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Relationship of Perceived Thirst to Measures of Hydration During and Following Exercise

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Lesley Willis Vandermark, PhD

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The purposes of these investigations were to describe the relationship of thirst to hydration during exercise, and to determine the impact of fluid volume on thirst following exercise. Eleven males completed four treadmill exercise trials of up to 180 minutes in a warm environment (35°C, 30% RH), then a 1-hour seated recovery. Two trials began euhydrated, and two hypohydrated. Fluid was given in one trial during exercise to match sweat rate. During recovery, participants were grouped to receive either a Small (1% of body mass (BM)) or Large (4% of BM) fluid bolus. Immediately post-exercise (IPE), there was a relationship between Thirst and body mass loss (BML) ($p<0.008$) and BML% ($p<0.009$). Moderate-high thirst was significantly related to IPE plasma osmolality (POsm; $R^2=0.142$, $p=0.037$). There was a significant relationship between low change in thirst and IPE POsm change ($R^2=0.317$, $p=0.010$), Copeptin change ($R^2=0.212$, $p=0.041$), and BML ($R^2=0.325$, p=0,009) and BML% ($R^2=0.356$, $p=0.006$). IPE thirst was correlated with IPE POsm ($\rho=0.600$, $p<0.001$), BML% ($\rho=-0.644$, $p<0.001$), and plasma volume change ($\rho=-0.326$, $p=0.040$). HyR trial fluid bolus in the Large group (2±1) resulted in lower Thirst than the Small group (7±2, $p<0.001$) after recovery. Large group only consumed fluid to replace 3.84±1.73% BML. There is a relationship between a low level of thirst and hydration before exercise, and a higher level of thirst and hydration after exercise. Fluid bolus decreased thirst to such a degree that body mass loss replacement was incomplete.

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Relationship of Perceived Thirst to Measures of Hydration During and Following Exercise

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Chapter 1

REVIEW OF THE LITERATURE

For decades a link between hydration and optimal physiological function has been examined in field and laboratory studies. Hydration has been linked to both performance and safety during exercise. Although the concept of hydrating is innate, drinking behavior is complex. Combinations of stressors such as sleep loss, dehydration, nutrition deficiency, and heat exposure are common in many scenarios, including the military and athletics. For example, a study of a military unit during an intense four-day field training exercise revealed that mood became more negative over the training period, and simple and complex cognition .[1] Additionally, stress hormone levels were elevated, indicating physiological as well as psychological degradation under these conditions.[1] These effects can lead to crucial errors which decrease overall performance and safety.[2] Hydration recommendations[3,4] exist from various medical and sport organizations, but without a true consensus.[5,6]

A. Hydration: Risks, Benefits, and Assessment Strategies during Exercise

Hydration as a state is controlled by homeostatic mechanisms that drive fluid loss and retention in connection with the central nervous and renal systems.[7,8] Figure 1.1, adapted from Cheuvront et al. (2013) describes different types of body water loss and the cascade of physiological responses that follow to conserve or acquire the water necessary to sustain body function.[8] Hydration as a process references total body water, which is not easily examined in non-laboratory settings. This process is of fluids lost and gained, and impacts physiological function and exercise performance. Hydration assessment provides information that, when interpreted appropriately, can improve health and exercise performance.

For clarity in the following text, definitions for hydration states and process are provided, from a former review of thermal physiology.[9] Euhydration is a steady state condition of normal body water, hypohydration is a steady-state of decreased body water, and hyperhydration is a steady state of increased body water. The process of dehydration is water loss leading to hypohydration, and rehydration is the process of adding water leading towards a state of euhydration.

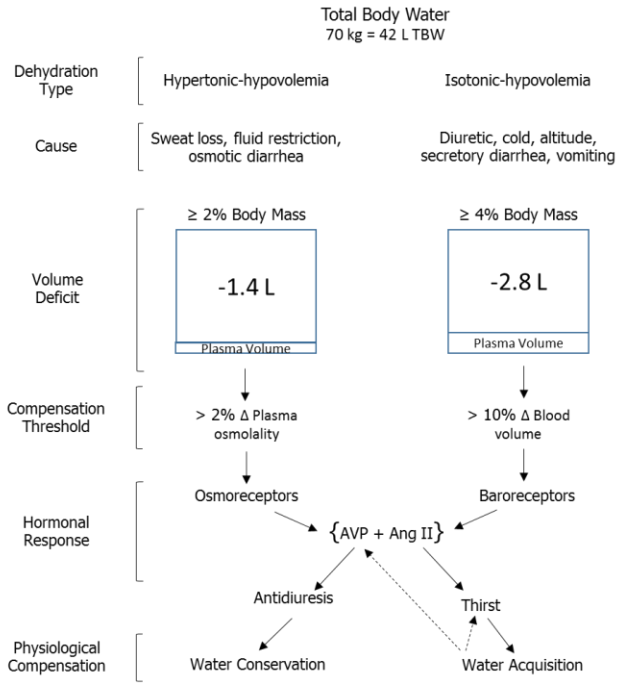

Figure 1.1. Adapted from Cheuvront, Kenefick, Charkoudian, and Sawka (2013).[8] Body water regulation in response to dehydration. Schematic includes the 2 major types of dehydration, their typical causes, and the estimated magnitude of dehydration required to stimulate a primary osmotic- or volume-dependent response for compensatory water conservation and acquisition. A change in TBW was equated with a change in body mass (1 L = 1 kg), whereby dehydration was expressed as a percentage of body mass. Plasma volume and TBW losses are depicted to scale as are their 1:10 and 1:5 ratios for hypertonic and isotonic hypovolemia, respectively. Dashed arrows represent negative feedback. Ang II, angiotensin II; AVP, arginine vasopressin; TBW, total body water.

Safety and Performance Considerations with Hydration during Exercise

Hydration during exercise has both health and safety, and performance implications. Thermoregulation and cardiovascular function are of primary health and safety concern. Thermoregulation is affected by hydration during exercise. There is a relationship between hypohydration and change in temperature, that higher levels of hypohydration are associated with higher exercising body temperatures when compared to the same exercise in a euhydrated state.[10] For example, a systematic review described body temperature as having a positive relationship with increased levels of dehydration, where for every 1% loss of body mass, body temperature is 0.25°C higher.[10] Increased body temperature with hypohydration greater than 1-2% corresponds with a reduction in sweating attributed to the effects of blood hyperosmolality.[11–13] Reduced sweating stifles dissipation of body heat via evaporation of sweat from the skin, which contributes to rise in body temperature. Even a small increase in body temperature impacts health and safety by putting an athlete at higher risk of experiencing a heat illness. Although thermoregulation is very commonly associated with hydration, cardiovascular function and performance (e.g. cognition, neuromuscular control, and aerobic performance) are also impacted by dehydration.

Cardiovascular function is affected by hydration during exercise. There is competition for blood flow between muscle and skin during exercise in a dehydrated state, with muscle typically losing out to the skin in favor of thermoregulation.[13–15] The competition is linked to a reduction in stroke volume and reduced blood volume, resulting in an overall reduction in cardiac output during intense aerobic exercise.[16,17] Heart rate has been shown to be increased during aerobic exercise while in a state of dehydration, in comparison to a state of euhydration.[16,18] A systematic review of heart rate and hydration state during aerobic exercise

uncovered a small elevation in exercising heart rate for every 1% body mass loss during aerobic exercise.[19] Changes in cardiac output are likely related to decreased blood volume with increased demand.

In addition to safety considerations, performance considerations related to hydration status may be of significant importance. Athletic performance has been known to suffer in many ways related to dehydration. Cognition, development of fatigue, and neuromuscular control can be heavily impacted by significant dehydration.[1,20–23] It appears that dehydration increases cardiovascular strain, which in turn produces fatigue with or without hyperthermia.[14,24] However, mild dehydration of less than 2% body mass loss has little effect on fatigue, potentially owing to the relatively little increased cardiovascular strain experienced at such a low level. A study of basketball players revealed that total fatigue was not different between players after a simulated basketball game until approximately 3-4% body mass loss.[23] Fatigue may also be influenced by the environment, where a greater heat stress elevates the perception of fatigue;[8] or altered muscle metabolism, again however, it appears that decrements do not become noticeable until greater than 2% body mass loss is achieved.

Hypohydration and hypothermia may have an impact on aerobic, but not anaerobic exercise.[25–27] In the endurance population, it appears that 2% body mass loss inhibits long distance (5,000 and 10,000 meter) running performance, but not shorter distance.[25,28] For example, distance runners performed a 12km race in the heat faster and with more even pacing in a hydrated state, compared to a hypohydrated state.[21] In a thermoneutral environment endurance exercise performance of 90 minutes did not decrease with dehydration less than 2%.[25]

Hypohydration and dehydration have been considered to impact cognition and mood during exercise. However, a study of military subjects who were approximately 3%

hypohydrated based on body mass deficit did not show any cognitive or mood deficits, suggesting that at lower levels of water loss there would be no decrement.[29] This may be beneficial during exercise, if under mild conditions, negative emotions are not perceived and cause little or no distraction. However, this may lead to further dehydration as no indication of need to hydrate (i.e. thirst) is perceived. Negative emotions have also been noted when individuals with commonly high daily fluid consumption are fluid restricted, where their low-drinking counterparts had improved emotions with additional fluid provision.[20]

However, this conflicts with evidence by Cian et al. (2005)[30] describing both passive heat exposure and exercise-induced dehydration, in comparison to a control condition, related to cognition and mood. Cognition deteriorated with greater than 2% body mass loss evidenced by higher ratings of fatigue, increased reaction time, and decreased short term memory, regardless of passive or exercise-induced method.[30] This suggests that dehydration, not exercise, is the cause of the cognitive change. Cognitive benefit was not evidenced when body mass was acutely replaced to less than 1%, indicating that although a physiological change was induced, psychological change was not.[30] Mood was positive and amiable regardless of passive or exercise-induced method, or fluid ingestion.[30]

Additionally, neuromuscular control has been shown to diminish under conditions of high body temperature, dehydration of >3%, and a combination of the two conditions. DiStefano et al. (2013)[22] demonstrated that jump landing mechanics, which are a widely accepted descriptor of lower extremity injury risk, are poorer under these conditions.[22] As another factor related to lower extremity injury, balance was negatively impacted by these factors as well. In a study of basketball players, very few drills were impaired at 2% body mass loss, which may be considered a tipping-point of sorts, where dehydration which progresses beyond this level

significantly impairs performance.[23] However, it may be reasonable to consider that less than 2% body mass loss does not impair skill performance.

Over-consumption of fluid can also be detrimental to safety during exercise. Over-consumption of water is considered the primary cause of hyponatremia in healthy individuals during exercise, due to a dilution of plasma sodium. Exercise associated hyponatremia (EAH) is defined as a blood sodium concentration of less than 135 mmol/L.[4] Plasma sodium may be reduced by either overconsumption of a significant amount of electrolyte deficient fluid, excretion of a significant amount of electrolyte in body fluid, or a combination of these two factors by which electrolytes are lost and not replaced. This results in significant impairment, and ultimately death in extreme or unrecognized cases. EAH is most common in endurance exercise and military type events,[4] where fluid replacement guidelines may be inadequately assessed. Current general fluid replacement guidelines do not account for individual variation (e.g. sweating rate, exercise type, environment) are potentially harmful.[3,31] Lack of clear fluid consumption guidelines may lead to inappropriate fluid consumption of either too much or too little, and exertional hyponatremia in some cases.[4,32]

Assessment of Hydration

Total body water is commonly estimated as approximately 73% of fat free body mass.[7,8,33–35] Assessment of total body water assumes the individual is normal, health, chronically euhydrated, and resting in mild environmental conditions.[7] Total body water is variable and not applicable in exercise settings, so other practices are used to assess hydration. As depicted in Table 1.1 from Armstrong (2005)[33] these methods vary in field applicability,

accuracy, and other factors. The most commonly used hydration measures of field research use blood and urine, and body mass as indicators of hydration state or change during exercise.

Table 1.1 From Armstrong et al. (2004).[33] Comparison of techniques of to assess hydration status.

Technique	Purpose	Instrument Cost	Time Required Per Analysis	Technical Expertise Required	Accuracy	Portability	Risk to Individual Health
Isotope dilution, isotope appearance	Fluid volume	3	3	3	1	3	2,3*
Neutron activation analysis	Fluid volume, whole body K+	3	3	3	1	3	2
Plasma and urine osmolality	Fluid concentration	3	2	3	1	3	urine, 1 plasma, 2
Hematocrit, hemoglobin	% plasma volume change	2	2	3	1	3	2
Bioelectrical impedance and BIS†	Fluid volume	2	3	2	2	2	1
Urine specific gravity	Body water change	2	1	1	2	1	1
Urine conductivity meter	Body water change	2	2	2	2	3‡	1
Urine color	Body water change	1	1	1	2	1	1
Body mass change§	Body water change	1	1	1	2	1	1
Rating of thirst	Body water change	1	1	1	3	1	1
	Key to ratings:	1 = small 2 = moderate 3 = great	1 = small 2 = moderate 3 = great	1 = little 2 = intermediate 3 = much	1 = high 2 = moderate 3 = low	1 = portable 2 = moderate 3 = not portable	1 = low 2 = moderate 3 = high

*Depending on the type of isotope involved (i.e., radioactive, stable, non-radioactive)
†Bioimpedance spectroscopy
‡Portable, hand-held meters are available[16]
§Using a floor scale

Blood and Urine

Blood markers such as osmolality and plasma volume change have been used during exercise. Plasma osmolality refers to the concentration of solutes in plasma measured by either vapor pressure or freezing point depression methods. Normal plasma osmolality is considered to be approximately 287 mOsm/kg among healthy, chronically well hydrated individuals.[33] But plasma osmolality lacks applicability as a long-term measure of whole body hydration state due

to the highly protected nature.[36–38] Plasma osmolality is protected by fluid retention (depicted in Figure 1.1) and stimulation of the renin-angiotensin-aldosterone system (RAAS). A small elevation (1-2%) in extracellular fluid osmolality stimulates.

In a review of commonly used analytical urine measures, Armstrong (2005)[33] described the applicability and diagnostic accuracy of urine markers. Urine measures have a unique challenge, where a difference between longitudinal and spot samples has been reported.[39] Diagnostic accuracy of spot samples is questioned due to the lagging response of renal function with acute body water change.[40] Urine volume, collected over 24-hours, refers to the urine concentrating ability of the kidney based on body water needs. Typical values are considered to be approximately 1.4 L/24-hr.[33,41] Urine volume, and measures (i.e. osmolality, specific gravity) taken from 24-hour urine collection have greater applicability for long-term hydration state.[39]

Normal euhydrated measures of urine osmolality are considered 442–1052 mOsm/kg. This measure is commonly used in laboratory analysis where an osmometer is available.[41] Urine specific gravity and urine osmolality have a highly correlated relationship, but are less correlated with plasma osmolality.[42] Urine color has been used with reasonable accuracy as a more field-expedient measure of urine concentration, but lacks precision for laboratory assessment.[42,43] It refers to the color of urine as measured against a validated 8-point index to determine approximate percent dehydration, where 1 color increase is approximately equal to 1% loss.[42] Normal euhydrated measures of urine color are considered to be approximately 3-7 on the index.[41]

Urine specific gravity is a measure that compares the density of urine to pure water, where a higher density is related to more solute, and thus a higher concentration of urine.

Normal euhydrated measures of spot-sample urine specific gravity are considered 1.013-1.020, with lower concentrations of 24-hour samples.[41]

Body Mass

Body mass change is one of the most acute measures of fluid loss, and is commonly used as a comparison to the previously described blood and urine markers.[44] Body mass change accounts for sweat and insensible fluid loss during exercise, and may be used to detect modest changes that would not meet the dehydration thresholds (e.g. changes in urine osmolality, hormone concentration). Assessment of body mass change requires few, and primarily portable tools, and does not require complex laboratory procedure. It measures fluid loss by change in body mass over time, and is commonly used in both laboratory and field research. Normal values can be established with a few consecutive days of baseline weighing.[41] However, long-term or chronic body mass change is not easily assessed in this method without careful experimental control, and assessment during an exercise bout is often inconvenient, as undressing is required.

Thirst

Commonly used hydration guidelines recommend mitigating fluid losses to less than 2% loss in body mass, however it is very common for athletes to function at 3-4% loss. Most recommendations consider that both fluid and electrolytes are important parts of an appropriate hydration plan, but how to consume those substances is left in question. A study of fluid replacement tactics reveal that "drinking to thirst" is very commonly used in endurance events.[45]

To avoid both impactful dehydration and hyponatremia, appropriate estimation of fluid needs is absolutely necessary in populations with the potential fluid loss. Numerous sport and labor safety organizations provide recommendations to appropriately measure dehydration

before and after activity, to then replace fluid lost. However, the most common methods described are not feasible for application during activity, and give no insight into preventing impactful dehydration. Measures of urine color and specific gravity, as well as body mass change may be generally useful before and after exercise in field scenarios; however, they provide little indication of hydration status during activity.

Sweat is the primary fluid lost during exercise, and thus sweat rate is often used to describe the total water lost over time during exercise. Understanding the approximate amount of water lost in a given time during exercise would allow the athlete or laborer to effectively replace fluids and stave off the negative impacts of dehydration. This method is relatively simple and cost effective, but comes with several assumptions. Sweat rate is dependent on both internal and external factors, many of which cannot be controlled.[46] It is impractical to test sweat rate under all conditions, and thus sweat rate is only applicable under the assumed set of conditions. While replacing fluid based on sweat rate may provide some useful general guideline to follow during activity, it may over- or under-estimate fluid needs. Moreover, fluid replacement guidelines for sweat rate based on gender and age provide even more room for error when replacing fluid.

In order to make appropriate hydration decisions without stopping exercise, an accurate field-expedient and efficient measure must be available. Thirst has been elucidated as a possible answer to this problem,[47] as it has no associated cost and could be used with minimal disruption in activity. Thirst has been studied in many contexts, commonly during exercise, and may present a method to quickly assess hydration status, and subsequently hydrate appropriately.

As thirst is driven by both physiological and psychological factors, assessment of thirst is challenging. However, a combination of methods and questions about perceptual factors

involved in thirst regulation (e.g. thirstiness, pleasantness to consume fluid, mouth feel and taste, gastrointestinal comfort, fatigue, perception of hydration status) are used in research (Figure 1.2).[48]

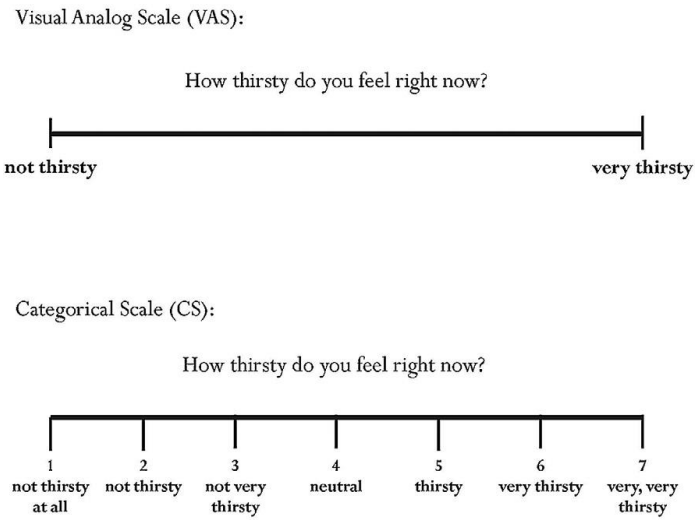

Figure 1.2. Reproduced from Millard-Stafford et al. (2012).[48] Representations of instruments used to assess human thirst: 10-cm visual analogue scale (top) and 7-point categorical scale (bottom).

Likert-type scales

There is not a validated and reliable gold standard for measuring thirst, as it is difficult to validate. Thirst is a perception that is linked to a combination of intrinsic and extrinsic factors that have some imperfect relationship. This is not the case with all perceptual indices. Thermal sensation indices, such as a heat scale used commonly in thermoregulation research,[49] can be compare to body temperatures to validate thermal perception. However, there are several commonly used indices that have been correlated with factors surrounding thirst, such as plasma osmolality, or concomitantly assess related factors such as gastrointestinal and oropharyngeal factors. Likert-type or categorical type scales are commonly used to measure perceptions during exercise, and this extends to thirst. A simple 1-9 point anchored Likert-type scale (Appendix A)

has been used in several publications relating perception of thirst to static and dynamic hydration state, during and following exercise.[50–55] This is a simplified way of analyzing thirst, assuming that it follows a linear trend, and that the distance between points on the scale is relative but not equal.

A series of Likert-type scales has been used to assess several aspects of thirst beyond simply asking the question "how thirsty do you feel?" This collection of 37 scales incorporates the understanding of thirst perception as having a relationship with other influencing factors such as gut fullness, mouth and throat dryness, and mouth taste.[56] In a study of exercise-induced progressive levels of dehydration, with subsequent ad libitum fluid replacement, the scales were used successfully to describe thirst related to plasma osmolality and body mass loss.[56]

Visual Analogue Scales

Visual analogue scales have been used to measure thirst. Measurement of change of thirst over time from baseline was performed in a study on hunger provocation following glucose administration.[57] In this case, participants would initially mark a 30-cm line, and would repeat this at time points following glucose administration. Assuming that individuals perceive thirst differently and that at baseline all participants were normally hydrated and not thirsty, differences in thirst were calculated to determine the relationship between thirst and hunger. While this method does account for individual variation, it would not be appropriate for situations where there is potential for beginning exercise in a hypohydrated state. Visual analogue scales can be used independently, or in groups of related questions. A study of thirst following high intensity intermittent exercise used two visual analogue scales of thirst and mouth dryness to characterize thirst following this type of exercise.[58]

A series of visual analogue scales on 10-cm lines addressing questions related to thirst were used to assess changes during water deprivation and rehydration.[59] Again, changes in rating were used to assess subjective ratings of thirst within each individual before and after a fluid bolus, and were positively correlated with changes in plasma osmolality.[59] Another series of five visual analogue scales about thirst and oropharyngeal comfort was used to describe thirst with combinations of hypohydration and water immersion in men.[36,60]

Uncommonly, drinking behavior and preconceived notions on drinking are assessed in relation to fluid intake. In the first study to examine a difference, Armstrong et al. (2014)[55] described drinking to thirst vs. ad libitum fluid intake by evaluating cyclists' perception of thirst and their own drinking behavior.[55] In this study, drinking to thirst (i.e. drinking only when perceiving a sensation of thirst) and ad libitum drinking provide similar fluid replacement results during exercise.

B. Thirst as a Factor of Hydration

Thirst is a bio-psychological variable, with roots in fluid regulatory hypothalamic stimulation, hormonal control, and is subjective to perceptual influences. There are several proposed mechanisms by which thirst is stimulated, including hypovolemia and cellular dehydration which both increase osmolality.[61–63] The sensation of thirst is not a solution to a less desirable state of hydration but does, in part, give rise to the behavior of drinking.

Physiological Control of Thirst

Central Control of Thirst in the Hypothalamus

The complex system of neural circuitry that responds to physiological changes in osmolality of plasma and cerebrospinal fluid, also receives sensory input from esophageal and gastrointestinal receptors to create a sensation which drives drinking behavior. Depicted in Figure 1.3, hypothalamic stimulation, specifically of osmoreceptors in the organum vasculosum of the lamina terminals (OVLT), subfornical organ (SFO), and median preoptic nucleus (MnPO) provides neuronal connection to the organs which produce arginine vasopressin (AVP).[64] Arginine Vasopressin has a critical role in fluid regulation and thirst. Farrell et al. (2011)[65] described increased cerebral blood flow during rehydration, supporting the theory that these hypothalamic organs may monitor change in hydration in real time. Further research on the influence of the vagus nerve, as well as impact of hormones on hypothalamic function and resulting change in thirst regulation, are ongoing and thus far inconclusive.[66,67]

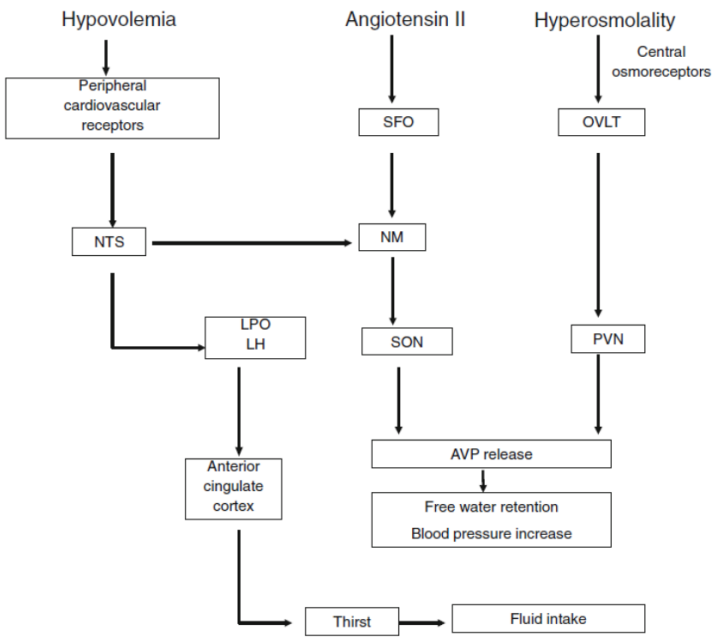

Figure 1.3. Reproduced from Stachenfeld, 2008. Schematic diagram of central regulation of body fluid regulation in response to acute changes in sodium and volume. AVP arginine vasopressin, LH lateral hypothalamus, LPO lateral preoptic nucleus, NM median preoptic nucleus, NTS nucleus of the solitary tract, OLT organum vasculosum of the laminal terminalis, PVN paraventricular nucleus, SFO subfornical organ, SON supraoptic nucleus.

