer-simulated exercise performance in the heat. External cooling application is an efficiency strategy to cool down the body and maintain exercise in the heat. Various cooling techniques were developed and have been well-researched, suggesting the efficacy of cooling down the body temperature and improving the exercise performance. However, not all cooling interventions can be applied in real-world sport settings due to various sporting rules and equipment accessibility. Therefore, we conducted a leg cooling intervention during half-time in a soccer-simulated exercise and this method was likely to be applicable in soccer. We hypothesized that leg cooling would decrease the perceptual thermal sensation and body temperature.

The last study (Chapter 6) examined the upper body heat dissipation in participants wear a novel synthetic shirt fabric while exercising in the heat with two types of simulated wind, electric cooling fan and ventilation vest. During exercise in the heat, clothing generally creates a microclimate between the skin and the shirt, producing more uncompensable heat. Clothing with greater evaporative rate is crucial to lower human thermoregulatory stress. Shirts made of new synthetic fabric have been proposed to promote the evaporative capacity required for heat dissipation. We hypothesized that the novel shirt has optimal evaporative and ventilation property compared to the standard shirt fabric material during exercise in the heat with the application of simulated wind.

1.1 References

1. Lim CL, Byrne C, Lee JK. Human thermoregulation and measurement of body temperature in exercise and clinical settings. *Ann Acad Med Singapore.* 2008;37(4):347-353.

2. Cheuvront SN, Kenefick RW, Montain SJ, Sawka MN. Mechanisms of aerobic performance impairment with heat stress and dehydration. *J Appl Physiol (1985).*

2010;109(6):1989-1995.

3. Armstrong LE, Johnson EC, Casa DJ, et al. The American football uniform: uncompensable heat stress and hyperthermic exhaustion. *J Athl Train.* 2010;45(2):117-127.

4. Veneroso CE, Ramos GP, Mendes TT, Silami-Garcia E. Physical performance and environmental conditions: 2014 World Soccer Cup and 2016 Summer Olympics in Brazil. *Temperature (Austin).* 2015;2(4):439-440.

5. Ely BR, Cheuvront SN, Kenefick RW, Sawka MN. Aerobic performance is degraded, despite modest hyperthermia, in hot environments. *Med Sci Sports Exerc.* 2010;42(1):135-141.

6. Goulet ED. Dehydration and endurance performance in competitive athletes. *Nutr Rev.* 2012;70 Suppl 2:S132-136.

7. Cheuvront SN, Carter R, 3rd, Sawka MN. Fluid balance and endurance exercise performance. *Current sports medicine reports.* 2003;2(4):202-208.

8. Judelson DA, Maresh CM, Farrell MJ, et al. Effect of hydration state on strength, power, and resistance exercise performance. *Med Sci Sports Exerc.* 2007;39(10):1817-1824.

9. Maxwell NS, Gardner F, Nimmo MA. Intermittent running: muscle metabolism in the heat and effect of hypohydration. *Med Sci Sports Exerc.* 1999;31(5):675-683.

10. Nybo L, Jensen T, Nielsen B, Gonzalez-Alonso J. Effects of marked hyperthermia with and without dehydration on VO(2) kinetics during intense exercise. *J Appl Physiol (1985).* 2001;90(3):1057-1064.

11. Gonzalez-Alonso J, Calbet JA. Reductions in systemic and skeletal muscle blood flow and oxygen delivery limit maximal aerobic capacity in humans. *Circulation.* 2003;107(6):824-830.

12. Arngrimsson SA, Stewart DJ, Borrani F, Skinner KA, Cureton KJ. Relation of heart rate to percent VO2 peak during submaximal exercise in the heat. *J Appl Physiol (1985).* 2003;94(3):1162-1168.

13. Wingo JE, Cureton KJ. Maximal oxygen uptake after attenuation of cardiovascular drift during heat stress. *Aviat Space Environ Med.* 2006;77(7):687-694.

14. Fritzsche RG, Switzer TW, Hodgkinson BJ, Coyle EF. Stroke volume decline during prolonged exercise is influenced by the increase in heart rate. *J Appl Physiol (1985).* 1999;86(3):799-805.

15. Coyle EF, Gonzalez-Alonso J. Cardiovascular drift during prolonged exercise: new perspectives. *Exerc Sport Sci Rev.* 2001;29(2):88-92.

16. Thompson PD, Arena R, Riebe D, Pescatello LS, American College of Sports M. ACSM's new preparticipation health screening recommendations from ACSM's guidelines for exercise testing and prescription, ninth edition. *Curr Sports Med Rep.* 2013;12(4):215-217.

17. Hubbard RW, Sandick BL, Matthew WT, et al. Voluntary dehydration and alliesthesia for water. *J Appl Physiol Respir Environ Exerc Physiol.* 1984;57(3):868-873.

18. Passe DH, Stofan JR, Rowe CL, Horswill CA, Murray R. Exercise condition affects hedonic responses to sodium in a sport drink. *Appetite.* 2009;52(3):561-567.

19. Kingsley M, Penas-Ruiz C, Terry C, Russell M. Effects of carbohydrate-hydration strategies on glucose metabolism, sprint performance and hydration during a soccer match simulation in recreational players. *J Sci Med Sport.* 2014;17(2):239-243.

20. Maughan RJ, Merson SJ, Broad NP, Shirreffs SM. Fluid and electrolyte intake and loss in elite soccer players during training. *Int J Sport Nutr Exerc Metab.* 2004;14(3):333-346.

21. Hasegawa H, Takatori T, Komura T, Yamasaki M. Combined effects of pre-cooling and water ingestion on thermoregulation and physical capacity during exercise in a hot environment. *J Sports Sci.* 2006;24(1):3-9.

22. Chaen Y, Onitsuka S, Hasegawa H. Wearing a Cooling Vest During Half-Time Improves Intermittent Exercise in the Heat. *Front Physiol.* 2019;10:711.

23. Tyler CJ, Sunderland C. Cooling the neck region during exercise in the heat. *J Athl Train.* 2011;46(1):61-68.

CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

Exercise in the heat brings the extra challenge to the human body by elevating body core temperature from metabolic heat production and environmental heat gain. In addition, high body core temperature poses a higher demand of heat dissipation for sweat evaporation.[1] Dehydration rapidly occurs when inadequate fluid consumption is present with excessive sweat evaporation during exercise. Generally, dehydration over 2% of body weight loss has been well recognized in research suggesting the impact of decreasing plasma volume and impaired exercise performance.[2] Consequently, body water loss resulting from dehydration significantly impairs sweat capacity inducing hyperthermia.

Exercise-induced hyperthermia is one of the critical factors leading to exercise cessation due to altered cardiovascular function and increased thermal strain. Previous research showed that an increase in body core temperature of 1°C has been suggested to increase heart rate by 9 bpm and decrease stroke volume by 11 ml.[3] These alterations of cardiovascular function significantly limit exercise duration and performance in the heat. A retrospective study reported that 137,580 runners participated in endurance races from March 2007 to November 2013, and 21 experienced serious heat stroke, including 2 fatal and 12 life threatening in Marathon races (10-21 km).[4] Therefore, if external cooling strategies are not applied or exercise unterminated, heat-related illness such as heat exhaustion, heat cramps, and even heat stroke commonly occurs.

Recently, various cooling interventions have been developed to reduce exercise-induced hyperthermia and to protect heat-related illness including cold water immersion,

cooling vest, neck cooling, and cold beverage consumption. In recent years, the cooling interventions and procedures have been popularly investigated. However, the effects of the cooling interventions on exercise performance still maintain contradicting results, possibly due to various procedures and different exercise modalities. Therefore, the purpose of this review is to provide basic knowledge of how the human body regulates exercise in the heat (thermoregulation) and what common concerns need to be considered, such as fluid balance, hyperthermia, and cardiovascular strain during heat exposure. Moreover, this review will demonstrate the various cooling interventions application before-, during-, and post-exercise on performance.

2.2 Human Thermoregulation

Humans are homeotherms, which means humans can maintain constant body temperature despite large variable environmental conditions. In other words, humans can adjust body temperature through oxygen transport, cellular metabolism, muscle contraction, and sweat evaporation relatively independent of environment. The hypothalamus functions as the thermostat of the body, and thermoreceptors in the hypothalamus regulate body temperature within the normal range between 36.5~37.5°C.[5] When exposed to a hot environment, the thermoreceptors send the nerve impulse to the spinal cord and hypothalamus. The anterior hypothalamus then stimulates sweat gland to produce sweat depending on metabolic heat production and ambient temperature for heat dissipation. In addition, the vasodilation of skin blood vessels is activated allowing increased heat loss out of the body.[1] The changes in body temperature regulated by hypothalamus represent the heat storage capacity of a human, and a greater heat storage capacity can represent a longer tolerance duration in the hot environment.

Heat storage can be estimated by heat gain and heat loss over a period of time. Both heat gain and loss can be generated from external or internal factors such as environmental temperature, metabolic heat production, sweat evaporation, and external cooling application.

2.2.1 Body Temperatures

In humans, skin and core temperature represent the body temperature. Skin temperature is affected by skin blood flow and ambient temperature, whereas core temperature is regulated by hypothalamus in the brain. Skin temperature refers to the temperature of the skin and subcutaneous tissue. Core temperature is the measured in the internal abdominal, thoracic, and/or cranial cavities. Generally, the core temperature can be measured by non-invasive techniques through oral, the axilla, the tympanic membrane, rectal and ingestible sensors[5,6]. During the exercise, body temperature rises because metabolic heat production dramatically accumulates resulting in heat gain. Lim et al. (2008) determined the effects of core temperature responses after performing a 45 min self-paced outdoor run under an ambient temperature of 30°C and relative humidity (RH) of 65%. Core temperature was assessed by using the ingestible sensor (VitalSense, Mini Mitter, Bend, OR) 8 hours before exercise. The results showed that exercise cessation occurred when the core temperature reach to 39.3~40.3°C (Figure 2.1).[5] Generally, the metabolic heat production remains in a steady-state level at the first 10-min of exercise.[7] However, as exercise continued, the evaporation requirement exceeds the maximal evaporation capacity leading to a gradually elevating core temperature.[8]

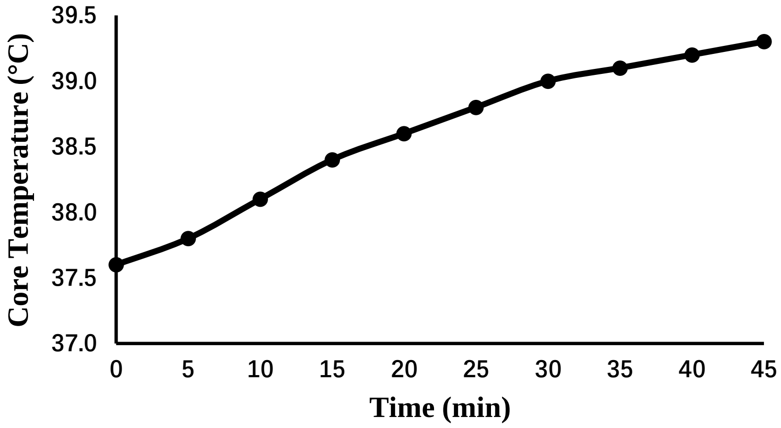


Figure 2.1. Core temperature during a 45-min self-paced outdoor run under ambient temperature of 30°C and 65% RH; Adapted from Lim et al. (2008)[5]

Hot environments consist of hot dry and hot humid conditions, which may have different effects on responses of skin and core temperature during exercise. Hot dry environment is RH less than 30% and hot humid is RH greater than 60%. Previous research showed exercise performance significantly decreased as RH elevated (60%~80% vs. 24%) under the same ambient temperature at ~30°C.[9] Gupta et al. (1984) compared the effects of hot dry and hot humid environments on physiological responses during a continuous work in heat acclimation individuals. The heat stress was monitored by web-bulb global temperature (WBGT), and exercise was performed on a braked bicycle ergometer at three workloads (65, 83, and 98 watts) under three environmental conditions: comfortable (CON; WBGT=23.5°C; RH=60%), hot dry (HD; WBGT=34.7°C; RH=30%), and hot humid (HH; WBGT=34.8°C; RH=60%). The results showed work duration was significantly reduced in HD and HH compared to CON, and also a significant reduction was found in HH compared to HD across three workloads (Table 2.1). In addition, skin temperature in HD condition showed a slight decrease to a steady level, whereas skin temperature in HH showed a linear rising across all

workloads. Core temperature was higher in HH and HD compared to CON, but core temperature reached to critical value (~38.5°C) 5-10 min earlier in HH compared to HD across three workloads.[10]

Table 2.1. Duration (min) in continuous work at different intensity in HH and HD conditions.

Conditions	65 Watts	82 Watts	98 Watts
CON	More than 90*	More than 90*	More than 90*
HD	85#	75#	41#
HH	45	36	23

*Significant different in CON compared to HH and HD; #Significant different in HD compared to HH; Adapted from Gupta et al. (1984)[10]

Another study conducted by Hayes et al. (2014) examining the effects of HH and HD on intermittent exercise performance.[11] Eleven males were randomly assigned to HH (33.7±0.5°C; 78.2±2.3%) and HD (40.2±0.2°C; 33.1±4.9%) with matched heat stress. Intermittent exercise consisted of a warm-up phase followed by 20×30s cycling bouts with 30s resting intervals. The results showed skin temperature was significantly higher in HD (37.2±0.8°C) compared to HH (36.2±0.7°C). However, the core temperature was not different between the two conditions. In addition, HH decreased work done by 10% and HD had an 8% reduction compared to temperate condition, but no differences was found between the two heat trials.[11] In this study, Hayes et al. (2014) found the responses of skin temperature and core temperature exhibited differently in HD and HH environmental conditions.[11] The possible explanation of this results could be the variability of fitness level or acclimation level between participants.[12] In addition, body heat stress might not continuously accumulate in the

intermittent exercise compared to continuous exercise. Thus, Hayes et al failed to observe core temperature differences between the two conditions (HH vs. HD). Thus, by comparing these two environmental conditions (HH vs. HD), high relative humidity increases sweat drippage or trapping on the skin and clothes instead of evaporation resulted in loss the function of cooling.[11,13] Therefore, HH condition may have a greater contribution to the hyperthermia and thermal strain by impairing sweat evaporation capacity.

Body temperature and heat dissipation demand are closely related to the duration and intensity of exercise and exacerbated by high ambient temperature and humidity. Heat dissipation can be achieved by increasing the skin blood flow, which decreases core temperature to maintain thermal balance. However, heat dissipation is less effective in hot humid compared to hot dry environmental condition, subsequently resulted in high body temperature and impairs exercise performance.[14] Core temperature is one of the limiting factors to impact exercise capacity. Monitoring core temperature while exercising in the heat is a critical assessment to prevent exercise reduction and heat injuries (>41°C). In addition, the assessments of body temperature and heat dissipation have the practical meaningful application to reflect heat balance in sports.

2.2.2 Heat Storage

Thermal physiologists had long been debated the accurate determination of human body heat storage representing the balance of heat gain and heat losses.[15] Three fundamental measurements have been developed: two rely on thermometry, and one is calorimetry. The first thermometry method is estimated by the product of the changes in body core temperature, the lean body mass, and the average specific heat of body tissue (3.47 kJ/kg/°C; Equation 2.1).[16,17]

Where ΔT_c is the total changes in core temperature (°C), and LBM represents the lean body mass in kg.

$$\textbf{Equation 2.1} \qquad S_{Tc} = \Delta T_c * LBM * 3.47 \text{ (kJ)}$$

The second thermometry method is estimated by the product of the changes in mean body temperature (T_b; Equation 2.2),[18] body mass, and the average specific heat of body tissue of 3.47 kJ/kg/°C (Equation 2.3).[19] Where T_b is mean body temperature estimated by mean skin temperatures (T_{sk}) and core temperature, ΔT_b is the changes of mean body temperature, and BM is the body mass in kg. On the other hand, the calorimetry method estimates heat storage with respect to time instead of changes in body temperature to reflect the balance of heat gain and heat loss.[20] In the equation 2.4, S refers to body heat storage, M is the metabolic rate, W is the external work rate, L is the respiratory heat exchange by convection, E is the heat loss by evaporation, K is the heat exchange by conduction, and R+C is the dry heat exchange by radiation and convention (all units represented as W/m^2).

$$\textbf{Equation 2.2} \qquad T_b = 0.8\ T_c + (1\text{-}0.8)\ T_{sk}$$

$$\textbf{Equation 2.3} \qquad S_{Tb} = \Delta T_b * BM * 3.47 \text{ (kJ)}$$

$$\textbf{Equation 2.4} \qquad S = M - W - E \pm L \pm K \pm (R + C)\ (W/m^2)$$

Vallerand et al. (1992) conducted a study to compare the three heat storage methods with passive heat exposure in 13 healthy participants. The participants were asked to rest in the climatic chamber with 30°C, 38% RH, and the wind speed at 0.4m/s for 90-min. After 90-min, the climatic chamber was increased to 50°C, 17% RH with the wind speed at 0.8-1.0m/s for another 90-min. Metabolic rates were measured at the last 5-10 min of each 90-min period by using O_2 and CO_2 analyzers. The skin temperature was assessed from ten sites of the body, and

the core temperature was measured by both rectal and auditory canal. The results showed in thermometry equations (Equation 2.2 and 2.3), when the core temperature was assessed by the auditory canal, the heat storage was not different compared to calorimetry. However, when using rectal measuring core temperature to determine body temperature changes, the heat storage was underestimated by up to 49% in thermometry equation compared to calorimetry. In addition, within S_{Tc} alone of thermometry (Equation 2.1), the differences between rectal and canal temperature to estimate heat storage was up to 67% differences (Table 2.2).[21]

Table 2.2. Thermometry heat storage estimated by the product change in core temperature and lean body mass.

	N	S_{Trec} (KJ)		S_{Tac} (KJ)	
		Mean	**SEM**	**Mean**	**SEM**
Men	6	114.6	16.2*	196.7	32.3*
Women	7	116.6	19.9*	190.3	24.2*

*Significant difference between heat storage estimated from S_{Trec} and S_{Tac}. S_{Trec} and S_{Tac} refer to heat storage calculated using core temperature of rectal and auditory canal, respectively; Adapted from Vallerand et al. (1992)[21]

Vallerand et al. (1992) pointed out that the heat storage estimated by S_{Tb} (Equation 2.3) resulted in a large variability in both men and women. This large variability could be due to the constant coefficient (0.8) used in equation 2.2 to calculate mean body temperature. Previous research showed that this constant coefficient varied from 0.65 to 0.9 depending on different environmental conditions, between participants (lean or obese) and laboratories.[22-24] In addition, the specific heat of body tissues might be varied between participants. The researchers have commonly used 3.47, but two different studies showed a different range of 2.93-3.77 kJ/kg/°C[25] and 3.01-3.47 kJ/kg/°C;[26] respectively. In partitional calorimetry, Vallerand et al suggested that

W and K were almost negligible because participants were in resting and contacted only with a suspended nylon-webbed cot. L (respiratory) and E (evaporation) can be accurately measured through the continuous changes in body mass, and R + C was determined by skin temperature and ambient temperature. The huge differences in heat storage estimations among those three equations might be due to the site chosen to represent core temperature (rectal vs. auditory canal). The rectal temperature may not respond quickly as the temperature rises up compared to the auditory canal during resting in the heat. Vallerand et al. (1992) showed heat storage underestimated by using rectal core temperature in both equations of thermometry compared to auditory canal.[21] Therefore, faster response in changing index of core temperature can better reflect heat storage in the passive heat exposure when consider using thermometry methods. Partitional calorimetry can be applied in not only during exercise in a compensable environment, but also cooling intervention on heat balance.[27]

2.3 Fluid Balance

In an 18-25°C environment, a healthy sedentary individual will have moderate fluid losses between 1.8-3.0 L/day.[28] During exercise in the hot and humid conditions, the demand of sweat evaporation is elevated leading to a sweat rate as greater as 3.0 L/hr.[29] Consequently, excessive sweat secretion results in large body fluid losses. If fluids are consumed inadequately, dehydration would rapidly occur during exercise in the heat. Thus, fluid intake is important to counterbalance the fluid losses to prevent dehydration. It is well recognized that dehydration as great as 2% body mass loss commonly occur in outdoor sports, such as American football, track, and soccer.[30] In addition, research showed that dehydration greater than 2% resulted in an at least 22% decrease in endurance performance.[31] Webster et al. (1990) found 5%

dehydration status caused a significant decrease in maximal cycling anaerobic power by 21.5% and anaerobic capacity by 9.8% under hot and humid conditions.[32] Previous research studies have been investigated in this popular topic area, and also have been drawn attention by athletes, coaches, and athletic training staff. Therefore, the background of fluid balance will be reviewed in this section, such as hydration assessments, sweat response, and beverage selection.

2.3.1 Hydration Assessment

The commonly used hydration assessments include body weight changes, urine indices, and blood electrolytes and osmolality. Body weight changes before and after exercise is a valid and simple method, and has been widely used to assess body fluid loss in research and sport events.[33] In addition, hydration status is assessed by urine indices including urine specific gravity (USG), urine color, and urine osmolality. Although urine indices cannot directly demonstrate dehydration state, it can be a good indicator of kidney function. Kidneys are the organs to regulate body water loss through urine output. In a dehydrated condition, water is reabsorbed by the body without electrolyte reabsorption leading to less urine output and higher urine concentration.[34] Subsequently, the values of urine osmolality and USG increase and urine color gets dark. Urine osmolality is the concentration of osmotic solutes in the urine and ranges from 50 to 1400 mOsm/kg. USG corresponds to the urine density indicating the weight of urine compared with an equal volume of distilled water, typically range from 1.007-1.030.[35] Higher values of urine osmolality and USG suggest hypertonic urine. The urine color is an another assessment by using a urine color chart contained a standardized color scale ranging from 1 (pale yellow) to 8 (dark brown).[36] Previous research showed three urinary markers were strongly and consistently correlated ($r = 0.73$-0.80) with each other (Table 2.3).[37]

Table 2.3. The changes of body weight and urine indices before and after 90-min physical activity.

Measures	**Before**	**After**	**P value**
Body weight (kg)	72.87 ± 14.54	72.07 ± 14.35	0.001
Urine Specific Gravity	1.014 ± 0.008	1.018 ± 0.007	0.001
Urine osmolality (mOsmol/kg)	599 (156-1,171)	642 (133-1,009)	0.05
Color (shade)	2.3 ± 0.9	3.1 ± 1.0	0.001

An Aggregate Urine Analysis Tool to Detect Acute Dehydration. The measurements for dehydration before and after 90min physical exercise; Adapted from Hahn et al, (2013)[37]

However, the disadvantage of using urine to assess hydration status is the large inter-individual variability. USG and urine color are also easily influenced by the number and size of the particles in the urine. In addition, urine specific gravity will be falsely elevated by glucose, proteins, and urea presenting in the urine.[38] Thus, dietary intake of the electrolytes and vitamin (B_2 and B_{12}) may affect the day-to-day values of those urinary markers (USG and urine color).[35,39] This disadvantage was further confirmed by Cutrufello et al. (2018) examined hydration assessments by USG among marathoners. Thirty-five marathon runners competed for an average time of 3.8 hr, and total body weight loss was approximate 1.6 kg. The USG significantly increased from 1.007 to 1.018. However, no correlations between the changes in USG and body mass loss was found.[40]

Besides urinary indices to reflect hydration status, blood markers can further define intracellular and extracellular hydration status. Blood osmolality and sodium concentration can represent intracellular hydration status, whereas total blood plasma volume indicates extracellular hydration status.[41] The plasma osmolality has a small deviation with a cut-off value for dehydration of 290 mOsm/kg. Previous research showed urinary indices failed to

correlate with blood markers to reflect the hydration status.[42-44] Hew-Butler et al. (2017) compared blood and urine for hydration status in 318 collegiate Division I athletes. Spot serum and urine were collected for osmolality and electrolytes. The data were categorized into dehydrated and hydrated based on the dehydration status defined as urine osmolality over 700 mOsmol/kg and USG over 1.020. According to the urine-based dehydration criteria, the study identified 55% of participants were dehydrated at the time of testing; however, no clinical meaningful differences were found in any blood markers (Table 2.4).[45]

Table 2.4. The difference in blood markers between urine-based hydration status.

Variable	Dehydrated	Hydrated
Serum [Na^+] (mmol/L)	139.9 ± 2.2	139.9 ± 2.1
Serum [K^+] (mmol/L)	4.4 ± 0.4	4.4 ± 0.3
Serum Osmolality (mOsmol/kg)	280.6 ± 8.3	280.6 ± 8.8
Urine Osmolality (mOsmol/kg)	893.4 ± 189.7	605.8 ± 299.0*
USG	1.022 ± 0.003	1.011 ± 0.004*
Thirst Rating (0-10)	4.3 ±1.9	4.4 ± 1.8

*Significant difference between two groups; Adapted from Hew-Butler et al. (2017)[45]

Previous research have been reported that a strong correlation was found within urine indices (USG and urine osmolality),[37] but not between urinary indices and blood markers.[42-45] The possible explanation could be the diet, the timing of fluid intake, and the renal responses to exercise, contributing to the different utility of urine indices as markers of water and sodium homeostasis.[39] Therefore, urine indices are the good indicators of kidney function, but may not be an ideal marker for determining hydration status. Researchers should be careful to conclude hydration status according to urinary indices. On the other hand, the acute body weight changes

can be equated with losses of body fluid with minimal error and can be as the best practical of monitoring hydration status before and after exercise in sports events.[39]

2.3.2 Sweat Response

During exercise, body heat dissipation and fluid losses are primary through sweat evaporation. Collecting and analyzing sweat during exercise not only provides information about how much fluid is lost, but also sweat electrolyte losses in a given specific time period. Sweat secreted onto the skin surface mainly contains water and electrolytes (sodium, potassium, and chloride), and is derived from the extra-cellular fluid. Excessive sweating during physical activity causes large fluid and electrolyte loss leading to the exercise performance reduction. The primary electrolyte in sweat is sodium, which is contained in large quantities and affects body fluid balance and plasma volume.[46] Research studies investigated sweat rates and sweat sodium concentrations across different sporting events. Results showed the sweat rate can vary in a range of 0.5-3.0 L/hr during training sessions under both warm and cool climates including soccer,[47] volleyball,[48] basketball,[49] and American football.[50] In addition, the average sweat sodium concentrations ([Na^+]) were from 40-60 mmol/L, whereas the range can be from 10-90 mmol/L across those sports events during an approximate 90-min practice.[29]

Baker et al. (2016) determined the normative data for regional sweat [Na^+] and whole body sweat rate in more than 500 athletes across various team sports (American football, baseball, basketball, soccer, tennis, and endurance including cycling, running, and triathlon) from cool to hot environmental conditions (15–50°C). The regional absorbent patch technique was used to collect sweat on the mid-forearm, and whole body sweat rate was estimated from pre- to post-exercise changes in body mass, corrected for fluid intake and total urine output.

The author suggested the season of the year, the age, and whole body sweat rate were the important factors to influence the variability of sweat [Na^+] across players.[51] Furthermore, between sports, absolute whole body sweat rate was significantly higher in American football players compared to other sports possibly due to large body mass, high exercise intensity, and high ambient temperature (Table 2.5), and this result was consistently with the previous research.[2]

Table 2.5. Age-adjusted mean (95% CI) of absolute and relative whole body sweat rate by different team sports.

	Absolute whole body sweat rate (L/hr)	**Relative whole body sweat rate (ml/kg/hr)**
American football	1.3 (1.2-1.5)*	14.3 (13.3-15.5)#
Baseball	1.0 (0.9-1.1)	11.3 (10.2-12.5)
Basketball	0.8 (0.7-1.0)	10.2 (0.1-11.4)
Soccer	0.9 (0.8-0.9)	14.3 (13.2-15.5)

*Significant difference in American football compared to other sports; #Significant difference in American football compared to baseball and basketball; The sample size was n=110 for American football, n=50 for baseball, n=39 for basketball, and n=99 for soccer; Adapted from Baker et al. (2016)[51]

The intra/intervariability of sweat rate and sweat [Na^+] has also been reported in prior studies, and mainly due to either sweat collection methodologies or individual factors.[52,53] For sweat collection methodology, the attached time duration and different regions of the body are the two primary factors to influence sweat rate and sweat [Na^+]. In the field hydration research, the absorbent patches were widely used across sports, but the adherence time varied between 15-30 min[54,55] and even up to 90-min.[29,47,56] However, one of the considerations is the short adherence time might not represent the sweat response during the entire practice duration.[57]

Although few studies have examined the effects of patch adherence time on sweat rate and sweat [Na^+], Dziedzic et al. (2014) found no significant difference in sweat [Na^+] on the forearm with 30-min patches attached compared to 70-min.[52] Weschler et al. (2008) suggested that a longer time (~90 min) of sweat patches adherence might falsely estimate the sweat results because of oversaturation of the patches. In addition, sweat results also can vary in different regions of the body. The sweat rate tends to be higher in the torso compared to extremities.[57] Patterson et al. (2000) reported that local sweat [Na^+] can be different as much as 80-120% across the body within participants. Besides methodology, the day-to-day variability of dietary sodium intake may also play in a role on sweat [Na^+] within participants.[58] Hargreaves et al. (1989) examined the effects of two weeks of sodium intake (50 vs. 150 mmol/day) on the ability to exercise (60% VO_{2max}) in a hot environment (35°C), and found no significant difference in exercise performance, sweat rate, heart rate, and plasma sodium between two groups.[59] They only found that low sodium intake had less sweat [Na^+] compared to high sodium intake. However, Koenders et al. (2016) investigated a similar study to determine the effects of 9 days reduced dietary sodium intake on the ability to exercise (55% VO_{2max}), and sweat [Na^+] was similar in regular sodium intake compared to reduced sodium intake (54.5±40.0 vs. 54.5±23 mmol/L) without reporting the total sodium intake in 9 days.[60]

Given sweat data (sweat rate and sweat [Na^+]) are critical to monitor during exercise in the heat. Future research is needed to keep investigating sweat collection techniques for attaching duration and regions to provide accurate practical information for fluid balance. Moreover, the factors impacting sweat variability have to be considered, such as exercise intensity, exercise duration, hydration status, heat acclimation, fitness level, and dietary sodium

intake.[53] Sweat data are useful information to estimate players' fluid and electrolyte loss, which can further aid to develop fluid replacement recommendations and sports beverages.

2.3.3 Beverage Selection

Drinking behavior and beverage selection before and during exercise are the main strategies to prevent dehydration, hyponatremia, thermoregulation stress, and cardiovascular stress. Drinking behaviors during exercise can be categorized as planned drinking and drinking to thirst or *ad libitum* ingestion. Planned drinking is the strategy based on the sweat rate and sweat electrolyte concentrations between individuals to customized fluid replacement recommendations. The main advantage of planned drinking is to prevent dehydration and excessive water consumption causing hyponatremia.[2,61] The strategy of drinking to thirst is interchangeable with *ad libitum* ingestion, which is defined as fluid consumed at any time with whatever desired volume according to individual percepts of thirst.[62] The purpose of this strategy is to use the thirst mechanism to guide fluid ingestion. Thirst mechanism is stimulated when plasma osmolality is greater than 290 mOsm/kg resulting in vasopressin secretion. Consequently, water is retained in the body to dilute the hypertonic plasma by increasing water intake (Figure 2.2).[63] Plasma osmolality can be elevated by either body water deficit or hypertonic fluid ingestion. Johannsen et al. (2009) investigated that the effects of chicken noodle soup (167 mmol/L Na^+) ingestion 45-min before exercise on total water intake during exercise. They found that plasma osmolality and total water intake were higher in chicken noodle soup consumption compared to water ingestion prior exercise, possibly due to thirst mechanism stimulation.[64]

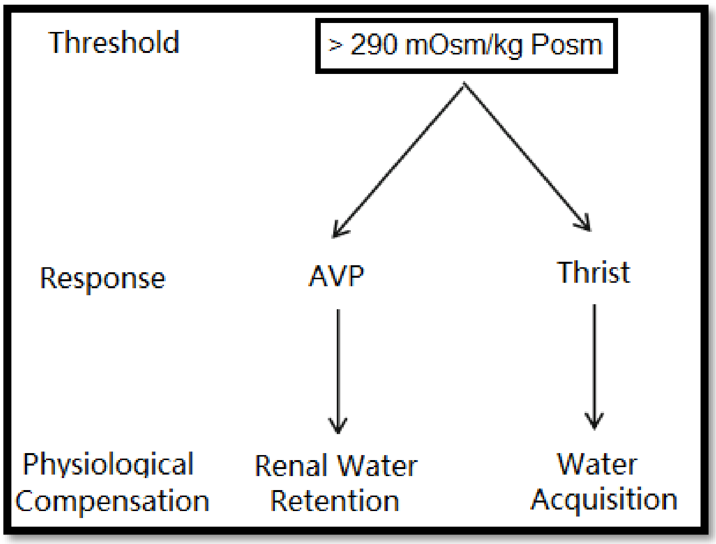


Figure 2.2. Body water regulation and thirst mechanism with the response of body weight deficit and plasma osmolality. Body water deficits over 2% results in an increase in plasma osmolality (Posm). Increasing in Posm over 2% (6 mmol/kg) is an osmotic threshold for renal water conservation and water acquisition (thirst), which regulating by osmoreceptors anti-diuretic hormone arginine vasopressin (AVP).

Beside drinking behavior, various sport beverages are developed based on the compensatory of carbohydrate and sweat sodium loss. Carbohydrate-electrolyte solution (CES) can help to maintain hydration status and exercise performance based on the consequences of fluid retention and carbohydrate replenishment.[65] Murray et al. (1999) compared the different effects between water consumption to those beverages contained 4%, 6%, and 8% carbohydrate (CHO) on fluid retention, and they found 6% CHO beverage had a greater gastric emptying rate compared to water and 8% carbohydrate beverage.[66] In addition, Osterberg et al. (2008) determined the dose effects of CHO (3%, 6%, and 12%) compared to water on fluid retention after 90-min exercise. All CHO beverages contained the same content of electrolyte (18 mmol/L [Na^+], 3 mmol/L [K^+], and 11 mmol/L [Cl^-]), and the results found beverages containing CHO contributed to greater fluid retention at post-exercise compared to water

ingestion only. However, the authors did not detect any dose effects, suggesting the combination of CHO with electrolyte might have the greatest effects on fluid retention after exercise.[67]

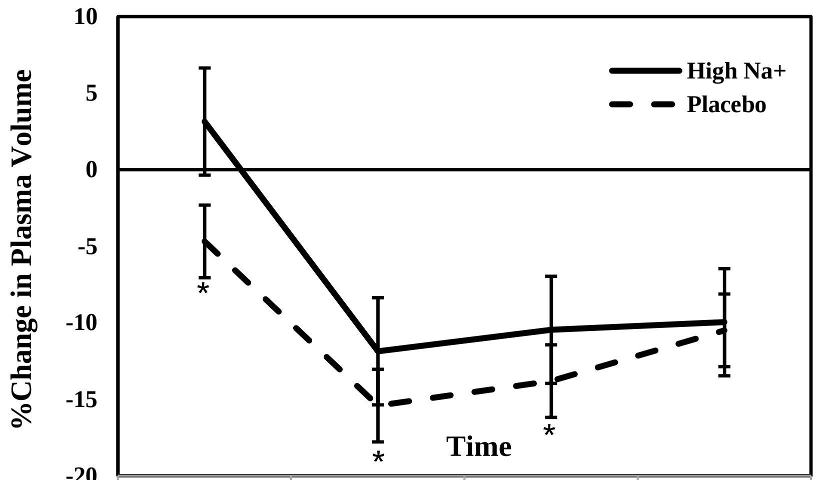
The beverage containing different amounts of sodium has investigated in previous research because of preventing hyponatremia. Anastasiou et al. (2009) examined the effects of high versus low sodium concentration (36.2 vs. 19.9 mmol/L) on serum sodium, plasma osmolality, plasma volume, and muscle cramping. The participants performed 3 hours of exercise consisting of alternative 30-min walking and cycling in the heat (30°C), and the amount of fluid intake was matched with total body mass loss. The data showed a lower serum sodium concentration (134.4 mmol/L) by consuming water only compared to both high sodium (137.3 mmol/L) and lower sodium beverages (136.7 mmol/L), and no difference was found between the two sodium beverages.[68] The perspective of this study was to prevent hyponatremia after prolonged exercise duration. However, those studies haven't investigated the effects of beverage selection on exercise performance. The importance of sodium ingestion to maintain exercise have also been drawn attention to researchers and athletic training. Johannsen et al. (2009) successfully found chicken noodle soup ingestion contained high sodium concentration before exercise improved exercising fluid intake.[64] Sims et al. (2007) examined the effects of high (164 mmol Na^+/L) and low sodium (10 mmol Na^+/L) beverages on the fluid balance of trained men exercising in the heat (32°C, 50% RH). The data showed that high sodium beverage ingestion increased plasma volume by 4.5%, whereas low sodium did not. In addition, higher sodium ingestion had greater time to exhaustion termination compared to low sodium ingestion (57.6±6.0 vs. 46.4±4.0 min; Table 2.6).[69]

Table 2.6. The effects of difference between high sodium and low sodium beverage ingestion.

	Time (min)	Change in PV (%)	Heart Rate (bpm)	Urine Output (ml)
High Na^+	57.9 ± 6.0*	4.5 ± 3.7*	157 ± 11	492 ± 197*
Low Na^+	46.4 ± 4.0	0.0 ± 0.5	161 ± 16	659 ± 168

*Significant different in high sodium compared to low sodium beverage; Time refers to exercise time to exhaustion or participants were terminated when core temperature reached to 39.5°C. Adapted from Sims et al. (2007)[69]

Another study conducted by Coles et al. (2004) to investigate the effects of sodium intake before exercise on hypervolemia, endurance performance and thermoregulation in college males. Fourteen male participants consumed a high sodium beverage (163.7 mmol/L Na^+) compared to no sodium beverage (placebo), and total fluid intake was equivalent to 10 ml/kg. The beverages were ingested in 3 equal portions over 30-min followed by a 15-min equilibration period before exercise. The results showed that high sodium beverage successfully improved plasma volume before exercise (Figure 2.3). Participants performed approximately 0.97 km further during a 15-min time trial cycling without any difference in sweat production, sweat rate, and body weight changes.[70]



	0	15	30	45
High Na+	3.14	-11.87	-10.47	-9.97
Placebo	-4.69	-15.42	-13.82	-10.5

Figure 2.3. A comparison of percent changes in plasma volume over time between high sodium beverage and placebo control. *Significant difference between two groups; Adapted from Coles et al. (2004)[70]

Those studies concluded that high sodium beverage ingestion (~160 mmol/L) before exercise increased plasma volume and exercise performance under warm and hot environmental conditions due to increased fluid retention and stimulated thirst reflex, a large benefit for the people with less aerobic fitness.[64,69-71] During exercise, beverages should be "planned drinking" based on the predicted fluid requirement for different exercise durations intensities, environmental conditions, and body sizes to maintain hydration status. CES will be optimal to compensate for glycogen depletion and electrolyte losses.[61] In addition, post-exercise of CES ingestion is necessary to help gastric emptying and fluid retention without dose effects differences. However, the high concentration of CHO or energy density in a beverage might decrease the rate of gastric emptying and gastrointestinal discomfort after exercise.[66] The appropriate percentage of CHO (3~6%) was recommended that had a greater effect on intracellular fluid retention related to glycogen storage.[67] Maughan and Shirreffs

(2010) also suggested that hypertonic beverages are recommended for pre-exercise, isotonic beverages during exercise, and hypotonic beverages for post-exercise.[72,73] Therefore, the appropriate beverage selection of CES with planned drink behavior would optimal fluid balance and exercise performance.

