Influence of peak menstrual cycle hormonal changes on restoration of fluid balance after induced dehydration.

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Running head: Menstrual cycle and rehydration after exercise.

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Abstract

The present study examined the impact of hormonal differences between late follicular (LF) and mid-luteal (ML) phases on restoration of fluid balance following dehydration. Ten eumenorreheic female participants were dehydrated by 2% of their body mass through overnight fluid restriction followed by exercise-heat stress. Trials were undertaken during the LF (between day 10 and 13 of the menstrual cycle) and ML phases (between day 18 and 23 of the menstrual cycle) with one phase repeated to assess reliability of observations. Following dehydration, participants ingested a volume equivalent to 100% of mass loss of a commercially available sports drink in 4 equal volumes over 30 minutes. Mean serum values for steroid hormones during the ML (estradiol (E_2):92±11pg/mL; progesterone:19±4ng/mL) and LF (estradiol (E_2):232±64pg/mL; progesterone:3±2ng/mL) were significantly different between phases. Urine tests confirmed no luteinizing hormone surge evident during LF trials. There was no effect of menstrual cycle phase on cumulative urine volume during the 3-h rehydration period (ML: 630(197-935) mL; LF: 649(180-845) mL) with percentage of fluid retained being 47(33-85)% on ML and 46(37-89)% on LF, *p=0.29*). There was no association between the progesterone:estradiol ratio and fluid retained in either phase. Net fluid balance, urine osmolality, and thirst intensity were not different between phases. No differences in sodium (ML: -61(-36-(-131)) mmol; LF: -73(-5-(-118)) mmol; p=*0.45*) or potassium (ML: -36(-11-(-80)) mmol; LF: -30 (-19-(-89)) mmol; *p=0.96*) balance were observed. Fluid replacement after dehydration does not appear to be affected by normal hormonal fluctuations during the menstrual cycle in eumenorreheic young women.

Key words: electrolytes; rehydration; exercise; heat stress; female

Introduction

Maintenance of fluid balance is a major consideration for recreational exercisers and athletes (Maughan et al. 1997). Since hypohydration, defined as the the uncompensated loss of body water, is known to influence exercise performance and health (Evans et al. 2017) it is recommended to start exercise in a euhydrated state, and to replace lost fluids on cessation of exercise, however, most of the research on this field has been done in males due to uncertainty about inclusion of females in relation to menstrual cycle phase effects on fluid balance. Many factors affect fluid balance and rehydration such as drink composition and volume (Shirreffs and Maughan 2000), but also it is believed that hormonal changes associated with the menstrual cycle can influence fluid retention (Fortney 1996).

Although fluid replacement after dehydration has been extensively studied in males, there have been few attempts to address the influence of different levels of endogenous, circulating steroid hormones on this topic in women. Key hormones such as progesterone and oestrogens could exert a profound control over the regulation of fluid and electrolyte balance (Stachenfeld, 2009; Stachenfeld 2008; Spruce et al. 1985; Vokes et al. 1988) but to date there is limited data examining these responses. Since oestrogens promote fluid retention while progesterone has an opposite action, it has been hypothesized that fluid and electrolyte balance in women may be affected by fluctuations of these steroid hormones during the normal menstrual cycle phases (Stachenfeld, 2008). Indeed, differences in urine volume have been reported in 24-hour collections between menstrual cycle phases, in spite of constant fluid intake (Claybaugh et al. 2000; Fong and Kretsch 1993). However, these observations raise the question of whether there is an acute effect of endogenous hormone status on fluid balance (Ormerod, 2011).

Yasuda et al. (2013) evaluated the effects of menstrual cycle phase on hydration status following

exercise in nine eumenorrheic female basketball players. The hydration status in the mid-follicular phase was similar to that of the mid-luteal phase. Similarly, the acute restoration of fluid balance after exercise-induced hypohydration was unaffected between mid-follicular and mid-luteal and late-luteal phases of the menstrual cycle in five healthy untrained eumenorrheic young women (Maughan et al. 1996). These findings suggest that post-exercise hydration/rehydration does not appear to be directly affected by the menstrual cycle hormonal fluctuations. However, neither of these previous studies measured hormone concentrations to verify phases, and to determine the magnitude of differences in the hormonal milieu. This omission could be important as the balance between the effects of oestrogens and those of progesterone at the mid-luteal or late-luteal versus the mid-follicular phase does not maximise the potential impact upon fluid balance / rehydration. Fluid retention is thought to occur near ovulation during the peak oestrogen surge and just before the onset of menses at a time when progesterone concentration declines (late-follicular phase). High progesterone during the mid-luteal phase would oppose any potential oestrogen action on fluid retention (Fortney, 1996) suggesting that the progesterone:oestradiol ratio may play a role in influencing fluid retention. Therefore, to fully evaluate any potential menstrual cycle phase effects it seems necessary to compare restoration of fluid balance during the late follicular versus the mid-luteal phase and to include hormonal validation. Thus, the aim of the present study was to determine whether any differences in restoration of fluid balance and fluid retention occurred between late follicular and mid-luteal phases, and to document any association between fluid retention and the progesterone:oestradiol ratio.