Hormonal Response Initiating and Perpetuating Thirst

Cellular dehydration occurs when fluid shifts out of the cells, plasma osmolality being the typical precursor, which stimulates thirst. This is likely due to an increase in transmembrane osmotic pressure, creating more potential for water to cross from intracellular fluid (ICF) to intravascular extracellular fluid (ECF) space. When solutes become more concentrated in plasma, water shifts from the ICF to the ECF via osmosis readily.[68] Hypertonic saline infusion causes increased sodium entering the plasma causes large fluid shifts out of the ICF.[69–73] Stimulation of the OVLT, SFO, and MnPO in the hypothalamus cause AVP production to encourage fluid conservation and fluid intake.[61] Three primary pathways lead to thirst[74] (Figure 1.4); it is important to note that thirst is not the sole determinant of drinking behavior.

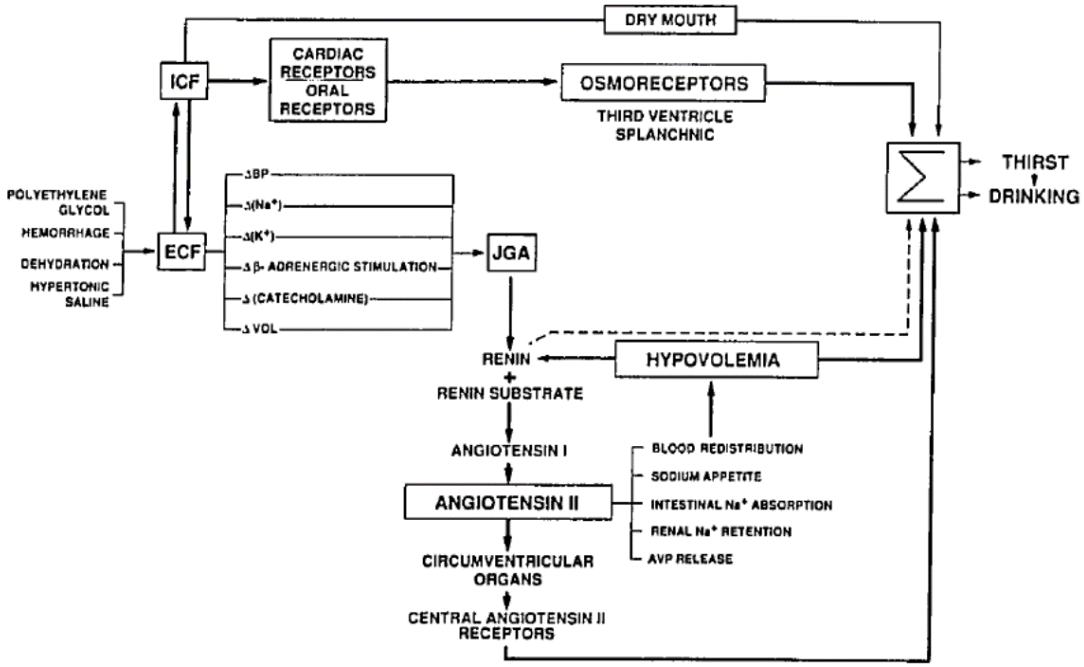

Figure 1.4. Reproduced from Greenleaf, 1992.[74] A model illustrating three major pathways- osmoreceptors, hypovolemia, angiotensin II – and associated factors for induction of fluid intake.

Arginine vasopressin is secreted after baroreceptor and osmoreceptor stimulation of the hypothalamus. Additionally, peripheral baroreceptors and the presence of angiotensin II may stimulate release of AVP. All of these systems combined create a complex of actions that lead to the production of AVP.

The action of AVP in fluid regulation is two-fold: renal water reabsorption and systemic vasoconstriction.[31,56,75] The threshold for thirst and AVP release are tightly linked, and thus sweating during exercise can stimulate thirst. Fluid intake which reduces ratings of thirst is followed by a concurrent reduction in AVP.[63] This concept that thirst and AVP follow a similar pattern is evident as Robertson (1984)[75] described AVP, thirst, and plasma osmolality, depicted in Figure 1.5. Thirst increases as plasma osmolality increases, and appears to be stimulated at a relatively low plasma osmolality,[76] but relatively high concentration of AVP.

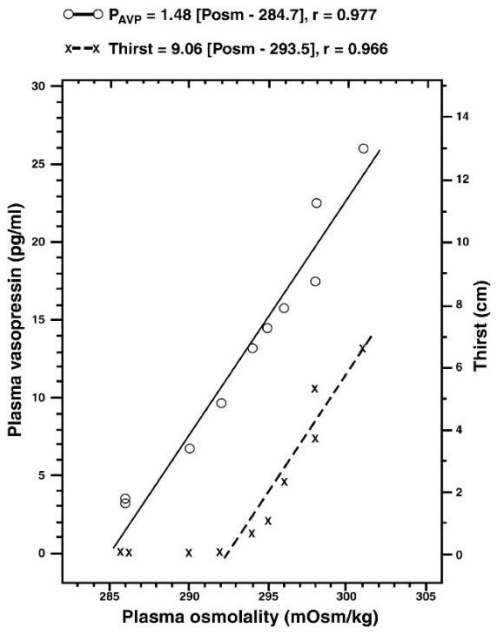

Figure 1.5. Reproduced from Robertson, 1984.[75] The relationship of plasma AVP (○) and thirst (x) to plasma osmolality in a healthy volunteer during infusion of 5% saline. The plasma AVP level was determined by radioimmunoassay. Thirst intensity was estimated by means of a geometric rating scale. The regression functions were calculated by least-squares analysis of all data pairs above the x-intercept.

Normal concentration of plasma AVP is approximately 1 pg/mL. In this graph, AVP appears at a plasma osmolality of approximately 290 mOsm/kg, and thirst is stimulated at an osmolality of approximately 294 mOsm/kg. At that plasma osmolality, AVP concentration is approximately 11 pg/ml, showing that thirst has a delay in stimulation.

Copeptin is a pre-pro-hormone synthesized in the hypothalamus, and stored following axonal transport in the posterior pituitary. During transport, it is cleaved into its major parts, vasopressin, neurophysin II, and copeptin. Although copeptin is inactive in the circulation, its concentrations during osmotic shifts in blood are very similar to those shifts observed with AVP, and are highly correlated as depicted in Figure 1.6.[77] Thus, although copeptin does not have a direct action to influence thirst, it can be used reasonably as a marker to describe the concentration of a more unstable hormone, AVP, which does influence thirst.[77–80]

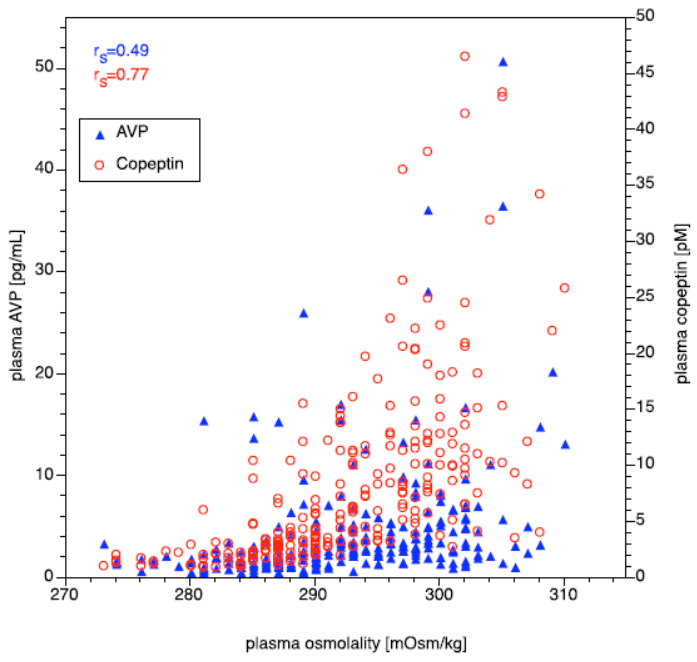

Figure 1.6. Reproduced from Balanescu et al. (2011).[77] Plasma AVP and copeptin concentrations measured during the individual water load-hypertonic saline tests are shown as scatter plot. r_s denotes Spearman's rank correlation coefficients.

With 24 hour water deprivation, there is an upward shift in plasma osmolality,[59] plasma sodium, and hematocrit. With oral rehydration, plasma osmolality decreased in as little as 5 minutes after drinking, and decreased to below pre-deprivation values within 15 minutes.[59] During water deprivation, subjective ratings of thirst increased. Thirst then rapidly decreased after drinking, becoming significantly different from before drinking by 2.5 minutes, although at this time only a small amount of fluid was ingested.[59] Thirst eventually returned to pre-deprivation levels between 5-30 minutes after fluid consumption was initiated.[59]

Plasma osmolality is kept within a tight range, between 280-292 mOsm/kg, and even small deviations within this range, as low as 2% increase corresponding to about 290 mOsm/kg[63,81] can activate the RAAS. However, the perceived thirst appears to vary between individuals,[61] eluding to the influence of non-physiological drivers of thirst. Although other components of the RAAS may be active or potentially elevated with fluid deprivation, such as antiogensin II and renin, they do not stimulate thirst directly, but satisfy it indirectly through hormonal regulation of fluid retention.[59,63]

Hypovolemia also plays a role in thirst stimulation. Depletion of the intravascular fluid causes decreased plasma volume, which commonly occurs with thermoregulatory sweating during exercise.[82] This decrease in plasma volume stimulates baroreceptors in the hypothalamus, and initiates the release of hormones to protect fluid loss and provide still appropriate circulation to perfuse necessary tissues.[62] In many cases hypovolemia leads to cellular dehydration by increasing the osmolality, which creates an environment to cause fluid shift between compartments.[83]

During head-out water immersion thirst is suppressed when a person has significant ECF volume depletion.[36,60] External hydrostatic pressure pushes fluid from the ICF to the ECF,

causing cellular dehydration, but decreasing plasma osmolality into the normal range by increasing plasma volume. The increased external hydrostatic pressure decreases the inherent stimulation of baroreceptors, limiting the activation of the RAAS. With concurrent water immersion and hypohydration, perceived thirst decreases without fluid intake; however, without hypohydration, fluid shift is not as dramatic, and there is no effect on thirst.

A review of studies describing the mechanisms which govern thirst aimed to further describe the complex actions of these hormones on thirst. Ratings of thirst are highly correlated with plasma osmolality during exercise in trained athletes, but not untrained athletes.[63] Factors such as metabolic disease, training status, and advancing age are shown to delay thirst due to altered handling of body water and altered AVP release.[63]

C. Factors Which Impact Thirst Perception and Drinking Behavior

Regulation of thirst is complex, including not only hormonal, but also physiological regulation. We can see that thirst during daily life and during exercise are somewhat separate phenomena. Indirectly, increased water consumption during daily life is associated with improved overall mood, lower ratings of thirst, and more positive emotion.[20] Additionally, decreased water consumption during daily life is associated with deteriorated mood and more negative emotion.[20] Importantly, these changes are directly related to typical water consumption habits, where those people who typically drink a lower amount of fluid do not experience significantly greater negative emotion in daily life. There must be the understanding that physiologically thirst may be generated based on hormonal and CNS responses of physiological states, but may not actually be perceived.

Evidence on thirst as a method for adequate fluid replacement is mixed,[84] and sometimes

confused with drinking behavior. Drinking behavior is, at its essence, driven by thirst but influenced by many other factors. Depicted in Figure 1.7, both intrinsic and extrinsic factors have a profound effect on the perception of thirst.[31,56] Thirst should stimulate what is termed voluntary fluid intake, however often athletes do not take in enough fluid to prevent dehydration, which is theoretically the primary driver of thirst. Oropharyngeal, gastrointestinal, and post-absorptive factors can influence thirst over time, but we propose that identification and modification of these factors could improve voluntary fluid intake when thirst is present. Involuntary dehydration, or the process by which adults to not fully replace lost fluid during exercise, can potentially be prevented by washing out perceptual interference.

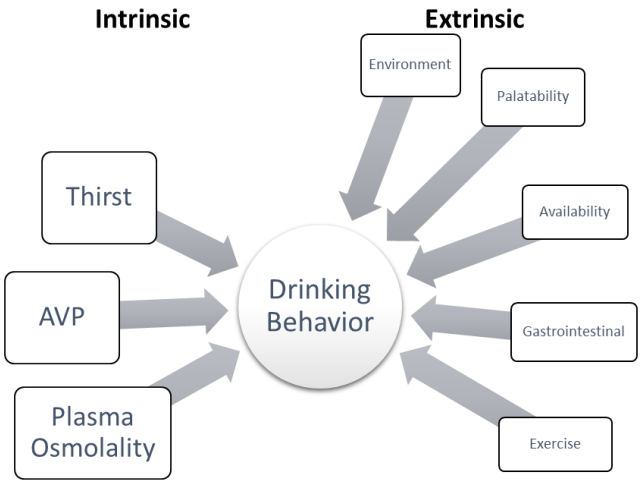

Figure 1.7. Factors affecting drinking behavior.

Environment

The environment potentially influences perception of thirst both during exercise and at rest. A warm environment likely stimulates thirst based on fluid balance in response to sweating. Cold exposure may reduce thirst sensation[63,85] by a similar mechanism as water immersion, increased central blood volume. In cold environments however, rather than a fluid compartment

shift more plasma is driven centrally by peripheral vasoconstriction to preserve body heat at rest. The use of cooling modalities during or after exercise may provide a similar, but not as dramatic, effect as a cold environment, driving blood out of the periphery.[86] The increased volume mitigates the thirst response by up to 40%, effectively deceiving the baroreceptors to delay release of AVP.[85] As plasma volume will not be reflective of body water balance in this environment, thirst may not be as sensitive to changes in hydration status at rest in a cold environment. However, exercise in the cold at higher intensities may not see the same reduction in thirst, likely due to the greater heat production and less blood centralization.[87] Mitigation of thirst may be controlled to some extent by wearing appropriate clothing so as to maintain heat balance during exercise, or assessing thirst prior to application of cooling modalities.

Although not related to the physical environment, the social and cultural environment may predispose one to suppress thirst. Consideration of religious rituals which control food and beverage consumption can profoundly affect hydration before, during, and after exercise. For example, observation of Ramadan during an athletic season can have profound impacts on daytime hydration and energy replacement, where no consumption may occur during daylight hours. However, because fluid consumption is allowed during darkness, athletes may use strategies to prevent cumulative hypohydration across the month.[88] This practice does not allow the sensation of thirst to dictate consumption of fluid, and thus need for fluid consumption via thirst cannot be assessed.

Availability of fluid

Availability of fluid is commonly associated with voluntary hydration, particularly in the labor and athletic settings.[89] If extra energy or time must be taken to locate or obtain fluid, it is theoretically more likely that an athlete or laborer will go without drinking until a greater level of

thirst is experienced. Availability may be considered in two respects: fluid proximity and container type.[61]

Fluid proximity has been studied in endurance sports, specifically cycling where fluid is attached to the bike frame and readily available for consumption. Before, twice during, and immediately following a 164-km cycling event in a hot environment thirst, as well as other field-expedient hydration markers, was assessed.[2,54,55] It was found that body mass was relatively stable (approximately 2 kg lost) over the course of the race, and although thirst did increase slightly from approximately 2 to 4 on the 9-point scale, it would also be considered mild. Although the physical conditions are stressful, one major proposed factor influencing fluid consumption in this case was the availability of water. Cyclists keep bottles of water within reach on a bike frame, and thus have the unique ability to consume fluid without stopping activity.

In a follow-up study,[53] total fluid intake and body mass were evaluated as predictors of thirst in a 164-km cycling event. However, thirst was unrelated to either measure, implying that what inspires an athlete to consume fluid is complex in nature, and that body mass change and body fluid balance are not the same. In cycling where fluid is readily available, athletes may be more likely to consume fluid based on factors unrelated to thirst. The same study[53] evaluated the relationship between duration of exercise and total fluid intake, amidst previous research proposing that endurance athletes are at greater risk of exertional hyponatremia due simply to the total fluid volume consumed during longer events. There was a positive relationship between duration and total fluid intake, but still with mild fluid loss (body mass change of -2.7±1.8%). This supports the notion that thirst may not be the only factor to drive an athlete to consume fluid during exercise.

Endurance running has been of particular concern in regards to drinking behavior during running. Fluid is not readily available during long distance running events. However, a study of heat acclimatized male runners performing treadmill running described that when fluid was readily available, only a mild level of dehydration was reached (< 1%).[90] Although carrying fluid has become more prevalent, there are inherent drawbacks to carrying enough fluid to prevent significant dehydration during long events.

Additionally, container type may affect drinking behavior or beverage preference by making fluid either more or less accessible.[61] It was shown that during rest, subjects consumed more fluid from a wide-mouthed container, such as a cup, than from small-mouthed containers such as bottles and cans. This may be due to a faster flow-rate of fluid out of the container allowing more rapid fluid consumption, and less required effort to access the fluid. Although cups of fluid are not typically available at most team setting sporting events, they are very common at endurance running and cycling events. However, research describing long distance running continue to report high levels of dehydration seen in finishers.[45,91–93]

Adding to this issue of availability is the concept of mobility and forethought about restroom usage. In elderly populations, thirst may be elevated however these individuals choose not to act upon thirst cues to prevent the need to use the restroom frequently. Mobility or issues with immediacy, such as incontinence, make people less likely to consume fluid according to thirst in order to prevent numerous trips to a restroom.[94]

Palatability

Palatability is generally thought to be a combination of several characteristics of a beverage which may have a varying level of pleasantness to the drinker. Consideration of factors that influence beverage palatability surely influence the choice to drink the beverage, regardless

of the presence of thirst. Sagawa described water immersion decreased thirst, and decreased palatability of fluid in terms of mouth feel and taste, feel of fullness, and fluid desirability, at a similar rate.[60] This suggests that these factors are closely tied to thirst, and that influence of fluid palatability is large.

Ali et al. (2011)[95] describes no preference for carbohydrate-electrolyte solutions over water before, during, or after exercise, but thirst quenching ability and overall liking of all beverages increased with exercise.[95] However, thirst was not measured, and thus actual thirst-altering quality of the beverage cannot be elucidated. Also, typical beverage preference was not controlled in this study, and thus change in individual preference during exercise cannot be elucidated. Beverage palatability can be affected by flavor, mouth feel, temperature, and typical beverage preference.[62]

Flavor and Content

Beverage content has been shown to provide a more favorable drinking experience, and thus result in higher rate of intake and total intake, and a reduction in body mass loss from exercise. (Vandermark systematic review, unpublished). These beverages fall into several categories: flavored but without caloric content, carbohydrate containing, electrolyte containing, acidic, and combinations thereof. Flavored beverages typically contain carbohydrate with or without electrolyte, and sometimes protein in varying amounts. Sweetness and saltiness, as well as additional flavor may affect palatability of fluid, influencing fluid consumption. Any flavor beyond water may provide some stimulus to drink as it commonly is associated with sweetness or energy consumption, but particular flavor preference is likely individual.[96] For flavored beverages, more diluted beverages are preferred and result in less body mass loss over strongly

flavored beverages, and result in lower ratings of thirst and higher ratings of palatability during labor in the heat.[62,89]

Electrolyte containing beverages have been shown to be similarly palatable as plain water, but appear to mitigate the effects of involuntary dehydration.[97] The inclusion of small doses of electrolyte in beverages during and after exercise has been found to increase both hydration during exercise as well as rehydration after.[98] In contrast, a study of children exercising in the heat found that they neither preferred a sports drink nor did they consume more of it than water, eluding to personal preference as a main confounder.[90] Electrolytes may provide a more palatable solution to lessen voluntary dehydration while still inspiring thirst. However the impact of specific individual components, such as electrolytes alone, is largely undescribed.[99–106]

Beverages which contain electrolytes can have a large effect on voluntary fluid intake and may impact thirst also. Even when salt content is low, it drives fluid shift from ICF to ECF by forcing regulation of plasma osmolality. This might also suppress thirst with the increase in volume and maintenance of osmolality, but causes cellular dehydration. However, while this may be the case in a relatively well hydrated individual, during recovery periods or rest before exercise when hypohydrated, salt content can help drive thirst by increasing plasma osmolality; it may also provide the lasting benefit of fluid retention with fluid consumption.[107]

Hypertonic beverages, such as pickle juice[108] and soup broth,[109] have been considered as potentially exaggerating thirst by the same mechanism. Although there appears to be no difference between these solutions, they may inspire more water consumption due to lesser palatability than water without a significant change in plasma osmolality.[108] Recommendations to rehydrate with these beverages should be taken with a grain of salt, however, as the lack of

change seen in plasma osmolality may be the due to cellular dehydration whereby the increased plasma sodium drives fluid out of the cell as a compensatory mechanism. Furthermore, these beverages had little effect on perceived thirst and are largely unpalatable during exercise. Access to pleasant tasting beverages, or at least not unpleasant tasting beverages, will increase voluntary fluid intake during exercise. Provision of more dilute, electrolyte containing beverage options if some flavor is preferred,[89] but also justifies the use of plain water to prevent significant voluntary dehydration during exercise in the heat.

Temperature

Fluid temperature is widely considered an influencing factor in fluid intake, where cooler fluids are preferable during exercise in warm environments.[61,110] Availability of cooler fluids results in approximately 50% greater volume consumption during exercise, regardless of beverage type.[62,110] Temperature preference during exercise is closer to 22°C than 6°C.[28,62,97]

However, this preference may be impacted by environment and exercise intensity. Most studies in which beverage temperature is assessed in relation to volume of fluid consumption have been performed in warm environments,[110] creating an external link between a preference for cool beverages and warm environments. However, it appears that in cases where cool beverages are not available, ambient temperature fluid consumption may be similar enough to prevent significant body mass loss.[96] Interestingly, perceived fluid temperature is variable, likely due again to environmental influence, and thus a range of temperatures is likely acceptable.[111] The recommendation that fluid available during exercise should be cool, but not very cold, in order to make it most appealing.

Mouth Feel

Relatively little information on fluid consumption and mouth feel or viscosity exists at this time, however beverages with a high viscosity or syrupy mouthfeel are typically less appealing during exercise leading into voluntary dehydration.[61] Fluid consumption following fluid deprivation was perceived to decrease thirst. In a study of males who underwent 24 hours of fluid deprivation, perception of mouth feel, mouth taste, and pleasantness to consume water were all rapidly decreased following the initiation of drinking water, and were sustained over time.[59] Questioning of reasons for rehydration following this deprivation period revealed that fluid consumption by participants was driven by the need to eliminate a "tacky" or dry mouth.[59] Additionally, participants recognized that only small amounts of fluid were required to suspend dry mouth feel.[59] Cold temperature, and a resultant very small amount of water, via sucking on ice chips following exercise induced dehydration has been shown to decrease thirst perception without a change in plasma osmolality or AVP.[112]

Additionally, mouth dryness has been linked to increased fluid palatability, leading to increased fluid consumption. Thirst and mouth feel may play a role in both initiation and cessation of drinking. Studies of mouth dryness after exercise where saliva was collected via cotton and not allowed to enter the oral cavity describe fluid consumption after this period with an increased volume, frequency, and number of bouts than when saliva was not collected.[113] Although a similar level of dehydration was reached between saliva and non-saliva conditions, more drinking occurred when no saliva was present in the mouth both at rest and during exercise.[113,114] This indicates that even the relatively small stimulus of salivary wetting of the mouth can cause a decrease in thirst and fluid consumption.

