Fluid balance, kidney function and acute kidney injury risk in healthy men and women during simulated work-related heat stress

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ABSTRACT

We tested the hypothesis that men are at greater risk of dehydration during simulated work-related heat stress (WrHS) compared to women independent of menstrual cycle phase. Twelve eumenorrheic women completed a WrHS simulation during the early follicular (EF), late follicular (LF) and mid-luteal (ML) phase of their menstrual cycle and twelve men completed one WrHS simulation. The WrHS simulation involved a four-hour exposure to 33.8±0.8°C, 54±1% relative humidity where participants walked on a treadmill for 30 min per hour at a rate of metabolic heat production of 5.2±0.7 W/kg (199±28 W/m^2). Participants drank a cool non-caloric sport drink ad libitum. Percent changes in nude body weight from pre- to post- WrHS did not differ between men (-0.5±0.5%) and women (EF: -0.5±0.9%; LF: -0.3±0.9%; ML: -0.3±0.7%) during any phase of the menstrual cycle (p≥0.4694). Relative fluid intake was higher in women during LF (43±18 mL/kg LBM; p=0.0148) and ML (43±16 mL/kg LBM; p=0.0048), but not EF (36±21 mL/kg LBM; p=0.1792) compared to men (24±5 mL/kg LBM). Relative urine output was higher in women (20±12 mL/kg LBM) compared to men (8±4 mL/kg LBM) across menstrual cycle phases (p≤0.0367). Relative total sweat loss did not differ between men (700±160 mL/m^2 BSA) and women (675±254 mL/m^2 BSA) during any phase of the menstrual cycle (p≥0.8458). While the way it was achieved differed, when cool fluids are available fluid balance is similarly achieved in men and women independent of the menstrual cycle, suggesting the risk of dehydration during WrHS is not modified by biological sex.

Keywords: ad libitum drinking, exercise, kidney function, fluid regulation, sex hormones

NEW AND NOTEWORTHY

Although men are often thought to be at greater risk of dehydration during work-related heat stress due to lower fluid intake, the impact of biological sex on fluid balance remains unclear. This study shows that when cool fluids are available, men and women maintain similar fluid balance regardless of menstrual cycle phase, suggesting sex does not influence dehydration risk during simulated work-related heat stress.

INTRODUCTION

Total body water reflects the balance between fluid intake from food and drink, and fluid output, which is modulated physiologically due to sweating, respiratory and metabolic water loss and urine production (1). Inadequate fluid intake relative to sweat loss is common during work-related heat stress, which underlies the risk for developing dehydration, a hypovolemic-hyperosmotic state (2). Hypovolemia and/or increases in plasma osmolality trigger the release of vasopressin and activation of the renin-angiotensin-aldosterone-system to stimulate thirst and promote renal water conservation (2). Sex hormones may be important modulators of the hormonal control of fluid conservation. For example, estrogen and testosterone cross the blood brain barrier and can bind with vasopressin-producing cells within the hypothalamus (3, 4), which can modify the synthesis and secretion of vasopressin (5). Thus, it is possible that the hormonal control of fluid regulation differs between men and women, given that women have higher estrogen and lower testosterone levels compared to men.

The effect of biological sex on fluid balance and regulation during work-related heat stress remains poorly understood. In general, it is suggested that men are at greater risk of becoming dehydrated compared to women because they tend to under drink when given free access to fluids (6). In contrast, women may actually be at greater risk of developing hyponatremia due to overdrinking of hypotonic fluids (7) and greater sensitivity to vasopressin (8). Furthermore, women are typically smaller in body size and generate less metabolic heat for a given task during physical work and, therefore, require less sweat loss (9). Thus, the water intake required to maintain fluid balance during work-related heat stress will often be less in women compared to men. Given the increased number of women entering the manual work labor force (10) and combat roles in the military (11), there may be a need to tailor work-related hydration recommendations more specific to women. We have demonstrated that the menstrual cycle has little influence on fluid balance in women during simulated work-related heat stress when fluids

78 are freely available (12). To our knowledge, whether fluid balance differs between men and
79 women has only been previously examined on one occasion in which during ~8.5 hours of
80 continuous walking exercise in a field-research setting women drank more fluid compared to
81 men, which resulted in a lower incidence of dehydration in women (6). Whether these findings
82 can be translated to a work-related heat stress scenario, and whether menstrual cycle phase
83 modifies any biological sex-related differences remains unknown. Therefore, the primary
84 purpose of this study was to test the hypothesis that men are at greater risk of dehydration
85 during simulated work-related heat stress compared to women independent of menstrual cycle
86 phase.
87
88 Furthermore, one of the main health risks of regular work-related heat stress is chronic kidney
89 disease of non-traditional causes (13), which is linked to recurrent episodes of acute kidney
90 injury (AKI) (14). Indeed, up to 15% of workers frequently encountering heat stress in the
91 workplace experience AKI (15). Observational evidence from the general population has
92 demonstrated that men are at greater risk for heat-induced hospitalized AKI (16) and reductions
93 in glomerular filtration rate (17) compared to women. These observations are generally
94 consistent with observations in the non-heat-stressed state where AKI mortality is twice as high
95 in men and premenopausal women have the lowest incidence of AKI (18). Whether this effect of
96 biological sex on AKI risk and kidney function translates to work-related heat stress scenarios,
97 and whether this effect is modified by menstrual cycle phase has never been explored.
98 Therefore, the secondary purpose of this study was to test the hypothesis that AKI risk provoked
99 by simulated work-related heat stress will be higher in men compared to women independent of
100 menstrual cycle phase.

METHODS

Participants

The data presented in this manuscript were collected concurrently with those previously reported, which tested a unique hypothesis (12). As previously stated (12), at the time of study design there were no direct data to inform a sample size calculations. That said, previous evidence indicated that the effect size (Cohen's d_z) of the impact of endogenous menstrual cycle hormones on circulating vasopressin concentrations was 0.707 (19). Thus, an *a priori* power calculation using this effect size was carried out using G-Power version 3.1.9.4 (20), which determined that 12 participants in each group were required to achieve 80% power with α=0.05 to detect a significant within-between interaction assuming a correlation coefficient among repeated measures of 0.7. Therefore, twelve healthy, eumenorrheic women and twelve healthy men between the ages of 18 to 34 years participated in this study. Participant characteristics upon screening are presented in Table 1. Participants were eligible if they self-reported no known cardiovascular, metabolic, renal, or neurological diseases. Additional eligibility criteria included being physically active, nonsmokers, not currently taking any type of hormonal contraceptive and/or had abstained from contraceptives for >6 months. Participating women were not pregnant, which was confirmed through a urine pregnancy test taken at each visit, self-reported to be normally menstruating and reported no menstrual cycle specific disorder as assessed by a menstrual cycle questionnaire. Participants were considered recreationally active as they all met minimum physical activity requirements of participating in at least 150 min / week of aerobic exercise (21).

All participants provided verbal and written informed consent after being fully informed of the experimental procedures and possible risks. This study was approved by the Indiana University Institutional Review Board (IRB #: 2004319694) and was carried out according to the most recent revision of the Declaration of Helsinki, except for registration in a database. To determine

menstrual cycle status and confirm study eligibility, we adhered to the recommended screening criteria for the determination of physiologically natural, menstrual cycles through confirmation of eumenorrheic menstrual cycle length (≥ 21 days and ≤ 35 days), presence of urinary luteinizing hormone (LH) surge, and basal body temperature exhibiting normal patterns, all of which were tracked for two whole menstrual cycles (22, 23). These three measures were our strict inclusion criteria to determine if women were eligible to complete the experimental protocol. Hormone levels were measured during the experimental visits as a tertiary measure to confirm menstrual cycle phase.

Details of menstrual cycle tracking are as follows. During the screening visit (Visit 1), participants were asked to track their menstrual cycles through the FitrWoman App (Orreco Ltd., Galway, Ireland) as a method to log when they started menstrual bleeding throughout the duration of the study. The study team had access to FitrWoman app data via a remote desktop as part of the FitrCoach platform (Orreco Ltd., Galway, Ireland). This minimized participant burden throughout the tracking process. Prior to experimental testing, oral temperature was measured for at least 2 cycles, every morning upon waking, using a digital thermometer (Medical Thermometer, B.Weiss Personal Care, Shenzhen, China) to provide a measure of the increase in body temperature from the follicular to the mid-luteal phase that should occur if ovulation is present. The women also took a daily urinary ovulation test (Ovukit Self-Test, Cen-Med Enterprise, New Brunswick, NJ) between days 10-20 of their menstrual cycle to detect the surge in luteinizing hormone that occurs on average 14 ± 1 day post-menses, to confirm the occurrence of ovulation. Blood samples were collected at the beginning of each test session and were retrospectively measured for serum estradiol (intra-assay CV: 10.6 ± 7.6%) and progesterone (intra-assay CV: 5.9 ± 2.2%) in women and testosterone concentrations (intra-assay CV: 7.8 ± 3.0%, inter-assay CV: 2.6 ± 1.7%) in women and men using commercially

available assay kits (Eagle Biosciences, Amherst, NH) to confirm the hormone profile within each phase. One woman in our study had contaminated baseline hormone samples but we were able to confirm that she had a normal menstrual cycle length, ovulated, and exhibited the fluctuating temperature pattern indicative of a eumenorrheic menstrual cycle profile.

Instrumentation and Measurements

Height was measured during the first screening visit using a stadiometer (Holtain Limited, UK) and recorded to the nearest cm and nude body mass was measured to the nearest g using a digital scale (Sauter, Balingen, Germany) at the beginning of each experimental visit. Body mass index (24) and body surface area (25) were calculated from height and weight. Lean body mass, fat mass and percent body fat were measured via dual-energy x-ray absorptiometry (Lunar IDXA, GE Healthcare, USA). Core temperature was measured continually with an ingestible telemetry capsule (n=10 women, n=7 men, HQ Inc., USA) ingested upon arrival at the laboratory (approximately one hour prior to exercise in the heat) or rectal temperature probe (n=2 women, n=5 men, Covidien, Medtronic, USA) the latter of which was measured when participants were contraindicated for ingesting the telemetry capsule. Weighted mean skin temperature was calculated from the measurement of skin temperature on the chest, triceps, thigh, and calf on the right side of the body (Thermochron iButtons, Maxim Integrated, USA) (26). Heart rate was continuously measured using a Polar heart rate monitor. Blood pressure was measured manually and in duplicate measures by the same operator during both rest and exercise for all experimental trials within each subject. Expired gases were sampled for volume, oxygen and carbon dioxide fractions, which were used to determine oxygen consumption and carbon dioxide production during exercise (Parvo Medics, USA), and the rate of metabolic heat production was calculated accordingly (27).

Percentage changes in nude body weight provided an index of fluid balance (28). Whole-body sweat loss was calculated from changes in nude body mass corrected for fluid intake and urine loss. Total fluid intake, urine output and whole-body sweat losses were assessed as absolute values and as normalized to lean body mass (fluid intake and urine output) or body surface area. Normalization was deemed necessary to ensure accurate comparisons between men and women given that both lean body mass and body surface area typically differ between men and women, while total body water is strongly influenced lean body mass (29) and that whole-body sweat rate is influenced by body surface area (30).

A standardized script was used to verbally explain ratings of thirst (from: 1-9), thermal comfort (from: 1-4) and thermal sensation (from: 1-7), which were measured using visual analog scales. Specifically, participants were asked *"how thirsty do you feel now?"* on a scale from 1-9, where 1 is *"not thirsty at all"* and 9 is *"very, very thirsty"* (31), *"how comfortable does the thermal state of your body feel?*" on a scale from 1-4, where 1 signifies *"comfortable"* and 4 signifies *"very uncomfortable"* and *"how does the temperature of your body feel?"* on a scale from 1-7, where 1 is *"cold"*, 4 is *"neutral"*, and 7 is *"hot"* (32).

