IMPACT OF SEX AND REHYDRATING FLUID ON PARAMETERS OF DEHYDRATION, REHYDRATION, AND ATHLETIC PERFORMANCE

by

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STATEMENT BY AUTHOR

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Dedication

This is for my mother and father. I could not, and would not, have done this without you. Your selfless support, and tireless dedication to seeing me succeed has been my backbone throughout college, and especially through graduate school.

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Abstract

Background.

In humans, total body water volume and osmolality are tightly regulated by various homeostatic mechanisms, triggered by deviations in osmolality. Heat and exercise are two stressors, which in combination can cause dehydration, and an increase in fluid osmolality, contributing to health detriments, as well as deficits in aerobic exercise performance. However, it is unclear whether dehydration affects muscular strength. Deep-ocean mineral water has been shown to have benefits on various physiological and pathophysiological conditions, including aerobic performance and muscle strength.

Objectives.

The aims of this study were to examine any sex differences that may exist in response to dehydration of 3% of body mass, rehydration with various fluids, and the consequences of dehydration and rehydration on muscle power and hydration status.

Design.

We used a counterbalanced, crossover study design, in which subjects (n=17, 9 males vs 8 females) performed a dehydrating exercise protocol until achieving a 3% body mass loss, and then rehydrated with either deep-ocean mineral water (**Deep**), mountain spring water (**Spring**), or a carbohydrate-based sports drink (**Sports**). Subjects completed the protocol three times, with each subject receiving the rehydrating fluid in a different order to control for order effects. Saliva samples were collected throughout the protocol to measure osmolality, and muscle strength was measured by peak torque leg extension at baseline, post-exercise, and post-rehydration.

Results.

We found no differences between men and women in baseline or peak salivary osmolality, or in the exercise-induced increase in osmolality. Male subjects took less time to reach 3% body mass loss than females, and females demonstrated lower sweat rates than males. Salivary osmolality returned to baseline after rehydration, with the **Deep** group exhibiting a significantly more rapid return to baseline, for both sexes, compared to **Sports** and **Spring**. Males generated greater peak torque extension than females at baseline, while both males and females displayed a similar significant deficit in this measure following dehydration. Peak torque recovery post-rehydration was significantly affected by fluid designation and sex, and a significant difference was seen between the **Deep** and the **Sports** groups in females.

Conclusions.

Males reached 3% body mass loss faster than females, while dehydration resulted in increased salivary osmolality and muscle strength deficits similarly for males and females. Deep-ocean mineral water had a significant beneficial effect on hydration recovery, for both males and females, compared to the other fluids. Recovery of muscle strength after rehydration was affected by fluid and sex, with the main driver being females.

Introduction

Background.

The volume of total body water in humans represents approximately 60% of total body weight in men and 50% of body weight in women, with some variability primarily due to differences in body composition [1].This volume is tightly regulated so that humans maintain a fairly constant osmolality (the concentration of solute in a solution) of body fluids [2]. Environmental factors like temperature, water and sodium ingestion, and body water loss can perturb body fluid osmolality, triggering both behavioral responses, and physiological mechanisms to restore homeostatic values of osmolality. For example, in humans, increases in osmolality activate a thirst response, causing us to seek water, and decreases our appetite for sodium. Hyperosmotic conditions also cause a release of vasopressin, or anti-diuretic hormone, which enhances water retention in the kidneys, returning osmolality back to the body's normal "set-point" of around 100 mmol/Kg [2].

Exercise in a hot environment, however, provides two concurrent stressors, and can overwhelm these homeostatic mechanisms, reducing total body water, and increasing osmolality of extracellular fluids (ECF). First, the body must deliver sufficient blood flow to the working muscle to meet the metabolic demands of the exercise. Second, the body attempts to maintain core temperature primarily by increasing sweat production for evaporative cooling, and by increasing cutaneous blood flow in an effort to dissipate heat to the environment [3]. Interestingly, despite females having a greater density and absolute number of heat-activated sweat glands [4], they have been shown to have a lower maximal sweat rate than males of the same size and fitness level [5, 6]. Sweat is drawn from the interstitial fluid and is hypotonic due to the reabsorption of various ions in the sweat duct. This results in both a decrease in total body

water and a concomitant increase in the osmolality of the interstitial fluid [7]. Consequently, increases in the osmolality of the interstitial fluid results in water being drawn from the plasma and intracellular stores to maintain equivalent osmolality in the interstitial fluid and intracellular fluid, as the two forces governing water distribution in the body are the hydrostatic and osmotic pressures [1, 2]. Osmotic fluctuations, if severe enough, can have serious health consequences, such as weakness, cardiac arrest, spasticity, coma, seizures, and death [2, 8]. It has also been well established that dehydration can impair an individual's ability to thermoregulate [9] and perform aerobic exercise, independent of thermal, dietary, or metabolic stresses [10, 11]. Dehydration increases cardiovascular strain by reducing blood volume through fluid loss, thereby decreasing stroke volume, and increasing heart rate. When core temperature increases as a result of exercise and dehydration, elevated skin blood flow displaces blood away from the central blood volume, exacerbating cardiovascular strain [11]. Increased core temperature also signals the central nervous system to reduce the drive to exercise, although the precise mechanism for this is unknown. Dehydration can also alter energy metabolism pathways in the muscles, causing muscle glycogen to be utilized much more quickly. These factors all result in greater perceived exertion and earlier time to fatigue, and therefore limit the ability of an individual to perform aerobic exercise [11]. Moreover, exercise in a hot environment amplifies dehydration, enhances the above effects, and accelerates performance deficits [11-13].

While dehydration definitely impairs aerobic exercise performance, it is inconclusive if dehydration affects measures of muscle strength and power, in male and female subjects [14, 15]. Many studies fail to make conclusive connections between dehydration and impaired muscle strength due to confounding effects of other performance-reducing factors, such as increased core or muscle temperature, or muscle fatigue [14, 15]. Interestingly, animals supplemented with

desalinated deep-ocean mineral water show benefit on various physiological and pathophysiological conditions, mostly attributed to the unique mineral and trace mineral content of this water compared to water from surface sources [16-20]. For an example of mineral composition of deep-ocean mineral water, see the comparison in **Table 1**. Miyamura et al demonstrated that deep-ocean mineral water improved factors related to hyperlipidemia and atherosclerosis, including plasma LDL cholesterol, plasma antioxidant activity, aortic lipid deposition, and foam cell formation in dietary-induced hyperlipidemia rabbits, compared to surface-level seawater and distilled water [18]. Katsuda et al reported an improvement in the hypertension-related factors of systolic and diastolic blood pressure, mean arterial pressure, and peripheral resistance, with no effect on total cholesterol or triglyceride levels in heritable hypercholesterolemic rabbits when fed deep-ocean mineral water compared to tap water [17]. In a 2014 study by Wang et al, deep-ocean mineral water was shown to increase the exercise performance of gerbils, compared to distilled water, measured by retention rates during a 90-minute treadmill exercise [20]. Many other physiological and health effects of deep-ocean mineral water have been supported by numerous other studies, including its potential to protect from obesity [21-25], prevent and treat diabetes [21, 22, 24, 26], and improve symptoms of skin conditions [24, 27, 28].

Similarly, a recent human study shows that deep-ocean water (662 m) taken from the coast of Hualien, Taiwan, improves recovery following dehydrating exercise, evidenced by accelerated recovery of aerobic capacity, increased lower-body muscle power performance, and significantly reduced levels of exercise-induced muscle damage markers compared to subjects drinking purified tap-water [16]. Considering the established connection between hydration status and exercise performance, these data suggest that deep-ocean mineral water may provide

Table 1. Fluid Comparison of Selected Nutrients

Nutrient	Kona Deep (deep-ocean mineral water)	Arrowhead (mountain spring water)*	Gatorade (carbohydrate-based sports drink)**
Sodium	85 mg/L	3 mg/L	450.9 mg/L
Chloride	150 mg/L	0-12 mg/L	408.3 mg/L
Potassium	4 mg/L	0-2.9 mg/L	126.8 mg/L
Magnesium	4.3 mg/L	1.4 mg/L	0 mg/L
Calcium	1.4 mg/L	4 mg/L	0 mg/L
Boron	0.65 mg/L	0 mg/L	0 mg/L
Bromide	540 µg/L	3.1 µg/L	0 µg/L
Chromium	2.2 µg/L	0 µg/L	0 µg/L
Carbohydrates	0 g/L	0 g/L	59.2 g/L

*Values are minimum ranges based on Arrowhead 2016 Water Analysis Report at www.nestle-watersna.com
**Values calculated based on amounts reported for Gatorade Thirst Quencher at www.gatorade.com

optimal rehydration for performance following high-intensity exercise in well-conditioned individuals. Therefore, deep-ocean mineral water may be helpful in the development of an efficient and optimal rehydration strategy, which could prove beneficial during high-intensity activities.

Objectives and Hypotheses.

The questions addressed by this study are 1) Do males and females differ in parameters of exercise-induced dehydration, or in the effects of dehydration on athletic performance? and 2) Is rehydration and recovery after exercise influenced by sex or rehydrating fluid? We hypothesize that rate of dehydration will be slower in females than in males, based on lower sweat rates in females, and that males and females will exhibit similar rises in ECF osmolality as a result of dehydration. In addition, we hypothesize that muscle strength will decrease as a result of dehydration, and that females will show a larger strength deficit than males, due to lower initial body fluid volumes. Finally, we hypothesize that deep-ocean mineral water will improve rehydration rate as well as muscle recovery after exercise, and this will not be different between males and females.

Consequently, one aim of this study was to examine if parameters affected by dehydration of 3% of body mass (including heart rate, body temperature, fluid osmolality, and rate of dehydration) due to exercise in the heat, are different between males and females. We also aimed to examine if the effects of dehydration on lower-body muscle power production, if any, are different between males and females. In addition, while it has been shown that rate of dehydration varies based on sex [6], we were interested if sex or rehydrating fluid had an effect on the rate of rehydration or muscle performance recovery after exercise. Dehydration and

rehydration with either deep-ocean mineral water (**Deep**), mountain spring water (**Spring**) or a carbohydrate-based sports drink (**Sports**) was measured by salivary osmolality, an established marker of hydration during intense exercise [29, 30].

Methods

Subjects.

Participants eligible for this study included females and males ages 20-25 years, non-smokers and well-conditioned with moderate to excellent aerobic capacity, as determined by a self-reported 3-6 hours of athletic conditioning per week. Subjects were free from medication, stimulants, nutritional supplements, or major health-related issues, as determined by the University of Arizona Health History Screening Questionnaire (UAHHSQ) (**Appendix A**). With a conservative estimate of salivary osmolality at baseline (100 mmol/Kg) compared to peak (150 mmol/Kg) during the experimental protocol with a standard deviation of 30, sample size was determined using a power of 0.8 at α=0.05 (6 or greater per group). This was also confirmed using power analysis of mean population serum and urinary osmolality. All subjects provided consent under protocols adhering to guidelines approved by the Institutional Review Board at the University of Arizona and in accordance with the Declaration of Helsinki (**Appendix B**). Subjects were asked to follow a normal diet while avoiding foods high in sodium 24 hours prior to study initiation, and to avoid any exercise 24 hours prior to each trial. In addition, subjects were required to abstain from alcohol consumption 36 hours prior to each exercise trial, as alcohol has diuretic effects, and may confound observed effects of dehydration. Subjects were also instructed to begin each trial in a euhydrated state (confirmed by baseline salivary osmolality), and to maintain a reasonably similar diet prior to each of the exercise trials.

Study design.