Methods

Ten healthy female volunteers were recruited to complete this investigation. Participants were able to take part if they were between 18 to 40 years old, had regular menstrual cycles (25-32 d), were not using hormonal contraceptive methods, and were physically active on a recreational basis (exercised 3-5 times per week for 60 minutes or more) but none engaged in any form of systematic training at

the time of the study (age: 25 (18-33) years, body mass: 63.1±8.6 kg, stature: 167.6±7.1 cm, menstrual cycle length: 28.5±1.6 days, cycling VO_{2max}: 44.4±4.7 ml/kg/min). In order to confirm the regularity and length of the menstrual cycle, all participants tracked their menstrual cyle over three cycles before undertaking the experimental trials. The experimental procedures were approved by the local Research Ethics Committee and all the participants were informed of the nature of the investigation before they gave their written consent to participate.

Preliminary testing

As a preliminary procedure, VO_{2max} was determined during an incremental exercise test on a cycle ergometer (participants began at 20W and the load was increased by 20W every 3 minutes until volitional fatigue) in order to characterise the participants and to establish the workload at which each individual exercised during the exercise-heat stress experimental trials (moderate intensity, calculated to be approximately 60% of VO_{2max} for each subject).

Experimental Trials

Each individual participated in three experimental trials, the first was completed either during the late follicular (LF) phase (between day 10 and 13 of the menstrual cycle), or in the mid-luteal (ML) phase (between day 18 and 23 of the menstrual cycle), a second trial was conducted in the opposite phase, and a third trial was a repeat of either the ML or LF trial, whichever was the first trial undertaken. Five participants started with and repeated the LF trial with the remaining 5 starting with and repeating the ML trial. In order to assess the stability of the participants' body mass before each trial, they were provided with a set of scales to record their nude body mass over the three days preceding each trial. The participants were also instructed to record their diet (food and fluid) and physical activity over the 24-h preceding the first trial, and to replicate those patterns for subsequent trials. No instructions were given to individuals as to what they could or could not eat during this period with the exception of abstaining from alcohol.

Participants were asked to attend the laboratory at 17:30 the evening before the exercise-induced dehydration session. They were instructed to drink 500 mL of water 2-h before arriving at the laboratory to ensure they were euhydrated. Upon arrival, participants emptied their bladder and provided a urine sample to assess osmolality. Subsequently, euhydrated nude body mass was measured. A single 5-mL blood sample was collected via venepuncture from an antecubital vein, and blood was dispensed into a serum tube for subsequent analysis of osmolality and hormones to verify hydration status and menstrual cycle phase. Additionally, thirst intensity was subjectively assessed through a 100-mm Visual Analogue Scale (VAS). Finally, participants were instructed to stop the ingestion of foods and fluids at 20:00 that evening in order to begin the dehydration process. After overnight dehydration and fasting, participants arrived at the laboratory at 08:00. They were first asked to empty their bladder and provide a urine sample. Nude body mass was measured and the body mass loss required to attain a 2% loss in relation to euhydrated nude body mass, measured the evening before, was calculated. Also, thirst intensity was assessed before commencing the exercise-heat stress induced dehydration protocol. A schematic of the study protocol can be found in Figure 1. When participants arrived for the LF phase trial, part of the urine collected was assessed using a LH (Luteinising Hormone) urine ovulation test kit (One Test®) in order to ensure that ovulation had not yet occurred. Prior to exercise, the skin of the participants' right mid-thigh area was cleaned with alcohol, rinsed with deionized water and then dried with paper towel in preparation for sweat absorbent pad (3M™) application (as described by Patterson et al, 2000) for subsequent measurement of sweat electrolyte losses (Na^+ and K^+) during exercise.

Participants exercised on a cycle ergometer in a warm environment (28 ºC, relative humidity 30%) at moderate intensity (~60% of VO_{2max}). Nude body mass was measured after 30-min of continuous exercise. Individuals then continued exercising for periods of 10-min, interspersed by short rest periods during which the participants towelled dry and nude body mass was recorded, to determine body mass loss, and allow for calculation of sweat rate. Once 2% of body mass loss was achieved the

exercise was stopped. The sweat patch was removed using clean forceps and placed in a sterile plastic filter tube. Participants then left the warm environment and sat quietly for a 15-min cool-down period and were given a further 15-min to shower. Subsequently, participants emptied their bladder into a urine container. Nude body mass was then measured in order to obtain the total body mass loss through dehydration, and thirst intensity was recorded.

The rehydration process then began in which a commercially available sports drink (6.4% carbohydrates, 25 mmol/L Na^+, 3.5 mmol/L K^+) was provided in a volume equivalent to 100% of body mass loss. The total volume was divided into four equal aliquots, each of which was consumed over a 7.5-min period to complete a 30-min drinking period. Immediately following the drinking period, thirst intensity was assessed and the participants were asked to provide a urine sample. Further urine samples were collected at 1, 2 and 3 h after the end of the rehydration period. For each urine sample, the participants emptied their bladder as completely as possible and the entire volume was collected. Nude body mass was measured 3-h after the rehydration period and thirst intensity was assessed for the last time.

Analytical procedures / calculations

Whole blood in the serum tube was allowed to clot before centrifugation (15-min.; 4ºC; 4000 X g). Part of the serum was dispensed into eppendorf tubes and stored at –70ºC for the subsequent measurement of estradiol (E2) and progesterone using commercially available enzyme-linked immunosorbent assays (ELISA). Progesterone:estradiol ratio was then calculated (Elgindy, 2011). The remaining serum was stored in a microcentrifuge tube at 4 ºC for measurement of osmolality within 1-2 days. Assays were performed according to manufacturer recommendations to determine serum concentration of estradiol (E2) (Abcam - ab108649) and progesterone (Abcam – ab108654). Sweat was extracted from the absorbent pad through centrifugation (15-min.; 4ºC; 4000 X g). The sweat obtained was placed into a microcentrifuge tube and stored at 4 ºC for subsequent electrolyte analyses.