Plasma aldosterone (intra-assay CV: 12.7 ± 6.9%, inter-assay CV: 10.3 ± 4.0%) and plasma copeptin (intra-assay CV: 2.9 ± 2.1%, inter-assay CV: 8.9 ± 8.6%), a surrogate measure of vasopressin release (33) were measured in duplicate using commercially available ELISA kits (Eagle Biosciences, USA; My BioSource, USA). Urine flow rate was calculated as urine volume divided by the time between each bladder void. Urine specific gravity was measured using refractometry (Atago, Tokyo, Japan). Urine and serum osmolality (Advanced Instruments, USA) and sodium concentrations (Medica Corporation, YSA) were measured in duplicate using commercially available systems. Serum and urine creatinine were measured via a clinical analyzer (COBAS INTEGRA 400+ Analyzer, Roche Diagnostics, USA). Glomerular filtration rate

was estimated from creatinine clearance (i.e., urinary creatinine x urine flow rate ÷ serum creatinine). Fractional excretion of sodium was calculated as 100 x ([urinary sodium x serum creatinine] ÷ [serum sodium x urinary creatinine]) [1]. Free water clearance was calculated as urine flow rate x (1-[urinary osmolality ÷ plasma osmolality]). Osmolar clearance was calculated as (urinary osmolality ÷ plasma osmolality) x urine flow rate [2].

Insulin-like growth factor-binding protein 7 (IGFBP7), a marker of G1 cell cycle arrest in the renal epithelium preferentially secreted in the renal proximal tubules (2), and tissue inhibitor metalloproteinase 2 (TIMP-2), a marker of G1 cell cycle arrest in the renal epithelium preferentially secreted in the renal distal tubules [1], were measured in the urine using commercially available assay kits (RayBiotech Life, USA). Consistent with the U.S. Food and Drug Administration approved indication in clinical settings, the product of urinary IGFBP7 and TIMP-2 ([IGFBP7·TIMP-2]) provided an index of AKI risk (2). Urinary [IGFBP7·TIMP-2] is presented over time and as the peak change from preexposure, which is consistent with the approved indication where time course is not considered [1]. To account for differences in urine concentration, [IGFBP7·TIMP-2] was normalized to urine specific gravity (i.e., $[IGFBP7 \cdot TIMP\text{-}2]_{USG}$)(34).

Carbon monoxide (CO) rebreathing was used to measure red blood cell volume and through this measure we calculated absolute plasma volume from hemoglobin concentration and hematocrit using methods previously reported in our laboratory (35, 36). Following at least 15 min of supine rest, the CO rebreathing procedures consisted of a bolus of 99.5% chemically pure CO in the amount of 1.0 mL CO / kg body weight delivered via a 100 mL syringe and inhaled by the participant. The participant rebreathed for 2 min from a closed spirometer system connected to a 5 L anesthetic bag filled with 100% oxygen while remaining in the supine

position. Before and at 4 min and 6 min after the start of the rebreathe, finger prick blood samples were collected. The obtained capillary blood was sampled by a blood gas analyzer (OSM3, Radiometer) for the determination of carboxy-hemoglobin concentration, which permitted the calculation of hemoglobin mass. Plasma volume was calculated from total blood volume subtracted by erythrocyte volume (37) and is presented as both absolute and normalized to body surface area to ensure accurate comparisons between men and women.

Renal blood velocity was measured via Doppler ultrasound (Toshiba Aplio 300, Canon Medical Systems, USA) in the distal segment of the right renal artery (renal artery) and in the middle portion of a segmental artery in the right kidney (segmental artery) as a surrogate for renal blood flow, using methods that have been thoroughly described previously (38). The segmental artery and the location of measurement within the renal and segmental arteries was the same at all time points within a participant. With participants in the left lateral recumbent position and employing the coronal approach, a phased-array transducer (2.5–3.5 MHz) was held in the same location for all measurements after marking the transducer location, which was marked with indelible ink during baseline measurements. In all instances, the focal zone was set to the artery's depth, and the insonation angle was <60°. Mean renal and segmental artery blood velocities were indexed from the waveform envelope by the time-averaged maximum velocity and reported as the average of three cardiac cycles (39–41). All renal measurements were obtained and extracted by the same sonographer (J.A.F.). Optimization and measurement of blood velocity in the renal and segmental arteries occurred within two min of when the transducer was replaced on the participant during each measurement timepoint. With this approach the within-subject test-retest coefficients of variation for blood velocity measurements were 2.8 ± 1.5% (renal artery) and 3.4 ± 1.3% (segmental artery) for the sonographer in this study. Given the depth of the renal and segmental arteries, it is not possible to accurately measure vessel wall diameter. However, renal blood velocity was interpreted to reflect changes

in renal blood flow as has been done previously (16, 26, 39, 42–47). This was deemed reasonable because pharmacologically induced changes in renal blood flow were due to changes in renal artery blood velocity and not diameter (47, 48). Renal and segmental artery blood velocity were normalized to mean arterial pressure to provide an index of vascular resistance (i.e., mean arterial pressure ÷ blood velocity).

Participants had free access to a cool (10-15°C) non-caloric sport drink (Gatorade Zero) during exercise in the heat, and the volume consumed was measured every hour. Ad libitum fluid intake permitted assessment of body fluid balance, which is a function of both fluid intake and output, and also to enhance the external validity of our protocol, given that the workplace hydration recommendations encourage cool fluids to be freely available throughout the workday, particularly in hot conditions (49). A non-caloric sport drink (Gatorade Zero) was used given the recommendations for electrolyte replacement when heat exposure exceeds 2 hours in duration (50) and to avoid any risk of hyponatremia. A non-caloric beverage was used to ensure that drinking behavior was not influenced by caloric intake. During the screening visit (Visit 1), each participant had the opportunity to choose their preferred flavor of non-caloric sport drink and drank that same flavor for each experimental visit. The nutritional information was identical between the flavors.

Experimental Protocol

Participating women completed three experimental trials, each during a different phase of the menstrual cycle [early follicular (EF, 3 ± 2 days), late follicular (LF, 13 ± 2 days), mid-luteal (ML, 21 ± 2 days)], in a randomized cross-over design and men completed one experimental visit. Blinding of the experimental conditions was not possible in this study, but participants were blinded from the research hypotheses. The order of experimental trials (Visits 2-4) was assigned in blocks of 3 where 1 participant begins visit 2 in the early follicular phase, 1

participant in the late follicular phase, and 1 participant in the mid-luteal phase in a randomized order. The experimental design is presented in Figure 1. The EF, LF, and ML phases were chosen because these menstrual cycle phases typically differ with regards to their progesterone and estrogen profiles – EF: low estrogen, low progesterone; LF: high estrogen, low progesterone; ML: low/moderate estrogen, high progesterone (51).

Men and women participants both reported to the temperature-controlled laboratory (21.1 ± 0.9°C) after abstaining from exercise, caffeine, and alcohol for 12 hours and food for 2 hours. Participants were encouraged to arrive at the laboratory well hydrated but were not given any specific fluid intake instructions. Participants were encouraged to maintain their normal exercise routines and diet throughout the duration of the experimental period. To ensure this was the case, a diet and fluid log was collected in the 24 hours preceding the experimental trial and for repeated visits to the laboratory (i.e., the women) were instructed to replicate the food and fluid intake prior to their subsequent visits. Moreover, participants were instructed to complete a 24-hour urine collection prior to the visit. To control for diurnal changes, each experimental visit was completed at the same time of day (+/- one hour) within a participant.

Upon arrival at the laboratory for each experimental visit, all participants completed a health history update questionnaire to ensure no relevant changes in their health history had occurred. Participants were then instructed to void their bladder and euhydration was confirmed using this urine sample (i.e., urine specific gravity <1.020) (52). If a participant did not meet the urine specific gravity requirement with this urine sample, they were given 250 mL of water, and their urine specific gravity was measured again 30 min after. If participants remained improperly hydrated the visit was rescheduled. Participants measured their nude body weight, consumed 250 mL of cool tap water to promote urine production and then body composition was measured using dual-energy x-ray absorptiometry. Then participants were instrumented with iButtons and

a heart rate monitor and laid in the supine position. Following 20 min of rest, pre-exercise measurements of heart rate, mean arterial pressure, core and skin temperatures, and renal blood velocity were taken, and culminated in the collection of a venous blood sample and voiding of the bladder. The 20 min of supine rest was necessary to control for plasma volume shifts associated with changes in posture (53).

Following pre-exercise data collection, all participants exercised in the heat for 4 hours in an environmental chamber maintained at 33.7 ± 0.3°C, 54 ± 1% relative humidity. Participants were fitted with a long sleeve button down shirt and long pants to mimic the outfit of many manual laborers. Four hours was chosen to simulate half of a workday. The selected environmental conditions elicit a wet bulb globe temperature of 28 ± 0°C, which approximates the environmental conditions encountered in outdoor occupations (49, 50, 54). During this 4-hour period, participants walked on a treadmill for 30 min every hour at a treadmill speed and grade to elicit a rate of metabolic heat production approximately 5.0 W/kg or 200 W/m^2. The treadmill speed and grade were established during the screening visit (Visit 1), and the external workload was maintained constant within and between each experimental trial. Exercise intensity was prescribed as normalized to body mass and body surface area to attempt to control for differences in core temperature and whole-body sweat rate between men and women (30), particularly given that, as expected, these groups differed in body size (Table 1). The resulting absolute rate of metabolic heat production was 300-400 W. Based on the Compendium of Physical Activities (55), this absolute rate of metabolic heat production represents the average workload regularly encountered in outdoor work-related settings that are regularly exposed to heat stress, e.g., construction [3]. Moreover, the prescribed work-rest ratio is in accordance with the NIOSH heat stress recommendations (50). Expired gases were collected and analyzed for 5 min at the start of each hour of exercise and reported as an average over the entire four-hour work-related heat stress simulation. Fluid intake was measured every 15 min, while measures of

heat strain were monitored continuously and reported here every 30 min. Urine was collected (if needed) during the rest periods. Participants completed visual analogue scales every 30 min to measure thermal comfort, thermal sensation and thirst.

Upon completion of exercise in the heat, participants returned to the moderate thermal environment for post-exercise measurements. Participants were laid in the supine position for 20 min of rest, to standardize for plasma volume shifts (53). Following this period, post-exercise measurements of heart rate, mean arterial pressure, core and skin temperatures and renal blood velocity were collected, a final blood draw and urine sample were obtained, and nude body weight was measured. This was followed by a carbon monoxide rebreathing procedure. The carbon monoxide rebreathing procedure was conducted after exercise in the heat to ensure that the administration of carbon monoxide did not interfere with measures during exercise in the heat and because there is no evidence that hemoglobin mass is altered by a single bout of exercise in the heat (56).

Statistical Analysis

Many of the data presented herein were presented in a previous study examining the effect of menstrual cycle phase on fluid balance (12). Thus, to prevent redundancy no comparisons were made between menstrual cycle phases unless noted otherwise (see below). To this end, pre-experimental trial data, indices of metabolic heat production, fluid balance, and AKI risk data were compared between men and women at each phase of the menstrual cycle using generalized least squares models. The generalized least squares models accounted for the fact that menstrual cycle phases data are not independent of one another, whereas comparisons between each menstrual cycle phase and the data from men were treated as independent. A priori established pairwise comparisons were made between men and women across each phase of the menstrual cycle adjusted for multiple comparisons using the Benjamini-Hochberg

method. Differences in participant characteristics between men and women were also compared using two-tailed unpaired t-tests. Data presented over time (e.g., core temperature, plasma volume, etc.) were analyzed using two-way linear mixed-effects models at each phase of the menstrual cycle with a repeated factor of time and an independent factor of group (men vs. women). To our knowledge, changes in indices of renal perfusion and urinary $[IGFBP7{\cdot}TIMP\text{-}2]_{USG}$ due to simulated work-related heat stress have not been reported across menstrual cycle phases. Thus, these variables, when presented over time, were analyzed using two-way linear mixed-effects models with repeated factors of time and menstrual cycle phase (EF, LF, ML) within the women only. The change in urinary $[IGFBP7{\cdot}TIMP\text{-}2]_{USG}$ from pre- to post- simulated work-related heat stress within women was analyzed using a one-way linear mixed-effects model with a repeated factor of menstrual cycle phase. In all instances, when the assumption of sphericity was violated the Geisser-Greenhouse correction was applied. All data were normally distributed as determined via visual inspection of the predicted versus actual residuals. If a mixed effects model revealed a significant interaction, pairwise comparisons were made with Sidak's test. Statistical analyses were carried out using GraphPad Prism software (version 10.6.1; La Jolla, CA), except for the generalized least squares models, which were conducted using the statistical package nlme() in R (v. 4.5.1). A priori statistical significance is set an $p \leq 0.05$. Where possible, exact p-values are reported. However, in instances referring to more than one pairwise comparison the highest (for comparisons where $p \leq 0.05$) or lowest (for comparisons where $p > 0.05$) p-values are reported using $\leq$ or $\geq$ symbols, respectively. Data are reported as mean ± SD and/or as mean and individual values.