We used a counterbalanced, crossover study design in which subjects (n=17) were randomized to begin in one of 3 experimental groups: Kona Deep® deep-ocean mineral water (**Deep**), Gatorade sports drink (**Sports**), or Arrowhead mountain spring water (**Spring**). A comparison of the mineral composition of these fluids is provided in **Table 1**. Each subject was required to complete the dehydrating exercise protocol 3 times, one trial for each hydration fluid, each separated by a period of at least 48 hours, to give participants adequate time to recover. After the first trial, subjects were randomized to a second group to complete the second arm of the study hydrating with one of the two remaining hydrating fluids. During the third trial, subjects rehydrated with the last remaining beverage.

Dehydration and hydration protocols.

A graphical summary of the dehydration and hydration protocol is illustrated in **Figure 1**. Euhydrated subjects were asked to remove any excess or loose clothing including shirts, athletic pants, shoes, and socks. Subjects were instrumented with a Polar™ heart rate monitor allowing continuous monitoring of heart rate. Baseline measurements of heart rate, body weight (using a digital scale) and tympanic temperature (Braun ThermoScan® PRO 4000) were collected prior to the initiation of the exercise dehydration protocol. In addition, stimulated and unstimulated saliva samples (detailed below) were taken to establish baseline osmolality values and to ensure all subjects were euhydrated at the start of each trial. Next, subjects initiated exercise using a Monark stationary cycle under moderate heat stress using heat lamps to provide warm conditions of about 30° C, since sweat rate is greatest at warm ambient temperatures [31]. Subjects were instructed to sustain about 60% of maximum heart rate and maintain 150-200 watts on the

Figure 1.

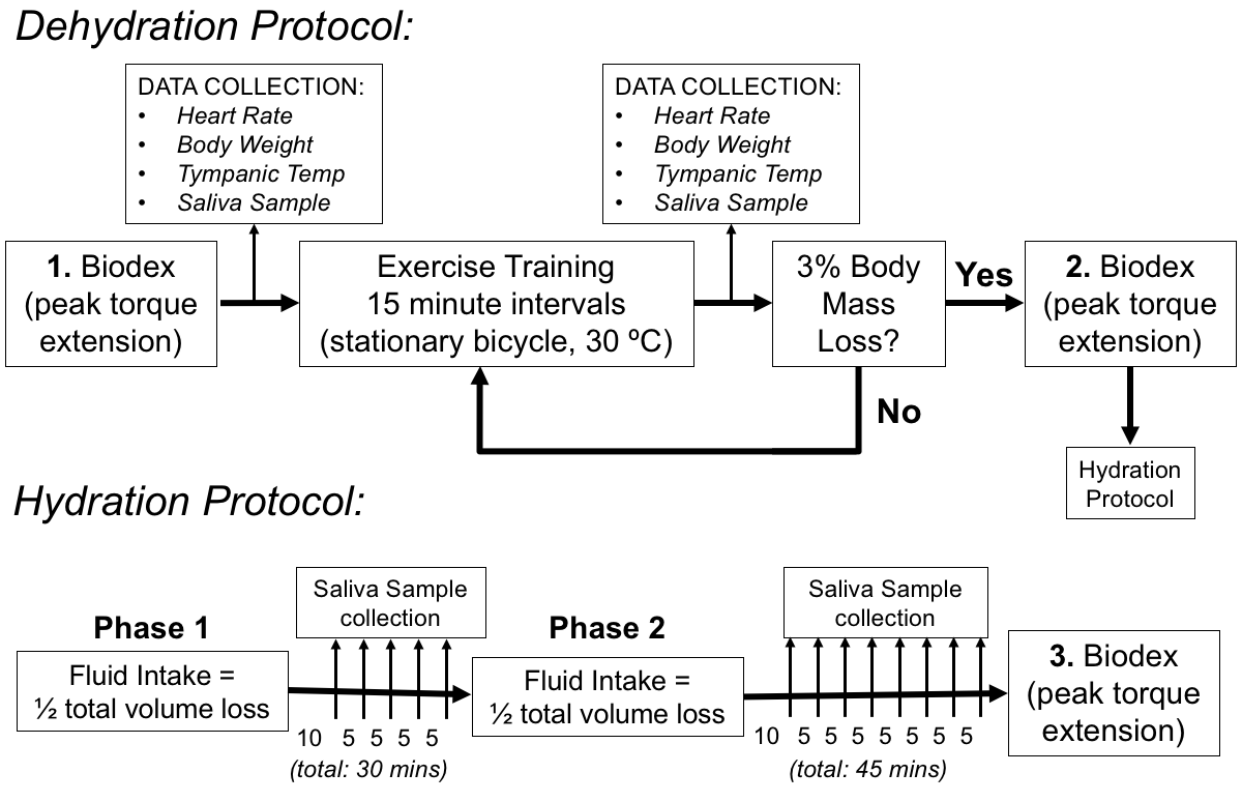

Figure 1: Experimental design and protocol. Dehydration Protocol: Euhydrated subjects were randomly assigned in a counterbalanced fashion to one of three groups (Deep, Sports, or Spring). Prior to data collection, subjects executed **1** of **3** peak torque extension maneuvers to obtain a baseline value. Following peak torque extension, parameters were collected (indicated in the panel labeled DATA COLLECTION) and exercise was initiated. To instigate safe and rapid dehydration, subjects initiated intense exercise using a stationary bicycle under moderate heat stress (32-35 °C). Exercise was continued for 15 minutes followed by collection of parameters. If subjects did not achieve 3% body mass loss, exercise was reinitiated for another 15 minutes. This cycle was continued until subjects lost a minimum 3% of body mass and subjects were not allowed to evacuate or intake any fluids. Upon completion of the Dehydration Protocol, subjects immediately executed the second (**2**) of **3** peak torque extension maneuvers to obtain a post-exercise value and transitioned to the **Hydration protocol**. **Hydration protocol:** Subjects were asked to consume 1 of 3 fluids at ½ of the total volume lost, which was determined as the ml equivalent of gram weight lost assuming 1 gram = 1 ml. **Phase 1:** Due to the confounding impact of an oral rinse on S_{osm}, 10 minutes following fluid intake a saliva sample was collected. Sample collection continued at 5 minute intervals until 30 minutes from the time of fluid intake. **Phase 2:** The remaining amount (½ of the total volume lost) of fluid was ingested followed by saliva sample collection 10 minutes later. Saliva collection continued at 5-minute intervals until 45 minutes from the second fluid intake. Immediately following the final saliva collection, subjects executed the third (**3**) of **3** peak torque extension maneuvers to obtain a post-hydration value.

stationary bicycle at a cadence greater than 70 rpm. Subjects self-monitored and adjusted watts by increasing rpm or resistance. Exercise bouts continued for 15 minutes, at which time subjects were asked to remove any excess or loose clothing as above, and towel-dry for data collection (body weight, heart rate, temperature, saliva samples). This protocol was repeated at 15-minute intervals until target dehydration, indicated by a body mass loss of 3% was achieved. Studies have indicated that greater than 2% body mass loss by dehydration results in a significant exercise deficit [11, 12, 16].

When 3% body mass loss was reached, subjects rehydrated by consuming a volume of fluid (**Deep**, **Sports**, or **Spring**) equal to their body mass loss, assuming that each 1 liter = 1 kg. To prevent hypervolemia, rehydration occurred in two phases. In the first phase, subjects consumed one-half of the total volume lost. Stimulated and unstimulated saliva samples were collected starting at 10 minutes after this initial rehydration phase, and at each subsequent 5-minute interval, for 30 minutes. After this 30-minute time period, the second phase occurred, in which subjects consumed the remainder of the fluid. Saliva samples were taken starting at 10 minutes after this second rehydration phase, and at each subsequent 5-minute interval, for 45 minutes.

Lower body muscle performance.

Peak torque leg extension measured by a Biodex™ System 3 dynamometer was used as a measure of lower body muscle performance. As illustrated in **Figure 1**, a peak torque extension maneuver was executed three times during the Dehydration and Hydration Protocols: 1.) baseline, prior to exercise initiation, 2.) post-exercise, following loss of 3% body mass, and 3.) post hydration, after the final saliva collection (75 minutes post-exercise). Subjects performed a

series of 3 maximal contractions of the left knee extensors using the Biodex™, and the average peak torque was determined from the 3 contractions. To maintain consistency, subjects performed this test oriented in the same position, and using the same hand grips on the machine for support during each of the measurements. Subjects were also vigorously encouraged to exert maximal effort on each measurement by the same individuals.

Salivary Osmolality.

Hydration status was monitored using salivary osmolality. Several different measurements can be used to assess hydration, including serum, saliva, and urine osmolality, and urine volume and specific gravity. The most appropriate measurement depends on the mode of dehydration, and the frequency of the measurement. Previous studies have demonstrated that, for repeated measurements during active dehydration (i.e. exercise) in the heat, salivary osmolality is an effective way to measure ECF osmolality, and is noninvasive [29, 30]. Saliva was collected from the oral cavity, first as a passive expectorant (unstimulated) [29, 32], and then following mechanical (stimulated) orofacial movement (chewing on a cotton swab). Both stimulated and unstimulated saliva samples were used, because it is not clear from the literature whether changes in salivary osmolality due to dehydration from exercise differs between the two collection methods. Stimulated saliva samples were spun down in a centrifuge for 10 minutes at 9000 rpm to collect saliva from the cotton swab. All samples, both stimulated and unstimulated were then vortexed to homogenize the samples, and salivary osmolality was measured using a dedicated vapor pressure osmometer (Wescor VAPRO® 5600). To maintain consistency, this was performed by the same three individuals, using 10 µl of each sample, and ach sample was run in triplicate. This was done immediately after sample collection to prevent sample spoilage.

In addition to daily calibrations, the osmometer was calibrated prior to each new biological sample.

Data and statistical analysis.

All values are presented as mean (SD). Body Mass Index (BMI) was calculated using the following equation:

$$\text{BMI} = \text{body weight (in kg)}/\text{height (in meters)}^2$$

Body Surface Area (BSA) was calculated based on the following equation [33]:

$$\text{BSA } (m^2) = \sqrt{(\text{Height (in cm) x body weight (in kg)})/3600}$$

To compare heart rate, BW, BMI, BSA, and tympanic temperature at baseline and peak, the measured values in each individual from the three arms of the study were averaged. The same method was used to calculate mean values for time to 3% body mass loss and sweat rate. Salivary osmolality (S_{osm}) was plotted against percent body mass loss; body mass loss was calculated as the difference in body mass after completion of the dehydrating exercise, from body mass at trial initiation. This value was divided by body mass at trial initiation and expressed as a percentage. For each individual, raw S_{osm} against body mass loss (%) was fit by linear regression. Differences between the groups were calculated using one-way analysis of variance with Bonferroni post hoc correction for multiple comparisons. The return of S_{osm} to

baseline during the Hydration Protocol was best fit by a mono-exponential (one-phase decay) model where,

$$S_{osm}=(S_{osm(peak)}- S_{osm(baseline)})^{Kt} + S_{osm(baseline)}$$

S_{osm}=Salivary osmolality; $S_{osm(baseline)}$= Salivary osmolality at baseline (approximate; calculated as plateau phase of best fit model); $S_{osm(peak)}$= Salivary osmolality at peak (S_{osm} at end of Dehydration Protocol). Time was adjusted such that the time at $S_{osm(peak)}$ was set to t=0 in order to normalize fit parameters. K=rate constant, tau (τ) =1/K, and $\tau_{1/2}$ = half-time S_{osm} recovery.

We used p Values of <0.05 to indicate statistical significance. Statistical calculations were calculated using commercially available software (GraphPad Prism version 5.0 for Mac OS X). Differences between averaged age, height, heart rate, BW, BMI, BSA, tympanic temperature, time to 3% body mass loss and sweat rate were determined using 2-way analysis of variance with post-hoc Bonferroni analysis. All other comparisons were completed using a repeated measures 2-way ANOVA followed by a post-hoc Bonferroni analysis.