2.4 Exercise and Heat Stress

During exercise, the rising temperature of the human body is primary generated by metabolic heat production from muscular activity. If the ambient temperature is higher, the extra heat gain would be added from environment. As heat evaporation requirement exceeds the maximal capacity of heat dissipation, a dramatically heat will be accumulated in the human body. Thus, thermal stress and physiological stress will be added from both exercise and environmental heat. Heat stress is when the human body is exposed to hot and humid environmental conditions with intense physical exertion causing high body temperature. Hyperthermia occurs when the body core temperature reaches to 38~39°C, which is a limiting factor of exercise performance. Therefore, exercise in the heat significantly results in high thermal strain and physiological strain. Both aerobic and anaerobic exercise performance are impaired significantly during exercise in hyperthermic condition compared to normothermic conditions.[74,75]

2.4.1 Exercise Performance

Time to exhaustion performance and time completion trial are commonly used to assess aerobic performance under heat stress.[74] Previous research demonstrated that heat exhaustion occurred when core temperature reached to 39.7°C, regardless of ambient temperature.[76] However, higher ambient temperature accelerates the rise in core temperature to

this critical value (39.7°C) resulting in a shortened exercise duration. One of the first studies conducted by Galloway and Maughan (1997) systematically examined the effects of different ambient temperatures on the capacity of prolonged cycling exercise performance. The ambient temperature consisted of 4, 11, 21, and 31°C with the same 70% RH, and exercise performance was determined by the time to exhaustion at a fixed intensity (70% VO_{2max}). The results showed that exercise performance was optimal at 11°C and shorter at 31°C with a 44% difference (93.5±6.2 vs. 51.6±3.7 min; respectively). In addition, exercise performance was not different between 4 and 21°C (81.4±9.6 min, 81.2±5.7 min, respectively), but the exercise duration was significantly longer than 31°C and shorter than 11°C. In addition, the sweat rate, heart rate, and rating perceived exertion were all significantly higher at 31°C compared to the ambient temperature of 4, 11, and 21°C.[77]

Another study conducted by MacDougall et al. (1974) investigated the effects of thermal condition on aerobic performance using a water-perfusion suit. Thermal conditions were classified as hypothermic, normothermic, and hyperthermic conditions. The normothermic condition was perfusing the suit water equal to the ambient temperature of 23°C. Hypothermia was perfusing the suit water at 18±1°C with high-speed fans to widen the internal-to-external temperature gradient. Then, hyperthermia condition was achieved by perfusing hot water adjusted at 2 min intervals to maintain water temperature equal to rectal temperature. The exercise was determined by running to exhaustion time at an intensity of 70% VO_{2max}. The results showed that exercise duration was 42-min (47%) shorter in hyperthermic condition compared to hypothermic condition, and 27-min (36%) shorter compared to normothermic condition. In addition, core temperature increased more rapidly in the

hyperthermic trial compared to both normothermic and hypothermic conditions.[78] Another addictive study conducted by Sawka et al. (1985) to investigate the effects of hyperthermia on VO_{2max} before and after heat acclimation. The VO_{2max} test was conducted in two different environmental conditions; moderate (21°C, 30% RH) and hot (49°C, 20% RH) both before and after heat acclimation. They found that VO_{2max} increased by 4% in both environmental conditions after heat acclimation. However, the authors observed a reduction in VO_{2max} with 8% before and 7% after acclimation in the hot environment compared to the moderate environment.[79] Table 2.7 also shows seven studies that investigated the effects of different ambient temperature on either oxygen uptake at the fixed intensity or VO_{2max} testing. The results showed three of them did not observed oxygen uptake changes,[80-82] and four observed a reduction by approximate 6-20%.[77,79,83,84] In these studies, typically the VO_{2max} testing protocols were lasting 15-20 min, which might or might not induce hyperthermia in that amount of time leading to the different results.[84]

Table 2.7. The effects of ambient temperature on oxygen uptake across the studies.

Studies	Ambient Temp	Duration	Intensity	Difference
William et al. (1962)[80]	21°C; ---- RH 35°C; ---- RH	45 min	225 Watts	-1%
Rowell et al. (1965)[81]	26°C; 37% RH 43°C; 30% RH	NA	VO_{2max} Test	-2%
Rowell et al. (1966)[82]	26°C; 40% RH 43°C; 30% RH	NA	VO_{2max} Test	-1%
Sakate et al. (1978)[83]	20°C; 50% RH 50°C; 50% RH	NA	VO_{2max} Test	-6%*
Sawka et al. (1985)[79]	21°C; 30% RH 49°C; 20% RH	NA	VO_{2max} Test	-8%*
Galloway et al. (1997)[77]	11°C; 70% RH 31°C; 70% RH	45 min	70%VO_{2max}	-12%*
Arngrimsson et al. (2004)[84]	25°C; 50% RH 45°C; 50% RH	20 min	33%VO_{2max}	-20%*

The difference of oxygen uptake reduction refers to comparison of high and low ambient temperature in each study. *Significant difference in hyperthermic condition compared to normothermic condition.

Heat stress-induced hyperthermia also impacts anaerobic performance. Tatterson et al. (2000) investigated the effects of heat stress on cycling performance. They found a 6.5% power output reduction during a maximal work 30-min cycling in hot ambient temperature (32°C) compared to normal temperature (23°C; Figure 2.4).[85] In addition, hyperthermia not only can be generated from high ambient temperature, but also can be generated from dehydration. Nybo et al. (2001) determined that whether marked hyperthermia alone or in combination with dehydration decreases the initial rate of rising in VO_2 consumption at 50% VO_{2max} and VO_{2max} during intense cycling exercise. Six endurance-trained male cyclists were asked to complete four maximal cycle ergometer exercise tests. The testing trials consisted of either euhydrated

or dehydrated (4% body mass) with normal core temperature (37.5±0.2°C) and skin temperature (30.8±0.6°C) compared to thermal strain conditions (core temperature: 38.5±0.2°C and skin temperature: 37.0±0.2°C). The results showed that at the first 60s 50%VO_{2max} intensity, all trials had the similar VO_2 consumption. After 60s, VO_2 was significantly lower in two hyperthermic groups (euhydrated + hyperthermia and dehydrated + hyperthermia) compared to other groups. VO_{2max} equally decreased by 16±1% in the two hyperthermic groups, and performance time duration was shorter by about one-half than that in normal temperature groups.[86]

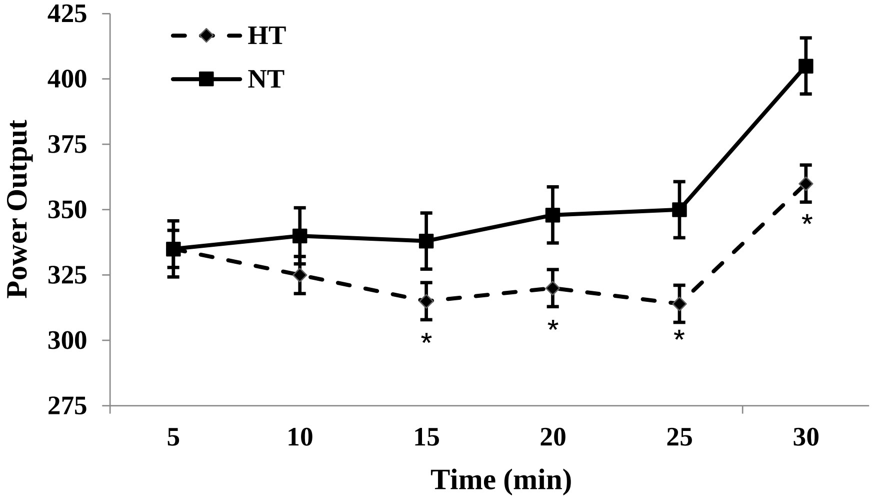


Figure 2.4. Power output during 30 min cycling time trial at 32°C (HT) and 23°C (NT) in elite road cyclists. *Significant difference between two conditions and the power output significantly declined after 15 min of cycling. Adapted from Tatterson et al (2000)[85]

High ambient temperature significantly impacts exercise capacity by shortening the exercise time duration compared to the normothermic condition. In addition, exercise capacity in the heat mainly depends on whether hyperthermia being induced. These studies in this section support that hyperthermia induced by either high ambient temperature or dehydration

can significantly degrade oxygen consumption. In addition, any factor that decreases VO_{2max} can directly impair aerobic performance due to changes of circulation blood flow and cardiovascular function.[74,84] Therefore, hyperthermia is one of the primary limiting factors to affect exercise capacity along with thermoregulation stress impairing cardiovascular function, regardless of ambient temperature, dehydration, and acclimation level of individuals.

2.4.2 Cardiovascular Strain

Maintaining cardiovascular function is crucial during exercise, which regulates both central and local blood flow to meet the energetic demands for muscular activity. Maximal oxygen consumption represents the aerobic exercise capacity. According to the Fick equation, VO_2 is directly influenced by cardiac output (Q) and arterial-venous oxygen concentration difference (a-vO_2 Diff) (Equation 2.5). Cardiac output can be also determined by the product of heart rate (HR) and stroke volume (SV; Equation 2.6).[1]

Equation 2.5 $VO_2 = Q * \text{a-v}O_2\ \text{Diff}$

Equation 2.6 $Q = HR * SV$

From rest to exercise, the Q can increase from 5 L/min to 20 L/min by increasing heart rate and stroke volume. Blood flow increases to deliver adequate oxygen and substrate to the active muscle as well as to the skin for heat dissipation.[87] However, blood flow to working muscle and skin results in a competition for the available Q due to the limited ability to meet the dual demands during intense exercise in the heat.[88] Thus, cardiovascular function is impaired leading to the early fatigue compared to normothermic condition. Loring Rowell and his colleagues were some of the first researchers to investigate cardiovascular function during exercise under heat in trained and untrained individuals. One of their studies was to determine

the VO_{2max} under two different environmental conditions (26°C; 40% RH vs. 43°C; 30% RH) in untrained and unacclimated men. Compared to normothermic conditions, the Q, SV, and central blood volume (CBV) significantly lowered by 6%, 20%, and 11% respectively, whereas heart rate was 18% higher.[82] Another study compared the changes in cardiovascular function before and after 11-12 days of heat acclimation under 48.4°C 15% RH. Heart rate decreased by 19% and SV volume increased by 21% without any changes in Q compared to before heat acclimation.[89] Another unique study conducted by Rowell et al. (1967) determined the central circulatory responses while skin temperature was changed in a square-wave pattern during uninterrupted exercise from low to moderate exercise intensity (26% to 64% VO_{2max}). Eleven untrained and unacclimation participants were separated into two groups (low vs. moderate intensity) to perform a 105-min continuous exercise. The exercise conducted a treadmill walking was divided into three 30-min periods and a fourth final 15-min period. The skin temperature was adjusted by water-perfusion suit starting at 21°C (period 1), to 40°C (period 2), 10°C (period 3), and 50°C (period 4). The data showed that the cardiovascular function was affected by different suit temperatures. As suit temperature went up to 40 and 50°C, the Q and HR increased while SV decreased in both low and high intensity exercise. However, when the suit temperature cool down to 10°C, the Q and HR decrease while SV increases back to the baseline level (21°C).[90] The influence of Q during exercise in the heat varied among studies possibly because of individual fitness level and different heat exposure protocols. These studies investigated by Rowell suggested that heating and cooling skin results in changes in peripheral circulation. Heat stress resulted in a lower CBV further reducing venous return and heart refilling to impair exercise capacity.[81,82,88,91]

Hyperthermia coupled with dehydration poses severe cardiovascular stress by increasing thermoregulatory demand for skin blood flow, decreasing SV, skin and local muscle blood flow during high intensity exercise.[87] Gonzalez-Alonso et al. (1997) quantified the cardiovascular stress produced either by hyperthermia alone, dehydration alone, or the combination of the two in trained endurance athletes. In the hyperthermia trial, the exercise started at core temperature elevated to 39.3°C with a euhydrated status and the dehydration trials consisted of 4% body mass loss by having participants exercise in a cool environment. Exercise was performed at a 72% VO_{2max} in a 35.5°C, 50% RH environment. The results showed that SV decreased by 8% and HR increased by 5% in the hyperthermia trial alone. In addition, the dehydration trial showed SV reduced by 7% and HR increased by 5%. In these two trials, Q was not significant differences compared to the control group. However, when hyperthermia and dehydration were combined, SV showed a 20% reduction and HR increased by 9% with a 13% Q reduction (Table 2.8).[3]

Table 2.8. Cardiovascular responses to moderately intense exercise with hyperthermia alone, dehydration alone, and combined hyperthermia and dehydration.

	Q (L/min)	**HR (bpm)**	**SV (ml/beat)**
Control (Hyperthermia)	21.1±0.8	164±4	130±8
Hyperthermia alone	20.4±0.7	172±4*	119±7*
Hyperthermia/Dehydration	18.4±0.7$^{\#}$	178±4$^{\#}$	104±6$^{\#}$
Control (Dehydration)	21.4±0.9	147±4	146±8
Dehydration alone	20.7±0.9	154±4*	136±7*

Q refers to cardiac output; HR refers to hear rate; and SV refers to stroke volume; $^{\#}$Significant difference in combined (hyperthermia/Dehydration) compared to other group; *Significant difference from corresponding control; Adapted from Gonzalez-Alonso et al. (1997)[3]

Most of the research agree that heat stress can significantly increase HR and decrease SV, whereas Q either decreases or no changes. The possible explanation could be Q highly depends on the magnitude of changing in HR and SV (Equation 2.6). The decrease in Q might be due to the small magnitude increase in HR and large magnitude decrease in SV, and vice-versa. In addition, the changes in Q is associated with exercise intensity, duration, fitness level, and acclimation level. In heat-acclimation and trained individuals might not observe the reduction in Q because the slightly reduction in SV can be compensated by elevated HR.[81,82,90,92] On the other hand, untrained and unacclimated individuals might experience more heat stress as a response of Q reduction. In addition, the reduction in SV and CBV reflects a redistribution of blood from core to the peripheral in the heat impairing venous turn and blood refilling to the heart. Furthermore, cardiovascular stress is even greater when extra stress combined with heat stress, such as prolonged high intensity exercise and dehydration.[3,91]

2.5 Cooling Interventions

As previously mentioned, the prolonged and intense exercise under hot and humid environmental conditions increases the risk of hyperthermia, dehydration, cardiovascular stress, and impairing exercise performance. Hyperthermia plays a role as a mediator between dehydration, cardiovascular stress, and exercise performance. Previous research investigated the interventions of heat stress-induced hyperthermia by lowering skin and core temperature to diminish cardiovascular stress and exercise performance reduction. Various cooling interventions are developed to reduce hyperthermia by decreasing high body temperature targeted by either whole body or specific body area in hot and humid environmental conditions. Typically, the cooling interventions are applied at pre-exercise, during exercise, and post-

exercise, including cold water immersion (CWI), cooling fans and vest, and cold beverage consumption. The purpose of pre-exercise cooling intervention is to lower the starting body core temperature, which increase the margin between initial body core temperature and critical limiting core temperature to prolong exercise duration in hot and humid conditions.[93] The strategy of during exercise cooling mainly focuses on lowering skin temperature and/or core temperature by cooling the specific body area or cold beverage ingestion.[94] However, the post-exercise cooling intervention is widely used to lower the tissue temperature and reduce exercise-induced muscle damage for recovery.[95]

2.5.1 Cold Water Immersion (CWI)

Cold water immersion is one of the most popular cooling methods that is applied to both pre- and post-exercise. Table 2.9 shows five studies that investigated the effects of pre-exercise CWI on exercise performance by determining the time to exhaustion at a fixed intensity[96-98] or the self-paced distance completed in a given amount of time.[99,100] Gonzalez-Alonso et al. (1999) investigated the effects of different initial body temperatures on exercise performance. The water immersion was applied at the temperature of 17, 36, and 40°C for 30-min before exercise to manipulate the participants' initial core temperature to 36, 37, and 38°C. The results found an inverse relationship between initial core temperature (36, 37, and 38°C) and time to exhaustion exercise performance (63±3, 46±3, and 28±2 min, respectively).[96] A similar study conducted by Hasegawa et al. (2004) examined the effects of 30-min of CWI pre-exercise cooling on exercise performance. The exercise performance was determined by the time to exhaustion at 80% VO_{2max} at 32°C, 80% RH. The initial core temperatures before exercise were 37°C and 37.5°C in pre-exercise cooling and control, respectively. Pre-exercise

cooling application successfully prolonged the exercise duration in the heat compared to control (CON: 317±50 vs. Cooling: 152±16 sec).[97] In addition, Siegel et al. (2011) found that CWI pre-exercise decreased initial body temperature and prolonged time to exhaustion exercise trial by 22%.[98] Two additional studies conducted pre-exercise cooling for 10-20-min CWI and exercise was performed in 30-min at self-paced. Kay et al. (1999) found the cycling distance completed increased by 6%,[99] and Duffield et al. (2010) found the cycling distance completed increased by 7% and power output by 11% after applying CWI pre-exercise compared to control.[100] Across these five studies, pre-exercise CWI around 30-min at 22-25°C was shown to successfully decrease initial body temperature resulting in a greater body heat storage capacity and prolonged exercise performance under hot and humid environmental conditions (Table 2.9).[96-100]

Table 2.9. The effects of pre-exercise CWI on exercise performance across studies.

Study	CWI	Initial Core Temp	Performance	Heat Storage
Gonzalez-Alonso et al. (1999)[96]	30 min	36°C 37°C 38°C	63±3 min 46±3 min 28±2 min	---
Hasegawa et al. (2004)[97]	30 min 25°C	37°C 37.5°C	317±50 sec 152±16 sec	72.9±3.4 W/m^2 94.1±3.1 W/m^2
Siegel et al. (2011)[98]	30 min 24°C	37°C 37.3°C	56.8±5.6 min 46.7±7.2 min	103.7±15.4 W/m^2 73.6±7.5 W/m^2
Kay et al. (1999)[99]	20 min 25°C	37°C 37.5°C	15.8±0.7 km 14.9±0.8 km	153.0±13.1 W/m^2 84.0±8.8 W/m^2
Duffield et al. (2010)[100]	20 min 22°C	37.4°C 37.8°C	19.3±1.3 km 18.0±1.4 km	---

*CWI refers to cold body water immersion; Performance refers to exercise performance determined by time to exhaustion or distance completed in given amount of time.

Post-exercise CWI is mainly applied in athletic events for recovery through changing the tissue temperature and blood flow to ameliorate exercise-induced hyperthermia and exercise induced muscle damage (EIMD). Lowering skin temperature by CWI causes vasoconstriction leading to decrease inflammation and edema to attenuate the onset of muscle soreness.[101] Roberts et al. (2015) conducted the first study to compare the effects of CWI to the active recovery on hemodynamics.[102] Cold water immersion was applied after resistance exercise for 10-min at 10°C, whereas active recovery was conducted a 10-min low-intensity cycling. The skin and muscle temperature were both below the pre-exercise value with CWI, whereas active recovery showed a lower skin and higher muscle temperature compared to pre-exercise value. For the hemodynamics, Q and SV decreased rapidly in CWI, but maintained elevated in active recovery. This reduction in Q was contributed by decreasing blood flow to peripheral regions to maintain core temperature during CWI. However, the increase in Q during active recovery was due to the continuous muscle contract.[102] These responses in Q to CWI and active recovery could be also explained by the activation of the sympathetic nervous system and parasympathetic nervous system.[103] For exercise performance, Roberts et al. (2015) also found that the isometric strength remained the same after CWI, and decreased after active recovery compared to pre-exercise.[102] Moreover, other studies have also shown either no effects or reduction in maximal isometric strength after implemented CWI following by resistance exercise. Pointon et al. (2011) conducted a 20-min whole body cooling with ice cuffs (0.5°C) and this cooling method had no effects on isometric muscle strength.[104] Bergh et al. (1979) previously pointed out that intense acute CWI was detrimental to short-term high-intensity sprint performance, probably because the dramatical lowering of body temperature

resulted in the loss of blood flow in muscle and impaired muscle contraction.[105] Thus, the isometric strength and short-term exercise performance will be affected by CWI procedures such as water temperature, duration, and body immersion area.[102]

Post-exercise CWI is beneficial for the training induced EIMD and optimal day-to-day recovery. Vieira et al. (2016) conducted a study to investigate the effects of different temperatures of CWI on EIMD after 5×20 drop jumps. The CWI protocol consisted of either 5°C (G5) or 15°C (G15) water temperature for 20-min immediately post-exercise. The symptoms of EIMD and muscle performance kept monitoring at 24, 48, 72, 96, and 168-hr after the cooling interventions. Drop jump exercise significantly increased EIMD in this study, and muscle isometric strength gradually recovered to pre-exercise level at 168-hr without any treatment effects. However, counter-movement jump was recovered 96-hr post-exercise in G5 and 72-hr in G15. The subjects reported muscle soreness gradually increased and reached to a maximum at 48-hr post-exercise in both groups. However, the G15 showed a lower soreness score during that period of time. In addition, the serum creatine kinase (CK) concentrations revealed that G15 had a lower CK level throughout recovery and were near to the pre-exercise levels at 72-hr, whereas CK levels in G5 never returned to the baseline level during 168-hr period of time.[95] Moreover, previous research suggested that post-exercise CWI reduced EIMD by decreasing the action of inflammatory cells to prevent secondary tissue damage.[106,107] This study also showed that post-exercise CWI at 15°C might be the optimal temperature for recovery from EIMD because intense cooling (longer duration plus colder water) may interfere blood flow to remove the metabolic byproducts and exacerbate the pro-inflammation response.[108-110]

In this section, the pre-exercise CWI at 20-25°C water temperature for 20-30 min decreases body temperature not only at the beginning of the exercise, but also during exercise. Those studies also showed that when body core temperature reached the critical value (38-39°C), exercise was terminated regardless of pre-cooling interventions. Therefore, lowering core temperature before and during exercise can increase the margin between initial core temperature and critical limiting core temperature. As the previous section 2.2.2 reviewed, the heat storage can be determined by the total change in body temperature (Equation 2.1-2.3). Thus, the heat storage capacity increases allowing delayed the onset of exercise-induced hyperthermia and fatigue.[111] Moreover, the post-exercise CWI with water temperature around 15°C is optimal to decrease body temperature facilitating recovery from heat stress, cardiovascular strain, and EIMD. The efficacy of post-exercise CWI for ameliorating EIMD is dependent on the cooling protocol and exercise modalities.[101] Warm water temperature around 10-15°C may be more appropriate compared to cold water temperature (0-5°C) due to further pro-inflammatory occurred after intense CWI.[95,108,109] Conflicted results among studies related to the effects of post-exercise CWI on muscle isometric strength and eccentric movement need to be further confirmed. In addition, localized muscle blood flow reduction caused by acute CWI appears to be detrimental for short-term high intensity exercise.[105] Therefore, pre-and post-exercise CWI is more effective and appropriate for the intermittent or endurance-based exercise.[101]

2.5.2 Cooling Vest and Neck Cooling

Cold water immersion successfully reduces body temperature in pre-exercise and enhances recovery post-exercise to optimal exercise performance with appropriate water

temperature and procedure used. However, the disadvantages of CWI would be the accessibility, transportable, and high cost of maintenance. Moreover, CWI is not applicable during exercise, in particular it cannot be used during the time out recovery of team sports. Thus, besides CWI, other cooling techniques have also been developed, such as cooling garments or vest, ice packs, and misted cooling fans. Tan et al. (2017) conducted a study to evaluate the cooling rate between various cooling techniques after exercise-induced hyperthermia (39.5°C). Data showed that the whole-body cooling vest (VEST) with cold water-perfusion had a greater cooling rate consequents lowering core temperature from 39.5 to 38°C (Table 2.10) similar to CWI (~0.20°C/min).[112] Thus, CV might be an interchangeable technique to CWI and more applicable during exercise.[113]

Table 2.10. The comparison of cooling rate of various cooling techniques.

Cooling Techniques	**Procedure**	**Cooling Rate (39-38.5°C)**	**Cooling Rate (38.5-38°C)**
Cooling Vest	Thermo-suit with ice water pumped at 2°C	0.23±0.10*	0.24±0.12*
Cooling Room	Cooling fan with 2m/s wind speed at 120cm away	0.15±0.08	0.07±0.07
Ice Pack	Pads were stored -11°C for 24 hr before, and attached on chest, back, and thigh	0.16±0.08	0.12±0.12#

*Significant different in cooling vest compared to others; #Significant difference between cooling room and ice-packs; Cooling rate was in °C/min. Adapted from Tan et al. (2017)[112]

In the past several years, VEST during exercise has been popularly investigated targeting either the whole body or specific area. Kenny et al. (2011) found that wearing whole body ice cooling vest during exercise decreased the sensation of thermal strain and increased

the time to exhaustion by 11%.[114] Furthermore, Arngrimsson et al. (2004) suggested that wearing VEST during an active warm up improved 5-km running performance by 13 sec (VEST: 1134±132 vs. CON: 1147±130 sec; $P<0.05$), and lowed pre-exercise HR by 11 bpm and thermal strain with averaged 0.6 unit (5-point scale) without further differences as exercise continued.[115] Two additional studies have been conducted to investigate the effects of VEST on firefighting activity, Barr et al. (2009) applied the VEST under firefight uniform during a 15-min resting interval between two 20-min exercise bouts of treadmill walking (5 mph, 7.5% grade). They found a significantly lowered core temperature, skin temperature, and HR in the cooling group compared to control, suggesting the VEST was worn significantly reducing thermal strain under heat stress.[116] Bennett et al. (1995) implemented a four-pack ice vest and six-pack ice vest in men dressed firefighting uniform, the performance was determined by 30-min cycling followed by 30-min walking on the treadmill. Similar results were found in that exercise tolerance time increased in both VEST groups by 29% compared to control trial, and six-packs ice vest showed a lower thermal strain only at the second 30-min exercise bout compared to four-packs ice vest.[117] However, one of the disadvantages of the VEST is the extra weight. In Kenny et al. (2011) study, the VEST had 7.5 kg weighed during an activity at 3 mph walking, and it is not feasible to wear it during prolonged competitive sports (>2 hr).

According to the cooling efficiency and application disadvantages in VEST, more cooling interventions have been developed targeting localized cooling skin area (e.g., neck and hand). Three studies conducted by Tyler et al (2010-2011) used either time to exhaustion trial or distance completed in 15-min to assess the effects of neck cooling (NC) after exercise-induced hyperthermia (~39.5°C). Across these three studies, exercise-induced hyperthermia

was implanted at 60-70% VO_{2max} in 75-90 min in a hot and humid environment (30°C; 50%RH). The results showed NC increased time to exhaustion trial by 13.5%[118] and increased 15-min of distance completed by 5-8%.[119,120] In examining the effects of NC on a repeated bout of exercise, Sunderland et al. (2015) found that NC significant improved peak power by 6% and reduced perceptual thermal sensation, but without any changes in body core temperature and serum cortisol level.[121]

Across these NC studies, investigators used self-paced performance tests and found reduced perceptual thermal strain and improve exercise performance without any changes in the physiologic variables (e.g., core temperature and hormone level). However, the mechanisms of NC improved exercise performance in the heat is still unknown. By contrast to the critical core temperature hypothesis, the central governor theory was developed suggesting that exercise limited in the heat because individuals are experiencing dangerously high core temperature.[76,96] The NC advanced exercise performance might be a possible explanation relative to this central governor theory, which reduced perceptual thermal strain leading to a faster self-paced during exercise.[118] In support by Lee et al. (2014) found NC method significant increased cognitive performance, reaction time, working memory, and alertness after exercise-induced hyperthermia.[122] The investigators proposed the potential mechanism might be NC efficiently decrease the blood temperature of carotid arteries flowing to the thermoregulatory center (hypothalamus), further decrease cerebral temperature.[123] However, this hypothesis has been only confirmed in a mathematical model, not in the human model yet.[124] However, neck cooling can be applied in self-paced exercise, whereas the whole-body cooling strategies (e.g., CWI and CV) may be applied better in fixed intensity exercise.

2.5.3 Ice Slurry Ingestion

Cold beverage consumption is another cooling intervention can be applied pre- and during exercise. Ice slurry (IS) ingestion is one of the cold beverages contained an icy mixture normally temperature around -1~0.5°C and can be used as a cooling intervention 30-min pre-exercise and/or during exercise. The first IS study on exercise performance was conducted by Siegel et al. (2010), and found IS ingestion before exercise with the amount of 7.5 g/kg can significantly lower core temperature by 0.5°C and prolonged exercise time by 19% compared to cold water ingestion (4°C).[125] This result was further verified by Dugas et al. (2011) suggesting IS ingestion before exercise was effective to prolong endurance exercise and lowering body core temperature allowing core temperature to rise higher before exhaustion compared to cold water drinks.[126] Ice slurry ingestion during exercise also showed a positive impact on performance. Naito et al. (2018) found IS ingestion during time break between tennis match can dramatically attenuate raised core temperature and thermal strain.[127] However, three studies found IS was effective at reducing core temperature before and during exercise without enhanced performance. Mejuto et al. (2018) suggested IS ingestion both before and during exercise was efficient at lowering core temperature, but did not improve 10-km time trial performance.[128] In addition, Gerrett et al. (2017) found the similar results in that IS ingestion with 7.5 g/kg at 30-min before exercise successfully decreased core temperature without seeing any improvement in self-paced intermittent exercise performance.[129] One of the unique studies conducted under Maunder et al. (2017) to examine the effects of *ad libitum* IS ingestion during a 40-km running. The rate of core temperature changes was lower with IS ingestion, but the performance and mean power were reduced by 2.5% and 2.2%, respectively. Surprisingly, the

ad libitum fluid ingestion volume was significantly lowered by 29.7% (370 ml) in IS ingestion compared to water consumption.[130]

Across these IS studies, IS ingestion successfully reduced core temperature because IS ingestion creates a larger heat sink by changing the physical state from ice to water. Melting 1 kg ice requires 334 kJ of energy absorbed without temperature changes. Thus, this unique thermodynamics characteristic in IS yields a larger heat storing capacity compared to water only, further affecting the core temperature.[125] However, contradicting results have been shown on exercise performance across the studies. Among those studies with exercise improvement, Siegel et al. (2010) pointed out that IS was consumed by mouth possibly leading to a decrease in brain temperature. Furthermore, one of the common limitations in the IS study is the placebo effects, by which the intervention trial cannot be blinded to the participants and those participants may believe the cooler intervention have a beneficial improvement in exercise performance.[126-128] On the other hand, Mejuto et al. (2018) and Gerrett et al. (2017) did not see any improvement in exercise performance and they pointed out that the exercise trial is not intense enough to create heat stress with failure to detect any effects from IS ingestion.[128,129] However, the mechanism behind IS ingestion is still unknown related to lower core temperature without exercise performance benefits, which is contrary to the other cooling techniques in this review (CWI and CV). In addition, IS ingestion during endurance exercise may not be practically applicable during exercise due to difficult ingestion and impaired breathing compared to fluid consumption.[130] Therefore, IS ingestion before exercise is effective at lowering body core temperature, and the effects on exercise performance need to be further investigated.

2.6 Conclusion and Future Direction

To conclude this review, human thermoregulation mechanism can maintain body temperature in a normal range, regardless of environments. The hypothalamus is the organ to regulate body temperatures by either activating sweat mechanism to decrease body temperature or metabolic activity to increase body temperature. As exercise in the heat, exercise cessation is occurred when the core temperature elevated to 39~40°C. Heat storage can be determined through calorimetry (metabolic heat production, convention, or conduction) and thermometry (changes in body temperature). Heat storage represents heat balance (heat gain or heat loss) during rest or exercise under variable environmental conditions. Thus, exercise performance and work capacity in hot and humid environments can be better understood.

During exercise in the hot and humid environmental conditions, dehydration commonly occur due to the large requirement of heat dissipation through sweat resulting in body fluid loss. Research well describe the fact that CES on fluid balance during exercise in the heat compared to only water consumption. The carbohydrate and electrolyte content in the beverage can compensate for glycogen depletion and electrolyte losses during exercise. However, individualizing beverages may be the future to investigate large inter-individual variability of sweat responses.

Cardiovascular strain combined thermal stress are essential contributors of early fatigue under heat conditions. Exercise in the heat significantly alters cardiovascular function and blood flow circulation. Given the amount of blood volume pumping more to the peripheral for heat dissipation cause a decline in CBV and blood refill to the heart compared to the normothermic condition. Thus, this blood redistribution further reduces stroke volume and

cardiac output leading to cardiovascular changes to impact oxygen consumption and exercise capacity.

In order to ameliorate cardiovascular stress, blood circulation changes, and elevated body temperature, various cooling interventions have been developed and investigated in recent years. Cold water immersion has been used widely in the team sport, and studies showed that appropriate cooling procedures bring large benefits with pre-exercise cooling and post-exercise recovery. For those sports or individuals with limited resources and facilities, the cooling vest would be more applicable for cooling in pre-and post-exercise. However, the extra weight of cooling vest is not ideal to wear during the exercise bout. Neck cooling and ice slurry ingestion are feasible to be applied during exercise, but the effects of these cooling strategies on exercise performance still remain equivocal. Therefore, future research is necessary to understand the facts of cooling interventions and specific procedures relative to different exercise modalities to reduce cardiovascular strain, thermal stress, and optimal exercise performance in the heat.

2.7 References

1. Brooks GA, Fahey TD, Baldwin KM. *Exercise physiology : human bioenergetics and its applications.* 4th ed. Boston: McGraw-Hill; 2005.

2. American College of Sports M, Sawka MN, Burke LM, et al. American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci Sports Exerc.* 2007;39(2):377-390.

3. Gonzalez-Alonso J, Mora-Rodriguez R, Below PR, Coyle EF. Dehydration markedly impairs cardiovascular function in hyperthermic endurance athletes during exercise. *J Appl Physiol (1985).* 1997;82(4):1229-1236.

4. Yankelson L, Sadeh B, Gershovitz L, et al. Life-threatening events during endurance sports: is heat stroke more prevalent than arrhythmic death? *J Am Coll Cardiol.* 2014;64(5):463-469.

5. Lim CL, Byrne C, Lee JK. Human thermoregulation and measurement of body temperature in exercise and clinical settings. *Ann Acad Med Singapore.* 2008;37(4):347-353.

6. Gisolfi CV, Mora F. *The hot brain : survival, temperature, and the human body.* Cambridge, Mass.: MIT Press; 2000.

7. Kenny GP, Webb P, Ducharme MB, Reardon FD, Jay O. Calorimetric measurement of postexercise net heat loss and residual body heat storage. *Med Sci Sports Exerc.* 2008;40(9):1629-1636.

8. Kenny GP, Reardon FD, Zaleski W, Reardon ML, Haman F, Ducharme MB. Muscle temperature transients before, during, and after exercise measured using an intramuscular multisensor probe. *J Appl Physiol (1985).* 2003;94(6):2350-2357.

9. Maughan RJ, Otani H, Watson P. Influence of relative humidity on prolonged exercise capacity in a warm environment. *Eur J Appl Physiol.* 2012;112(6):2313-2321.

10. Sen Gupta J, Swamy YV, Pichan G, Dimri GP. Physiological responses during continuous work in hot dry and hot humid environments in Indians. *Int J Biometeorol.* 1984;28(2):137-146.

11. Hayes M, Castle PC, Ross EZ, Maxwell NS. The influence of hot humid and hot dry environments on intermittent-sprint exercise performance. *Int J Sports Physiol Perform.* 2014;9(3):387-396.

12. McLellan TM, Cheung SS, Selkirk GA, Wright HE. Influence of aerobic fitness on thermoregulation during exercise in the heat. *Exerc Sport Sci Rev.* 2012;40(4):218-219; author reply 220.

13. Cheung SS. *Advanced environmental exercise physiology.* Champaign, IL: Human Kinetics; 2010.

14. Shvartz E, Saar E, Meyerstein N, Benor D. A comparison of three methods of acclimatization to dry heat. *J Appl Physiol.* 1973;34(2):214-219.

15. Henane R, Bittel J, Viret R, Morino S. Thermal strain resulting from protective clothing of an armored vehicle crew in warm conditions. *Aviat Space Environ Med.* 1979;50(6):599-603.

16. Cadarette BS, Levine L, Rock PB, Stephenson LA, Kolka MA. Effects of atropine on thermoregulatory responses to exercise in different environments. *Aviat Space Environ Med.* 1986;57(11):1050-1055.

17. Gonzalez RR, Kluger MJ, Stolwijk JA. Thermoregulatory responses to thermal transients in the rabbit. *Am J Physiol.* 1974;227(6):1292-1298.

18. Vallerand AL, Michas RD, Frim J, Ackles KN. Heat balance of subjects wearing protective clothing with a liquid- or air-cooled vest. *Aviat Space Environ Med.* 1991;62(5):383-391.

19. Holmer I. Body cooling with ice for warm-water diving operations. *Undersea Biomed Res.* 1989;16(6):471-479.

20. Bittel J, Henane R. Comparison of thermal exchanges in men and women under neutral and hot conditions. *J Physiol.* 1975;250(3):475-489.

21. Vallerand AL, Savourey G, Hanniquet AM, Bittel JH. How should body heat storage be determined in humans: by thermometry or calorimetry? *Eur J Appl Physiol Occup Physiol.* 1992;65(3):286-294.

22. Colin J, Timbal J, Houdas Y, Boutelier C, Guieu JD. Computation of mean body temperature from rectal and skin temperatures. *J Appl Physiol.* 1971;31(3):484-489.

23. Colin J, Timbal J, Boutelier C. [Experimental determination of the equation permitting the calculation of the mean body temperature in neutral and hot environment]. *J Physiol (Paris).* 1971;63(3):229-231.

24. Livingstone SD. Calculation of mean body temperature. *Can J Physiol Pharmacol.* 1968;46(1):15-17.

25. Burton AC. Human calorimetry II. The average temperature of the tissues of the body. *J Nutr.* 1935;9:264-280.

26. Hardy JD, Dubois EF. Basal metabolism, radiation, convection and vaporisation at temperatures of 22 to 35° C. *J Nutr.* 1938;1-5:477-497.

27. Cramer MN, Jay O. Partitional calorimetry. *J Appl Physiol (1985).* 2019;126(2):267-277.

28. Grandjean AC, Reimers KJ, Buyckx ME. Hydration: issues for the 21st century. *Nutr Rev.* 2003;61(8):261-271.

29. Duffield R, McCall A, Coutts AJ, Peiffer JJ. Hydration, sweat and thermoregulatory responses to professional football training in the heat. *J Sports Sci.* 2012;30(10):957-965.

30. Goulet ED. Dehydration and endurance performance in competitive athletes. *Nutr Rev.*

2012;70 Suppl 2:S132-136.

31. Armstrong LE, Curtis WC, Hubbard RW, Francesconi RP, Moore R, Askew EW. Symptomatic hyponatremia during prolonged exercise in heat. *Med Sci Sports Exerc.* 1993;25(5):543-549.

32. Webster S, Rutt R, Weltman A. Physiological effects of a weight loss regimen practiced by college wrestlers. *Med Sci Sports Exerc.* 1990;22(2):229-234.

33. Armstrong LE, Costill DL, Fink WJ. Influence of diuretic-induced dehydration on competitive running performance. *Med Sci Sports Exerc.* 1985;17(4):456-461.

34. Jequier E, Constant F. Water as an essential nutrient: the physiological basis of hydration. *Eur J Clin Nutr.* 2010;64(2):115-123.

35. Baron S, Courbebaisse M, Lepicard EM, Friedlander G. Assessment of hydration status in a large population. *Br J Nutr.* 2015;113(1):147-158.

36. Armstrong LE, Soto JA, Hacker FT, Jr., Casa DJ, Kavouras SA, Maresh CM. Urinary indices during dehydration, exercise, and rehydration. *Int J Sport Nutr.* 1998;8(4):345-355.

37. Hahn RG, Waldreus N. An aggregate urine analysis tool to detect acute dehydration. *Int J Sport Nutr Exerc Metab.* 2013;23(4):303-311.

38. Baron S, Courbebaisse M, Lepicard EM, Friedlander G. Assessment of hydration status in a large population. *The British journal of nutrition.* 2014:1-12.

39. Cheuvront SN, Kenefick RW, Zambraski EJ. Spot Urine Concentrations Should Not be Used for Hydration Assessment: A Methodology Review. *Int J Sport Nutr Exerc Metab.* 2015;25(3):293-297.

