5-2022

Examining the Effects of Oral Contraceptive Use on Thermoregulation

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Health, Sport, and Exercise Science

by

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May 2022
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This dissertation is approved for recommendation to the Graduate Council.

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Abstract

Purpose: The purpose of this investigation was to evaluate the effects of combined (estradiol and progestin) monophasic oral contraceptive pill (OCP) use on thermoregulation. Further, we sought to evaluate OCP use on acute rehydration post-exercise in the heat using recommended rehydration guidelines. **Methods:** Eleven healthy, aerobically trained (VO_{2peak} = 47.8 ± 4.7 mL/kg/min), long term female oral contraceptive users completed a familiarization trial and two experimental days separated by ≥ 7 days. The two experimental trials were identical except for the hormone dosing phase. One trial was completed during the third week of active pill dosing (ACT) and one during the placebo week (PLA) of their normally prescribed OCPs. Participants completed 90 minutes of cycling in 30ºC and 55% relative humidity and a rehydration protocol. Exercise intensity was set at 55% of the wattage attained during the final stage of the VO_2peak test. $T_{rec,}$ heart rate, blood pressure and perceptual measurements were recorded every 15-minutes. Body mass change was measured continuously and recorded every 15-minutes and subsequently used to provide water (warmed to 38°C) to replace sweat losses. Water was given to the participant to match 50% of sweat loss at each time point. Metabolic data (VO_2) wattage and cadence was collected at 30-minutes, 60-minutes, 75-minutes and at the cessation of exercise (Hashimoto et al., 2016) to ensure work intensity was kept constant. 125% of fluid lost was replaced via a recovery beverage and water post-exercise. **Results:** T_{re} demonstrated a significant main effect difference for ACT to be greater than PLA compared to PLA ($F_{1.55,15.53}$ = 74.019, $P < 0.001$). There was also a significant main effect for T_{re} to increase over time, regardless of trial ($F_{1,10}$ = 24.064, $P < 0.001$). There was not an interaction of time x trial ($F_{1.96,19.62}$ = 1.822, $P = 0.189$) for T_{re}. There was no difference in overall change in temperature (baseline to maximum temperature) between trials (ACT: 1.3 ± 0.5ºC, PLA: 1.4 ± 0.4ºC, t_{10} = -

0.588, $P = 0.570$). Post-exercise, there was no difference between trials in the amount of fluid consumed (ACT: 1007 ± 256 mL; PLA: 921 ± 448 mL, $t_{10} = 0.874$, $P = 0.403$). There was no difference in spot sample USG assessed 3-h post-trial between trials ($t7 = -0.743$, $P = 0.487$). Likewise, 3-h spot sample urine osmolality was not different between trials ($t7 = - 1.177$, $P = 0.287$). Urgency to void at 3-h post-trial was not different between groups ($t7 = - 1.000$, $P = 0.351$). Perception of thirst was not different 3-h post-trial ($t7 = - 0.3859$, $P = 0.711$).

Conclusion: We demonstrated a significant difference in core temperature elevation during the ACT trial. However, in following recommended hydration guidelines, OCP phase has no effect on fluid retention post-exercise in the heat.

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I. Introduction

As women continue to participate more in sport, contribute to the workforce, and enlist as military personnel, there is an increased need for specific safety guidelines and hydration and nutrition recommendations (Giersch et al., 2019; Sims & Heather, 2018). To outline proper recommendations, understanding female specific physiology should be prioritized. Often, heat and hydration research is conducted on men. Most of the existing literature attributes differences in physiology to body size discrepancies mainly due to total body surface area and body composition variability between sexes. While these physical differences do exist, research in this area has ignored the potential influences of the female menstrual cycle. Unfortunately, this assumption underserves half of the human population.

The impact of female hormones on fluid regulation is not fully understood. While it is known that female reproductive hormones impact volume regulatory physiology (Stachenfeld, 2008), the pursuit to fully understand the differences of hormonal fluctuations and fluid balance throughout the menstrual cycle has been lackluster. It has become common practice to exclude females in fluid balance research due to physiological fluctuations throughout the menstrual cycle. There are differences due to the menstrual cycle but these differences are not fully understood (Sims & Heather, 2018), and therefore, are not well-controlled in methodological design. When female participants are included in hydration or fluid balance studies, it is common to limit testing during the early follicular phase of the menstrual cycle. During this phase, estrogen and progesterone are at their lowest concentrations (Constantini et al., 2005; Sims & Heather, 2018; Stachenfeld, DiPietro, et al., 1999; Stachenfeld & Taylor, 2014). The logic is that lower concentrations of circulating female hormones reveal outcomes similar to males without considering the effect that hormonal fluctuations may have throughout the menstrual cycle.