Results

Age and Body Morphometrics.

A summary of subject characteristics is provided in **Table 2**. Age-matched female and male study participants meeting eligibility criteria were further selected for equivalent fitness levels based on a self-reported 3-6 hours of athletic conditioning per week. The study population engaged in primarily dynamic activities including cycling, running, hockey, soccer, and triathlons. At the time of the study, female participants (n=8) were 22.1 ± 2.1 years of age, which was not statistically different from male participants (n=9) who were 23.6 ± 2.2 years of age. Female subjects were significantly shorter when compared to male counterparts (167.6 ± 2.9 cm vs 181.1 ± 4.6 cm). For each of the three trials, subjects maintained their baseline body weight (BW). Since there were no significant differences in baseline BW, the values for BW, body-mass index (BMI), and body surface area (BSA) represent the averaged baseline value of the three trials for each subject. Considering the significant sex difference in height, BW (65.2 ± 10.2 kg vs. 76.0 ± 8.6 kg) and BSA (1.74 ± 0.11 m^2 vs 1.95 ± 0.14 m^2) were significantly less in females compared to males. However, this difference was eliminated in the calculated BMI; females had a BMI of 23.3 ± 3.0 kg/m^2 while males had a BMI of 23.2 ± 3.4 kg/m^2.

Heart rate and body temperature.

Dehydration due to water loss can impact heart rate [11]. Therefore, we wished to assess whether dehydration during the exercise protocol impacted heart rate differently in female and male athletes. Accordingly, heart rate was monitored throughout the exercise and post-exercise periods. Again, the values for heart rate represent the averaged values for each subject over the three trials as there were no significant differences among the three trials. Although baseline

heart rate trended higher in females (85.8 ± 6.4 bpm) compared to males (80.4 ± 16.3 bpm), the trend did not reach significance.

Because each subject was equipped with a heart rate monitor, we were able to monitor heart rate throughout the exercise protocol. For each 15-minute bout of exercise, we recorded peak heart rate and subsequently averaged these values to arrive at a single peak heart rate. As expected, peak heart rate during exercise was significantly elevated for both women (176.4 ± 11.7 bpm) and men (178.9 ± 9.7 bpm) over baseline values but did not display any differences between the sexes. These data are summarized in **Table 3,** and also displayed in **Figure 2A**.

In addition, sex differences may exist in thermoregulation following heat stress [5]. Therefore, tympanic temperature as an indicator of core temperature was also recorded throughout the exercise protocol. Again, the peak temperature for each subject was not different across the three trials, so averaged values of tympanic temperature were determined as described for peak heart rate. Despite being subjected to exercise and moderate heat stress, both female (97.9 ± 0.5 °F vs. 99.2 ± 0.7 °F) and male (97.7 ± 0.8 °F vs. 99.3 ± 0.7 °F) participants maintained body temperature to a similar extent, displaying no significant increase in body temperature during the exercise protocol. These data are summarized in **Table 3,** and also displayed in **Figure 2B**.

Table 2. Subject Characteristics

	Females (n=8)	Males (n=9)	P value
Age (yrs)	22.1 ± 2.1	23.6 ± 2.2	0.2080
Height (cm)	167.6 ± 2.9	181.1 ± 4.6*	<0.0001
BW (kg)	65.2 ± 10.2	76.0 ± 8.6*	0.0464
BMI (kg/m^2)	23.3 ± 3.0	23.2 ± 3.4	0.9728
BSA (m^2)	1.74 ± 0.11	1.95 ± 0.14*	0.0083

Table 3. Peak heart rate and body temperature (tympanic) during the exercise trial

	Females (n=8)		Males (n=9)	
	BL	**Peak**	**BL**	**Peak**
Heart Rate (bpm)	85.8 ± 6.4	176.4 ± 11.7*	80.4 ± 16.3	178.9 ± 9.7*
Tympanic Temp (℉)	97.9 ± 0.5	99.2 ± 0.7	97.7 ± 0.8	99.3 ± 0.7

Figure 2.

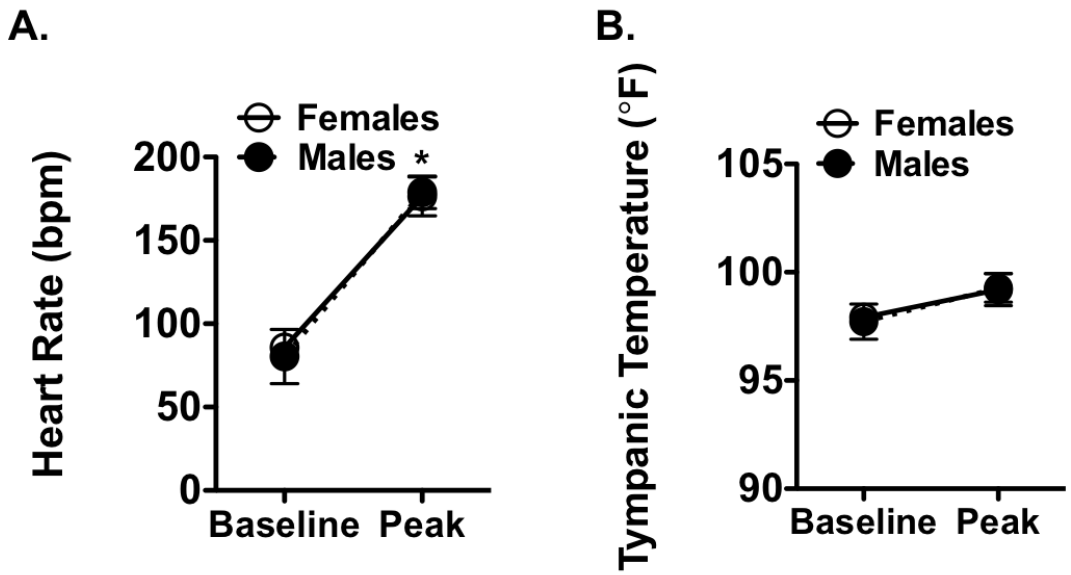

Figure 2: Heart rate and tympanic temperature during the dehydrating exercise protocol. **A**: Subjects were equipped with a heart rate monitor, which was checked at each DATA COLLECTION interval. Because individual heart rate did not vary in the three trials, we determined an average value for each subject. Peak heart rate was the averaged value from the peak heart rate during each 15-minute interval. A two-way ANOVA demonstrated a significant ($p<0.001$) impact of exercise (**Baseline** vs. **Peak**) but not sex (**Female** vs. **Male**) on heart rate in beats per minute (bpm). Furthermore, there was no interaction of sex and exercise on heart rate. Post-hoc analysis showed significant elevation (*$p<0.001$) of heart rate in females and males. **B**: Tympanic temperature (as an indicator of core body temperature) at **Baseline** and **Peak** in **Females** and **Males**. Average values were used for comparison and no significant differences were found. Data presented as mean ± standard deviation (S.D.).

Exercise and Salivary Osmolality.

A goal of this study was to achieve at least a 3% loss in body mass from the exercise protocol in order to determine the impact of different fluid intake on exercise performance and rehydration. At the same time, we wanted to monitor hydration status throughout the experimental protocol and identify any sex dimorphisms. Although several markers of hydration such as plasma and urine have been used previously, saliva is also considered to be a useful and accurate indicator of hydration status even though individual variation may be large [11, 29, 30, 32, 34]. At regular intervals, saliva was collected from the oral cavity as a passive expectorant (unstimulated) [29, 32] and following mechanical (stimulated) oralfacial movement (chewing). For each salivary sample (unstimulated and stimulated), we determined salivary osmolality (S_{osm}) and plotted S_{osm} against the percent of body mass lost (**Figure 3**). For display purposes, we represent the data as binned samples ± standard deviation (S.D.).

Stimulated S_{osm} was significantly (positively) correlated with percent of body mass loss for both females and males. As illustrated in **Figure 3**, S_{osm} progressively increased during exercise bouts paralleling lost body mass. The increasing S_{osm} was primarily through increased water loss through sweat, which indicates significant dehydration. Importantly, the relationship of S_{osm} and percent body mass loss was not different between females and males.

Because every participant performed the exercise trial three times per the counterbalanced study design, we wished to compare stimulated and unstimulated S_{osm} in each arm of the exercise trial completed by the subjects. The experimental protocol required subjects to be euhydrated at the beginning of each exercise bout. Using a repeated measures 2-way ANOVA followed by a post-hoc Bonferroni analysis, we compared the three study arms, sex, and the interaction between study arms and sex. It should be noted that grouping based on fluid

designation (**Deep**, **Sports**, or **Spring**) only represents the initial arm since a specific rehydrating fluid has not been introduced at this point in the study protocol. No significant differences in baseline S_{osm} among study groups based on fluid designation were detected, validating that subjects began each arm of the three trials at the same hydration level (**Figure 4**). Importantly, baseline S_{osm} was not effected by sex in the stimulated (females 94.39 ± 14.90 vs males 113.00 ± 63.84), or the unstimulated (females 94.06 ± 26.62 vs males 95.51 ± 33.27) saliva samples, and there was no interaction between study group designation and sex. We conclude that female and male subjects started each of the three trials at the same level of hydration. Peak S_{osm} (stimulated and unstimulated) was taken as the value for S_{osm} once subjects reached at least 3% body mass loss. Similar to baseline, peak S_{osm} was not significantly impacted by either study group designation or sex in the stimulated (females 180.29 ± 60.37 vs males 256.96 ± 104.57), or the unstimulated (females 235.04 ± 105.99 vs males 297.14 ± 102.39) saliva samples. Moreover, there was no significant interaction between these factors on peak S_{osm} (**Figure 4**).

Again, considering no significant effect of study group designation or sex at baseline or peak, we calculated an average S_{osm}, which represents the averaged value from the three trial arms, for each subject. Using a two-way ANOVA, we were able to assess whether there was a difference in baseline and peak S_{osm} (simulated or unstimulated) and whether this was impacted by the sex of the subject. Baseline or Peak S_{osm} was not different when comparing females and males. At the completion of 3% body mass loss, all subjects achieved a significant increase in averaged S_{osm} over baseline values. However, this elevation in S_{osm} was not affected by the sex of the subject (**Figure 5A, 5B**).

Figure 3.

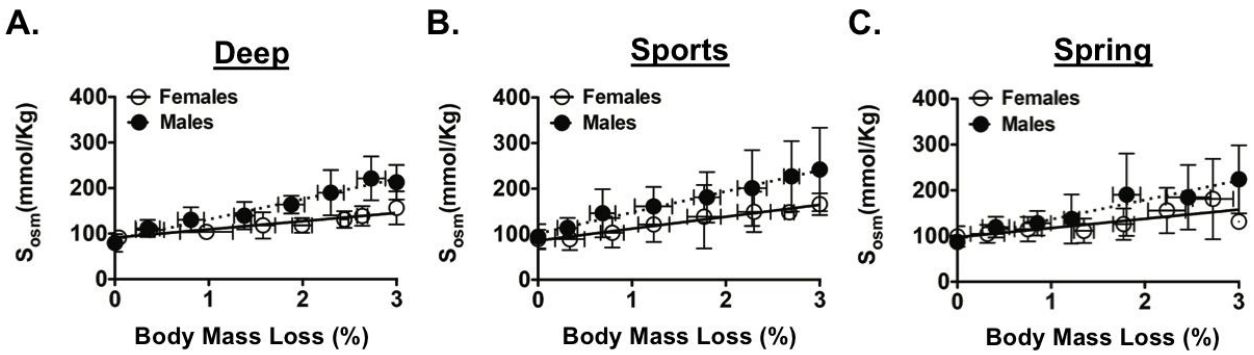

Figure 3: Salivary osmolality as a function of body mass loss. Salivary osmolality (S_{osm} mmol/Kg) was determined at regular intervals throughout the dehydration protocol. S_{osm} was plotted as a function of change in body mass percentage in each of the three groups: Deep (**A**), Sports (**B**) or Spring (**C**) groups. Data presented as binned samples ± standard deviation (S.D.).