Urine samples obtained during the study were collected in a 1-L plastic container. The total mass of the sample in the container (to the nearest 0.1 g) was assessed to determine urine volume. A 5-mL aliquot was then dispensed into a plain screw-capped tube. Urine samples were stored at 4 ºC prior to analysis of osmolality, sodium and potassium concentration. Duplicate measurements of serum and urine osmolality were made using the freezing point depression method (Löser Micro-Digital Osmometer M15). Sodium and potassium of urine, sweat and drink samples were determined in duplicate by flame photometry (Jenway PFP7 Flame Photometer).

Net fluid balance was calculated from body mass loss, the volume of fluid ingested, and the volume of urine excreted. Electrolyte (Na^+ and K^+) balance was determined from ingested electrolytes, sweat losses, and urine losses.

Statistical analyses

Statistical analyses were conducted using IBM SPSS Statistics statistical software package version 23 (IBM Corporation, Armonk, NY, USA). Data are presented as means±SD, or median (range) as appropriate following Shapiro-Wilks analysis for normality of distribution. 95% confidence intervals for mean differences are presented where possible. To identify differences in normally distributed results, two-way (time-by-phase) repeated measures ANOVA were employed, and paired t-tests, where appropriate. For non-parametric analysis Wilcoxon tests were used instead of t-tests. The effect size based on using Cohen's d with threshold values for trivial, small, moderate, large, very large, and extremely large effects set at <0.2, 0.2. 0.6, 1.2, 2.0, and 4.0 (Hopkins et al 2009) was reported along with a written description for the main outcome (percentge of fluid retained). Test re-test reliability for duplicate trials was assessed through Intraclass Correlation Coefficient (ICC) and coefficient of variation (CV). The Associations between progesterone and estradiol ratios and fluid retention were investigated through Pearson's correlation. For the purpose of hypothesis testing, the 95% level of confidence was predetermined as the minimum criterion to denote a statistical difference ($p < 0.05$).

Results

Body mass was stable over the three days preceding the trials (day 1: 63.1±8.1 kg, day 2: 63.2±8.2 kg, day 3: 63.2±8.4 kg). The evening euhydrated body mass measurements did not differ between menstrual cycle phases (ML:63.2±8.4 kg; LF:63.4±8.2 kg). There were no differences overnight fluid losses (ML: 610±228mL; LF: 650±222mL), degree of dehydration achieved following exercise heat stress (ML: 2.0±0.1%; LF: 2.0±0.1%), or estimated sweat rate during exercise (ML: 28±8mL/min; LF: 28±6mL/min).

Cumulative urine volume, net fluid balance, percentafe of fluid retained

There was no effect of menstrual cycle phase on the urine volume excreted over the 3-h follow-up period after rehydration (*p=0.33;* Figure 2A) or on net fluid balance (*p=0.33*) (Figure 2B). The percentage of the ingested fluid which was retained at the end of the 3-h follow-up period after rehydration was 47(33-85)% and 46(37-89)% in the ML and LF trials, respectively (*p=0.29*). The mean difference (95% CI) in percentage of fluid retained between the ML and LF trials was +3.5(-16.2, +23.2)% with an effect size (Cohen's d) of 0.1 (trivial effec).

Electrolyte balance, urine osmolality and thirst

There was no difference in sodium (*p=0.45*) or potassium (*p=0.96*) balance between the ML and LF trials (Figure 3). The mean difference (95% CI) in sodium between the ML and LF trials was +6.9 (-28.1, -41.8) mmol. The mean difference (95% CI) in percentage of fluid retained between the ML and LF trials was +0.7 (-18.4, +19.8) mmol. There was no effect of menstrual cycle phase on urine osmolality (*p=0.37*) (Figure 4A). Thirst intensity was not affected by menstrual cycle phase (*p=0.40;* Figure 4B).

Verification of phase, hormonal milieu, and reliability of response

Mean and individual values for estradiol and progesterone are shown in Figure 5. These data confirm that participants were in LF and ML phases on trial days. The absence of detection of an LH surge also confirmed that the LF trial occurred prior to ovulation when oestrogen remains high and progesterone is low. Despite finding significant differences *($p<0.05$*) in hormone concentration (estradiol, progesterone) between phases there were no differences in the percentage of fluid retained. The progesterone:estradiol ratio in the ML phase (207±37) was higher than in the LF phase (14±11), indicating the expected estradiol dominance during the LF phase. However, no association between progesterone:estradiol ratio and percentage of fluid retained was observed in either the ML (r=0.41, *$p=0.91$*) or the LF (r=-0.39, *$p=0.26$*) phase.

Test re-test reliability data between duplicate trials on the ML and LF phases is shown in Table 1. These data indicate that the key outcome measures have good test–retest reliability based on ICC, and variation around the mean is acceptable with CV% of 9-11%.