RESULTS

Pre-experimental trial measures

Pre-experimental trial measures are presented in Table 2. To summarize, 24-hour urine volume ($p \geq 0.07720$), 24-hour urine specific gravity ($p \geq 0.2282$), urine osmolality ($p \geq 0.2529$) and urine sodium concentration ($p \geq 0.1639$) did not differ between men and women during any phase of the menstrual cycle. Independent of menstrual cycle phase, while percent body fat did not statistically differ between men and women ($p \geq 0.0846$) and lean body mass ($p < 0.0001$) was lower in women compared to men. Core temperature did not differ between men and women, independent of menstrual cycle phase ($p \geq 0.0642$).

Thermoregulatory and cardiovascular responses to simulated work-related heat stress

Indices of the average rate of metabolic heat production are presented in Table 3. To summarize, while the absolute rate of metabolic heat production (in Watts) was lower in women compared to men during any phase of the menstrual cycle ($p \leq 0.0095$), the rate of metabolic heat production when normalized to body mass ($p \geq 0.4476$) or body surface area ($p \geq 0.2175$) did not differ between men and women during any phase of the menstrual cycle. Core and mean skin temperature data are presented in Figure 2. To summarize, the change in core temperature did not differ between men and women in the EF ($p=0.3501$) LF ($p=0.4330$), or ML ($p=0.1792$) phase of the menstrual cycle. Mean skin temperature did not differ between men and women during any phase of the menstrual cycle (main effect of group: $p \geq 0.1632$). Heart rate and mean arterial pressure data are presented in Figure 3. To summarize, while both heart rate and mean arterial pressure changed as a function of time and the corresponding work-to-rest intervals (main effect of time: $p < 0.0001$), neither of these variables differed between groups (time x group: $p \geq 0.0602$).

Fluid balance responses to simulated work-related heat stress

Indices influencing fluid balance are presented in Figure 4. To summarize, neither the percent change in body weight (all pairwise comparisons: $p \geq 0.4694$) nor the absolute change in body weight (all pairwise comparisons: $p \geq 0.3503$) differed between men and women during any phase of the menstrual cycle. While absolute fluid intake did not differ between men and women during any phase of the menstrual cycle (all pairwise comparisons: $p \geq 0.2275$), fluid intake normalized to lean body mass was higher in women during the LF ($p=0.0148$) and ML ($p=0.0048$) phases of the menstrual cycle compared to men. Urine output normalized to lean body mass was higher in women compared to men across all phases of the menstrual cycle (all pairwise comparisons: $p \leq 0.0367$), whereas total sweat loss normalized to body surface area did not differ between men and women during any phase of the menstrual cycle (all pairwise comparisons: $p \geq 0.8458$).

Hydration and fluid regulation responses to simulated work-related heat stress

Hydration measures and fluid regulation hormones are presented in Table 4. To summarize, plasma volume did not change differently between men and women during any phase of the menstrual cycle (time x group: $p \geq 0.4981$). Despite no differences pre- simulated work-related heat stress (pairwise comparisons: $p \geq 0.3180$), urine specific gravity was lower in women across the menstrual cycle compared to men (pairwise comparisons: $p \leq 0.0367$). While copeptin concentrations were higher in women compared to men (main effect of group: $p \leq 0.0006$), aldosterone concentration (time x group: $p \geq 0.5651$) did not differ over time between men and women during any phase of the menstrual cycle.

Perceptual responses to simulated work-related heat stress

Perceptual responses are presented in Figure 5. To summarize, thirst sensation (all time x group: $p \geq 0.0501$) and whole-body thermal comfort (all time x group: $p \geq 0.6728$) and thermal

sensation (all time x group: p≥0.3446) did not change differently between men and women during any phase of the menstrual cycle.

Kidney function and perfusion responses to simulated work-related heat stress

Indices of kidney function and fluid conservation are presented in Table 5. To summarize, urine osmolality following simulated work-related heat stress was generally lower in women compared men during the early follicular (main effect of group: p=0.0636), late follicular (main effect of group: p=0.0460), and mid-luteal phases (pairwise comparison: p=0.0305) of the menstrual cycle. Compared to men, the fractional excretion of sodium was lower in women during the EF phase (main effect of group: p=0.0121) simulated work-related heat stress but did not differ between groups during LF (main effect of group: p= 0.2188) or ML (main effect of group: p=0.9543) phases. In general, markers of kidney function, including urine flow rate (pairwise comparisons: p≥0.1693), creatinine clearance (time x group: p≤0.3898), and free water clearance (pairwise comparisons: p≥0.0667), did not change differently between men and women across any phase of the menstrual cycle during simulated occupational heat stress. Indices of renal perfusion are presented in Table 6. To summarize, neither renal (time x group: p≥0.0617) nor segmental (time x group: p≥0.1257) artery vascular resistance changed differently over time between men and women across any phase of the menstrual cycle and did not differ between menstrual cycle phases within women (main effect of phase: p≥0.2495).

Acute kidney injury risk in response to simulated work-related heat stress

Acute kidney injury risk data are presented in Figure 6. Due to the inability to fit samples on the standard curve and no additional sample remaining to establish dilution factors, urinary $[IGFBP7{\cdot}TIMP\text{-}2]_{USG}$ in women is presented as n=11 in LF and ML phases of the menstrual cycle, and n=12 in EF and the men. To summarize, within the women the increase in urinary $[IGFBP7{\cdot}TIMP\text{-}2]_{USG}$ following simulated work-related heat stress was greater in EF compared

to LF ($p=0.0106$) but did not differ between the other menstrual cycle phases (pairwise comparisons: $p \geq 0.3431$). Urinary $[IGFBP7 \cdot TIMP\text{-}2]_{USG}$ did not differ between men and women across any phase of the menstrual cycle (all pairwise comparisons: $p \geq 0.4378$).

DISCUSSION

The primary purpose of this study was to test the hypothesis that men are at greater risk of dehydration during simulated work-related heat stress when fluids are consumed ad libitum compared to women independent of menstrual cycle phase. The data presented do not support this hypothesis. To highlight, percent changes in nude body weight, a marker of changes in total body water (28), did not differ between men and women in any phase of the menstrual cycle (Figure 4). Thus, when fluids are freely available during simulated work-related heat stress fluid balance is achieved in men and women independent of the menstrual cycle, suggesting that the risk of dehydration is not modified by biological sex.

The secondary purpose of this study was to test the hypothesis that AKI risk provoked by simulated work-related heat stress will be higher in men compared to women independent of menstrual cycle phase. Again, the data presented do not support this hypothesis. Specifically, urinary $[IGFBP7 \cdot TIMP\text{-}2]_{USG}$, a marker of AKI risk (2), did not differ between men and women in any phase of the menstrual cycle (Figure 6). Interestingly, however, when assessing urinary $[IGFBP7 \cdot TIMP\text{-}2]_{USG}$ within women across the menstrual cycle, the magnitude of AKI risk provoked by simulated work-related heat stress was higher in the early follicular phase of the menstrual cycle compared to the late follicular and mid-luteal phases (Figure 6). Therefore, while AKI risk may be slightly modified by menstrual cycle phase in women, AKI risk does not differ between men and women during simulated work-related heat stress when fluids are freely available.

Fluid balance during simulated work-related heat stress in men and women

By design (Table 2), the thermoregulatory (Figure 2) and cardiovascular (Figure 3) responses to the four-hour work-related heat stress simulation did not differ between men and women, except for a moderately higher blood pressure before and after the work-related heat stress simulation

(Table 6). A non-caloric sports drink was freely available throughout. It is notable, therefore, that the magnitude of dehydration during simulated work-related heat stress was minimal. Specifically, the average percent changes in nude body weight did not exceed 1% in both men and women independent of menstrual cycle phase (Figure 4). This finding, together with the observations that plasma volume was well maintained in both men and women and serum osmolality was sustained in normal ranges (Table 4), indicates that fluid balance was similarly maintained between groups. Interestingly, however, the way fluid balance was achieved differed between men and women. For example, despite no differences in sweat loss (Figure 4) or thirst perceptions (Figure 5) between men and women, women generally drank more fluid, but this was compensated for by an increase in urine output (Figure 4). This is reflected by women producing more dilute urine throughout simulated work-related heat stress, as evidenced by lower urine specific gravity (Table 4) and urine osmolality (Table 5). While this finding is novel in the context of work-related heat stress and the comparisons between men and women across the menstrual cycle, this observation is generally consistent with previous work demonstrating that water intake is higher in female rats following water deprivation compared to male rats (57). Similarly, additional work in humans demonstrates that fluid intake in women is higher than in men during 8.5 hours of walking exercise (6). That said, in this previous study sweat loss was greater in men, resulting in a higher incidence of dehydration in men compared to women (6). Thus, the current study demonstrates that in a controlled setting in which fluid losses were similar, the incidence of dehydration between men and women is minimal.

While plasma aldosterone did not differ between men and women, plasma copeptin (a surrogate marker of vasopressin (58, 59) was higher in women during each phase of the menstrual cycle compared to men (Table 4). This finding contrasts with previous observations indicating that men have slightly higher circulating copeptin concentrations compared to women, even when well hydrated (59, 60). However, estrogen, which is almost always higher in women compared

to men independent of menstrual cycle phase, likely lowers the osmotic operating point for body fluid regulation (61). Thus, for a given serum osmolality a higher copeptin concentration might be expected in women. Nevertheless, vasopressin stimulates both enhanced renal water reabsorption and thirst (58). Thus, it is possible that the increased circulating copeptin (i.e., vasopressin) in women played a role in the greater fluid consumption. That said, we acknowledge that this response may have been observed in the absence of the heightened copeptin concentrations given the evidence of heightened sensitivity to a given circulating vasopressin concentration in women (8). Moreover, it is notable that despite apparent differences between men and women in circulating copeptin (Table 4), the way fluid balance was maintained (Figure 4) and the small but consistently lower measures of renal artery vascular resistance (Table 6) (likely contributed to by higher blood pressure in the men (Table 6)), indices of kidney function (e.g., creatinine clearance, free water clearance, etc.) generally did not change differently throughout simulated work-related heat stress between men and women (Table 5). The exception to this conclusion was that the fractional excretion of sodium was higher in women in the early follicular phase of the menstrual cycle (Table 5). This finding is supported by previous observations that urinary sodium excretion is heightened in women compared to men during both the follicular and luteal phases of the menstrual cycle (5). The reason why we only observed heightened sodium excretion during the early follicular phase of the menstrual cycle remains unclear but is likely related to differences in the experimental protocol where the previous work involved hypertonic saline infusion (5) and our protocol involved a four-hour work-related heat stress simulation while permitting voluntary drinking of a non-caloric sports drink.

AKI risk during simulated work-related heat stress in men and women

A secondary purpose of this study was to explore the effects of biological sex on AKI risk and to identify whether menstrual cycle phase modifies this risk. To this end, we have identified that

urinary [IGFBP7·TIMP-2]$_{USG}$ did not differ between men and women in any phase of the menstrual cycle (Figure 6). These data contrast findings in observational studies demonstrating that men are at greater risk for heat-induced hospitalized AKI compared to women (16, 62). That said, given the primary role of renal blood flow in the etiology of heat-induced AKI risk (2, 63), the findings presented here are supported by previous observations that renal vascular control during heat stress does not differ between women in the early follicular phase of the menstrual cycle and men (64). It should be noted, however, that by design the magnitude of hyperthermia largely did not differ between men and women (Figure 2). Thus, given that women are often smaller and lighter, and that men and women often must carry out the same absolute work task on a job site, it is feasible that in the workplace women might be rendered more hyperthermic (65), which could place them at a heightened risk of AKI (66, 67). Moreover, the magnitude of dehydration was minimal in the current study and did not differ between men and women (Figure 4). Therefore, it remains unknown whether AKI risk differs between men and women across the menstrual when both men and women become dehydrated.