Figure 4.

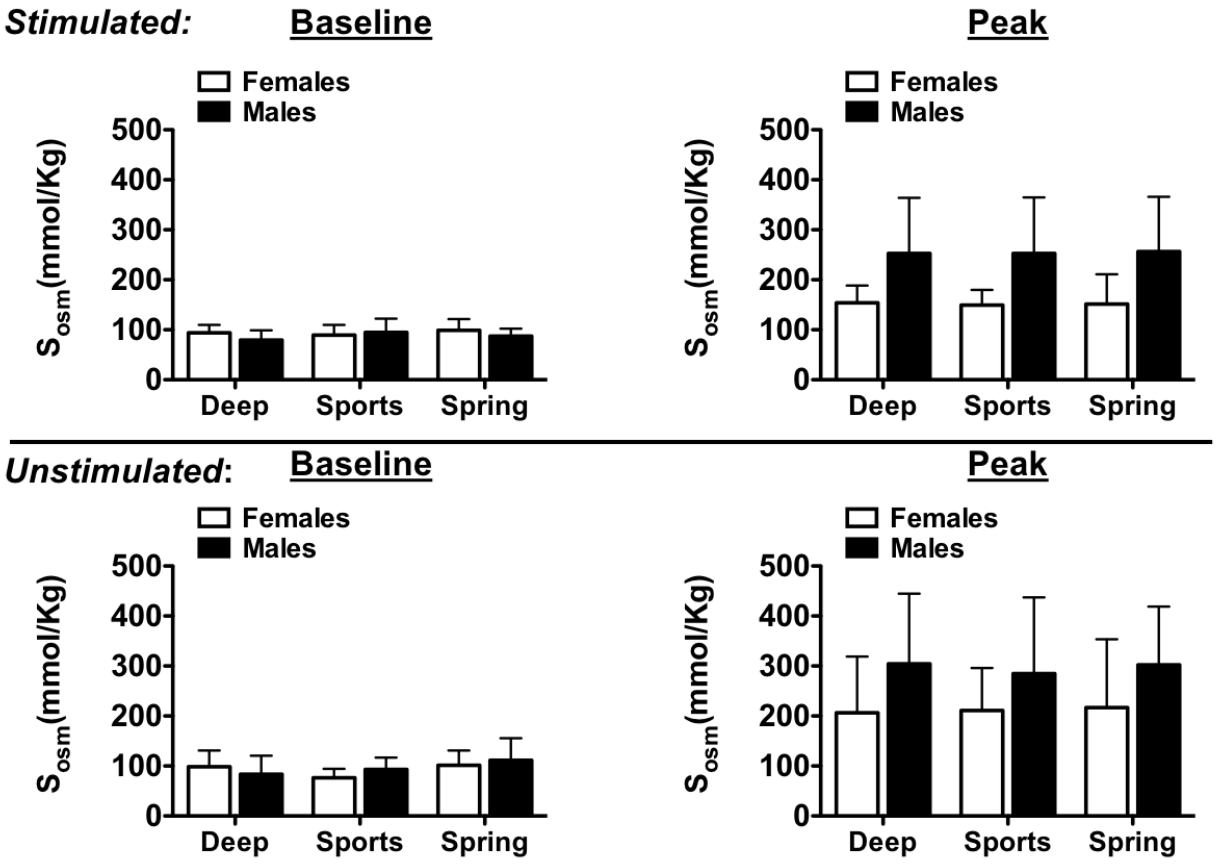

Figure 4: Salivary osmolality at baseline and peak dehydration. Bar graph representation of salivary osmolality (S_{osm} mmol/Kg) at the indicated timepoints in the **Deep**, **Sports** and **Spring** groups collected as either stimulated or unstimulated salivary. **Top Panel:** Stimulated S_{osm} (mmol/Kg) at **Baseline** and **Peak** in both **Females** and **Males**. **Bottom Panel:** Unstimulated S_{osm} (mmol/Kg) at **Baseline** and **Peak** in both **Females** and **Males**. A repeated measures two-way ANOVA indicated no significant differences in S_{osm} (**Stimulated** or **Unstimulated**) when comparing trial groups (**Deep**, **Sports** and **Spring**) or between **Females** and **Males**. Data presented as mean ± S.D.

Figure 5.

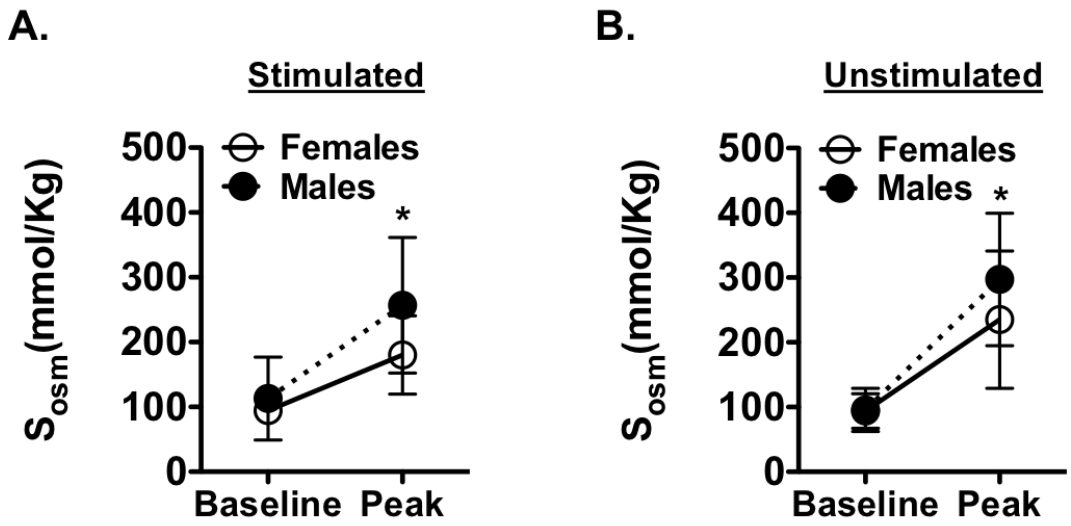

Figure 5: Impact of dehydrating exercise on salivary osmolality. Individual measures of salivary osmolality were averaged from the three trials. Salivary osmolality (S_{osm} mmol/Kg) was significantly elevated (*$p<0.001$; Females and Males) at **Peak** compared to **Baseline** collected as either **Stimulated** (**A**) or **Unstimulated** (**B**). No difference between **Females** and **Males** was detected nor was there an interaction between sex and timepoint of data collection. Two-way ANOVA with post-hoc Bonferroni analysis. Data presented as mean ± S.D.

Time to Body Mass Loss.

As stated above, the primary endpoint of this study was to achieve at least a 3% loss in body mass. Considering this endpoint, we did not rigorously monitor power output while on the stationary bicycle. However, we required subjects to maintain between 150 to 200 watts as detailed in the Methods section, which was primarily self-monitored. Returning to a repeated measures two-way ANOVA, we compared the expended time for subjects to achieve a 3% body mass loss in each study arm (study group designation) and whether this time was different for females and males. We were not surprised to find that subjects (female and male) did not differ in the time to achieve a 3% body mass loss between the 3 trials, indicating consistency in exercise performance (**Figure 6A**). Interestingly, we found a significant effect ($P<0.01$) of sex on this measured parameter with no interaction between study group designation and sex. Subsequent comparison of the mean values for each subject demonstrated that males took less time (90.0 ± 18.3 minutes; $P<0.01$) to reach the required body mass loss when compared to females (127.1 ± 20.0 minutes) (**Figure 6B**).

Assuming that body mass loss during the exercise period was due to water loss, we calculated sweat rate using the absolute mass of lost body mass and the total time to reach 3% body mass loss (**Figure 6C**). Based on the averaged values of sweat rate for each subject, females (15.3 ± 3.2 ml/min; $p<0.01$) demonstrated lower sweat rates compared to males (25.2 ± 7.8 ml/min), consistent with the greater time to achieve 3% body mass loss in the female group.

Impact of Fluid on S_{osm} Recovery (Rehydration).

Starting at similar baseline S_{osm}, subjects demonstrated a progressive, but significant, increase in S_{osm} concomitant with a loss in body mass from exercise. Moreover, these values in

S_{osm} were not significantly different between females and males. Next, we wished to determine how fluid type affected the rate of hydration recovery. Once subjects reached the appropriate loss in body mass, they were asked to consume a volume of fluid (**Deep**, **Sports**, or **Spring**) equal to their body mass loss in two phases as described in the Methods. Because previous work demonstrated that a simple oral rinse returns S_{osm} to baseline values and eliminates the diagnostic value of saliva as a hydration marker [29], we first determined that 10 minutes following fluid intakes removes the confounding effect of an oral rinse. Then, we collected saliva samples (simulated and unstimulated) every 5 minutes until the second phase of fluid intake. Ten minutes following the second fluid ingestion, saliva was sampled and continued every 5 minutes for 75 minutes of total saliva collection time.

As mentioned above, peak S_{osm} (stimulated and unstimulated) was taken as the value when subjects reached at least 3% body mass loss. Peak S_{osm} steadily declined and returned to the baseline, euhydrated S_{osm} values before completion of the saliva collection time. Using a mono-exponential model to fit these data, we determined the rate constant (K), time constant (τ), and the time constant half-life ($\tau_{1/2}$) for each subject in each trial (**Figure 7A**). We compared fit parameters with a repeated measures two-way ANOVA and found a significant effect of rehydrating fluid ($p<0.0001$) that was not impacted by sex. Post-hoc analysis revealed that K, τ, and $\tau_{1/2}$ determined for the **Deep** group were significantly different from both **Sports** and **Spring** groups in both females and males (**Figures 7B-7D**). These values are presented in **Table 4**. An elevated rate constant (K) and subsequent faster time constant (τ) and time constant half-life ($\tau_{1/2}$) suggests a more rapid return to baseline S_{osm}, or rehydration when subjects consumed Kona Deep. The same trend of significance was seen whether S_{osm} was taken from the stimulated or the unstimulated samples.

Figure 6.