Discussion

To our knowledge, this is the first study that has evaluated the effect of the LF vs. ML phases of the menstrual cycle on fluid and electrolyte restoration after induced dehydration. The LF phase is characterised by peak levels of oestrogen and low progesterone versus the ML phase where progesterone and oestrogen are both high. Previous studies reporting no effect of menstrual cycle phase on body fluid balance (Maughan et al. 1996, Yasuda et al. 2013) may have failed to detect differences due to phase selection in which oestrogen was not high, or its actions were counterbalanced by high progesterone (Fortney 1996). Furthermore, the absence of hormonal verification of phase in these previous studies did not allow examination of the potential impact of progesterone to estradiol ratio on fluid retention. Despite choosing more extreme hormonal phase differences in the present study we did not observe any impact of LF vs ML phase upon fluid balance restoration.

The absence of any phase difference in restoration of fluid balance is consistent with the observations of Maughan et al. (1996), and adds to the small body of evidence that acute restoration of fluid balance between menstrual cycle phases is not different. Previous work has suggested a tendency toward significant free water retention during periods of high oestrogen (Ormerod 2011; Stachenfeld 2008; Stachenfeld et al. 1999; Vokes et al. 1988; Spruce et al. 1985; Forsling et al. 1981). It has been suggested that progesterone may have opposing actions in the regulation of fluid balance during the menstrual cycle (Calzone et al. 2001; Fortney 1996). However, in assessing these different influences of the hormonal milieu, our data suggest that within the range of hormone responses observed in the present study there is no impact upon short term fluid balance. The lack of effects on cumulative urine output, net fluid balance, percentage of fluid retained, electrolyte balance, urine osmolality, or thirst intensity between phases provides robust evidence for a lack of menstrual cycle phase effects.

It is worth noting that oral contraceptives alter the naturally occurring ovarian cycle by changing the internal hormonal milieu (Elliot-Sale and Hicks 2018), this altered status reflects a significant down regulation of endogenous sex hormones thereby negating the fluctuations in hormone concentration seen in eumenorrheic females (Elliot-Sale et al. 2013). Stachenfeld et al. (1999) investigated the oestrogen effects on body fluid regulation, dehydration, and rehydration through the administration of oral contraceptives. These authors reported that there was an osmotically induced antidiuretic hormone (ADH) secretion and thirst stimulation during dehydration, but there were no changes in body fluid regulation during dehydration or subsequent *ad libitum* rehydration. Although their study indicated a role of oestrogens in the osmotic regulation of ADH their oral contraceptive doses delivered a much higher oestradiol concentration than endogenously produced oestrogens at any time point during the menstrual cycle, and they also contained progestins.

To better isolate the effects of either oestradiol or progesterone on fluid regulatory systems, Stachenfeld (2008) subsequently utilised a gonadotropin-releasing hormone (GnRH) agonist or antagonist to suppress sex hormones, and then administered them to attain levels similar to those

occurring over a normal menstrual cycle in young women. Even though oestradiol was found once again to alter the threshold of osmotically induced ADH release and thirst onset, as well as producing alterations in the sodium-regulating hormones, water and sodium regulation seemed only minimally affected by estradiol administration (Stachenfeld and Keefe 2002). These data combined with those from the present study suggest that while oestrogen has primary effects on some aspects of body fluid regulation, it has no effect on overall regulation of fluid balance after induced dehydration. In addition, progesterone does not have a major effect on osmotic regulation of ADH, thirst (Calzone et al. 2001; Vokes et al. 1988; Stachenfeld et al. 1999; Stachenfeld and Keefe 2002; Stachenfeld 2008) or fluid balance. These findings likely explain the lack of difference in fluid retention during the LF compared to the ML phases in the present study.

Since sodium is the major ion in the extracellular fluid, it is intuitive that sodium losses should be replaced if plasma volume is to be maintained during exercise or restored after exercise (Shirreffs et al. 2004), thus it is important to consider sodium balance when assessing hydration status. Stachenfeld et al. (1999, 2002) have reported lower sodium excretion during the luteal phase of the menstrual cycle, however, these studies have not assessed sodium balance and therefore the outcomes relating to sodium excretion could be due to differences in intake. In the present study, we did not observe differences in net sodium balance between the menstrual cycle phases evaluated.

Evidence suggests that when the sodium content of the ingested beverage is low (<23 mmol/L) a large urinary excretion is stimulated if a large volume of fluid is consumed. However, with the ingestion of a large volume of a high sodium beverage (61 mmol/L) a larger proportion of fluid is retained, resulting in a better net fluid balance (Shirreffs et al. 1996). We used a commercially available sports drink that had a relatively low sodium (25 mmol/L) content which could add water in excess of solute to the blood and consequently increase blood volume and lower osmolality (Maughan and Leiper, 1995). These two effects suppress the release of ADH and consequently urine output is stimulated (Maughan et al 1996). The fraction of the ingested fluid retained in this study was comparable to that

found in a previous studies (Maughan et al. 1996) where the participants ingested a beverage with a similar sodium content. It could be proposed that by increasing the sodium concentration of the beverage a greater proportion of the ingested fluid would be retained. However, as highlighted by Maughan et al. (1996) the effect of the composition of the ingested fluid on fractional retention is likely far greater than any variations across the menstrual cycle.

Although it has been reported that greater water retention could be induced by oestrogen dominance (Stachenfeld, 2009), the present data demonstrate that during the LF phase (oestrogen dominance) there was no difference in fluid retention during rehydration compared with ML. Furthermore, no association between the progesterone:estradiol ratio and fluid retention was observed indicating that within the range of hormone values obtained in the present study there was no relationship with fluid retention. Nevertheless, our study did not discriminate between women experiencing pre-menstrual water retention and/or tendency to present pre-menstrual syndrome; both of these conditions could have an effect on fluid retention due to alterations on the progesterone:estradiol ratio. (Bäckstrom et al. 1976).