An interesting and somewhat unexpected finding was that AKI risk in women was higher during the early follicular phase compared to the late follicular and mid-luteal phases of the menstrual cycle (Figure 6). The rationale underlying this observation at the present time is unclear as, to our knowledge, we know of no observational or experimental data to support this observation. However, given that vasopressin appears to play an important role in heat-induced AKI risk (68) and that the follicular phase of the menstrual cycle is associated with a heightened sensitivity to vasopressin, it is feasible that the actions of vasopressin may explain the heightened AKI risk in the early follicular phase of the menstrual cycle. By contrast, however, this apparent heightened sensitivity to vasopressin did not translate to greater reductions in free water clearance in the present study (Table 5). Thus, future research is required to understand the mechanisms by which heat-induced AKI risk is heightened in the early follicular phase of the menstrual cycle.

Methodological considerations

In addition to those presented in our previous study (12), there are a few methodological considerations to consider when interpreting the data presented herein. First, to enhance internal validity the present study was conducted in a lab-controlled environment over a four-hour period and at a constant rate of metabolic heat production. We therefore acknowledge that this contrasts what often occurs on the job where during half of a typical workday fluctuations in environmental conditions and the rate of metabolic heat production would be anticipated (69, 70). Second, since the examination of fluid balance and kidney-related indices occurred after a simulated half workday, it remains unknown if these responses would be the same if the duration had been extended (e.g., 6-12 hours, overnight). Third, the men were only tested one time and not three times, like the women who participated in this study. While this is an acknowledged limitation, this decision was made due to time and financial constraints and given that there is little evidence that the thermal or fluid balance responses to exercise in the heat would differ in men tested three times separated by a few weeks (71). Fourth, the assessment of renal blood velocity took place at least 20 min following the cessation of the four-hour work-related heat stress simulation. Thus, it is unlikely that the measured renal hemodynamics reflected what was happening during the work-related heat stress simulation. Finally, the participants in this study self-reported to be healthy and aged 20-40 years. Thus, it may not be broadly generalizable to the general population of workers exposed to heat stress that are aged across the entire adult lifespan [4] or carry traditional risk factors that may further challenge the fluid balance and the kidneys during work-related heat stress (e.g., hypertension, diabetes, obesity) [5].

Perspectives

588 Approximately 2.4 billion workers, or ~70% of the global workforce, are frequently exposed to
589 work-related heat stress, with over 90% of these exposures occurring outside of a heat wave
590 (13). Moreover, an increasing number of women are entering the manual labor workforce (10).
591 Work-related heat stress is associated with an increased risk of morbidity and mortality, while
592 dehydration is considered an aggravating factor (13). Despite acknowledgement that hydration
593 during work-related heat stress may differ between men and women, the current National
594 Institute of Occupational Safety and Health (50), American College of Sports Medicine (52), and
595 National Athletic Trainers Association (72) hydration recommendations do not address
596 biological sex, while the menstrual cycle is not mentioned as a potential modifying factor. The
597 findings presented here, together with our previous work (12), suggest that neither biological
598 sex nor the menstrual cycle are risk factors for the development of dehydration during work-
599 related heat stress. Thus, our work indicates that hydration recommendations do not require
600 adjusting for biological sex or the menstrual cycle. Nevertheless, additional work should
601 consider focusing on field settings where factors outside of the fundamental physiological
602 rationale may differentially influence fluid loss (due to sweating) and fluid intake (due to drinking)
603 in women and men during work-related heat stress. For example, access to bathrooms has
604 been demonstrated to be a barrier to fluid intake in women (73). Thus, additional research is
605 required to ensure adequate protection of both men and women who frequently encounter work-
606 related heat stress.
607
608 *Conclusion*
609 The present study demonstrates that when cool fluids are freely available during a four-hour
610 work-related heat stress simulation fluid balance is achieved in men and women independent of
611 the menstrual cycle, suggesting that the risk of dehydration is not modified by biological sex.
612 The present study also demonstrates that while AKI risk may be slightly elevated in the early
613 follicular phase of the menstrual cycle in women, AKI risk does not differ between men and

614 women during simulated work-related heat stress when fluids are freely available. Collectively,
615 our results indicate that when men and women have free access to cool fluids during simulated
616 work-related heat stress fluid balance, kidney function and AKI risk do not differ.

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JAF has received consultant fees from Orreco Ltd. and GB is employed by Orreco Ltd. ZJS has received consultant fees from Otsuka Holdings Co., Ltd. Otherwise, no other potential conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Conceived and designed research: JAF, SJC, RFC, BJM, TM, ZJS

Performed experiments: JAF, CSG, RA, ZJS

Analyzed the data: JAF, TBB, ZJS

Interpreted results of experiments: JAF, CSG, RA, SJC, RFC, BJM, GB, TM, ZJS

Prepared figures: JAF, ZJS

Drafted manuscript: JAF, ZJS

Edited and revised manuscript: JAF, CSG, RA, TBB, SJC, RFC, BJM, GB, TM, ZJS

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Figure Legends:

Figure 1: Schematic of experimental design.

Figure 2: Core temperature (top) and mean skin temperature (bottom) over time and the change (Δ) in these variables from the start of exercise. Data are presented as Mean ± SD and individual values where possible. At each menstrual cycle phase, data presented over time were analyzed via two-way linear mixed effects models. For change data, data were analyzed using generalized least squares models, with a priori pairwise comparisons between men and women across each phase of the menstrual cycle. Pairwise comparisons were adjusted for multiple comparisons using the Benjamini-Hochberg method. In all instances, no comparisons were made between menstrual cycle phases within the women because these data have been presented previously (12). Data are - women: n=12, men n=12.

Figure 3: Heart rate (top) and mean arterial pressure (bottom) over time. Data are presented as Mean ± SD. At each menstrual cycle phase, data presented over time were analyzed via two-way linear mixed effects models. No comparisons were made between menstrual cycle phases within the women because these data have been presented previously (12). Data are - women: n=12, men n=12.

Figure 4: Indices influencing fluid balance. Data are presented as Mean ± SD and individual values. Data were analyzed using generalized least squares models, with a priori pairwise comparisons between men and women across each phase of the menstrual cycle. Pairwise comparisons were adjusted for multiple comparisons using the Benjamini-Hochberg method. In all instances, no comparisons were made between menstrual cycle phases because these data have been presented previously (12). Data are - women: n=12, men n=12. LBM: lean body mass.

Figure 5: Thirst sensation (top) and whole-body thermal comfort (middle) and thermal sensation (bottom) over time. Data are presented as Mean ± SD. At each menstrual cycle phase, data presented over time were analyzed via two-way linear mixed effects models. No comparisons were made between menstrual cycle phases within the women because these data have been presented previously (12). Data are - women: n=12, men n=12.

Figure 6: Acute kidney injury risk over time and the change (Δ) in these variables from pre-simulated work-related heat stress. Data are presented as Mean ± SD and individual values where possible. Data presented over time were analyzed via two-way linear mixed effects models across menstrual cycle phase within women only or within each menstrual cycle phase for comparisons between men and women. For change data, data in women across the menstrual cycle were analyzed using a one-way repeated measures linear mixed effects model. Change data were also analyzed using generalized least squares models, with a priori pairwise comparisons between men and women across each phase of the menstrual cycle. Pairwise comparisons were adjusted for multiple comparisons using the Benjamini-Hochberg method. Data are - women - EF: n=12, LF: n=11; ML: n=11, men - n=12.

Tables:

Table 1: Participant characteristics at screening

	Women	Men	p-value
Age (y)	26 ± 5	25 ± 5	0.5851
Height (cm)	164 ± 4	180 ± 4	<0.0001
Body mass (kg)	64.6 ± 6.7	84.6 ± 10.8	<0.0001
Body mass index (kg/m^2)	24 ± 2	26 ± 4	0.0686
Body surface area (m^2)	1.7 ± 0.1	2.1 ± 0.1	<0.0001
Mean arterial pressure (mmHg)	87 ± 6	94 ± 3	0.0006
Menstrual cycle length (days)	30 ± 3	--	--
Day of luteinizing hormone surge	15 ± 1	--	--

Data are presented as Mean ± SD. Data are - women: n=12, men n=12. Data analyzed via two-tailed unpaired t-test.

Table 2: Pre-experimental trial measures

	Women			Men
	EF	LF	ML	
24-hour urine analysis				
Volume (mL)	1673 ± 985	1931 ± 829	1820 ± 699	2587 ± 784
Specific gravity	1.012 ± 0.006	1.010 ± 0.004	1.011 ± 0.004	1.009 ± 0.004
Osmolality (mOsm/kg)	443 ± 220	402 ± 158	439 ± 149	344 ± 143
Sodium (mmol/L)	83 ± 45	91 ± 37	92 ± 40	64 ± 32
Body composition				
Body fat (%)	32 ± 6	31 ± 6	32 ± 6	25 ± 8
Lean body mass (kg)	44.6 ± 4.7 *	44.5 ± 4.6 *	44.4 ± 5.0 *	62.4 ± 3.8
Thermal measures				
Core temperature (°C)	37.1 ± 0.3	36.8 ± 0.6	37.2 ± 0.3	37.0 ± 0.2
Mean skin temperature (°C)	32.3 ± 0.8 *	32.5 ± 0.8	32.5 ± 0.9	33.0 ± 0.8
Sex hormones				
Estradiol (ng/mL)	65.1 ± 15.9	111.2 ± 45	116.8 ± 29.1	--
Progesterone (ng/mL)	0.6 ± 0.5	1.4 ± 3.1	6.6 ± 4.1	--
Testosterone (ng/mL)	1.4 ± 0.3 *	1.5 ± 0.4 *	1.5 ± 0.4 *	18.5 ± 20.1

Data are presented as mean ± SD. All data are men: n=12, women: n=12 except hormone levels due to non-physiological hormone levels in one participant. Data were analyzed using generalized least squares models, with a priori pairwise comparisons between men and women across each phase of the menstrual cycle. Pairwise comparisons were adjusted for multiple comparisons using the Benjamini-Hochberg method. In all instances, no comparisons were made between menstrual cycle phases because these data have been presented previously (12). * indicates significantly different from Men ($p<0.0001$).

Table 3: Indices of the average rate of metabolic heat production

	Women			Men
	EF	LF	ML	
Watts	338 ± 60 *	326 ± 53 *	344 ± 50 *	424 ± 61
Watts per kg	5.2 ± 0.8	5.0 ± 0.6	5.4 ± 0.9	5.0 ± 0.7
Watts per m^2	197 ± 32	190 ± 24	201 ± 29	207 ± 26

Data are presented as Mean ± SD. Data are - women: n=12, men n=12. Data were analyzed using generalized least squares models, with a priori pairwise comparisons between men and women across each phase of the menstrual cycle. Pairwise comparisons were adjusted for multiple comparisons using the Benjamini-Hochberg method. In all instances, no comparisons were made between menstrual cycle phases because these data have been presented previously (12). * indicates significantly different from Men ($p \leq 0.0023$).