Indirectly mouth feel has been attributed to satiety after fluid consumption in studies of oral versus intravenous rehydration.[51,50,52,115] In a study where hydration status was controlled

via intravenous catheterized infusion of saline, thirst was also controlled via mouth rinsing. It appeared that the presence of a water mouth rinse during exercise reduced the perception of thirst, but did not completely abolish it.[70] This implies that the wet mouth feeling was enough to suppress thirst in the short-term, which falls in line with previous research.[63,116] This gives rise to the notion that incomplete fluid replacement may suppress thirst during exercise, but will not completely shut it out so that thirst may still be considered to indicate need for additional fluid consumption.

Gastrointestinal

Stomach distention has a profound effect on our willingness to consume fluid, and relates even to negative feelings such as nausea. Beverage type may affect stomach fullness; sugar and electrolyte containing drinks inspire higher levels of fullness than water, leading to decreased drive to consume additional fluid.[90,111] In a study of hypohydrated and euhydrated people exposed to fluid-restricted exercise, stomach fullness was much less in the hypohydrated group, which consequently took in more fluid during a rehydration period.[60] There is a tight, if not completely understood relationship between nausea and AVP, giving rise to an indirect relationship between thirst and nausea.[117] More discovery of how AVP and nausea interact can lead to better planning to appropriately assess thirst.

In a study involving rehydration following a 24-hour fluid deprivation period, participants described that rehydration was limited by the feeling of stomach fullness.[59] It appeared through perception questionnaires that fullness increased rapidly with initiation of fluid consumption.[59] In a study involving fluid extraction after consumption, gastrointestinal fullness following fluid consumption was avoided by extracting the consumed fluid. Thirst was still decreased, indicating an independent role of gastrointestinal and oropharyngeal factors.[118] It is

unclear in the literature whether sensation of intake volume can be accurately detected by humans.

Exercise

Although fluid replacement during exercise is often discussed in relation to dehydration, there is a lack of evidence describing the impact of exercise itself on thirst, however theoretical models exist. Thirst is theoretically increased by exertion, due to the presence of causational factors previously described. With high intensity exercise, for example, there is a body water shift from the extracellular to intracellular space associated with a rise in blood osmolality, as well as AVP release.[119,120] Kenefick et al. (2004)[85] described exercise in a cold environment as modulating thirst perception in comparison to exercise in a temperate environment by causing vasoconstriction and a lack of central volume decrease, thus inhibiting AVP release (Figure 1.8).

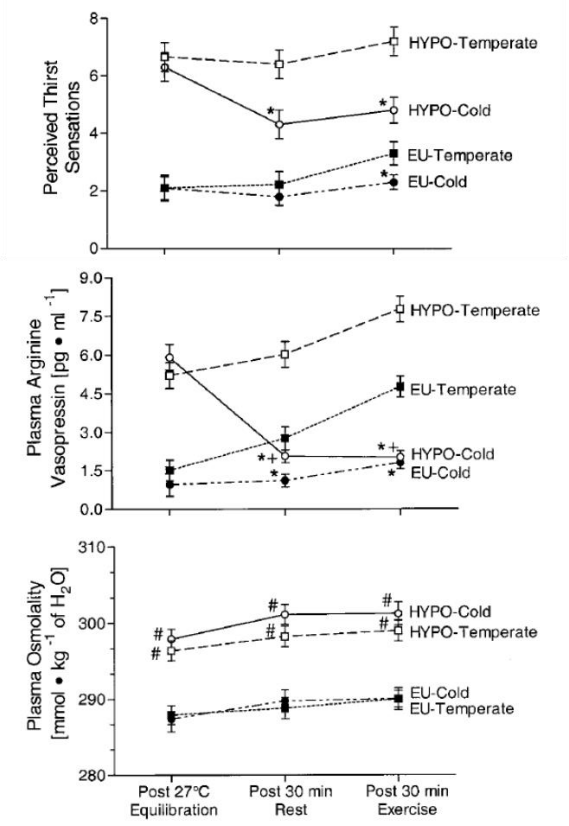

Figure 1.8. Compiled from Kenefick et al. (2004).[85] Perceived thirst, plasma AVP and plasma osmolality vs. time (min) during exercise in cold and temperature environments with hypo or euhydration. Data are presented as Mean±SE. # denotes significant difference of hypohydration conditions from euhydration conditions at respective time points; * denotes cold conditions significantly different from temperate conditions at respective time points.

Analysis of exercise type describes that thirst during exercise is increased with a range of intensities.[58,72,87,121] Fluid consumption and sensations of thirst during high intensity intermittent exercise were associated with a rapid increase in serum osmolality and AVP levels. While thirst sensations occurred at low exercise intensity in the same study, it was to a lesser degree in accordance with diminished change in serum osmolality and AVP.[121] Mears and Shirreffs (2014)[87] found that thirst during and following moderate intensity exercise in a cold environment was blunted in comparison to a hot environment. However, this speaks highly to the impact of external environment on intrinsic factors of thirst regulation rather than the effect of exercise.[87] Low intensity exercise in the heat is sufficient to elicit change in plasma osmolality, how thirst and fluid intake in this case had a greater relationship with pre-exercise hydration state than with markers which changed over time. This suggests that low-intensity exercise is not a large enough stimulus itself to dictate thirst response during exercise.

Additionally, a change in beverage preference, and fluid palatability is seen during moderate intensity exercise. During exercise, three formulations of a carbohydrate electrolyte solution was given to participants before, during, and after exercise, and evaluated based on factors related to pleasantness (sweetness, saltiness, thirst quenching ability, and overall liking) on visual analogue scales.[122] Intensity of sweetness increased over exercise, and saltiness decreased. Overall liking and thirst quenching ability increased during exercise regardless of beverage type, likely due to presence of some level of thirst.[122] This indicates that beverage preference changes due to exercise, becoming greater for saltiness theoretically to inherently return body water back to the extracellular space.

D. Thirst as a Viable Indicator of Fluid Needs

Thirst has not been proven to be a feasible measure of need for fluid consumption during exercise in the athletic population, more-so than other measures of hydration such as urine analysis and estimation of body water loss. One benefit of the utilization of thirst is the possibility for real time hydration assessment. Primary factors which influence thirst can be controlled with appropriate planning to allow the physiological drivers of thirst to function naturally. Finally, understanding that perception of thirst is individual to each person, and a measure of thirst should not be used as an absolute measure of body fluid, but rather as an indicator of change in hydration status.

Lack of thirst does not indicate a hydrated state.[36,60,74,123] Additionally, presence of thirst does not indicate significant dehydration.[63,74,116] There is strong evidence for the presence of thirst indicating some level of body fluid loss. However, we understand that thirst has a delayed onset commonly understood to be about 2% dehydration, and thus the lack of thirst does not indicate euhydration.[75,124] Outside influence of beverage preference, exercise type and intensity, and physical environment may all confound human perception of thirst.[97,103,110]

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Chapter 2

RELATIONSHIP OF THIRST TO HYDRATION STATE DURING EXERCISE

Relationship of Thirst to Hydration State during Exercise

ABSTRACT

Purpose: To determine the relationship of thirst to hydration markers during exercise. Methods: Ten healthy, recreationally active males (Mean±SD; age: 22±3 years; height: 179±6 cm; body mass: 73.5±10.6 kg; body fat: 11.7±3.8%; VO_{2max}: 54.08±5.26 $ml \cdot kg^{-1} \cdot min^{-1}$) completed four exercise trials in a randomized, counterbalanced order. Participants started two trials in a euhydrated state (EuD, EuR), and twotrials in a hypohydrated state (HyD, HyR). Fluid was replaced to replace loss calculated from sweat in the EuR trial only. Fluid was not replaced in the EuD, HyD, and HyR trials. Exercise consisted of intermittent, moderate intensity aerobic treadmill exercise in cycles of walking, running, and rest. Thirst perception was measured using a Likert scale (Thirst) pre-exercise and immediately post-exercise (IPE). Nude body mass was assessed pre-exercise and IPE. A blood draw was performed pre-exercise and IPE. Results: There was a significant relationship between Thirst level < 4 and pre-exercise body mass change (R^2=0.286, p=0.006) and percent change (R^2=0.287, p=0.006). Immediately post-exercise, there was a significant relationship between Thirst and body mass change (p<0.008) and percent change (p<0.009), regardless of Thirst level. Thirst level ≥ 4 was significantly related to IPE plasma osmolality (R^2=0.142, p=0.037). There was no significant relationship between Thirst and Copeptin pre-exercise (p>0.263) or IPE (p>0.272). There was a significant relationship between change in Thirst level < 4 and IPE plasma osmolality change (R^2=0.317, p=0.010), Copeptin change (R^2=0.212, p=0.041), and nude body mass change (R^2=0.325, p=0,009) and percent change (R^2=0.356, p=0.006). Conclusion: There appears to be a relationship between a low level of thirst and hydration before exercise, and a higher level of thirst and hydration after exercise. There is also a relationship between change in thirst and change in hydration state.

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INTRODUCTION

Greater than 2% body mass loss during exercise results in performance decrements related to cognition,[1,2] thermoregulation,[3–5] cardiovascular function,[6,7] and neuromuscular control and detailed skill performance[8,9] Athletes and clinicians should limit dehydration during exercise as a method of performance and safety enhancement. Fluid recommendations by the American College of Sports Medicine and National Athletic Trainers' Association recommend fluid replacement based on individual factors which influence water loss during exercise.[10,11] These factors include sweat rate, exercise intensity, environment, and equipment or clothing worn during exercise. There are currently no reasonable methods for assessing hydration status in real-time during exercise,[12] and given the previously listed factors, determination of fluid needs during exercise can be difficult to determine. Thirst presents an interesting potential option to the hydration assessment problem, as thirst is a psychological sensation linked to changes in physiology, specifically related to hydration state. During rest, thirst perception has been extensively linked to blood osmolality, and release of hormone mediators of body fluid regulation, such as arginine vasopressin (AVP) [13–15] or copeptin.[16] In classic studies of post-exercise recovery, thirst perception appears to be responsive to differing hydration states.[17,18] Access during exercise has mixed results, where free access during exercise may be adequate[19] or inadequate.[20,21] Thus, drinking to thirst is often a recommended drinking behavior during exercise. Studies of non-prescribed drinking in endurance exercise indicates that performance is not decreased by thirst-driven drinking in comparison to prescribed drinking,[22] even when body mass was not maintained within 2% loss.[23] However, this can be misleading, as often it is unclear if prescribed drinking is based on appropriate recommendations, or if drinking in non-prescribed scenarios is truly dictated by thirst in the absence of other external stimuli.

Recent research on fluid intake following moderate intensity exercise described that ad libitum fluid intake during and following exercise was sufficient to keep body mass within 2% loss, in both warm and cold environments.[24] Additionally, delayed consumption of fluid did not change ratings of thirst, but

did sustain them for a longer period, and resulted in consumption of a similar amount of fluid as when water was immediately available following exercise.[19] Thirst during and following high intensity activity appears to be unrelated to changes in blood osmolality,[19] leaving the impression that other factors have influence on thirst perception during exercise. Therefore, the purpose of this study is to determine if the relationship of hydration differs between a low and moderate-high level of thirst during exercise. We hypothesize that a moderate-high level of thirst will have a relationship with post-exercise hydration state, and a change in thirst will have a relationship to change in hydration state following exercise.

METHODS

Participants

Ten recreationally active males volunteered to participate in this study, which was approved by the Institutional Review Board at the University of Connecticut. Prior to obtaining written informed consent (Appendix D), participants were informed of study procedures and possible risks associated with participation in this study. Participants were included based on the following inclusion criteria: male, aged 18-35; reported being recreationally active defined as >30 minutes of exercise on 4-5 days per week; and VO_{2max} assessed as >45 $ml \cdot kg^{-1} \cdot min^{-1}$. Participants were excluded based on the following exclusion criteria: fever or current illness at the time of testing; history of cardiovascular, metabolic, or respiratory disease; current musculoskeletal injury that limits physical activity; or exertional heat illness in the past 3 years. Satisfaction of these criteria was determined from a medical history questionnaire (Appendix E).

Experimental Design and Procedures

This lab study followed a randomized, counterbalanced, crossover design. Study was conducted in the Human Performance Laboratory at the University of Connecticut, in an

environmental chamber (Model 200, Minus Eleven, Inc., Malden MA) set at an ambient temperature at 35.2±1.1°C and 31.4±6.3% relative humidity. Data collection occurred between February - May 2016. Each participant visited the laboratory for (Figure 2.1): 1 familiarization visit, 3 baseline body mass visits, and 4 exercise trials.

Familiarization Visit

Baseline and familiarization visits occurred prior to the first exercise trial to familiarize participants to study procedures, and collect normative data on participant characteristics. Participants were instructed to arrive euhydrated to the laboratory. Upon arrival to the laboratory, height was measured. Body fat was assessed via Lange skinfold calipers (Beta Technology, Santa Cruz, CA) using the 7-site method described by the American College of Sports Medicine.[25] Participants performed a maximal oxygen uptake (VO_{2max}) test following a modified Bruce protocol[26] on a motorized treadmill (Commercial 2950, NordicTrack, Logan, UT) in a thermoneutral environment to establish appropriate aerobic intensity during the exercise trials and assess satisfaction of inclusion criterion. Exercise intensities were derived from the maximum velocity in the VO_{2max} test.

Following completion of the VO_{2max} test and a brief rest period, sweat rate and electrolyte content was assessed to determine fluid replacement volumes during exercise. The sweat rate assessment was performed in the environmental chamber at approximately 35°C, approximately 30% relative humidity. Prior to entering the environmental chamber, nude body mass was measured (Defender 5000, Ohaus, Parsippany, NJ). Participants entered the environmental chamber and rested for 15 minutes. During this time, participants were familiarized with the Thirst scale (Appendix A) to be used throughout the study, as well as a diet log (Appendix C).

Participants performed an exercise protocol which was the same as one cycle of exercise completed during an exercise trial to obtain an accurate sweat rate. Exercise consisted of 8-minutes at 40% VO_{2max}, 8 minutes at 60% VO_{2max}, and 8-minutes at 40% VO_{2max} on a motorized treadmill, followed by 6 minutes of rest. After exercise, participants exited the environmental chamber, wiped away any remaining sweat, and provided a final nude body mass. The difference between pre-test nude body mass and post-test nude body mass was used to calculate sweat rate for 30 minutes.

Baseline Visits

To gain insight into normative body mass, a 3-day baseline body mass occurred on three consecutive days following the familiarization visit. Prior to each baseline body mass session, participants were instructed to eat according with their normal diet, hydrate well, avoid alcohol, and record everything consumed on a diet log. The diet log was entered into a food processing program to determine macronutrients, electrolytes and moisture (Nutritionist Pro, version 5, Axxya Systems, LLC, Redmond, WA). They were also instructed to collect all urine in a provided urine jug for 24 hours, and provide a small urine sample in a clean urine cup prior to bedtime on the night prior to each visit. In the morning of each day of the 3-day period, participants arrived at the laboratory in a fasted state, returned the urine jug, bedtime urine cup, and diet log from the previous 24 hours. Urine was analyzed for urine specific gravity (Usg; TS400, Reichert Technologies, Buffalo, NY), osmolality (UOsm; Model 3320, Advanced Instruments freezing-point depression Osmometer, Norwood, MA), color,[27] 24-hr sample and bedtime sample. Twenty-four hour urine volume was measured by combining bedtime and 24-hour samples prior to weighing (Ranger 3000, Ohaus, Parsippany, NJ). Nude body mass was recorded. This process was repeated for each participant for three consecutive days. Three-day

baseline nude body mass was averaged, and used as a comparison to all nude body mass measures for exercise trials to describe change and percent change. These same urine collection and diet log completion procedures described here were completed the day prior to each exercise trial as well.

Exercise Trials

Each exercise trial was scheduled with no less than 5 days between exercise trials, and participants were asked to maintain normal diet and exercise routines throughout the study. A general timeline for the study, and basic description of trial procedures is depicted in Figure 2.1. Trial conditions were a crossover design, and randomized and counter balanced (Table 2.1).

Participants inserted a rectal probe to monitor body temperature (4600 series, Measurement Specialties, Hampton, VA) and a heart rate strap to monitor heart rate (digital 2.4 Timex heart rate monitor, Timex Group USA, Middlebury, CT) to determine safety cut offs, as described later, for the duration of the exercise trial. Participants entered the environmental chamber (35.21±1.09°C, 31.39±6.32% relative humidity) and sat for a 15-minute equilibration period, after which exercise commenced.

Exercise consisted of up to six, 30-minute cycles (24-minutes total of treadmill exercise: 16 minutes at 40% VO_{2max}, 8 minutes at 60% VO_{2max} at 2% incline, and 6 minutes of rest), equaling up to 3 hours of exercise performance. For the EuR trial, where participants minimized fluid loss, participants were given a volume of water to consume matching sweat rate per 30-minutes as assessed in the familiarization trial. For conditions needed as part of a larger study, three trials (EuD, HyR, HyD), participants progressively dehydrated by fluid restriction for the duration of exercise.

Exercise was terminated at one of the following criteria: rectal temperature reaching 39.99°C, participant requested, altered or uneven gait, heart rate > age-predicted maximum heart rate (220-age) for 5 minutes, 3 hours of exercise completed, or 5% body mass loss. At the termination of exercise, perceptual, hydration, and blood measures were taken, and a 1-hour passively seated recovery period commenced.

Hydration Assessment

For 24 hours prior to each exercise trial, participants collected urine in a clean urine jug, and completed a diet log as described in baseline body mass visits. Urine was analyzed in the same fashion as described in the baseline visit section. For euhydrated trials (EuR and EuD), participants were asked to consume an additional 500 mL of water before and 500 mL of water after sleep the night before the exercise trials to ensure appropriate hydration status. For hypohydrated trials (HyR and HyD), participants underwent a 22-hour fluid restriction to attain a hypohydrated state, including instruction to avoid high fluid containing foods (such as soup, yogurt, or large amounts of raw fruits or vegetables) and all beverages. Participants were asked to avoid alcohol, have similar dietary intake between lab visits, with the exception of fluid and high fluid containing foods, and recorded intake on a diet log for 24 hours prior to the scheduled lab visit. Operational definitions for euhydrated and hypohydrated states can be found in Table 2.2. For euhydrated trials, if euhydration criteria were not met, participants were rescheduled and given further instruction on appropriate fluid consumption to establish a euhydrated state prior to the rescheduled trial. Body mass, urine analysis, and blood analysis were used to assess hydration throughout the study.

Nude body mass was assessed before exercise (PRE) and immediately following a one-hour recovery period. To determine immediately post-exercise (IPE) nude body mass, minimally clothed body mass immediately following exercise was described in reference to urine excreted and mass of clothing. Change in nude body mass is described as the difference from average baseline nude body mass.

Thirst Perception

Thirst perception was collected using two indices. A measure of thirst (Thirst) was collected on a one-point anchored scale from 1-9, where 1 indicated "not thirsty at all," and 9 indicated "very, very thirsty" PRE and IPE (Appendix A).[20,28,29]

Blood Analysis

During each exercise trial, a blood sample was drawn PRE after a 15 minute seated rest, and IPE. At each of these time points, the participant was seated and 7 ml blood was drawn from an antecubital vein using an aseptic technique by a researcher trained to perform blood draws. Plasma osmolality (POsm) and plasma volume change were analyzed immediately from a lithium heparin-treated tube, then the K_2 EDTA-treated tube sample was prepared and stored for later analysis of copeptin. Both tubes were inverted 8-10 times immediately following the blood draw.

Hematocrit (Hct), hemoglobin (Hb), and POsm were assessed immediately. Hematocrit and Hb were used to assess change in plasma volume over time using the equation from Dill and Costill (1974).[30] Hct was analyzed from lithium heparin-treated whole blood using non-heparinized microcapillary tubes, and centrifuged at room temperature and 10,000 rpm for 5 minutes. Hb was assessed using the HemoCue Hb 201+ (HemoCue America, Brea, CA). The lithium heparin-treated blood collection tube was centrifuged at room temperature and 5,000 rpm

for 15 minutes to attain plasma. Plasma osmolality was assessed using the freezing point depression method. Samples were analyzed in duplicate (or more in cases of > 3 mOsm/kg difference in readings) and averaged to determine plasma osmolality.

To prepare for copeptin analysis, 1.5 mL of whole blood collected in K2 EDTA-treated collection tubes and 78 µl of aprotinin (From Bovine Lung, approx.. 10,000 KIU/mL; Fisher Scientific, Fair Lawn, NJ, USA), at a concentration of 0.6TIU/ml of blood were added to a centrifuge tube, then gently rocked 4-5 times to ensure appropriate mixing. Blood was then centrifuged at 4°C and 1,600g for 15 minutes. Plasma was then aliquoted into microcentrifuge tubes and stored at -80°C for up to 3 months for later analysis.

Frozen plasma samples containing K_2 EDTA and aprotinin were transferred to the diagnostic laboratory. For measurement of copeptin, plasma was used in an extraction-free competitive enzyme immunoassay (EK-065-32, Phoenix Peptides, Burlingame, CA). All samples were analyzed in duplicate. Description of assay procedures and standard curve is found in Appendix F. The minimum detectable concentration was 0.12 ng/ml, with a linear range of 0.12-2.79 ng/ml, and an upper limit of 100 ng/ml. Absorbance optical density was read at 450nm on a microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA) using SoftMax Pro software (version 5.3, Molecular Devices, Sunnyvale, CA). The standard curve was used to interpolate concentration from absorbance (GraphPad, Prism, version 7 for Windows, 2016). Intra-assay CV% was calculated to be less than 8.7%, and the inter-assay CV% was calculated as 7.9%.

Statistical Analysis

Data were analyzed for the following exercise time points: PRE and IPE. Baseline variables to establish normative data were analyzed using a one-way repeated measures analysis

of variance (RM-ANOVA) to determine if differences between baseline days were present for hydration variables.

Piecewise regression was performed to assess the relationship of hydration and thirst variables to adjust for non-linearity in the model. A breakpoint of thirst level 4 was used to partition the data into low and moderate-high segments. Estimates of slope were calculated for each segment. For all analyses, *a priori* alpha level was set at $p \leq 0.05$

RESULTS

Sample Characteristics

Participant characteristics were as follows: Mean±SD: age: 22±3 years; height: 179±6 cm; body mass: 73.53±10.62 kg; body fat: 11.7±3.8%; VO_{2max}: 54.08±5.26 $ml \cdot kg^{-1} \cdot min^{-1}$. Sweat rate, as determined in the familiarization trial, was 0.90±0.87 $L \cdot hr^{-1}$ during exercise. Sweat electrolytes as determined from whole body washdown were as follows: sodium (3.7±1.2 $mEq \cdot L^{-1}$), potassium (0.6±0.2 $mEq \cdot L^{-1}$), and chloride (2.6±1.6 $mEq \cdot L^{-1}$).

There were no differences in nude body mass between baseline visits ($F_{(2, 29)}$=0.000, p=1.000). Hydration status on baseline days derived from 24-hour urine variables was considered euhydrated on all three days based on the operational definitions in Table 2.2, and was not different between baseline visits (Table 2.3), therefore, baseline data were pooled for all following analyses. Baseline nutrition variables analyzed by one-way ANOVA (nutrition component by day) did not differ across the three day baseline period ($p>0.05$).

Thirst and Hydration during Exercise

Regressions of thirst and pre-exercise hydration state are presented with scatterplots in figure 2.2. Regressions of thirst and post-exercise hydration state are presented with scatterplots in figure 2.3. Regressions of change in thirst and post-exercise hydration state are presented with

scatterplots in figure 2.4. Several regressions described a negative y-intercept, and will be further investigated. Regressions of thirst and pre-exercise urine variables were performed, however no significant regression was revealed.

DISCUSSION

The purpose of the present study was to describe the relationship of thirst to measures of hydration during exercise. We hypothesized that thirst will have a relationship with hydration before and after exercise. To test this hypothesis, we used a crossover design with repeated measures of hydration and perceptual variables before, during, and following exercise.

Major findings in the present study were: 1) There appears to be a relationship between a low level of thirst and hydration before exercise, 2) there is a relationship between a higher level of thirst and hydration after exercise, and 3) there is a relationship between change in thirst and change in hydration state.