40. Cutrufello PT, Dixon CB, Zavorsky GS. Hydration assessment among marathoners using urine specific gravity and bioelectrical impedance analysis. *Res Sports Med.* 2016;24(3):234-242.

41. Mange K, Matsuura D, Cizman B, et al. Language guiding therapy: the case of dehydration versus volume depletion. *Ann Intern Med.* 1997;127(9):848-853.

42. Bartok C, Schoeller DA, Sullivan JC, Clark RR, Landry GL. Hydration testing in collegiate wrestlers undergoing hypertonic dehydration. *Med Sci Sports Exerc.* 2004;36(3):510-517.

43. Kovacs EM, Senden JM, Brouns F. Urine color, osmolality and specific electrical

conductance are not accurate measures of hydration status during postexercise rehydration. *J Sports Med Phys Fitness.* 1999;39(1):47-53.

44. Popowski LA, Oppliger RA, Patrick Lambert G, Johnson RF, Kim Johnson A, Gisolf CV. Blood and urinary measures of hydration status during progressive acute dehydration. *Med Sci Sports Exerc.* 2001;33(5):747-753.

45. Hew-Butler TD, Eskin C, Bickham J, Rusnak M, VanderMeulen M. Dehydration is how you define it: comparison of 318 blood and urine athlete spot checks. *BMJ Open Sport Exerc Med.* 2018;4(1):e000297.

46. Shirreffs SM, Sawka MN. Fluid and electrolyte needs for training, competition, and recovery. *J Sports Sci.* 2011;29 Suppl 1:S39-46.

47. Kilding AE, Tunstall H, Wraith E, Good M, Gammon C, Smith C. Sweat rate and sweat electrolyte composition in international female soccer players during game specific training. *Int J Sports Med.* 2009;30(6):443-447.

48. Zetou E, Giatsis G, Mountaki F, Komninakidou A. Body weight changes and voluntary fluid intakes of beach volleyball players during an official tournament. *J Sci Med Sport.* 2008;11(2):139-145.

49. Broad EM, Burke LM, Cox GR, Heeley P, Riley M. Body weight changes and voluntary fluid intakes during training and competition sessions in team sports. *Int J Sport Nutr.* 1996;6(3):307-320.

50. Davis JK, Baker LB, Barnes K, Ungaro C, Stofan J. Thermoregulation, Fluid Balance, and Sweat Losses in American Football Players. *Sports Med.* 2016;46(10):1391-1405.

51. Baker LB, Barnes KA, Anderson ML, Passe DH, Stofan JR. Normative data for regional sweat sodium concentration and whole-body sweating rate in athletes. *J Sports Sci.* 2016;34(4):358-368.

52. Dziedzic CE, Ross ML, Slater GJ, Burke LM. Variability of measurements of sweat sodium using the regional absorbent-patch method. *Int J Sports Physiol Perform.* 2014;9(5):832-838.

53. Baker LB. Sweating Rate and Sweat Sodium Concentration in Athletes: A Review of Methodology and Intra/Interindividual Variability. *Sports Med.* 2017;47(Suppl 1):111-128.

54. Shirreffs SM, Aragon-Vargas LF, Chamorro M, Maughan RJ, Serratosa L, Zachwieja JJ. The sweating response of elite professional soccer players to training in the heat. *Int J Sports Med.* 2005;26(2):90-95.

55. Stofan JR, Zachwieja JJ, Horswill CA, Murray R, Anderson SA, Eichner ER. Sweat and sodium losses in NCAA football players: a precursor to heat cramps? *Int J Sport Nutr Exerc Metab.* 2005;15(6):641-652.

56. Maughan RJ, Merson SJ, Broad NP, Shirreffs SM. Fluid and electrolyte intake and loss in elite soccer players during training. *Int J Sport Nutr Exerc Metab.* 2004;14(3):333-346.

57. Weschler LB. Sweat electrolyte concentrations obtained from within occlusive coverings are falsely high because sweat itself leaches skin electrolytes. *J Appl Physiol (1985).* 2008;105(4):1376-1377.

58. Patterson MJ, Galloway SD, Nimmo MA. Variations in regional sweat composition in normal human males. *Exp Physiol.* 2000;85(6):869-875.

59. Hargreaves M, Morgan TO, Snow R, Guerin M. Exercise tolerance in the heat on low and normal salt intakes. *Clin Sci (Lond).* 1989;76(5):553-557.

60. Koenders EE, Franken CPG, Cotter JD, Thornton SN, Rehrer NJ. Restricting dietary sodium reduces plasma sodium response to exercise in the heat. *Scand J Med Sci Sports.* 2017;27(11):1213-1220.

61. Kenefick RW. Drinking Strategies: Planned Drinking Versus Drinking to Thirst. *Sports Med.* 2018;48(Suppl 1):31-37.

62. Hew-Butler T, Verbalis JG, Noakes TD, International Marathon Medical Directors A. Updated fluid recommendation: position statement from the International Marathon Medical Directors Association (IMMDA). *Clin J Sport Med.* 2006;16(4):283-292.

63. Hew-Butler T, Rosner MH, Fowkes-Godek S, et al. Statement of the Third International Exercise-Associated Hyponatremia Consensus Development Conference, Carlsbad, California, 2015. *Clin J Sport Med.* 2015;25(4):303-320.

64. Johannsen NM, Lind E, King DS, Sharp RL. Effect of preexercise electrolyte ingestion on fluid balance in men and women. *Med Sci Sports Exerc.* 2009;41(11):2017-2025.

65. Kingsley M, Penas-Ruiz C, Terry C, Russell M. Effects of carbohydrate-hydration strategies on glucose metabolism, sprint performance and hydration during a soccer match simulation in recreational players. *J Sci Med Sport.* 2014;17(2):239-243.

66. Murray R, Bartoli W, Stofan J, Horn M, Eddy D. A comparison of the gastric emptying characteristics of selected sports drinks. *Int J Sport Nutr.* 1999;9(3):263-274.

67. Osterberg KL, Pallardy SE, Johnson RJ, Horswill CA. Carbohydrate exerts a mild

influence on fluid retention following exercise-induced dehydration. *J Appl Physiol (1985).* 2010;108(2):245-250.

68. Anastasiou CA, Kavouras SA, Arnaoutis G, et al. Sodium replacement and plasma sodium drop during exercise in the heat when fluid intake matches fluid loss. *J Athl Train.* 2009;44(2):117-123.

69. Sims ST, van Vliet L, Cotter JD, Rehrer NJ. Sodium loading aids fluid balance and reduces physiological strain of trained men exercising in the heat. *Med Sci Sports Exerc.* 2007;39(1):123-130.

70. Coles MG, Luetkemeier MJ. Sodium-facilitated hypervolemia, endurance performance, and thermoregulation. *Int J Sports Med.* 2005;26(3):182-187.

71. Sims ST, Rehrer NJ, Bell ML, Cotter JD. Preexercise sodium loading aids fluid balance and endurance for women exercising in the heat. *J Appl Physiol (1985).* 2007;103(2):534-541.

72. Maughan RJ, Shirreffs SM. Development of hydration strategies to optimize performance for athletes in high-intensity sports and in sports with repeated intense efforts. *Scand J Med Sci Sports.* 2010;20 Suppl 2:59-69.

73. Urdampilleta A, Gomez-Zorita S, Soriano JM, Martinez-Sanz JM, Medina S, Gil-Izquierdo A. Hydration and chemical ingredients in sport drinks: food safety in the European context. *Nutr Hosp.* 2015;31(5):1889-1899.

74. Cheuvront SN, Kenefick RW, Montain SJ, Sawka MN. Mechanisms of aerobic performance impairment with heat stress and dehydration. *J Appl Physiol (1985).* 2010;109(6):1989-1995.

75. Girard O, Brocherie F, Bishop DJ. Sprint performance under heat stress: A review. *Scand J Med Sci Sports.* 2015;25 Suppl 1:79-89.

76. Nielsen B, Hales JR, Strange S, Christensen NJ, Warberg J, Saltin B. Human circulatory and thermoregulatory adaptations with heat acclimation and exercise in a hot, dry environment. *J Physiol.* 1993;460:467-485.

77. Galloway SD, Maughan RJ. Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. *Med Sci Sports Exerc.* 1997;29(9):1240-1249.

78. MacDougall JD, Reddan WG, Layton CR, Dempsey JA. Effects of metabolic hyperthermia on performance during heavy prolonged exercise. *J Appl Physiol.* 1974;36(5):538-544.

79. Sawka MN, Young AJ, Cadarette BS, Levine L, Pandolf KB. Influence of heat stress and acclimation on maximal aerobic power. *Eur J Appl Physiol Occup Physiol.* 1985;53(4):294-298.

80. Williams CG, Bredell GA, Wyndham CH, et al. Circulatory and metabolic reactions to work in heat. *J Appl Physiol.* 1962;17:625-638.

81. Rowell LB, Blackmon JR, Martin RH, Mazzarella JA, Bruce RA. Hepatic clearance of indocyanine green in man under thermal and exercise stresses. *J Appl Physiol.* 1965;20(3):384-394.

82. Rowell LB, Marx HJ, Bruce RA, Conn RD, Kusumi F. Reductions in cardiac output, central blood volume, and stroke volume with thermal stress in normal men during exercise. *J Clin Invest.* 1966;45(11):1801-1816.

83. Sakate T. The effect of air temperature on physical working capacity. *J Hum Ergol (Tokyo).* 1978;7(2):127-134.

84. Arngrimsson SA, Petitt DS, Borrani F, Skinner KA, Cureton KJ. Hyperthermia and maximal oxygen uptake in men and women. *Eur J Appl Physiol.* 2004;92(4-5):524-532.

85. Tatterson AJ, Hahn AG, Martin DT, Febbraio MA. Effects of heat stress on physiological responses and exercise performance in elite cyclists. *J Sci Med Sport.* 2000;3(2):186-193.

86. Nybo L, Jensen T, Nielsen B, Gonzalez-Alonso J. Effects of marked hyperthermia with and without dehydration on VO(2) kinetics during intense exercise. *J Appl Physiol (1985).* 2001;90(3):1057-1064.

87. Gonzalez-Alonso J, Crandall CG, Johnson JM. The cardiovascular challenge of exercising in the heat. *J Physiol.* 2008;586(1):45-53.

88. Rowell LB. Human cardiovascular adjustments to exercise and thermal stress. *Physiol Rev.* 1974;54(1):75-159.

89. Rowell LB, Kraning KK, 2nd, Kennedy JW, Evans TO. Central circulatory responses to work in dry heat before and after acclimatization. *J Appl Physiol.* 1967;22(3):509-518.

90. Rowell LB, Murray JA, Brengelmann GL, Kraning KK, 2nd. Human cardiovascular adjustments to rapid changes in skin temperature during exercise. *Circ Res.* 1969;24(5):711-724.

91. Crandall CG, Gonzalez-Alonso J. Cardiovascular function in the heat-stressed human.

Acta Physiol (Oxf). 2010;199(4):407-423.

92. Rowell LB, Brengelmann GL, Murray JA, Kraning KK, 2nd, Kusumi F. Human metabolic responses to hyperthermia during mild to maximal exercise. *J Appl Physiol.* 1969;26(4):395-402.

93. Lee DT, Haymes EM. Exercise duration and thermoregulatory responses after whole body precooling. *J Appl Physiol (1985).* 1995;79(6):1971-1976.

94. Stevens CJ, Taylor L, Dascombe BJ. Cooling During Exercise: An Overlooked Strategy for Enhancing Endurance Performance in the Heat. *Sports Med.* 2017;47(5):829-841.

95. Vieira A, Siqueira AF, Ferreira-Junior JB, et al. The Effect of Water Temperature during Cold-Water Immersion on Recovery from Exercise-Induced Muscle Damage. *Int J Sports Med.* 2016;37(12):937-943.

96. Gonzalez-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):1032-1039.

97. Hasegawa H, Takatori T, Komura T, Yamasaki M. Combined effects of pre-cooling and water ingestion on thermoregulation and physical capacity during exercise in a hot environment. *J Sports Sci.* 2006;24(1):3-9.

98. Siegel R, Mate J, Watson G, Nosaka K, Laursen PB. Pre-cooling with ice slurry ingestion leads to similar run times to exhaustion in the heat as cold water immersion. *J Sports Sci.* 2012;30(2):155-165.

99. Kay D, Taaffe DR, Marino FE. Whole-body pre-cooling and heat storage during self-paced cycling performance in warm humid conditions. *J Sports Sci.* 1999;17(12):937-944.

100. Duffield R, Green R, Castle P, Maxwell N. Precooling can prevent the reduction of self-paced exercise intensity in the heat. *Med Sci Sports Exerc.* 2010;42(3):577-584.

101. Ihsan M, Watson G, Abbiss CR. What are the Physiological Mechanisms for Post-Exercise Cold Water Immersion in the Recovery from Prolonged Endurance and Intermittent Exercise? *Sports Med.* 2016;46(8):1095-1109.

102. Roberts LA, Muthalib M, Stanley J, et al. Effects of cold water immersion and active recovery on hemodynamics and recovery of muscle strength following resistance exercise. *Am J Physiol Regul Integr Comp Physiol.* 2015;309(4):R389-398.

103. Al Haddad H, Laursen PB, Ahmaidi S, Buchheit M. Influence of cold water face

immersion on post-exercise parasympathetic reactivation. *Eur J Appl Physiol.* 2010;108(3):599-606.

104. Pointon M, Duffield R, Cannon J, Marino FE. Cold application for neuromuscular recovery following intense lower-body exercise. *Eur J Appl Physiol.* 2011;111(12):2977-2986.

105. Bergh U, Ekblom B. Influence of muscle temperature on maximal muscle strength and power output in human skeletal muscles. *Acta Physiol Scand.* 1979;107(1):33-37.

106. Merrick MA. Secondary injury after musculoskeletal trauma: a review and update. *J Athl Train.* 2002;37(2):209-217.

107. Puntel GO, Carvalho NR, Amaral GP, et al. Therapeutic cold: An effective kind to modulate the oxidative damage resulting of a skeletal muscle contusion. *Free Radic Res.* 2011;45(2):125-138.

108. White GE, Rhind SG, Wells GD. The effect of various cold-water immersion protocols on exercise-induced inflammatory response and functional recovery from high-intensity sprint exercise. *Eur J Appl Physiol.* 2014;114(11):2353-2367.

109. Lee EC, Watson G, Casa D, et al. Interleukin-6 responses to water immersion therapy after acute exercise heat stress: a pilot investigation. *J Athl Train.* 2012;47(6):655-663.

110. Tseng CY, Lee JP, Tsai YS, et al. Topical cooling (icing) delays recovery from eccentric exercise-induced muscle damage. *J Strength Cond Res.* 2013;27(5):1354-1361.

111. Marino FE. Methods, advantages, and limitations of body cooling for exercise performance. *Br J Sports Med.* 2002;36(2):89-94.

112. Tan PM, Teo EY, Ali NB, et al. Evaluation of Various Cooling Systems After Exercise-Induced Hyperthermia. *J Athl Train.* 2017;52(2):108-116.

113. Armstrong LE, Crago AE, Adams R, Roberts WO, Maresh CM. Whole-body cooling of hyperthermic runners: comparison of two field therapies. *Am J Emerg Med.* 1996;14(4):355-358.

114. Kenny GP, Schissler AR, Stapleton J, et al. Ice cooling vest on tolerance for exercise under uncompensable heat stress. *J Occup Environ Hyg.* 2011;8(8):484-491.

115. Arngrimsson SA, Petitt DS, Stueck MG, Jorgensen DK, Cureton KJ. Cooling vest worn during active warm-up improves 5-km run performance in the heat. *J Appl Physiol (1985).* 2004;96(5):1867-1874.

116. Barr D, Gregson W, Sutton L, Reilly T. A practical cooling strategy for reducing the physiological strain associated with firefighting activity in the heat. *Ergonomics.* 2009;52(4):413-420.

117. Bennett BL, Hagan RD, Huey KA, Minson C, Cain D. Comparison of two cool vests on heat-strain reduction while wearing a firefighting ensemble. *Eur J Appl Physiol Occup Physiol.* 1995;70(4):322-328.

118. Tyler CJ, Sunderland C. Cooling the neck region during exercise in the heat. *J Athl Train.* 2011;46(1):61-68.

119. Tyler CJ, Wild P, Sunderland C. Practical neck cooling and time-trial running performance in a hot environment. *Eur J Appl Physiol.* 2010;110(5):1063-1074.

120. Tyler CJ, Sunderland C. Neck cooling and running performance in the heat: single versus repeated application. *Med Sci Sports Exerc.* 2011;43(12):2388-2395.

121. Sunderland C, Stevens R, Everson B, Tyler CJ. Neck-cooling improves repeated sprint performance in the heat. *Front Physiol.* 2015;6:314.

122. Lee JK, Koh AC, Koh SX, Liu GJ, Nio AQ, Fan PW. Neck cooling and cognitive performance following exercise-induced hyperthermia. *Eur J Appl Physiol.* 2014;114(2):375-384.

123. Haymaker; W. Blood supply of the human hypothalamus. *The Hypothalamus.* 1969:210-218.

124. Sukstanskii AL, Yablonskiy DA. Theoretical limits on brain cooling by external head cooling devices. *Eur J Appl Physiol.* 2007;101(1):41-49.

125. Siegel R, Mate J, Brearley MB, Watson G, Nosaka K, Laursen PB. Ice slurry ingestion increases core temperature capacity and running time in the heat. *Med Sci Sports Exerc.* 2010;42(4):717-725.

126. Dugas J. Ice slurry ingestion increases running time in the heat. *Clin J Sport Med.* 2011;21(6):541-542.

127. Naito T, Sagayama H, Akazawa N, Haramura M, Tasaki M, Takahashi H. Ice slurry ingestion during break times attenuates the increase of core temperature in a simulation of physical demand of match-play tennis in the heat. *Temperature (Austin).* 2018;5(4):371-379.

128. Mejuto G, Chalmers S, Gilbert S, Bentley D. The effect of ice slurry ingestion on body temperature and cycling performance in competitive athletes. *J Therm Biol.*

2018;72:143-147.

129. Gerrett N, Jackson S, Yates J, Thomas G. Ice slurry ingestion does not enhance self-paced intermittent exercise in the heat. *Scand J Med Sci Sports.* 2017;27(11):1202-1212.

130. Maunder E, Laursen PB, Kilding AE. Effect of ad Libitum Ice-Slurry and Cold-Fluid Ingestion on Cycling Time-Trial Performance in the Heat. *Int J Sports Physiol Perform.* 2017;12(1):99-105.

CHAPTER 3. THE EFFECTS OF FIELD POSITION ON FLUID BALANCE AND ELECTROLYTE LOSSES IN NCAA DIVISION I FEMALE SOCCER PLAYERS

3.1 Introduction

Competitive soccer players continuously move during match play, covering large distances (8-12km) using both aerobic and anaerobic systems. Due to the nature of the sport, soccer players have limited access to fluids and time to rehydrate as there are no timeouts and minimal player substitutions.[1] The requirements for high intensity efforts combined with limited fluid availability puts athletes at high risk for dehydration, which can be further exacerbated by warm/hot and humid environmental conditions.[2] The Fédération Internationale de Football Association (FIFA) declares hydration breaks at the 30th and 75th minutes of 90 minutes match when environmental stress, assessed via wet-bulb globe temperature (WBGT), is greater than 31°C. In addition, according to the new rules proposed by National Collegiate Athletic Association (NCAA) in 2019, players are required to take hydration breaks between 25-30 minutes and 70-75 minutes when WBGT reaches to 30°C. Without these hydration breaks on hot days loss of body water enhanced by sweating may lead to excessive of electrolytes loss (such as sodium), significantly impairing athletic performance by altering cardiovascular function and thermoregulation.[3,4] Insufficient electrolyte consumption to replace losses can result in greater serum osmolality and lower plasma volume, thus effecting exercise performance by elevating heart rate.[5,6] Consequentially, dehydration (≥2% loss of body weight) during soccer increases an athlete's risk of poorer physical and cognitive performance,[7] heat-related illness, and injury.[8,9]

While guidelines exist to protect athletes from illness and injury as a result of

dehydration, current fluid replacement guidelines are not sport-specific and do not detail intake differences between sexes despite known differences in drinking behavior and thermo-regulation.[4,10] Tremendous growth in youth and competitive soccer among females warrants the development of hydration guidelines to better protect females from the challenges of the environment and sport. As more females join the sport more may be at greater risk for dehydration due to unspecific fluid replacement guidelines and lack of hydration strategies. Thus, dehydration has been found to be more prevalent in female soccer in the previous studies.[11-13]

Soccer players perform high-intensity (sprinting, running, and cruising) and low-intensity (walking, jogging, and standing) movements in a 90 min play, but movements vary depending on position.[14] Forwards spend a larger proportion of time sprinting and cruising compared to mid-fielders or defenders, mimicking high intensity interval type activity. Forwards tend more distance in sprinting compared to mid-fielders and defenders,[15] thus may have greater fluid replacement needs. Mid-fielders tend to spend more time running reflecting continuous aerobic activity. Defenders and goalkeepers exhibit more low intensity type activity, with defenders spending the majority of the match jogging and walking, and goalkeepers primarily standing.[14] Previous study found elite female soccer players performed average total distance ~8.5km and average sprint distances 14.9m.[16] and total distance covered was influenced by playing position.[17-19] For example, Gabbett et al. (2008) found mid-fielders covered ~600m more than defenders and ~1000m more than forwards, suggesting mid-fielder's continual activity requires more fluid replacement than defenders or goalkeepers.[20] Therefore, the differences in exercise characteristics (intensity and distance) by position may change

hydration replacement requirements. Specialized hydration strategy to a position, taking into account the environment and exercise characteristics, may enhance the individual and team performance. To our knowledge, no study has examined the hydration status and changes during exercise differentiated by soccer positions in female. Therefore, the purpose of this study was to determine the fluid balance, sweat rate and electrolyte losses in NCAA Division I female soccer players across field positions. This study also aimed to examine the effect of environmental stress (i.e. warm and cool practices) positional differences in these main outcomes. We hypothesized more active positions on hotter days would have greater fluid loss, fluid intake, sweat rate, and electrolyte losses than less active positions.

3.2 Methods

3.2.1 Participants

Eighteen players were recruited from an NCAA Division I collegiate female soccer team (19.2±1.0yr; mean±SD). According to on-field positions, players were grouped as follows: 3 forwards (FW), 7 mid-fielders (MD), 5 defenders (DF), and 3 goalkeepers (GK). Players were free of any chronic condition and injury and were cleared by their team physician to participate in play. The study was approved by the University Institutional Review Board, and all players signed a written informed consent prior to any assessments.

3.2.2 Experiment Design

The study took place over 7 weeks of field practice in the spring off-season training camp (March to mid-April). Players had team field training on every Tuesday and Thursday morning (0730-0900) and Friday afternoon (1530-1700), for a total of 14 practices evaluated. Due to the accessibility of players and training schedule, 2 of the 3 practices per week were

observed. Training sessions (~90 minutes total) consisted of a 10- to 15-min warm-up and 4-5 sets of 15-min scrimmages with 1- to 2-min rest. During practices, players were provided beverages *ad libitum* with self-selected assess to water or carbohydrate-electrolyte solution (CES). The beverages were provided by athletic trainers and each position and player had equal access to both water and CES throughout the entire practice. Practice assessments included environmental conditions and physiological monitoring of heart rate (HR), body weight, fluid intake, and sweat and urine samplings. Study investigators had no influence on the training or sport-specific performance testing (pre-determined by coaching staff). Medical and athletic training staff were present throughout each practice. Environmental conditions were measured before and after practice and consisted of wet-bulb globe temperature (WBGT8758 Vernon Hills, IL US) and field surface temperature (IR Thermometer; Extech IR200 Nashua, NH US).

3.2.3 Body Weight

Body weight was assessed using a TANITA scale with a sensitivity of 0.1 kg (TBF-300 Arlington Heights, IL) before and after each practice session. Players wore only their practice jersey after toweling dry and urinating. Fluid balance was calculated as the change in body weight, accounting for fluid intake and urine output. Hydration status would be categorized by euhydration (Eu; >0% body weight), mild dehydration (Mi; <-1% body weight), moderate dehydration (Mo; -1~-2% body weight), and severe dehydration (Se; >-2% body weight).

3.2.4 Fluid Intake

Fluid intake was monitored throughout practice using labeled, pre-weighed bottles weighed on food preparation scales (TANITA KD-160BK; Arlington Heights, IL). Participants self-selected a beverage to drink *ad libitum* throughout the training period. The type of beverages consumed was recorded (water or CES), and the bottles were weighed after training session to estimate overall fluid intake. Players were encouraged to ingest all fluids that entered the mouth so accurate measures of total fluid intake could be determined.

3.2.5 Urine Samples

Urine samples were collected for immediate analysis before and after each practice session. USG was measured using by Pocket Refractometer (ATAGO CO., LTD, US). Urine was also analyzed for electrolyte concentrations (sodium [Na^+], potassium [K^+], and chloride [Cl^-]) using ion-selective probes (MEDICA EasyLyte; Bedford, MA).

3.2.6 Sweat Samples

Sweat was collected on the lower back using the technical absorbent patch technique.[21] Alcohol spray and dry towel were used to clean the skin surface before the sweat patch was attached. Sweat patches were removed at the first resting interval after 60min of each practice to avoid patch over-saturation. The local sweat rate (LSR) was calculated by weight differences of the absorbent patch before and after training divided by the product of the sweat patch area and time duration ($g/cm^2 \cdot hr$). Sweat was analyzed for electrolyte concentrations (sodium [Na^+], potassium [K^+], and chloride [Cl^-]) similarly to urine.

3.2.7 Heart Rate

Continuous HR monitoring was conducted with the use of a Bioharness BH3 (Zephyr; Annapolis, MD) worn around the chest during each practice. For a given practice, 11 of the 18 players wore a Bioharness as designated by the head coach and included FW (n=3), MD (n=5), and DF (n=3). Average heart rate (HR_{ave}) and maximal heart rate (HR_{max}) were determined for the time during training. Average exercise intensity was calculated as the HRmean divided by HR_{max}, multiplied by 100 (%HR_{max}). HR_{max} was determined by the maximum HR across 14 days of practice within each player.

3.2.8 Statistical Analysis

Data were analyzed using JMPro 14 (SAS Inc., Cary, NC). Cool days were categorized by a WBGT <18°C and ≥18°C as warm days. Outcome variables included body weight loss, fluid intake, LSR, sweat and urine electrolytes, USG, and HR. One and two-way analysis of variance (ANOVA) was used to analyze outcome variables in different positions (FW, MD, DF, and GK) and differences between temperature with post-hoc tests to examine group differences. Chi-square was used to analyze the hydration status by positions. Pearson product moment correlations were used to examine the relationships between outcome variables. Data are presented as mean ± standard deviation (SD) and statistical significance was accepted at $P<0.05$.

3.3 Results

Eighteen players (19±1y) were observed across 14 days of practice. Practices were held on 6 cool days (4 morning, 2 evening) and 8 warm days (7 morning, 1 evening; Table 1). Player compliance was 90% with 9 players missing practice due to injury or researchers did

not have reasonable access to the player. Across the 9 players missing practice, a total of 9 cool day and 17 warm day practices were missed. Two players missed 6 practices (5 Cool, 7 Warm) while the remaining 7 players missed no more than 3 practices (4 Cool, 10 Warm). Sweat samples (n=8) were eliminated due to sample contamination or drop off during practice. Loss of urine samples from pre (n=7) to post (n=13) practice can be attributed to players running late prior to practice or late to class after practice. The mean WBGT and humidity across all practices were 18.3±3.1°C (range: 10.2-23.6°C) and 63.9±17.3% (range: 28.7-86.0%), respectively.

Table 3.1. Data collection and environmental conditions.

	Cool Environment	**Warm Environment**	**All**
Practices (n)	6	8	14
Pre urine samples (n)	94	125	219
Post urine samples (n)	94	119	213
Sweat samples (n)	93	125	218
Ambient temperature (°C)	19.7±2.8	22.3±1.4*	21.2±2.4
Humidity (%)	48.1±12.8	75.8±6.5*	64.1±16.7
WBGT (°C)	15.8±2.7	20.3±1.5*	18.4±3.0

WBGT, wet bulb globe temperature. *significant difference between cool and warm environment (all P<0.001).

Proportion of different hydration status by positions across all practices was displayed in Figure 1. Overall, most of players exhibited in Mi by 51% with remaining 26.7% of players in Eu, 20.5% of players in Mo, and only 1.9% of players in Se. In addition, DF exhibited the

most practices in Eu (11.9%). MD was the only position to exhibit Se (1.9%) and had the greatest proportion of practices Mo (11.0%). Within the position, greater proportion of practices moderately dehydration was found in 24% of FW and 27% of MD, whereas 12% of GK and 14% of DF.

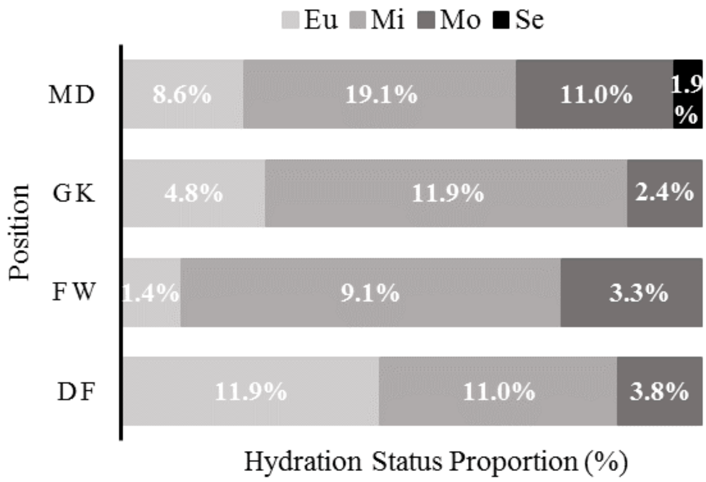


Figure 3.1. Hydration status in different positions across 14 practices. Light grey bars are euhydration status (Eu >0% body weight); Grey bars are mild dehydration status (Mild) categorized by <-1% body weight. Dark grey bars are moderate dehydration status (Mod) categorized by -1~-2% body weight; Black bars are severe dehydration status (Sev) categorized by >-2% body weight. DF defenders, FW forwards, GK goalkeepers, MD mid-fielders.

Markers of fluid balance across all practices and different by positions are presented in Table 2. Body weight loss was significantly lower in DF compared to FW, MD, and GK (P<0.001). In addition, DF had the greatest total fluid intake (728±369g) which was characterized by the greatest CES (525±351g) and least water (203±264g) ingestion compared to all other positions (P<0.001). In addition, total fluid intake was significantly associated with hydration status across positions (r=0.45; P<0.001). Mean pre-practice USG was 1.020±0.008 (range 1.003~1.037), with FW showing the greatest pre-practice USG. Urine electrolytes pre-

practice were not different between positions ($P>0.05$), except for [Na^+] was greater in FW compared to DF and MD ($P=0.02$). Post-practice FW, DF and GK demonstrated greater USG compared to MD ($P<0.001$). MD also had the lowest urine [Na^+] compared to DF and FW. GK had significantly lowest sweat [Na^+] compared to FW, MD, and DF ($P<0.001$). FW had significantly greater sweat [Na^+] compared MD and greater sweat [Cl^-] compared to MD and DF ($P<0.001$). Sweat [K^+] was greatest in DF compared to FW and MD ($P=0.03$). However, LSR was similar across all practices and all positions (Table 2).

Ambient temperature in the present study was highly variable across the 14 practices (18.3±3.1°C range: 10.2-23.6°C). Therefore, WBGT was categorized in cool (<18°C) and warm (≥18°C)) practice to gauge environmental heat stress on the main outcomes (Table 1). Cool and warm days did not affect the hydration status, total fluid intake, and sweat electrolyte concentrations ($P>0.05$). In addition, these outcomes exhibited the similar results across the different positions in cool and warm environments (Figure 2A and B). However, warm environment ($0.31\pm0.14 g/cm^2 \cdot hr$) resulted in a significantly greater LSR compared to cool environment ($0.24\pm0.12 g/cm^2 \cdot hr$; $P<0.001$). By positions, MD ($0.33\pm0.13\ g/cm^2 \cdot hr$) and DF ($0.31\pm0.15 g/cm^2 \cdot hr$) exhibited significantly greater LSR in warm compared to cool environment (MD: $0.25\pm0.13 g/cm^2 \cdot hr$ and DF: $0.22\pm0.11 g/cm^2 \cdot hr$; both $P=0.01$). FW and GK also exhibited greater LSR in warm environment, but not significance (Figure 2C).

An ancillary subset of 11 players wore heart rate monitors during 14 practices (3 FW, 5 MD, 3 DF). HR_{ave} was similar between positions (FW: 162±17 bpm, MD: 162±14 bpm, and DF: 161±16 bpm; $P=0.9$). MD had significantly greater HR_{max} (216±18 bpm) compared to FW (207±10 bpm) and DF (209±19 bpm; $P=0.04$). In addition, FW had significantly greater

exercise intensity (74.5±8.2% HR_{max}) compared to MD (67.6±5.8% HR_{max}) and DF (68.8±7.3% HR_{max}; $P<0.001$). In addition, exercise intensity (% HR_{max}) was significantly associated with sweat [Na^+] ($r=0.31$; $P=0.04$) across positions.

Table 3.2. Physical and Physiological characteristics of participants by field positions across training practices.

	FW (n=3)	MD (n=7)	DF (n=5)	GK (n=3)	All (n=18)	P value
Sample Size (n)	30	90	58	40	218	---
Age (years)	20±1	19±1	19±1	19±1	19±1	0.09
Weight (kg)	71.0±6.9§	63.5±4.9§†	63.9±6.0§†	82.7±6.1	68.5±9.0	0.001
Height (m)	1.69±0.05§‡	1.66±0.05§	1.68±0.09§‡	1.74±0.03	1.68±0.07	0.001
Fluid Balance						
Body weight loss (%)	-0.6±0.5*	-0.7±0.6*	-0.3±0.6	-0.5±0.5*	-0.5±0.6	<0.001
Water intake (g)	356±239*	357±275*§	203±264	473±367*	333±296	<0.001
CES intake (g)	233±248*§	242±256*§	525±351	80±179*	288±312	<0.001
Total fluid intake (g)	589±225*	607±273*	728±369	559±340*	630±312	0.03
Urine Analysis						
Pre USG	1.023±0.007‡	1.017±0.009	1.021±0.007‡	1.020±0.007	1.020±0.008	0.002
Pre [Na^+] (mmol/L)	178.5±64.8	133.1±72.4†	141.5±59.0†	151.4±69.3	144.8±68.6	0.02
Pre [K^+] (mmol/L)	42.8±17.0	43.0±31.0	51.4±27.7	56.8±39.9	47.7±30.7	0.07
Pre [Cl^-] (mmol/L)	158.4±57.8	128.0±71.9	132.2±54.8	141.8±75.9	135.7±66.9	0.16
Post USG	1.021±0.008‡	1.017±0.008	1.022±0.006‡	1.021±0.007‡	1.020±0.008	0.001
Post [Na^+] (mmol/L)	143.2±54.7	96.6±55.6†*	123.1±58.5	104.9±50.0†	111.3±57.4	0.001
Post [K^+] (mmol/L)	57.6±24.9	50.0±31.8	58.1±22.9	60.7±30.4	55.1±28.6	0.16
Post [Cl^-] (mmol/L)	140.6±57.3‡	95.1±58.3	120.9±55.2‡	121.4±65.6‡	112.7±60.5	0.001
Sweat Analysis						
Sweat [Na^+] (mmol/L)	55.7±16.3§	46.7±15.7†§	50.1±10.7§	21.2±6.6†	44.2±17.4	<0.001
Sweat [K^+] (mmol/L)	4.9±1.4*	5.3±0.9*	5.8±1.3	5.3±1.0	5.4±1.1	0.03
Sweat [Cl^-] (mmol/L)	46.1±15.2§	37.2±14.6†§	38.8±10.3†§	14.4±5.4†	34.7±16.0	0.001
LSR ($g/cm^2 \cdot hr$)	0.25±0.14	0.30±0.14	0.27±0.14	0.28±0.13	0.28±0.14	0.42

FW, forwards; MD, midfielders; DF, defenders; GK, goalkeepers; BSA body surface area, CES carbohydrate-electrolyte solution, USG urine specific gravity, LSR local sweat rate. *Significantly different compared to DF (P<0.05). §Significantly different compared to GK (P<0.05). ‡Significantly different compared to MD (P<0.05). †Significantly different compared to FW (P<0.05).

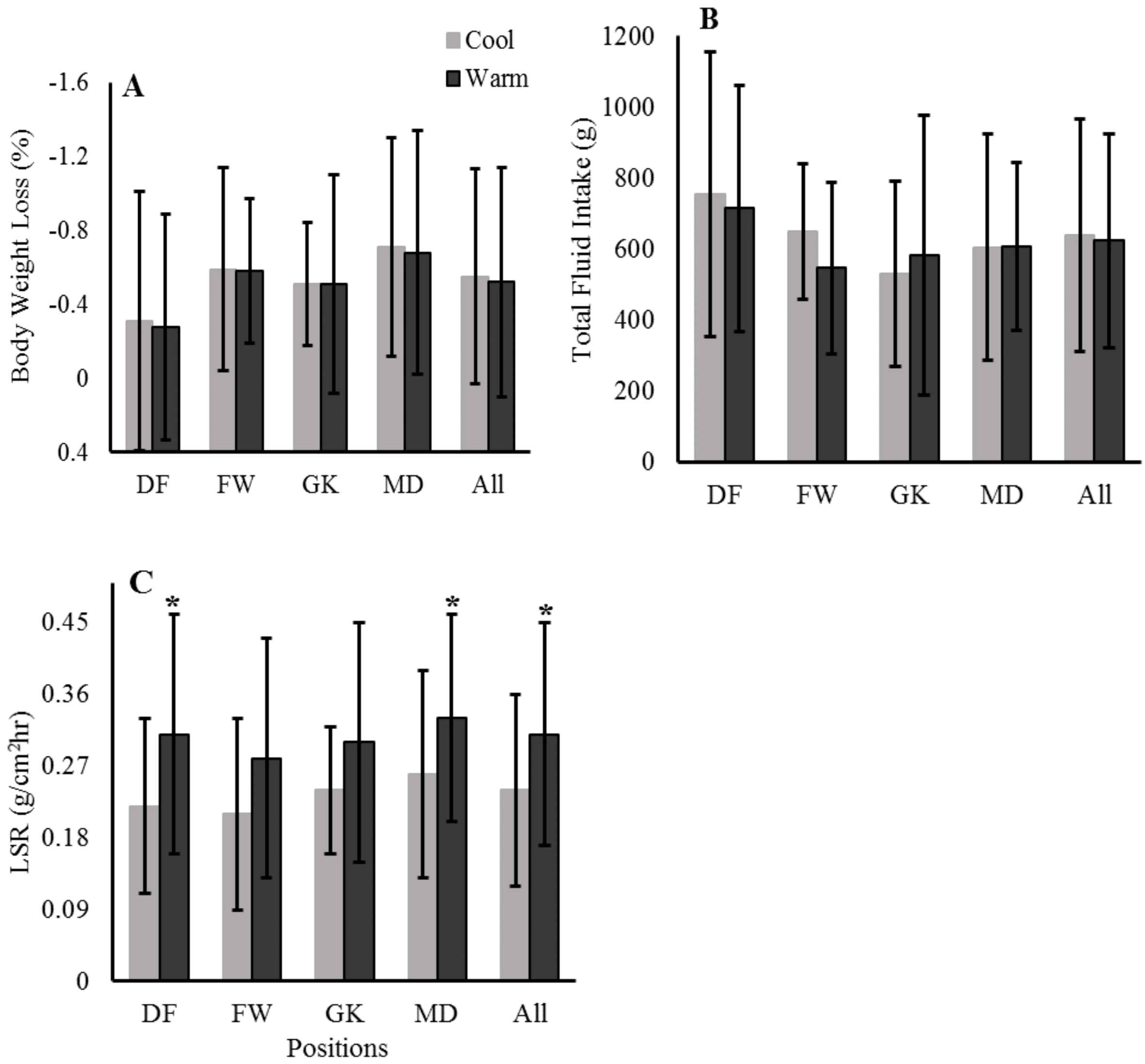


Figure 3.2. The effects of cool and warm temperatures on body weight loss (A), total fluid intake (B), and LSR (C) by different positions. Cool temperature was categorized by WBGT less than 18°C (light grey) and warm temperature was WBGT between 18-24°C (dark grey). DF defenders, FW forwards, GK goalkeepers, MD mid-fielders, All across all positions. *Significant different in LSR between cool and warm temperature in DF, MD, and across all positions (P<0.05). LSR local sweat rate.