940 **Table 4:** Hydration measures and fluid regulation hormones

	Women			Men	Linear mixed-effects models		
	EF	LF	ML		EF vs. Men	LF vs. Men	ML vs. Men
Plasma volume (mL)					Time: p=0.0036 Group: p=0.3769 Time x Group: p=0.7678	Time: p=0.0018 Group: p=0.1825 Time x Group: p=0.6894	Time: p=0.0045 Group: p=0.1134 Time x Group: p=0.9995
Pre	3839 ± 909	3396 ± 1014	3189 ± 740	4759 ± 3211			
Post	4014 ± 843	3579 ± 1063	3335 ± 745	4905 ± 3208			
Plasma volume (mL/m^2)					Time: p=0.0041 Group: p=0.9235 Time x Group: p=0.5305	Time: p=0.0016 Group: p=0.5422 Time x Group: p=0.4981	Time: p=0.0068 Group: p=0.3448 Time x Group: p=0.7809
Pre	2221 ± 510	1988 ± 627	1863 ± 440	2282 ± 1408			
Post	2324 ± 487	2091 ± 635	1947 ± 440	2351 ± 1403			
Urine specific gravity					Time: p=0.0002 Group: p=0.0603 Time x Group: p=0.0509	Time: p=0.0003 Group: p=0.0427 Time x Group: p=0.1554	Time: p=0.0009 Group: p=0.1197 Time x Group: p=0.0133
Pre	1.004 ± 0.004	1.003 ± 0.005	1.005 ± 0.006	1.007 ± 0.007			
Post	1.007 ± 0.005 *	1.006 ± 0.006	1.006 ± 0.006 *	1.013 ± 0.005			
Serum osmolality (mOsm/kg)					Time: p=0.0003 Group: p=0.1328 Time x Group: p=0.5749	Time: p=0.0047 Group: p=0.0032 Time x Group: p=0.2638	Time: p=0.0003 Group: p=0.0011 Time x Group: p=0.4793
Pre	290 ± 6	288 ± 2 *	287 ± 5 *	292 ± 2			
Post	286 ± 3	287 ± 3	283 ± 4 *	289 ± 3			
Plasma copeptin (pg/mL)					Time: p=0.3415 Group: p=0.0006 Time x Group: p=0.7351	Time: p=0.6531 Group: p=0.0005 Time x Group: p=0.1371	Time: p=0.8067 Group: p<0.0001 Time x Group: p=0.2988
Pre	98 ± 30	109 ± 39	125 ± 42	62 ± 7			
Post	99 ± 32	104 ± 36	120 ± 42	64 ± 8			
Plasma aldosterone (pg/mL)					Time: p=0.8691 Group: p=0.2431 Time x Group: p=0.4981	Time: p=0.4929 Group: p=0.0443 Time x Group: p=0.9095	Time: p=0.3363 Group: p=0.0827 Time x Group: p=0.5651
Pre	163 ± 93	165 ± 68	395 ± 294	236 ± 100			
Post	182 ± 177	148 ± 77	349 ± 233	224 ± 123			

941 Data are presented as Mean ± SD. Data are - women: n=12, men n=12, except for indices of
942 plasma volume where n=11 for the men due to equipment failure during carbon monoxide
943 rebreathing test. At each menstrual cycle phase, data analyzed via two-way linear mixed effects
944 models. No comparisons were made between menstrual cycle phases because these data have
945 been presented previously (12). * Indicates significantly different from Men (p≤0.0367).
946

947 **Table 5:** Indices of kidney function and fluid conservation

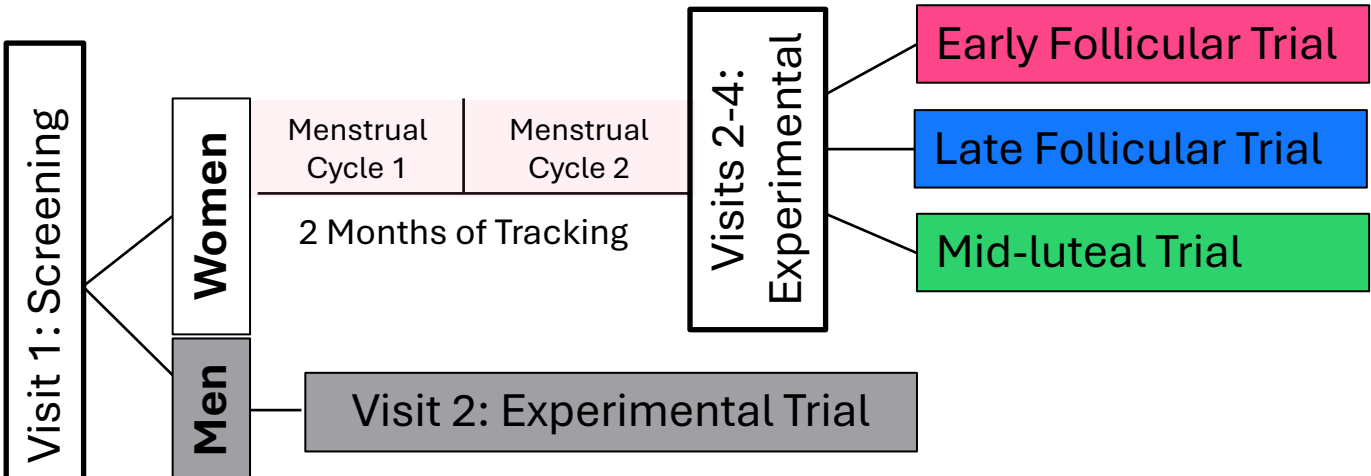
	Women			Men	Linear mixed-effects models		
	EF	LF	ML		EF vs. Men	LF vs. Men	ML vs. Men
Urine flow rate (mL/min)					Time: p<0.0001 Group: p=0.5828 Time x Group: p=0.0542	Time: p=0.0001 Group: p=0.3127 Time x Group: p=0.1476	Time: p<0.0001 Group: p=0.9718 Time x Group: p=0.0096
Pre	5.3 ± 2.4	5.9 ± 3.3	4.4 ± 2.9	6.0 ± 3.5			
Post	3.3 ± 3.1	3.6 ± 3.0	3.3 ± 2.4	1.6 ± 1.1			
Serum creatinine (mg/dL)					Time: p=0.0486 Group: p<0.0001 Time x Group: p=0.8689	Time: p=0.7917 Group: p=0.0005 Time x Group: p=0.2338	Time: p=0.6066 Group: p=0.0004 Time x Group: p=0.2230
Pre	0.74 ± 0.09 *	0.78 ± 0.12 *	0.79 ± 0.12 *	0.95 ± 0.09			
Post	0.77 ± 0.12 *	0.77 ± 0.14 *	0.79 ± 0.12 *	0.97 ± 0.09			
Urine creatinine (mg/dL)					Time: p=0.0041 Group: p=0.9235 Time x Group: p=0.5305	Time: p=0.0016 Group: p=0.5422 Time x Group: p=0.4981	Time: p=0.0068 Group: p=0.3448 Time x Group: p=0.7809
Pre	30.0 ± 35.4	39.4 ± 69.8	45.2 ± 50.4	54.8 ± 74.2			
Post	61.1 ± 54.1	71.0 ± 81.6	51.9 ± 60.1	117.2 ± 62.2			
Creatinine clearance (mL/min)					Time: p=0.0834 Group: p=0.1155 Time x Group: p=0.7734	Time: p=0.1168 Group: p=0.0273 Time x Group: p=0.7720	Time: p=0.1727 Group: p=0.0193 Time x Group: p=0.3898
Pre	142 ± 30	134 ± 22	127 ± 24	158 ± 19			
Post	131 ± 21	124 ± 30	124 ± 32	145 ± 38			
Urine osmolality (mOsm/kg)					Time: p=0.0002 Group: p=0.0636 Time x Group: p=0.0618	Time: p=0.0022 Group: p=0.0460 Time x Group: p=0.1291	Time: p=0.0105 Group: p=0.1286 Time x Group: p=0.0026
Pre	174 ± 141	160 ± 154	234 ± 224	267 ± 263			
Post	248 ± 168	230 ± 209	221 ± 189 *	455 ± 211			
Free water clearance (mL/min)					Time: p=0.0008 Group: p=0.2201 Time x Group: p=0.1130	Time: p=0.0012 Group: p=0.0578 Time x Group: p=0.3247	Time: p=0.0010 Group: p=0.3246 Time x Group: p=0.0168
Pre	3.1 ± 2.5	3.8 ± 2.9	2.4 ± 2.9	2.8 ± 3.2			
Post	1.6 ± 2.8	1.9 ± 2.6	1.8 ± 2.2	-0.4 ± 1.0			
Osmolar clearance (mL/min)					Time: p<0.0001 Group: p=0.0480 Time x Group: p=0.1805	Time: p<0.0001 Group: p=0.0181 Time x Group: p=0.1321	Time: p=0.0001 Group: p=0.0066 Time x Group: p=0.0256
Pre	2.4 ± 0.7	2.2 ± 0.8	2.0 ± 0.6 *	3.2 ± 1.0			
Post	1.7 ± 0.6	1.6 ± 0.6	1.6 ± 0.4	2.0 ± 0.8			
Serum sodium (mmol/L)					Time: p>0.9999 Group: p=0.3101 Time x Group: p=0.6633	Time: p=0.5029 Group: p=0.8192 Time x Group: p=0.3128	Time: p=0.0935 Group: p=0.0150 Time x Group: p=0.0448
Pre	141 ± 1	141 ± 1	140 ± 1	140 ± 1			
Post	141 ± 1	140 ± 1	139 ± 1 *	141 ± 1			
Urine sodium (mmol/L)					Time: p=0.0419 Group: p=0.4848 Time x Group: p=0.4331	Time: p=0.4684 Group: p=0.1255 Time x Group: p=0.0152	Time: p=0.6301 Group: p=0.4105 Time x Group: p=0.0172
Pre	57 ± 51	41 ± 29	47 ± 47	41 ± 34			
Post	67 ± 44	29 ± 21 *	33 ± 29	61 ± 28			
Fractional excretion of sodium (%)					Time: p=0.0002 Group: p=0.0121 Time x Group: p=0.8548	Time: p=0.0057 Group: p=0.2188 Time x Group: p=0.3116	Time: p=0.0001 Group: p=0.9543 Time x Group: p=0.3404
Pre	1.1 ± 0.6	1.1 ± 1.0	0.7 ± 0.4	0.8 ± 0.3			
Post	0.8 ± 0.3	0.5 ± 0.4	0.5 ± 0.2	0.4 ± 0.2			

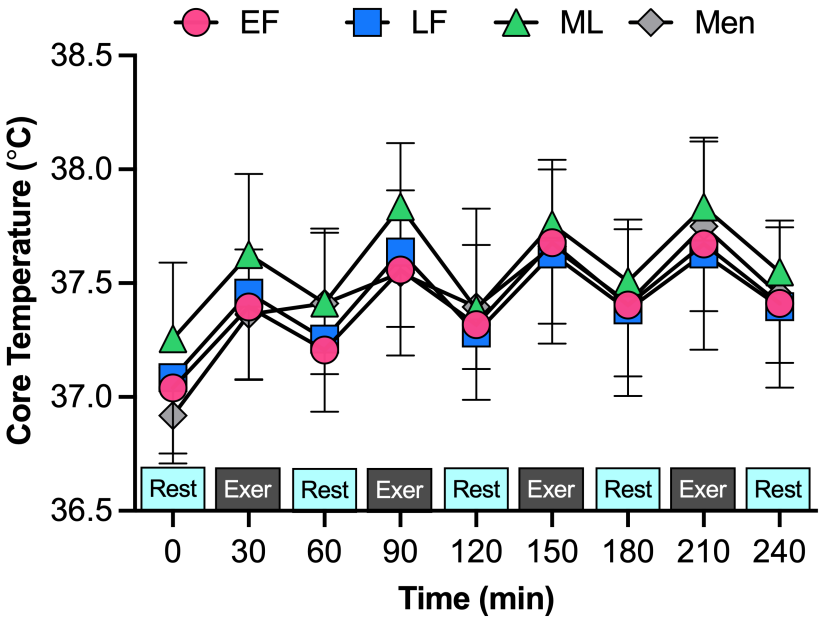
948 Data are presented as Mean ± SD. Data are - women: n=12, men n=12. Data presented over
949 time were analyzed via two-way linear mixed effects models. No comparisons were made
950 between menstrual cycle phases because most of these data have been presented previously
951 (12). * Indicates significantly different from Men ($p \leq 0.0379$).
952