These findings are in contrast with previous research which described an extended period of exercise-induced dehydration the day prior to the experimental trial as having such large effects on hormonal and perceptual markers that the exercise responses were indistinguishable.[20] However, the present study used a passive hypohydration period, which resulted in both body mass and hematologic, but not urinary, differences pre-trial. The change in body mass achieved in the previous study (~3% body mass loss)[20] was not as large in magnitude, and thus may have exhibited blunted responses as compared to the present study (~5% body mass loss). With this, we cannot suggest that the change in thirst was related to change in hydration state unrelated to exercise.

The impact of exercise on thirst has not been well described. In the present study, we attempted to characterize this response by limiting dehydration due to sweating in exercise by

replacing fluid loss. One exercise trial was constructed to provide no change in hydration state (EuR), which was successfully achieved. We found no change among any hydration measures or thirst perception in this trial. However, we recognize that the method by which we limited dehydration inherently impacts thirst by allowing oral fluid consumption and potentially inciting oropharyngeal responses.[31,32] Although thirst changes during rest with saline-induced osmotic shifts,[33] future research should examine infusion of fluid rather than oral consumption to determine the impact of exercise on thirst perception.

The piecewise regression of thirst and hydration variables in the present study showed different slopes for low and moderate-high levels of thirst (e.g. PRE nude body mass % change: low thirst: y=1.862-0.734x; moderate-high thirst: y=-0.720-0.102x). Previous literature has not attempted to distinguish a difference between a low and moderate-high level of thirst, and the relationship to hydration status. This describes that the perception of thirst is probably not linearly related to hydration at both a low and high level of thirst. Rather, as described in the present study, a moderate-high level of thirst was related to a higher plasma osmolality (R^2=0.142) and percent body mass change (R^2=0.378) after exercise.

In the present study, a low level of change in thirst was related to a small change in hydration variables, but a moderate-high change in thirst was not related to a moderate-high level change in hydration variables. A low (< 4 points) change in thirst perception was related to percent body mass loss (R^2=0.356), change in plasma osmolality (R^2=0.317), and change in copeptin concentration (R^2=0.212) after exercise. The interpretation of this relationship is unclear, but supports the concept that thirst may not have a linear relationship with hydration or change in hydration state during exercise.

Plasma volume change was not related to thirst at any time point. However, the significant relationship between plasma osmolality and a moderate-high level of thirst post-exercise supports the theory that thirst is related to an osmotic shift.[17] In the present study a level of thirst 4 and greater had a significant relationship with post-exercise plasma osmolality ($p=0.037$). A plasma osmolality of 295 mOsm/kg has been described as the threshold for thirst.[34] The results of the present study suggest that this threshold plasma osmolality may be related to a moderate-high level of thirst, but that some lower level of thirst may exist below that threshold. In the present study, a low level of thirst was present with a lower plasma osmolality, but did not have a significant relationship ($p=0.091$) after exercise. These results suggest that thirst of a moderate-high level (e.g. ≥ 4 on the 1-9 Likert scale) may be a better indicator of hydration state than thirst of a low level.

Thirst perception has been described in reference to changes in plasma osmolality causing release of AVP. Copeptin has been used to describe AVP in previous literature, as it is more stable and easier to analyze than AVP.[35–37] However, a relationship between copeptin and thirst has not been described in previous literature. In the present study, copeptin was not related to thirst perception before exercise ($p>0.263$) or after exercise ($p>0.272$), but a change in thirst was related to a change in copeptin. The copeptin concentrations observed in the present study are far less than those observed following an ultramarathon,[16] but a lack of research in lower intensity exercise prevents further comparison. Future research should investigate the relationship between thirst and copeptin during exercise.

CONCLUSIONS

Thirst perception has a relationship with hydration state before and after exercise. However, the relationship appears stronger at a low level of thirst before exercise, and a moderate-high level of thirst after exercise. Therefore, moderate-high level of thirst may indicate some level of dehydration following exercise. A low-level change in thirst was related to change in hydration state, but the meaning of this relationship is unclear. Also, copeptin did not have a significant relationship to thirst, further investigation of this preprohormone in relation to thirst and exercise should occur.

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TABLES AND FIGURES

Trial Acronym	Pre-Exercise Hydration State	Exercise Condition
EuR	Euhydrated	Fluid intake = sweat rate
EuD	Euhydrated	⃠ fluid intake
HyR	Hypohydrated	⃠ fluid intake
HyD	Hypohydrated	⃠ fluid intake

Table 2.1. Description of trial hydration conditions.

The trial description in this figure is used to denote each trial from here forward. Fluid intake during exercise was in accordance with sweat rate, and only occurred in the EuR trial.

	Euhydration	Hypohydration
24-hr Usg	≤1.020	≥ 1.021
24-hr UOsm	≤ 500 mOsm/kg	> 500 mOsm/kg
Fluid consumption	Consumed 1 L overnight	Restricted for 22 hours
% nude body mass loss	≤ 1%	~ 1%

Table 2.2. Operational definitions of pre-trial hydration states.

Note: % nude body mass loss is defined as difference of pre-exercise nude body mass from baseline average nude body mass. UOsm, urine osmolality; Usg, urine specific gravity.

Table 2.3. Urine hydration measures from the three-day baseline.

	Bedtime			24-hour			
	UOsm (mOsm/kg)	Usg	Color	UOsm (mOsm/kg)	Usg	Color	Volume (L)
Mean±SD	689±281	1.020±0.008	4±1	513±210	1.015±0.006	4±1	2.23±1.02
95%CI	584, 794	1.017, 1.023	3, 5	435, 592	1.013, 1.017	3, 4	1.85, 2.61
p-value	0.479	0.462	0.561	0.392	0.319	0.544	0.674

Note: Each measure was averaged to determine three-day value. Data are represented as bedtime and 24-hour samples. For 24-hour sample, bedtime sample was analyzed, then added to the 24-hour collection for analysis. UOsm, urine osmolality; Usg, urine specific gravity

Table 2.4. Variables used to determine pre-trial hydration state. All urine measures are derived from 24-hour urine collection. Body mass change was determined as pre-trial nude body mass difference from average baseline nude body mass, representing difference over 24-hours. Data are presented as Mean±SD [95% CI].

	Urine Volume (L)	Usg	Urine Color	UOsm (mOsm/kg)	Body Mass change (kg)	Body Mass change (%)	Dietary moisture (g)	POsm (mOsm/kg)
EuD	2.82±1.39 [1.83, 3.82]	1.012±0.005 [1.009, 1.015]	3±1 [3, 4]	417±181 [288, 547]	0.73±0.52* [0.36, 1.10]	1.02±0.80* [0.45, 1.59]	3396±2186 [1833, 4960]	299±2* [295, 304]
EuR	2.71±1.17 [1.88, 3.55]	1.012±0.006 [1.008, 1.016]	3±1 [2, 4]	418±218 [262, 573]	0.48±0.67* [-0.003, 0.96]	0.60±0.94* [-0.07, 1.27]	4194±2258* [2578, 5809]	290±2* [287, 294]
HyD	1.87±1.02 [1.14, 2.60]	1.016±0.006 [1.017, 1.020]	4±1 [3, 5]	570±223 [411, 729]	-0.83±0.83† [-1.42, -0.24]	-1.40±0.71† [-1.92, -0.89]	1356±1115‡ [559, 2153]	309±3† [302, 315]
HyR	2.05±1.05 [1.30, 2.80]	1.014±0.006 [1.010, 1.018]	3±1 [3, 4]	504±226 [343, 666]	-0.67±0.69† [-1.16, -0.17]	-0.89±0.91† [-1.55, -0.24]	1352±1447‡ [317, 2387]	310±2† [304, 315]

Note: *significantly different from hypohydrated trials (HyD and HyR; $p<0.05$). †significantly different from euhydrated trials (EuD and EuR; $p<0.05$). ‡significantly different from EuR trial only ($p<0.05$). Usg, urine specific gravity; UOsm, urine osmolality; POsm, plasma osmolality.

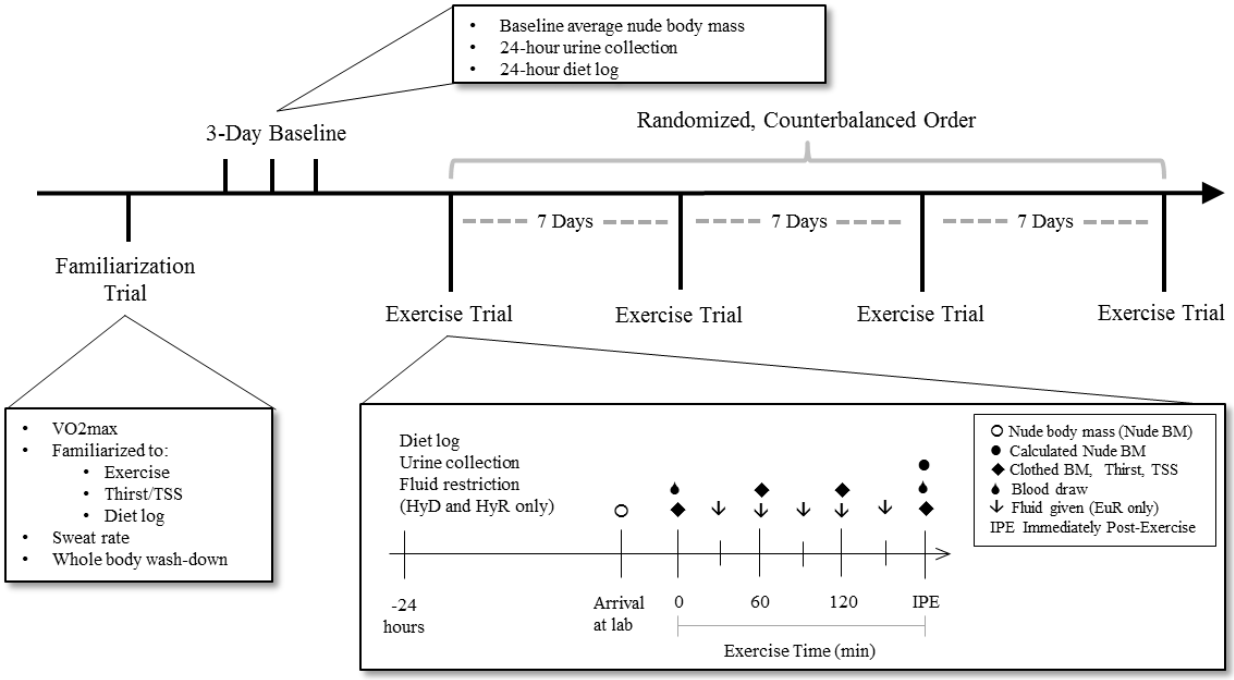
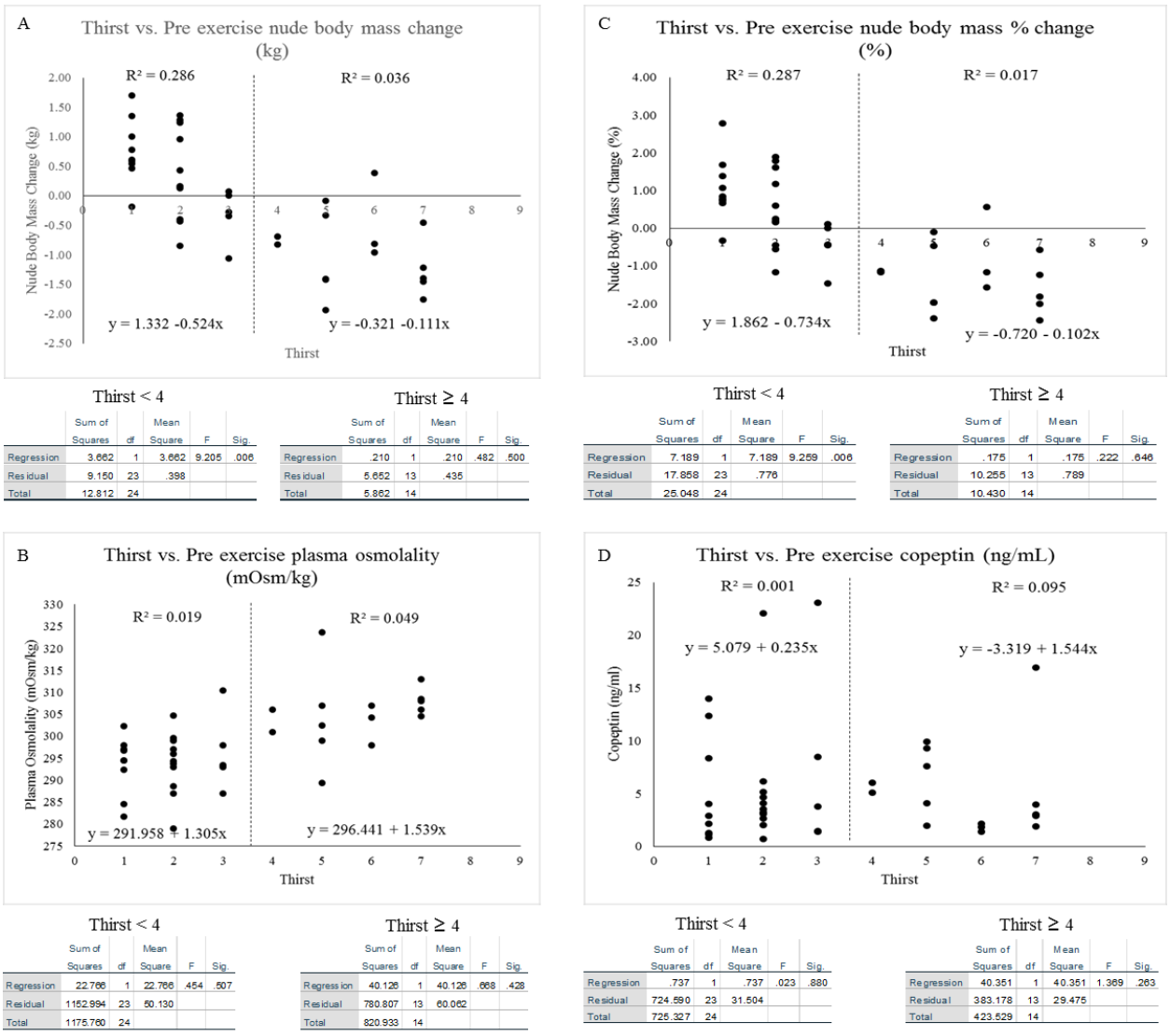


Figure 2.1. Timeline of laboratory testing and description of basic study procedures. Familiarization trials could have been separated from 3-day baseline visits for up to two weeks. Three-day baseline occurred over three consecutive days where measurements were taken in the morning at approximately the same time each day. Exercise trials were separated by approximately 7 days, but no less than 5 days. Exercise trial order refers to hydration state, presented in Table 2.1.



Thirst < 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	3.662	1	3.662	9.205	.006
Residual	9.150	23	.398		
Total	12.812	24			

Thirst ≥ 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	.210	1	.210	.482	.500
Residual	5.652	13	.435		
Total	5.862	14			

Thirst < 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	7.189	1	7.189	9.259	.006
Residual	17.858	23	.776		
Total	25.048	24			

Thirst ≥ 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	.175	1	.175	.222	.646
Residual	10.255	13	.789		
Total	10.430	14			

Thirst < 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	22.766	1	22.766	.454	.507
Residual	1152.994	23	50.130		
Total	1175.760	24			

Thirst ≥ 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	40.126	1	40.126	.668	.428
Residual	780.807	13	60.062		
Total	820.933	14			

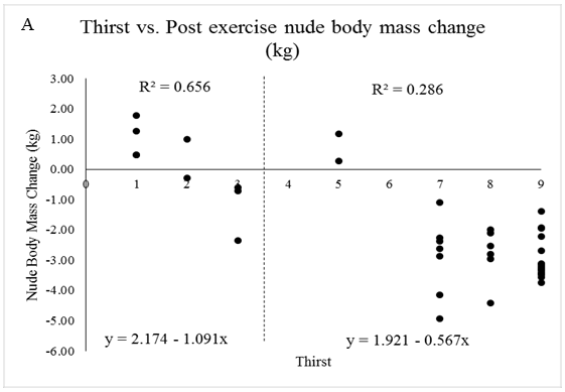
Thirst < 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	.737	1	.737	.023	.880
Residual	724.590	23	31.504		
Total	725.327	24			

Thirst ≥ 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	40.351	1	40.351	1.369	.263
Residual	383.178	13	29.475		
Total	423.529	14			

Figure 2.2. Scatterplots of pre-trial thirst and hydration variables: A) nude body mass change (kg), B) plasma osmolality (mOsm/kg), C) nude body mass change (%), and D) plasma copeptin (ng/ml). The dotted line represents the point of separation between regressions with thirst level less than 4, and thirst level 4 and greater. The slope equation and ANOVA table is presented for each regression.

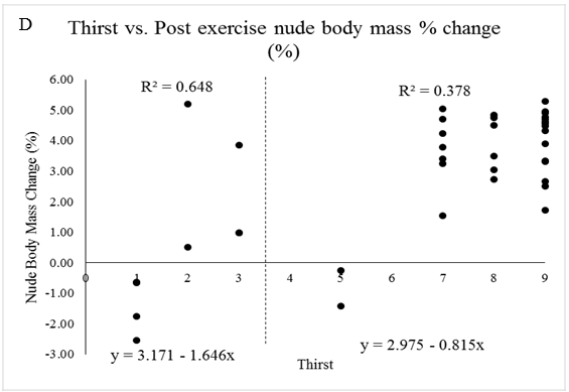


Thirst < 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	8.204	1	8.204	13.369	.008
Residual	4.296	7	.614		
Total	12.500	8			

Thirst ≥ 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	13.107	1	13.107	11.599	.002
Residual	32.771	29	1.130		
Total	45.878	30			

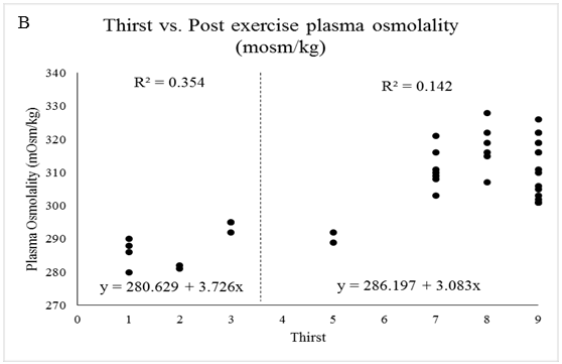


Thirst < 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	18.660	1	18.660	12.900	.009
Residual	10.125	7	1.446		
Total	28.785	8			

Thirst ≥ 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	27.070	1	27.070	17.601	.000
Residual	44.602	29	1.538		
Total	71.672	30			

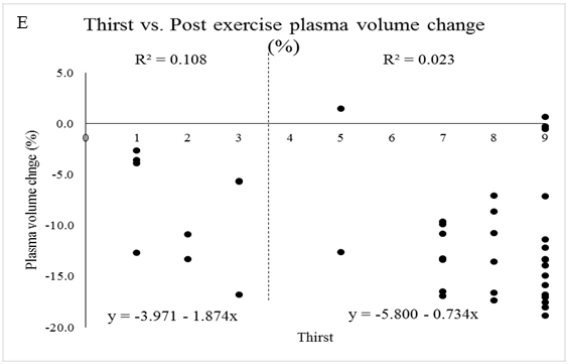


Thirst < 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	95.629	1	95.629	3.839	.091
Residual	174.371	7	24.910		
Total	270.000	8			

Thirst ≥ 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	386.991	1	386.991	4.789	.037
Residual	2343.202	29	80.800		
Total	2730.194	30			

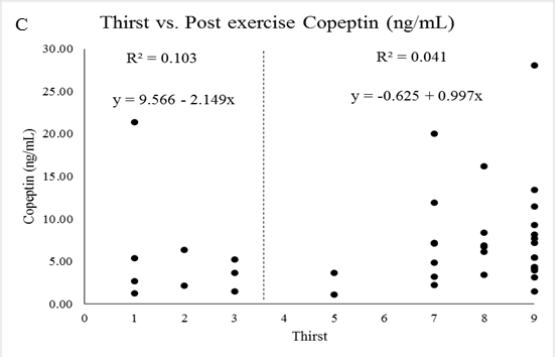


Thirst < 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	24.198	1	24.198	.850	.387
Residual	199.271	7	28.467		
Total	223.469	8			

Thirst ≥ 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	21.923	1	21.923	.676	.418
Residual	940.993	29	32.448		
Total	962.915	30			



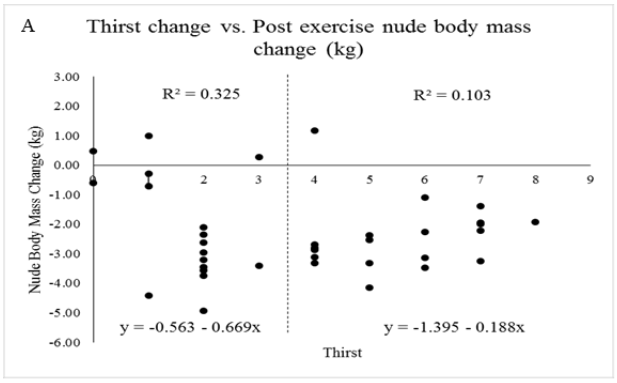
Thirst < 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	31.819	1	31.819	.806	.399
Residual	276.397	7	39.485		
Total	308.217	8			

Thirst ≥ 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	40.446	1	40.446	1.254	.272
Residual	935.555	29	32.261		
Total	976.001	30			

Figure 2.3. Scatterplots of IPE thirst and hydration variables: A) nude body mass change (kg), B) plasma osmolality (mOsm/kg), C) plasma copeptin (ng/ml), D) nude body mass change (%), and E) plasma volume change (%). The dotted line represents the point of separation between regressions with thirst level less than 4, and thirst level 4 and greater. The slope equation and ANOVA table is presented for each regression.

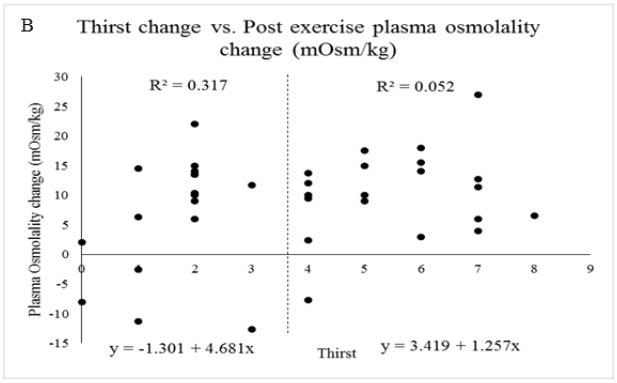


Thirst < 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	13.305	1	13.305	8.656	.009
Residual	27.666	18	1.537		
Total	40.971	19			

Thirst ≥ 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	1.162	1	1.162	2.076	.167
Residual	10.072	18	.560		
Total	11.234	19			

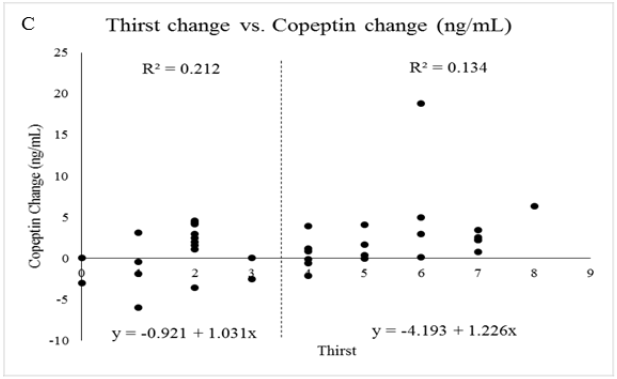


Thirst < 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	651.784	1	651.784	8.349	.010
Residual	1405.166	18	78.065		
Total	2056.950	19			

Thirst ≥ 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	51.391	1	51.391	.987	.334
Residual	937.159	18	52.064		
Total	988.550	19			

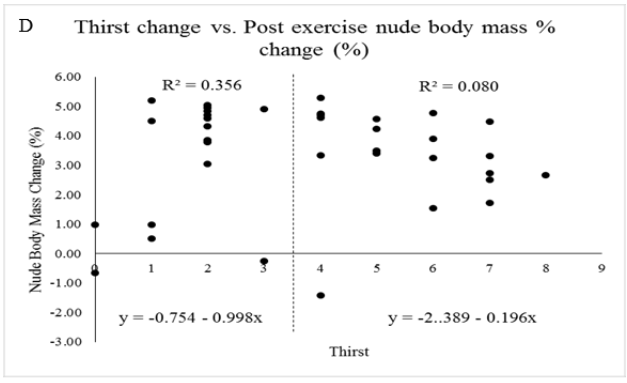


Thirst < 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	31.637	1	31.637	4.848	.041
Residual	117.454	18	6.525		
Total	149.092	19			

Thirst ≥ 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	49.565	1	49.565	2.776	.113
Residual	321.337	18	17.852		
Total	370.902	19			

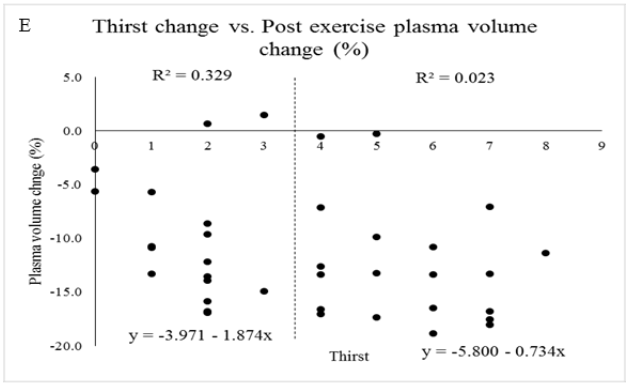


Thirst < 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	29.620	1	29.620	9.932	.006
Residual	53.681	18	2.982		
Total	83.301	19			

Thirst ≥ 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	1.261	1	1.261	1.559	.228
Residual	14.559	18	.809		
Total	15.820	19			



Thirst < 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	24.198	1	24.198	.850	.387
Residual	199.271	7	28.467		
Total	223.469	8			

Thirst ≥ 4

	Sum of Squares	df	Mean Square	F	Sig.
Regression	21.923	1	21.923	.676	.418
Residual	940.993	29	32.448		
Total	962.915	30			

Figure 2.4. Scatterplots of change in thirst and IPE hydration variables: A) nude body mass change (kg), B) plasma osmolality (mOsm/kg), C) plasma copeptin (ng/ml), D) nude body mass change (%), and E) plasma volume change (%). The dotted line represents the point of separation between regressions with thirst level less than 4, and thirst level 4 and greater. The slope equation and ANOVA table is presented for each regression.