3.4 Discussion

Uniquely, the present study examined the positional differences in fluid balance, sweat electrolytes and sweat rate, considering the differences of on-field physical demands by position. We observed players were on average mildly dehydrated (-0.5%BW) after practice.

Before practice, FW, MD, and GK on average exhibited slight dehydration (USG >1.020), which persisted throughout practice. Interestingly, we found DF had a significantly greater total fluid intake resulting in a better fluid balance compared to other positions. Across all practices, all positions spent a small proportion of practices euhydrated. MD showed the greatest risk for moderately and severely dehydration. FW exhibited greater sweat sodium and chloride concentrations, whereas GK had the lowest compared to other positions. Comparing practices on warm and cool days, we found little effect on fluid balance or sweat electrolytes. Cool and warm environments significantly affected LSR but had no influence on drinking behaviors or hydration status between positions.

3.4.1 Overall Hydration Status

In the present study, the hydration status of players was similar to the previous female soccer hydration studies regardless of the skill level.[12,13] Kilding et al. (2009) found players were mildly dehydrated (-0.6%BW) with a similar fluid intake during game specific training to the present study.[12] Gibson et al. (2012) found players experienced greater dehydration (-0.8% BW) but had a smaller fluid intake.[13] Both studies were performed in cooler environments (9-14 ºC) and did not examine differences in positions. The present study had less fluid balance deficit compared to previous research potentially due to the greater total fluid intake and environment conditions. Cool environmental conditions may decrease sweat loss and physiological drive for fluid consumption.[22] Warmer temperatures may have provoked players to drink more *ad libitum*. However, the present study found total fluid intake was not different between cool and warm days. Large variation of total fluid intake was found between and

within positions. Thus, the hydration status was likely associated with total fluid intake regardless of environmental conditions. Variation of total fluid intake between positions might be associated with the other factors, such as personal drink behavior or exercise intensity rather. For example, DF seemed to prefer CES beverages more than FW and MD in the present study.

3.4.2 Positional Difference

By positions, DF had a significantly greater total fluid intake resulted in a better fluid balance compared to other positions. In the previous research, on-field running distances had been proposed that is one of the factors to affect total fluid intake in soccer players.[23] In addition, previous study suggested that athletes who perform more runs might not tolerance the ingestion of large volumes of fluids during exercise.[24] As mentioned earlier, MD and FW required more sprints and runs compared to DF in the matches and training. Thus, fluid that can increase gastric emptying may be more important to FW and MD, facilitating the total fluid intake. Our study demonstrated MD were at greatest risk for moderate to severe dehydration. In addition, FW spent the least proportion of practices euhydrated. We examined on-field heart rate to assess exercise intensity in a subset of assigned players (3 FW, 5 MD, and 3 DF). Exercise intensity appeared greater in FW (75% HR_{max}) compared to MD (68% HR_{max}) and DF (67% HR_{max}), which is consisted with previous research[17]. MD exhibited greatest maximal heart rate during the practices, potentially suggesting more continuous on-field runs. Therefore, these positional differences suggest characteristics of exercise performed varies each position which may influence hydration status.

FW exhibited greater sweat sodium and chloride electrolyte concentrations, whereas GK had the lowest. Sweat sodium concentration have been shown to elevate as exercise intensity increases.[25] GK is considered a less active position compared to other positions, possibly resulting in the lowest sweat electrolyte concentrations. As support, a moderate relationship (0.31; p=0.004) between sweat sodium concentration and exercise intensity was seen in the present study, suggesting exercise intensity is a factor associated with sweat responses. The mean sweat sodium concentration in the present study was similar to previous studies despite different techniques.[12,13,26] We applied sweat patches to the lower back for 60 minutes to easily adhere and remove from players without interfering with their practice. Low back sweat sodium concentration has been associated with whole-body sweat sodium concentration,[27] suggesting the validity of our results. By limiting sweat patch duration to 60 minutes oversaturation was prevented, which can falsely elevate sweat electrolyte concentrations.[28] Therefore, the technique of sweat collection in the present study was a fast and reliable method, which provided a valuable application for the on-field team sports with assessing large number of players.

3.4.3 Warm and Cool Environments

There was no difference in LSR observed between positions across all practices. Previous studies have reported sweat rate cannot easily be explained by fluid intake, pre-exercise hydration status, and variations in body mass within players.[22,29] However, our results showed LSR was affected by cool and warm environments, mainly exhibited by MD and DF. GK and FW also exhibited greater LSR in warm environment, but not significance. During

exercise in the warm environment, the temperature gradient between skin temperature and air temperature is lower than that players expose in cool environment. In addition, the temperature gradient between core and the environment is also reduced. Consequently, cooling mechanism of hypothalamus is activated to increase skin blood flow leading to a greater rate of sweat production for evaporative heat loss.[30] Therefore, cool and warm environments significantly affected LSR across positions in the present study and this finding needs to be confirmed in the future study.

3.4.4 Implications

This study was a field research to investigate the fluid balance in female soccer players and also grouped by different positions. Laboratory settings may not duplicate environmental conditions and methods for actual outdoor team sports.[23] Importantly, in soccer matches, the movements of soccer players are based on the ball movement, so physical effort could be affected leading to different outcomes between laboratory and field studies.[31] Our on-field study provided important findings relative to hydration status, which can generalize to real-life practices in collegiate female soccer players under unique environmental condition. However, no running distance data were measured in the present study. Previous study suggested that total distance covered is one of the best predictors for the hydration status in NCAA Division I soccer players during the pre-season training.[32] In our study, HR was collected to at minimum demonstrate physical effort. Thus, this data can further aid to explain the differentiated hydration status of each position in collegiate female soccer players. In addition, players' menstrual cycle, which has been showed to associate with fluid fluctuations and fluid balance,

was not recorded.[33] In addition. whole-body sweat rate was not estimated because the urine volume was not measured. Collegiate athletes have several demands on time (class, tutoring, study hall, etc.) leaving quick transitions from academic to practice. There was limited time to assess player measurements such as attaching sweat patches, weighing water bottles or collect urine in order to not influence player's training schedule. Therefore, when assessing a large number of players real-time, on-field the quickest and most practical method should be applied.

3.4.5 Conclusion

In the present study, a large proportion of players exhibited in mildly dehydration before and after practice, with average ~600g total fluid intake across practices. FW and MD appeared to be at the greatest risk of moderate dehydration, potentially associated with their high intensity activity. Of note, severe dehydration only occurred in MD, suggesting hydration requirements vary by position. DF exhibited a greater total fluid intake resulting in a smaller fluid balance deficit. FW had the greatest sweat sodium concentration, whereas GK had lowest, suggesting exercise intensity and beverage type are factors to consider when developing a hydration strategy. LSR was not affected by total fluid intake or hydration status when comparing positions. However, LSR appeared to be influenced by cool and warm environmental conditions. This is the first study to describe the positional differences in hydration status of collegiate female soccer players. Water and sodium replacement which is necessary during and after practice should be tailored to the position. In addition, hydration strategies should be in place before practices and matches to ensure players are continuing to fuel their body for the demands of the sport.

3.5 References

1. Sporis G, Jukic I, Ostojic SM, Milanovic D. Fitness profiling in soccer: physical and physiologic characteristics of elite players. *Journal of strength and conditioning research / National Strength & Conditioning Association.* 2009;23(7):1947-1953.

2. Guttierres APM, Natali AJ, Vianna JM, Reis VM, Marins JCB. Dehydration in Soccer Players After a Match in the Heat. *Biology of Sport.* 2011;28(4):249-254.

3. Maughan RJ, Merson SJ, Broad NP, Shirreffs SM. Fluid and electrolyte intake and loss in elite soccer players during training. *International journal of sport nutrition and exercise metabolism.* 2004;14(3):333-346.

4. Sawka MN, Burke LM, Eichner ER, Maughan RJ, Montain SJ, Stachenfeld NS. American College of Sports Medicine position stand. Exercise and fluid replacement. *Medicine and science in sports and exercise.* 2007;39(2):377-390.

5. Shirreffs SM, Sawka MN. Fluid and electrolyte needs for training, competition, and recovery. *Journal of sports sciences.* 2011;29 Suppl 1:S39-46.

6. Armstrong LE, Costill DL, Fink WJ. Influence of diuretic-induced dehydration on competitive running performance. *Medicine and science in sports and exercise.* 1985;17(4):456-461.

7. Nuccio RP, Barnes KA, Carter JM, Baker LB. Fluid Balance in Team Sport Athletes and the Effect of Hypohydration on Cognitive, Technical, and Physical Performance. *Sports medicine.* 2017;47(10):1951-1982.

8. Davis JK, Laurent CM, Allen KE, et al. Influence of Dehydration on Intermittent Sprint Performance. *Journal of strength and conditioning research / National Strength & Conditioning Association.* 2015;29(9):2586-2593.

9. Goulet ED. Dehydration and endurance performance in competitive athletes. *Nutrition reviews.* 2012;70 Suppl 2:S132-136.

10. McDermott BP, Anderson SA, Armstrong LE, et al. National Athletic Trainers' Association Position Statement: Fluid Replacement for the Physically Active. *Journal of athletic training.* 2017;52(9):877-895.

11. Mauricio Castro-Sepulveda, Javier Astudillo, Pablo Letelier, Zbinden-Foncea H. Prevalence of Dehydration Before Training Sessions, Friendly and Official Matches in Elite Female Soccer Players. *Journal of human kinetics.* 2016;50:79-84.

12. Kilding AE, Tunstall H, Wraith E, Good M, Gammon C, Smith C. Sweat rate and sweat electrolyte composition in international female soccer players during game specific training. *International journal of sports medicine.* 2009;30(6):443-447.

13. Gibson JC, Stuart-Hill LA, Pethick W, Gaul CA. Hydration status and fluid and sodium balance in elite Canadian junior women's soccer players in a cool environment. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme.* 2012;37(5):931-937.

14. Orendurff MS, Walker JD, Jovanovic M, Tulchin KL, Levy M, Hoffmann DK. Intensity and duration of intermittent exercise and recovery during a soccer match. *Journal of strength and conditioning research / National Strength & Conditioning Association.* 2010;24(10):2683-2692.

15. Mohr M, Krustrup P, Andersson H, Kirkendal D, Bangsbo J. Match activities of elite women soccer players at different performance levels. *Journal of strength and conditioning research / National Strength & Conditioning Association.* 2008;22(2):341-349.

16. Davis JA, Brewer J. Applied physiology of female soccer players. *Sports medicine.* 1993;16(3):180-189.

17. Datson N, Drust B, Weston M, Jarman IH, Lisboa PJ, Gregson W. Match Physical Performance of Elite Female Soccer Players During International Competition. *Journal of strength and conditioning research / National Strength & Conditioning Association.* 2017;31(9):2379-2387.

18. Mara JK, Thompson KG, Pumpa KL, Morgan S. Quantifying the High-Speed Running and Sprinting Profiles of Elite Female Soccer Players During Competitive Matches Using an Optical Player Tracking System. *Journal of strength and conditioning research / National Strength & Conditioning Association.* 2017;31(6):1500-1508.

19. Lockie RG, Moreno MR, Lazar A, et al. The Physical and Athletic Performance Characteristics of Division I Collegiate Female Soccer Players by Position. *Journal of strength and conditioning research / National Strength & Conditioning Association.* 2018;32(2):334-343.

20. Gabbett TJ, Mulvey MJ. Time-motion analysis of small-sided training games and competition in elite women soccer players. *Journal of strength and conditioning research / National Strength & Conditioning Association.* 2008;22(2):543-552.

21. Morris NB, Cramer MN, Hodder SG, Havenith G, Jay O. A comparison between the

technical absorbent and ventilated capsule methods for measuring local sweat rate. *Journal of applied physiology.* 2013;114(6):816-823.

22. Maughan RJ, Shirreffs SM, Merson SJ, Horswill CA. Fluid and electrolyte balance in elite male football (soccer) players training in a cool environment. *Journal of sports sciences.* 2005;23(1):73-79.

23. Godek SF, Bartolozzi AR, Peduzzi C, et al. Fluid consumption and sweating in National Football League and collegiate football players with different access to fluids during practice. *Journal of athletic training.* 2010;45(2):128-135.

24. Brouns F. Gastric emptying as a regulatory factor in fluid uptake. *International journal of sports medicine.* 1998;19 Suppl 2:S125-128.

25. Baker LB, De Chavez PJD, Ungaro CT, et al. Exercise intensity effects on total sweat electrolyte losses and regional vs. whole-body sweat [Na(+)], [Cl(-)], and [K(+)]. *European journal of applied physiology.* 2019;119(2):361-375.

26. Shirreffs SM, Sawka MN, Stone M. Water and electrolyte needs for football training and match-play. *Journal of sports sciences.* 2006;24(7):699-707.

27. Patterson MJ, Galloway SD, Nimmo MA. Variations in regional sweat composition in normal human males. *Exp Physiol.* 2000;85(6):869-875.

28. Weschler LB. Sweat electrolyte concentrations obtained from within occlusive coverings are falsely high because sweat itself leaches skin electrolytes. *Journal of applied physiology.* 2008;105(4):1376-1377.

29. Shirreffs SM, Aragon-Vargas LF, Chamorro M, Maughan RJ, Serratosa L, Zachwieja JJ. The sweating response of elite professional soccer players to training in the heat. *International journal of sports medicine.* 2005;26(2):90-95.

30. Galloway SD, Maughan RJ. Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. *Medicine and science in sports and exercise.* 1997;29(9):1240-1249.

31. Drust B, Atkinson G, Reilly T. Future perspectives in the evaluation of the physiological demands of soccer. *Sports medicine.* 2007;37(9):783-805.

32. Sekiguchi Y, Adams WM, Curtis RM, Benjamin CL, Casa DJ. Factors influencing hydration status during a National Collegiate Athletics Association division 1 soccer preseason. *Journal of science and medicine in sport / Sports Medicine Australia.*

2019;22(6):624-628.

33. Claybaugh JR, Sato AK, Crosswhite LK, Hassell LH. Effects of time of day, gender, and menstrual cycle phase on the human response to a water load. *Am J Physiol Regul Integr Comp Physiol.* 2000;279(3):R966-973.

CHAPTER 4. A LONGITUDINAL STUDY OF HEMATOLOGICAL RESPONSES, HYDRATION STATUS, AND ON-FIELD RUNNING PERFORMANCE IN NCAA DIVISION I AMERICAN FOOTBALL PLAYERS WITH SICKLE CELL TRAIT

4.1 Introduction

Sickle cell trait (SCT) is a genetic condition characterized by the presence of hemoglobin S (HbS), which makes up approximately 40% of total hemoglobin.[1] The molecular structure of HbS reduces its oxygen affinity, resulting in less oxygen delivery to active muscle and contributing to local hypoxia, which is vital for optimal performance in athletes.[1,2] SCT is seen in 7%~9% of African Americans and up to 40% in Western Africans.[3] Previous studies have investigated the effects of acute exercise on SCT individuals, suggesting SCT carriers had greater blood viscosity and red blood cell rigidity at rest, during intensive exercise, and subsequent recovery.[4] In addition, Tripette et al. (2010) demonstrated that hyperviscosity should be considered a risk factor associated with exercise-related sudden death in SCT carriers.[5] The physiological and environmental stress during exercise including exercise-induced acidosis, dehydration, heat stress, and regional hypoxemia, promote hematological changes that can increase the risk of microcirculatory disorders, cardiovascular complications, and fatality in SCT carriers.[4]

American football players with SCT have an increased risk of complications due to the exhaustive nature of practice and competitions. In addition, geographical location with hot and humid environmental conditions provide an extra challenge to the players with SCT. Previous reports showed that NCAA Division I football players with SCT experience a higher percentage of heat-related illness and exertional-related death with up to 16-fold to 21-fold

compared to non-SCT players.[2,6] From 2000~2010, 16 deaths have occurred during sport-related conditioning and games in Division I football players, among which 10 (63%) were SCT carriers. Hematological alterations, inflammation, and vascular adhesion have been proposed as potential mediators of the microcirculatory changes that induce sickle cell anemia.[7,8] Although a clear relationship exists between SCT and risk of exertional-related sudden death in collegiate American football, the pathophysiology of sickling-induced hematological alterations is still unknown.[9] According to the NCAA precaution guidelines, the main prevention of sickling crisis is educating players to avoid heat stress and dehydration. The conditioning regimen must be implemented slowly and gradually allowing players have enough resting intervals. In addition, athletic training staff are required to identify the signs and symptoms of sickle cell crisis and be able to differentiate with heat cramp.[10] To our knowledge, no study has investigated the effects of training and competitions across the entire season on players with SCT, and it is necessary to assess the fitness test and blood works periodically during the season. Therefore, the purpose of this study was to conduct a longitudinal study examining hematological responses, hydration status, and on-field running performance in NCAA Division I football players with SCT.

4.2 Methods

4.2.1 Participants

Twenty NCAA Division I football players were recruited in two consecutive years (10 SCT and 10 position-matched controls) from a university located in the southeast. The physical characteristics of players are displayed in Table 1. The protocol and informed consent were

approved by the university Institutional Review Board (IRB) and all participants signed informed consents before any assessments were completed.

Table 4.1. Physical characteristics of participants by groups.

	SCT (n=10)	CON (n=10)
Age (yr)	19.8±1.6 (18~23)	21.1±1.2 (19~23)
Height (cm)	186.7±5.4 (177.8~193.1)	190.9±4.9 (180.3~198.1)
Weight (kg)	110.6±28.4 (80.0~154.7)	109.9±24.7 (84.5~145.2)
Hemoglobin-A (%)	57.3±2.5 (55.4~64.0)*	95.2±0.4 (94.8~95.8)
Hemoglobin-A2 (%)	3.5±0.2 (3.3~3.7)	2.9±0.2 (2.5~3.1)
Hemoglobin-F (%)	0.4±0.2 (0.1~0.8)	0.5±0.2 (0.2~0.9)
Hemoglobin-S (%)	37.9±2.4 (31.4~40.1)*	0
Hemoglobin-C (%)	0	0

Data are presented as Mean±SD (Range), *SCT* sickle cell trait; *CON* control group with normal hemoglobin. *Significant difference between groups.

4.2.2 Study Design and Visits

Blood was drawn from the players at three visits: 1) before pre-season training camp started (pre-camp), 2) pre-season training camp finished (post-camp/pre-season), and 3) at end of the season (post-season). There were 17 consecutive daily scrimmages between pre-camp and post-camp and 13 weekly in-season competitions between post-camp and post-season (Figure 1). Physiological monitoring was conducted at each daily scrimmage practice in the pre-season training camp, included hydration status (urine and sweat) and on-field running performance. Pre-camp data collection served as the baseline measurements. The follow-up measurements examined the alterations associated with practice and competitive season.

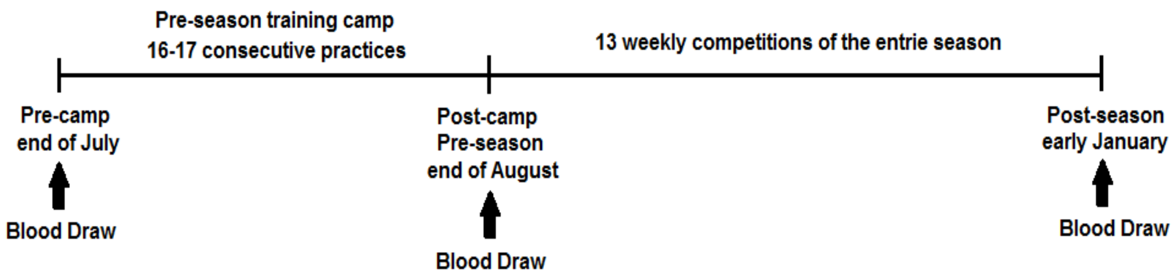


Figure 4.1. The time-point of the blood draws throughout the entire football season.

4.2.3 Blood samples

Each participant had 10 ml of whole blood (EDTA) and 10 ml of serum drawn at each time point to measure hemoglobin variants, a chemistry panel 26 (CM26) and complete blood cell counts (CBC) associated with sickle cell trait.

4.2.4 Measures of Hydration Status

Environmental conditions were measured before and after practice using wet-bulb globe temperature (WBGT; IL, USA). Body weight was assessed using a standard scale (TANITA, Arlington Heights, IL, USA) with a sensitivity of 0.1 kg before and after each practice session. Weight was taken in minimal clothing after toweling dry. The change in body weight was used to determine overall hydration status and the changes of fluid balance. Urine samples were collected before and after each practice session for urine specific gravity (USG; ATAGO CO., LTD, USA). Urine electrolyte concentrations (sodium [Na^+], potassium [K^+], and chloride [Cl^-]) were assessed by ion-selective probes (*MEDICA* EasyLyte; Bedford, MA, USA). Sweat was collected from the lower back region using the technical absorbent patch technique.[11] Alcohol spray and dry towels were used to clean the skin surface before the sweat

patch was attached. Sweat was collected at the first resting interval after 60 min of practice. The local sweat rate (LSR) was calculated by weight differences of the absorbent material before and after training divided by the product of the sweat patch area and time duration ($g/cm^2 \cdot hr$). Sweat patches were centrifuged and the resultant sweat was analyzed for electrolyte concentrations (sodium [Na^+], potassium [K^+], and chloride [Cl^-]) similarly to the urine.

4.2.5 On-field Running Performance

On-field running distance was measured using the Polar Team Pro System (Kempele, Finland). The device provided heart rate data (HR), the time spent in different heart rate intensity zones (50~59%, 60~69%, 70~79%, 80~89%, and 90~100% HR_{max}), total running distance, and running distance at various speed zones (3~6.9, 7~10.9, 11~14.9, 15~18.9, and >19km/h). The main outcomes were to investigate the running distance and heart rate during practices.

4.2.6 Statistical Analysis

Data were analyzed using JMPro 14 (SAS Inc., Cary, NC, USA). Two-way repeated measures analysis of variance (RM ANOVA) was used to analyze hemoglobin electrophoresis, CBC, and CM26 between two groups (SCT and CON) with position matched paired t-test. In addition, paired t-test was used to analyze outcome variables of hydration and running performance between two groups (SCT and CON), including fluid balance, LSR, sweat and urine electrolyte concentrations, USG, urine color, running distance and HR. Data are presented as mean ± standard deviation (SD) and statistical significance was set at $P<0.05$.

4.3 Results

4.3.1 Hydration Status

Hydration markers derived from urine and sweat samples were collected during 17 practices in the pre-season training camp under averaged 26.8±1.9°C (WBGT) and 68.9±11.1% relative humidity. The hydration markers were presented in Table 2, showed no difference in body weight changes between two groups (SCT: -0.9±0.9 vs. CON: -1.3±1.1kg; P=0.16). However, SCT players had a significantly lower pre- and post-practice urine electrolyte concentrations ([Na^+], [K^+], and [Cl^-]). In addition, a significantly lower pre- and post-practice USG was observed in SCT (1.019±0.006) compared to CON (1.026±0.008; P<0.001). Sweat sodium (SCT: 62.3±22.6 vs. CON: 45.9±17.6 mmol/L; P<0.001) and potassium concentrations (SCT: 4.7±1.2 vs. CON: 4.3±0.9 mmol/L; P=0.002) were significant greater in SCT compared to CON. However, LSR was not difference (SCT: 0.40±0.15 vs. CON: 0.42±0.16g/cm^2·hr; P=0.32).

Table 4.2. Football pre-season camp practice hydration markers by groups.

Variables	Measurements (n=17)	SCT (n=9)	CON (n=9)	P value
Body Weight Loss (kg)	n=12	-0.9 (-1.1~-0.7)	-1.3 (-1.5~-1.1)	0.16
Body Weight Change (%)	n=12	-0.8 (-0.9~-0.6)	-1.0 (-1.2~-0.9)	0.25
Pre-Urine Na^+ (mmol/L)	n=15	98.4 (92.8~103.9)*	159.6 (148.8~170.4)	<0.001
Pre-Urine K^+ (mmol/L)	n=15	37.2 (34.0~40.4)*	55.8 (50.8~60.8)	0.007
Pre-Urine Cl^- (mmol/L)	n=15	88.5 (82.6~94.4)*	150.2 (140.3~160.1)	<0.001
Pre USG	n=15	1.019 (1.019~1.020)*	1.026 (1.025~1.027)	0.004
Post-Urine Na^+ (mmol/L)	n=14	72.0 (67.1~76.8)*	116.6 (107.4~125.7)	0.006
Post-Urine K^+ (mmol/L)	n=14	37.4 (35.3~39.5)*	63.8 (59.2~68.4)	<0.001
Post-Urine Cl^- (mmol/L)	n=14	57.1 (52.1~62.1)*	93.7 (85.2~102.1)	0.02
Post USG	n=14	1.020 (1.019~1.021)*	1.030 (1.028~1.031)	<0.001
Sweat Na^+ (mmol/L)	n=14	62.3 (58.2~66.4)*	45.9 (42.7~49.1)	0.01
Sweat K^+ (mmol/L)	n=14	4.7 (4.5~4.9)	4.3 (4.1~4.5)	0.29
Sweat Cl^- (mmol/L)	n=14	54.0 (50.2~57.7)*	39.2 (36.2~42.1)	0.03
Local Sweat Rate (g/cm^2hr)	n=14	0.40 (0.38~0.43)	0.42 (0.39`0.42)	0.32

Pre; pre-practice, Post; post-practice, SCT; sickle cell players, CON position-matched control. *Significant difference in SCT compared to CON. Data were presented as mean (95% CI) of averaged 17 practices in the pre-season training camp.

Table 4.3. Completed blood count file during the entire football season by different groups.

Biomarkers	Reference	SCT (n=10)	CON (n=10)	Pre-camp	Post-camp	Post-season
RBC (10^6)	4.5~5.9	5.2±0.2	5.0±0.6	4.9±0.4	5.1±0.5$^{\$}$	5.2±0.5$^{\$}$
Hb (g/dL)	14~18	14.4±0.7	14.4±0.8	14.1±0.6	14.5±0.7$^{\$}$	14.7±0.7$^{\$}$
Hematocrit (%)	42~52	42.4±2.0	42.4±2.2	41.5±1.8$^{\&}$	42.2±1.9$^{\&}$	43.8±1.9
MCV (fL)	80~94	81.9±2.9	86.2±8.5	84.1±6.8$^{\#}$	83.5±6.5	84.6±6.9$^{\#}$
MCH (pg)	27~31	27.8±1.2	29.4±3.3	28.6±2.6	28.7±2.6	28.5±2.7
MCHC (g/dL)	32~36	34.0±0.7	34.1±0.8	34.0±0.7	34.3±0.8	33.7±0.6$^{\&}$
RDW (%)	11.5~14.5	14.3±0.9*	13.4±0.9	14.0±1.0	13.9±0.9	13.7±0.9
Platelet Count (10^3)	150~450	207.2±49.5	256.0±65.1	226.1±53.6	234.1±61.6	238.2±75.3
WBC (10^3)	3.6~8.9	5.6±1.6*	6.9±2.2	6.9±2.4	5.7±1.4	6.1±1.9
Neutrophil (%)	42.2~75.2	53.7±12.2	53.6±12.3	55.1±10.1	56.7±12.6	48.6±12.7
Lymphocyte (%)	20.5~51.1	32.6±8.1	35.2±11.0	32.6±8.6	32.5±10.6	36.9±9.6
Monocytes (%)	1.7~9.3	9.3±3.1	8.4±2.7	8.9±3.1$^{\#}$	7.9±2.0	9.8±3.3$^{\#}$
Eosinophils (%)	0~10	4.1±4.8	2.1±1.5	3.4±4.0	2.2±2.2	3.8±4.4
Basophils (%)	0~0.8	0.8±0.4	0.7±0.2	0.6±0.2	0.7±0.4	0.9±0.4$^{\&}$
Numbers of Neutrophil (10^3)	1.5~5.9	3.1±1.3	3.8±2.0	3.9±2.2	3.4±1.4	2.9±1.3
Numbers of Lymphocyte (10^3)	1.2~3.5	1.8±0.5*	2.3±0.9	2.2±0.6	1.8±0.5	2.2±1.2
Numbers of Monocytes (10^3)	0.1~0.6	0.5±0.2	0.6±0.2	0.6±0.2$^{\#}$	0.4±0.1	0.6±0.2$^{\#}$
Numbers of Eosinophils (10^3)	0~0.7	0.2±0.2	0.2±0.1	0.2±0.2	0.1±0.1	0.2±0.2
Numbers of Basophils (10^3)	0~0.2	0.02±0.04	0.04±0.05	0.02±0.04	0.03±0.04	0.05±0.05
Numbers of Reticulocytes (%)	0.9~2.3	1.3±0.5	1.3±0.5	1.3±0.5	1.3±0.5	1.2±0.5

SCT sickle cell trait, RBC red blood cells, Hb hemoglobin, MCV mean corpuscular volume, MCH mean corpuscular volume, MCHC mean corpuscular hemoglobin concentration, RDW red cell distribution width, WBC white blood cell count. Data presented as mean±SD. *Significant difference in SCT compared to CON; $^{\$}$Significant difference in pre-camp; $^{\#}$Significant difference in pre-season/post-camp; $^{\&}$Significant difference in post-season.

Table 4.4. Chemistry panel 26 profile during the entire football season by different groups.

Biomarkers	Reference	SCT (n=10)	CON (n=10)	Pre-camp	Post-camp	Post-season
BUN (mg/dL)	8~23	15.6±3.1	17.9±3.5	18.6±3.5	16.2±3.3$	14.9±2.1$
Creatinine (mg/dL)	0.9~1.3	1.2±0.2	1.2±0.2	1.3±0.2	1.2±0.2	1.2±0.2
Sodium (mmol/L)	136~147	137.3±1.3	137.9±1.3	138.1±1.2	137.3±1.5$	137.5±1.2$
Chloride (mmol/L)	101~111	103.7±1.4	103.6±1.4	103.9±1.3	103.7±1.2	103.3±1.8
Potassium (mmol/L)	3.7~5.0	4.3±0.4	4.3±0.3	4.2±0.3#	4.5±0.3	4.2±0.4#
Carbon Dioxide (mmol/L)	25~33	24.3±1.8	24.1±3.6	24.4±1.7&	25.4±1.5&	22.7±4.0
Uric Acid (mg/dL)	2.6~7.5	7.3±1.0*	6.1±0.6	6.8±1.1	6.7±1.2	6.7±0.9
Total Protein (g/dL)	5.8~7.9	7.3±0.3	7.4±0.4	7.2±0.3&	7.3±0.3&	7.5±0.2
Albumin (g/dL)	4.1~5.4	4.4±0.3	4.4±0.3	4.4±0.3	4.4±0.3	4.4±0.2
Calcium (mg/dL)	8.9~10.4	9.4±0.3	9.4±0.2	9.4±0.3	9.4±0.3	9.4±0.2
Magnesium (mg/dL)	1.7~2.4	2.1±0.1	2.1±0.1	2.1±0.1	2.1±0.1$	2.1±0.1$
Total Bilirubin (mg/dL)	0.2~1.5	0.9±0.3	1.0±0.4	0.9±0.4	0.9±0.4	1.0±0.3
CPK (IU/L)	38~333	955.2±797.2	718.0±509.5	1327.2±876.2	512.8±239.2$	647.3±388.3$
LDH (IU/L)	82~195	197.4±72.1	218.5±44.7	210.8±50.7	209.3±65.3	203.4±67.5
AST (IU/L)	13~47	37.9±15.5	35.0±9.4	42.6±17.2	31.6±6.6$	35.0±9.6$
ALT (IU/L)	10~60	28.0±10.9	29.2±11.6	31.9±11.2	26.2±9.9$	27.7±12.3$
ALK Phosphate (IU/L)	32~130	85.3±25.3	74.3±21.7	81.1±26.8	77.8±22.6	80.6±23.4
GGT (IU/L)	5~63	18.7±10.0	17.7±12.2	19.6±10.1	17.2±11.7	17.9±11.8
Amylase (U/L)	28~100	105.9±45.7	87.9±48.4	110.9±57.9	88.9±40.8$	89.2±39.7$
Iron (ug/dL)	50~160	83.3±29.5	92.7±38.3	69.7±25.7	97.6±31.6$	97.6±38.3$
Triglycerides (mg/dL)	44~201	89.8±51.9	89.9±55.1	77.8±46.2#	108.7±67.2	71.9±32.6#
HDL (mg/dL)	30~70	52.4±13.8	61.3±11.0	55.3±12.4	56.7±14.3	58.7±13.0
LDL (mg/dL)	66~165	102.0±20.7	82.8±19.4	91.1±24.6	90.0±17.5	95.4±24.3

BUN blood urine nitrogen, CPK creatine kinase, LDH Lactate Dehydrogenase, AST aspartate amino transferase, ALT alanine amino transferase, ALK alkaline phosphate, GGT gamma-glutamyl transferase. Data presented as mean±SD. *Significant different in SCT compared to CON; $Significant difference in pre-camp; #Significant difference in pre-season/post-camp; &Significant difference in post-season.

4.3.2 Blood Biomarkers

Hemoglobin-electrophoresis showed that SCT players had an average of 57.3±2.5% HbA and 37.9±2.4% HbS, and the percentages of HbA and HbS were consistent throughout the entire season (Table 1). CBC profile is displayed in Table 3 and exhibited a significant lower number of white blood count (WBC; SCT: 5.6±1.6 vs. CON: $6.9±2.2×10^3$; P=0.02) and lymphocytes (SCT: 1.8±0.5 vs. $2.3±0.9×10^3$; P=0.01) in SCT compared to CON. In addition, SCT had a significant greater red cell distribution width (RDW) compared to CON (14.3±0.9 vs. 13.4±0.9%; P=0.02) across all time points (Figure 2a). Platelet count was significantly lower in SCT compared to CON only at post-season (197.9±48.0 vs. $278.7±78.1×10^3$; P=0.03). Data from the CM26 can be found in Table 4 and showed uric acid was greater in SCT compared to CON throughout the entire season (SCT: 7.3±1.0 vs. 6.1±0.6mg/dL; P=0.001; Figure 2b). Serum CPK level was higher at pre-camp (1327.2±876.2IU/L) compared to post-camp (512.8±239.2IU/L) and post-season (647.3±388.3IU/L; P<0.001) across two groups. Moreover, SCT had a greater serum CPK level at pre-camp compared to CON (SCT: 1617.0±1034.8 vs. 1037.4±602.8IU/L; P=0.03; Figure 3a). Lastly, SCT had a significantly lower blood iron concentration at pre-season (86.0±24.0ug/dL) compared to CON (109.2±35.1ug/dL; P<0.01).

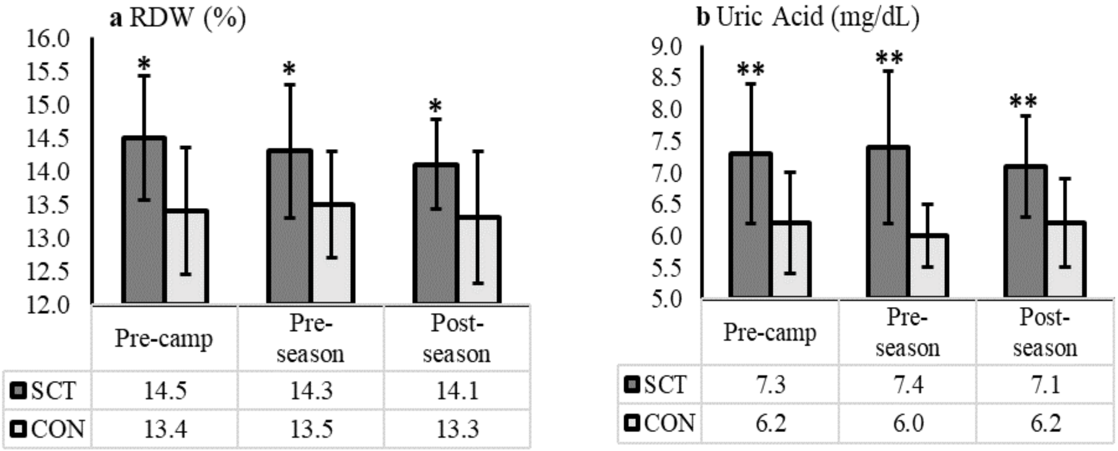


Figure 4.2. The red blood cell distribution width (RDW; a) and uric acid (b) across the visits by groups (SCT and CON). Dark grey bars are sickle cell trait players (SCT), and light grey bars are position-matched control group (CON). SCT players exhibited significantly greater RDW and uric acid across all visits compared to CON; *Significant differences P<0.05; **Significant differences P<0.01

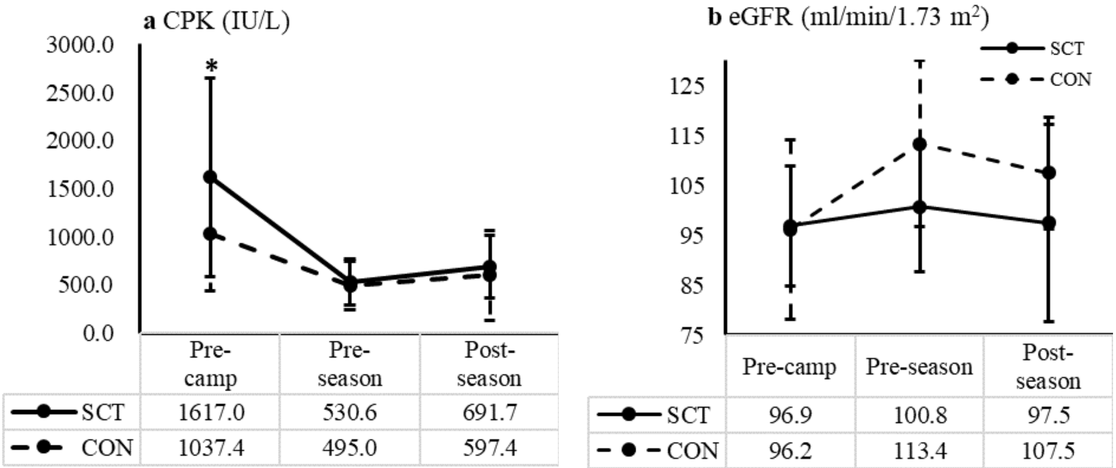


Figure 4.3. The CPK (creatine phosphokinase; a) and eGFR (estimated glomerular filtration rate; b) across the visits by groups (SCT and CON). Solid line indicates sickle cell trait players (SCT), and dash line indicates position-matched control group (CON). CPK levels were found significantly greater in SCT (1617.0±1034.8 IU/L) at pre-camp compared to CON (1037.4±602.8 IU/L; P=0.04). The eGFR was lower in SCT at pre-season and post-season but not significant compared to CON (all P>0.1). The eGFR was estimated by the equation from Shlipak et al. (2013)[20]

4.3.3 On-field Running Performance

Running distance and HR data were averaged cross 17 practices of pre-season camp training. SCT had a significantly lower HR_{max} (SCT: 196.2±18.3 vs. 208.4±20.2bpm; P=0.001) and higher on-field exercise intensity (SCT: 67.1±7.6 vs. 64.1±8.0% HR_{max}; P=0.004) compared to CON. Total running distance was approximately 485 meters (14%) less in SCT compared to CON during the training (SCT: 3520.7±1223.6 vs. 4005.7±1513.9 m; P=0.009). In addition, SCT ran less distance when at speeds of 7~10.9, 11~14.9, and 15~18.9km/h compared to CON (Figure 4). However, exercise time spent in different HR intensity zones (50~59%, 60~69%, 70~79%, 80~89%, and 90~100% HR_{max}) were similar between two groups (all P>0.05).