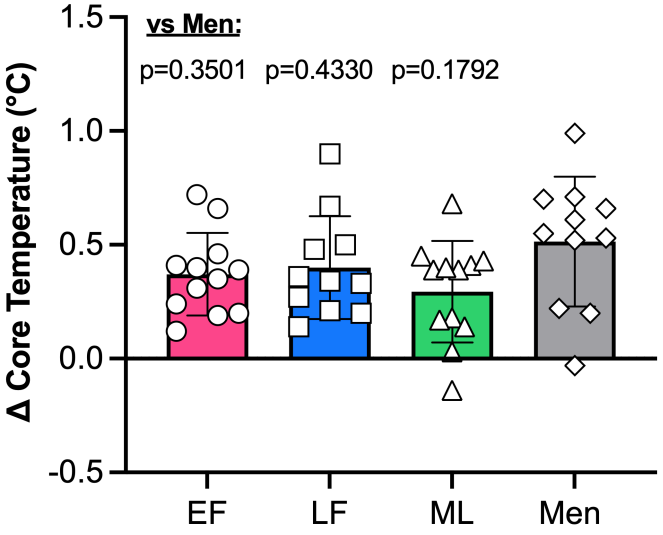
Table 6: Indices of renal perfusion

	Women			Men	Linear mixed-effects models			
	EF	LF	ML		Women only	EF vs. Men	LF vs. Men	ML vs. Men
Heart rate (bpm)					Time: p=0.0241 Phase: p=0.1272 Time x Phase: p=0.4723	Time: p=0.0011 Group: p=0.8834 Time x Group: p=0.3466	Time: p=0.0004 Group: p=0.9680 Time x Group: p=0.5852	Time: p=0.0014 Group: p=0.5286 Time x Group: p=0.3436
Pre	62 ± 9	61 ± 11	65 ± 10	60 ± 10				
Post	67 ± 11	68 ± 12	70 ± 12	70 ± 11				
Mean arterial pressure (mmHg)					Time: p=0.0760 Phase: p=0.6370 Time x Phase: p=0.8883	Time: p=0.3559 Group: p=0.0113 Time x Group: p=0.5598	Time: p=0.4635 Group: p=0.0055 Time x Group: p=0.7310	Time: p=0.4704 Group: p=0.0051 Time x Group: p=0.7451
Pre	87 ± 9	86 ± 7	86 ± 9	95 ± 4				
Post	90 ± 8	88 ± 9	88 ± 7	96 ± 10				
Renal artery blood velocity (cm/s)					Time: p=0.0600 Phase: p=0.9222 Time x Phase: p=0.0235	Time: p=0.0085 Group: p=0.0080 Time x Group: p=0.0907	Time: p=0.0226 Group: p=0.0067 Time x Group: p=0.2835	Time: p=0.6410 Group: p=0.0090 Time x Group: p=0.3848
Pre	46 ± 8	48 ± 11	52 ± 10	38 ± 10				
Post	55 ± 14	53 ± 10	51 ± 14	40 ± 8				
Segmental artery blood velocity (cm/s)					Time: p=0.1289 Phase: p=0.3827 Time x Phase: p=0.3388	Time: p=0.0289 Group: p=0.8691 Time x Group: p=0.3292	Time: p=0.1594 Group: p=0.1912 Time x Group: p=0.1391	Time: p=0.0050 Group: p=0.7020 Time x Group: p>0.9999
Pre	20 ± 4	22 ± 4	20 ± 4	19 ± 4				
Post	21 ± 5	22 ± 3	22 ± 3	21 ± 3				
Renal artery vascular resistance (mmHg/cm/s)					Time: p=0.2051 Phase: p=0.8705 Time x Phase: p=0.0787	Time: p=0.0162 Group: p=0.0024 Time x Group: p=0.8346	Time: p=0.0297 Group: p=0.0015 Time x Group: p=0.9818	Time: p=0.5616 Group: p=0.0017 Time x Group: p=0.0617
Pre	1.9 ± 0.4	1.9 ± 0.4	1.7 ± 0.4	2.7 ± 0.7				
Post	1.7 ± 0.4	1.7 ± 0.4	1.8 ± 0.5	2.5 ± 0.6				
Segmental artery vascular resistance (mmHg/cm/s)					Time: p=0.4127 Phase: p=0.2495 Time x Phase: p=0.3454	Time: p=0.1780 Group: p=0.2577 Time x Group: p=0.1628	Time: p=0.1257 Group: p=0.0119 Time x Group: p=0.1257	Time: p=0.0150 Group: p=0.0964 Time x Group: p=0.8126
Pre	4.5 ± 0.8	4.1 ± 0.8	4.5 ± 0.9	5.1 ± 0.9				
Post	4.5 ± 1.0	4.1 ± 0.5	4.2 ± 0.7	4.7 ± 1.0				

Data are presented as Mean ± SD. Data are - women: n=12, men n=12. Data presented over time were analyzed via two-way linear mixed effects models across menstrual cycle phase within women only or within each menstrual cycle phase for comparisons between men and women. * indicates significantly different from Men ($p \leq 0.0433$).



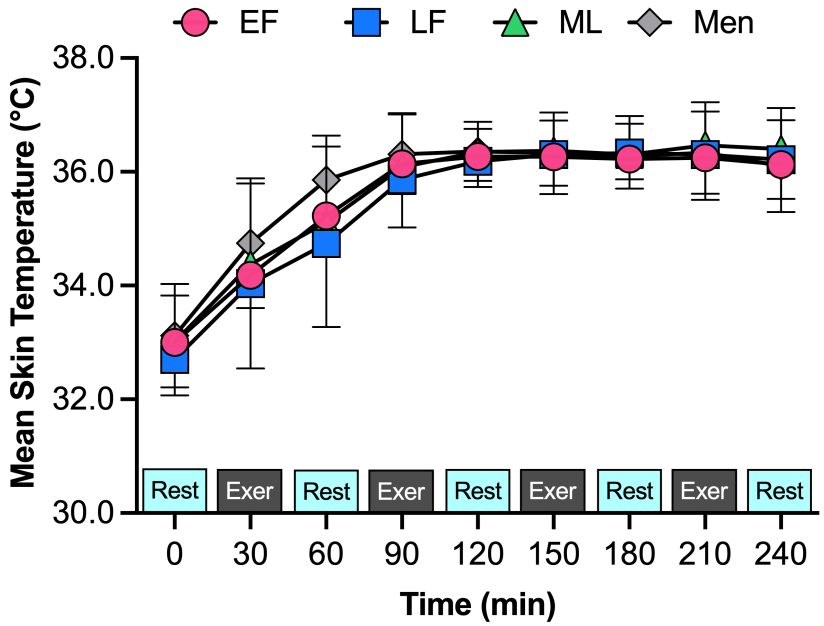


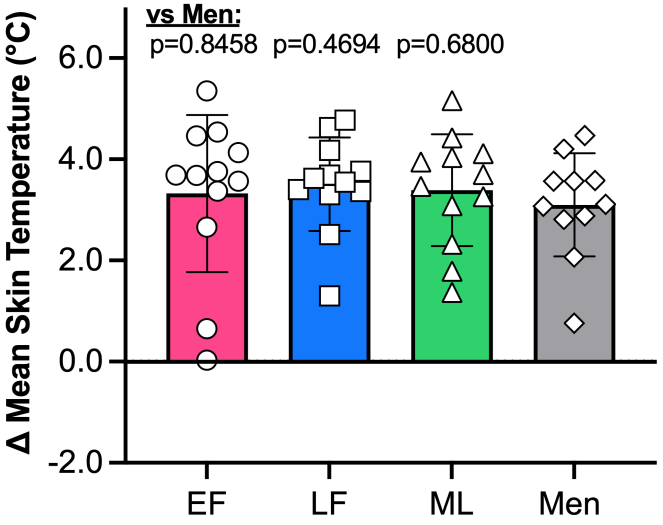


Early Follicular (EF):
Time: p<0.0001
Group: p=0.7756
Time x Group: p=0.1002

Late Follicular (LF):
Time: p<0.0001
Group: p=0.8033
Time x Group: p=0.0210

Mid Luteal (ML):
Time: p<0.0001
Group: p=0.2297
Time x Group: p=0.0117

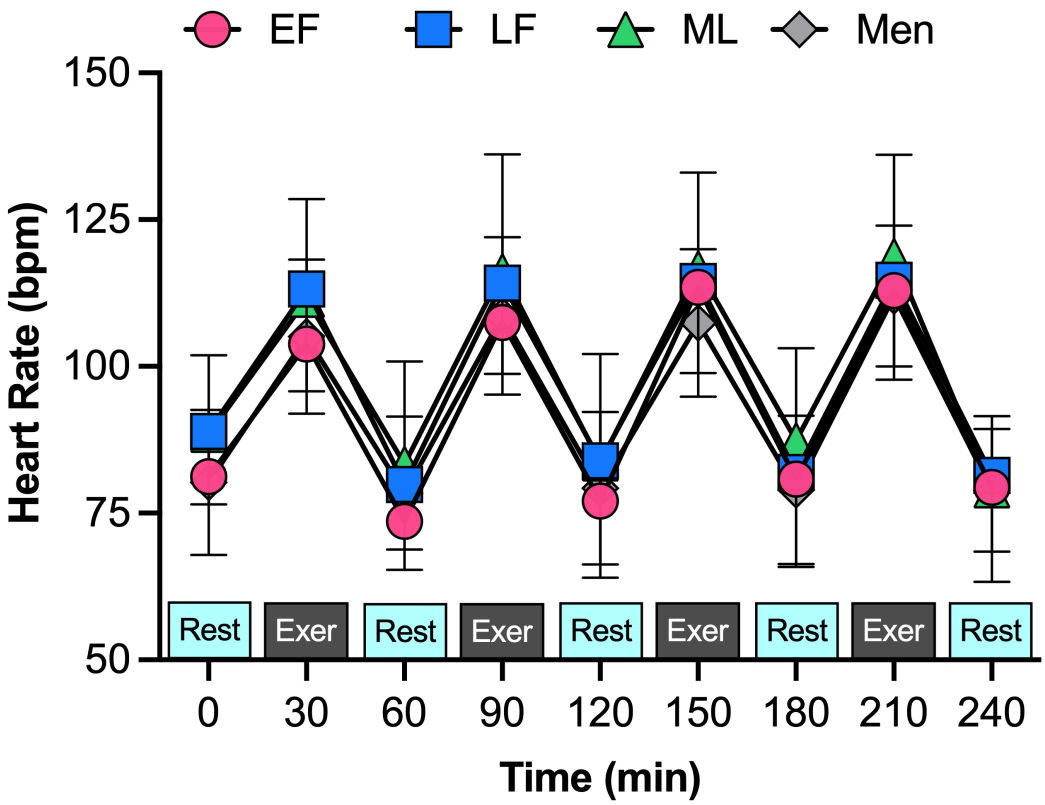




Early Follicular (EF):
Time: p<0.0001
Group: p=0.3732
Time x Group: p=0.7623

Late Follicular (LF):
Time: p<0.0001
Group: p=0.1632
Time x Group: p=0.0602

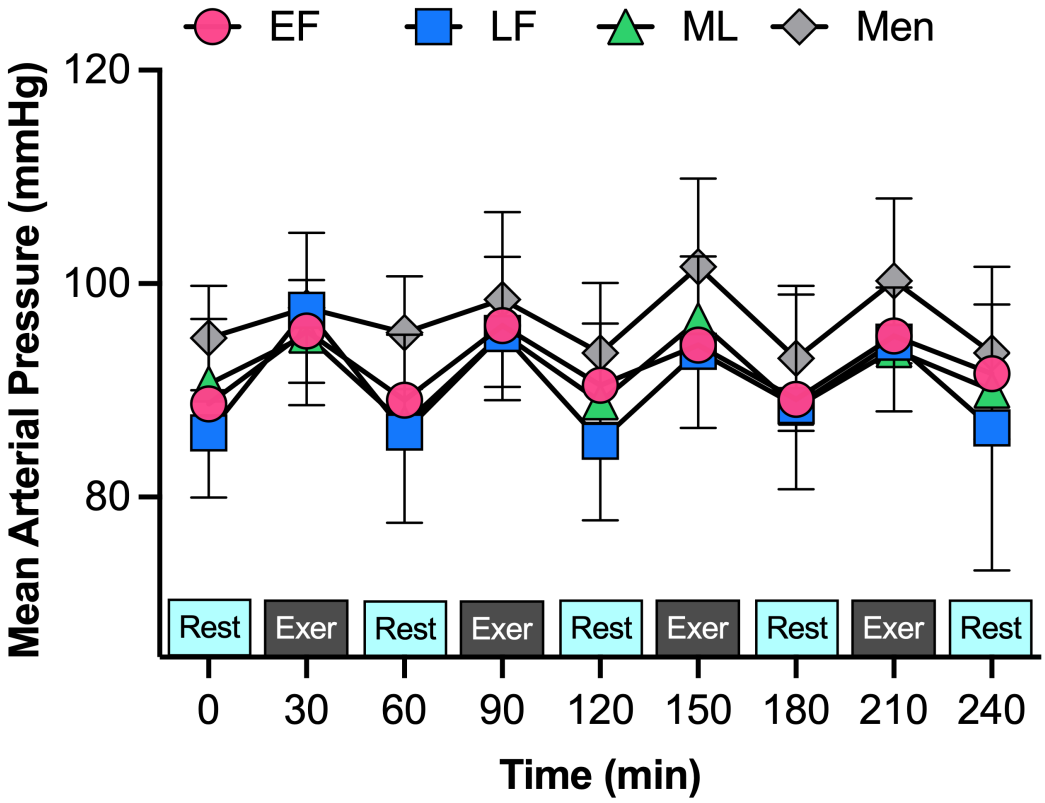
Mid Luteal (ML):
Time: p<0.0001
Group: p=0.6659
Time x Group: p=0.2892