In conclusion, this study provides further robust evidence suggesting that restoration of fluid and electrolyte balance after dehydration is not affected by the normal menstrual cycle in healthy active eumenorrheic young women. Concerns over the inclusion of females in fluid balance studies due to the potential effects of menstrual cycle hormonal fluctuations on fluid retention appear unwarranted. However, since many active female individuals and athletes can present with amenorrhoea or oligomenorrhoea due to disturbances in hormonal status, the responses of those individuals may be different (Baker, 1981). Therefore, studies on rehydration and fluid balance in women who experience disturbances of their menstrual cycles or those taking different kinds of hormonal contraceptives are still needed in the literature. Nevertheless, from a practical perspective it seems there is no reason to exclude healthy active eumenorrheic young women, alongside males, when conducting acute fluid balance studies.

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373 Table 1. Test-retest reliability for duplicate trials performed during ML (n=5) and LF (n=5) phases.

Outcome	ML		LF	
	CV (%)	*ICC (CI)*	*CV (%)*	*ICC (CI)*
Cumulative urine output	9.1	0.9 (0.6,0.9)	7.3	0.9 (0.7,0.9)
Net fluid balance	9.1	0.9 (0.6,0.9)	7.3	0.9 (0.7,0.9)
Percentage of fluid retained	11.2	0.8 (0.2,0.9)	8.1	0.8 (0.1, 0.9)

374 ML: Mid-Luteal. LF: Late Follicular. CV: Coefficient of Variation. ICC: Intraclass Correlation

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Figure Legends

Figure 1. Schematic of the study protocol.

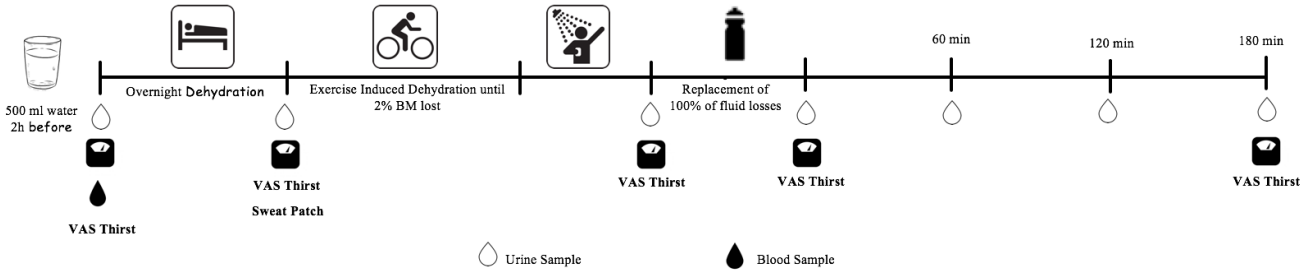
Figure 2. Cumulative Urine Output (A) at each time point in the follow-up period after rehydration on trials conducted in the mid-luteal (ML) and late follicular (LF) phases of the menstrual cycle; and Net Fluid Balance (B) calculated from the volume of sweat loss, fluid ingested and urine output at different times points (EU: euhydrated, OD: after overnight dehydration, EID: following exercise-induced dehydration) during the trials conducted in the mid-luteal (ML) and late follicular (LF) phases of the menstrual cycle. All values are mean±SD. No differences were observed between trials.

Figure 3. Electrolyte Balance calculated from the electrolyte content in the sports drink ingested and the electrolytes lost in sweat and urine during the protocol on the trials conducted in the mid-luteal (ML) and late follicular (LF). All values are mean±SD with individual data points shown. No differences were observed between trials.