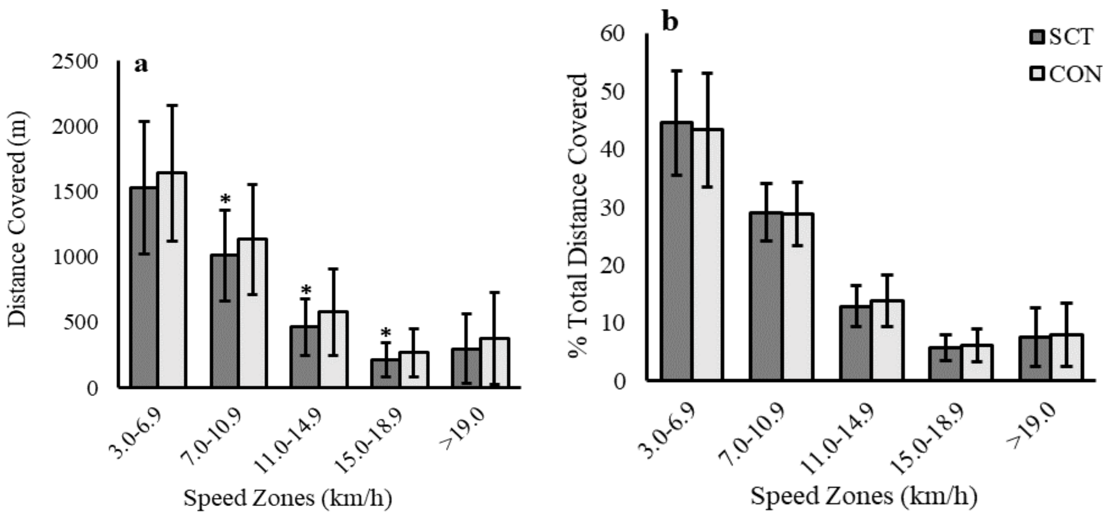


Figure 4.4. Distance ran (a) and the proportion of total distance (b) at different speed zones by groups (SCT vs. CON). Dark grey bars are sickle cell trait players (SCT), and light grey bars are control group (CON). SCT ran significantly less distance compared to CON at 7-10.9km/h (SCT: 1011.9±350.7 vs. CON: 1134.4±422.8m; P=0.02), 11-14.9 km/h (SCT: 463.8±213.9 vs. CON: 580.7±330.5m; P=0.001), and 15-18.9km/h (SCT: 215.2±130.9 vs. CON: 269.2±181.7M; P=0.01). The proportion of distance ran in total distance was similar across all speed zones.

4.4 Discussion

This is the first study to examine longitudinal changes in hematological parameters in SCT compared to CON. During the pre-season training camp, there were no statistically significant differences in fluid balance or LSR between groups. However, SCT players exhibited significantly lower pre- and post-practice urine electrolyte concentrations and USG. Higher sweat electrolyte concentrations were also observed in SCT compared to CON. Blood biomarkers revealed SCT players had 37.9% (31.4~40.1%) of HbS and greater RDW values compared to CON throughout the season. In addition, SCT players had significantly higher serum uric acid compared to CON throughout the entire season. Lastly, SCT players had a significantly higher CPK level at pre-camp and less running distance covered during practices compared to CON.

Our recent research focused on hydration and heat-related illness for those players who frequently compete in hot and humid environments. SCT players are at great risk of exercise-associated sickle cell collapse due to dehydration and heat stress.[9] In the present study, fluid balance was not statistically different between groups, but both groups were hypohydrated with approximate -1% body weight loss and elevated USG throughout the training. However, pre-and post-practice USG and urine electrolyte concentrations were significantly lower in SCT players compared to CON. Recently, our athletic training staff and coaches kept educating SCT players on the importance of fluid replacement before and during exercise. This result was possibly associated with greater total fluid consumed by SCT compared to CON. In addition, higher sweat sodium concentration was found in SCT players, suggesting a possible

physiological difference between SCT and CON players that need to be corroborated in future research.

The finding that SCT players had concomitant elevations in HbS and RDW is consistent with previous studies, which suggested HbS was associated with higher RDW value, indicating the greater degree of red cell anisocytosis and larger variation of red blood cell morphology.[12,13] In addition, this finding further confirms the physiological elevations in HbS among asymptomatic SCT players. Although, the immune cell counts appeared differently between the two groups, the values remained in the normal reference range in both groups and do not appear clinically different.

Surprisingly, serum uric acid remained significantly higher throughout the season in SCT players compared to CON, suggesting either overproduction or insufficient clearance. The potential mechanism has been proposed that serum uric acid is inversely associated with urine clearance rather than to the overproduction of uric acid.[14] Diamond et al. (1976) suggested that uric acid clearance was highly associated with renal excretion approaching the sum of new purine biosynthesis and dietary purine intake.[15] Previous studies have also reported that renal manifestations are commonly observed with an impaired urinary concentration in SCT carriers.[16-18] In our data, lower pre- and post-practice urine electrolyte concentrations in SCT players also possibly indicated less renal excretion to remove waste products through the urine. We also estimated the glomerular filtration rate (eGFR) based on serum creatinine.[19] We found that SCT players showed a trend for having lower eGFR at post-camp (pre-season) and post-season compared to CON, but these differences did not reach the level of statistical significance

(all P>0.1; Figure 3b). On the other hand, serum uric acid remained higher in both groups approaching the upper reference value. The previous study reported that serum uric acid is an antioxidant that contributed to >50% capacity of the blood.[20] Thus, serum uric acid may play protective role against exercise-induced oxidative stress.[21] In addition. previous studies showed that uric acid increased in response to the exercise, such as short-distance running[22] and ultramarathon racing.[23] The exercise-induced higher uric acid maybe associated with increased purine oxidation and subsequent formation of uric acid can be functioned as an antioxidant molecule.[24] In the present study, our participants were long-term highly trained elite collegiate American football players, and greater serum uric acid might be associated with the exercise effects. To our knowledge, no studies had been reported high serum uric acid in SCT players. However, the present study cannot explain whether greater serum uric acid observed in SCT players was associated with alterations of renal function. Future studies should further assess urine creatinine allowing to estimate fractional excretion of urine electrolyte concentrations, which can further aid to explain the capability of urine clearance in SCT players.

Another important consideration is the effect of exercise on muscle damage in SCT players because previous studies frequently suggested exertional rhabdomyolysis have been involved in SCT carriers.[25] Exertional rhabdomyolysis is a syndrome caused by the severe breakdown of skeletal muscle tissue.[26] Exertional rhabdomyolysis should be considered in NCAA football players with SCT, who are frequently exposed to demanding high-intensity exercise in hot and humid environmental conditions. In addition, laboratory tests have shown that serum CPK levels elevate when rhabdomyolysis occurs.[27] The present study found that

serum CPK level had 3-5 times higher than the upper reference limits in both groups across the visits. Furthermore, both groups had a significantly higher serum CPK level at pre-camp compared to other visits. Moghadam-Kia et al. (2016) showed serum CPK level could increase as much as 30 times of upper limit within 24 hours of strenuous exercise.[28] In addition, previous studies pointed out that muscle mass and total body mass were associated with serum CPK level.[29] This information further explained the greater serum CPK level in the present study, possibly associated with large body mass, especially muscle mass of our participants. Of note, serum CPK levels in the present study were greater at pre-camp in both groups and was significantly greater in SCT players. The possible explanation could be that date selection of blood draw in the pre-camp and subsequent of greater CPK level maybe a factor of resistance training. As such, this increase in serum CPK levels could be linked to methodological and sampling issues. Interestingly, both groups had a similar serum CPK level at post-camp. However, on-field running performance showed that SCT players had an average 681m less in total distance running on the last day of practice, which is 24-hour before post-camp blood draw. By averaging the running distance in 17 practices, SCT players had 485m (14%) less averaged running distance compared to CON. In addition, the distance running in all speed zones was less in SCT, but specially noted at speed zones of 7-10, 11-14.9, and 15-18.9km/h compared to CON. However, the proportion of distance running in different speed zones to the total distance was similar between the two groups. In addition, the time spent in training was similar between groups across different intensity zones. These findings suggested SCT players had a lower running performance compared to CON across the similar intensity zones. In

addition, SCT players might require longer resting intervals during training due to the nature of deoxygenated form of HbS, possibly resulting in greater muscle damage.[4,30] Although, the serum CPK level at pre-camp cannot explain the differences in muscle damage between two groups, the running distance and post-camp CPK level might indicate the differential effects of exercise on muscle breakdown between two groups. The present study cannot conclusively conclude that there is greater risk of muscle damage in SCT players compared to CON players. Knowing this might help athletic trainers and physicians to better detect how to distinguish the differences between sickle cell crisis and heat cramps. In the future, urine myoglobin can be measured as another indicator of muscle damage to further understand the difference in muscle breakdown between SCT and CON players.

The strength of the present study is the first to determine the alterations of hematology, hydration, and on-field running performance in elite collegiate football players with SCT in a longitudinal study design. Thompson et al. (2013) suggested that much of the published information on SCT and exertion injuries consisted of older data or only case series.[31] The results in the present study can provide further scope on this topic for laboratory-based research, which could focus on the renal function and muscle damage in SCT players. In addition, the present study will provide valuable information for the NCAA to develop additional strategies to prevent sickling crisis and exertional-related injuries during conditioning or competitions. The limitation of the present study is fluid intake was not measured during the camp training practices and we cannot conclude lower urine electrolytes was associated with more fluid intake or other factors. In addition, other important biomarkers were not assessed, such as urine

creatinine and urine myoglobin concentrations for determining the renal function and muscle breakdown in different groups. Lastly, the date chosen for blood draw at the pre-camp time point could be an issue to interpret the serum CPK level. However, this is the field-based study and the day chosen for blood draw was the most convenient according to players training schedule.

In conclusion, the present study showed that SCT players had similar fluid balance compared to CON but had a lower urine electrolyte concentrations and greater sweat sodium concentrations during training camp. The blood biomarker showed SCT players exhibited 31~40% HbS of hemoglobin and greater red blood cell distribution width. In addition, serum uric acid remained greater in SCT players throughout the entire season. Lastly, on-field running distance during training was 14% less in SCT players compared to position-matched controls. The results of this study can help athletic training staff and physicians better monitor SCT players during exhaustive training and competitions. Future research is worth exploring in the laboratory-based to examine the effects of exercise on the alterations of hematological biomarkers in players with SCT to further verify the results of the present study.

4.5 References

1. Le Gallais D, Bile A, Mercier J, Paschel M, Tonellot JL, Dauverchain J. Exercise-induced death in sickle cell trait: role of aging, training, and deconditioning. *Med Sci Sports Exerc.* 1996;28(5):541-544.

2. Eichner ER. Sickle cell considerations in athletes. *Clin Sports Med.* 2011;30(3):537-549.

3. Gozal D, Thiriet P, Mbala E, et al. Effect of different modalities of exercise and recovery on exercise performance in subjects with sickle cell trait. *Med Sci Sports Exerc.* 1992;24(12):1325-1331.

4. Connes P, Sara F, Hardy-Dessources MD, et al. Effects of short supramaximal exercise on hemorheology in sickle cell trait carriers. *Eur J Appl Physiol.* 2006;97(2):143-150.

5. Tripette J, Loko G, Samb A, et al. Effects of hydration and dehydration on blood rheology in sickle cell trait carriers during exercise. *Am J Physiol Heart Circ Physiol.* 2010;299(3):H908-914.

6. Eichner ER. Sickle cell trait in sports. *Curr Sports Med Rep.* 2010;9(6):347-351.

7. Tripette J, Connes P, Hedreville M, et al. Patterns of exercise-related inflammatory response in sickle cell trait carriers. *Br J Sports Med.* 2010;44(4):232-237.

8. Tripette J, Hardy-Dessources MD, Sara F, et al. Does repeated and heavy exercise impair blood rheology in carriers of sickle cell trait? *Clin J Sport Med.* 2007;17(6):465-470.

9. Loosemore M, Walsh SB, Morris E, Stewart G, Porter JB, Montgomery H. Sudden exertional death in sickle cell trait. *Br J Sports Med.* 2012;46(5):312-314.

10. Eichner ER. Sickle cell trait. *J Sport Rehabil.* 2007;16(3):197-203.

11. Morris NB, Cramer MN, Hodder SG, Havenith G, Jay O. A comparison between the technical absorbent and ventilated capsule methods for measuring local sweat rate. *J Appl Physiol (1985).* 2013;114(6):816-823.

12. Webster P, Castro O. Red cell distribution width in sickle cell disease. *Ann Clin Lab Sci.* 1986;16(4):274-277.

13. Olujohungbe A, Garvey B. Red cell distribution width (RDW) in sickle cell disease. *Clin Lab Haematol.* 1993;15(1):72-73.

14. Nugent CA, Tyler FH. The renal excretion of uric acid in patients with gout and in nongouty subjects. *J Clin Invest.* 1959;38:1890-1898.

15. Diamond HS, Neisel A, Kaplan D. Renal tubular transport of urate in man. *Bull Rheum Dis.* 1976;27(1):876-881.

16. Tsaras G, Owusu-Ansah A, Boateng FO, Amoateng-Adjepong Y. Complications associated with sickle cell trait: a brief narrative review. *Am J Med.* 2009;122(6):507-512.

17. Heller P, Best WR, Nelson RB, Becktel J. Clinical implications of sickle-cell trait and

glucose-6-phosphate dehydrogenase deficiency in hospitalized black male patients. *N Engl J Med.* 1979;300(18):1001-1005.

18. Statius van Eps LW, Pinedo-Veels C, de Vries GH, de Koning J. Nature of concentrating defect in sickle-cell nephropathy. Microradioangiographic studies. *Lancet.* 1970;1(7644):450-452.

19. Shlipak MG, Matsushita K, Arnlov J, et al. Cystatin C versus creatinine in determining risk based on kidney function. *N Engl J Med.* 2013;369(10):932-943.

20. Glantzounis GK, Tsimoyiannis EC, Kappas AM, Galaris DA. Uric acid and oxidative stress. *Curr Pharm Des.* 2005;11(32):4145-4151.

21. de Oliveira EP, Burini RC. High plasma uric acid concentration: causes and consequences. *Diabetol Metab Syndr.* 2012;4:12.

22. Westing YH, Ekblom B, Sjodin B. The metabolic relation between hypoxanthine and uric acid in man following maximal short-distance running. *Acta Physiol Scand.* 1989;137(3):341-345.

23. Mastaloudis A, Leonard SW, Traber MG. Oxidative stress in athletes during extreme endurance exercise. *Free Radic Biol Med.* 2001;31(7):911-922.

24. Tekin A. Xanthine oxidase and uric acid response to a 6-week pre-season training programme in male athletes. *African Journal of Pharmacy and Pharmacology.* 2010;4(8):511-515.

25. Harrelson GL, Fincher AL, Robinson JB. Acute exertional rhabdomyolysis and its relationship to sickle cell trait. *J Athl Train.* 1995;30(4):309-312.

26. Nelson DA, Deuster PA, Carter R, 3rd, Hill OT, Wolcott VL, Kurina LM. Sickle Cell Trait, Rhabdomyolysis, and Mortality among U.S. Army Soldiers. *N Engl J Med.* 2016;375(5):435-442.

27. Zhang MH. Rhabdomyolosis and its pathogenesis. *World J Emerg Med.* 2012;3(1):11-15.

28. Moghadam-Kia S, Oddis CV, Aggarwal R. Approach to asymptomatic creatine kinase elevation. *Cleve Clin J Med.* 2016;83(1):37-42.

29. Wong ET, Cobb C, Umehara MK, et al. Heterogeneity of serum creatine kinase activity among racial and gender groups of the population. *Am J Clin Pathol.* 1983;79(5):582-

586.

30. Vincent L, Oyono-Enguelle S, Feasson L, et al. Effects of regular physical activity on skeletal muscle structural, energetic, and microvascular properties in carriers of sickle cell trait. *J Appl Physiol (1985).* 2012;113(4):549-556.

31. Thompson AA. Sickle cell trait testing and athletic participation: a solution in search of a problem? *Hematology Am Soc Hematol Educ Program.* 2013;2013:632-637.

CHAPTER 5. THE EFFECTS OF HALF-TIME COOLING INTERVENTIONS ON MALE SOCCER-SIMULATED INTERMITTENT EXERCISE PERFORMANCE

5.1 Introduction

Soccer is an outdoor sport characterized by high intensity intermittent exercise with players performing multiple sprints, runs, walks, and stands. In addition, soccer players are frequently exposed to hot and humid environmental conditions with ambient temperature at ~30°C accompanied with high relative humidity in excess of 60%.[1,2] Hot and humid environmental conditions bring extra challenge on players causing elevated core and skin temperature resulting in a high thermal stress.[3] Core temperature elevated to 39°C had been observed in competitive soccer games under elevated heat conditions causing the performance reduction and exercise cessation.[4] Chmura et al. (2017) found sprint performance and total distance covered were significantly impaired when the ambient temperature exceeded 22°C with the relative humidity greater than 60% or when the ambient temperature exceeded 28°C regardless of relative humidity.[4,5] In addition, Nassis et al. (2015) suggested that soccer players exhibited a reduced number of sprints and increased passing rate with unchanged running speed during the competition in the heat conditions.[6]

Cooling interventions have been recently investigated for the purpose of reducing thermal strain and ameliorating physiological stress in intermittent exercise under heat conditions, suggesting the potential application in soccer events.[7] Previous studies showed cooling interventions applied during the warm-up phase before exercise significantly attenuated the increased thermal perceptual responses and improved total distance covered in intermittent exercise.[8,9] Wang et al. (2019) found that leg and thigh are the key body segments

to be cooled and affected thermal sensation in hot environments.[10] In addition, metabolic heat production is mostly generated from leg muscle during running and sprinting under the heat. Castle et al. (2006) observed pre-exercise leg cooling with ice packs significantly improved the power output of repeated sprints by 4% compared to no cooling in hot and humid conditions.[11] In addition, Hayashi et al. (2004) suggested leg cooling applied during recovery period can significantly reducing thermal and cardiorespiratory stress in the subsequent moderate exercise.[12] Although leg cooling had been reported to impair muscle power output due to cold exercise muscles,[13,14] leg cooling with appropriate temperature setting during exercise can potentially decrease thermal sensation and body temperature.[15] Therefore, the purpose of this study was to determine the effects of cooling interventions applied during the half-time on a soccer-specific intermittent exercise. We hypothesized that leg cooling application decreases the perceptual thermal sensation and body temperature.

5.2 Methods

5.2.1 Participants

Five male participants were recruited in this study (averaged age: 19.8±2.2 yr, weight: 79.9±19.1 kg, height: 180.4±7.4 cm, VO_{2max}: 50.5±4.2 ml/kg/min; Table 1). Inclusion criteria was healthy participants aged 18~30 yr and VO_{2max} greater than 45 ml/kg/min. Exclusion criteria were those with known cardiovascular, pulmonary or metabolic disease, or signs/symptoms categorized by the American College of Sports Medicine (ACSM).[16] In addition, no alcohol consumption and strenuous exercise 24-hr before experiment visits. This study was approved by university Institutional Review Board and inform consent was given to

the participants before any assessment.

Table 5.1. The physical characteristics of the participants (n=5).

Variable	Mean±SD	Range
Age (yr)	19.8±2.2	18–23
Height (cm)	180.4±7.4	173–190
Weight (kg)	79.9±19.1	62.2–110.2
VO2max (ml/kg/min)	50.5±4.2	45.0–56.7

5.2.2 Experiment Design

Participants completed a total 4 laboratory visits including pre-exercise screening visit, familiarization visits and two main exercise cooling trials in a randomized cross-over design. The main exercise cooling trials were conducted in a controlled climate environment chamber at a temperature of 30°C and 60% relative humidity. The exercise was a soccer-simulated intermittent exercise, consisting of two 45 min halves separated by a 15-min half-time. The cooling interventions were applied during the half-time period for 10 min with leg cooling (LEG) or no cooling (CON). The exercise tests were separately at least 4 days and the participants were required to complete at the same of the day with the same clothes.

5.2.3 Experiment Protocol

Anthropometrics were measured for each participant in body height and weight during the pre-screening visit. Maximal oxygen consumption (VO_{2max}) was assessed on a treadmill using indirect calorimetry (Parvo Medics TrueOne 2400, US) under normal room temperature 20~22°C. The test started at lower speed and grade, which were gradually increased in a regular

time increments for every 3 min until participants volitional fatigue. The VO_{2max} value was used to determine the inclusion/exclusion criteria and prescribe the speed during intermittent exercise.

Familiarization visit was to walk through the entire experiment settings with participants to negate the order effects of the main experiment trials. The food logs were provided to the participants to record food items and water intake 24-hour before familiarization visit. After participants arrived at the testing lab, study investigators reviewed the food logs with participants using 24-hour recalls form in an electronic format. The 24-hour recalls form was saved and printed to participants that ensure the similar diet can be followed before each experiment trial. The data of familiarization trial did not include in the statistical analysis.

The intermittent exercise was designed to simulate the demands of soccer physical characters including sprinting, running, walking, and standing. The time proportion of the physical characters was suggested by previous research.[17] The intermittent exercise consisted 30-s maximal sprinting at the speed of 80~90% VO_{2max}, 90-s running at the speed of 60% VO_{2max}, 150-s walking at the speed of 30% VO_{2max} and standing for 30-s (Figure 1). After 90 min of the exercise, participants kept performing the extra sets of intermittent exercise until volitional exhaustion or the core temperature reaches to 40°C. The number of sets was used to determine exercise performance and the number of sets was blinded to participants until three experiment trials finished. Participants had a 5 min warm up on the treadmill at speed of 8 km/h with 3 sets of sprints at self-selected speed. The entire exercise protocol had two 45 min halves

separated by 15 min half time. Each half contains 9 sets of 5 min intermittent exercise. Water was provided to participant at 10~15 ml/kg *ad libitum* during the entire exercise.

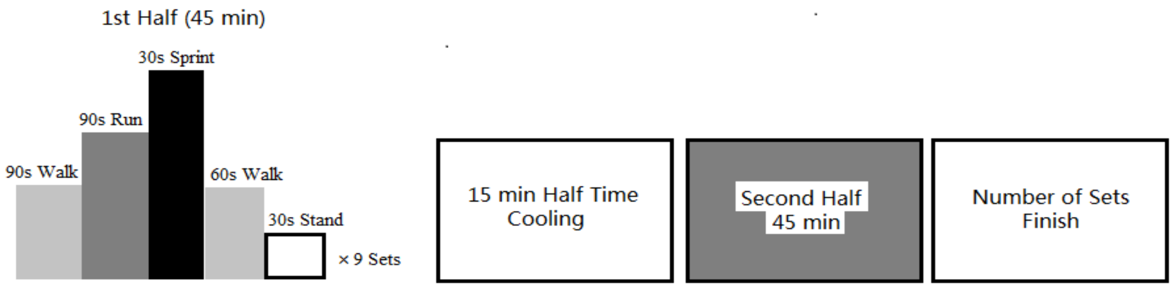


Figure 5.1. Soccer-Specific Intermittent exercise protocol.

5.2.4. Cooling Interventions

Cooling interventions was applied for 10 min during half-time and cooling trials were taken place under normal room temperature 20~22°C with participants in seated position. Leg cooling was used a temperature-controlled device (Kelvi Inc.) covering quadriceps and hamstring at temperature of 15°C for both legs. In the control trial, participants were in the seated position under normal ambient temperature with no cooling device was applied.

5.2.5 Physiological Measures

Body weight will be measured before and after exercise with minimal clothing at each visit to estimate hydration status. Pre-exercise urine specific gravity (USG) was measured to ensure participants were well hydrated before each exercise trial. Heart rate monitor (Garmin 920XT) was worn around the participants' chest to record continuous heart rate throughout the entire experiment trial. Mean HR (HR_{mean}) was determined by the average running HR and sprinting HR of each set and maximal HR (HR_{max}) was also determined of each set.

Core temperature was measured continuously by using an ingestible sensor (HQI Technologies; Palmetto, FL, USA). Participant were required to swallow the sensor 4-5 hours before exercise trial to assure it reaches the intestinal tract and negates the potential impact of beverage intake. Skin temperature probes (BIOPAC System) were attached to the skin at 3 sites of the body: forearm, abdominal, and calf. In addition, auditory canal temperature was measured by using a probe attached near the tympanic membrane and insulated from ambient temperature by a cotton earplug[18]. Mean skin temperature and body temperature were calculated according to the Burton's formula[19]:

$$\text{Mean Skin Temperature} = 0.5\times T_{trunk} + 0.36\times T_{leg} + 0.13\times T_{forearm}$$

$$\text{Body Temperature} = 0.64\times T_{core} + 0.36\times T_{mean\ skin}$$

Leg muscle tissue saturation index (TSI) was measured using a portable wireless near-infrared spectroscopy probe (NIRs; PortaMon; Artinis Medical Systems, Zetten, The Netherlands) to assess the % oxygen saturation of hemoglobin/myoglobin. The NIRs probe was attached with the surgical tape over the central part of lateral gastrocnemius muscle of the leg. The probe and the skin were covered by a strap wrapping around the leg to stabilize during exercise and also to prevent contamination from the ambient light.[20] The lowest TSI value measured during the sprinting phase represents the maximal oxygen desaturation at each set across the entire intermittent exercise.

5.2.6 Perceptual Measures

Whole body thermal sensation and rating perceived exertional scale (RPE) were taken at every set during the standing phase. Thermal sensation was also taken at each minute during

the cooling period. RPE was used the Borg 6-20 scale and thermal sensation scale was used a 9-point scale that ranged from -4 (very cold) to 4 (very hot).[21]

5.2.7 Statistical Analysis

Data were analyzed using JMPro 14 (SAS Inc., Cary, NC). Independent t-test with two-way repeated measure ANOVA was used to analyze the outcomes between the cooling trials. The outcome variables included exercise performance, heart rate, core temperature, skin temperature, tissue saturation index (TSI), and the perceptual measures. Data were presented as mean ± standard deviation (SD) and statistical significance was accepted at $P<0.05$.

5.3 Results

The changes in outcome variables across the entire intermittent exercise is displayed in Table 2. HR_{mean} and HR_{max} gradually increased as exercise continued and were significantly greater at 45 min compared to resting. However, no significant differences in HR_{mean} and HR_{max} were detected between LEG and CON during any exercise halves (all P=0.2). During cooling phase, HR_{mean} dropped by 39% in LEG and 37% in CON with no differences observed (P=0.6).

Mean skin, body temperature, and RPE were not different in two groups during any period (all P>0.5). Core temperature was not different in two groups during exercise phase, however; was significant lower in LEG (37.1±1.6˚C) compared to CON (37.6±1.0˚C, P=0.01) in cooling phase. In addition, a significant lowered auditory canal temperature was found in the LEG compared to CON during cooling phase (LEG: 34.1±1.3˚C vs. CON: 35.1±0.6˚C; P=0.001), and second half (LEG: 34.8±1.1˚C vs. CON: 35.5±0.5˚C; P=0.008).

Table 5.2. The difference between LEG and CON across the exercise halves and cooling phase.

	First Half			Half-Time Cooling			Second Half		
	LEG	**CON**	**P value**	**LEG**	**CON**	**P value**	**LEG**	**CON**	**P value**
HR_{mean} (bpm)	159±14	150±14	0.2	97±15	94±16	0.6	164±15	154±13	0.2
Core (˚C)	37.3±1.3	37.6±0.5	0.4	37.1±1.6*	37.6±1.0	0.01	37.5±1.3	37.7±0.5	0.8
Canal (˚C)	35.0±0.7	35.3±0.5	0.2	34.1±1.3*	35.1±0.6	0.001	34.8±1.1*	35.5±0.5	0.008
Mean Skin (˚C)	33.6±0.6	33.6±0.9	0.9	32.6±0.5	32.5±0.9	0.9	32.9±0.5	33.0±1.2	0.8
Body Temp (˚C)	36.0±0.9	36.3±0.6	0.1	35.5±1.1	35.8±0.8	0.1	35.9±1.0	36.0±0.6	0.4
Thermal	2.1±0.8	2.0±0.8	0.9	-0.4±0.9*	0.1±0.9	0.008	1.9±1.1	2.2±0.8	0.3
RPE	10±3	11±3	0.7	N/A	N/A	N/A	11±4	12±3	0.6
Min-TSI (%)	43.5±5.5	46.2±10.6	0.1	71.4±4.1	69.5±5.5	0.3	47.2±8.0	48.7±7.7	0.4
Max-TSI (%)	73.4±3.5	72.5±3.8	0.3	74.2±4.1	73.5±4.7	0.7	72.4±3.7	71.9±3.0	0.6

Canal-auditory canal temperature; Thermal-thermal sensation; RPE-rating of perceived exertion; TSI-tissue saturation index; N/A-not applicable.

LEG application at the first 10 min during the half-time successfully decreased mean leg skin temperature by 4.3°C (Pre-cool 33.5±1.4 vs. Post-cool 29.2±2.3°C), whereas mean leg skin temperature dropped by 0.3°C in CON (Pre-cool 33.3±0.8 vs. Post-cool 33.0±0.8°C). The mean leg skin temperature was significantly lowered in the LEG compared to CON after cooling phase (P=0.02; Figure 2). In addition, thermal sensation was significantly lowered in LEG (-0.4±0.9) compared to CON (0.1±0.9; P=0.008) during the cooling phase and was significantly correlated with auditory canal temperature changes (r=0.76; P<0.001). TSI decreased from resting value 60~73% to 37~50% as exercise continued in the sprinting phase and increased or remained above the resting value during standing recovery phase. Between the groups, no significant difference was found across all the time points (all P>0.1, Figure 3A and B).

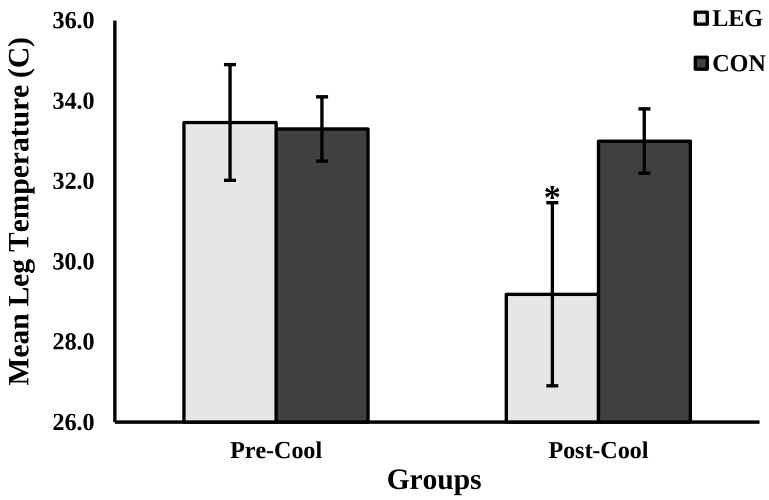


Figure 5.2. The mean skin leg temperature before and after cooling intervention. LEG, leg cooling; CON, no cooling. *Significant lower mean leg skin temperature in LEG (29.2±2.3°C) compared to CON (33.0±0.8°C; P=0.02).

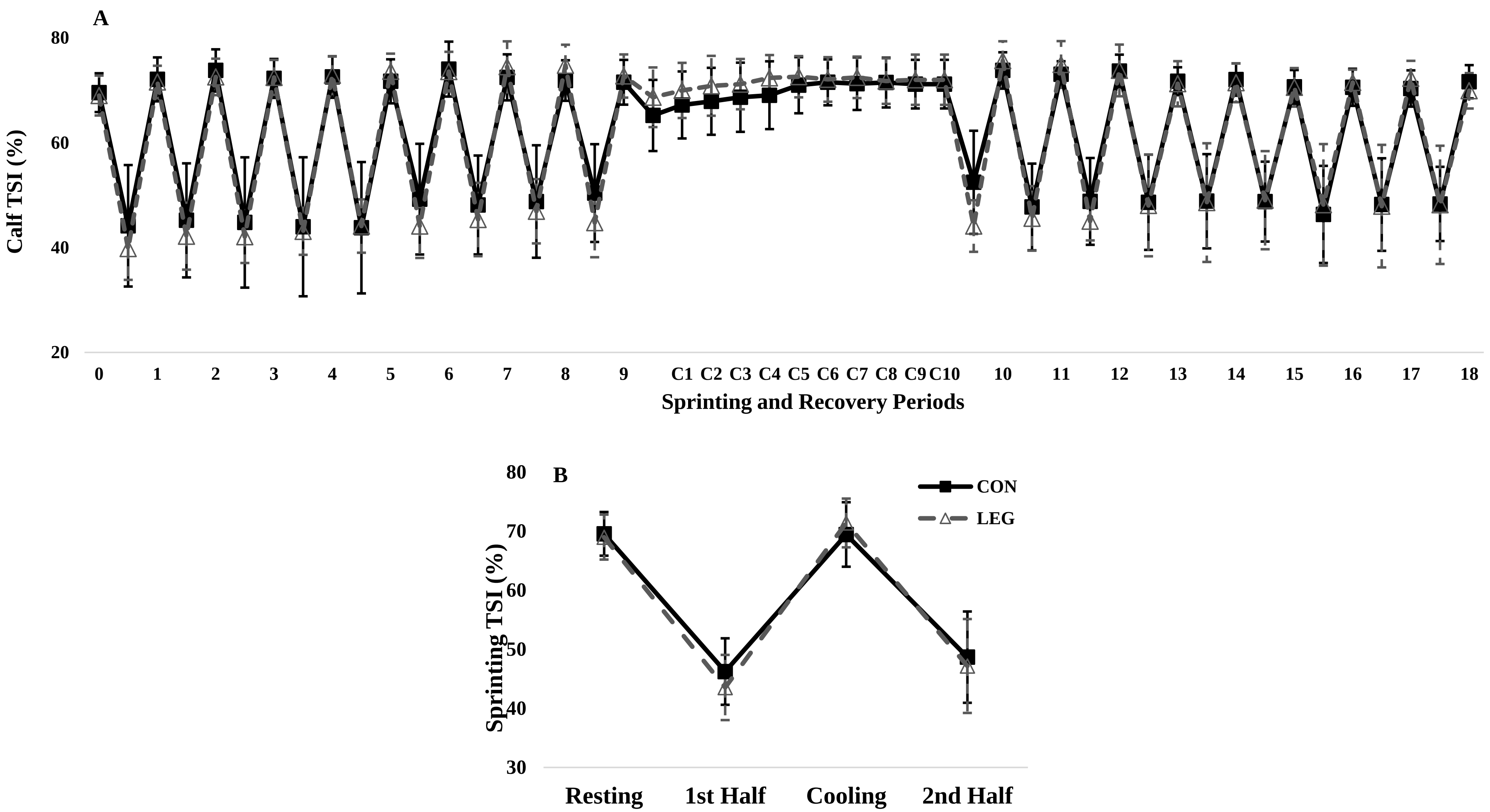


Figure 5.3. min and max TSI changes at each set in both halves and 10 min cooling phase (A) and mean TSI across exercise and cooling phase (B). Min TSI represented the sprinting phase and max referred to standing recovery phase of each intermittent set. No significant difference was found between two groups across exercise and cooling phase.

5.4 Discussion

The main purpose of this study was to examine the effects of half-time leg cooling on a soccer-simulated intermittent exercise. The present study conducted a 10 min 15℃ leg cooling maneuver that successfully decreased leg temperature by 4.3℃ during half-time period. The main findings of this study were that leg cooling resulted in significant lowering core temperature, auditory canal temperature and perceptual thermal sensation compared to CON during the cooling phase. In addition, a moderately correlation was found between the change in canal temperature and thermal sensation. However, mean skin, body temperature, heart rate, RPE and TSI were not difference between the two groups.

The present study applied the leg cooling on the thigh area for 10 min during the half-time period after core temperature elevated in the first half of intermittent exercise. Previous study concerned that leg cooling could decrease the lower limb force development and high intensity exercise performance due to cold the muscle temperature.[22] However, Bristow et al. (1994) found that whole leg immersion in 8℃ water for 20 min did not induce muscle temperature reduction due to greater volume of the leg muscles.[23] In the present study, the cooling device was set up at 15℃ and resulted in a 4.3℃ reduction in skin temperature. Therefore, the above evidence suggested our cooling maneuver was appropriate to cool down the skin not the leg muscles.

In this study, leg cooling significantly decreased core temperature, auditory canal temperature, and thermal sensation compared to CON during the cooling phase. A similar study conducted by Hayashi et al. (2004), they suggested leg cooling during recovery period can

significantly decreased core temperature and thermal strain.[12] During exercise in the heat, active vasodilation of the blood vessels increases the skin blood flow, which contributes to core and canal temperature elevation.[24] Leg cooling induced local cutaneous vasoconstriction which could decrease the magnitude of core and canal temperature elevated. As a result, we observed a lower core and auditory canal temperature.[25,26] Another main finding was that the changes of auditory canal temperature were significantly associated with thermal sensation. Cabanac et al. (1987) suggested that a reduction in auditory canal is an evidence of selective brain cooling.[27] Measuring the temperature of tympanic membrane has been suggested that is a non-invasive method to assess the carotid artery temperature.[18] Carotid artery is one of the main arteries to supply the blood to brain, forming the anterior and middle cerebral arteries to the anterior circulation of the forebrain.[28] Therefore, the reduction of auditory canal temperature induced by leg cooling suggested the selective brain cooling to affect the cold thermal perception. On the other hand, no correlation was observed between the changes of core temperature and thermal sensation, probably because core temperature change was more likely to correlate with hot thermal sensation rather than cold thermal sensation.[29] However, we did not find leg cooling to affect the heart rate during cooling and subsequent intermittent exercise, which was in contrast to Hayashi et al. (2004).[12] The possible explanation could be that our participants were highly trained individuals, who had the faster heart rate recovery and it's hard to observe the cooling effects.[30]

NIRs is a non-invasive technique to assess the muscle blood volume, blood flow, and TSI by detecting the mixed hemoglobin oxygenation saturation in the microvasculature of the

muscle tissue.[31] The resting gastrocnemius TSI value of the present was comparable with the previous research, suggesting validation of our NIRs setting, which the near-infrared light penetrates skin, subcutaneous fat and underlying the muscles.[31,32] In the present study, the TSI patterns in responses to the intermittent exercise was similar to the previous study conducted by Rodriguez et al. (2019).[33] At the onset of sprinting phase of each set, a rapid decrease in TSI was observed. This suggested that the oxygen extraction likely increased in the gastrocnemius. During the walking and standing phase, TSI tended to increase and remained above the resting level as a consequence of reperfusion and tissue re-oxygenation accompanied with increasing in total hemoglobin (Figure 3A).[31,34] In addition, TSI was identical between the two groups across the exercise sets and during the cooling phase. However, Wakabayashi et al. (2018) suggested that leg cooling induced a significant lower TSI.[35] The lowering skeletal muscle temperature could result in a less blood perfusion and an increase of deoxygenated hemoglobin.[36] The cooling induced lower muscle temperature can decrease muscle blood flow or O_2 delivery relative to O_2 consumption.[35-37] TSI was not different between the cooling intervention in the present study could be cooling the thigh might not see the further effects on the blood flow in the distal gastrocnemius muscle. Second, muscle oxygenation was not continuously compromised due to reoxygenation capacity between sprint bouts in the intermittent exercise. Cardiac output can still meet the O_2 demands of both respiratory and locomotor muscles and may not detect the further impact by cooling interventions. Lastly, our cooling maneuver did not induce muscle temperature reduction as we applied a 10 min 15°C cooling temperature compared to Wakabayashi et al. (2018) conducted a 30 min 12°C water

immersion.