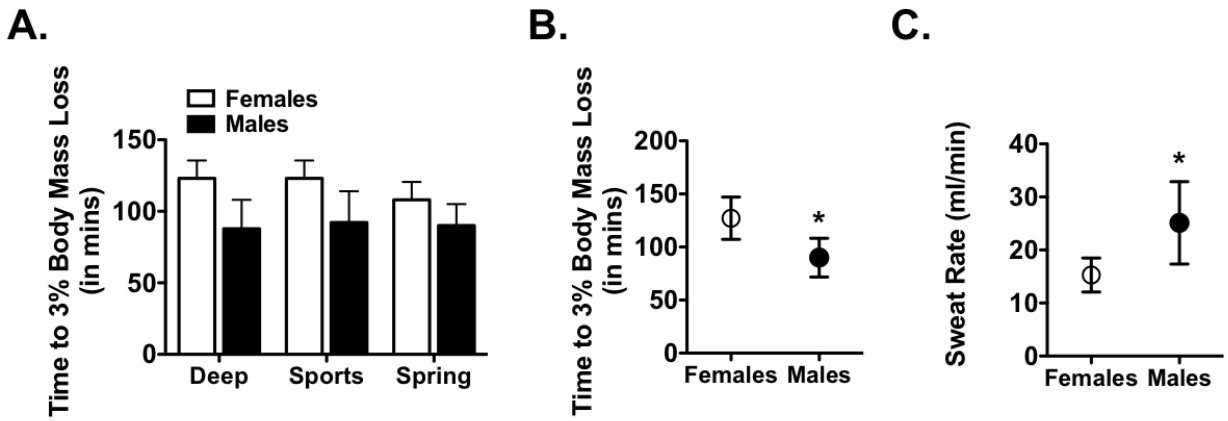

Figure 6: Time required to achieve target dehydration indicated by a 3% body mass loss. A: Bar graph representation of the time required to reach a loss of body mass that was at least 3% starting body mass in the **Deep**, **Sports** and **Spring** groups for **Females** and **Males**. A repeated measures two-way ANOVA indicated no significant differences in **Time to 3% Body Mass Loss** among the experimental groups but did find a significant effect of sex ($p<0.01$). **B:** Averaged values across experimental groups for **Time to 3% Body Mass Loss** in **Females** and **Males**. (*$p<0.01$; **Females** vs. **Males**) **C:** Averaged values across experimental groups for **Sweat Rate** (ml/min; total volume lost in ml over total time to 3% body mass loss;) in **Females** and **Males**. (*$p<0.01$; **Females** vs. **Males**). Two-way ANOVA with post-hoc Bonferroni analysis. Data presented as mean ± S.D.

Figure 7.

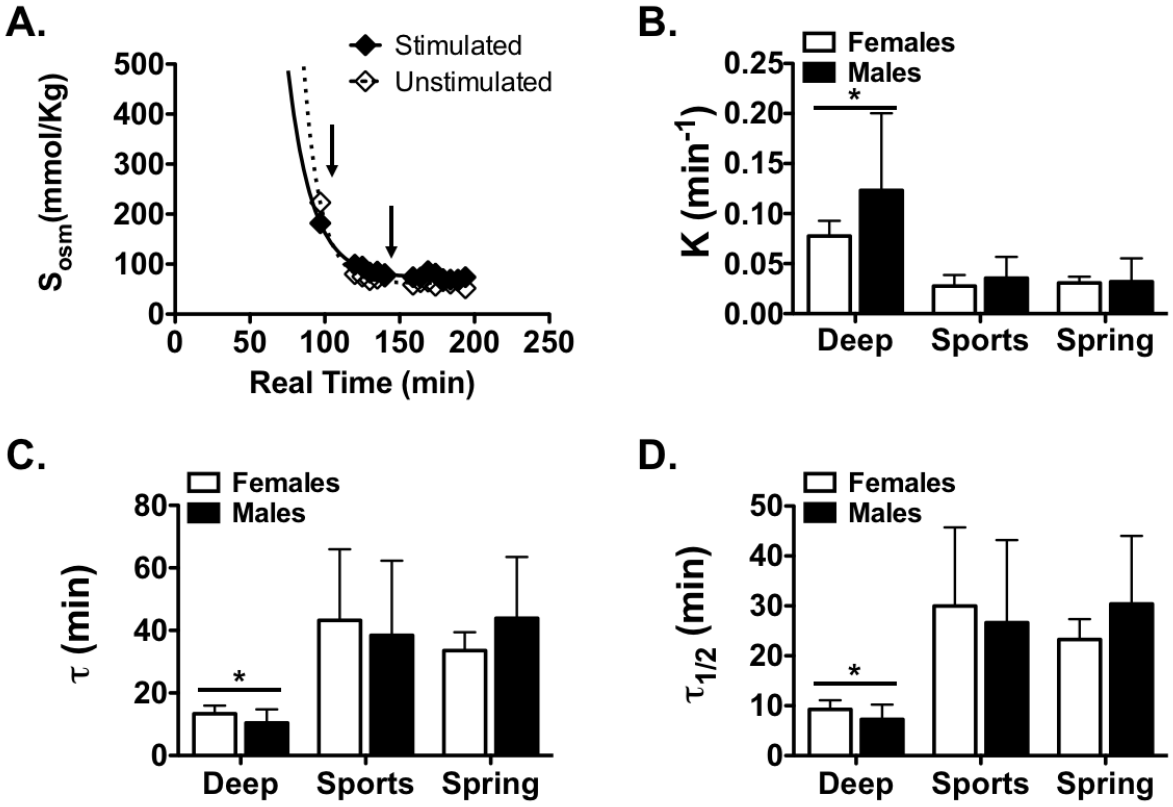

Figure 7: Rate of salivary osmolality recovery during fluid hydration following dehydrating exercise protocol. Salivary osmolality was fit with a single exponential decay (one-phase decay) starting with peak salivary osmolality against real time. **A:** representative one-phase decay fit to salivary osmolality recovery during fluid hydration. Fluid was ingested in two phases indicated by the arrows. **B-D:** Bar graph representation of one-phase decay fit parameters (**K**, **tau (τ)**,and $\tau_{1/2}$) in **Females** and **Males** from the **Deep**, **Sports** and **Spring** groups. A repeated measures two-way ANOVA determined a significant impact of fluid on rate parameters of hydration that was not impacted by sex. Post -hoc Bonferroni analysis indicated a significant difference in the **Deep** group compared to both **Sports** and **Spring** groups (*$p<0.0001$ compared to **Sports** and **Spring** groups). One-phase decay equation: $S_{osm}=(S_{osm(peak)}- S_{osm(baseline)})^{Kt} + S_{osm(baseline)}$; K=rate constant, tau (τ) =1/K, and $\tau_{1/2}$ = half-time S_{osm} recovery. Data presented as mean ± S.D.

Table 4. Recovery of Salivary Osmolality (S_{osm})	**Females (n=8)**	**Males (n=9)**
Stimulated S_{osm}		
Deep		
K (min^{-1})	0.077 ± 0.015	0.123 ± 0.077
τ [37]	13.3 ± 2.6	10.5 ± 4.3
$\tau_{1/2}$ [37]	9.2 ± 1.8	7.2 ± 3.0
Sports		
K (min^{-1})	0.028 ± 0.011	0.035 ± 0.021
τ [37]	43.2 ± 22.7	38.5 ± 23.8
$\tau_{1/2}$ [37]	30.0 ± 15.8	26.6 ± 16.5
Spring		
K (min^{-1})	0.031 ± 0.006	0.032 ± 0.023
τ [37]	33.6 ± 5.9	43.9 ± 19.6
$\tau_{1/2}$ [37]	23.3 ± 4.1	30.4 ± 13.6
Unstimulated S_{osm}		
Deep		
K (min^{-1})	0.085 ± 0.017	0.106 ± 0.0.38
τ [37]	12.3 ± 2.7	10.3 ± 2.7
$\tau_{1/2}$ [37]	8.5 ± 1.8	7.2 ± 1.8
Sports		
K (min^{-1})	0.028 ± 0.013	0.030 ± 0.014
τ [37]	42.0 ± 16.6	32.9 ± 9.6
$\tau_{1/2}$ [37]	29.1 ± 11.5	22.8 ± 6.6
Spring		
K (min^{-1})	0.028 ± 0.011	0.032 ± 0.023
τ [37]	42.1 ± 13.3	40.0 ± 16.1
$\tau_{1/2}$ [37]	29.1 ± 19.1	27.7 ± 11.2

Dehydration, Rehydration, and Exercise Performance.

Target body mass loss of 3% results in a significant performance deficit [11, 12, 16]. To monitor performance, study subjects performed a series of 3 maximal contractions of the left knee extensors using a Biodex™ dynamometer, which measures peak torque extension. We implemented this maneuver prior to exercise (**Baseline**), immediately after exercise (**Post-Exercise**), and after the final rehydrating phase (**Post-Hydration**). These data are summarized in **Table 5**.

Overall, males generated greater peak torque extension at baseline when compared to females (308.33 ± 57.6 vs 172.8 ± 10.5). Despite considerable variability in peak torque extension among individuals, female and male subjects completed the peak torque extension maneuver similarly at the beginning (**Baseline**) of each trial arm. To quantify the impact of dehydration on performance in each individual, we determined the percent loss of peak torque extension at the end of 3% body mass loss relative to baseline peak torque extension. Immediately following loss of 3% body mass (**Post-Exercise**), subjects displayed a significant performance deficit in peak torque extension, but this deficit (expressed as a percent loss from baseline peak torque) was not different between females and males in any of the three groups. (**Figure 8A**). We performed a repeated measures two-way ANOVA to compare fluid designation and sex as the main factors. Although subjects in each study group failed to fully recover **Baseline** peak torque extension following rehydration, there was a significant effect of the rehydrating fluid and sex ($p<0.01$) on the percent peak torque recovery (**Figure 8B**). Post-hoc analysis identified a significant difference between the **Deep** and **Sports** groups ($p<0.05$) in females.

Table 5. Peak torque Extension Normalized to Body Weight (%N-m)

	Females (n=8)			Males (n=8)		
	Baseline	**Post-Exercise**	**Post-Hydration**	**Baseline**	**Post-Exercise**	**Post-Hydration**
Deep	171.4 ± 44.7	169.1 ± 25.0	162.1 ± 24.2	295.7 ± 10.9	264.0 ± 43.6	265.0 ± 50.1
Sports	186.6 ± 34.9	179.6 ± 24.1	163.1 ± 44.5	316.7 ± 37.9	282.9 ± 52.9	272.8 ± 51.0
Spring	176.7 ± 35.6	167.8 ± 34.8	161.4 ± 42.1	317.4 ± 69.5	269.5 ± 54.8	279.6 ± 57.8

Figure 8.

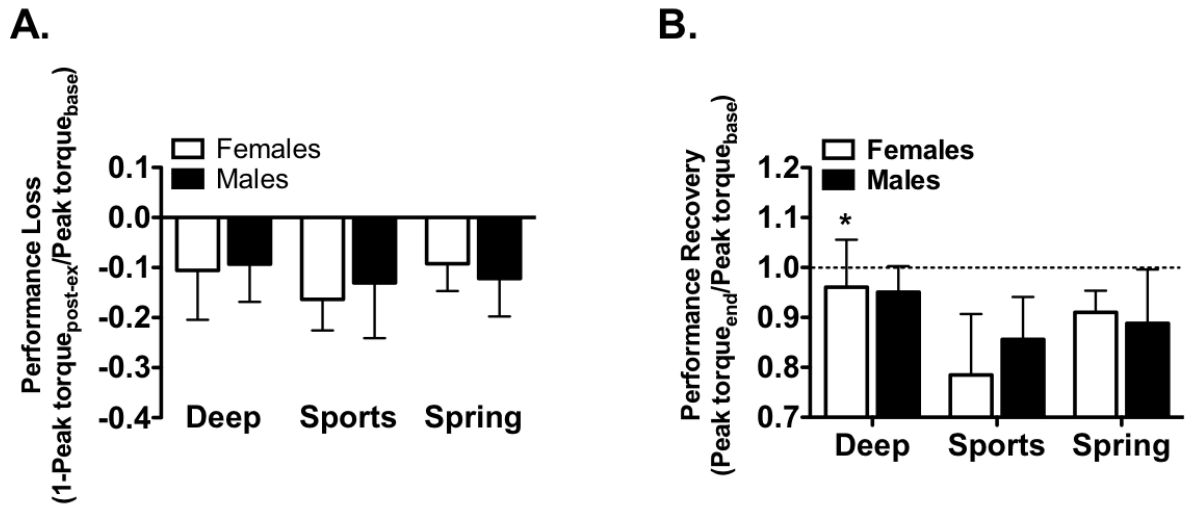

Figure 8: Impact of dehydration and hydration on lower body muscle performance. Peak torque extension using Biodex™ was determined prior to and immediately following exercise protocol, and then 75 minutes post exercise and following complete rehydration of the study fluid. **A:** Bar graph representation of the decrease in peak torque extension at the completion of the dehydration protocol expressed as percentage lost from baseline peak torque (1- Peak $Torque_{post\text{-}ex}$/Peak $Torque_{base}$). A repeated measures two-way ANOVA determined that all experimental groups (**Deep**, **Sports** and **Spring**) experienced a significant loss of peak torque extension following the dehydration ($p<0.01$) but were not different from each other or between sexes. **B:** Bar graph representation of the recovery in peak torque extension at the completion of the hydration protocol expressed as percentage recovery to baseline peak torque (Peak $Torque_{end}$/Peak $Torque_{base}$). A repeated measures two-way ANOVA determined a significant impact of hydrating fluid ($p<0.05$) and sex ($p<0.05$). Post-hoc Bonferroni analysis indicated a significant difference between the **Deep** and **Sports** groups in **Females** (*$p<0.05$). Peak $Torque_{post\text{-}ex}$: peak torque extension at 3% body mass loss; Peak $Torque_{base}$: peak torque extension at baseline prior to initiation of dehydrating exercise; Peak $Torque_{end}$: peak torque extension at the end of hydration protocol. Data presented as mean ± S.D.