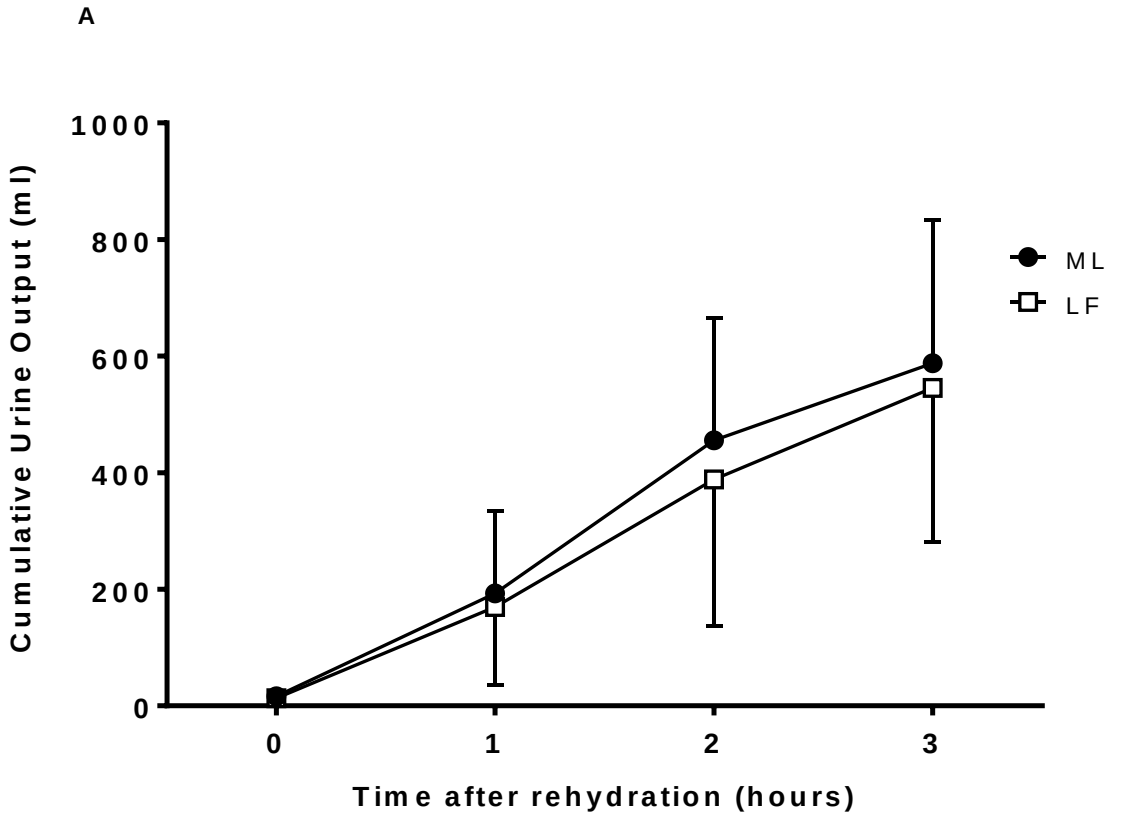
Figure 4. Urine Osmolality (A) at different time points (EU: euhydrated, OD: after overnight dehydration, EID: following exercise-induced dehydration) over the trials conducted in the mid-luteal (ML) and late follicular (LF) phases of the menstrual cycle ; and VAS Thirst Intensity (B) at different time points (EU: euhydrated, OD: after overnight dehydration, EID: following exercise-induced dehydration) over the trials conducted in the mid-luteal (ML) and late follicular (LF) phases of the menstrual cycle. All values are mean±SD. No differences were observed between trials.

Figure 5. Serum estradiol E_2 (pg/mL) (A) and progesterone (ng/mL) (B) values during the trials conducted in the mid-luteal (ML) and late follicular (LF) phases of the menstrual cycle. All values are mean±SD with individual data points shown. Trials were significantly different for both hormones.* indicates difference between trials.

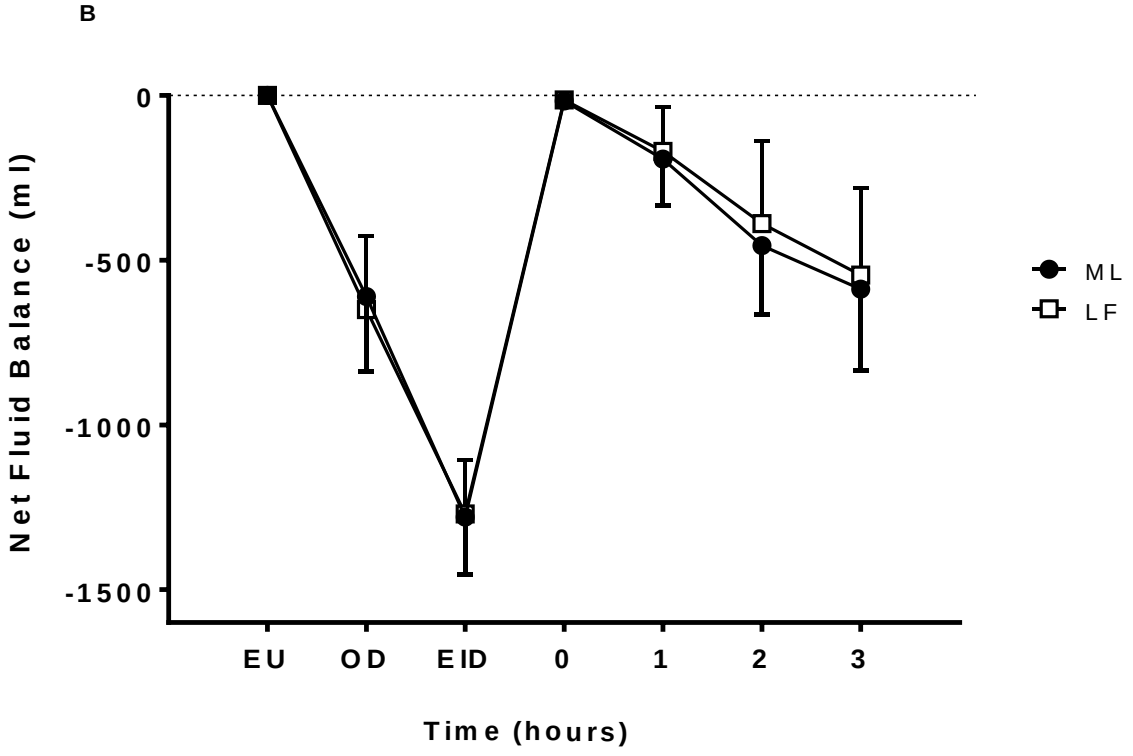
Figure 1


500 ml water
2h before
Overnight Dehydration
Exercise Induced Dehydration until
2% BM lost
Replacement of
100% of fluid losses
60 min
120 min
180 min
VAS Thirst
VAS Thirst
Sweat Patch
VAS Thirst
VAS Thirst
VAS Thirst
Urine Sample
Blood Sample