A strength of this study was our intermittent exercise protocol simulated the soccer exercise demands, suggesting our cooling maneuver can be potentially applied in the soccer players to ameliorate perceptual thermal stress and elevated core temperature during exercise in the heat. In addition, the present study had well controlled for the potential impact variables, such as fluid intake, diet, and exercise clothing. A limitation of the present study was a small sample size. Second, we recruited highly trained individuals with VO_{2max} around 50 ml/kg/min. Participants recovered quicker between the bouts and the intermittent exercise routine may not continuously accumulate the core temperature in the first half, subsequently, less cooling effects can be detected during the second half. Lastly, the novel coronavirus pandemic in the US influence the number of participants willing to perform the extra sets to examine the effects of cooling intervention on the exercise performance.

Half-time leg cooling for 10 min at temperature of 15°C significantly decreased the core temperature, auditory canal temperature, and whole-body thermal sensation. Auditory canal temperature was significantly correlated with whole-body thermal sensation. TSI was not different between the two groups. The above evidence suggested our cooling maneuver was effective and successful lowering the whole-body thermal sensation without cooling the muscles. Future research should include an exercise routine that elevates core temperature in the first half. Consequently, the cooling effects can be examined whether affect these outcome variables during cooling phase and the second half to verify the results of the present study.

5.5 References

1. Coker NA, Wells AJ, Gepner Y. The Effect of Heat Stress on Measures of Running Performance and Heart Rate Responses During A Competitive Season in Male Soccer Players. *J Strength Cond Res.* 2018.

2. Ruddock AD, Thompson SW, Hudson SA, James CA, Gibson OR, Mee JA. Combined active and passive heat exposure induced heat acclimation in a soccer referee before 2014 FIFA World Cup. *Springerplus.* 2016;5:617.

3. Maughan RJ, Otani H, Watson P. Influence of relative humidity on prolonged exercise capacity in a warm environment. *Eur J Appl Physiol.* 2012;112(6):2313-2321.

4. Ozgunen KT, Kurdak SS, Maughan RJ, et al. Effect of hot environmental conditions on physical activity patterns and temperature response of football players. *Scand J Med Sci Sports.* 2010;20 Suppl 3:140-147.

5. Chmura P, Konefal M, Andrzejewski M, Kosowski J, Rokita A, Chmura J. Physical activity profile of 2014 FIFA World Cup players, with regard to different ranges of air temperature and relative humidity. *Int J Biometeorol.* 2017;61(4):677-684.

6. Nassis GP, Brito J, Dvorak J, Chalabi H, Racinais S. The association of environmental heat stress with performance: analysis of the 2014 FIFA World Cup Brazil. *Br J Sports Med.* 2015;49(9):609-613.

7. Duffield R, Dawson B, Bishop D, Fitzsimons M, Lawrence S. Effect of wearing an ice cooling jacket on repeat sprint performance in warm/humid conditions. *Br J Sports Med.* 2003;37(2):164-169.

8. Thomas G, Cullen T, Davies M, Hetherton C, Duncan B, Gerrett N. Independent or simultaneous lowering of core and skin temperature has no impact on self-paced intermittent running performance in hot conditions. *Eur J Appl Physiol.* 2019;119(8):1841-1853.

9. Duffield R, Marino FE. Effects of pre-cooling procedures on intermittent-sprint exercise performance in warm conditions. *Eur J Appl Physiol.* 2007;100(6):727-735.

10. Wang L, Tian Y, Kim J, Yin H. The key local segments of human body for personalized heating and cooling. *J Therm Biol.* 2019;81:118-127.

11. Castle PC, Macdonald AL, Philp A, Webborn A, Watt PW, Maxwell NS. Precooling leg muscle improves intermittent sprint exercise performance in hot, humid conditions. *J*

Appl Physiol (1985). 2006;100(4):1377-1384.

12. Hayashi K, Honda Y, Ogawa T, Wada H, Kondo N, Nishiyasu T. Effects of brief leg cooling after moderate exercise on cardiorespiratory responses to subsequent exercise in the heat. *Eur J Appl Physiol.* 2004;92(4-5):414-420.

13. Bergh U, Ekblom B. Influence of muscle temperature on maximal muscle strength and power output in human skeletal muscles. *Acta Physiol Scand.* 1979;107(1):33-37.

14. Sargeant AJ. Effect of muscle temperature on leg extension force and short-term power output in humans. *Eur J Appl Physiol Occup Physiol.* 1987;56(6):693-698.

15. Edwards RH, Harris RC, Hultman E, Kaijser L, Koh D, Nordesjo LO. Effect of temperature on muscle energy metabolism and endurance during successive isometric contractions, sustained to fatigue, of the quadriceps muscle in man. *J Physiol.* 1972;220(2):335-352.

16. Thompson PD, Arena R, Riebe D, Pescatello LS, American College of Sports M. ACSM's new preparticipation health screening recommendations from ACSM's guidelines for exercise testing and prescription, ninth edition. *Curr Sports Med Rep.* 2013;12(4):215-217.

17. Aldous JW, Akubat I, Chrismas BC, et al. The reliability and validity of a soccer-specific nonmotorised treadmill simulation (intermittent soccer performance test). *J Strength Cond Res.* 2014;28(7):1971-1980.

18. Bittel JH, Livecchi-Gonnot GH, Hanniquet AM, Poulain C, Etienne JL. Thermal changes observed before and after J.-L. Etienne's journey to the North Pole. Is central nervous system temperature preserved in hypothermia? *Eur J Appl Physiol Occup Physiol.* 1989;58(6):646-651.

19. Ramanathan NL. A New Weighting System for Mean Surface Temperature of the Human Body. *J Appl Physiol.* 1964;19:531-533.

20. Rittweger J, Moss AD, Colier W, Stewart C, Degens H. Muscle tissue oxygenation and VEGF in VO-matched vibration and squatting exercise. *Clin Physiol Funct Imaging.* 2010;30(4):269-278.

21. Olesen BW, Brager GS. A better way to predict comfort. *Ashrae J.* 2004;46(8):20-+.

22. Crowley GC, Garg A, Lohn MS, Van Someren N, Wade AJ. Effects of cooling the legs on performance in a standard Wingate anaerobic power test. *Br J Sports Med.*

1991;25(4):200-203.

23. Bristow GK, Sessler DI, Giesbrecht GG. Leg temperature and heat content in humans during immersion hypothermia and rewarming. *Aviat Space Environ Med.* 1994;65(3):220-226.

24. Wyss CR, Brengelmann GL, Johnson JM, Rowell LB, Niederberger M. Control of skin blood flow, sweating, and heart rate: role of skin vs. core temperature. *J Appl Physiol.* 1974;36(6):726-733.

25. Johnson JM, Rowell LB, Brengelmann GL. Modification of the skin blood flow-body temperature relationship by upright exercise. *J Appl Physiol.* 1974;37(6):880-886.

26. Brengelmann GL, Johnson JM, Hermansen L, Rowell LB. Altered control of skin blood flow during exercise at high internal temperatures. *J Appl Physiol Respir Environ Exerc Physiol.* 1977;43(5):790-794.

27. Cabanac M, Germain M, Brinnel H. Tympanic temperatures during hemiface cooling. *Eur J Appl Physiol Occup Physiol.* 1987;56(5):534-539.

28. Snell RS. *Clinical neuroanatomy.* 7th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2010.

29. Fujii N, Aoki-Murakami E, Tsuji B, et al. Body temperature and cold sensation during and following exercise under temperate room conditions in cold-sensitive young trained females. *Physiol Rep.* 2017;5(20).

30. McDonald KG, Grote S, Shoepe TC. Effect of training mode on post-exercise heart rate recovery of trained cyclists. *J Hum Kinet.* 2014;41:43-49.

31. Quaresima V, Komiyama T, Ferrari M. Differences in oxygen re-saturation of thigh and calf muscles after two treadmill stress tests. *Comp Biochem Physiol A Mol Integr Physiol.* 2002;132(1):67-73.

32. Boushel R, Langberg H, Green S, Skovgaard D, Bulow J, Kjaer M. Blood flow and oxygenation in peritendinous tissue and calf muscle during dynamic exercise in humans. *J Physiol.* 2000;524 Pt 1:305-313.

33. Rodriguez RF, Townsend NE, Aughey RJ, Billaut F. Muscle oxygenation maintained during repeated-sprints despite inspiratory muscle loading. *PLoS One.* 2019;14(9):e0222487.

34. Quaresima V, Homma S, Azuma K, et al. Calf and shin muscle oxygenation patterns and femoral artery blood flow during dynamic plantar flexion exercise in humans. *Eur J Appl Physiol.* 2001;84(5):387-394.

35. Wakabayashi H, Osawa M, Koga S, et al. Effects of muscle cooling on kinetics of pulmonary oxygen uptake and muscle deoxygenation at the onset of exercise. *Physiol Rep.* 2018;6(21):e13910.

36. Gregson W, Black MA, Jones H, et al. Influence of cold water immersion on limb and cutaneous blood flow at rest. *Am J Sports Med.* 2011;39(6):1316-1323.

37. Koga S, Rossiter HB, Heinonen I, Musch TI, Poole DC. Dynamic heterogeneity of exercising muscle blood flow and O2 utilization. *Med Sci Sports Exerc.* 2014;46(5):860-876.

CHAPTER 6. UPPER BODY HEAT DISSIPATION WEARING A NOVEL SYNTHETIC MATERIAL SHIRT DURING EXERCISE IN THE HEAT

6.1 Introduction

The human body can maintain a constant core temperature balance between heat gain and heat loss. During exercise in the heat, metabolic heat production and hot environments are the major factors contributing to heat gain in the human body, whereas heat loss mainly depends on sweat evaporation. In addition, the rate of sweat evaporation plays an important role to cool down the human body due to an increased demand of heat loss. Berglund et al. (1977) pointed out that the clothing and airflow velocity had significantly impact on water evaporation rate to affect human thermoregulation.[1]

Clothing generally represents a layer of insulation and acts as the barrier of heat transfer by increasing the evaporation resistance.[2,3] In military and sport, clothing potentially limits evaporation from the skin due to an inhibitory microclimate between the skin and environment.[4,5] Therefore, the effects of clothing on evaporation rate is critical consideration to decrease the thermoregulatory stress. The fabrics that improve the evaporative characteristics had been suggested to ameliorate thermal stress by lowering skin and core temperatures.[5] In addition, the airflow is considered to decrease convective heat dissipation and increase evaporation by lowering the water vapor pressure surrounding the human body.[6] In outdoor activities, increased airflow velocity had been proposed to decrease the thermoregulatory stress.[7] Munson et al. (1978) suggested that airflow velocity increasing from 0.25 to 0.5 m/s resulted in a 50% reduction in total thermal insulation of clothing.[8] Thus, many shirt fabric materials and aeration systems are being developed to negate this limitation from

current clothing, potentially being introduced to the athletic and military apparel market. Therefore, the purpose of this study was to determine the effects of a novel t-shirt fabric material on upper body heat dissipation during exercise in a warm environment with and without simulated wind electric fan versus the novel ventilated vest. We hypothesized that the novel shirt has an improved evaporative characteristic compared to the standard fabric resulting in greater heat dissipation during exercise in the heat.

6.2 Methods

6.2.1 Participants

Eight healthy male participants were recruited in the study (age: 25±3yr; height: 171.6±7.4cm; weight: 79.2±14.2kg; Mean±SD) and physical characteristics were displayed in Table 1. Exclusion criteria was those with known cardiovascular, pulmonary or metabolic disease, or signs/symptoms categorized by the American College of Sports Medicine (ACSM).[9] The study protocol was approved by the university Institutional Review Board, and all participants signed the written informed consent prior to any assessments.

Table 6.1. Physical characteristics of the participants (n=8).

Age (yr)	25.1±2.6
Height (cm)	171.7±6.8
Body Weight (kg)	79.2±13.7
Surface Area (m^2)	1.87±0.34

6.2.2 Experiment Design

The study was a 2×2 cross-over design to determine the effects of two t-shirt fabric materials (standard vs. novel) with and without simulated wind (electric fan vs. ventilated vest) on body temperature during exercise in the heat. Participants were randomly assigned to 4 exercise trials: standard+fan (S+F), novel+fan (N+F), standard+vest (S+V), and novel+vest (N+V). Heat dissipation was monitored by differences in core and skin temperature, and local sweat rates during exercise in the heated environment.

6.2.3 Exercise

Each exercise trial consisted of a 60-minute steady-state bout of exercise on a cycle ergometer (Monark, Sweden) in a heated environment (~30°C, ~30-45% RH). During the exercise bout, pedal rate on the cycle ergometer was fixed at 60 rpm and the resistance factor necessary to elicit an individualized estimated metabolic rate of 45 W/m^2 body surface area, using ACSM cycle equations.[10] This workload is considered moderate to vigorous intensity exercise across the age and sex normative data for the participants. At the first 30-min, participants exercised in the trial specific shirt with no external simulated wind. In the second 30-min, the fan was used to simulate wind speed equivalent to 2m/s applied to the chest or the ventilated vest was worn to simulate wind. The ventilated vest had a 10×15cm ventilation area covered the mid-chest and mid-back. Between the two 30-min exercises, a 5-min break was given to the participants allowing investigators to adjust the fan or ventilated vest.

6.2.4 Physiological Measures

During the exercise testing, body temperature was assessed by core and skin

temperatures. Core temperature (T_{Core}) was measured continuously by using an ingestible sensor (HQ Technologies; Palmetto, FL, USA). Participant required to ingest the sensor 4-5 hours before exercise trial to assure it reaches the intestinal tract and negates the potential impact of beverage intake. Skin temperatures (T_{Skin}) were measured continuously at 5 sites of the body (BIOPAC System), the upper chest, sternum at the ziphoid process (mid-chest), forearm (reference temperature), mid-scapular (upper-back), and middle of the back (mid-back). Continuous heart rate (HR) monitoring was conducted with the use of a Bioharness BH3 (Zephyr; Annapolis, MD, USA) worn around the chest during the exercise. Sweat was collected on the lower back in the first of 30 min using the technical absorbent patch technique.[11] Alcohol spray and dry towel were used to clean the skin surface before the sweat patch was attached. The local sweat rate (LSR) was calculated by weight differences of the absorbent patch before and after training divided by the product of the sweat patch area and time duration ($g/cm^2 \cdot hr$). In addition, the perceptual measures were collected every 5-min for subjective ratings of perceived exertion (Borg 6-20 Scale) and thermal comfort (+5 good; -5 bad).

6.2.5 Statistical Analysis

Data were analyzed using JMPro 14 (SAS Inc., Cary, NC, USA). One and two-way repeated measures analysis of variance (ANOVA) across groups and the exercise time. Significant effects were evaluated using Student t post-hoc analysis. The main outcome variables included core and skin temperatures, heart rate, local sweat rate, and perceptual measures. Data were presented as mean ± standard deviation (SD) and statistical significance was accepted at $P<0.05$.

6.3 Results

Across all trials, the environments of the heated room were identical (29.4±0.4°C and 32.0±2.6% RH; all $P>0.05$), and all the outcome variables were similar in the first 30-min, including body temperatures (core and skin), HR, LSR, and perceptual measures. In the final 30-min when the simulated wind was applied, fan trials had a lower averaged skin temperature (31.5±1.5 vs. 33.3±1.2°C; $P<0.001$) and HR (127±15 vs. 134±15 bpm; $P=0.002$). In addition, the perceptual measures showed fan trials had a lower RPE (9±1 vs. 10±2; $P<0.001$) and better feeling (3±2 vs. 2±1; $P=0.05$) compared to the vest, regardless of the shirt fabric materials.

Across all the trials, mean weighted T_{Skin} was lowest in the N+F (31.3±1.2°C) compared to other trials (F+V: 33.4±1.1°C; S+F: 31.7±1.7°C; S+V: 33.2±1.2°C; $p<0.01$). In addition, N+F (30.8±1.1°C) had a significantly T_{Skin} of averaged mid-chest and back (inside of ventilation area) compared to N+V (32.1±1.9°C), S+F (31.5±2.3°C), and S+V (32.1±1.9°C; $P=0.003$, Figure 1A). However, T_{Skin} of averaged upper chest and back (outside of ventilation area) was lower in fan trials compared to vest, regardless of the fabric materials (Figure 2B). In perceptual measures, similar trends were observed for lower RPE (main effect of ventilation $P=0.007$) and better feeling (main effect of ventilation $P=0.001$) with the fan trials during the final 30 minutes when ventilation was applied. In particular, the N+F had the lowest RPE and the best overall feeling compared to other trials (Figure 2). However, no significant differences were found in T_{Core} and HR across the trials.

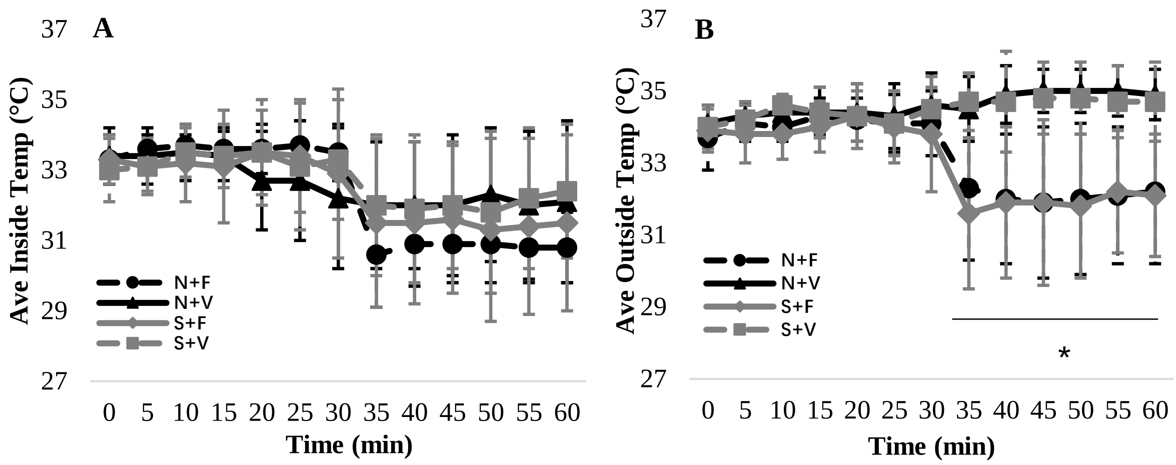


Figure 6.1. The averaged skin temperature inside (mid-chest and mid-back; A) and outside (upper-chest and back; B) the ventilation area. The black circle dash line was novel shirts with fan (N+F), the black triangle solid line refers to novel shirt with vest (N+V), the grey diamond solid line refers to standard with fan (S+F), and the grey square dash line represents standard shirt with vest (S+V). *significant effects in ventilation type (fan vs. vest; P<0.01).

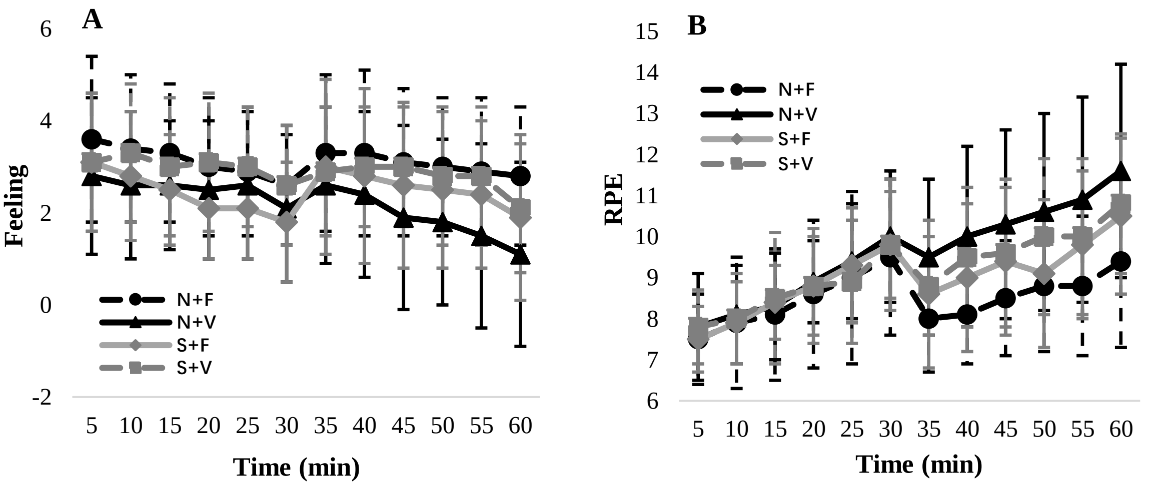


Figure 6.2. The ratings of perceived exertion (A) and overall feelings (B) across the trials. The black circle dash line was novel shirts with fan (N+F), the black triangle solid line refers to novel shirt with vest (N+V), the grey diamond solid line refers to standard with fan (S+F), and the grey square dash line represents standard shirt with vest (S+V).

By comparing with two shirt materials (Standard vs. Novel), no significant differences were found in fan trials across the main outcomes (Table 2). However, a tread of decreasing in average mid-chest and back temperature was observed in the novel material compared to the

standard (30.8±1.1 vs. 31.5±2.3°C; P=0.07). When the ventilation vest was applied, a greater increase in core temperature (0.47±0.24 vs. 0.31±0.35°C; P=0.01) in the novel compared to the standard material. In addition, overall feeling was worse in the novel compared to the standard material (2±2 vs. 3±1; P=0.01). The skin temperatures and HR were not significant difference between the two fabric materials (Table 3).

Table 6.2. The changes of variables in different shirt fabric materials with and without fan applied.

	Fan off (0~30 min)		**Fan on (30~60 min)**	
	Novel	**Standard**	**Novel**	**Standard**
Delta Core (°C)	0.24±0.23	0.26±0.23	0.49±0.22	0.48±0.28
HR (bpm)	120±23	120±20	125±19	128±10
Ave Inside (°C)	33.6±0.6	33.2±1.4	30.8±1.1	31.5±2.3
Ave Outside (°C)	34.1±0.7	34.0±0.9	31.9±2.1	31.9±2.0
Weighted Skin (°C)	33.7±0.5	33.5±0.8	31.3±1.2	31.7±1.7
RPE	8±2	9±1	9±2	9±2
Feelings	3±1	2±1	3±2	3±2

PSI physiological strain index; RPE ratings of perceived exertion; Ave Inside (averaged mid-chest and back); Ave Outside (averaged upper chest and back)

Table 6.3. The changes of variables in the shirt fabric materials with and without vest applied.

	Vest off (0~30 min)		**Vest on (30~60 min)**	
	Novel	**Standard**	**Novel**	**Standard**
Delta Core (°C)	0.23±0.23	0.22±0.25	0.47±0.24*	0.31±0.35
HR (bpm)	122±24	118±21	136±17	132±14
Ave Inside (°C)	33.0±1.3	33.3±1.2	32.1±1.9	32.1±1.9
Ave Outside (°C)	34.3±0.7	34.3±0.7	35.0±0.7	34.7±1.0
Weighted Skin (°C)	33.5±0.6	33.5±0.8	33.4±1.1	33.2±1.2
RPE	9±2	9±1	10±2	10±2
Feelings	3±2	3±1	2±2*	3±1

PSI physiological strain index; RPE ratings of perceived exertion; Ave Inside (averaged mid-chest and back); Ave Outside (averaged upper chest and back)

6.4 Discussion

The purpose of this study was to determine the effects of the shirt fabric materials on the upper body heat dissipation with or without the simulated wind (fan vs. vest). The main finding of the present study was no fabric material effects were observed in the first 30 min without simulated wind. Fan trials had an overall better upper body dissipation compared to ventilation vest trials, appearing in lowered averaged skin temperature and HR, regardless of shirt fabric materials. Across all the trials, N+F exhibited a significant lower averaged skin temperature and RPE, and better overall feeling compared to other trials. In the fan trials, a tread of lower skin temperature in the chest region was observed in novel fabric shirt compared to the standard. In addition, in the ventilation vest trials, the novel shirt material had a greater elevated in core temperature and worse overall feeling compared to the standard material.

In the first 30 min when no simulated wind was applied, the main outcome variables were similar, indicating a good control of the environmental conditions and exercise intensities across all trials. In addition, we did not observe any significant differences in core and skin temperatures, HR, LSR, and perceptual measures between the shirt fabric materials. This finding of the present study agreed with previous studies, suggesting no difference in thermoregulation for either clothing fibers or constructions when little airflow surrounds.[12-14] In contrast, several studies did find that the synthetic fabric resulted in a lower skin temperature during a soccer simulated match and high intensity exercises.[15,16] The conflict in results could be explained by the fact that the exercise bout in the present study was of moderate intensity, which did not produce a sufficient heat load to further detect the beneficial of fabric materials

in lowering body temperatures.[12] Piwonka et al. (1967) showed rectal temperature and exercise heart rate were significantly increased as environmental temperature elevated to 40~50°C in the trained individuals. In addition, Bishop et al. (2013) suggested in a cool to moderate environment, the clothing lowered skin temperature might be overwhelmed with elevated sweat rates and higher exercise intensity.[17] Therefore, the environmental condition (~29°C) and exercise intensity in the present was not sufficient to increase the physiological stress and the perceptual thermal stress of the participants.

The present study applied two types of simulated wind, electric fan and the ventilation vest in the final 30 min exercise. Comparing with two ventilation types, fan trials exhibited better physiological responses and perceptual measures compared to ventilation vest, regardless of the shirt fabric materials. This is reasonable that electric fan covers most of the upper body and head area, whereas ventilation vest covers 10×15 cm in the front and back of the upper body. In the fan trials, we did not find any significant differences in main outcomes between two fabric materials. Interestingly, we observed a tread of lowering skin temperature in the chest region where inside the ventilation area in N+F compared to S+F. Watkins et al. (1984) suggested clothing fabric materials, which can enhance ventilation through the clothing, potentially reduce thermal insulation with evaporative and convective airflow.[18] This finding indicated that our novel fabric material might potentially have enhanced evaporative and ventilation through the clothing based on the construction of the fabric.[19,20] In addition, Sawka et al. (2012) suggested that a reduction in microenvironment of any skin region could possibly lower heat strain during exercise in the heat.[21] Therefore, participants might receive the

advantage to lower skin temperature and enhance the tolerance during exercise in the heat wearing our novel fabric shirt in the outdoor activities or sports.

In the ventilation vest trials, we found the novel fabric material resulted in a greater increase in core temperature and a worse overall feeling compared to the standard material, which contradicts to our hypothesis. When the exercise stopped, we observed that the novel fabric shirt under the ventilation vest area had a low moisture regain, whereas a greater moisture regains in the standard shirt. Previous study showed moisture fabric had been proposed to affect thermoregulatory responses during exercise in the heat.[22-24] In addition, Kwon et al. (1998) found that during a moderate exercise in the heat, low moisture fabric shirt had a higher skin and body temperatures compared to average moisture shirt material with the application of 1.5m/s wind.[24] These cooling effects are from conduction and convection in the moisture clothing by contacting the body and surrounding airflow. On the other hand, a drier clothing indicates a superior property for water evaporation.[4] Pascoe et al. (1994) suggested that clothing attenuates the evaporation of sweat from the skin reducing the cooling efficiency. Clothing that has greater evaporative capacity can provide beneficial during exercise in the heat.[4,25] Moreover, a drier clothing could enhance thermal comfort due to less clinginess of clothing and contract with the skin.[1,26,27] When the participants worn a ventilation vest, more uncompensable heat was produced. Thus, the sweat regains in the novel fabric shirt evaporated to the vest pocket instead of the air, in the consequence of elevating the microclimate temperature between the shirt and vest pocket. The greater evaporation character of our novel fabric shirt might comprise the conductive cooling efficiency in the microclimate between shirt

and ventilation vest pocket.[4] Thus, given the evidence above, when the thermal load and exercise intensity is sufficient, our novel shirt material potentially reduce the skin temperature and thermal stress and increased overall feeling when the airflow surrounds. However, this is not a conclusive observation, and more study need to be done.

The present study showed a trend that potential effects of different shirt fabric materials on the upper body heat dissipation in two types of simulated wind. We found when surrounding airflow is near 2m/s, our novel fabric shirt material has better evaporative and ventilation capacity through the clothing by detecting lowered skin temperature, RPE and better overall feeling. In addition, our novel shirt showed a tread to lower the microenvironment temperature of the chest region. The findings have noticeable application that individuals can receive advantage by wearing the novel shirt in the outdoor activities or sports, especially under high humid environment condition or for the individuals who sweat a lot. However, the limitation of the present study was the ventilation vest pocket is small. This makes it less comparable with electric cooling fan, which covers large body surface area. We believe this novel ventilation vest has the potential implication for the military event. However, more study needs to be done to optimal the ventilation vest on body heat dissipation and thermal stress because we found an opposite result. In addition, our moderate exercise intensity and environment conditions only elevated core temperature by 0.4°C from 37.3 to 37.7°C in the first 30 min. Lastly, participants cannot be blinded from the shirt fabric materials due the inside construction of the knit sizes. If the novel shirt does not have significant effects on upper body heat dissipation, participants tend to prefer the original standard one because they may wear

the similar material for a long time.

To summarize, in the first 30 min, no shirt fabric effects were observed. During the final 30 min, our novel shirt with application of electric cooling fan had greatest impact on upper body heat dissipation, mainly appearing in lower averaged skin temperature and RPE with better overall feeling. In addition, our novel shirt with application of ventilation vest showed a tread to lower skin temperature in the chest region, where inside the ventilation pocket. Future research should aim to increase the environmental stress (increase ambient and radiant heat exposure) or exercise intensity to detect the differences. In addition, investigators can conduct additional trial to create a misting microenvironment in the ventilation vest pocket or increase the ventilation vest area to optimize evaporative heat losses.

6.5 References

1. Berglund LG, Gonzalez RR. Evaporation of sweat from sedentary man in humid environments. *J Appl Physiol Respir Environ Exerc Physiol.* 1977;42(5):767-772.

2. Kenney WL, Wilmore JH, Costill DL, Wilmore JH. *Physiology of sport and exercise.* 5th ed. Champaign, IL: Human Kinetics; 2012.

3. Burton AC, Edholm OG. *Man in a cold environment; physiological and pathological effects of exposure to low temperatures.* New York,: Hafner Pub. Co.; 1969.

4. Gavin TP. Clothing and thermoregulation during exercise. *Sports Med.* 2003;33(13):941-947.

5. Nielsen R, Endrusick TL. Thermoregulatory responses to intermittent exercise are influenced by knit structure of underwear. *Eur J Appl Physiol Occup Physiol.* 1990;60(1):15-25.

6. Brown SL, Banister EW. Thermoregulation during prolonged actual and laboratory-simulated bicycling. *Eur J Appl Physiol Occup Physiol.* 1985;54(1):125-130.

7. Nielsen R, Olesen BW, Fanger PO. Effect of physical activity and air velocity on the

thermal insulation of clothing. *Ergonomics.* 1985;28(12):1617-1631.

8. Munson DM, Hayter R. An overview of clothing as it relates to the physiology of human comfort. . *Am Society Heat Refrigerat Air Cond Eng Trans.* 1978;84:713-718.

9. Thompson PD, Arena R, Riebe D, Pescatello LS, American College of Sports M. ACSM's new preparticipation health screening recommendations from ACSM's guidelines for exercise testing and prescription, ninth edition. *Curr Sports Med Rep.* 2013;12(4):215-217.

10. Pescatello LS, American College of Sports Medicine. *ACSM's guidelines for exercise testing and prescription.* 9th ed. Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins Health; 2014.

11. Morris NB, Cramer MN, Hodder SG, Havenith G, Jay O. A comparison between the technical absorbent and ventilated capsule methods for measuring local sweat rate. *J Appl Physiol (1985).* 2013;114(6):816-823.

12. Gavin TP, Babington JP, Harms CA, Ardelt ME, Tanner DA, Stager JM. Clothing fabric does not affect thermoregulation during exercise in moderate heat. *Med Sci Sports Exerc.* 2001;33(12):2124-2130.

13. Laing RM, Sims ST, Wilson CA, Niven BE, Cruthers NM. Differences in wearer response to garments for outdoor activity. *Ergonomics.* 2008;51(4):492-510.

14. Wickwire PJ, Bishop PA, Green JM, et al. Physiological and comfort effects of a commercial "cooling cap" worn under protective helmets. *J Occup Environ Hyg.* 2009;6(8):455-459.

15. Dai XQ, Imamura R, Liu GL, Zhou FP. Effect of moisture transport on microclimate under T-shirts. *Eur J Appl Physiol.* 2008;104(2):337-340.

16. Roberts B, Waller T, Caime M. Thermoregulatory response to base layer garments during treadmill exercise. . *Int J Sports Sci Eng.* 2007;1:29-38.

17. Bishop P, Balilonis G, Davis J, Zhang Y. Ergonomics and comfort in protective and sport clothing: A brief review. *J Ergon.* 2013(S2):1-7.

18. Watkins SM. *Clothing : the portable environment.* 1st ed. Ames, Iowa: Iowa State University Press; 1984.

19. Gonzales BR, Hagin V, Guillot R, Placet V, Groslambert A. Effects of polyester

jerseys on psycho-physiological responses during exercise in a hot and moist environment. *J Strength Cond Res.* 2011;25(12):3432-3438.

20. Sperlich B, Born DP, Lefter MD, Holmberg HC. Exercising in a hot environment: which T-shirt to wear? *Wilderness Environ Med.* 2013;24(3):211-220.

21. Sawka MN, Cheuvront SN, Kenefick RW. High skin temperature and hypohydration impair aerobic performance. *Exp Physiol.* 2012;97(3):327-332.

22. Havenith G, Richards MG, Wang X, et al. Apparent latent heat of evaporation from clothing: attenuation and "heat pipe" effects. *J Appl Physiol (1985).* 2008;104(1):142-149.

23. Kicklighter T, Edsall J, Martin M. Effect of moisture-wicking garments on temperature regulation during exercise. *International Journal of Athletic Therapy & Training.* 2011;16(6):9-13.

24. Kwon A, Kato M, Kawamura H, Yanai Y, Tokura H. Physiological significance of hydrophilic and hydrophobic textile materials during intermittent exercise in humans under the influence of warm ambient temperature with and without wind. *Eur J Appl Physiol Occup Physiol.* 1998;78(6):487-493.

25. Pascoe DD, Shanley LA, Smith EW. Clothing and exercise. I: Biophysics of heat transfer between the individual, clothing and environment. *Sports Med.* 1994;18(1):38-54.

26. Davis JK, Bishop PA. Impact of clothing on exercise in the heat. *Sports Med.* 2013;43(8):695-706.

27. Gwosdow A, Stevens J, Berglund L, Stolwijk J. Skin friction and fabric sensations in neutral and warm environments. *Test Res J.* 1986;56:574-580.

CHAPTER 7. CONCLUSION

The purpose of this dissertation was to investigate the effects of hot and humid environmental conditions on exercise, preventing heat-related illness and exercise performance decrement. The first study presented in this dissertation (Chapter 3) was conducted on collegiate female soccer players. It suggested that most players were in a mild hypohydration status during the off-season training camp in warm weather. The player position was the potential factor that influenced drinking behavior and hydration status. However, we found that environmental conditions have the greater effect on the sweat rate than hydration status and drinking behavior. Future research should focus on the positional effects on hydration status in soccer players due to the known differential of positional physical exertion demands.

In Chapter 4, the football players with sickle cell trait exhibited a 31~40% of hemoglobin S and greater red blood cell distribution width. Moreover, they had a similar fluid balance compared to non-sickle cell players. Interestingly, we observed a greater serum uric acid, urine electrolyte concentration and sweat sodium concentration in sickle cell trait players. Future research should investigate the laboratory-based to verify the result of this study. Although, sickle cell trait is a benign condition, more research should be done to prevent the players developing sickling injuries during exercise in the heat.

In Chapter 5, a pilot study examined the leg cooling on the soccer simulated intermittent exercise performance. We did not find that leg cooling enhanced the exercise performance, possibly due to less thermal load for participants. However, we found a significantly lower auditory canal temperature, core temperature and whole-body thermal

sensation during cooling phase. This suggested that if the thermal stress is sufficient, participants can receive the advantages to lower body temperature by the application of leg cooling. Future research should aim to conduct on-field research to examine whether our cooling maneuver can apply in the real-world soccer game, and whether soccer players can cool down the body efficiently during the half-time period promoting the exercise performance in the second half.

Lastly, chapter 6 aimed to determine the upper body heat dissipation wearing different shirt fabric materials with two types of simulated wind. The novel shirt fabric material has greater evaporative and ventilation capacity through clothing and a significant skin temperature reduction was observed with the application of the cooling fan. In addition, ratings of perceived exertion and overall subjective feeling were promoted. However, in the ventilation vest trials, we found the opposite effects that our novel shirts elevated the core temperature and lowered overall feeling. This finding could be due to low moisture regain of our novel shirt. Future study should optimal the ventilation vest by either increasing the ventilation area or misting the ventilation area to further detect the cooling efficiency of our novel shirt.

In summary, during exercise in hot and humid environmental conditions, exercise performance is dramatically decremented due to an increase thermoregulatory stress and alterations in cardiovascular function. Heat-related illnesses can frequently occur when players are in hypohydration state or hyperthermia condition. Monitoring the hydration status and body temperatures are necessary to promote athlete health. Therefore, this dissertation provides the

extended knowledge of the exercise precautions in the heat, developing fluid balance assessing techniques and the external body cooling maneuvers.

APPENDIX. STUDY FORMS

1.1 LSU IRB Approval

ACTION ON PROTOCOL APPROVAL REQUEST

Institutional Review Board
Dr. Dennis Landin, Chair
130 David Boyd Hall
Baton Rouge, LA 70803
P: 225.578.8692
F: 225.578.5983
irb@lsu.edu
lsu.edu/research

TO: Neil Johannsen
Kinesiology

FROM: Dennis Landin
Chair, Institutional Review Board

DATE: December 19, 2019

RE: IRB# 4308

TITLE: The effects of half-time cooling interventions on male soccer-simulated intermittent exercise performance

New Protocol/Modification/Continuation: New Protocol

Review type: Full _X_ Expedited ____ **Review date:** 12/13/2019

Risk Factor: Minimal _______ Uncertain _X____ Greater Than Minimal_______

Approved ___X___ **Disapproved** __________

Approval Date: 12/13/2019 **Approval Expiration Date:** 12/12/2020

Re-review frequency: (annual unless otherwise stated)

Number of subjects approved: 20

LSU Proposal Number (if applicable):

By: Dennis Landin, Chairman DLandin

**PRINCIPAL INVESTIGATOR: PLEASE READ THE FOLLOWING –
Continuing approval is CONDITIONAL on:**

1. Adherence to the approved protocol, familiarity with, and adherence to the ethical standards of the Belmont Report, and LSU's Assurance of Compliance with DHHS regulations for the protection of human subjects*
2. Prior approval of a change in protocol, including revision of the consent documents or an increase in the number of subjects over that approved.
3. Obtaining renewed approval (or submittal of a termination report), prior to the approval expiration date, upon request by the IRB office (irrespective of when the project actually begins); notification of project termination.
4. Retention of documentation of informed consent and study records for at least 3 years after the study ends.
5. Continuing attention to the physical and psychological well-being and informed consent of the individual participants, including notification of new information that might affect consent.
6. A prompt report to the IRB of any adverse event affecting a participant potentially arising from the study.
7. Notification of the IRB of a serious compliance failure.
8. SPECIAL NOTE: **Make sure you use bcc when emailing more than one recipient.**

**All investigators and support staff have access to copies of the Belmont Report, LSU's Assurance with DHHS, DHHS (45 CFR 46) and FDA regulations governing use of human subjects, and other relevant documents in print in this office or on our World Wide Web site at http://www.lsu.edu/irb*

1.2 Consent Forms

CONSENT TO PARTICIPATE IN A RESEARCH STUDY
INFORMED CONSENT

Title of Study: The effects of half-time cooling interventions on male soccer-simulated intermittent exercise performance.

1- Purpose of the Study:

The main aim of this study is to determine the effects of half-time cooling interventions on male soccer-simulated intermittent exercise performance in hot and humid environmental conditions. In addition, we also want to examine the differences between the cooling applications of neck cooling and leg cooling on the responses of blood flow (skin and muscle), body temperature (core and skin), and perceptual thermal sensation.