Early Follicular (EF):
Time: p<0.0001
Group: p=0.9504
Time x Group: p=0.5294

Late Follicular (LF):
Time: p<0.0001
Group: p=0.3129
Time x Group: p=0.8621

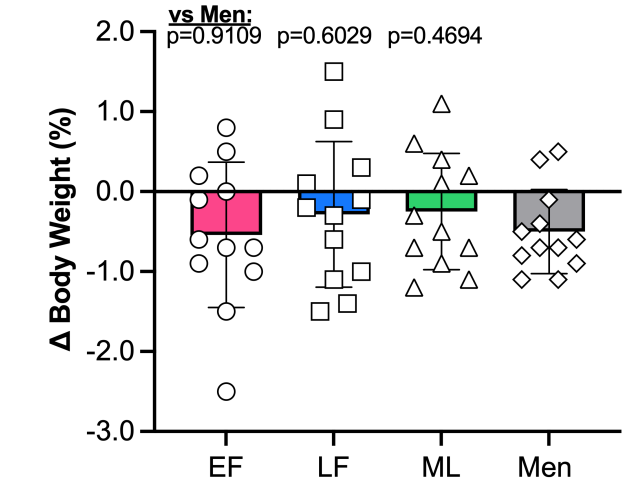
Mid Luteal (ML):
Time: p<0.0001
Group: p=0.2195
Time x Group: p=0.8013

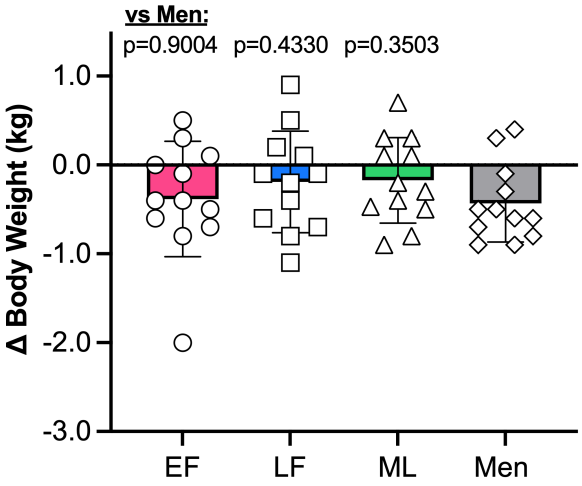


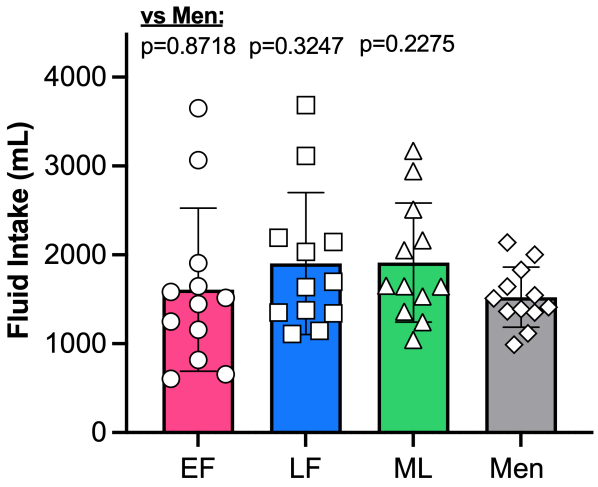
Early Follicular (EF):
Time: p<0.0001
Group: p=0.0583
Time x Group: p=0.4377

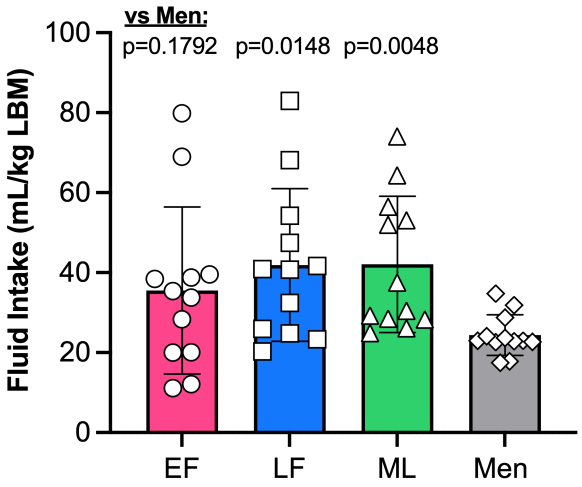
Late Follicular (LF):
Time: p<0.0001
Group: p=0.1632
Time x Group: p=0.0602

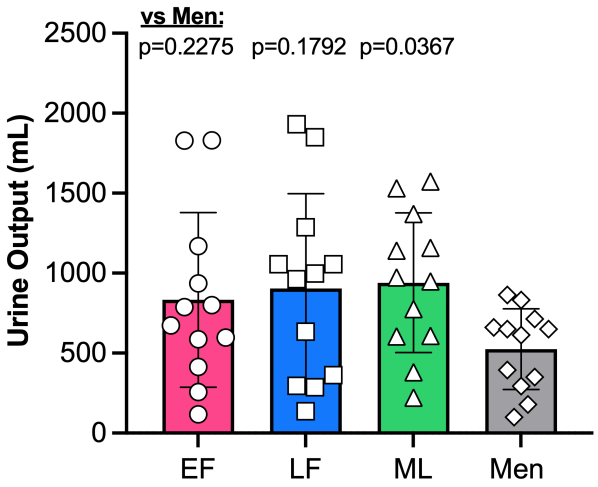
Mid Luteal (ML):
Time: p<0.0001
Group: p=0.0459
Time x Group: p=0.5544

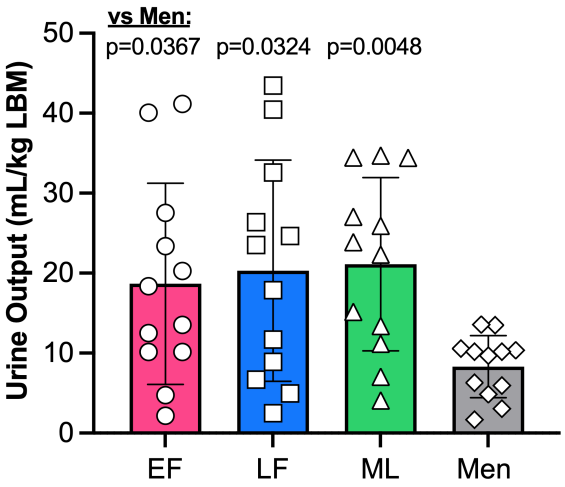


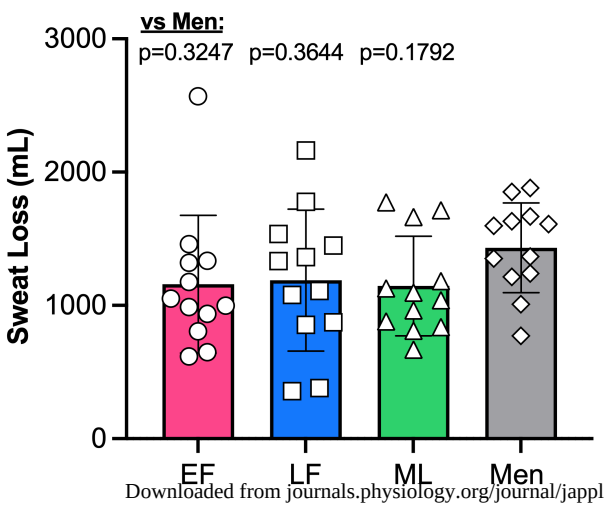


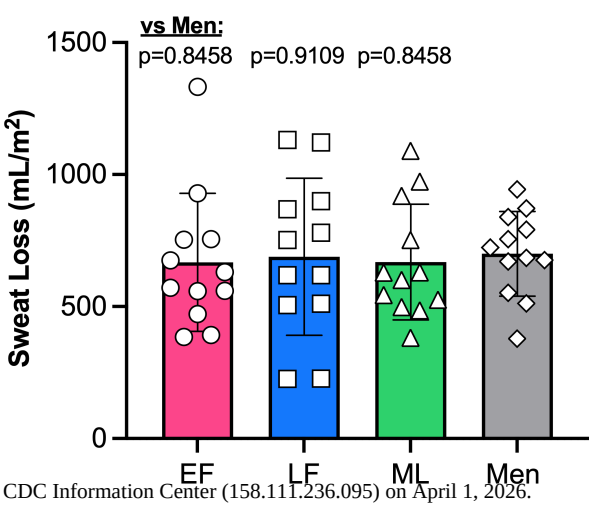


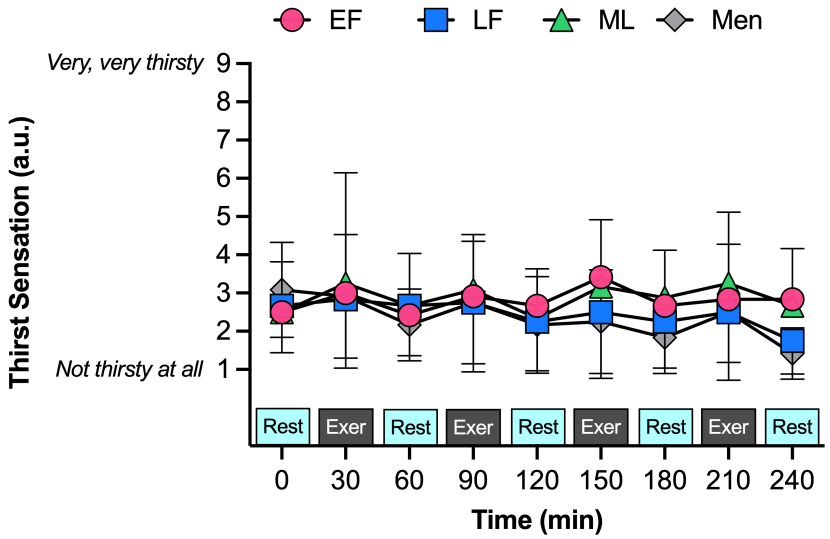








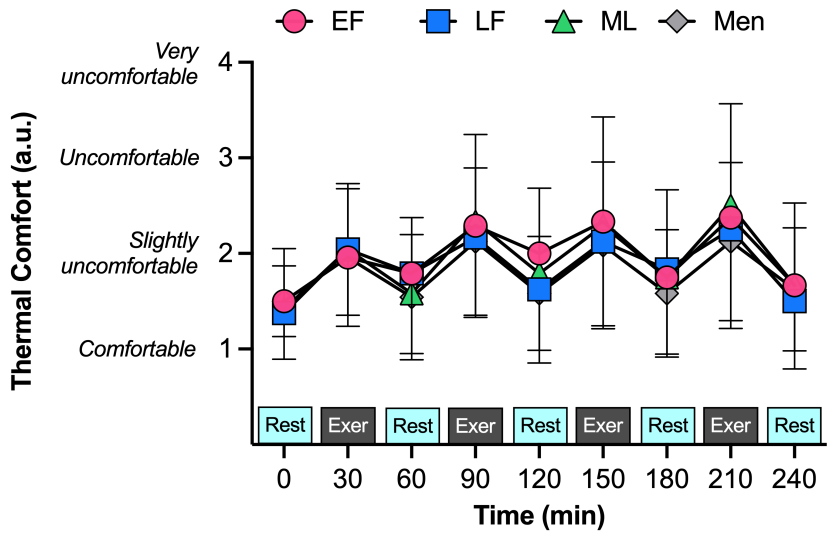




Early Follicular (EF):
Time: p=0.0463
Group: p=0.3494
Time x Group: p=0.0501

Late Follicular (LF):
Time: p=0.0004
Group: p=0.7968
Time x Group: p=0.5606

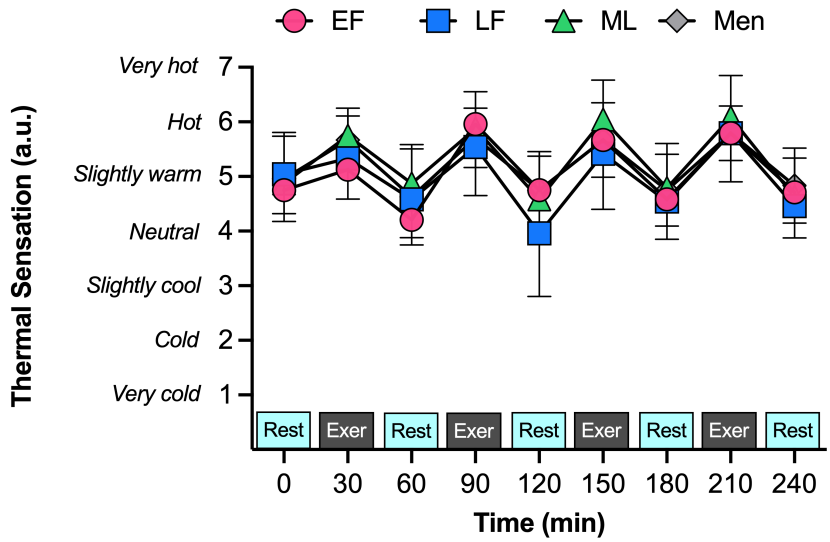
Mid Luteal (ML):
Time: p=0.0288
Group: p=0.2962
Time x Group: p=0.1377