Discussion

The goals of the study were to evaluate male and female differences in dehydration physiology, and then to determine if hydration recovery was dependent on sex or rehydrating fluid. We found that males tended to reach 3% body mass loss faster than females, and males and females both showed similar increases in salivary osmolality due to dehydration. Males and females also exhibited similar deficits in muscle strength after the dehydration protocol. Deep-ocean mineral water had a significant beneficial effect on hydration recovery, for both males and females, compared to mountain spring water and the sports drink. Recovery of muscle strength after rehydration was affected by fluid and sex, with a significant difference seen between the **Deep** and the **Sports** groups in females.

Age and Body Morphometrics

For each subject, body weight did not change across each of the three trials Weight measurements and calculations (body mass, body mass index (BMI), and body surface area) represent an average across the three trials for each subject. Males had a significantly greater height, body weight, and body surface area than females, while females had a higher ratio of body surface area to body mass, consistent with reports from other studies [5, 6]. Average age and BMI were not different between males and females. The lower relative body surface area of females is thought to be one of the reasons that females have a greater density of sweat glands than males. Humans acquire their maximum number of sweat glands at a very early age, and the amount of growth which occurs after that age determines how spread out one's sweat glands are. Since females typically grow less than males, their sweat glands are typically more concentrated than males, due to the relative lower body surface area [4]. Despite females having greater

density and absolute number of sweat glands than males [4], they typically exhibit lower maximal sweat rates than males [5, 6]. Based on the averaged values of sweat rate for each subject, females (15.3 ± 3.2 ml/min; $p<0.01$) demonstrated lower sweat rates compared to males (25.2 ± 7.8 ml/min), consistent with the greater time to achieve 3% body mass loss in the female group.

Heart rate and body temperature.

During the exercise dehydration protocol, we constantly monitored and recorded heart rate in order to assess the effect of progressive dehydration due to exercise on the increase in heart rate. Given that the only distinction between the three study arms was the rehydrating fluid, we did not expect to see any differences in any of the pre-hydration parameters (heart rate, body temperature, sweat rate, time to 3% dehydration, body morphometrics, and salivary osmolality during dehydration) within each subject between the three trials, which held true for all listed parameters. Since each subject's baseline and peak heart rate values were not different across the three trials, we were able to average the values from the three trials to get representative baseline and peak heart rates for each subject. As the purpose of this study was not to measure the isolated effects of exercise or dehydration on heart rate, we do not report a compounded effect of dehydration and exercise versus exercise alone, only that the combination of the two factors produced significantly increased heart rates in both males and females compared to baseline, as expected. Although we did not isolate the effect of dehydration on heart rate from that of exercise on heart rate, previous studies have extensively reported that dehydration exacerbates the heart rate-increasing effects of exercise [11, 35]. In addition, we assessed whether the impact of exercise and dehydration on heart rate was different for male and female athletes. Comparing

heart rate values in men and women, we saw no significant differences between the two in terms of baseline, peak, or the increase in heart rate throughout the exercise phase. This is consistent with many studies in the literature reporting statistically similar increases in heart rate for women and men[36-39], although at least one study reports a greater increase in peak heart rate in men than in women in response to exercise in the heat [40].

Since it has been shown that sex differences may exist in thermoregulatory capabilities following heat stress and exercise [5], we also assessed body temperature throughout the exercise protocol. Subjects were exposed to lamps during exercise to maintain temperature at a value greater than room temperature. We compared baseline and peak values, and assessed whether these were different between men and women. We used tympanic temperature as our measure of body temperature, as studies have validated that tympanic temperature is a reasonably accurate estimate of core temperature [41]. Like heart rate, values of baseline and peak tympanic temperature represent averaged values across the three studies for each subject. Although much literature supports the idea that exercise, heat, and dehydration impair thermoregulation [5, 11, 13], we only saw a slight and insignificant increase in tympanic temperature throughout the exercise phase for men and women.

We hypothesized that a dehydration of 3% body mass loss would be sufficient to elicit significant negative effects in thermoregulation, and therefore significant increases in body temperature upon reaching this level of dehydration. In fact, several studies show that even a dehydration of 2% body mass loss is sufficient to cause impaired thermoregulation as well as exercise performance decrements [11, 12, 16]. Many factors can play into this inconsistency. One review reports that exercise in temperate environments, and exercise times of less than 90 minutes did not result in thermal strain [11], and several of the subjects in our study completed

the exercise phase of each trial in 90 minutes of exercise or less. Other studies suggest that the specific effects of dehydration, including thermoregulatory effects, depend on the specific method and conditions of dehydration, and the training status of the athlete [11, 14]. We did attempt to control for training status of the athletes by matching subjects for fitness level based on self-report, but individual differences in these factors could have had a role in the overall lack of temperature increase.

More likely, heat acclimation may be at least partially responsible for this observation. Acclimation to exercising in hot conditions may provide the athlete with the benefit of expanded erythrocyte volume, and plasma volume, both of which have the potential to improve thermoregulatory ability in athletes [35]. We did not account for heat acclimation in this study, but it is reasonable to infer that some or all of the athletes in the study likely had some experience exercising in the heat, as they live in Southern Arizona, a region with a hot, dry climate throughout most of the year. We saw no significant difference in tympanic temperature between males and females, in baseline or peak values. This was not surprising, because although males and females differ in some specific aspects of thermoregulation during exercise in the heat, it is thought that females and males are able to maintain body temperature with similar efficiency [5]. Although women have markedly lower sweat rates than men, they possess superior evaporative cooling efficiency, due to their larger average BSA:BM ratio, so thermoregulatory ability of men and women is essentially balanced by these factors [5, 6].

Exercise and Salivary Osmolality.

To assess the subjects' hydration status throughout the exercise protocol, we measured stimulated and unstimulated salivary osmolality, as outlined in the Methods, at baseline and at

15-minute intervals during exercise, and plotted these values against the percent body mass loss achieved at each time point for each subject. Peak salivary osmolality was determined as the measured osmolality when subjects reached 3% body mass loss. As with the other pre-hydration parameters, no differences existed in baseline, peak, or the increase in salivary osmolality relative to body mass loss, between the three study trials for each subject. Again, this was expected since the rehydrating beverage was not introduced until the rehydrating phase. Values were averaged across the three trials for each subject. For each subject, we compared average baseline salivary osmolality with average peak salivary osmolality, and found a significant increase from baseline to peak in each subject, male and female, and in both stimulated and unstimulated saliva samples. Sweat is hypotonic due to reabsorption of ions in sweat ducts, meaning subjects were losing more water than solute. This causes an increase in body fluid osmolality as water loss through sweat increases [7]. Accordingly, we observed a significant positive correlation between salivary osmolality and percent body mass loss during the exercise phase. This indicates that the body mass loss observed was primarily due to dehydration. Next, we compared salivary osmolality values in males and females, because we were interested if there were any sex differences in hydration status throughout the exercise protocol. Similar to when we compared salivary osmolality between the three study groups, we found no significant difference in baseline, peak, or the increase in salivary osmolality relative to body mass loss, between men and women, and there was no significant interaction between study group and sex on these values. These findings indicate to us that subjects began all trials in a euhydrated state, and men and women began and ended the exercise phase with the same hydration status.

Time to Body Mass Loss.

Time was recorded throughout the entire protocol for each subject, and total time to achieve a 3% reduction in body mass was compared between the 3 trials, and between men and women. As stated, group designation should have no effect on time to dehydration for each individual, as each trial was identical in procedure up until dehydration was achieved. Indeed, we saw no significant difference in time to dehydration between the three groups, so each subject's time from each of the three trials were averaged. Furthermore, there were no interactions between group designation and sex. This is an important observation, because it indicates that exercise performance was consistent for each subject throughout the study. While group designation should have had no effect on dehydration time, it was possible for dehydration times to have varied within a subject, due to varying power output. This could have been different across the three trials for each subject based on inconsistencies in factors that were not well-controlled for, such as motivation, diet, sleep, or mood surrounding each of the three trials [42-45]. Fortunately, our results indicate that this was not the case, and there was no significant variation within each subject in times to dehydration. Men in this study took significantly less time to reach a 3% body mass loss than women. One possible explanation for this is that men may have had higher average power output on the stationary cycle. As the desired outcome of the exercise phase was simply that the subjects achieved a 3% reduction in body mass, we did not measure power output, and so we cannot confirm any difference in power between men and women. However, informal/anecdotal observation during the study protocol suggests sex differences in power output. We instructed the subjects to maintain 150-200 watts of power, which subjects self-monitored, and constantly adjusted during exercise by altering the resistance of the cycle, and it was noted that men tended to use greater resistance throughout the workout,

which would be consistent with research stating that men tend to have a higher aerobic workload capacity than that of women [6]. Another factor contributing to this sex difference in dehydration time is sweat rate. Based on the literature, women have lower average maximal sweat rate than men [5, 6], and this would definitely increase time to dehydration.

Since we were able to determine that the body mass loss which occurred during the exercise phase was primarily due to fluid loss, we calculated sweat rate in each subject by dividing the actual amount of weight lost by the total time to achieve 3% body mass loss for each of the three trials. Similar to time to reach dehydration, sweat rate was not different between the three trials for each subject, so the three calculated sweat rates for each subject were averaged. Comparing these averages, we confirmed previous findings [5, 6] that women have significantly lower sweat rates than men, and this was not affected by group designation. We confirm that a lower sweat rate in women is likely a large contributor to the increased time to reach dehydration compared to men. Reasons for lower observed sweat rates in females have been explored in other studies. Females have a greater density of sweat glands than males [4], and it is thought that one reason for this may be the lower relative body surface area of females compared to males. Humans acquire their maximum number of sweat glands at a very early age, and the amount of growth which occurs after that age determines the density of one's sweat glands. Since females typically grow less than males, their sweat glands are typically more concentrated than males, due to the relative lower body surface area [4]. While females have a higher density of sweat glands than males [4], the efficiency and maximal sweat rate of each gland is dependent in part on gland size [3, 46]. Although it is still unclear if females naturally have smaller average sweat gland sizes than males, those who are described as poor sweaters do exhibit much smaller gland sizes than those described as good sweaters [46]. In addition, sweat gland size can increase as an

adaptation to endurance training, and this effect seems to be more exaggerated in males than in females [47].