2-Study Procedures:

The study for which you are volunteering will take about 3-4 weeks to complete and will have 3 phases:

1. Screening Visit and VO_{2max} Testing (visit 1)
2. Familiarization Trial (visit 2)
3. Experimental Trials (visits 3-5)

Study Timeline and Visits

Participants enrolling in the study will be required to make 5 visits to the exercise testing laboratory or the environmental laboratory at LSU, including 1 screening visit and VO_{2max} testing about (30~60 min), 1 familiarization trial, and 3 experimental trials. We estimate that each participant will take 3 to 4 weeks to complete the full study with ~2 hours commitment for each visit.

<u>Screening Visit – 30 min</u>

At this visit, our study staff will explain the informed consent to you and the procedures that will be performed. If you choose to sign the informed consent, the following tests and procedures will be performed to determine if you qualify to participate in this research study.

- You will be asked questions about your:
    - Demographics
    - Medical history and Physical Activity Readiness (PAR-Q+)
    - Medication use

- You will be asked to perform the following tests:
    - Height and weight
    - VO_{2max} Test

Familiarization trial (90 minutes)

You will perform a familiarization trial to walk through the entire experimental procedure (Figure 1) under normal environmental conditions (~70°F). Food logs will be provided to you to record the item and amount of food and water intake 24 hours before this trial. After you arrive at testing lab, our study staff will review the food logs with you and type in a 24 hr recall food logs. The 24 hr recall food logs will be printed and you will follow the same diet before each exercise trial. Heart rate monitor will be worn during the exercise. Meanwhile, our study staff will explain the exercise protocol and potential measurements during exercise. As exercise starts, heart rate will be measured continuously. RPE and thermal sensation will be measured during the standing phase.

Exercise Bouts – 3 Exercise trials (2 hours each)

You will be randomized to 3 exercise testing trials with a different cooling intervention during the half-time of the intermittent exercise separated by about 1 week (minimum 4 days). A core temperature pill will be ingested 4-5 hours before each testing period. The three interventions include 1) neck cooling, 2) leg cooling, and 3) no cooling.

Exercise Test in Heat Environmental Chamber (Figure 1)

Each exercise test consists of 90 min of intermittent exercise followed by extra sets of intermittent exercise on the treadmill to elicit volitional fatigue. The room will be heated to ~30°C (86°F) and 60% relative humidity during the testing. The first 45 min (1st half) consists 9 sets of 5 min intermittent exercise with sprints, runs, walks, and stands, similarly to the familiarization trial. Each 5 min intermittent exercise contains 150s walking, 90s running, 30s sprinting and 30s standing (Figure 1). After the 1st half is finished, a 15 min break (half-time) will be given for cooling interventions an fluid intake. The second 45 min (2nd half) is the same as first half. The extra sets of intermittent exercise will be performed after 90 min until voluntary exhaustion or core temperature 40°C (104°F). After exercise is finished, an extra 10 min of hands cooling (Kelvi Inc.) will be provided to reduce the core temperature. Water will be provided at 5 ml/kg in each half between and 10 ml/kg during the half time. The water provided must be ingested during the time block provided. The following measures will be conducted during the exercise testing period:

- Diet: The same diet will be recommended before each experiment trial according to the diet that are record in the familiarization trial. Our study staff will give you the hard copies of your dietary intake form that ensure the same diet can be

followed.

- Body weight: Body weight will be measured before and after exercise to determine overall sweat loss, and body weight will be measured after urinating and toweling dry.

- Urine specific gravity: Urine sample will be collected before exercise to determine the hydration status.

- Heart rate: You will wear a heart rate monitor around the chest for the duration of the exercise test. The device will continuously detect heart rate throughout the exercise.

- Body temperature: Core and skin temperature will be continuously monitored during the entire exercise. The exercise test will be discontinued if your core temperature excess of 40°C (104°F) or the participant is symptomatic for heat exhaustion.

- Cooling interventions: Cooling intervention will be applied during the half time for 10 min with either neck cooling, leg cooling, and no cooling. Neck cooling will use a temperature controlled cooling device to wrap around the neck and head, and leg cooling will use a temperature controlled cooling device (Kelvi Inc.) to cool down the quadriceps and hamstrings. In control trial, participants only have a seated position outside chamber without cooling applied.

- Skin blood flow: Skin blood flow probe will be applied for 1 min while participants at rest with the seated position at pre-exercise as baseline, 1 min rest of the first half, 1 min at the beginning of the half time, 1 min after the cooling, and 1 min after exercise finished.

- fNIRs: Leg muscle blood flow will be used a portable NIRs probe (PortaMon; Artinis Medical System) is attached with a surgical tape over the central part of the lateral gastrocnemius muscle of one leg. The probe and the skin will be covered with a strap wrapping around the leg to stabilize and to prevent contamination from the ambient light.

- Other measurements: Whole body thermal sensation, neck and leg thermal sensation were taken at each intermittent set. The thermal sensation scale will used 9 points scale that ranged from -4 (very cold) to 4 (very hot). Ratings of perceived exertion (RPE; Borg 6-20 scale) will be taken at each set during the standing phase.

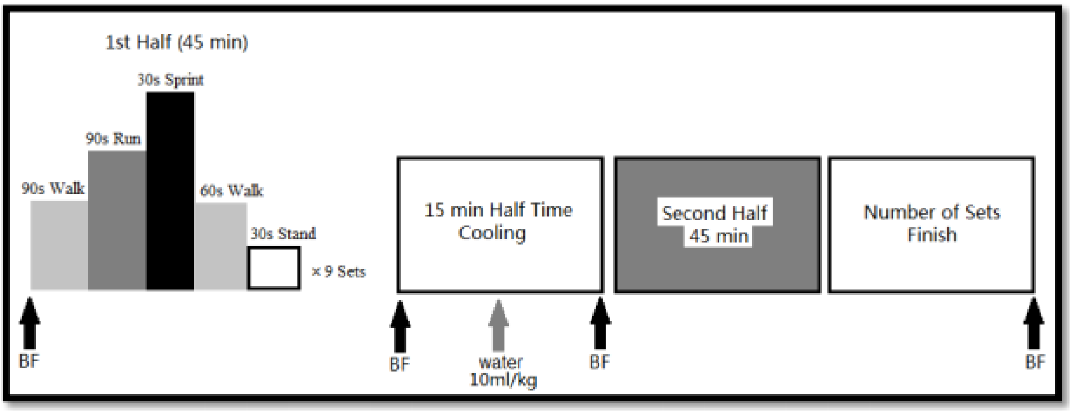


Figure 1. Intermittent Exercise Protocol. BF: blood flow (skin and leg muscle)

3- Investigators:

The following investigator will be available for questions about this study:

Principal Investigator: Neil M. Johannsen, Ph.D.
Phone: 225-578-5314
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Haoyan Wang
Phone: 225-328-8984
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Neil Johannsen, Ph.D. and Haoyan Wang direct this study. We expect up to have 5 visits including, 1 screening visit, 1 familiarization trial, and 3 experimental trials with total ~9 hours.

4- Performance Site:

This study will take place at Louisiana State University-Baton Rouge Campus.

5- Participant Inclusion:

Study staff will discuss with you the requirements for participation in this study. It is important that you are completely truthful with the staff about your health history. You should not participate in this study if you do not meet all the qualifications.

You are eligible for this study if you are:

- Men aged 18-30 years
- Maximal oxygen uptake 45~60 ml/kg/min
- Healthy (No Known Disease/No uncontrolled Disease)
- Body mass index (BMI) < 30 kg/m^2
- Answer all "no" on the PAR-Q form
- Willing to participate in a research study that will take 3 to 4 weeks.
- Willing to participate in exercise trials in a heated environment chamber
- Willing to wear physiological monitors (ex. heart rate monitor) during the exercise bouts.
- Willing to ingest a pill to determine core (body) temperature and attach skin temperature probes prior to and maintain during the exercise tests
- Willing to be attached skin temperature probes on abdomen, calf, forearm, neck and auditory canal
- Willing to wear blood flow probes attached to the forearm, leg and head
- Willing to apply the cooling material during the half-time of the exercise

You are **NOT eligible** for this study if you have any of the following conditions:

- HIV
- Hepatitis B
- Hepatitis C
- Uncontrolled CVD/ arrhythmia
- COPD
- Emphysema
- Exercise Induced asthma/bronchospasm
- Cerebral Palsy
- Multiple Sclerosis
- Cystic Fibrosis
- Amyotrophic lateral sclerosis
- Diabetes Mellitus
- Uncontrolled Thyroid disorder (controlled = 6 months of medication)
- Epilepsy
- Osteo/Rheumatoid arthritis
- Heat Intolerance
- Unresolved orthopedic injury of any kind
- Any other condition or known disease that can be exacerbated by exercise in a heated environment

Medications/Non-Drug Therapies

- Diuretics
- Beta-blocker
- Antipsychotic
- Other medications that may affect fluid balance, thirst, or heat tolerance

Lifestyle

- Consumption > 3 drinks/day of any alcoholic beverage
- Smoker (Former smokers must be smoke free for 12 months)
- Donated blood within the past 6 weeks
- Maximal oxygen uptake less than 45 ml/kg/min
- Answer any "Yes" on the statement of PAR-Q form

6- Risks/Discomforts:

Exercise testing in the heat: All exercise testing is completed in accordance with the American College of Sports Medicine's Guidelines for Exercise Testing and Prescription as well as the American Heart Association. There is minimal risk of injury or a cardiovascular event during testing. We believe the use of a highly trained staff, a pretest review of your associated risk factors including the Physical Activity Readiness Questionnaire (PAR-Q) and medical screening by your doctor, and well-defined emergency procedures minimize the risk of an event during testing. The exercise will be discontinued if your core temperature measured by core temperature pill exceeds 40°C (104°F). You may also experience muscle fatigue, weakness, soreness and/or muscle pulls or tears. All tests are conducted in the presence of trained staff. In addition, all staff are trained in BLS (basic life support-CPR) and/or ACLS (advanced cardiac life support). In the event of an emergency, you would be treated appropriately and transported to the nearest acute care medical-surgical facility via Emergency Medical Services.

Body temperature:
The pill used to detect core body temperature should not enter an MRI due to a risk of internal injury. We will provide you with a wrist band to be worn for 48 hours after the ingestion of the pill to be sure medical personnel know of the pill in case of emergency.

Loss of Confidentiality: Completing questionnaires may result in a breach in confidentiality of personal data. You will be assigned ID numbers and information that could identify you will not appear in publications.
Unknown risks: In addition to the risk listed above, you may experience a previously unknown risk or side effect.

7- Benefits:
We cannot promise any benefits from your being in the study. However, possible benefits include:

- Information about your general health
- Knowledge of your cardiovascular fitness
- An understanding of your exercise performance and physical activity

8-Alternatives to Participation:
There are no alternatives to the study described in this consent. You have the choice at any time not to participate in this research study. If you choose not to participate, any benefits to which you are entitled will not be affected in any way.

9- Injury/Illness or Questions:
If you have any questions about your rights as a research volunteer, you should call Dennis Landin, Ph.D., Institutional Review Board Office at 225-578-8692. If you have any questions about the research study or think you have a research-related injury or medical illness, contact Neil Johannsen, Ph.D. at 225-578-5314 during regular working hours.

10-Privacy:
Every effort will be made to maintain the confidentiality of your study records. Results of the study may be published; however, we will keep your name and other identifying information private. Other than as set forth above, your identity will remain confidential unless disclosure is required by law.

11-Early Study Withdrawal:
Neil Johannsen, Ph.D. can withdraw you from the study for any reason or for no reason. You may withdraw from the study at any time without penalty. Possible reasons for withdrawal include injury, the presence of an old or existing injury that may be deemed risky, sufficient medical history deemed too risky for testing. The sponsor of the study may end the study early.

12-Additional Information:
During the course of this study there may be new findings from this or other research that may affect your willingness to continue participation. Information concerning any such new findings will be provided to you.

13-Charges for Participation:

None

14-Payments for Participation:

You will receive $25 for each completed exercise bout for a total of $100. This amount will be paid upon the completion of the study (takes ~3-4 weeks) or upon withdraw from the study for partial payments.

15- Compensation for study-related injury or medical illness:

No form of compensation for medical treatment or for other damages (i.e., lost wages, time lost from work, etc.) is available from Louisiana State University. In the event of injury or medical illness resulting from the research procedures in which you participate, you will be referred to a treatment facility. Medical treatment may be provided at your own expense or at the expense of your health care insurer (e.g., Medicare, Medicaid, Blue Cross-Blue Shield, Dental Insurer, etc.) which may or may not provide coverage.

16- Signatures:

The study has been discussed with me and all my questions have been answered. I understand that additional questions regarding the study should be directed to the study investigators. I agree with the terms above and acknowledge that I have been given a copy of the consent form.

____

Printed Name of Volunteer

____________________________________ ______________

Signature of Volunteer Date

____

Date of Birth of Volunteer

____ ______________

Signature of Person Administering Informed Consent Date

Neil M. Johannsen, Ph.D.

Principal Investigator

1.3 Study Protocol

Study Protocol

I) Title

The effects of half-time cooling interventions on male soccer-simulated intermittent exercise performance.

II) Overview

Soccer players are frequently exposed in the hot and humid environmental conditions with the ambient temperature around 30°C (86°F) accompanied with high relative humidity in excess of 60%.[1,2] Hot and humid environmental conditions bring extra challenge on players causing elevated core and skin temperature resulting in a high thermal stress.[3] Core temperature reaching to the upper limit value of 39°C had been observed in competitive soccer games under the heat conditions causing the performance reduction and exercise cessation. Sprint performance and total distance covered were significantly impaired when the ambient temperature exceeded 22°C (72°F) with the relative humidity greater than 60% or when the ambient temperature exceeded 28°C (82°F) regardless of relative humidity.[4,5] Cooling interventions have been recently investigated for the purpose of reducing thermal strain and ameliorating physiological stress in intermittent exercise under heat conditions, suggesting the potential application in soccer events. However, fewer study had examined the effects of cooling interventions on soccer-specific intermittent exercise.

III) Purpose

The main aim of this study is to determine the effects of half-time cooling interventions on male soccer-simulated intermittent exercise performance in hot and humid environmental conditions. In addition, we also want to examine the differences between the cooling applications of neck cooling and leg cooling on the responses of blood flow (skin and muscle), body temperature (core and skin), and perceptual thermal sensation.

IV) Inclusion/Exclusion

The target study group will be 9-12 heathy men aged 18 to 25 years. High-risk individuals according to the American College of Sports Medicine (ACSM) includes those with known cardiovascular, pulmonary, or metabolic disease, or sign/symptoms suggestive of disease will be excluded.[6] Potential participants will be included or excluded based on the following criteria:

a) Inclusion Criteria

- Men aged 18-30 years
- Maximal oxygen uptake ranged 45~60 ml/kg/min
- Healthy (No Known Disease/No uncontrolled Disease)
- Body mass index (BMI) < 30 kg/m^2
- Answer all "no" on the PAR-Q form
- Willing to participate in a research study that will take 3 to 4 weeks.
- Willing to participate in 4 exercise trials in a heated environment chamber
- Willing to wear physiological monitors (ex. heart rate monitor) during the exercise bouts.
- Willing to ingest a pill to determine core (body) temperature and attach skin temperature probes prior to and maintain during the exercise tests
- Willing to be attached skin temperature probes on abdomen, calf, forearm, neck and auditory canal
- Willing to wear blood flow probes attached to the forearm, leg and head
- Willing to apply the cooling material during the half-time of the exercise

b) Exclusion Criteria

i) Diseases

- HIV
- Hepatitis B
- Hepatitis C
- Uncontrolled CVD/ arrhythmia
- COPD
- Emphysema
- Exercise Induced asthma/bronchospasm
- Cerebral Palsy
- Multiple Sclerosis
- Cystic Fibrosis
- Amyotrophic lateral sclerosis
- Diabetes Mellitus
- Uncontrolled Thyroid disorder (controlled = 6 months of medication)
- Epilepsy
- Osteo/Rheumatoid arthritis
- Heat Intolerance
- Unresolved orthopedic injury of any kind
- Any other condition or known disease that can be exacerbated by exercise in a heated environment

ii) Medications/Non-Drug Therapies

- Diuretics
- Beta-blocker
- Antipsychotic
- Other medications that may affect fluid balance, thirst, or heat tolerance

iii) Lifestyle

- Consumption > 3 drinks/day of any alcoholic beverage
- Smoker (Former smokers must be smoke free for 12 months)
- Donated blood within the past 6 weeks
- Maximal oxygen uptake less than 45 ml/kg/min
- Answer any "Yes" on the statement of PAR-Q form

V) <u>Main Study Outcomes</u>

This main outcome is to determine the effects of cooling interventions on the soccer-simulated intermittent exercise performance in the heat. The exercise performance will be determined by the number of intermittent sets after cooling interventions. The secondary outcomes will examine the responses of body temperature (skin and core), blood flow (skin and muscle), and perceptual thermal sensation while different cooling interventions applied.

a) Exercise Test in Heat Environment Chamber

The exercise testing will be conducted in the heat chamber at temperature of 30°C (86°F) and 60% relative humidity. The intermittent exercise will simulate the demands of soccer physical characters. Participants will warm up for 5 minutes before the exercise starting with the speed at 8 km/h and 3 sets of speed self-selected sprints on a treadmill. The intermittent exercise consists of two 45 min halves separated by 15 min half-time for cooling applications. Each half contains 9 sets of 5 min intermittent exercises. Each set

of intermittent exercise consists 30-s maximal sprints with the speed at 80~90% VO_{2max}, 90-s runs at the speed of 60% VO_{2max}, 150-s walking at the speed of 30% VO_{2max}, and 30-s standing (Figure 1). After 90 min of the exercise, participant keeps performing the extra sets of intermittent exercise until voluntary exhaustion or the core temperature reaches to 40°C (104°F). After exercise is finished, an extra 10 min hands cooling (Kelvi Inc.) will be provided to reduce the core temperature. The number of sets will be used to determine exercise performance. The number of sets will be blinded to participant until 3 experiment trials finishes.

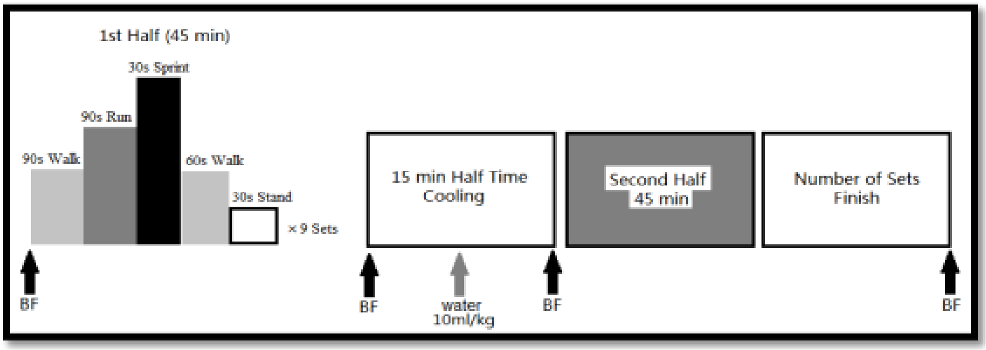

Figure 1. Intermittent Exercise Protocol; BF: blood flow (skin and leg muscle)

b) Physiology Measures

Body weight will be measured using a standard scale before and after exercise with minimal clothing at each visit. The change in body weight will be used to estimate sweat loss. Heart rate monitor will be worn throughout the entire exercise.

Urine sample will be collected in the urine cup before exercise. Urine specific gravity (USG) will be measure to ensure the euhydration status before exercise with the value less than 1.020. Urine sample will not be stored for the further analysis.

Body Core temperature will be measured continuously by using an ingestible sensor (HTI Technologies; Palmetto, FL). Participants are required to ingest core temperature pills 4-5 hours before exercise trial to assure it reaches the intestinal tract and negates the potential impact of beverage intake on core temperature readings. Mean skin temperature will be calculated from 4 sites of skin temperatures: trunk (right abdomen), forearm (mid-point of fullest muscle belly), and calf (mid-point of two gastrocnemius).[7] In addition, neck temperature will be measured at back of the neck and outside the cooling area. Auditory canal temperature will be measured by using skin temperature probe attached near the tympanic membrane and insulated from ambient temperature by a cotton earplug.[8]

Skin blood flow will be measured using a laser probe Doppler flowmeter placed on the largest circumferences of the forearm (Perimed, Stockholm, Sweden) with double sided tape.

Functional near-infrared spectroscopy (fNIRs) will be used to determine the blood flow of the leg muscle by measuring the differential concentration of oxygenated hemoglobin (HbO2) and deoxygenated hemoglobin (Hb). The portable wearable wireless NIRs probe will be placed on leg. For leg muscle blood flow, the NIRs probe will be placed on the central part of the lateral gastrocnemius of one leg with surgical tape. In addition, the

probe will be secured in place by an elastic strap to stabilize on the location and to prevent the contamination from the ambient light.[9]

c) **Perceptual Measures**
Whole body thermal sensation, neck and leg thermal sensation will be measured by using the thermal sensation scale with 9 points ranged from -4 (very cold) to 4 (very hot). Ratings of perceived exertion (RPE) will be used Borg 6-20 scale.

VI) Study Timeline and Visits

Participants enrolling in the study will be required to make 5 visits to the exercise testing in the heat chamber of LSU, including 1 screening visit and VO_{2max} testing (30~60 min), 1 familiarization trial, and 3 experiment trials in a crossover randomized order. We estimate that each participant will take 3 to 4 weeks to complete the full study with ~2 hours of each exercise trial.

a) **Screening Visit (30~60 min)**
During the screening visit, participants will be given the informed consent, if signed, will undergo medical screening, anthropometrics, and VO_{2max} measures.

- Informed consent: The informed consent will be explained to participants by study staff. Once procedures have been explained and participants have had the opportunity to ask questions, they will have the option to sign the informed consent.

- Medical Screening: All participants will complete a PAR-Q form, medical history questionnaires, and medication inventory. **Individuals who mark an answer other than "No" on the PAR-Q will be excluded from the study.**

- Anthropometrics: Body height and weight will be assessed.

- Maximal Exercise Test: This test may or may not on the same day of screening visit. Aerobic fitness will be assessed while running on the treadmill. Participants will warm up on the treadmill at a low speed. After warm up, the initial speed and grade of the treadmill at a low intensity, and slowly increase in a regular time increments every 2 to 4 minutes unit participants are unable to continue running. **VO_{2max} is lower than 45 ml/kg/min will be excluded from the study.**

b) **Familiarization Visit**
One familiarization trial will be given to participants to walk through the entire experiment settings. Food logs will be provided to the participants to record the items and the amount of food and water intake 24 hours before exercise. After participant arrives the testing lab, study staff will use 24-hour recalls in an electronic format review the logs with participants to ensure nothing will miss. The food records will save and print that participants can follow the similar diet before each experiment trial. In addition, 5 min resting will be given to participant for wearing heart rate monitor. Meanwhile, the study staff will explain the exercise protocol and potential measurements during exercise. As exercise starts, heart rate will be measured continuously. RPE and thermal sensation will be measured at each set during the standing phase. After 1st half of exercise, 15 min half-time will be given to participant followed by the second half of exercise, which is the same as first half. The data of familiarization trial will not include in the statistical analysis. This visit is to ensure the participant familiarizes the protocol and procedures and negates the order effects of the main experiment trials.

c) Randomization and Exercise Testing (2 hours each visits)

Participants will be randomized to 3 exercise testing trials with the different cooling interventions. Core temperature pill is ingested 4-5 hours before each visit. The three interventions include 1) neck cooling, 2) leg cooling, and 3) no cooling. The exercise tests will be separated by at least 4 days. Each exercise test will consist of a 90-min intermittent exercise plus an extra sets of intermittent exercise in the heat chamber. Each 45 minutes of the intermittent exercise has 9 sets of 5 min intermittent exercise. The half-time has 15 minutes and 10 minutes will be applied for cooling interventions. The extra sets of intermittent exercise will be performed after 90 min until voluntary exhaustion or core temperature 40°C (104°F). After exercise is finished, an extra 10 min of hands cooling will be provided to reduce the core temperature. Water will be allowed to ingest at 5 ml/kg in each half and 10 ml/kg at the half-time. The water provided must be ingested during the time block provided.

- Intermittent exercise: Intermittent exercise will consist of 90 min on the treadmill followed by the same intermittent exercise until participant voluntary fatigue or core temperature reaches to 40°C (104°F).

- Body weight: Body weight will be measured before and after exercise to determine overall sweat loss, and body weight will be measured after urinating and toweling dry.

- Urine specific gravity: Urine sample will be collected before exercise to determine the hydration status.

- Heart rate: Participants will wear a heart rate monitor around the chest for the duration of the exercise test. The device will continuously detect heart rate throughout the exercise.

- Body temperature: Core and skin temperature will be continuously monitored during the entire exercise. The exercise test will be discontinued if a participant's core temperature excess of 40°C (104°F) or the participant is symptomatic for heat exhaustion.

- Cooling interventions: Cooling interventions will be applied during half time for 10 min with either neck cooling, leg cooling (Kelvi Inc.) and no-cooling (control). Neck cooling will use a cooling device that wraps around the neck and head, and leg cooling will use the cooling device to cool down the quadriceps and hamstrings. In the control trial, participants are only in a seated position outside chamber without cooling applied.

- Skin Blood flow: Skin blood flow probe will be applied for 1 min while participants at rest with the seated position at pre-exercise as baseline, 1 min rest of the first half, 1 min at the beginning of the half time, 1 min after the cooling, and 1 min after exercise finished.

- fNIRs: Leg muscle blood flow will be used a wireless portable monitor wearing on the leg during the exercise.

- Other measurements: Whole body thermal sensation, neck and leg thermal sensation were taken every 4 min and 1 min rest. Ratings of perceived exertion (RPE; Borg 6-20 scale) will be taken at every 4 min in all trials.

VII) Statistical Analysis

Power analysis was used to calculate the sample size (GPower) at 80% power and α=0.05. Assuming the effect size of intervention of the experimental procedure ranges from 0.4~0.6, the sample size range will be from 9~12.

Data will be analyzed using JMPro 14 (SAS Inc., Cary, NC). One-way analysis of variance (ANOVA) will be used to analyze outcome variables in different trials. The outcome variables include exercise performance, heart rate, core temperature, body temperature, blood flow, and the perceptual measures. Data will present as mean ± standard deviation (SD) and statistical significance will be accepted at $p<0.05$.

VIII) Expected Outcomes

We hypothesize that cooling trials can prevent exercise decrements and prolong exercise duration under heat conditions compared to no cooling. In addition, we expect to observe neck cooling affects perceptual thermal sensation, and alter auditory canal temperature. Leg cooling alters skin blood flow, muscle temperature, and core temperature.

Table 1. Schedule of Assessment

	Screening Visit	Familiarization Trial	Experiment Trials
Procedure (Time)	**Visit 1**	**Visit 2**	**Visit 3-5**
Informed Consent	×		
PAR-Q and Medical History	×		
Medication Inventory	×		
Height	×		
Weight	×	×	×
Maximal Oxygen Uptake	×		
Randomization	×		
Food and Fluid Record		×	×
Urine Specific Gravity		×	×
Intermittent Exercise		×	×
Core Temperature			×
Skin Temperature		×	×
Skin blood flow		×	×
fNIRs (leg muscle blood flow)		×	×
Perceptual Measures		×	×

<u>**References**</u>

1. Coker NA, Wells AJ, Gepner Y. The Effect of Heat Stress on Measures of Running Performance and Heart Rate Responses During A Competitive Season in Male Soccer Players. *J Strength Cond Res.* 2018.
2. Ruddock AD, Thompson SW, Hudson SA, James CA, Gibson OR, Mee JA. Combined active and passive heat exposure induced heat acclimation in a soccer referee before 2014 FIFA World Cup. *Springerplus.* 2016;5:617.
3. Maughan RJ, Otani H, Watson P. Influence of relative humidity on prolonged exercise capacity in a warm environment. *Eur J Appl Physiol.* 2012;112(6):2313-2321.
4. Ozgunen KT, Kurdak SS, Maughan RJ, et al. Effect of hot environmental conditions on physical activity patterns and temperature response of football players. *Scand J Med Sci Sports.* 2010;20 Suppl 3:140-147.
5. Chmura P, Konefal M, Andrzejewski M, Kosowski J, Rokita A, Chmura J. Physical activity profile of 2014 FIFA World Cup players, with regard to different ranges of air temperature and relative humidity. *Int J Biometeorol.* 2017;61(4):677-684.
6. Thompson PD, Arena R, Riebe D, Pescatello LS, American College of Sports M. ACSM's new preparticipation health screening recommendations from ACSM's guidelines for exercise testing and prescription, ninth edition. *Curr Sports Med Rep.* 2013;12(4):215-217.
7. Ramanathan NL. A New Weighting System for Mean Surface Temperature of the Human Body. *J Appl Physiol.* 1964;19:531-533.
8. Bittel JH, Livecchi-Gonnot GH, Hanniquet AM, Poulain C, Etienne JL. Thermal changes observed before and after J.-L. Etienne's journey to the North Pole. Is central nervous system temperature preserved in hypothermia? *Eur J Appl Physiol Occup Physiol.* 1989;58(6):646-651.
9. Inglis EC, Iannetta D, Murias JM. Evaluating the NIRS-derived microvascular O2 extraction "reserve" in groups varying in sex and training status using leg blood flow occlusions. *PLoS One.* 2019;14(7):e0220192.

LIST OF REFERENCES

Al Haddad H, Laursen PB, Ahmaidi S, Buchheit M. Influence of cold water face immersion on post-exercise parasympathetic reactivation. *Eur J Appl Physiol*. 2010;108(3):599-606.

Aldous JW, Akubat I, Chrismas BC, et al. The reliability and validity of a soccer-specific nonmotorised treadmill simulation (intermittent soccer performance test). *J Strength Cond Res*. 2014;28(7):1971-1980.

American College of Sports M, Sawka MN, Burke LM, et al. American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci Sports Exerc*. 2007;39(2):377-390.

Anastasiou CA, Kavouras SA, Arnaoutis G, et al. Sodium replacement and plasma sodium drop during exercise in the heat when fluid intake matches fluid loss. *J Athl Train*. 2009;44(2):117-123.

Armstrong LE, Costill DL, Fink WJ. Influence of diuretic-induced dehydration on competitive running performance. *Med Sci Sports Exerc.* 1985;17(4):456-461.

Armstrong LE, Crago AE, Adams R, Roberts WO, Maresh CM. Whole-body cooling of hyperthermic runners: comparison of two field therapies. *Am J Emerg Med*. 1996;14(4):355-358.

Armstrong LE, Curtis WC, Hubbard RW, Francesconi RP, Moore R, Askew EW. Symptomatic hyponatremia during prolonged exercise in heat. *Med Sci Sports Exerc*. 1993;25(5):543-549.

Armstrong LE, Johnson EC, Casa DJ, et al. The American football uniform: uncompensable heat stress and hyperthermic exhaustion. *J Athl Train.* 2010;45(2):117-127.

Armstrong LE, Soto JA, Hacker FT, Jr., Casa DJ, Kavouras SA, Maresh CM. Urinary indices during dehydration, exercise, and rehydration. *Int J Sport Nutr.* 1998;8(4):345-355.

Arngrimsson SA, Petitt DS, Borrani F, Skinner KA, Cureton KJ. Hyperthermia and maximal oxygen uptake in men and women. *Eur J Appl Physiol*. 2004;92(4-5):524-532.

Arngrimsson SA, Petitt DS, Stueck MG, Jorgensen DK, Cureton KJ. Cooling vest worn during active warm-up improves 5-km run performance in the heat. *J Appl Physiol (1985).* 2004;96(5):1867-1874.

Arngrimsson SA, Stewart DJ, Borrani F, Skinner KA, Cureton KJ. Relation of heart rate to

percent VO2 peak during submaximal exercise in the heat. *J Appl Physiol (1985).* 2003;94(3):1162-1168.

Baker LB, Barnes KA, Anderson ML, Passe DH, Stofan JR. Normative data for regional sweat sodium concentration and whole-body sweating rate in athletes. *J Sports Sci*. 2016;34(4):358-368.

Baker LB, De Chavez PJD, Ungaro CT, et al. Exercise intensity effects on total sweat electrolyte losses and regional vs. whole-body sweat [Na(+)], [Cl(-)], and [K(+)]. *Eur J Appl Physiol*. 2019;119(2):361-375.

Baker LB. Sweating Rate and Sweat Sodium Concentration in Athletes: A Review of Methodology and Intra/Interindividual Variability. *Sports Med*. 2017;47(Suppl 1):111-128.

Baron S, Courbebaisse M, Lepicard EM, Friedlander G. Assessment of hydration status in a large population. *Br J Nutr*. 2015;113(1):147-158.

Barr D, Gregson W, Sutton L, Reilly T. A practical cooling strategy for reducing the physiological strain associated with firefighting activity in the heat. *Ergonomics*. 2009;52(4):413-420.

Bartok C, Schoeller DA, Sullivan JC, Clark RR, Landry GL. Hydration testing in collegiate wrestlers undergoing hypertonic dehydration. *Med Sci Sports Exerc*. 2004;36(3):510-517.

Bennett BL, Hagan RD, Huey KA, Minson C, Cain D. Comparison of two cool vests on heat-strain reduction while wearing a firefighting ensemble. *Eur J Appl Physiol Occup Physiol*. 1995;70(4):322-328.

Bergh U, Ekblom B. Influence of muscle temperature on maximal muscle strength and power output in human skeletal muscles. *Acta Physiol Scand*. 1979;107(1):33-37.

Berglund LG, Gonzalez RR. Evaporation of sweat from sedentary man in humid environments. *J Appl Physiol Respir Environ Exerc Physiol*. 1977;42(5):767-772.

Bishop P, Balilonis G, Davis J, Zhang Y. Ergonomics and comfort in protective and sport clothing: A brief review. *J Ergon*. 2013(S2):1-7.

Bittel J, Henane R. Comparison of thermal exchanges in men and women under neutral and hot conditions. *J Physiol*. 1975;250(3):475-489.

Bittel JH, Livecchi-Gonnot GH, Hanniquet AM, Poulain C, Etienne JL. Thermal changes observed before and after J.-L. Etienne's journey to the North Pole. Is central nervous system temperature preserved in hypothermia*? Eur J Appl Physiol Occup Physiol*. 1989;58(6):646-651.

Boushel R, Langberg H, Green S, Skovgaard D, Bulow J, Kjaer M. Blood flow and oxygenation in peritendinous tissue and calf muscle during dynamic exercise in humans. *J Physiol*. 2000;524 Pt 1:305-313.

Brengelmann GL, Johnson JM, Hermansen L, Rowell LB. Altered control of skin blood flow during exercise at high internal temperatures. *J Appl Physiol Respir Environ Exerc Physiol*. 1977;43(5):790-794.

Bristow GK, Sessler DI, Giesbrecht GG. Leg temperature and heat content in humans during immersion hypothermia and rewarming. *Aviat Space Environ Med*. 1994;65(3):220-226.

Broad EM, Burke LM, Cox GR, Heeley P, Riley M. Body weight changes and voluntary fluid intakes during training and competition sessions in team sports. *Int J Sport Nutr*. 1996;6(3):307-320.

Brooks GA, Fahey TD, Baldwin KM. Exercise physiology : human bioenergetics and its applications. 4th ed. Boston: McGraw-Hill; 2005.

Brouns F. Gastric emptying as a regulatory factor in fluid uptake. *International journal of sports medicine*. 1998;19 Suppl 2:S125-128.

Brown SL, Banister EW. Thermoregulation during prolonged actual and laboratory-simulated bicycling. *Eur J Appl Physiol Occup Physiol*. 1985;54(1):125-130.

Burton AC, Edholm OG. Man in a cold environment; physiological and pathological effects of exposure to low temperatures. New York,: Hafner Pub. Co.; 1969.

Burton AC. Human calorimetry II. The average temperature of the tissues of the body. *J Nutr*. 1935;9:264-280.

Cabanac M, Germain M, Brinnel H. Tympanic temperatures during hemiface cooling. *Eur J Appl Physiol Occup Physiol*. 1987;56(5):534-539.

Cadarette BS, Levine L, Rock PB, Stephenson LA, Kolka MA. Effects of atropine on thermoregulatory responses to exercise in different environments. *Aviat Space Environ Med*. 1986;57(11):1050-1055.

Castle PC, Macdonald AL, Philp A, Webborn A, Watt PW, Maxwell NS. Precooling leg muscle improves intermittent sprint exercise performance in hot, humid conditions. *J Appl Physiol (1985)*. 2006;100(4):1377-1384.

Chaen Y, Onitsuka S, Hasegawa H. Wearing a Cooling Vest During Half-Time Improves Intermittent Exercise in the Heat. *Front Physiol.* 2019;10:711.

Cheung SS. Advanced environmental exercise physiology. Champaign, IL: Human Kinetics; 2010.

Cheuvront SN, Carter R, 3rd, Sawka MN. Fluid balance and endurance exercise performance. *Current sports medicine reports.* 2003;2(4):202-208.

Cheuvront SN, Kenefick RW, Montain SJ, Sawka MN. Mechanisms of aerobic performance impairment with heat stress and dehydration. *J Appl Physiol (1985).* 2010;109(6):1989-1995.

Cheuvront SN, Kenefick RW, Zambraski EJ. Spot Urine Concentrations Should Not be Used for Hydration Assessment: A Methodology Review. *Int J Sport Nutr Exerc Metab*. 2015;25(3):293-297.

Chmura P, Konefal M, Andrzejewski M, Kosowski J, Rokita A, Chmura J. Physical activity profile of 2014 FIFA World Cup players, with regard to different ranges of air temperature and relative humidity. *Int J Biometeorol*. 2017;61(4):677-684.

Claybaugh JR, Sato AK, Crosswhite LK, Hassell LH. Effects of time of day, gender, and menstrual cycle phase on the human response to a water load. *Am J Physiol Regul Integr Comp Physiol*. 2000;279(3):R966-973.

Coker NA, Wells AJ, Gepner Y. The Effect of Heat Stress on Measures of Running Performance and Heart Rate Responses During A Competitive Season in Male Soccer Players. *J Strength Cond Res*. 2018.

Coles MG, Luetkemeier MJ. Sodium-facilitated hypervolemia, endurance performance, and thermoregulation. *Int J Sports Med*. 2005;26(3):182-187.

Colin J, Timbal J, Boutelier C. [Experimental determination of the equation permitting the calculation of the mean body temperature in neutral and hot environment]. *J Physiol (Paris)*. 1971;63(3):229-231.

Colin J, Timbal J, Houdas Y, Boutelier C, Guieu JD. Computation of mean body temperature from rectal and skin temperatures. *J Appl Physiol*. 1971;31(3):484-489.

Connes P, Sara F, Hardy-Dessources MD, et al. Effects of short supramaximal exercise on hemorheology in sickle cell trait carriers. *Eur J Appl Physiol*. 2006;97(2):143-150.

Coyle EF, Gonzalez-Alonso J. Cardiovascular drift during prolonged exercise: new perspectives. *Exerc Sport Sci Rev.* 2001;29(2):88-92.

Cramer MN, Jay O. Partitional calorimetry. *J Appl Physiol (1985)*. 2019;126(2):267-277.

Crandall CG, Gonzalez-Alonso J. Cardiovascular function in the heat-stressed human. *Acta Physiol (Oxf)*. 2010;199(4):407-423.

Crowley GC, Garg A, Lohn MS, Van Someren N, Wade AJ. Effects of cooling the legs on performance in a standard Wingate anaerobic power test. *Br J Sports Med*. 1991;25(4):200-203.

Cutrufello PT, Dixon CB, Zavorsky GS. Hydration assessment among marathoners using urine specific gravity and bioelectrical impedance analysis. *Res Sports Med*. 2016;24(3):234-242.

Dai XQ, Imamura R, Liu GL, Zhou FP. Effect of moisture transport on microclimate under T-shirts. *Eur J Appl Physiol*. 2008;104(2):337-340.