Chapter 3

RESPONSE OF THIRST PERCEPTION TO DIFFERENT FLUID BOLUSES FOLLOWING EXERCISE

Response of Thirst Perception to Different Fluid Boluses Following Exercise

Abstract:

Thirst decreases in response to fluid consumption, however the impact of fluid volume on thirst is unknown. Purpose: To investigate the change in thirst perception impact of volume of fluid consumed following fluid intake after exercise. Methods: Eleven recreationally-active males (age: 22±3yr; height: 178±6cm; mass: 73.43±10.44kg; VO_{2max}: 54.3±5.4 $ml \cdot kg^{-1} \cdot min^{-1}$) completed four trials of ≤180 minutes of moderate-intensity exercise. For two trials, participants began euhydrated (EuR, EuD), and two were hypohydrated (HyR, HyD). During exercise fluid was replaced to match sweat rate (EuR only). Following exercise, a 60-minute recovery period commenced. In two trials (EuR, HyR), participants were split into groups which received fluid to match either Small (20%) or Large (80%) percent of body mass loss, but consumption was not required. Thirst perception was recorded every 10 minutes. Results: Participants began recovery with -0.2±0.9% (EuR) and 4.2±0.8% (HyR) body mass loss. Thirst remained low without change in the EuR trial. Thirst decreased following fluid consumption in both groups in the HyR trial, but was non-significant ($p=0.209$) and increased at 30 minutes in the Small group. Thirst in the Large group decreased over time ($p<0.001$), and remained low despite fluid consumption to replace only 2.66±0.69% of nude body mass ($p\leq0.001$), although they were given enough to replace 3.36±0.57%. Conclusions: Small fluid volumes will decrease thirst short-term, but a large volume will decrease thirst independent of amount of fluid loss replaced. Disparity between thirst perception and replaced fluid loss indicates potential for long-term hypohydration in the absence of a fluid replacement plan.

Key words: rehydration, involuntary dehydration, hypohydration, copeptin

Abstract Word Count: 250/250

INTRODUCTION

Rehydration following an exercise bout is a commonly debated issue in for physically active individuals. Incomplete rehydration following activity is impactful in two ways: leading to short-term hypohydration for the start of a subsequent bout of exercise, or leading to chronic, long term hypohydration. In a free living population, daily fluid consumption can have a large disparity between physiological need and desire to consume fluid. It has been reported that a majority of the population drinks less than 1 L/day,[1] leading to chronic hypohydration and elevation of arginine vasopressin (AVP) and copeptin, which has recently been linked to chronic disease progression in the long term (Guelinckx, in press), and performance and safety decrement in the short term.[2–7]

Several populations of physically active individuals are at risk of losing body mass due to thermoregulatory sweating during periods of activity. Heat-exposed workers reportedly consumed chilled and ambient temperature beverages at approximately the same rate *ad libitum* during exercise. However, these workers chose to only consume enough fluid to leave them at approximately 1% body mass loss throughout the day. This is corroborated by evidence of American football players and youth soccer players which described sustained and progressive fluid loss over days of exercise in American football players which was not addressed by *ad libitum* drinking.[8,9] This phenomenon has been termed involuntary dehydration.[10,11]

Thirst is one of the most basic bio-psychological factors, and one of few which begins as a perception which leads to behavior, such as drinking.[12] The sensation of thirst leads a human to be more cognizant of factors related to drinking fluids. But, it is not known if thirst has the ability to adapt to real-time change in hydration status by adjusting expectations and the seeking out of fluids for consumption.

Oropharyngeal and gastrointestinal factors have been investigated in the literature, and are found to be influencers of thirst perception among other factors.[13–17] Rehydration via indirect (intravenous) and direct (oral consumption) has shown to provide some impact on thirst in comparison to control, but direct consumption has a clear difference in satiety of thirst.[2] Additionally, mouth wetting without fluid consumption provides significant impetus to decrease thirst.[16,18,19] Studies of rehydration procedure following dehydrating exercise have determined that a combined approach of rapid rehydration and oral consumption best provided an expedient return of cardiovascular function following exercise.[20] It was also determined that *ad libitum* drinking did not provide adequate stimulus to completely replace body mass loss, leaving participants still greater than 2% loss after 60 minutes of unimpaired access to fluids.[20] However, short-term thirst reduction may not be enough to mask the deleterious effects of dehydration during exercise.[21]

While oral stimulation plays a role in immediate thirst reduction, change in plasma osmolality is necessary for sustained reduction in thirst perception.[13,18,19] This has a direct impact on AVP, and thus copeptin, production tied to osmotic change.[22,23] However, there is some delay where thirst is not perceived until a blood osmolality of approximately 294 mOsm/kg is reached, indicating that the relationship is not perfect, likely due to the heavy influence of external factors.[22,24]

Although investigation of thirst and fluid intake following exercise has been established, the adaptation of thirst perception to fluid volume is lacking in the current literature. Therefore, the purpose of this study was two-fold: to investigate the change in thirst perception following fluid intake after exercise, and to investigate a relationship between thirst perception and volume of fluid consumed. We hypothesized that thirst would have a reflexive decrease following fluid

consumption with a subsequent gradual increase over time, and volume of fluid consumed would have a large impact on thirst perception throughout the process.

METHODS

Participants

Eleven recreationally active males volunteered to participate in this study, which was approved by the Institutional Review Board at the University of Connecticut. Prior to obtaining written consent (Appendix D), participants were informed of possible risks associated with participation in the study. Participants were included based on the following inclusion criteria: aged 18-35; recreationally active defined as >30 minutes of exercise 4-5 days per week; VO_{2max} > 45 $ml \cdot kg^{-1} \cdot min^{-1}$. Participants were excluded based on the following exclusion criteria: fever or current illness at the time of testing; history of cardiovascular, metabolic, or respiratory disease; current musculoskeletal injury that limits their physical activity; exertional heat illness in the past 3 years. A medical history questionnaire was used to determine satisfaction of these criteria (Appendix E).

Experimental Design and Procedures

This lab study followed a randomized, counterbalanced, semi-cross over design, which took place in the thermal physiology laboratory in an environmental chamber (Model 200, Minus Eleven, Inc., Malden MA) set at an a warm environment (35°C ambient temperature and 30% relative humidity) in the Human Performance Laboratory (HPL) at the University of Connecticut. Data collection took approximately 3 months, from February to May, 2016. Each participant visited the laboratory a total of eight times for three types of laboratory testing sessions described below: one familiarization trial, three baseline body mass trials, and four

exercise trials. Each exercise trial was separated by at least five days to allow for recovery prior to the next testing session. The study timeline is depicted in Figure 3.1.

Familiarization Visit

Prior to completing the exercise trials, participants completed familiarization tasks to become familiar with study procedures and collect information necessary for exercise trials. Participants were instructed to arrive euhydrated to the laboratory. Upon arrival to the laboratory, participant's height, and body fat was measured. Body fat was measured with Lange skinfold calipers (Beta Technology, Santa Cruz, CA) using the 7-site method described by the American College of Sports Medicine.[25]

Participants performed a maximal oxygen uptake (VO_{2max}) test following a modified Bruce protocol on a treadmill in a thermoneutral environment to establish appropriate aerobic intensity during the exercise trials and assess satisfaction of inclusion criterion.[26] Velocity at VO_{2max} (vVO_{2max}) was used to determine walking and running intensities in the exercise trials.

Following completion of the VO_{2max} test and a brief rest period, sweat rate and electrolyte content was assessed to determine fluid replacement volumes during exercise and sweat constituents. The sweat rate assessment was performed in the environmental chamber at ~35°C, ~30% relative humidity. Prior to entering the environmental chamber, participants showered without soap and changed into a set of clothes which had been washed without detergent. Nude body mass was then measured (Defender 5000, Ohaus, Parsippany, NJ). Participants entered the environmental chamber and were familiarized with the perceptual indices used throughout the study (Thirst and Thirst Sensation Scale; Appendices A and B, respectively) as well as a diet log (Appendix C) during a 15-minute equilibration period. Perceptual indices are described in detail in the exercise trials section.

Participants then performed an exercise protocol which was the same as one cycle of exercise completed during an exercise trial to obtain an accurate sweat rate (8 minutes at 40% VO_{2max}, 8 minutes at 60% VO_{2max}, 8 minutes at 40% VO_{2max}, and 6 minutes of rest). The participants were instructed to wipe sweat with a towel washed without detergent to capture sweat and prevent dripping. After exercise, participants exited the environmental chamber and provided a final nude body mass to calculate sweat rate.

Baseline Body Mass

Following the familiarization visit, a 3-day baseline body mass was performed on three consecutive days to provide a measure of average body mass. Prior to each baseline body mass session, participants were instructed to eat and drink normally, hydrate well, avoid alcohol, and record everything consumed on a diet log. Diet logs were analyzed using a food processing program to determine kilocalories, macronutrients, sodium, potassium, and food moisture (Nutritionist Pro, version 5, Axxya Systems, LLC, Redmond, WA).

Participants were also instructed to collect all urine in a provided urine jug for 24 hours. On the night prior to each visit, participants collected a small urine sample in a clean urine cup prior to bedtime. In the morning of each day of the 3-day period, participants arrived at the laboratory in a fasted state, returned the urine jug, bedtime urine cup, and diet log from the previous 24 hours. Nude body mass was recorded. Urine from the bedtime cup was analyzed for urine specific gravity ($U_{sg;}$ TS400, Reichert Technologies, Buffalo, NY), osmolality (UOsm; Model 3320, Advanced Instruments freezing-point depression Osmometer, Norwood, MA), color[27], then added to the 24-hour collection jug for the same analyses as well as total volume.

Exercise Trials

Each exercise trial was scheduled with approximately seven days between exercise trials, and participants were asked to maintain normal diet and exercise routines for the duration of the study. Trial conditions were randomized and counter balanced between subjects. Recovery hydration group entry was randomized, but remained the same in both recovery trials. Abbreviations for trial conditions are included in Table 3.1, with the trial conditions, and will be used to refer to trials individually hereafter.

Exercise

During all exercise trials, participants wore undergarments, athletic shorts, shirt, and socks, and typical athletic shoes. Participants inserted a rectal probe to monitor body temperature (4600 series, Measurement Specialties, Hampton, VA) and a heart rate strap to monitor heart rate (digital 2.4 Timex heart rate monitor, Timex Group USA, Middlebury, CT) to determine safety cut offs, as described later, for the duration of the exercise trial. Participants entered the environmental chamber (35.2±0.6°C, 31.4±2.3% relative humidity) and sat for a 15-minute equilibration period. Following equilibration, baseline measures were recorded, then exercise commenced.

Exercise consisted of 6, 30-minute cycles (8 minutes at 40% VO_{2max}, 8 minutes at 60% VO_{2max} and 8 minutes at 40% VO_{2max} at 2% incline, and 6 minutes of rest.) For the EuR trial where participants minimized fluid loss, participants were given a volume of water to consume matching sweat rate every 30 minutes as assessed in the familiarization trial. For the trials where participants progressively dehydrated (EuD, HyD, HyR), fluid was restricted for the duration of exercise. Exercise was terminated at one of the following criteria: rectal temperature reaching 39.99°C, participant requested, altered or uneven gait, heart rate > age-predicted maximum heart

rate (220-age) for 5 minutes, 3 hours of exercise completed, or 5% body mass loss. At the termination of exercise, recovery commenced.

Recovery

During the one-hour recovery portion of the trial, participants rested passively in a chair for 60 minutes in the environmental chamber. For trials with recovery fluid replacement (EuR, HyR), participants were randomly allocated to fluid groups to receive either a Large (80% of body mass loss) or Small (20% of body mass loss) bolus of water based on the difference between average baseline and post-exercise body mass. Participants were encouraged to consume the bolus of fluid, but were not required to do so. For trials without fluid replacement, participants were not permitted to consume any fluids.

Hydration Assessment

For 24 hours prior to each exercise trial, participants collected urine and completed a 24-hour diet log. Urine analysis was performed with the same procedures as described in the baseline visit section. For hypohydrated trials (HyD and HyR), participants underwent 22 hours of fluid restriction to attain hypohydration, including instruction to avoid high fluid containing foods (i.e. soup, yogurt, large amounts of raw fruits or vegetables) as well as all beverages. For euhydrated trials (EuD and EuR), participants were reminded to consume fluids the day before, consume 500 mL the night before and 500 mL the morning of the study visit to ensure proper hydration. Participants were asked to avoid alcohol, have similar dietary intake between lab visits, with the exception of fluid and high fluid containing foods, and recorded intake on a diet log. Criterion definitions for euhydration and hypohydration can be found in Table 3.2. For euhydrated trials, if euhydration criteria were not met, participants were rescheduled and given

further instruction on appropriate fluid consumption to establish euhydration prior to the rescheduled trial. Body mass, urine analysis, and blood analysis were used to assess hydration throughout the study.

Body mass was assessed in two ways: minimally clothed and nude. Minimally clothed body mass was assessed every 30 minutes in the environmental chamber during breaks in exercise. Participants wiped away excess sweat and provided a body mass wearing only socks, shorts, and undergarments. Nude body mass was assessed before exercise, and immediately following the recovery period. To determine post-exercise nude body mass, minimally clothed body mass immediately following exercise was described in reference to urine excreted and mass of clothing. Change in nude body mass is described as the difference from average baseline nude body mass unless otherwise described.

Thirst Perception

Thirst perception was collected using the Thirst and Thirst Sensation Scale (TSS) indices. Thirst was collected before, and every 3 minutes during recovery (Appendix A). The thirst scale assessed perceived thirst on a one-point anchored likert-type scale from 1-9, where 1 indicated "not thirsty at all," and 9 indicated "very, very thirsty."[2,28,29] The TSS was collected before, and every 10 minutes during recovery (Appendix B). The TSS is modified from previous literature.[17,30] It is a series of six 100 mm visual analogue scales intended to assess thirst and related sensations along a continuum from not present to extremely present. Higher scores for each item indicated higher intensity of the sensation. Thirst sensation scale data is represented as a total score (TSS_{Total}) which is the sum of all six visual analogue scales. For purposes of direct comparison, Thirst scale data at minutes 0, 9, 21, 30, 39, 51, and 60 were used to compare to the 10-minute intervals of the TSS_{Total}.

Blood Analysis

Before exercise, as well as before and after the recovery period, blood was drawn from an antecubital vein using an aseptic technique by a researcher trained to perform blood draws. The 7-ml blood sample at both time points was used to determine hydration from plasma volume change and plasma osmolality (POsm), as well as copeptin. Blood for POsm and plasma volume change was analyzed immediately from a lithium heparin-treated tube, and blood drawn into a K_2 EDTA-treated tube was prepared for later analysis of copeptin. Both tubes were inverted 8-10 times immediately following the blood draw.

Hematocrit (Hct), hemoglobin (Hb), and POsm were assessed immediately. Hematocrit and Hb were used to assess change in plasma volume over time using the equation from Dill and Costill (1974). Hematocrit was analyzed from lithium heparin-treated whole blood using non-heparinized microcapillary tubes, and centrifuged at room temperature and 10,000 rpm for 5 minutes. Hemoglobin was assessed using the HemoCue Hb 201+ (HemoCue America, Brea, CA). The lithium heparin-treated blood collection tube was centrifuged at room temperature and 5,000 rpm for 15 minutes. Plasma osmolality was assessed using the freezing point depression method (model 3320, Advanced Instruments, Norwood, MA). Samples were analyzed in duplicate (or more in cases of >3 mOsm/kg difference in readings) and averaged to determine POsm.

Fifteen-hundred microliters of K_2 EDTA-treated whole blood and 78 µl of aprotinin (from bovine lung, approx. 10,000KIU/mL; Fisher Scientific, Fair Lawn, NJ, USA), at a concentration of 0.6TIU/ml of blood were added to a microcentrifuge tube, then gently rocked 4-5 times to ensure appropriate mixing. Blood was then centrifuged at 4°C and 4,000 rpm for 15

minutes. Plasma was then aliquoted into microcentrifuge tubes and stored at -80°C for up to 3 months for later analysis.

Frozen plasma samples containing K_2 EDTA and aprotinin were transferred to the diagnostic laboratory. For measurement of copeptin, plasma was used in an extraction-free competitive enzyme immunoassay (EK-065-32, Phoenix Peptides, Burlingame, CA). All samples were analyzed in duplicate. Description of assay procedures and standard curve is found in Appendix F. Absorbance optical density was read at 450nm on a microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA) using SoftMax Pro software (version 5.3, Molecular Devices, Sunnyvale, CA). The curve was used to interpolate concentration from absorbance (GraphPad, Prism, version 7 for Windows, 2016). Intra-assay CV% was calculated to be less than 8.7%, and the inter-assay CV% was calculated as 7.9%.

Statistical Analysis

A three-way (group x trial x time) mixed (between-within-within) analysis of variance (ANOVA) was conducted to compare differences of time and trial by rehydration group on Thirst perception and hydration variables. Analysis of studentized residuals determined normality when no residual was greater than ± 3 standard deviations. Sphericity was assessed by Mauchly's test of sphericity ($p>0.05$). If sphericity was violated, Greenhouse-Geisser correction was applied. When a significant interaction was determined, analysis of simple two-way interactions of trial on each level of time for each group was determined. When simple main effects of the two-way interaction were present, simple simple main effects with Bonferroni adjustments were interpreted to determine significant simple simple comparisons.

A two-way (group x trial) mixed ANOVA was conducted to compare difference of trial by group on Thirst perception and hydration variables. Analysis of studentized residuals

determined normality when no residual was greater than ± 3 standard deviations. Mauchley's test was used to determine if the assumption of sphericity was met. A Greenhouse-Geisser correction was applied when sphericity was violated ($p>0.05$). When a significant two-way interaction was determined, a one-way ANOVA was used to determine simple main effects.

One-way ANOVA of group in the EuR and HyR trials was used for fluid replacement variables to determine if differences by group existed. For three-way mixed ANOVA analyses, a Bonferroni correction was applied to the alpha level adjusted for the number of independent analyses run in the simple two-way ANOVA. Mauchley's test was used to determine if the assumption of sphericity was met. A Greenhouse-Geisser correction was applied when sphericity was violated ($p>0.05$).

Spearman rank-order correlations were used to analyze the relationship of ordinal thirst variables (Thirst and TSS_{Total}) to hydration and fluid variables during the recovery period. For all analyses, *a priori* alpha level was set at $p\leq0.05$. Data are presented as Mean±SD, unless otherwise stated.

RESULTS

Participant Characteristics

Characteristics for participants overall and in each fluid group are presented in Table 3.3. Analysis of participant characteristics revealed no statistically significant differences between fluid replacement groups on any characteristic except VO2max ($p=0.021$). Participants started the recovery period in similar hydration state within each trial, regardless of hydration group (Table 3.4).

Nude Body Mass

Two way mixed ANOVA revealed no significant interaction of group and trial ($F_{(3, 27)}$=1.379, p=0.270) for total nude body mass loss from average baseline to post-recovery. There was a significant main effect for total nude body mass loss, defined the percent difference between post-recovery nude body mass and baseline average body mass, by trial ($F_{(3, 27)}$=63.024, p<0.001), where total nude body mass loss in the EuR trial was significantly less than all other trials (-0.11±0.84 kg, p<0.005), and total nude body mass loss in the HyD trial (-3.81±0.66 kg) was significantly greater than all other trials (p<0.001). There was not a significant main effect for total nude body mass loss by recovery group.

Two-way mixed ANOVA revealed no significant interaction of group and trial ($F_{(1.819, 16.37)}$=0.833, p=0.442) for total nude body mass percent loss from average baseline to post-recovery. There was a significant main effect for total nude body mass percent loss by trial ($F_{(1.819, 16.370)}$=64.388, p<0.001) where total nude body mass loss in the EuR trial was significantly less than all other trials (-0.16±1.18%, p<0.001), and total nude body mass loss in the HyD trial was significantly greater than all other trials (-5.48±0.81%, p<0.009). There was also a significant main effect for total nude body mass loss by fluid group ($F_{(1, 9)}$=5.855, p=0.039) where the Small fluid group had greater total nude body mass loss percent than the Large fluid group (p<0.039).

Fluid Consumed

Participants were given fluid to consume during the recovery period starting after the Pre time point of trials EuR and HyR. Amount of fluid consumed in the EuR trial (0.05±0.08 L) was less than in the HyR trial (1.49±1.12 L), independent of fluid group, and more fluid was

consumed in the Large fluid group independent of trial (p=0.001). Participants sometimes chose not to consume all fluid given after exercise, and thus the percent of fluid given that participants consumed was analyzed via two-way ANOVA group by trial (EuR and HyR only). There was not a statistically significant difference in group by trial analysis of percentage of fluid consumed ($F_{(1, 9)}$=4.486, p=0.063), however, some difference did exist where incomplete fluid replacement only occurred in the HyR trial (Small: 99.6±0.9%; Large 85.1±15.1%).

Fluid consumed as a percentage of nude body mass loss are presented in Figure 3.2. In the EuR trial, several participants had not lost body mass as a result of appropriate fluid replacement, and thus were not given fluid to consume in the recovery period. All participants consumed 100% of the fluid given in the EuR trial, regardless of fluid group. One-way ANOVAs of differences between groups for amount of fluid given, consumed, and percentages of each, did not reveal a significant interaction in the EuR trial (p>0.457). One-way ANOVA of fluid given in the HyR trial revealed that the Large fluid group was given significantly more fluid than the Small fluid group (p<0.001). Additionally, the Large fluid group consumed significantly more fluid than the small fluid group (p=0.001). In the HyR trial, the Small fluid group was given enough fluid to replace up to 0.77±0.19% of nude body mass, and 99.6±0.9% of that fluid was consumed, which was significantly less than the HyR trial (p≤0.001). However, in the HyR trial the Large fluid group was given enough fluid to replace up to 3.36±0.57% of nude body mass, and only replaced fluid corresponding to 2.66±0.69% of nude body mass.

Plasma Osmolality and Volume Change

Three-way mixed ANOVA (2x4x2) revealed a significant of trial by time by group ($F_{(3, 27)}$=8.120, p=0.001) for POsm from pre to post recovery. There was a statistically significant simple two-way interaction of trial by time for POsm in the Large fluid group ($F_{(2.586,}$

$_{12.929)}$=11.842, p=0.001), but not the Small fluid group ($F_{(2.508, 10.034)}$=1.187, p=0.356). Analysis of POsm at the pre-recovery time point in the Large fluid group revealed significant simple simple main effects ($F_{(3, 15)}$=30.334, p<0.001). At the pre-recovery time point, POsm in the EuR trials was significantly less than all others (p<0.015). Additionally, POSm in the EuD trial was significantly less than in the HyR (p=0.044), but not HyD (p=0.087) trials. Analysis of the post-recovery time point revealed statistically significant simple simple main effects ($F_{(3, 15)}$=17.253, p<0.001). At the post-recovery time point, POsm in the EuR trial was significantly less than in the EuD (p=0.007) and HyD (p=0.004) trials, but not HyR (p=0.140) trial. POsm at the post-recovery time point in the EuD, HyD, and HyR trials were not different (p<0.068), and the HyR trial was not different from any other trial (p<0.068).