Another contributing factor to sex differences in sweat rate may be the difference in average total body water for men and women. Women have, on average, lower total body water than men [1], and it has been seen that lower volumes of body water may negatively affect sweat rate compared to higher relative volumes [3]. Therefore, it has been suggested that women regulate their body fluid loss from sweat more tightly than men, due to their lower body fluid volume [47]. This effect may also be influenced by a woman's menstrual status [14], which we attempted to capture using the health questionnaire, but was not well-controlled for when assessing sweat rate. Differences in total body fluid between men and women could also differentially affect muscle performance, as lower initial body water volume has been shown to result in greater performance deficits due to dehydration [11]. This will be discussed in the later section on dehydration, rehydration, and exercise performance.

Impact of Fluid on S_{osm} Recovery (Rehydration).

During the exercise phase, salivary osmolality increased as a function of body mass reduction from fluid loss. After the exercise phase, when subjects reached dehydration, defined by a 3% body mass loss, we wanted to assess the effects of the three rehydrating fluids (**Deep**, **Spring**, and **Sports**) on re-hydration rate, defined as the time for salivary osmolality to return to baseline values during the rehydration phase. Based on previous findings which suggest improvements in aerobic performance and muscle strength as a result of deep-ocean mineral water supplementation [16, 20], we hypothesized that Kona deep-ocean mineral water (**Deep**) would improve the rate of hydration recovery, and improve muscle strength after rehydration,

relative to the other rehydrating fluids (**Sports** and **Spring**). By measuring salivary osmolality (both stimulated and unstimulated), as outlined in the Methods section, over the 75-minute period of progressive rehydration, we found that each subject's salivary osmolality returned to baseline values after the rehydration period of each trial. This decrease in salivary osmolality as a result of increased hydration was not surprising, given that dehydration had the opposite effect. By fitting each subject's values of salivary osmolality at every time point during the rehydration phase of each of the three trials to a mono-exponential model, we determined a rehydration curve for each subject, from each trial.

For each subject, we then compared these rehydration curves between the three trials. Results indicated that the **Deep** group showed a significantly more rapid return to baseline salivary osmolality than the **Sports** and **Spring** groups, as evidenced by an elevated rate constant (K), and faster time constant (τ) and time constant half-life ($\tau_{1/2}$) on a mono-exponential (one-phase decay) model, indicating more efficient hydration capabilities of this fluid. This effect was seen when analyzing the stimulated as well as the unstimulated saliva samples, and was not influenced by sex. No significant differences in these parameters were seen between the **Sports** and **Spring** groups. This study is the first, to our knowledge, to show an improvement in hydration recovery due to rehydration with a deep-ocean mineral water compared to mountain spring water or a carbohydrate-based sports drink. We propose that these effects may be due to the unique mineral content of the deep-ocean mineral water (**Table 1**), although precise mechanisms will need to be explored in future studies. Furthermore, we propose that the hydration-enhancing effects of deep-ocean mineral water may be partially responsible for the improved athletic performance effects of deep-ocean mineral water seen in our study, and in other studies [16, 20, 34], though these studies did not measure markers of hydration.

Dehydration, Rehydration, and Exercise Performance.

In order to determine whether dehydration, and then rehydration with various fluids affected muscle performance, we measured lower-body muscle strength on a Biodex™ dynamometer. Subjects performed this test at the start of each trial, before the exercise phase (**Baseline**), after the exercise phase, when a 3% body mass loss was achieved (**Post-Exercise**), and after the 75-minute rehydrating phase (**Post-Hydration**). Baseline values indicate that males generated greater peak extension torque than females, and there was no significant difference in these values between study groups, for males or females. Similarly, post-exercise values were not different between groups for males or females. Baseline and post-exercise values were averaged across the three trials for each subject, and muscle performance deficit was determined as a percent loss from baseline peak torque. Post-exercise values were significantly lower for all subjects, male and female, and there was no difference in muscle performance deficit between males and females. This is contrary to the prediction that we might see increased performance deficits in females, compared to males, as a result of their lower relative initial body fluid volume [11]. Using a repeated measures two-way ANOVA, we compared post-exercise peak torque with post-hydration values based on fluid designation and sex. Although none of the subjects in any of the three groups were able to recover full baseline muscle performance after rehydration, we did see a significant fluid effect that was sex dependent ($p<0.01$). Post-hoc analysis revealed a significant difference between the **Deep** and the **Sports** groups in females. Although post-hoc analysis was not able to identify specific differences between any other groups, these results indicate that there is a beneficial effect of deep-ocean mineral water on muscle strength recovery after a dehydrating exercise, and that females are the main driver of this effect.

In a 2007 review, Judelson et al outlined important criteria related to the ability of a study to accurately assess the effects of dehydration on muscle performance. In brief, valid measures of strength decrement due to impaired hydration status should be isolated from exacerbating or masking factors. Exacerbating factors include alterations in body mass not due to dehydration (such as by calorie restriction), increases in muscle and/or core temperature, and muscle fatigue caused by the study protocol. Masking factors include training status of the subjects [48-51], impact of menstrual cycles (in females), and the impact of body mass-based tests (such as vertical jumping) that preclude a direct relationship between hydration and muscle performance [14]. Our study showed a decrease in muscle strength due to dehydration independently from increases in core temperature, as subjects' core temperatures did not increase significantly throughout the protocol.

However, we cannot claim that this effect on muscle performance was necessarily independent on an increase in temperature of the working muscle, since we did not measure muscle temperature. Muscle temperature, although to a much lesser extent than core temperature, has been shown to elicit decrements in muscle strength [14, 52-54]. This could have been a confounding factor in this instance, since muscle temperature likely increased as a result of the exercise as well as the moderate heat stress to subjects, and we had no way to assess whether the muscle had sufficiently cooled before the post-exercise strength test.

Muscle fatigue due to the exercise protocol could have been another confounding factor, as we did not allow sufficient recovery time between the exercise and the strength test. However, we did maintain consistency between subjects, and between trials, in terms of the relative times for the strength measurements. Each subject performed the post-exercise muscle strength test on the Biodex™ immediately following 3% body mass loss during each trial. In order to mitigate

the effects of muscle fatigue on muscle strength, other studies have assessed muscle strength due to passive hydration [53, 55], but since our goal was specifically to assess the effects from dehydration due to exercise, this would not be appropriate. Allowing some time for recovery from exercise before having subjects complete the strength test may be a more practical approach in future studies assessing the effects of dehydrating exercise on muscle strength. We also failed to adequately control for menstrual status in females, adding that as a masking factor. We can, however, conclude with reasonable confidence, that the deficit we observed in muscle performance was isolated from the effect of body mass loss, as the body mass loss that did occur was due primarily to the dehydration. In addition, we reasonably controlled for training status of the subjects by matching them by self-reported fitness level to make sure subjects are at least similar within our study. Our measure of strength was appropriate, since it did not rely on subjects' ability to move their own body weight. These types of exercises will become less strenuous as the subjects lose body mass, and this can interfere with measures of muscle strength [14].

To conclude, well-conditioned subjects undergoing dehydrating exercise exhibited significant increases in heart rate and salivary osmolality, and significant decreases in body mass and lower-body muscle performance, with no differences based on sex. Males produced greater lower-body muscle power than females, and females exhibited lower sweat rates than males, and therefore took longer to reach dehydration of 3% body mass loss. Kona deep-ocean mineral water improved the rate of rehydration after the dehydrating exercise for both males and females, and improved muscle performance recovery, which was mainly driven by a female sex effect. Although dehydration due to exercise did result in reduced muscle performance in males and females, we cannot confirm that dehydration had an isolated effect on muscle strength, due to

possible confounding factors. Future studies would aim to adjust for these factors to more directly measure the effects of dehydration on muscle strength. For example, allowing sufficient time for muscles to recover after exercise before measuring muscle strength would mitigate the confounding effects of muscle fatigue on muscle performance. This recovery time would also allow muscles to cool, thereby diminishing the confounding effects of elevated muscle temperature on muscle strength.

Future studies may alter aspects of the exercise protocol, including lengthening the follow-up period to 24-48 hours and monitoring subjects throughout. This would provide insight into the lasting effects of deep-ocean mineral water on hydration and exercise performance. This would be very applicable to athletes participating in sports with competitions on multiple consecutive days, giving the athletes only a short period for recovery between competitions. Testing the hydrating effects of deep-ocean mineral water in non-dehydrating conditions may also be more relevant since, in practical scenarios, athletes are usually hydrating to some extent during exercise, and not reaching 3% body mass loss from sweat. Adding a measure of aerobic performance, such as maximal oxygen consumption (VO_2 max), or a Wingate anaerobic test (WAnT), could provide supporting evidence for the effects of deep-ocean mineral water on aerobic capacity. Measuring aerobic capacity as well as muscle strength covers a wider range of athletic performance, and can help these findings become more generalizable. Utilizing other methods of measuring hydration, such as serum osmolality, could help clarify the physiology related to osmolality of different fluid compartments. When osmolality of interstitial fluid rises, fluid is drawn from intracellular stores, as well as from plasma [1]. When this rise in osmolality is due to fluid loss from sweat during exercise, this results in decreased blood volume, which negatively affects performance [11]. Whether or not this affects serum osmolality is unclear. All

of these changes to the current protocol could potentially make the results more applicable to a wider range of athlete situations. Other future studies may involve uncovering the mechanisms behind the hydration-enhancing properties of deep-ocean mineral water through cell or animal studies. For example, we conceptualized an experiment, in which an intestinal cell line, such as Caco-2, could be grown in culture and exposed to various fluids. We would then measure expression of aquaporin (water transport) channels in the intestinal cells. This would be done under the hypothesis that the mineral composition of deep-ocean mineral water may be enhancing expression of these channels, thereby enhancing water uptake in the gut. These types of future studies will help elucidate physiological mechanisms for the impact of fluid mineral composition on rehydration, and practical utility for this information.

Appendix A: University of Arizona Health History Screening Questionnaire (UAHHSQ)

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UNIVERSITY OF ARIZONA
HEALTH HISTORY SCREENING QUESTIONNAIRE (UAHHSQ)

Please complete thoroughly and accurately.

Date ____/____/____

Name:_____ Ethnicity:_____

Address:_____City:_____State:_____Zip:_____

Date of Birth: ____/____/____ Age:_____ Phone #:_____

Email:_____@_____

Emergency contact information: Name:_____ Phone #:_____

_____

Personal healthcare provider to contact in case of an emergency:

Name_____ Phone #:_____

City:_____

CARDIOVASCULAR HEALTH HISTORY

Have you ever been diagnosed with or had any of the following?

Heart Attack?	Yes	No
Heart Surgery ?	Yes	No
Cerebrovascular accident (e.g. Stroke)?	Yes	No
Transient Ischemic Attack (TIA)?	Yes	No
Carotid Artery Disease?	Yes	No
Cardiac Catheterization?	Yes	No
Coronary Angioplasty?	Yes	No
Pacemaker/Implantable Cardiac Device?	Yes	No
Irregular Heart Rate/Heart Rhythm Disturbance?	Yes	No
Atrial Fibrillation?	Yes	No
Heart Valve Disease?	Yes	No
Heart Failure?	Yes	No

Heart Murmur?	Yes	No
Heart Transplantation?	Yes	No
Congenital Heart Disease?	Yes	No

Have you ever experienced any of the following symptoms:

Chest discomfort with exertion?	Yes	No
Unreasonable breathlessness?	Yes	No
Dizziness, fainting, or blackouts?	Yes	No
Syncope (loss of consciousness)?	Yes	No
Hypoxia (low oxygen levels)?	Yes	No
Do you currently take heart medications?	Yes	No

If yes, what?_____

Have you been diagnosed with diabetes (Type 1 or Type 2) or problems with blood sugar levels?	Yes	No

If yes, please note Type 1 or Type 2_____

If you circled yes to any of the above statements in this section, consult your physician or other appropriate health care provider before engaging in exercise. You may need to use a facility with a medically qualified staff.