Early Follicular (EF):
Time: p<0.0001
Group: p=0.4800
Time x Group: p=0.6728

Late Follicular (LF):
Time: p<0.0001
Group: p=0.7967
Time x Group: p=0.7254

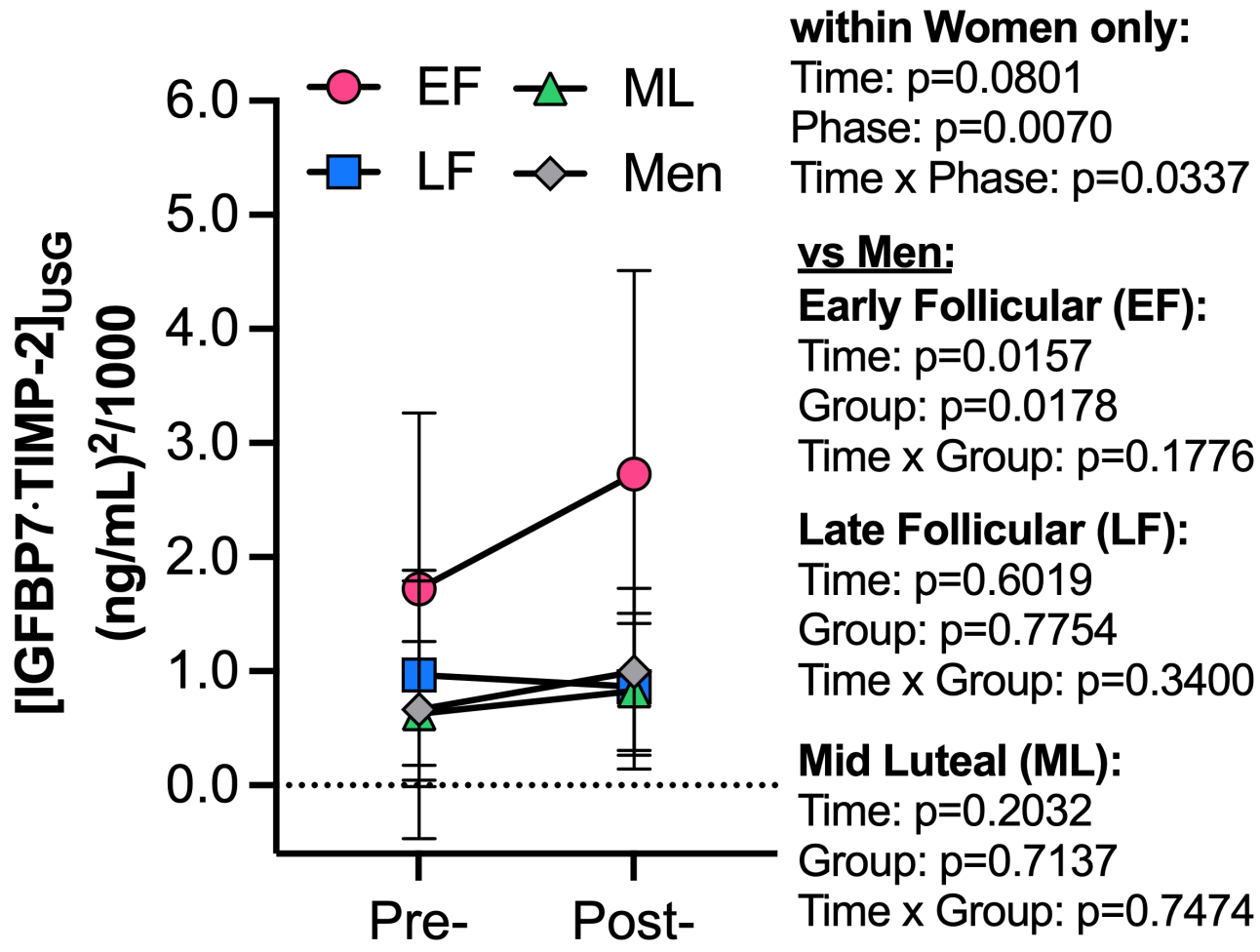
Mid Luteal (ML):
Time: p<0.0001
Group: p=0.6197
Time x Group: p=0.6986

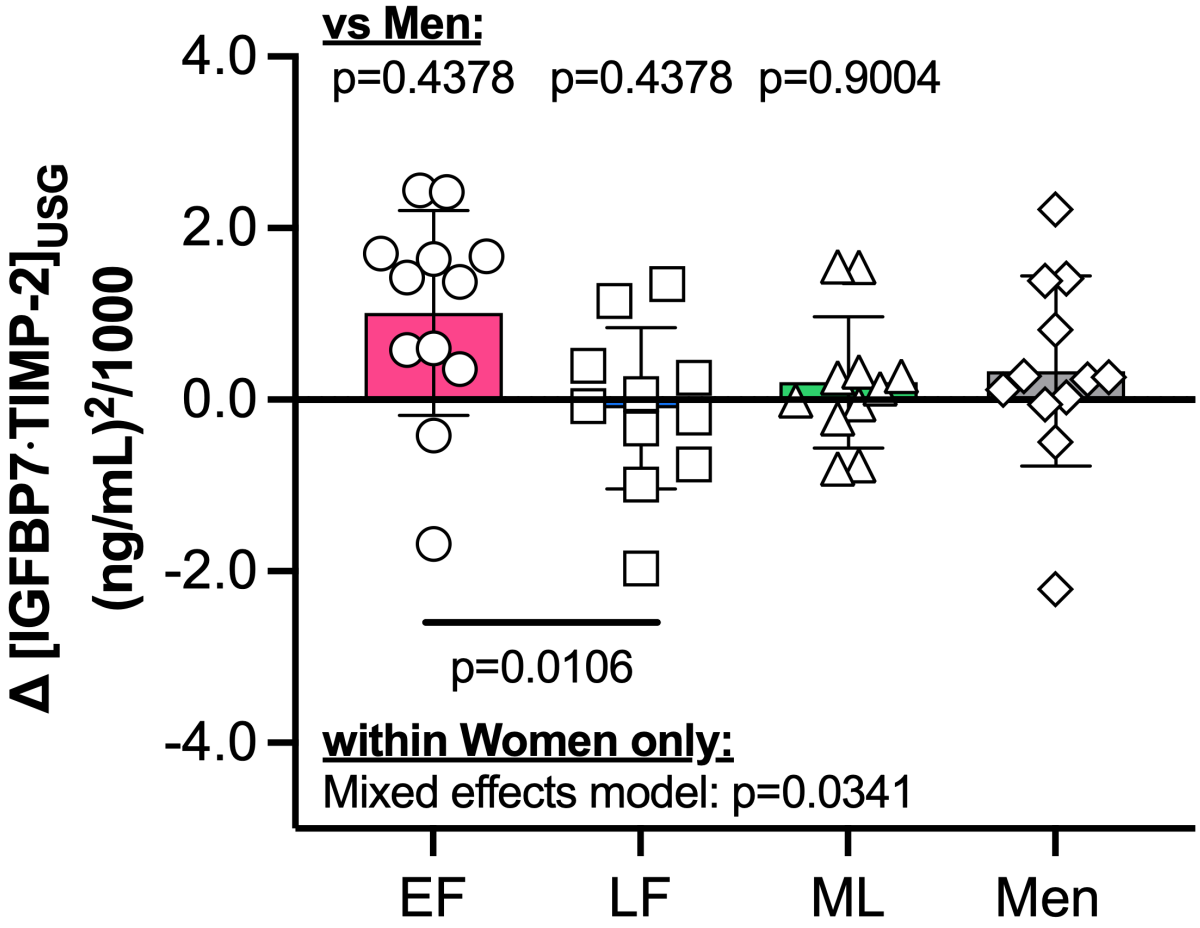


Early Follicular (EF):
Time: p<0.0001
Group: p=0.5328
Time x Group: p=0.5068

Late Follicular (LF):
Time: p<0.0001
Group: p=0.2721
Time x Group: p=0.3446

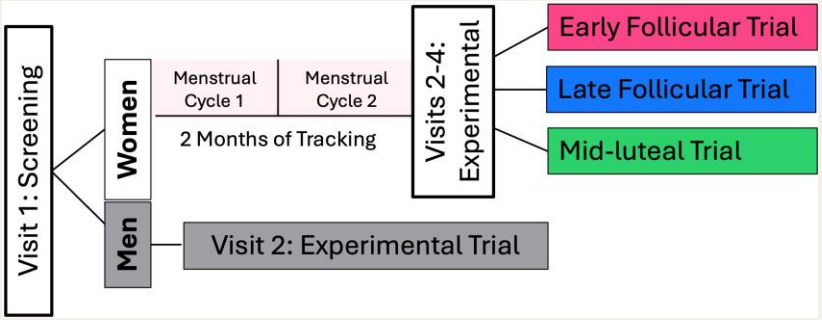
Mid Luteal (ML):
Time: p<0.0001
Group: p=0.6866
Time x Group: p=0.4577



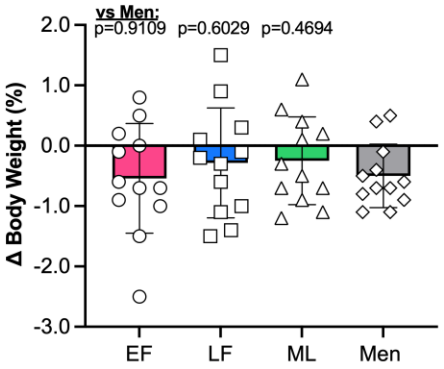


Fluid balance, kidney function and acute kidney injury risk in healthy men and women during simulated work-related heat stress

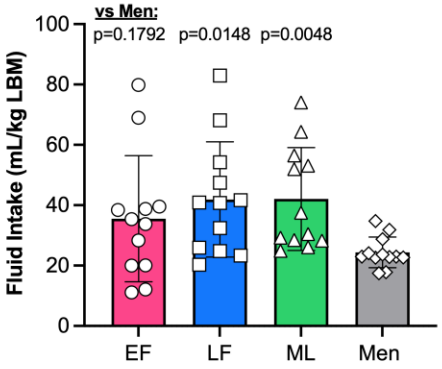
METHODS

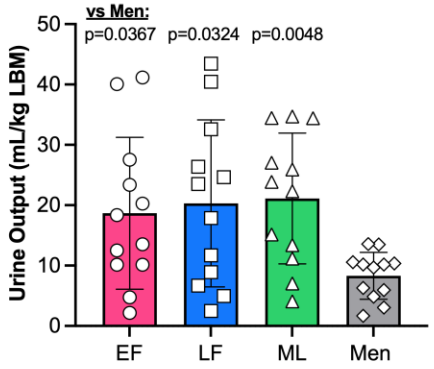


OUTCOME



Fluid balance was similarly maintained but the way fluid balance was achieved differed between men and women, with women drinking more, which was compensated for by an increase in urine output.





CONCLUSION When cool fluids are available, men and women maintain similar fluid balance regardless of menstrual cycle phase, suggesting sex does not influence dehydration risk during simulated work-related heat stress .