Two-way mixed ANOVA revealed a statistically significant interaction of group and trial on POsm change from pre to post-recovery ($F_{(3, 27)}$=8.098, p=0.001). In the HyR trial, change in POsm was significantly greater in the Large (-13±4 mOsm/kg) than in the Small fluid group (0±6 mOsm/kg, p=0.002), but not in any other trial. Two-way mixed ANOVA revealed a statistically significant interaction of group and trial on POsm percent change from pre to post-recovery ($F_{(3, 27)}$=7.683, p=0.001). In the HyR trial, percent change in POsm was significantly greater in the Large (-4.0±1.1%) fluid group than in the Small fluid group (-0.02±1.9%, p=0.002), but not in any other trial.

Two-way mixed ANOVA revealed no significant interaction of group and trial on plasma volume change from pre to post-recovery ($F_{(3, 27)}$=0.406, p=0.750). There was no significant main effect of trial (p=0.705), or main effect of rehydration group (p=0.451).

Copeptin

Three-way mixed ANOVA of copeptin revealed a significant interaction of group and trial ($F_{(1.413, 12.719)}$=8.752, p=0.007). There was not a significant simple two-way interaction of time by trial in the Large fluid group ($F_{(3, 15)}$=0.840, p=0.493), but there was a significant interaction in the Small fluid group ($F_{(1.148, 4.592)}$=9.292, p=0.030). Analysis of copeptin in the Small fluid group at the pre-recovery time point did not reveal significant simple simple main effects ($F_{(1.124, 4.495)}$=3.965, p=0.109). Analysis of copeptin in the Small fluid group at the post-recovery time point revealed statistically significant simple simple main effects ($F_{(3, 12)}$=5.976, p=0.010), however no statistically significant simple simple comparisons existed. Hydration variables are described by trial in Table 3.5.

Copeptin was correlated with POsm at the pre-recovery time point (ρ=0.373, p=0.013), as well as the post-recovery time point (ρ=0.408, p=0.006). However, copeptin was not correlated with the plasma volume percent change during exercise (ρ=0.155, p=0.314), nor with the plasma volume percent change during the recovery period (ρ=0.041, p=0.789).

Thirst Perception

Thirst and TSS data at 10-minute intervals are presented in Figure 3.3. Three-way mixed ANOVA (2x4x7) revealed a significant interaction of trial by time by group ($F_{(3.457, 24.200)}$=8.478, p<0.001) for Thirst. There was a statistically significant simple two-way interaction between trial and time for Thirst in the Large replacement group ($F_{(3.282, 13.127)}$=35.351, p<0.001), but not the Small replacement group ($F_{(2.230, 6.689)}$=1.993, p=0.209). Analysis of Thirst on trials at the pre-recovery time point (0 minutes) in the Large group revealed a significant simple simple main effect ($F_{(3, 15)}$=36.023, p<0.001). Thirst at 0 minutes in the EuR was significantly lower than all other trials (3±2, p<0.012), and EuD (8±1), HyD (8±1), and HyR were not different (9±1,

p>0.555). Thirst data was recorded every 3 minutes (Figure 3.4) during the recovery period, in order to potentially characterize a decrease and re-establishment of thirst perception following fluid ingestion.

Analysis of trials at the 10 minute time point in the Large group revealed a significant simple simple main effect ($F_{(3, 15)}$=23.231, p<0.001). Thirst at 10 minutes in the EuR (3±2) and HyR (5±2) trials were not different (p=1.000), nor was thirst at 10 minutes in the EuD (8±1) and HyD (9±1, p=1.000), however EuR and HyR were significantly lower than EuD and HyD (p<0.016). Analysis for simple simple main effects for the Large replacement group were performed for 20, 30, 40, 50, and 60 minutes and all remained significant (p<0.05). Additionally, pairwise analysis continued the same significant and non-significant relationships without exception at all time points.

Data for TSS_{Total} are represented in Figure 3.3. Three-way mixed ANOVA (2x4x7) revealed a significant interaction of trial by time by group ($F_{(3.426, 23.984)}$=3.263, p=0.34) for TSS_{Total}. There was a statistically significant simple two-way interaction between trial and time for TSS_{Total} in the Large replacement group ($F_{(1.236, 4.945)}$=23.315, p=0.004), but not the Small replacement group ($F_{(2.648, 7.945)}$=1.867, p=0.215). Analysis of TSS_{Total} at the pre-recovery time point in the Large group revealed a significant simple simple main effect ($F_{(3, 15)}$=132.431, p<0.001). At 0 minutes, TSS_{Total} in the EuR was significantly lower than all other trials (128±38, p<0.001); and EuD (393±12), HyD (425±34), and HyR were not different (409±26, p>0.262).

Analysis of TSS_{Total} at the 10-minute time point in the Large group revealed a significant simple simple main effect ($F_{(1.260, 6.298)}$=30.743, p=0.001). At the 10-minute time point, TSS_{Total} in the EuR trial (127±42) was less than EuD (393±14, p<0.001) and HyD (431±38, p=0.001). However, TSS_{Total} in the HyR trial was not different from any other trial (300±126, p>0.064).

Analysis of TSS_{Total} at the 20-minute time point in the Large group revealed a significant simple simple main effect ($F_{(1.320, 6.599)}$=18.851, p=0.003). At the 20-minute time point, TSS_{Total} in the EuR trial (139±66) was less than EuD (391±11, p=0.002) and HyD (438±48, p=0.005) trials; and TSS_{Total} in the HyD was greater than HyR (270±144, p=0.048).

Analysis of 30 minute time point in the Large group revealed a significant simple simple main effect ($F_{(1.145, 4.581)}$=31.089, p=0.003). At the 30 minute time point, TSS_{Total} in the EuD trial (387±15) and HyD (437±49) were not different (p=1.000), nor were EuR (124±52) and HyR (202±126) different (p=1.000), however TSS_{Total} in the EuD and HyD trials was significantly higher than EuR and HyR (p<0.045) in the Large group. Analysis for simple simple main effects for the Large replacement group were performed for 40, 50, and 60 minutes and all remained significant (p<0.05). Additionally, pairwise analysis continued the same significant and non-significant relationships as at the 30 minute time point without exception.

Thirst Perception and Hydration

Spearman correlations of thirst perception and hydration variables are presented in Table 3.6. At the pre-recovery time point, thirst perception was significantly correlated with nude body mass change and plasma osmolality (p<0.01), but not to plasma volume change during exercise. Thirst perception was also correlated with copeptin concentration (p<0.05). At the post-recovery time point, thirst perception was significantly correlated with nude body mass change and plasma osmolality (p<0.01), but not with change in plasma osmolality or volume. Post-recovery copeptin was correlated with thirst perception (p<0.05). Additionally, the volume of fluid consumed during the recovery period was significantly correlated with thirst perception (p<0.05). Relationship of thirst perception to overall plasma volume change nude body mass change (%), and post-recovery plasma osmolality and copeptin are presented in Figure 3.5.

DISCUSSION

The purpose of this study was to investigate the change in thirst perception following fluid intake after exercise, and to investigate a relationship between thirst perception and volume of fluid consumed. The main findings of this study were that hydration status changed over the course of recovery in the two trials where fluid was consumed. Thirst perception was reduced or eliminated in the group which consumed the large bolus of fluid, but remained elevated in the group which consumed the small bolus of fluid. Also, although thirst perception was decreased, participants in the group which was given the large bolus of fluid did not consume the entire bolus, and replaced less fluid than would be required to return to a euhydrated state.

To our knowledge, no study has assessed the impact of a prescribed fluid volume on thirst perception following a dehydrating bout of exercise. It is commonly accepted that consumption of fluid decreases thirst perception via a number of physiological and perceptual channels. Fluid volume touches on several of these specifically, where gastrointestinal and oropharyngeal sensory pathways are likely stimulated, as well as mouth feel and taste impacted. Classic research by Figaro and Mack (1997) suggested that the oropharyngeal region monitors fluid intake rate and volume, thus reducing thirst as fluid is consumed to innately prevent over-consumption. Fluid was either infused directly to the stomach, bypassing the mouth and esophagus, or consumed ad libitum. Thirst was not sated when fluid was directly infused, suggesting some role of mouth or esophagus sensory stimulation independent of changes in plasma osmolality.[13] The present study adds to this concept, as participants chose not to consume enough fluid to completely rehydrate, but saw a reflexive decrease in thirst perception over time with oral fluid consumption. Although there was a resultant decrease in plasma

osmolality which positively correlated with thirst perception, plasma volume changed during this fluid consumption period, but was not correlated with thirst perception.

In the present study, consumption of a small amount of fluid did not result in a significant reduction in thirst. This would seem to refute the theory of mouth feel or taste as a large impacting factor of thirst perception during rest. A randomized control trial of thirst perception with or without a series of mouth wetting but not fluid consumption techniques described that thirst could be sated without fluid consumption for some period of time less than 30 minutes.[18] This is additive to the slight dip in thirst that was perceived in the present study, where following even a small amount of fluid consumption, thirst perception initially decreased, and subsequently began a steady increase within 30 minutes. This would appear to implicate some level of oropharyngeal stimulation by fluid consumption as responsible for decreased thirst perception without plasma osmolality change.

Consumption of a large amount of fluid resulted in a large decrease in thirst in the present study. However, not all participants consumed all the fluid they were given, which in its entirety was not sufficient to completely replace body mass loss. This has the potential to lead to incomplete replacement of fluid prior to a subsequent bout of exercise. This has also been termed involuntary dehydration,[10,11] and is seen in both laboratory and field research settings. Classic research described that following intense aerobic exercise leading to graded hypohydration, *ad libitum* fluid replacement was incomplete.[11] However, the fluid given for replacement in this study was a carbohydrate-electrolyte solution, which may not have been preferred to water by all participants. We see in other literature that temperature[31] and fluid composition preference[32] may influence fluid intake.

More recent research describing rehydration modes as being important for fluid replacement following exercise found that *ad libitum* drinking of a flavored, non-caloric beverage did not provide adequate replacement of body mass lost during exercise. Similarly with the present study, a remaining deficit following an hour of recovery and fluid access occurred. Additionally, thirst perception was decreased to a level similar to intravenous and prescribed oral rehydration methods,[20] further supporting the results of the present study where thirst was decreased regardless of the amount of exercise-induced fluid loss. Additionally, thirst during a subsequent bout of exercise was decreased by oral fluid replacement, but not to as large a degree with intravenous fluid replacement in the first 15 minutes of exercise.[2] However, immediately following exercise, intravenous fluid replacement provided no difference in ratings of thirst from a control, no fluid consumption, condition.[2]

Plasma osmolality in the present study was reduced over the 60-minute recovery period in the present study. In previous research, a strong link of thirst related to plasma osmolality change has been established.[22,24] In the present study, thirst was related to plasma osmolality over the course of an hour. However, previous literature of high intensity intermittent exercise describes thirst unrelated to blood osmolality, where reduced osmolality due to cessation of exercise did not serve to reduce sensation of thirst when fluid was withheld for a short period of time.[15] Additionally, thirst was decreased in response to fluid intake, independent of the timing of fluid intake,[15] which is supportive of the responses in the present study.

In the present study, copeptin was related to plasma osmolality both before and after the recovery period. Production of copeptin in the present study has a relationship to thirst following exercise. However, emerging evidence suggests that copeptin may be a better predictor of long-term, sustained fluid loss and not short term fluid loss.[33] In the present study, this evidence may

be additive, but not supportive, of this concept where relatively short term dehydration (3 hours in the EuD trial) was included in the positive relationship of thirst perception to copeptin the same as in the longer term hypohydration (22-hours with 3 hours of exercise in the HyD and HyR trials).

With reported sweat rates of greater than 2 L/hr,[34,35] complete replacement during exercise may be uncomfortable due to large gastrointestinal load coupled with exercise. Thus, the post-exercise recovery period is an optimal time to rehydrate prior to a subsequent exercise bout. The present study found that fluid was not entirely replaced during the first hour, likely due to decreased thirst perception. If inhibited thirst perception was sustained, this could lead to lack of complete rehydration prior to a subsequent exercise bout. This is a problem from both performance and safety perspectives.[36,37,2,5,4,38]

Therefore, this reduced perception of thirst in the absence of complete rehydration is a realistic problem. There is the potential for an athlete who loses a significant amount of body mass and has only a short period of time before the next exercise bout, to not replace fluid loss. This is common in tournament play situations, where back to back games are not uncommon, as well as preseason practices which include double sessions. In the present study, we saw that after 60 minutes of access to fluids, the large fluid consumption group did not consume all fluids given, leaving a greater than 2% deficit in nude body mass. We may be able to attribute this lack of complete fluid consumption to an immediate reduction in thirst perception, and maintenance thereof. Similarly, a small fluid bolus had little impact on thirst for a short period of time, leaving participants hypohydrated and still perceiving thirst. Further research of timed fluid boluses over a series of short periods could lead to more fluid replacement, as thirst may be reestablished enough to elicit further drinking.

CONCLUSIONS

Thirst decreases in response to fluid ingestion following exercise. However, thirst perception appears to decrease more readily than body mass loss is replaced, such that incomplete replacement occurs. This may predispose physically active individuals to risk of chronic dehydration if fluid replacement beyond thirst after exercise does not occur. Further research is needed on timing of fluid bolus to determine if an optimal fluid consumption strategy can be devised which is dictated by thirst, but replaces a larger amount of fluid loss.

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TABLES AND FIGURES

Table 3.1. Trial hydration conditions. Fluid group: high, replace 80% loss; low, replace 20% loss.

Trial	Pre-Exercise	During Exercise	Recovery	Fluid Group
EuR	Euhydrated →	Fluid intake = sweat rate →	Replace fluid loss	Large n=6 / Small n=5
EuD	Euhydrated →	⊘fluid intake →	No fluid replacement	
HyR	Hypohydrated →	⊘fluid intake →	Replace fluid loss	Large n=6 / Small n=5
HyD	Hypohydrated →	⊘fluid intake →	No fluid replacement	

Table 3.2. Operational definition of pre-trial hydration states.

	Euhydration	Hypohydration
24-hr Usg	≤1.020	≥ 1.021
24-hr UOsm	≤ 500 mOsm/kg	> 500 mOsm/kg
Fluid consumption	Consumed 1 L overnight	Restricted for 22 hours
% nude body mass loss	≤ 1%	~ 1%

Note: % nude body mass loss is defined as difference from baseline average nude body mass. UOsm-urine osmolality

Table 3.3. Participant characteristics.

		Fluid Group	
	Overall	Large	Small
Age (yr)	22±3 [20, 24]	23±3 [20, 27]	20±2 [18, 23]
Height (cm)	178±6 [174, 183]	182±7 [174, 189]	174±2 [172, 177]
Nude body mass (kg)	73.43±10.44 [66.42, 80.45]	78.82±10.40 [67.91, 89.73]	66.97±6.47 [58.94, 75.00]
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	54.3±5.4 [50.6, 58.0]	51.1±4.8 [46.1, 56.1]	58.2±3.3* [54.0, 62.3]
Sweat rate (L/hr)	0.89±0.91 [0.28, 1.50]	1.27±0.37 [0.89, 1.66]	0.42±1.19 [1.05, 1.90]
Body fat (%)	11.6±3.9 [8.9, 14.1]	12.2±4.7 [7.2, 17.1]	10.9±3.1 [7.0, 14.7]

Note: Data are presented as Mean ± SD [95% CI]. Large: replace 80% of loss; Small: replace 20% of loss.
*Significantly greater than the Large group ($p<0.05$).

Table 3.4. Hydration state at the beginning of the recovery period.

	POsm (mOsm/kg)		Nude body mass loss (kg)		Nude body mass loss (%)		Plasma volume change (%)		Copeptin (ng/ml)	
	Small	Large	Small	Large	Small	Large	Small	Large	Small	Large
EuD	307±11	306±4	-2.4±0.6	-2.2±1.1	3.6±0.7	2.7±1.0	-14.5±5.8	-14.1±2.7	7.6±5.3	7.4±6.6
EuR	286±6	289±5	0.2±0.6	0.8±0.9	-0.2±0.9	-1.0±1.2	-7.5±4.4	-5.9±5.7	7.3±8.1	6.9±7.4
HyD	313±7	316±9	-3.2±0.5	-3.6±0.7	4.8±0.6	4.5±0.4	-12.7±4.8	-12.6±6.4	12.4±9.4	6.0±1.7
HyR	315±10	315±7	-2.8±0.5	-3.5±0.6	4.2±0.8	4.5±0.6	-14.2±3.5	-9.7±8.0	5.2±3.1	5.6±4.2

Note: Data are presented as Mean±SD. Nude body mass loss represents loss during exercise from average baseline body mass. Plasma volume change represents plasma volume change during exercise. Plasma osmolality (POsm) and copeptin represent pre-recovery time point. No significant differences between groups for any variable.

Table 3.5. Measures of hydration during and following the recovery period.

	Plasma volume change (%)		Plasma osmolality		Plasma osmolality change (mOsm/kg)		Plasma osmolality change (%)		Nude body mass change (kg)		Nude body mass change (%)		Copeptin (ng/ml)	
	Small	Large	Small	Large	Small	Large	Small	Large	Small	Large	Small	Large	Small	Large
EuD	-11.1±5.6	-10.8±4.1	303±8	307±6	8±3	13±6	2.7±1.1	4.5±2.0	-2.67±0.98	-2.72±1.16	-3.9±1.3	-3.4±1.1	7.3±4.8	6.1±5.4
EuR	-4.0±4.2	-5.2±3.6	286±6	292±2	-5±7	-3±6	-1.8±2.3	-1.0±1.9	-0.31±0.54	0.05±1.05	-0.5±0.9	0.1±1.4	6.9±7.4	2.4±1.2
HyD	-10.8±3.8	-10.3±5.7	310±8	312±6	10±2	7±6	3.3±0.7	2.3±2.0	-3.65±0.53	-3.94±0.77	-5.5±0.8	-5.0±0.5	11.59.8	6.2±4.2
HyR	-10.6±3.8	-7.4±5.9	315±7	302±7	12±9	-1±4	3.8±2.8	-0.4±1.3	-2.47±0.31	-1.72±0.46	-3.7±0.6	-2.2±0.5	16.3±10.3	5.6±2.6

Note: Data are presented as Mean±SD. Change in all variables represents change from pre-exercise to post-recovery. Plasma osmolality (POsm) and copeptin represent the post-recovery time point.

Table 3.6 Spearman correlations of Thirst with hydration variables a) pre-recovery and b) post-recovery time points.

a)	Nude body mass change (kg)	Nude body mass change (%)	Plasma osmolality (mOsm/kg)	Plasma osmolality change (mOsm/kg)	Plasma osmolality change (%)	Plasma volume change (%)	Copeptin (ng/ml)
Thirst	-0.569**	-0.582**	0.569**	0.434**	0.416**	-0.276	0.306*
TSS_{Total}	-0.549**	-0.608**	0.670**	0.447**	0.424**	-0.236	0.469**

b)	Nude body mass change (kg)	Nude body mass change (%)	Plasma osmolality (mOsm/kg)	Plasma osmolality change (mOsm/kg)	Plasma osmolality change (%)	Plasma volume change (%)	Copeptin (ng/ml)	Fluid consumed (L)	Fluid consumed (%)
Thirst	-0.690**	-0.687**	0.622*	0.089	0.107	-0.367*	0.313*	-0.431**	-0.431**
TSS_{Total}	-0.641**	-0.690**	0.674**	0.160	0.173	-0.275	0.437**	-0.344*	-0.340*

Note: * denotes significance at the $p<0.05$ level; ** denotes significance at the $p<0.01$ level. Fluid consumed (%) represents the amount of fluid consumed as a percentage of nude body mass. a) nude body mass change represents change during exercise from baseline average nude body mass. Plasma osmolality and volume change represent change during exercise. Plasma osmolality and copeptin represent the pre-recovery time point. b) nude body mass change represents change of post-recovery from baseline average nude body mass. Plasma osmolality and volume change represent overall change from the pre-exercise time point to the post-recovery time point. Plasma osmolality and copeptin represent measures at the post-recovery time point.

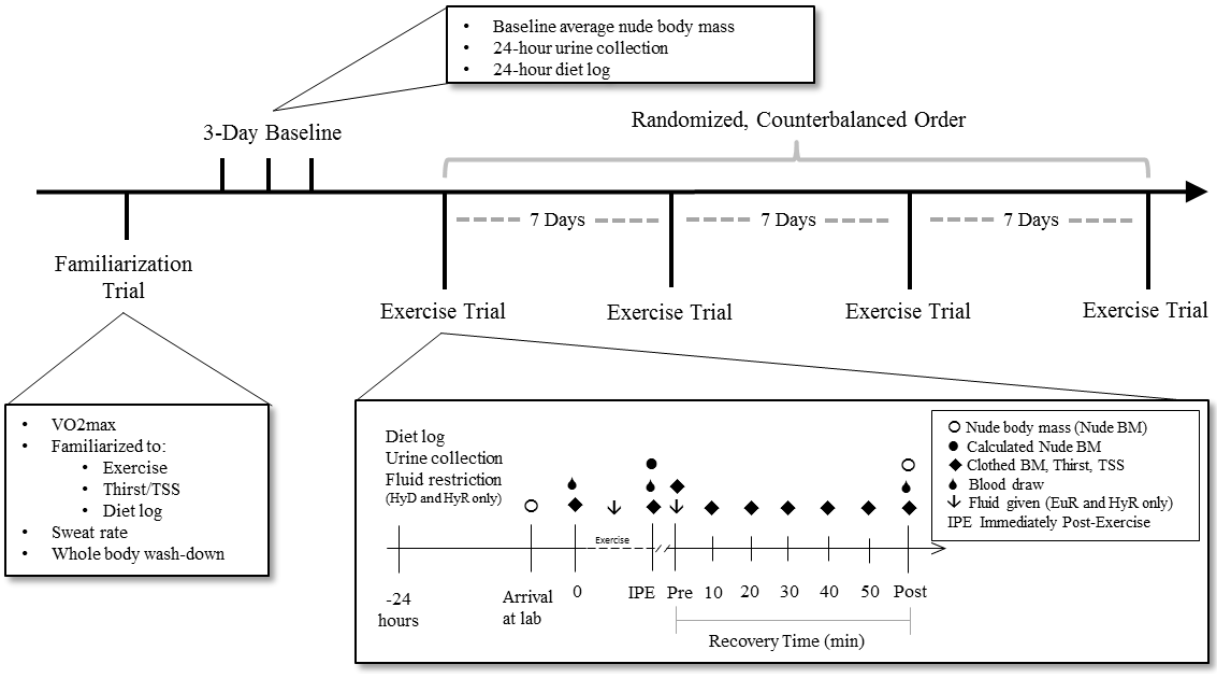


Figure 3.1. Timeline of laboratory testing, and basic description of study procedures for each type of visit. The familiarization trial was separated from the three-day baseline visits by up to two weeks. The three-day baseline visits occurred on three consecutive days prior to the first exercise trial. Each exercise trial was separated by approximately 7 days (no less than 5 days), and were performed in a randomized, counterbalanced order by trial type (Table 3.1.)

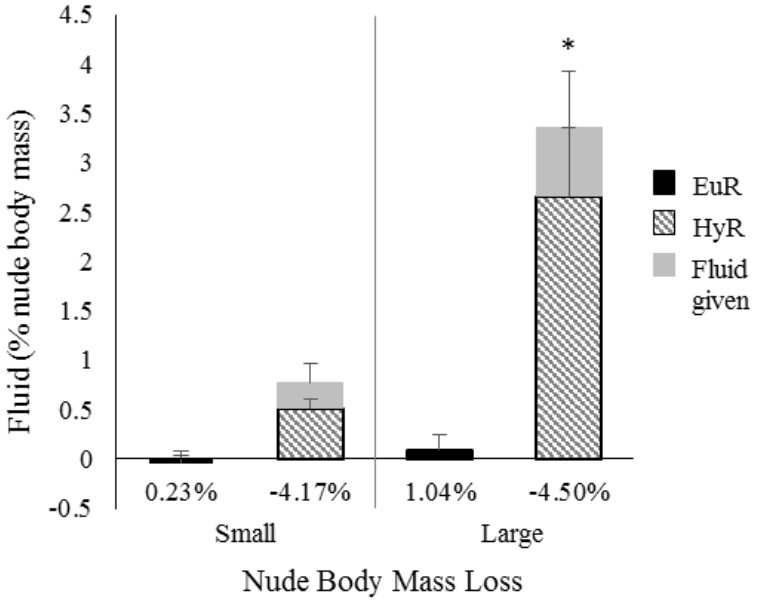


Figure 3.2. Fluid consumed as a percentage of nude body mass loss. Solid and striped bars represent fluid consumed during recovery in the EuR and HyR trials. Grey bars represent the additional fluid given, but not consumed. *denotes significant difference from Small group.