CARDIOVASCULAR RISK FACTORS

Are you a male over 45 years old?	Yes	No
Are you a female over 55 years old?	Yes	No
Have you had a hysterectomy?	Yes	No
Have you had both of your ovaries surgically removed?	Yes	No
Are you postmenopausal?	Yes	No
Do you currently smoke or have you quit within the last six months?	Yes	No
Is your blood pressure greater than 140/90 mm Hg?	Yes	No
	I Don't Know	

If known, what is your blood pressure? _____/_____ mm Hg

Do you currently take blood pressure medications?	Yes	No
Do you currently take any medications for your heart?	Yes	No
Is your total blood cholesterol level greater than 200 mg/dl?	Yes	No
	I Don't Know	

Do you know your cholesterol level? Yes No

If yes, Total Cholesterol _____

LDL _____

HDL _____

Triglycerides _____

Do you have a close blood relative who has suffered a heart attack or had any kind of heart surgery before the age of 55 (for father or brother) or age 65 (for mother or sister)? Yes No

Are you more than 20 pounds overweight? Yes No

I Don't Know

Are you physically inactive (i.e., do you get less than 30 minutes of physical activity less than three times a week)? Yes No

Have you had a recent surgery (in the past 2 years)? Yes No

Have you had an exercise stress test, heart catheterization, or echocardiogram? Yes No

If yes, please explain_____

_____

To the best of your knowledge, is there any reason that might make it **unsafe** for you to participate in exercise? Yes No

If you circled yes to two or more of the statements in the above section you should consult your physician or other appropriate health care provider before engaging in exercise. You might benefit from using a facility with a professionally/medically qualified exercise program and staff.

To the best of my knowledge, the information I have provided above is an accurate assessment of my health and medical history.

Name of Participant **Participant's Signature** **Date**

Name of Administering Staff **Signature of Staff Member** **Date**

Please stop here. The remainder of this Health History Screening Questionnaire will be administered to you by one of our staff.

STAFF: Administer the remaining portion of the UAHHSQ.

GENERAL MEDICAL HISTORY

Height:_____ Weight:_____ BMI (calculated):_____

Circle One

Do you drink alcohol? Yes No

If yes, how many drinks per week?_____

Are you taking any prescription or over-the-counter medication? Yes No

If yes, what medication and what dosage?_____

_____

Do you take any vitamins, supplements, or herbal/homeopathic medications? Yes No

If yes, what type and what dosage?_____

_____

Has your body weight been stable over the past 6 months? Yes No

If no, please explain_____

Have you been on a recent diet or a prescribed diet? Yes No

If yes, please explain_____

Have you been diagnosed with asthma, exercise-induced asthma, reactive airway disease, chronic obstructive pulmonary disease (COPD), or any other respiratory disease? Yes No

If yes, please describe:_____

_____

_____

Have you ever been diagnosed with cancer? Yes No

If yes, please describe when and what type:_____

_____

Have you ever undergone a lymphectomy? Yes No

If yes, please describe when and why?_____

_____

Do you have musculoskeletal problems that limit your physical activity such as walking? Yes No

Do you have concerns about your safety when you exercise or exert yourself? Yes No

Have you ever experienced burning or cramping sensations in your l legs when walking short distances? Yes No

Do you have any other health problems, illnesses, diseases, infections, surgeries, allergies, or hospitalizations? Yes No

If yes, please explain_____

_____

_____

FAMILY HISTORY

Please check all that apply

Family Member	High Blood Pressure	Diabetes Type I or II	Heart Diseases	Comments
Mother				If yes, was it before the age of 65? **Yes** **No**
Father				If yes, was it before the age of 65? **Yes** **No**
Sibling				Gender: Age:
Sibling				Gender: Age:
Paternal Grandmother				Age:
Paternal Grandfather				Age:
Maternal Grandmother				Age:
Maternal Grandfather				Age:

FOR FEMALES ONLY:

Are you pre-____, peri-____ or post-____ menopausal?

If premenopausal, are you using **any form** of contraception (birth control) or hormone therapy for any reason? Yes No

If yes, why and what type?_____

If you are premenopausal:

Are you pregnant?	Yes	No	I Don't Know
Could you be pregnant?	Yes	No	I Don't Know
Are you trying to become pregnant?	Yes	No	

If you are peri- or postmenopausal:

For how long?_____

When was your last menstrual period?

_____

Have you had a hysterectomy w/ or w/out ovary removal?	Yes	No
Have you had an oophorectomy without removal of your uterus?	Yes	No
Are you currently taking any type of hormone replacement therapy or using any form of contraception (birth control)?	Yes	No

If yes, what type?_____How long?_____Dosage

Name of Administering Staff	**Signature of Staff Member**	**Date**

Adapted from *ACSM's Guidelines for Exercise Testing and Prescription*. Philadelphia: Lippincott Williams & Wilkins, 2013.

Appendix B: Subject Consent Form

ARIZONA

The University of Arizona Consent to Participate in Research

Study Title: The Impact of Post-exercise Hydration with Deep Sea Mineral Water on Exercise Performance, Rehydration, and Recovery.

Principal Investigator: John Konhilas (PI) and Douglas Keen (co-PI)

This is a consent form for research participation. It contains important information about this study and what to expect if you decide to participate. Please consider the information carefully. Feel free to discuss the study with your friends and family and to ask questions before making your decision whether or not to participate.

Why is this study being done?

The purpose of this study is to determine if rehydration and exercise recovery is accelerated by consuming Kona Deep mineral seawater after exercise-induced dehydration, relative to rehydration with commercially available spring water or Gatorade. This study involves research using human subjects as voluntary participants.

What will happen if I take part in this study?

If you take part in this study, you will be asked to participate in three separate exercise/rehydration trials. Each trial will be separated by at least 48 hours. During each trial you will be asked to participate in an exercise-challenge protocol (stationary biking) under warm conditions (30°C) until 3-5% of your body mass is lost from exercise-induced dehydration (mainly through sweat). After this exercise protocol you will be asked to consume a rehydration fluid, the volume (in milliliters) of which will be equal to 1.5 times the total body mass you lost (in kg). The order of the rehydration fluid for trials 1, 2 and 3 will be randomized to either Kona Deep mineral seawater, commercially available spring water, or Gatorade to be consumed in a unique order. During each trial, the following parameters will be measured and/or collected to evaluate hydration status and exercise performance:

- Body mass
- Blood pressure and heart rate
- Tympanic temperature
- VO_2-60% maxHR
- Urine and salivary samples

You will be asked to perform a series of 3 maximal contractions of the left knee extensors on a Biodex machine as a measure of peak power production before the exercise protocol, immediately after the exercise protocol, and after rehydration. The night prior to each trial and on the morning of each trial you will be asked to consume 1.5L of commercially available bottled (non-spring) water to ensure that you are properly hydrated. You will be asked to

follow a normal diet while avoiding foods high in sodium and alcohol for 24 hours before each trial.

How long will I be in the study?
Each trial will be separated by at least 48 hours. Therefore, completion of all three trials will require a minimum commitment of one week. However, participants may reasonably expect trials to be separated by a week and should consider a commitment of 1 month.

How many people will take part in this study?
This study will involve the participation of approximately 15 male subjects and 15 female subjects.

Can I stop being in the study?
Your participation is voluntary. You may refuse to participate in this study. If you decide to take part in the study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your usual benefits. Your decision will not affect your future relationship with The University of Arizona. If you are a student or employee at the University of Arizona, your decision will not affect your grades or employment status.

What risks, side effects or discomforts can I expect from being in the study?

Participation in this study does not pose more than minimal risk. The risks associated with this study are similar to the risks present during normal physical exertion and may include physical fatigue, exhaustion, and dehydration. There are no anticipated psychological, social, legal, or economic risks associated with this study.

What benefits can I expect from being in the study?
You may or may not benefit as a result of participating in this study. Subjects may benefit from the moderate aerobic exercise required in this study, exercise being an important part of an active, healthy lifestyle.

What other choices do I have if I do not take part in the study?
You may choose not to participate in this study without penalty or loss of benefits to which you are otherwise entitled.

Will my study-related information be kept confidential?

Only the Principal Investigator, Dr. Konhilas, co-Principal Investigator, Dr. Keen, and the researcher, Ms. McKee, will have access to the materials containing personal identification of the participants throughout all steps of the experiment (recruitment, consent process, and research procedures). Participants will be assigned a subject code that will appear in the participants' record. The key to participant codes will be held separately from the data.

ARIZONA

Participant codes will not be used in any paper presentations or manuscripts resulting from the research. Participants' identities will be known only to the PI, co-PI and researcher. All data will be encrypted and stored electronically on a password protected desktop computer. The password will only be known to the PI and to the researcher. Data will be stored for 6 years after publication date.

Efforts will be made to keep your study-related information confidential. However, there may be circumstances where this information must be released. For example, personal information regarding your participation in this study may be disclosed if required by state law.

Also, your records may be reviewed by the following groups:

- Office for Human Research Protections or other federal, state, or international regulatory agencies
- The University of Arizona Institutional Review Board

What happens if I am injured because I took part in this study?

If you suffer an injury from participating in this study, you should seek treatment. The University of Arizona has no funds set aside for the payment of treatment expenses for this study. You will be provided with any new information that develops during the course of the research that may affect your decision whether or not to continue participation in the study.

When may participation in the study be stopped?

Participants may be withdrawn from the study at any time if it is determined that they have any of the exclusion criteria listed in the application form. Participants withdrawn from the study by the PI will be verbally informed and provided with an explanation for removal from the study. There are no consequences to the participant to withdraw or to be withdrawn from the study.

What are the costs of taking part in this study?

Participation will involve two meetings and three experimental trials. Each meeting will take approximately 30 minutes and will be separated by at least 36 hours. The first meeting will be to describe the experimental objectives, experimental protocols, and to discuss informed consent. The second meeting will be to authorize informed consent and to schedule experimental trial dates. Each experimental trial will require approximately 3 hours of participation time and will be separated by at least 48 hours. There are no additional costs associated with participation.

Will I be paid for taking part in this study?

Subjects will be compensated for their time. Each subject will receive $50 presented as a cheque upon completion of the study. By law, payments to subjects may be considered taxable income.

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Who can answer my questions about the study?
For questions, concerns, or complaints about the study you may contact John Konhilas (PI) Konhilas@email.arizona.edu, Douglas Keen (co-PI) dkeen@email.arizona.edu, or Laurel McKee (Researcher) lamckee@email.arizona.edu, 520-626-7677

For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research team, you may contact the Human Subjects Protection Program at 520-626-6721 or online at http://orcr.arizona.edu/hspp.

If you are injured as a result of participating in this study or for questions about a study-related injury, you may contact John Konhilas (PI).

An Institutional Review Board responsible for human subjects research at The University of Arizona reviewed this research project and found it to be acceptable, according to applicable state and federal regulations and University policies designed to protect the rights and welfare of participants in research.

Signing the consent form

I have read (or someone has read to me) this form, and I am aware that I am being asked to participate in a research study. I have had the opportunity to ask questions and have had them answered to my satisfaction. I voluntarily agree to participate in this study.

I am not giving up any legal rights by signing this form. I will be given a copy of this form.

Printed name of subject	Signature of subject
	AM/PM
	Date and time

Investigator/Research Staff

I have explained the research to the participant or the participant's representative before requesting the signature(s) above. There are no blanks in this document. A copy of this form has been given to the participant or to the participant's representative.

Printed name of person obtaining consent	Signature of person obtaining consent
	AM/PM
	Date and time

HSPP Use Only:
Human Subjects Protection Program
Consent Form T302a v 2015-01

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