422 **Figure 2**

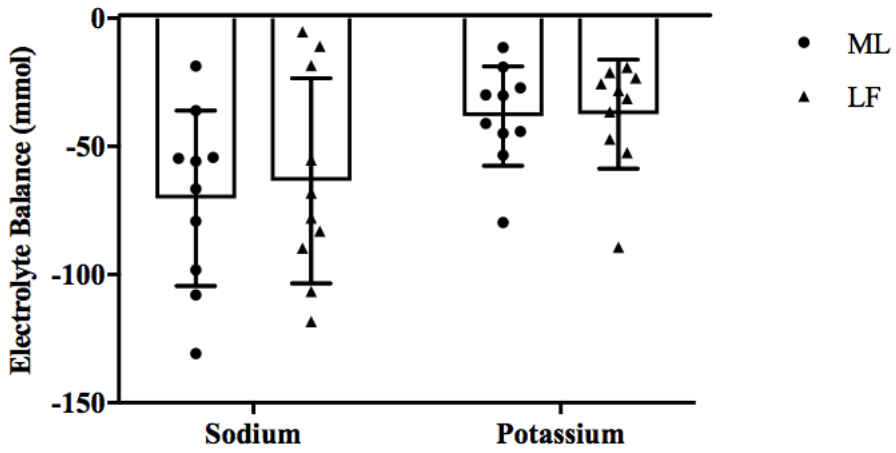


423

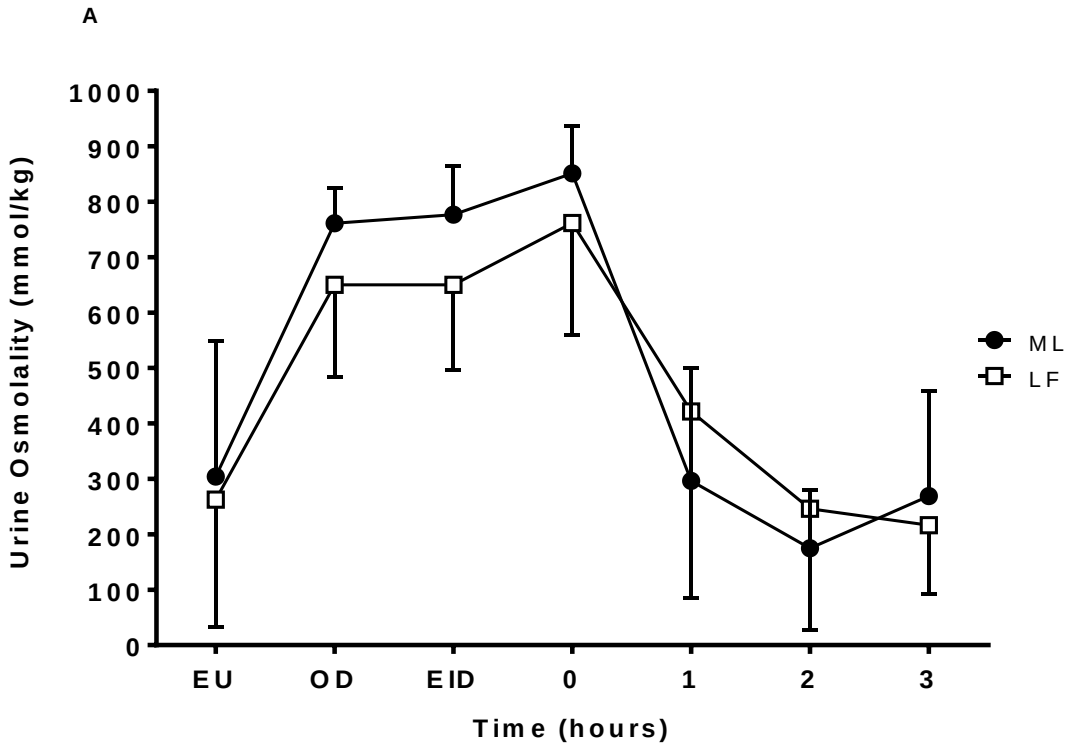


424

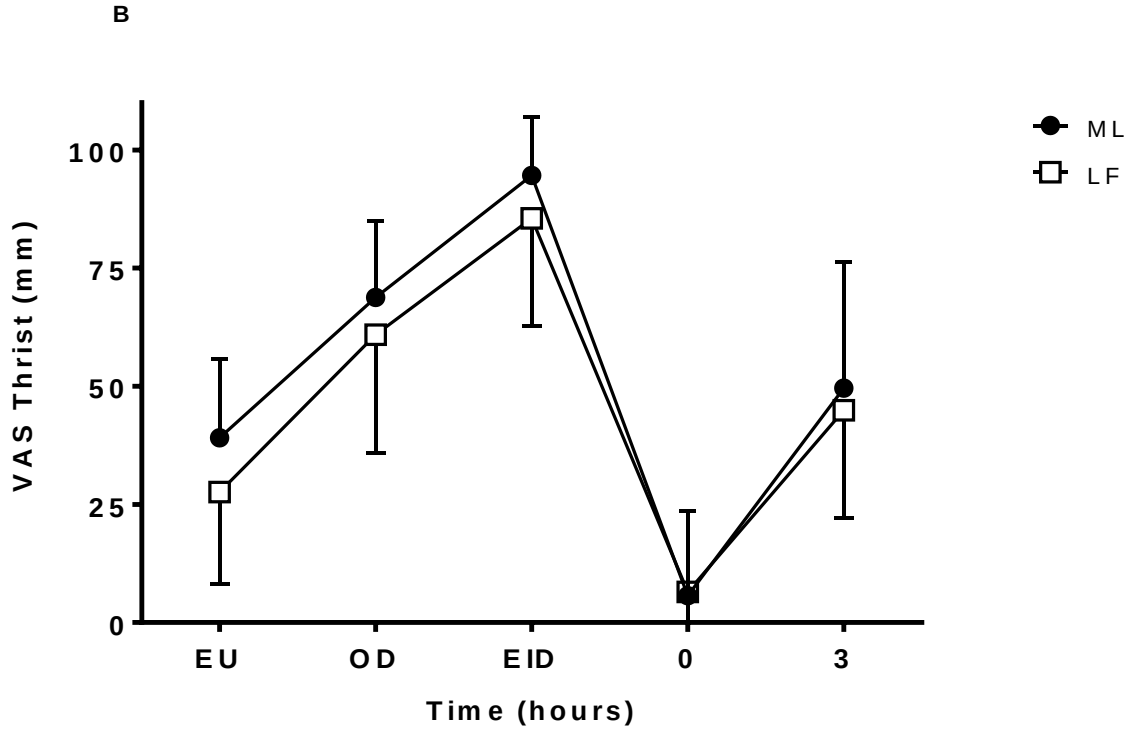
Figure 3


0
-50
-100
-150
Electrolyte Balance (mmol)
Sodium
Potassium
ML
LF

442 **Figure 4**