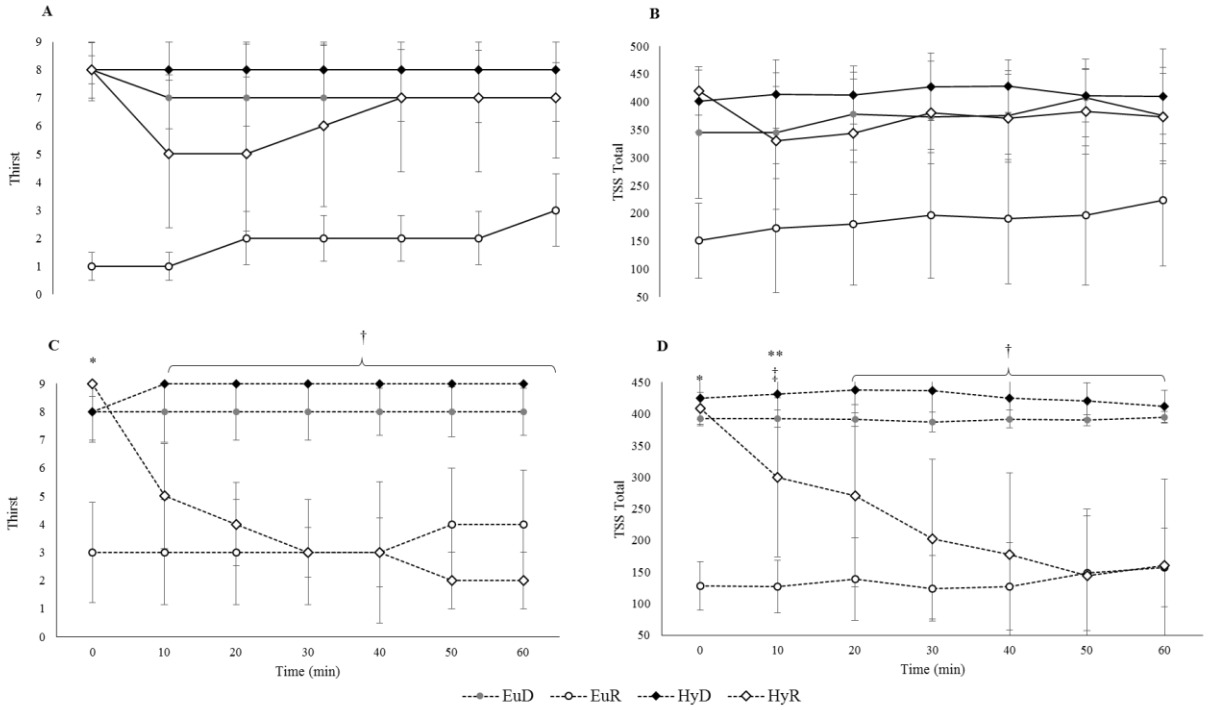


Figure 3.3. Thirst and TSS_{Total} during recovery. Solid lines (graphs A and B) indicate the Small rehydration group, dashed lines (graphs C and D) indicate the Large rehydration group. *EuR significantly different than all other trials ($p<0.05$), † EuR and HyR different than EuD and HyD ($p<0.05$), ‡ EuR significantly different than EuD and HyD ($p<0.05$), **HyD significantly different than HyR ($p<0.05$). Note: $TSS_{Total,}$ Thirst Sensation Scale total score.

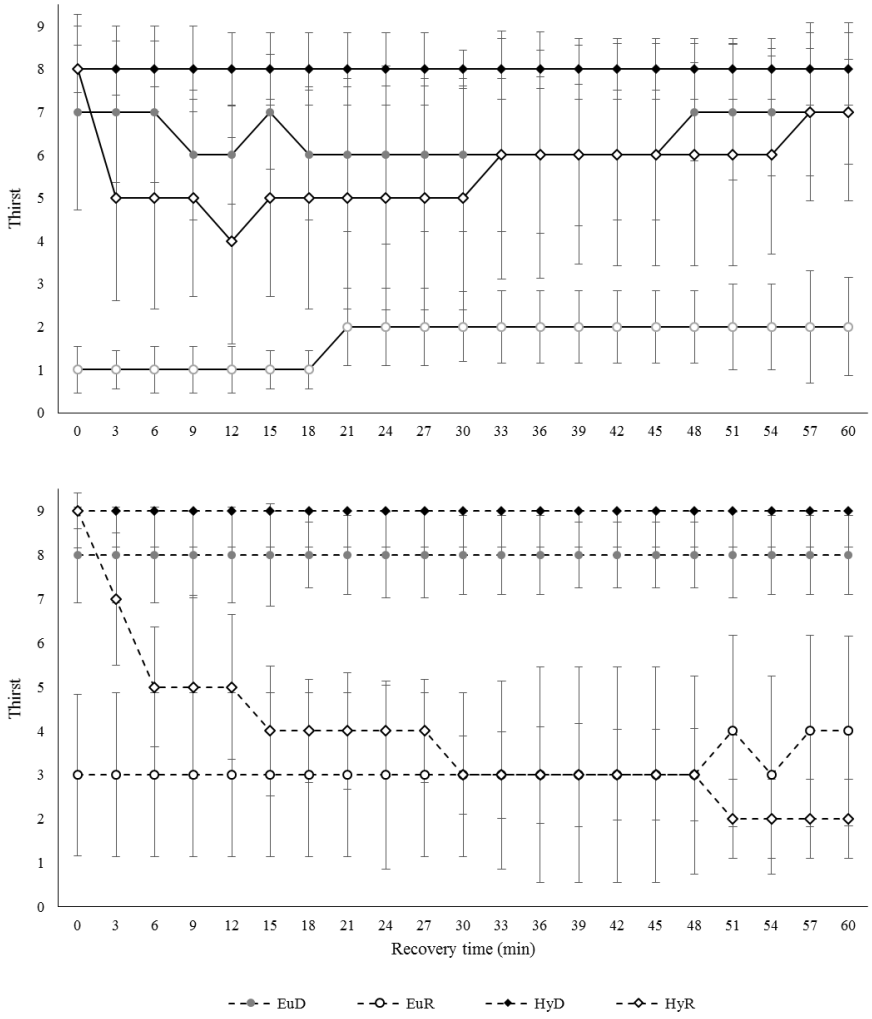


Figure 3.4. Thirst recorded at short intervals during seated recovery. Data are presented as Mean±SD. Solid lines represent thirst ratings from the Small fluid group; dashed lines represent thirst ratings from the Large fluid group. Thirst was recorded every 3 minutes. In the EuR and HyR trials, a fluid bolus (according to fluid group) was given at the 0-minute time point, and participants were allowed to consume it at a self-dictated rate, and volume.

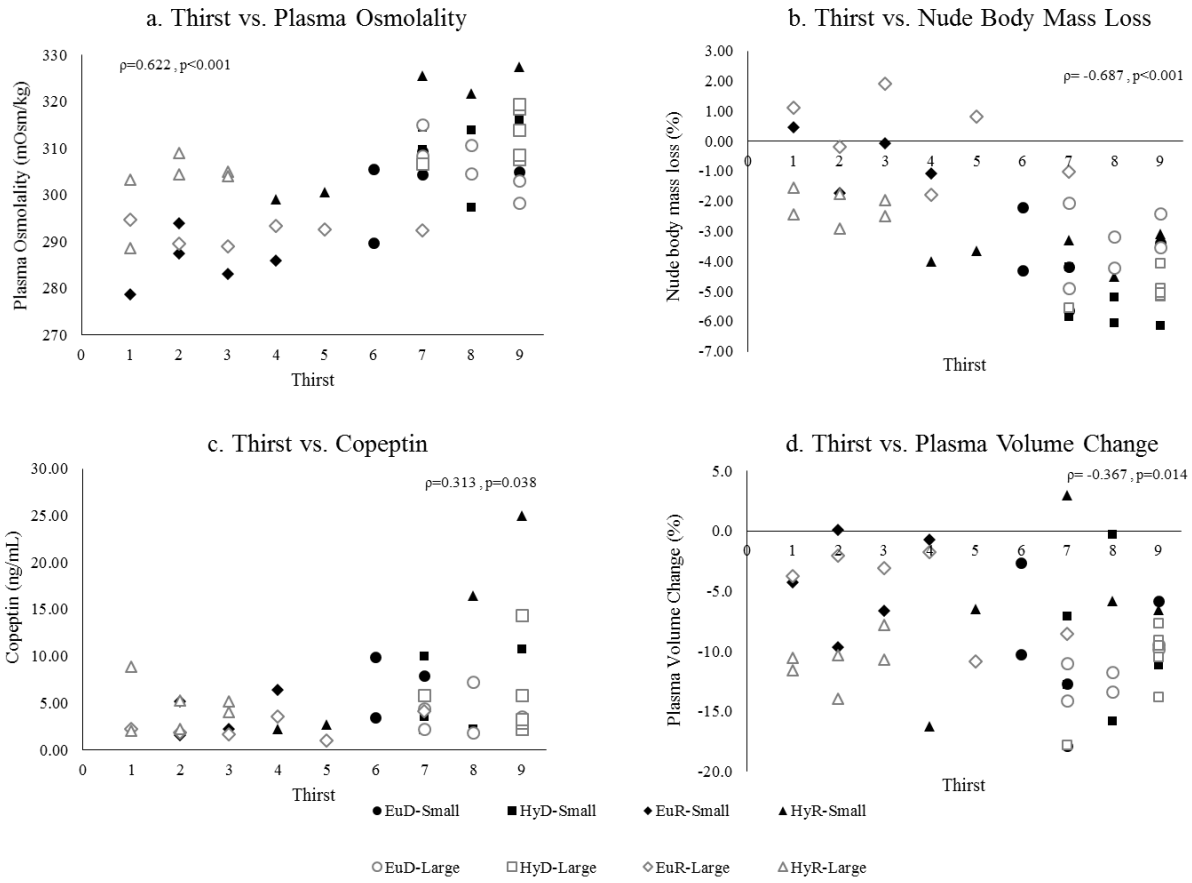


Figure 3.5. Scatter plots of thirst on hydration measures. Thirst represented in each graph is immediately post-exercise (IPE). Correlation statistic derived from Spearman's rho (ρ). a. plot of post-exercise plasma osmolality on IPE thirst, significantly correlated ($p<0.001$); b. plot of percent nude body mass loss from baseline average body mass on IPE thirst, significantly correlated ($p<0.001$); c. plot of post-exercise copeptin on IPE thirst, not significantly correlated ($p=0.094$); d. plot of percent plasma volume change from pre to post exercise on IPE thirst, significantly correlated ($p=0.040$).

Chapter 4

SUMMARY AND FUTURE DIRECTIONS

The purpose of these investigations was to determine the relationship of hydration markers to thirst perception during moderate-intensity exercise in a temperate environment and to determine the response of thirst to fluid intake at different volumes following exercise. The main findings suggest that thirst perception responds to hydration state change during and following exercise, however is not sensitive enough to determine exact fluid needs during or following exercise. During exercise, thirst increased with progressive dehydration, and was elevated similarly regardless of amount of body mass lost. Thirst was correlated with hydration measures over time due to a similar linear increase in thirst perception, plasma osmolality, and copeptin. However, thirst perception at the end of exercise was the same when any level of dehydration occurred, regardless of how much.

Additionally, following exercise, any fluid consumption decreased thirst for a short period of time. When only a small volume of fluid was consumed, thirst then increased, but not to pre-fluid consumption levels. This indicates that a small amount of fluid impacted thirst, but not so much that thirst was stifled with continued hypohydration. When a large volume of fluid was consumed, thirst decreased and remained absent or nearly absent despite that subjects had not replaced all fluid lost during exercise. We conclude that thirst is insufficient to replace exercise-induced fluid losses.

Appendices

Appendix A.

Thirst Scale

1 Not Thirsty At ALL

2

3 A Little Thirsty

4

5 Moderately Thirsty

6

7 Very Thirsty

8

9 Very, Very Thirsty

Appendix B.

Thirst Sensation Scale

How thirsty do you feel right now?

Not at all thirsty — Very thirsty

How pleasant would it be to drink some water right now?

Very unpleasant — Very pleasant

How dry does your mouth feel right now?

Not at all dry — Very dry

How would you describe the taste in your mouth?

Normal — Very unpleasant

How full does your stomach feel right now?

Not at all full — Very full

How sick to your stomach do you feel right now?

Not at all sick — Very sick

Adapted from *Sandick, Engell, and Maller* (1984) and *Rolls et al.* (1980)

Appendix C.

Dietary Food Record

Please record your dietary intake so that we can accurately assess your daily intake.

- **All** foods and beverages consumed should be recorded for *24 hours*.

- **Be very specific**. Make sure you include:
  - the **type** of food/beverage
  - the **amount** of each food/beverage
  - the **preparation method** (i.e., fried, baked)
  - the **brand name** of the food (if applicable)
  - the **time** it was eaten
  - if applicable, the restaurant you ate it at (i.e., Subway, Applebees, Red Robin)

  - Record food/beverage consumption **after each meal/snack** instead of waiting until the end of the day.

  - Indicate the **time of your workout** on this food record to identify pre, during, and post workout intake, as well as your habitual intake.

  - **If you prefer, save labels** from packages of food you eat and return them with your food record forms (this will greatly assist and enhance our analysis of your true nutrient intake).

  - Use nutrient descriptors (e.g., low-fat, low-carb, fat-free, light, reduced calorie, etc.).

  - Be sure to **include all ingredients**. For example, a sandwich should include the types and amounts of bread, meat, cheese, vegetables, dressing, etc

  - **Include miscellaneous items** such as condiments (ketchup, salad dressing, mayonnaise, jams, creams, sugar), and chewing gum.

Date:_____

BAD Example

Time	Detailed food/beverage description: brand name, restaurant, method of preparation, flavor, condiments, etc.	Amount	Calories (if known; from food label? Y/N)
7:30 am	Oatmeal	1 bowl	
	Low fat milk	8 oz	
	Omelette with ham and cheddar cheese	3 eggs	
	Orange juice	12 oz	
11:30am	Turkey sandwich	1	
	Regular chips	1 bag	
	Tomato soup	1 ½ cups	
	Dunkin Donuts Coffee	medium	
2:00 pm	Yogurt	1 container	
6:20 pm	Salad	1 large	
	Chicken breast chunks	Large handful	
	Frozen mixed vegetables	1 cup	
	Red wine	1 glass	
9:05pm	Popcorn	2 cups	
	Ice cream	¾ cup	
	water	1 bottle	

Date:_____ # GOOD Example

Time	Detailed food/beverage description: brand name, restaurant, method of preparation, flavor, condiments, etc.	Amount	Calories (if known; from food label? Y/N)
7:30 am	Regular oatmeal- Quick 1 minute Quaker oats made with 2 cups water	1 cup oats dry	
	Hood LightBlock 1% lowfat milk, vitamins A, C, & D (fortified)	8 oz	
	Omelette with 2 Tbsp Hillshire Farm diced ham and 2 oz Cabot 50% reduced fat sharp cheddar cheese	3 whole eggs (large)	
	Tropicana Pure Premium orange juice with calcium & vitamin D	12 oz	
11:30am	Turkey sandwich: Arnold 100% whole wheat bread Butterball extra thin sliced hroasted smoked turkey breast (deli meat) Kraft mayonnaise Iceberg lettuce	2 slices 7 slices 1 Tbsp 1 leaf	
	Lays classic potato chips	1 oz bag	
	Campbell's Select soup-Tomato Garden flavor	1 ½ cups	
	Dunkin Donuts Medium Coffee made with cream, 1 splenda packet	14 oz	
2:00 pm	Dannon fruit on the bottom yogurt- strawberry	1- 6 oz container	
	Bear Naked Banana Nut flavored Granola	¼ cup	
6:20 pm	Salad: 2 cups romaine lettuce medium red tomato cucumber Pepperidge Farm Zesty Italian croutons, Newman's Own Ranch dressing	1 large 3 slices 3 slices 8 2 Tbsp	
	Tyson premium chunk chicken breast (canned chunks)	4 oz.	

Subject #: _____ Date:_____

Time	Detailed food/beverage description: brand name, restaurant, method of preparation, flavor, condiments, etc.	Amount	Calories (from food label? Y/N)

Comments:

Appendix D.

Consent Form for Participation in a Research Study

UCONN
UNIVERSITY OF CONNECTICUT

Principal Investigator: Douglas J. Casa, PhD, ATC
Student Researcher: William M. Adams, MS, ATC, Lesley W. Vandermark, MS, ATC
Study Title: Monitoring Hydration and Sweat Electrolyte Concentration Using Halo Novel Wearable Technologies In Males
Sponsor: Halo Wearables, LLC

Introduction

You are invited to participate in a research study to determine the validation of non-invasive wearable technology on measuring hydration status and sweat electrolyte concentration during exercise. You are being asked to participate because you are a recreationally active individual (exercises regularly >30 minutes 4-5 times per week).

This consent form will give you the information you will need to understand why this study is being done and why you are being invited to participate. It will also describe what you will need to do to participate and any known risks, inconveniences or discomforts that you may have while participating. We encourage you to take some time to think this over and to discuss it with your family, friends and doctor. We also encourage you to ask questions now and at any time. If you decide to participate, you will be asked to sign this form and it will be a record of your agreement to participate. You will be given a copy of this form.

Why is this study being done?

The purpose of this study is to investigate the validity of non-invasive technology in determining hydration status after 6 bouts of 24 minutes of exercise in the heat. This is a non-medical device intended to give the wearer feedback on his/her hydration status. If this device is found to be valid in tracking hydration status during and after exercise in the heat, military, athletics, and industrial settings may benefit for having the potential of having field expedient non-invasive measures of hydration status. The company intends for this device to be marketed for collegiate and professional athletes.

Research questions include:

1. Does a novel wearable device with hydration sensors accurately track hydration status during exercise in the heat?
2. What is the relationship between thirst and markers of hydration status with progressive dehydration during exercise?
3. Are individuals able to replace fluid losses after exercise-induced dehydration for subsequent performance tasks?

UCONN IRB
Approved On 4/21/16
Approved Until 6/25/16
Approved By [illegible]

This research may lead to the development of a commercial product. This product may have economic benefit to Halo Wearables, LLC. If such a product is developed, Halo Wearables, LLC does not intend for you to share in the economic benefit.

What are the study procedures? What will I be asked to do?

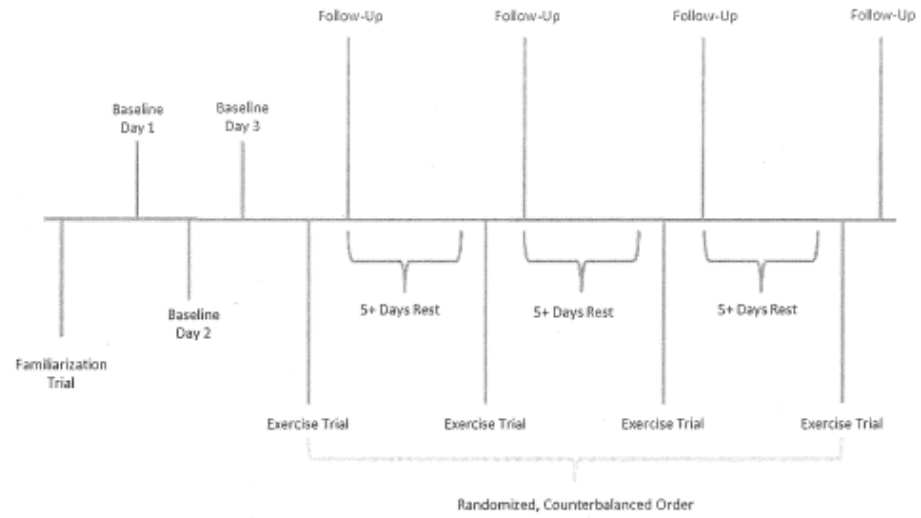
You may be included if you are a male between the ages of 18-35 and perform regular exercise at a minimum of four to five times per week for greater than 30 minutes. You will be screened via medical history questionnaire to ensure that you meet the following criteria: 1) no chronic health problems, 2) no fever or current illness at the time of testing, 3) no history of cardiovascular, metabolic, or respiratory disease, 4) no current musculoskeletal injury that limits their physical activity, 5) no history of exertional heat illness in the past 3 years, and 6) do not consume more than 3 alcoholic beverages per day, or more than 21 alcoholic beverages per week. Additionally, during the familiarization visit, you will perform a test to determine if you meet the fitness criteria set at VO_{2max} >45 ml/kg/min.

If you agree to take part in this study, you will be asked to partake in 11 visits; three consecutive baseline hydration days (approximately 10 minutes each day) one familiarization session (approximately 120 minutes), followed by 4 testing sessions (approximately 300 minutes each (5 hours), with four follow-up sessions the day following the four testing sessions (approximately 60 minutes) totaling 30 hours over the entire study. The four testing sessions will be conducted in a random order and are described below.

The four sessions will consist of:

1. Subjects will arrive normally hydrated, exercise while receiving fluid, and rest while receiving fluid.
2. Subjects will arrive normally hydrated, exercise without fluid, and then rest without receiving fluid.
3. Subjects will arrive less hydrated, exercise without fluid, and then rest while receiving fluid.
4. Subjects will arrive less hydrated, exercise without fluid, and rest without receiving fluid.

During all research sessions, you will be asked to insert a rectal probe to measure body temperature and wear a heart rate monitor. You will be asked provide urine samples, blood samples and body weights so we can accurately assess your hydration level. Below is a diagram of the study timeline to show you the order and types of trials described in the following section. Also included is a table of the types of data that will be collected during each type of trial.



Measure	Familiarization		Body Composition	Exercise Trial					
				Exercise			Recovery		
	Familiarization Trial	Baseline Days		Pre	During	Post	During (every 10 min)	Post	Follow-Up
24 hr. urine collection		X		X					X
Urine volume		X		X					X
Urine specific gravity	X	X		X				X	X
Urine color	X	X		X				X	X
Urine osmolality		X		X				X	X
Nude body mass	X	X		X				X	X
Clothed body mass				X	X (every 24 min)	X	X		
Skinfold Assessment			X						
Height	X								
Diet log		X		X					X
BESS/ LESS	X			X				X	X
Sweat rate/Washdown	X								
VO2max test	X								X
ESQ/ Thirst Sensation	X			X	X (every 24 min)	X	X	X	
KSQ	X			X					X
PSQI	X								
RPE/Thirst/Fatigue/Thermal	X			X	X (every 10 min)	X	X (every 3 min)	X	X
Heart rate/Rectal Temp	X			X	X (every 10 min)	X	X	X	X
Blood draw (10 mL)				X		X		X	X
Finger Stick	X								X
Sleep Quality		X		X					X
EIS/PPG				X	X (every 24 min)	X	X	X	

Familiarization Session:

The familiarization session will be composed of two parts: a familiarization session followed by 3-day baseline hydration visits. For the three day baseline hydration status assessment, you will collect your urine for a 24 hour period each day, and an additional sample at bedtime, in containers that we provide to you, and then arrive to the laboratory fasted and provide us a body mass and complete a brief sleep diary. You will also be asked to complete a diet log and wear a

Jawbone UP3 activity tracking device at night on each of the three days. You will then receive a clean urine jug and cup, a cleaned activity tracking device, and a new diet log for the following day. This will be completed for three consecutive days, and each visit will take less than 30 minutes to complete.

Before the familiarization trial, we will provide you a set of instructions to help you prepare for the trial. During the familiarization testing session you will arrive to the laboratory and provide us a body mass, and urine sample to assess hydration status. We will measure your height and also instruct you on the proper method for inserting the rectal temperature probe, which will be used during all exercise trials for safety monitoring. You will also be familiarized with the environmental symptoms questionnaire (ESQ), Thirst Sensation Scale (TSS), Karolinska Sleep Diary (KSD), Pittsburgh Sleep Quality Index (PSQI), thirst scale, fatigue scale, thermal scale, OMNI scale, diet log, and sleep diary that you will be doing during the exercise sessions. You will also be instructed on the use of the activity tracker, which will be used to track the quality of your sleep during the 3-day baseline and the night after your exercise trials prior to the follow up visit.