Datson N, Drust B, Weston M, Jarman IH, Lisboa PJ, Gregson W. Match Physical Performance of Elite Female Soccer Players During International Competition. *Journal of strength and conditioning research / National Strength & Conditioning Association*. 2017;31(9):2379-2387.

Davis JA, Brewer J. Applied physiology of female soccer players. *Sports Med*. 1993;16(3):180-189.

Davis JK, Baker LB, Barnes K, Ungaro C, Stofan J. Thermoregulation, Fluid Balance, and Sweat Losses in American Football Players. *Sports Med*. 2016;46(10):1391-1405.

Davis JK, Bishop PA. Impact of clothing on exercise in the heat. *Sports Med*. 2013;43(8):695-706.

Davis JK, Laurent CM, Allen KE, et al. Influence of Dehydration on Intermittent Sprint Performance. *Journal of strength and conditioning research / National Strength & Conditioning Association*. 2015;29(9):2586-2593.

de Oliveira EP, Burini RC. High plasma uric acid concentration: causes and consequences. *Diabetol Metab Syndr*. 2012;4:12.

Diamond HS, Neisel A, Kaplan D. Renal tubular transport of urate in man. *Bull Rheum Dis*. 1976;27(1):876-881.

Drust B, Atkinson G, Reilly T. Future perspectives in the evaluation of the physiological demands of soccer. *Sports Med*. 2007;37(9):783-805.

Duffield R, Dawson B, Bishop D, Fitzsimons M, Lawrence S. Effect of wearing an ice cooling jacket on repeat sprint performance in warm/humid conditions. *Br J Sports Med*. 2003;37(2):164-169.

Duffield R, Green R, Castle P, Maxwell N. Precooling can prevent the reduction of self-paced exercise intensity in the heat. *Med Sci Sports Exerc*. 2010;42(3):577-584.

Duffield R, Marino FE. Effects of pre-cooling procedures on intermittent-sprint exercise performance in warm conditions. *Eur J Appl Physiol*. 2007;100(6):727-735.

Duffield R, McCall A, Coutts AJ, Peiffer JJ. Hydration, sweat and thermoregulatory responses to professional football training in the heat. *J Sports Sci*. 2012;30(10):957-965.

Dugas J. Ice slurry ingestion increases running time in the heat. *Clin J Sport Med*. 2011;21(6):541-542.

Dziedzic CE, Ross ML, Slater GJ, Burke LM. Variability of measurements of sweat sodium using the regional absorbent-patch method. *Int J Sports Physiol Perform*. 2014;9(5):832-838.

Edwards RH, Harris RC, Hultman E, Kaijser L, Koh D, Nordesjo LO. Effect of temperature on muscle energy metabolism and endurance during successive isometric contractions, sustained to fatigue, of the quadriceps muscle in man. *J Physiol*. 1972;220(2):335-352.

Eichner ER. Sickle cell considerations in athletes. *Clin Sports Med*. 2011;30(3):537-549.

Eichner ER. Sickle cell trait in sports. *Curr Sports Med Rep*. 2010;9(6):347-351.

Eichner ER. Sickle cell trait. *J Sport Rehabil*. 2007;16(3):197-203.

Ely BR, Cheuvront SN, Kenefick RW, Sawka MN. Aerobic performance is degraded, despite modest hyperthermia, in hot environments. *Med Sci Sports Exerc.* 2010;42(1):135-141.

Fritzsche RG, Switzer TW, Hodgkinson BJ, Coyle EF. Stroke volume decline during prolonged exercise is influenced by the increase in heart rate. *J Appl Physiol (1985).* 1999;86(3):799-805.

Fujii N, Aoki-Murakami E, Tsuji B, et al. Body temperature and cold sensation during and following exercise under temperate room conditions in cold-sensitive young trained females. *Physiol Rep*. 2017;5(20).

Gabbett TJ, Mulvey MJ. Time-motion analysis of small-sided training games and competition in elite women soccer players. *Journal of strength and conditioning research / National Strength & Conditioning Association*. 2008;22(2):543-552.

Galloway SD, Maughan RJ. Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. *Med Sci Sports Exerc*. 1997;29(9):1240-1249.

Galloway SD, Maughan RJ. Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. *Medicine and science in sports and exercise*. 1997;29(9):1240-1249.

Gavin TP, Babington JP, Harms CA, Ardelt ME, Tanner DA, Stager JM. Clothing fabric does not affect thermoregulation during exercise in moderate heat. *Med Sci Sports Exerc*. 2001;33(12):2124-2130.

Gavin TP. Clothing and thermoregulation during exercise. *Sports Med*. 2003;33(13):941-947.

Gerrett N, Jackson S, Yates J, Thomas G. Ice slurry ingestion does not enhance self-paced intermittent exercise in the heat. *Scand J Med Sci Sports*. 2017;27(11):1202-1212.

Gibson JC, Stuart-Hill LA, Pethick W, Gaul CA. Hydration status and fluid and sodium balance in elite Canadian junior women's soccer players in a cool environment. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme*. 2012;37(5):931-937.

Girard O, Brocherie F, Bishop DJ. Sprint performance under heat stress: A review. *Scand J Med Sci Sports*. 2015;25 Suppl 1:79-89.

Gisolfi CV, Mora F. The hot brain : survival, temperature, and the human body. Cambridge, Mass.: MIT Press; 2000.

Glantzounis GK, Tsimoyiannis EC, Kappas AM, Galaris DA. Uric acid and oxidative stress. *Curr Pharm Des*. 2005;11(32):4145-4151.

Godek SF, Bartolozzi AR, Peduzzi C, et al. Fluid consumption and sweating in National Football League and collegiate football players with different access to fluids during practice. *Journal of athletic training*. 2010;45(2):128-135.

Gonzales BR, Hagin V, Guillot R, Placet V, Groslambert A. Effects of polyester jerseys on psycho-physiological responses during exercise in a hot and moist environment. *J Strength Cond Res.* 2011;25(12):3432-3438.

Gonzalez RR, Kluger MJ, Stolwijk JA. Thermoregulatory responses to thermal transients in the rabbit. *Am J Physiol*. 1974;227(6):1292-1298.

Gonzalez-Alonso J, Calbet JA. Reductions in systemic and skeletal muscle blood flow and oxygen delivery limit maximal aerobic capacity in humans. *Circulation.* 2003;107(6):824-830.

Gonzalez-Alonso J, Crandall CG, Johnson JM. The cardiovascular challenge of exercising in the heat. *J Physiol*. 2008;586(1):45-53.

Gonzalez-Alonso J, Mora-Rodriguez R, Below PR, Coyle EF. Dehydration markedly impairs cardiovascular function in hyperthermic endurance athletes during exercise. *J Appl Physiol (1985)*. 1997;82(4):1229-1236.

Gonzalez-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):1032-1039.

Goulet ED. Dehydration and endurance performance in competitive athletes. *Nutr Rev.* 2012;70 Suppl 2:S132-136.

Gozal D, Thiriet P, Mbala E, et al. Effect of different modalities of exercise and recovery on exercise performance in subjects with sickle cell trait. *Med Sci Sports Exerc*. 1992;24(12):1325-1331.

Grandjean AC, Reimers KJ, Buyckx ME. Hydration: issues for the 21st century. *Nutr Rev*. 2003;61(8):261-271.

Gregson W, Black MA, Jones H, et al. Influence of cold water immersion on limb and cutaneous blood flow at rest. *Am J Sports Med*. 2011;39(6):1316-1323.

Guttierres APM, Natali AJ, Vianna JM, Reis VM, Marins JCB. Dehydration in Soccer Players After a Match in the Heat. *Biology of Sport*. 2011;28(4):249-254.

Gwosdow A, Stevens J, Berglund L, Stolwijk J. Skin friction and fabric sensations in neutral and warm environments. *Test Res J*. 1986;56:574-580.

Hahn RG, Waldreus N. An aggregate urine analysis tool to detect acute dehydration. *Int J Sport*

Nutr Exerc Metab. 2013;23(4):303-311.

Hardy JD, Dubois EF. Basal metabolism, radiation, convection and vaporisation at temperatures of 22 to 35° C. *J Nutr*. 1938;1-5:477-497.

Hargreaves M, Morgan TO, Snow R, Guerin M. Exercise tolerance in the heat on low and normal salt intakes. *Clin Sci (Lond)*. 1989;76(5):553-557.

Harrelson GL, Fincher AL, Robinson JB. Acute exertional rhabdomyolysis and its relationship to sickle cell trait. *J Athl Train*. 1995;30(4):309-312.

Hasegawa H, Takatori T, Komura T, Yamasaki M. Combined effects of pre-cooling and water ingestion on thermoregulation and physical capacity during exercise in a hot environment. *J Sports Sci.* 2006;24(1):3-9.

Havenith G, Richards MG, Wang X, et al. Apparent latent heat of evaporation from clothing: attenuation and "heat pipe" effects. *J Appl Physiol (1985).* 2008;104(1):142-149.

Hayashi K, Honda Y, Ogawa T, Wada H, Kondo N, Nishiyasu T. Effects of brief leg cooling after moderate exercise on cardiorespiratory responses to subsequent exercise in the heat. *Eur J Appl Physiol*. 2004;92(4-5):414-420.

Hayes M, Castle PC, Ross EZ, Maxwell NS. The influence of hot humid and hot dry environments on intermittent-sprint exercise performance. *Int J Sports Physiol Perform*. 2014;9(3):387-396.

Haymaker; W. Blood supply of the human hypothalamus. *The Hypothalamus*. 1969:210-218.

Heller P, Best WR, Nelson RB, Becktel J. Clinical implications of sickle-cell trait and glucose-6-phosphate dehydrogenase deficiency in hospitalized black male patients. *N Engl J Med*. 1979;300(18):1001-1005.

Henane R, Bittel J, Viret R, Morino S. Thermal strain resulting from protective clothing of an armored vehicle crew in warm conditions. *Aviat Space Environ Med*. 1979;50(6):599-603.

Hew-Butler T, Rosner MH, Fowkes-Godek S, et al. Statement of the Third International Exercise-Associated Hyponatremia Consensus Development Conference, Carlsbad, California, 2015. *Clin J Sport Med*. 2015;25(4):303-320.

Hew-Butler T, Verbalis JG, Noakes TD, International Marathon Medical Directors A. Updated fluid recommendation: position statement from the International Marathon Medical

Directors Association (IMMDA). *Clin J Sport Med*. 2006;16(4):283-292.

Hew-Butler TD, Eskin C, Bickham J, Rusnak M, VanderMeulen M. Dehydration is how you define it: comparison of 318 blood and urine athlete spot checks. *BMJ Open Sport Exerc Med*. 2018;4(1):e000297.

Holmer I. Body cooling with ice for warm-water diving operations. *Undersea Biomed Res*. 1989;16(6):471-479.

Hubbard RW, Sandick BL, Matthew WT, et al. Voluntary dehydration and alliesthesia for water. *J Appl Physiol Respir Environ Exerc Physiol.* 1984;57(3):868-873.

Ihsan M, Watson G, Abbiss CR. What are the Physiological Mechanisms for Post-Exercise Cold Water Immersion in the Recovery from Prolonged Endurance and Intermittent Exercise? *Sports Med*. 2016;46(8):1095-1109.

Jequier E, Constant F. Water as an essential nutrient: the physiological basis of hydration. *Eur J Clin Nutr*. 2010;64(2):115-123.

Johannsen NM, Lind E, King DS, Sharp RL. Effect of preexercise electrolyte ingestion on fluid balance in men and women. *Med Sci Sports Exerc*. 2009;41(11):2017-2025.

Johnson JM, Rowell LB, Brengelmann GL. Modification of the skin blood flow-body temperature relationship by upright exercise. *J Appl Physiol*. 1974;37(6):880-886.

Judelson DA, Maresh CM, Farrell MJ, et al. Effect of hydration state on strength, power, and resistance exercise performance. *Med Sci Sports Exerc.* 2007;39(10):1817-1824.

Kay D, Taaffe DR, Marino FE. Whole-body pre-cooling and heat storage during self-paced cycling performance in warm humid conditions. *J Sports Sci*. 1999;17(12):937-944.

Kenefick RW. Drinking Strategies: Planned Drinking Versus Drinking to Thirst. *Sports Med*. 2018;48(Suppl 1):31-37.

Kenney WL, Wilmore JH, Costill DL, Wilmore JH. Physiology of sport and exercise. 5th ed. Champaign, IL: Human Kinetics; 2012.

Kenny GP, Reardon FD, Zaleski W, Reardon ML, Haman F, Ducharme MB. Muscle temperature transients before, during, and after exercise measured using an intramuscular multisensor probe. *J Appl Physiol (1985).* 2003;94(6):2350-2357.

Kenny GP, Schissler AR, Stapleton J, et al. Ice cooling vest on tolerance for exercise under

uncompensable heat stress. *J Occup Environ Hyg*. 2011;8(8):484-491.

Kenny GP, Webb P, Ducharme MB, Reardon FD, Jay O. Calorimetric measurement of postexercise net heat loss and residual body heat storage. *Med Sci Sports Exerc*. 2008;40(9):1629-1636.

Kicklighter T, Edsall J, Martin M. Effect of moisture-wicking garments on temperature regulation during exercise. I*nternational Journal of Athletic Therapy & Training*. 2011;16(6):9-13.

Kilding AE, Tunstall H, Wraith E, Good M, Gammon C, Smith C. Sweat rate and sweat electrolyte composition in international female soccer players during game specific training. *Int J Sports Med*. 2009;30(6):443-447.

Kingsley M, Penas-Ruiz C, Terry C, Russell M. Effects of carbohydrate-hydration strategies on glucose metabolism, sprint performance and hydration during a soccer match simulation in recreational players. *J Sci Med Sport.* 2014;17(2):239-243.

Koenders EE, Franken CPG, Cotter JD, Thornton SN, Rehrer NJ. Restricting dietary sodium reduces plasma sodium response to exercise in the heat. *Scand J Med Sci Sports*. 2017;27(11):1213-1220.

Koga S, Rossiter HB, Heinonen I, Musch TI, Poole DC. Dynamic heterogeneity of exercising muscle blood flow and O2 utilization. *Med Sci Sports Exerc*. 2014;46(5):860-876.

Kovacs EM, Senden JM, Brouns F. Urine color, osmolality and specific electrical conductance are not accurate measures of hydration status during postexercise rehydration. *J Sports Med Phys Fitness*. 1999;39(1):47-53.

Kwon A, Kato M, Kawamura H, Yanai Y, Tokura H. Physiological significance of hydrophilic and hydrophobic textile materials during intermittent exercise in humans under the influence of warm ambient temperature with and without wind. *Eur J Appl Physiol Occup Physiol*. 1998;78(6):487-493.

Laing RM, Sims ST, Wilson CA, Niven BE, Cruthers NM. Differences in wearer response to garments for outdoor activity. *Ergonomics*. 2008;51(4):492-510.

Le Gallais D, Bile A, Mercier J, Paschel M, Tonellot JL, Dauverchain J. Exercise-induced death in sickle cell trait: role of aging, training, and deconditioning. *Med Sci Sports Exerc*. 1996;28(5):541-544.

Lee DT, Haymes EM. Exercise duration and thermoregulatory responses after whole body

precooling. *J Appl Physiol (1985)*. 1995;79(6):1971-1976.

Lee EC, Watson G, Casa D, et al. Interleukin-6 responses to water immersion therapy after acute exercise heat stress: a pilot investigation. *J Athl Train*. 2012;47(6):655-663.

Lee JK, Koh AC, Koh SX, Liu GJ, Nio AQ, Fan PW. Neck cooling and cognitive performance following exercise-induced hyperthermia. *Eur J Appl Physiol*. 2014;114(2):375-384.

Lim CL, Byrne C, Lee JK. Human thermoregulation and measurement of body temperature in exercise and clinical settings. *Ann Acad Med Singapore.* 2008;37(4):347-353.

Livingstone SD. Calculation of mean body temperature. *Can J Physiol Pharmacol*. 1968;46(1):15-17.

Lockie RG, Moreno MR, Lazar A, et al. The Physical and Athletic Performance Characteristics of Division I Collegiate Female Soccer Players by Position. *Journal of strength and conditioning research / National Strength & Conditioning Association*. 2018;32(2):334-343.

Loosemore M, Walsh SB, Morris E, Stewart G, Porter JB, Montgomery H. Sudden exertional death in sickle cell trait. *Br J Sports Med.* 2012;46(5):312-314.

MacDougall JD, Reddan WG, Layton CR, Dempsey JA. Effects of metabolic hyperthermia on performance during heavy prolonged exercise. *J Appl Physiol*. 1974;36(5):538-544.

Mange K, Matsuura D, Cizman B, et al. Language guiding therapy: the case of dehydration versus volume depletion. *Ann Intern Med*. 1997;127(9):848-853.

Mara JK, Thompson KG, Pumpa KL, Morgan S. Quantifying the High-Speed Running and Sprinting Profiles of Elite Female Soccer Players During Competitive Matches Using an Optical Player Tracking System. *Journal of strength and conditioning research / National Strength & Conditioning Association*. 2017;31(6):1500-1508.

Marino FE. Methods, advantages, and limitations of body cooling for exercise performance. *Br J Sports Med*. 2002;36(2):89-94.

Mastaloudis A, Leonard SW, Traber MG. Oxidative stress in athletes during extreme endurance exercise. *Free Radic Biol Med*. 2001;31(7):911-922.

Maughan RJ, Merson SJ, Broad NP, Shirreffs SM. Fluid and electrolyte intake and loss in elite soccer players during training. *Int J Sport Nutr Exerc Metab.* 2004;14(3):333-346.

Maughan RJ, Merson SJ, Broad NP, Shirreffs SM. Fluid and electrolyte intake and loss in elite soccer players during training. *International journal of sport nutrition and exercise metabolism*. 2004;14(3):333-346.

Maughan RJ, Otani H, Watson P. Influence of relative humidity on prolonged exercise capacity in a warm environment. *Eur J Appl Physiol*. 2012;112(6):2313-2321.

Maughan RJ, Shirreffs SM, Merson SJ, Horswill CA. Fluid and electrolyte balance in elite male football (soccer) players training in a cool environment. *Journal of sports sciences*. 2005;23(1):73-79.

Maughan RJ, Shirreffs SM. Development of hydration strategies to optimize performance for athletes in high-intensity sports and in sports with repeated intense efforts. *Scand J Med Sci Sports*. 2010;20 Suppl 2:59-69.

Maunder E, Laursen PB, Kilding AE. Effect of ad Libitum Ice-Slurry and Cold-Fluid Ingestion on Cycling Time-Trial Performance in the Heat. *Int J Sports Physiol Perform*. 2017;12(1):99-105.

Mauricio Castro-Sepulveda, Javier Astudillo, Pablo Letelier, Zbinden-Foncea H. Prevalence of Dehydration Before Training Sessions, Friendly and Official Matches in Elite Female Soccer Players. *Journal of human kinetics*. 2016;50:79-84.

Maxwell NS, Gardner F, Nimmo MA. Intermittent running: muscle metabolism in the heat and effect of hypohydration. *Med Sci Sports Exerc.* 1999;31(5):675-683.

McDermott BP, Anderson SA, Armstrong LE, et al. National Athletic Trainers' Association Position Statement: Fluid Replacement for the Physically Active. *Journal of athletic training*. 2017;52(9):877-895.

McDonald KG, Grote S, Shoepe TC. Effect of training mode on post-exercise heart rate recovery of trained cyclists. *J Hum Kinet*. 2014;41:43-49.

McLellan TM, Cheung SS, Selkirk GA, Wright HE. Influence of aerobic fitness on thermoregulation during exercise in the heat. *Exerc Sport Sci Rev*. 2012;40(4):218-219; author reply 220.

Mejuto G, Chalmers S, Gilbert S, Bentley D. The effect of ice slurry ingestion on body temperature and cycling performance in competitive athletes. *J Therm Biol*. 2018;72:143-147.

Merrick MA. Secondary injury after musculoskeletal trauma: a review and update. *J Athl Train*.

2002;37(2):209-217.

Moghadam-Kia S, Oddis CV, Aggarwal R. Approach to asymptomatic creatine kinase elevation. Cleve *Clin J Med*. 2016;83(1):37-42.

Mohr M, Krustrup P, Andersson H, Kirkendal D, Bangsbo J. Match activities of elite women soccer players at different performance levels. *Journal of strength and conditioning research / National Strength & Conditioning Association*. 2008;22(2):341-349.

Morris NB, Cramer MN, Hodder SG, Havenith G, Jay O. A comparison between the technical absorbent and ventilated capsule methods for measuring local sweat rate. *Journal of applied physiology*. 2013;114(6):816-823.

Munson DM, Hayter R. An overview of clothing as it relates to the physiology of human comfort. *Am Society Heat Refrigerat Air Cond Eng Trans*. 1978;84:713-718.

Murray R, Bartoli W, Stofan J, Horn M, Eddy D. A comparison of the gastric emptying characteristics of selected sports drinks. *Int J Sport Nutr*. 1999;9(3):263-274.

Naito T, Sagayama H, Akazawa N, Haramura M, Tasaki M, Takahashi H. Ice slurry ingestion during break times attenuates the increase of core temperature in a simulation of physical demand of match-play tennis in the heat. *Temperature (Austin)*. 2018;5(4):371-379.

Nassis GP, Brito J, Dvorak J, Chalabi H, Racinais S. The association of environmental heat stress with performance: analysis of the 2014 FIFA World Cup Brazil. *Br J Sports Med*. 2015;49(9):609-613.

Nelson DA, Deuster PA, Carter R, 3rd, Hill OT, Wolcott VL, Kurina LM. Sickle Cell Trait, Rhabdomyolysis, and Mortality among U.S. Army Soldiers. *N Engl J Med*. 2016;375(5):435-442.

Nielsen B, Hales JR, Strange S, Christensen NJ, Warberg J, Saltin B. Human circulatory and thermoregulatory adaptations with heat acclimation and exercise in a hot, dry environment. *J Physiol*. 1993;460:467-485.

Nielsen R, Endrusick TL. Thermoregulatory responses to intermittent exercise are influenced by knit structure of underwear. *Eur J Appl Physiol Occup Physiol*. 1990;60(1):15-25.

Nielsen R, Olesen BW, Fanger PO. Effect of physical activity and air velocity on the thermal insulation of clothing. *Ergonomics*. 1985;28(12):1617-1631.

Nuccio RP, Barnes KA, Carter JM, Baker LB. Fluid Balance in Team Sport Athletes and the Effect of Hypohydration on Cognitive, Technical, and Physical Performance. *Sports medicine*. 2017;47(10):1951-1982.

Nugent CA, Tyler FH. The renal excretion of uric acid in patients with gout and in nongouty subjects. *J Clin Invest*. 1959;38:1890-1898.

Nybo L, Jensen T, Nielsen B, Gonzalez-Alonso J. Effects of marked hyperthermia with and without dehydration on VO(2) kinetics during intense exercise. *J Appl Physiol (1985)*. 2001;90(3):1057-1064.

Olesen BW, Brager GS. A better way to predict comfort. *Ashrae J*. 2004;46(8):20-+.

Olujohungbe A, Garvey B. Red cell distribution width (RDW) in sickle cell disease. *Clin Lab Haematol*. 1993;15(1):72-73.

Orendurff MS, Walker JD, Jovanovic M, Tulchin KL, Levy M, Hoffmann DK. Intensity and duration of intermittent exercise and recovery during a soccer match. *Journal of strength and conditioning research / National Strength & Conditioning Association*. 2010;24(10):2683-2692.

Osterberg KL, Pallardy SE, Johnson RJ, Horswill CA. Carbohydrate exerts a mild influence on fluid retention following exercise-induced dehydration. *J Appl Physiol (1985)*. 2010;108(2):245-250.

Ozgunen KT, Kurdak SS, Maughan RJ, et al. Effect of hot environmental conditions on physical activity patterns and temperature response of football players. *Scand J Med Sci Sports*. 2010;20 Suppl 3:140-147.

Pascoe DD, Shanley LA, Smith EW. Clothing and exercise. I: Biophysics of heat transfer between the individual, clothing and environment. *Sports Med*. 1994;18(1):38-54.

Passe DH, Stofan JR, Rowe CL, Horswill CA, Murray R. Exercise condition affects hedonic responses to sodium in a sport drink. *Appetite*. 2009;52(3):561-567.

Patterson MJ, Galloway SD, Nimmo MA. Variations in regional sweat composition in normal human males. *Exp Physiol*. 2000;85(6):869-875.

Pescatello LS, American College of Sports Medicine. ACSM's guidelines for exercise testing and prescription. 9th ed. Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins Health; 2014.

Pointon M, Duffield R, Cannon J, Marino FE. Cold application for neuromuscular recovery following intense lower-body exercise. *Eur J Appl Physiol*. 2011;111(12):2977-2986.

Popowski LA, Oppliger RA, Patrick Lambert G, Johnson RF, Kim Johnson A, Gisolf CV. Blood and urinary measures of hydration status during progressive acute dehydration. *Med Sci Sports Exerc*. 2001;33(5):747-753.

Puntel GO, Carvalho NR, Amaral GP, et al. Therapeutic cold: An effective kind to modulate the oxidative damage resulting of a skeletal muscle contusion. *Free Radic Res*. 2011;45(2):125-138.

Quaresima V, Homma S, Azuma K, et al. Calf and shin muscle oxygenation patterns and femoral artery blood flow during dynamic plantar flexion exercise in humans. *Eur J Appl Physiol*. 2001;84(5):387-394.

Quaresima V, Komiyama T, Ferrari M. Differences in oxygen re-saturation of thigh and calf muscles after two treadmill stress tests. *Comp Biochem Physiol A Mol Integr Physiol*. 2002;132(1):67-73.

Ramanathan NL. A New Weighting System for Mean Surface Temperature of the Human Body. *J Appl Physiol*. 1964;19:531-533.

Rittweger J, Moss AD, Colier W, Stewart C, Degens H. Muscle tissue oxygenation and VEGF in VO-matched vibration and squatting exercise. *Clin Physiol Funct Imaging*. 2010;30(4):269-278.

Roberts B, Waller T, Caime M. Thermoregulatory response to base layer garments during treadmill exercise. *Int J Sports Sci Eng*. 2007;1:29-38.

Roberts LA, Muthalib M, Stanley J, et al. Effects of cold water immersion and active recovery on hemodynamics and recovery of muscle strength following resistance exercise. *Am J Physiol Regul Integr Comp Physiol*. 2015;309(4):R389-398.

Rodriguez RF, Townsend NE, Aughey RJ, Billaut F. Muscle oxygenation maintained during repeated-sprints despite inspiratory muscle loading. *PLoS One*. 2019;14(9):e0222487.

Rowell LB, Blackmon JR, Martin RH, Mazzarella JA, Bruce RA. Hepatic clearance of indocyanine green in man under thermal and exercise stresses. *J Appl Physiol*. 1965;20(3):384-394.

Rowell LB, Brengelmann GL, Murray JA, Kraning KK, 2nd, Kusumi F. Human metabolic responses to hyperthermia during mild to maximal exercise. *J Appl Physiol*.

1969;26(4):395-402.

Rowell LB, Kraning KK, 2nd, Kennedy JW, Evans TO. Central circulatory responses to work in dry heat before and after acclimatization. *J Appl Physiol*. 1967;22(3):509-518.

Rowell LB, Marx HJ, Bruce RA, Conn RD, Kusumi F. Reductions in cardiac output, central blood volume, and stroke volume with thermal stress in normal men during exercise. *J Clin Invest*. 1966;45(11):1801-1816.

Rowell LB, Murray JA, Brengelmann GL, Kraning KK, 2nd. Human cardiovascular adjustments to rapid changes in skin temperature during exercise. *Circ Res*. 1969;24(5):711-724.

Rowell LB. Human cardiovascular adjustments to exercise and thermal stress. *Physiol Rev*. 1974;54(1):75-159.

Ruddock AD, Thompson SW, Hudson SA, James CA, Gibson OR, Mee JA. Combined active and passive heat exposure induced heat acclimation in a soccer referee before 2014 FIFA World Cup. *Springerplus*. 2016;5:617.

Sakate T. The effect of air temperature on physical working capacity. *J Hum Ergol (Tokyo)*. 1978;7(2):127-134.

Sargeant AJ. Effect of muscle temperature on leg extension force and short-term power output in humans. *Eur J Appl Physiol Occup Physiol*. 1987;56(6):693-698.

Sawka MN, Burke LM, Eichner ER, Maughan RJ, Montain SJ, Stachenfeld NS. American College of Sports Medicine position stand. Exercise and fluid replacement. *Medicine and science in sports and exercise*. 2007;39(2):377-390.

Sawka MN, Cheuvront SN, Kenefick RW. High skin temperature and hypohydration impair aerobic performance. *Exp Physiol*. 2012;97(3):327-332.

Sawka MN, Young AJ, Cadarette BS, Levine L, Pandolf KB. Influence of heat stress and acclimation on maximal aerobic power. *Eur J Appl Physiol Occup Physiol*. 1985;53(4):294-298.

Sekiguchi Y, Adams WM, Curtis RM, Benjamin CL, Casa DJ. Factors influencing hydration status during a National Collegiate Athletics Association division 1 soccer preseason. *Journal of science and medicine in sport / Sports Medicine Australia*. 2019;22(6):624-628.

Sen Gupta J, Swamy YV, Pichan G, Dimri GP. Physiological responses during continuous work in hot dry and hot humid environments in Indians. *Int J Biometeorol*. 1984;28(2):137-146.

Shirreffs SM, Aragon-Vargas LF, Chamorro M, Maughan RJ, Serratosa L, Zachwieja JJ. The sweating response of elite professional soccer players to training in the heat. *Int J Sports Med*. 2005;26(2):90-95.

Shirreffs SM, Sawka MN, Stone M. Water and electrolyte needs for football training and match-play. *J Sports Sci*. 2006;24(7):699-707.

Shirreffs SM, Sawka MN. Fluid and electrolyte needs for training, competition, and recovery. *J Sports Sci*. 2011;29 Suppl 1:S39-46.

Shirreffs SM, Sawka MN. Fluid and electrolyte needs for training, competition, and recovery. *J Sports Sci*. 2011;29 Suppl 1:S39-46.

Shlipak MG, Matsushita K, Arnlov J, et al. Cystatin C versus creatinine in determining risk based on kidney function. *N Engl J Med*. 2013;369(10):932-943.

Shvartz E, Saar E, Meyerstein N, Benor D. A comparison of three methods of acclimatization to dry heat. *J Appl Physiol*. 1973;34(2):214-219.

Siegel R, Mate J, Brearley MB, Watson G, Nosaka K, Laursen PB. Ice slurry ingestion increases core temperature capacity and running time in the heat. *Med Sci Sports Exerc*. 2010;42(4):717-725.

Siegel R, Mate J, Watson G, Nosaka K, Laursen PB. Pre-cooling with ice slurry ingestion leads to similar run times to exhaustion in the heat as cold water immersion. *J Sports Sci*. 2012;30(2):155-165.

Sims ST, Rehrer NJ, Bell ML, Cotter JD. Preexercise sodium loading aids fluid balance and endurance for women exercising in the heat. *J Appl Physiol (1985)*. 2007;103(2):534-541.

Sims ST, van Vliet L, Cotter JD, Rehrer NJ. Sodium loading aids fluid balance and reduces physiological strain of trained men exercising in the heat. *Med Sci Sports Exerc*. 2007;39(1):123-130.

Snell RS. Clinical neuroanatomy. 7th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2010.

Sperlich B, Born DP, Lefter MD, Holmberg HC. Exercising in a hot environment: which T-shirt to wear? *Wilderness Environ Med*. 2013;24(3):211-220.

Sporis G, Jukic I, Ostojic SM, Milanovic D. Fitness profiling in soccer: physical and physiologic characteristics of elite players. *Journal of strength and conditioning research / National Strength & Conditioning Association*. 2009;23(7):1947-1953.

Statius van Eps LW, Pinedo-Veels C, de Vries GH, de Koning J. Nature of concentrating defect in sickle-cell nephropathy. Microradioangiographic studies. *Lancet*. 1970;1(7644):450-452.

Stevens CJ, Taylor L, Dascombe BJ. Cooling During Exercise: An Overlooked Strategy for Enhancing Endurance Performance in the Heat. *Sports Med*. 2017;47(5):829-841.

Stofan JR, Zachwieja JJ, Horswill CA, Murray R, Anderson SA, Eichner ER. Sweat and sodium losses in NCAA football players: a precursor to heat cramps? *Int J Sport Nutr Exerc Metab*. 2005;15(6):641-652.

Sukstanskii AL, Yablonskiy DA. Theoretical limits on brain cooling by external head cooling devices. *Eur J Appl Physiol*. 2007;101(1):41-49.

Sunderland C, Stevens R, Everson B, Tyler CJ. Neck-cooling improves repeated sprint performance in the heat. *Front Physiol*. 2015;6:314.

Tan PM, Teo EY, Ali NB, et al. Evaluation of Various Cooling Systems After Exercise-Induced Hyperthermia. *J Athl Train*. 2017;52(2):108-116.

Tatterson AJ, Hahn AG, Martin DT, Febbraio MA. Effects of heat stress on physiological responses and exercise performance in elite cyclists. *J Sci Med Sport*. 2000;3(2):186-193.

Tekin A. Xanthine oxidase and uric acid response to a 6-week pre-season training programme in male athletes. *African Journal of Pharmacy and Pharmacology*. 2010;4(8):511-515.

Thomas G, Cullen T, Davies M, Hetherton C, Duncan B, Gerrett N. Independent or simultaneous lowering of core and skin temperature has no impact on self-paced intermittent running performance in hot conditions. *Eur J Appl Physiol*. 2019;119(8):1841-1853.

Thompson AA. Sickle cell trait testing and athletic participation: a solution in search of a problem? *Hematology Am Soc Hematol Educ Program*. 2013;2013:632-637.

Thompson PD, Arena R, Riebe D, Pescatello LS, American College of Sports M. ACSM's new preparticipation health screening recommendations from ACSM's guidelines for exercise testing and prescription, ninth edition. *Curr Sports Med Rep.* 2013;12(4):215-217.

Tripette J, Connes P, Hedreville M, et al. Patterns of exercise-related inflammatory response in sickle cell trait carriers. *Br J Sports Med*. 2010;44(4):232-237.

Tripette J, Hardy-Dessources MD, Sara F, et al. Does repeated and heavy exercise impair blood rheology in carriers of sickle cell trait? *Clin J Sport Med*. 2007;17(6):465-470.

Tripette J, Loko G, Samb A, et al. Effects of hydration and dehydration on blood rheology in sickle cell trait carriers during exercise. *Am J Physiol Heart Circ Physiol*. 2010;299(3):H908-914.

Tsaras G, Owusu-Ansah A, Boateng FO, Amoateng-Adjepong Y. Complications associated with sickle cell trait: a brief narrative review. *Am J Med*. 2009;122(6):507-512.

Tseng CY, Lee JP, Tsai YS, et al. Topical cooling (icing) delays recovery from eccentric exercise-induced muscle damage. *J Strength Cond Res*. 2013;27(5):1354-1361.

Tyler CJ, Sunderland C. Cooling the neck region during exercise in the heat. *J Athl Train.* 2011;46(1):61-68.

Tyler CJ, Sunderland C. Neck cooling and running performance in the heat: single versus repeated application. *Med Sci Sports Exerc*. 2011;43(12):2388-2395.

Tyler CJ, Wild P, Sunderland C. Practical neck cooling and time-trial running performance in a hot environment. *Eur J Appl Physiol*. 2010;110(5):1063-1074.

Urdampilleta A, Gomez-Zorita S, Soriano JM, Martinez-Sanz JM, Medina S, Gil-Izquierdo A. Hydration and chemical ingredients in sport drinks: food safety in the European context. *Nutr Hosp*. 2015;31(5):1889-1899.

Vallerand AL, Michas RD, Frim J, Ackles KN. Heat balance of subjects wearing protective clothing with a liquid- or air-cooled vest. *Aviat Space Environ Med*. 1991;62(5):383-391.

Vallerand AL, Savourey G, Hanniquet AM, Bittel JH. How should body heat storage be determined in humans: by thermometry or calorimetry? *Eur J Appl Physiol Occup Physiol*. 1992;65(3):286-294.

Veneroso CE, Ramos GP, Mendes TT, Silami-Garcia E. Physical performance and environmental conditions: 2014 World Soccer Cup and 2016 Summer Olympics in Brazil. *Temperature (Austin)*. 2015;2(4):439-440.

Vieira A, Siqueira AF, Ferreira-Junior JB, et al. The Effect of Water Temperature during Cold-Water Immersion on Recovery from Exercise-Induced Muscle Damage. *Int J Sports Med*. 2016;37(12):937-943.

Vincent L, Oyono-Enguelle S, Feasson L, et al. Effects of regular physical activity on skeletal muscle structural, energetic, and microvascular properties in carriers of sickle cell trait. *J Appl Physiol (1985)*. 2012;113(4):549-556.

Wakabayashi H, Osawa M, Koga S, et al. Effects of muscle cooling on kinetics of pulmonary oxygen uptake and muscle deoxygenation at the onset of exercise. *Physiol Rep*. 2018;6(21):e13910.

Wang L, Tian Y, Kim J, Yin H. The key local segments of human body for personalized heating and cooling. *J Therm Biol*. 2019;81:118-127.

Watkins SM. Clothing : the portable environment. 1st ed. Ames, Iowa: Iowa State University Press; 1984.

Webster P, Castro O. Red cell distribution width in sickle cell disease. *Ann Clin Lab Sci*. 1986;16(4):274-277.

Webster S, Rutt R, Weltman A. Physiological effects of a weight loss regimen practiced by college wrestlers. *Med Sci Sports Exerc*. 1990;22(2):229-234.

Weschler LB. Sweat electrolyte concentrations obtained from within occlusive coverings are falsely high because sweat itself leaches skin electrolytes. *J Appl Physiol (1985)*. 2008;105(4):1376-1377.

Weschler LB. Sweat electrolyte concentrations obtained from within occlusive coverings are falsely high because sweat itself leaches skin electrolytes. *J Appl Phy*. 2008;105(4):1376-1377.

Westing YH, Ekblom B, Sjodin B. The metabolic relation between hypoxanthine and uric acid in man following maximal short-distance running. *Acta Physiol Scand*. 1989;137(3):341-345.

White GE, Rhind SG, Wells GD. The effect of various cold-water immersion protocols on exercise-induced inflammatory response and functional recovery from high-intensity

sprint exercise. *Eur J Appl Physiol*. 2014;114(11):2353-2367.

Wickwire PJ, Bishop PA, Green JM, et al. Physiological and comfort effects of a commercial "cooling cap" worn under protective helmets. *J Occup Environ Hyg*. 2009;6(8):455-459.

Williams CG, Bredell GA, Wyndham CH, et al. Circulatory and metabolic reactions to work in heat. *J Appl Physiol*. 1962;17:625-638.

Wingo JE, Cureton KJ. Maximal oxygen uptake after attenuation of cardiovascular drift during heat stress. *Aviat Space Environ Med.* 2006;77(7):687-694.

Wong ET, Cobb C, Umehara MK, et al. Heterogeneity of serum creatine kinase activity among racial and gender groups of the population. *Am J Clin Pathol*. 1983;79(5):582-586.

Wyss CR, Brengelmann GL, Johnson JM, Rowell LB, Niederberger M. Control of skin blood flow, sweating, and heart rate: role of skin vs. core temperature. *J Appl Physiol*. 1974;36(6):726-733.

Yankelson L, Sadeh B, Gershovitz L, et al. Life-threatening events during endurance sports: is heat stroke more prevalent than arrhythmic death? *J Am Coll Cardiol*. 2014;64(5):463-469.

Zetou E, Giatsis G, Mountaki F, Komninakidou A. Body weight changes and voluntary fluid intakes of beach volleyball players during an official tournament. *J Sci Med Sport*. 2008;11(2):139-145.

Zhang MH. Rhabdomyolosis and its pathogenesis. *World J Emerg Med*. 2012;3(1):11-15.

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