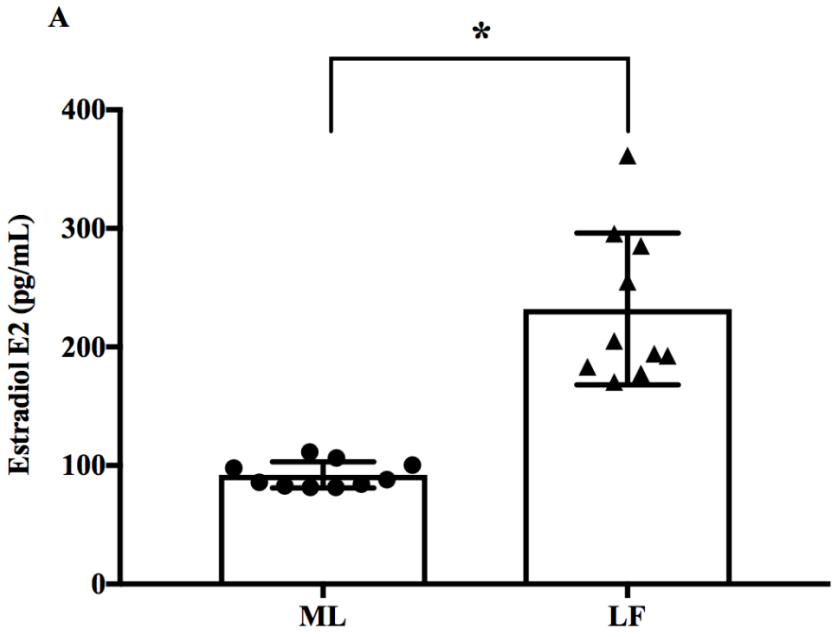


443



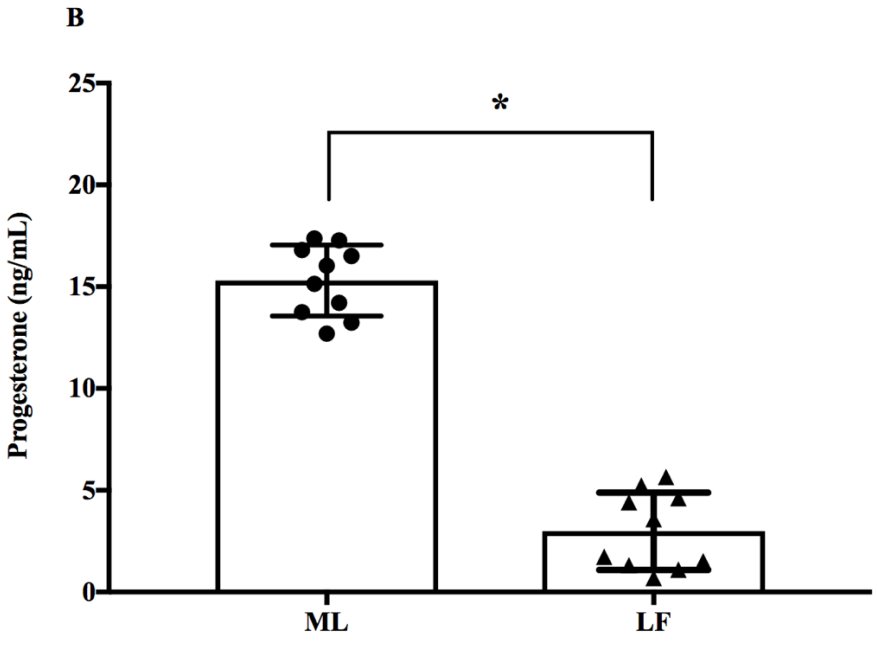
444

445 **Figure 5**



ML: Midluteal
LF: Late Follicular

446



ML: Midluteal
LF: Late Follicular

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