VO_{2max}

After these baseline measures, you will be familiarized with the balance tasks that you will be asked to perform during the exercise sessions and during the next day follow-up after the exercise sessions. You will then perform a maximal oxygen uptake (VO2max) test on a treadmill in a cool environment. This test will give us an estimate of your cardiorespiratory fitness level, which we will use as an inclusion criteria, as well as to guide your intensity during the exercise trials. After warming up with a walk on the treadmill the test will begin by having you jog and incrementally increase your speed every few minutes until exhaustion. There will be a researcher available to ensure proper spotting while you are running on the treadmill. Before and after the VO2max test you will have a finger stick, which will draw a small amount of blood from the end of your finger, to assess lactate in your blood.

During this session, we will also measure your sweat rate by having you exercise on a treadmill in the heat. The treadmill exercise will also familiarize you to the exercise sessions in regards to the intensity of exercise that will be performed on the treadmill, which will be based on the results of your VO_{2max} test.

Sweat Rate and Body Mass

Sweat rate will be calculated during the familiarization session. You will be asked to perform 30 minutes of exercise on the treadmill in the warm environmental chamber (~95°F, ~30% relative humidity) after showering briefly and changing clothes. You will not be permitted to drink water during this time so the researchers can get an accurate measure for the amount of fluid losses via sweat you lose during exercise. We will use your pre-exercise body mass and post-exercise body mass to calculate the amount of fluid you lost via sweat to calculate your sweat rate in the units of liters per hour (L/hr).

Following the sweat rate test, the electrolyte composition of your sweat will be analyzed with a whole body wash-down. To do this, we will rinse you with clean water and collect it along with your clothes and towels that you used during exercise to get a sample of your sweat.

Body mass will be calculated by having you provide a nude body mass. You will be asked to enter a private, locked room adjacent to the laboratory where the scale will be located. You will be asked to remove your clothing and step on the scale while the researcher records the measure from outside the locked door. Privacy will be afforded to you in that you will be behind a closed, solid door that does not allow others to see through. The familiarization session will take approximately 2 hours to complete.

Body Composition

On a date that we schedule during your time in the study, you will have your body composition measured by skinfold thickness assessment. This will be done with skinfold calipers at seven-sites on your body by a trained researcher. This will take approximately 5 minutes.

Exercise Session

Depending on the exercise trial, you will be asked to arrive to the laboratory in two hydration states. For the days that you will arrive normally hydrated, you will be asked to consume 500mL of water the night before the trial before going to bed and then again prior to arrival to the laboratory. This is to ensure proper hydration at the beginning of the trial. To test this, you will provide a urine sample in a clean urine cup. If you are not well hydrated you will drink an additional 500mL of water. For the trials in which you will be less hydrated, you will be asked to restrict fluid intake for 22 hours prior to your arrival to the laboratory. We will also ask that you limit your intake of water-heavy foods (soup, raw fruit and vegetables, cereal, etc.). Additionally, we will ask you to refrain from consuming alcohol for 24 hours before any laboratory visit. You will be given instructions on how to prepare for these exercise sessions.

For 24 hours before each visit, you will be asked to collect all of your urine in a container that we provide to you, and fill out a diet log. Additionally, you will be asked to collect a small sample of urine in a cup that we provide for you at bedtime the night before your visit. The night before your visit, you'll be asked to wear the activity tracker to record your sleep quality. When you arrive to the laboratory to pick up these items the day before your exercise trial, we will measure your nude body mass.

You will privately insert a rectal thermometer and nude body mass will be recorded. You will wear a heart rate monitor and will wear your own undergarments, shirt, shorts, socks, and shoes. Before entering the environmental chamber, you will perform the balance tasks that you were familiarized to during the familiarization testing session.

You will enter the warm environmental chamber (~95°F, ~30% relative humidity) and sit for 15 minutes to become used to the heat. After the 15-minute equilibration period and prior to exercise you will provide a blood sample. Blood samples will be taken by a trained researcher and used to assess hydration in several ways, looking at plasma volume and osmolality, as well as hormone markers such as lactate, copeptin, creatine kinase, and myoglobin. You will then

complete the ESQ, TSS, sleep diary, fatigue, thirst, thermal, KSD, and OMNI scales. You will be fitted with the hydration sensor, worn like a watch. After, you will perform cycles of treadmill exercise consisting of 24 minutes walking/running (8 minute walk, 8 minute run, and 8 minute walk) at a set percentage of your cardiorespiratory fitness level followed by 6 minutes of rest: This cycle will continue for a total of 6 times (3 hours) unless one of the following occurs: 1) your rectal temperature reaches 40°C (104°F), 2) you requests to stop, 3) you have an altered or uneven gait, 4) your heart rate > age-predicted maximum heart rate (220-age) for 5 minutes, 5) 3 hours of exercise, or 6) your reach a set percentage of body mass loss before the 3 hour mark.. Body mass, hydration status, and the hydration sensor will be measured during the break periods from exercise. Heart rate and rectal temperature will be continuously monitored as a safety precaution. For trial 1, you will be given fluid at a rate of your sweat rate to consume throughout exercise. For trials 2, 3, and 4 you will be restricted from drinking fluid for the duration of exercise.

After the completion of exercise, you will complete the perceptual scales and then you will provide another blood sample. You will then passively rest in a chair for 60 minutes after exercise for post exercise recovery. For trials 1 and 3, you will replace up to 1% fluid loss based on the difference between pre exercise and post exercise body mass. For trials 2 and 4, you will not be permitted to drink any fluids. Thirst, OMNI, Thermal, and Fatigue will be recorded every 3 minutes, and TSS every 10 minutes. After 60 minutes, you will complete the ESQ, and provide a last blood sample before exiting the environmental chamber.

After completion of the post exercise recovery period, you will towel off as much as possible, exit the environmental chamber, remove the heart rate monitor, hydration sensor, and rectal thermometer, which will then be properly cleaned and disinfected. You will provide a urine sample for hydration assessment and a final nude body mass will be measured. You will also perform balance tasks prior to leaving the laboratory.

For the follow-up testing the day following each trial, you will be instructed to collect your urine in a clean urine jug, and provide a bedtime urine sample in a clean urine cup, that we will provide for you. You will be given instructions for how you should hydrate after the exercise trials. You will also wear the activity tracker the night after your exercise trial to measure your sleep quality. You will also be asked to complete a diet log from the time you leave the laboratory until you return the following day. You will then be asked to return to the laboratory approximately 24 hours after the completion of your exercise session for hydration assessment, performance tasks, a blood draw before and a finger stick after the performance tasks. The performance tasks will include the balance tasks and a VO_{2max} test.

What other options are there?

Your participation in this study is voluntary. You may choose not to participate in this research study.

What are the risks or inconveniences of the study?

The risks of participation in this study are as follows. It is possible that you will experience musculoskeletal injury, exercise-induced muscle cramps, or delayed onset muscle soreness. It is possible that you will strain a muscle, sprain a ligament or tendon, or incur a stress fracture in bone. There is a risk of redness, irritation, or infection from venipunctures/finger sticks, and there is a risk associated with blood draws or finger pricks such as infection at the site of skin puncture. Other possible risks include: (a) a fall during the treadmill running/walking protocol (b) although very unlikely, it is possible that a disturbance of heart rhythm or sudden cardiac arrest will occur, (c) the risk of symptomatic exertional heatstoke is very low, due to the continuous physiological monitoring of rectal temperature and clinical signs and symptoms, (d) rectal thermometry may cause discomfort with insertion, removal, and movement of the device, (e) discomfort caused by grasping technique used for skinfold measurement, (f) the risk of survey fatigue is moderate because you will complete each several times during each trial. There are no known risks of using the activity tracker or hydration sensor.

Risk Mitigation: The following steps will be taken to limit the aforementioned risks.

1. To mitigate risks related to exercise, we will be involving young, healthy participants who have been screened for contraindications to vigorous exercise and a history of heat illnesses.
2. To mitigate risks related to heat illness, we will do the following:
    a. Education about the symptoms and signs of heat exhaustion and heatstroke, with instructions to stop exercise if these symptoms or signs develop.
    b. Monitoring rectal temperature, heart rate, as well as clinical signs and symptoms.
    c. If deemed necessary (core temperature reaches 40°C (104 °F) or there is obvious central nervous system dysfunction indicating possible exertional heat stroke), cold water cooling will be available to decrease body temperature.
3. To mitigate risks related to exercise in a hot environment, we have set the following criteria for cessation of exercise. Exercise testing will be terminated if *one* of the following criteria is met: (a) signs and symptoms indicate that your health or safety is compromised; (b) you verbally discloses that you choose to stop the training session. (c) rectal temperature > 40°C (104 °F); (d) signs and symptoms of heat exhaustion or heatstroke. (Note: it is very unlikely that any participant will reach one of these safety limits, considering the ambient temperature and exercise protocol. A certified athletic trainer, with expertice in the prevention, recognition, and treatment of exertional heat illness, will be on site during each exercise session.)
4. Risks associated with musculoskeletal injury have been addressed as follows:

a. Musculoskeletal injury risks will be minimized by thorough instruction of the task to you by one of the researchers as well as time to practice exercise tasks prior to performance.
b. If a musculoskeletal injury occurs, you will have access to a certified athletic trainer for treatment between exercise sessions.

5. To mitigate risks associated with blood draws, an HPL trained researcher trained in blood draws will perform all venipunctures using universal precautions and an aseptic technique to decrease the likelihood of infection. Total blood drawn over the course of this study will be roughly 144 mL, which is one third of the amount of blood given during a single blood donation. All finger sticks will be done using an aseptic technique and universal precautions to decrease the likelihood of infection.
6. To mitigate risks associated with cardiovascular events, one or more laboratory personnel trained in CPR and the use of an automatic external defibrillator (a device that electrically stimulates the heart to restore rhythm in people who are experiencing cardiac arrest) will be present during all exercise sessions. Furthermore, an AED is in the laboratory for expedient access. In the unlikely event of an emergency during exercise (e.g., incidence of 1 in 250,000 for arrhythmia), 911 will be called and a physician, Jeffrey Anderson, will be alerted. Dr. Anderson will be informed of all testing schedules, prior to performance tests.
7. Emergency protocols for handling a cardiovascular incident and/or hyperthermia are in place and all personnel (including certified athletic trainers among the graduate student investigators) are trained in rapid response, including the use of EMT services (telephone 911). A Kinesiology faculty member will be present in the Kinesiology department for all testing sessions.
8. A board certified/Connecticut license certified athletic trainer will be present for all testing sessions in which you will be exercising in the environmental chamber.
9. If you experiences any minor issues during the exercise trials, the researchers will monitor you and may allow you to continue with the remainder of the exercise trials if the issue is an isolated event and you wishes to continue participation in the study.

What are the benefits of the study?

The study does not provide any specific benefit to you. The proposed study will increase knowledge of the potential field application of using non-invasive wearable technology to measure hydration status and sweat electrolyte concentration. The results may introduce opportunities field expedient methods to assess hydration status, as none currently exist, to minimize performance deficits and safety implications. Your participation in this study may benefit the general population, by allowing researchers, athletic trainers and medical professionals working in athletics, industrial and military settings to have a non-invasive way of assessing hydration status to guide hydration strategies during exercise and physical activity.

Will I receive payment for participation? Are there costs to participate?

You will be compensated monetarily for your time and effort. Compensation will be determined based on your time commitment in addition to the nature of the exercise sessions you will be completing (i.e., exercise in the heat). If you take part in this study, you will receive up to $616 as monetary compensation. As we anticipate a small amount of attrition, payment has been pro-rated as shown below.

Phase Completed	Monetary Allotment
Familiarization	$50
Testing Sessions: 4 sessions x $100/session	$400
Follow-Up Sessions: 3 sessions x $16/session	$64
Completion Bonus	$102
Total Possible Compensation	**$616**

No course credit will be given for participation. You will receive compensation at the end of you involvement in the study. For example, if you complete the familiarization trials and one testing session but chooses to withdraw will receive $150 at that time; whereas if you complete the entire experimental protocol will receive $616 upon completion of the last testing session. You will not be able to keep the Jawbone UP3 activity tracker after completion of the study. This research may lead to the development of a commercial product. This product may have economic benefit to UConn and Halo Wearbles, LLC. If such a product is developed, UConn and Halo Wearables, LLC do not intend for you to share in the economic benefit.

How will my personal information be protected?

All information collected as part of this study will remain confidential. Recorded information will remain in a locked cabinet in the Korey Stringer Institute offices. When information is entered into computer databases (that is password protected), the information will not include any identifiable information. You will only be identified by a participant number on data sheets. There will only be one master list of the participant numbers that will be stored in a locked cabinet in the principal investigator's office. Information will be accessible only by the principal investigator and the student researchers. Your information, the information listed on the one copy of the master list with participant numbers and will be kept on file for three years per federal regulations.

All electronic files (e.g., database, spreadsheet, etc.) containing deidentified information will be password protected. Any computer hosting such files will also have password protection to prevent access by unauthorized users. Only the members of the research staff will have access to the passwords. Data that will be shared with others will be coded as described above to help protect your identity. At the conclusion of this study, the researchers may publish their findings. Information will be presented in summary format and you will not be identified in any publications or presentations.

The data retrieved from the Jawbone activity tracker will be shared with the company (Jawbone) in order to supplement their mass data pool to enhance their proprietary algorithms used to measure and monitor activity and sleep. The only data that will be shared with the company is the information that is specific to only the device and the variables that the device's sensors are measuring (heart rate, bioimpedance, skin temperature, etc.). The data will be transmitted to Jawbone via the cloud. No information related to you specifically will be shared with the company (age, gender, or any other demographic data) to ensure confidentiality.

You should also know that the UConn Institutional Review Board (IRB) and the Office of Research Compliance may inspect study records as part of its auditing program, but these reviews will only focus on the researchers and not on your responses or involvement. The IRB is a group of people who review research studies to protect the rights and welfare of research participants.

What happens if I am injured or sick because I took part in the study?

In the event you become sick or injured during the course of the research study, immediately notify the principal investigator or a member of the research team. If you require medical care for such sickness or injury, your care will be billed to you or to your insurance company in the same manner as your other medical needs are addressed.

However, if you believe that your illness or injury directly resulted from the research procedures of this study, you may be eligible to file a claim with the State of Connecticut Office of Claims Commissioner. For a description of this process, contact the Office of Research Compliance at the University of Connecticut at 860-486-8802.

Can I stop being in the study and what are my rights?

Your participation in this study is voluntary. You do not have to be in this study if you do not want to. If you agree to be in the study, but later change your mind, you may drop out at any time. There are no penalties or consequences of any kind if you decide that you do not want to participate. You will be notified of all significant new findings during the course of the study that may affect your willingness to continue. If necessary, you may be withdrawn from the study at any time. Examples of withdrawal considerations are safety/medical concerns, missed appointments, non-adherence to procedures, disruptive behavior during study procedures, and/or adverse reactions.

Whom do I contact if I have questions about the study?

Take as long as you like before you make a decision. We will be happy to answer any question you have about this study. If you have further questions about this study or if you have a research-related problem, you may contact the principal investigator, Douglas Casa, 860-420-9150, William Adams, 724-840-8772, or Lesley Vandermark, 814-558-1788. If you have any questions concerning your rights as a research participant, you may contact the University of Connecticut Institutional Review Board (IRB) at 860-486-8802.

Documentation of Consent:

I have read this form and decided that I will participate in the project described above. Its general purposes, the particulars of involvement and possible risks and inconveniences have been explained to my satisfaction. I understand that I can withdraw at any time. My signature also indicates that I have received a copy of this consent form.

Participant Signature:	Print Name:	Date:
Signature of Person Obtaining Consent	Print Name:	Date:

Appendix E.

HUMAN PERFORMANCE LABORATORY MEDICAL HISTORY QUESTIONNAIRE

Study _____ Subject # _____

Name _____ Sex ____ Age _____ DOB _____

Street _____

City _____ State ____ Zip _____ Phone _____

Email _____

PLEASE ANSWER ALL OF THE FOLLOWING QUESTIONS AND PROVIDE DETAILS FOR ALL "YES" ANSWERS IN THE SPACES AT THE BOTTOM OF THE FORM.

YES	NO		
☐	☐	1.	Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
☐	☐	2.	Has your doctor ever denied or restricted your participation in sports or exercise for any reason?
☐	☐	3.	Do you ever feel discomfort, pressure, or pain in your chest when you do physical activity?
☐	☐	4.	In the past month, have you had chest pain when you were not doing physical activity?
☐	☐	5.	Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	6.	Does your heart race or skip beats during exercise?
☐	☐	7.	Has a doctor ever ordered a test for you heart? (i.e. EKG, echocardiogram)
☐	☐	8.	Has anyone in your family died for no apparent reason or died from heart problems or sudden death before the age of 50?
☐	☐	9.	Have you ever had to spend the night in a hospital?
☐	☐	10.	Have you ever had surgery?
☐	☐	11.	Please check the box next to any of the following illnesses with which you have ever been diagnosed or for which you have been treated.

☐ High blood pressure	☐ Elevated cholesterol	☐ Diabetes
☐ Asthma	☐ Epilepsy (seizures)	☐ Kidney problems
☐ Bladder Problems	☐ Anemia	☐ Heart problems
☐ Coronary artery disease	☐ Lung problems	☐ Chronic headaches

YES	NO		
☐	☐	12.	Have you ever gotten sick because of exercising in the heat? (i.e. cramps, heat exhaustion, heat stroke)
☐	☐	13.	Have you had any other significant illnesses not listed above?
☐	☐	14.	Do you currently have any illness?
☐	☐	15.	Do you know of any other reason why you should not do physical activity?
		16.	Please list all medications you are currently taking. Make sure to include over-the-counter medications and birth control pills.

Drugs/Supplements/Vitamins	Dose	Frequency (i.e. daily, 2x/day, etc.)

DETAILS: _____

_____

_____

17. Please list all allergies you have.

Substance	Reaction

18 Do you have a family history of any of the following problems? If yes, note who in the space provided.

☐ High blood pressure		☐ Heart disease	
☐ High cholesterol		☐ Kidney disease	
☐ Diabetes		☐ Thyroid disease	

19 Please check the box next to any of the following body parts you have injured in the past and provide details.

☐ Head		☐ Hip		☐ Calf/shin	
☐ Neck		☐ Thigh		☐ Shoulder	
☐ Upper back		☐ Knee		☐ Upper arm	
☐ Lower back		☐ Ankle		☐ Elbow	
☐ Chest		☐ Foot		☐ Hand/fingers	

YES	NO		
☐	☐	20	Have you ever had a stress fracture?
☐	☐	21	Have you ever had a disc injury in your back?
☐	☐	22	Has a doctor ever restricted your exercise because of an injury?
☐	☐	23	Do you currently have any injuries that are bothering you?

24 Do you consider your occupation as?

- ☐ Sedentary (no exercise)
- ☐ Inactive-occasional light activity (walking)
- ☐ Active-regular light activity and/or occasional vigorous activity (heavy lifting, running, etc.)
- ☐ Heavy Work-regular vigorous activity

25 List your regular physical activities

Activity	How often do you do it?	How long do you do it?	How long ago did you start?

ADDITIONAL DETAILS:

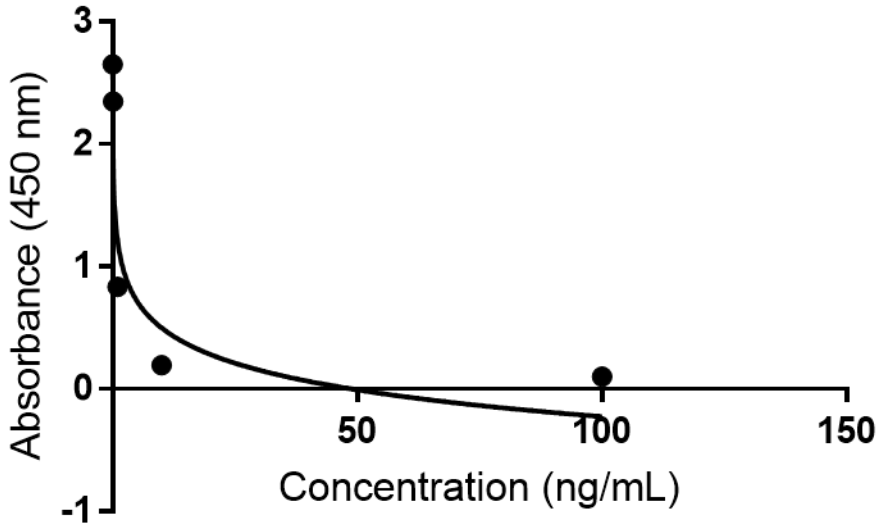
Appendix F.

Copeptin Assay Procedure

Fifty microliters of plasma was added per well to a 96-well plate, then 25 µl primary antibody and 25 µl biotinylated peptide was added per well, covered in foil, and incubated at room temperature on an orbital shaker at approximately 400 rpm for 2 hours. Following the first incubation, the plate was washed with 350 µl prepared buffer per well 4 times and blotted dry, then 100 µl SA-HRP solution per well was added, covered in foil, and incubated at room temperature on an orbital shaker at approximately 400 rpm for 1 hour. Following the second incubation, the plate was washed with 350 µl prepared buffer per well 4 times and blotted dry, then 100 µl TMB substrate per well was added, covered in foil, and incubated at room temperature on an orbital shaker at approximately 400 rpm for 40 minutes. The reaction was terminated with 100 µl/well of 2N HCL immediately following the third incubation period. Absorbance optical density was read at 450nm on a microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA) using SoftMax Pro software (version 5.3, Molecular Devices, Sunnyvale, CA). The minimum detectable concentration was 0.12 ng/ml, with a linear range of 0.12-2.79 ng/ml, and an upper limit of 100 ng/ml. Standard curve was established using the following equation for a semilog line:

$Y = Yintercept + slope*log(X)$

The curve was used to interpolate concentration from absorbance (GraphPad, Prism, version 7 for Windows, 2016).



Appendix G.

Thirst Sensation Scale data analysis.

Table 1. Thirst Sensation Scale individual components means and standard deviations during a) exercise and b) recovery.

a)	Pre-exercise		60 minutes		120 minutes		180 minutes		IPE	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Thirst	37	31	53	34	66	37	71	40	75	36
Pleasantness	61	22	68	27	75	31	78	32	80	29
Dryness	39	28	53	35	67	37	67	40	71	37
Taste	25	27	44	33	58	38	63	40	66	39
Fullness	37	20	31	21	26	22	22	25	21	23
Sickness	11	16	15	21	20	23	23	24	24	25
Total	209	101	265	122	312	135	323	140	338	134

b)	Pre-recovery		30 minutes		60 minutes	
	Mean	SD	Mean	SD	Mean	SD
Thirst	74	37	65	37	67	36
Pleasantness	80	29	74	29	75	30
Dryness	70	39	61	38	63	37
Taste	65	37	57	38	57	39
Fullness	22	23	27	25	22	24
Sickness	22	24	25	30	21	29
Total	334	131	309	141	306	140

Table 2. Thirst sensation scale individual components correlations with Nude Body Mass Loss (%) and plasma osmolality pre-exercise, IPE, and post-recovery.

		Body mass loss (%)			Plasma osmolality		
		Pre-exercise	IPE	Post-recovery	Pre-exercise	IPE	Post-recovery
Thirst	R	-.752**	-.839**	-.672**	.519**	.811**	.597**
	Sig. (2-tailed)	0.000	0.000	0.000	0.001	0.000	0.000
Pleasantness	R	-.679**	-.799**	-.645**	.633**	.782**	.543**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.000
Dryness	R	-.707**	-.853**	-.764**	.565**	.784**	.602**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.000
Taste	R	-.646**	-.790**	-.747**	.452**	.772**	.593**
	Sig. (2-tailed)	0.000	0.000	0.000	0.004	0.000	0.000
Fullness	R	0.208	.332*	0.172	-0.150	-0.234	-0.037
	Sig. (2-tailed)	0.204	0.036	0.290	0.362	0.146	0.819
Sickness	R	-0.205	-0.253	-.366*	0.056	0.216	0.262
	Sig. (2-tailed)	0.210	0.115	0.020	0.737	0.181	0.103
Total	R	-.742**	-.850**	-.769**	.556**	.825**	.644**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.000

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).