Exercising in the heat requires cardiovascular and thermoregulatory adjustments to maintain both core body temperature and mean arterial pressure (González-Alonso et al., 2000; Montain et al., 1995; Montain & Coyle, 1992; Sawka et al., 2001; Wendt et al., 2007). When dehydration is combined with exercise in the heat, there is substantial impairment in stroke volume due to total blood volume reductions (González-Alonso et al., 2000). Cardiac output (CO) is reduced during dehydration compared to an euhydrated state (González-Alonso et al., 1997). This reduction in CO results in a reduction in muscle blood flow, which leads to sacrificed performance (Casa et al., 2010; González-Alonso et al., 1998). To prevent heat illness and maintain performance, individual fluid recommendations have been proposed (McDermott et al., 2017). Yet, little research has been collected in female participants.

It has been proposed that sex steroids affect thermoregulation by changing the regulated hypothalamic temperature, or "set-point" (Stachenfeld et al., 2000). Animal studies have shown that estrogen and progesterone act on sex steroid binding neurons in the preoptic, anterior hypothalamus (Silva & Boulant, 1986). Progesterone is highest during the luteal phase of the menstrual cycle and is associated with an increase in resting core body temperature of 0.3-0.8°C (Baker et al., 2020; Charkoudian & Johnson, 1999; Charkoudian & Stachenfeld, 2016; Constantini et al., 2005; Kolka & Stephenson, 1989; Pivarnik et al., 1992; Rogers & Baker, 1996). Physiological outcomes during the midluteal phase would therefore be attributed to an influence of elevated sex hormones.

However, further complicating female physiology studies in dehydration and thermoregulation is oral contraceptive (OC) use. According to the National Center for Health Statistics data from the 2015-2017 National Survey of Family Growth, 64.9% of the 72.2 million women aged 15-49 in the U.S. were using contraception (Daniels, 2015). Of the reversible

methods of contraception, the most common was oral contraceptive pills (12.6%) within the previously mentioned age range (Daniels, 2015). Stachenfeld et al. (2018) reported that >90% of European and U.S. females are currently taking or previously have taken oral contraceptives. In athletes, the prevalence of females taking combined, monophasic oral contraceptives is 50% (Lei et al., 2019). Fluid balance studies suggest OCs containing estrogen (combined hormone OCs) increase osmotically induced AVP and thirst during dehydration and *ad libitum* rehydration, even though there were no changes in water retention (Stachenfeld, Silva, et al., 1999); confirming the hypothesis that estrogen is responsible for decreasing the plasma osmotic threshold. Thermoregulation studies suggest the rightward shift of the thermoregulatory set-point is maintained with OC use, this finding is exacerbated with exercise in hot and humid environments (Lei et al., 2019).

To date, there is insufficient data on the effects of OC use on fluid balance and thermoregulatory outcomes. Females are likely to be at no more of a disadvantage during exercise in the heat when compared to male counterparts (Charkoudian & Stachenfeld, 2016). However, a better understanding of female physiology may lead to advantages in sport and work performance, establish better criteria for determining heat intolerance, and potentially lead to better safety guidelines as more females play sports, join the military or occupational workforce.

Specific Aims

Aim 1. Identify the effect of monophasic oral contraceptive pill use on thermal physiology variables (core temperature, skin temperature, total sweat loss and sweat rate).

Research Hypothesis 1. Exogenous levels of progesterone increase core body temperature in eumenorrheic females. It is expected that participants may reach a higher core temperature during active pill dosing compared to placebo pill dosing due to the thermoregulatory set-point shift.

Aim 2. Identify the effects of monophasic oral contraceptive pill use on acute rehydration post-exercise in the heat.

Research Hypothesis 2: Previous research in our lab suggests no difference in fluid retention or turnover in oral contraceptive users between active pill dosing versus placebo pill dosing in resting conditions. Because fluid loss via sweat will be replaced via water consumption during exercise, it is expected there will not be significant differences between active and placebo pills on OC users in fluid volume turnover post-exercise.

II. Literature Review

Fluid Balance in Resting Conditions

In the human body, water constitutes 50-70% of total body weight (Sawka et al., 2005). Variability in total body water (TBW) is due to differences in body composition as water composes 65-75% of the weight of muscle and about 10% of fat (McArdle et al., 2015). Because body fat has a low water content compared to muscle, individuals with greater body fat have a decreased percentage of TBW compared to total body mass (McArdle et al., 2015). TBW is distributed into two fluid compartments: intracellular and extracellular. The intracellular fluid compartment is larger and contains about 62%-65% of TBW, whereas the extracellular compartment is made up of interstitial and plasma spaces, containing 30%-35% of TBW. Blood plasma accounts for nearly 20% of extracellular fluid (McArdle et al., 2015).

Approximately 5%-10% of TBW is replaced daily in the absence of exercise. This turnover includes respiratory water loss, influenced by environmental conditions (temperature and humidity), pulmonary ventilation, urine, and feces. Metabolic water is formed via oxidation of substrates but is offset by respiratory water loss. (McArdle et al., 2015). Urine output per day is approximately 1-2 L, and varies depending on food and fluid consumption (Armstrong et al., 2010; McArdle et al., 2015) and may be less in females (Armstrong et al., 2012). Urine output is the primary avenue to regulate net body water balance in resting conditions (McArdle et al., 2015; Sawka et al., 2005)

Net body water balance (loss = gain) is maintained because of thirst and hunger drive and *ad libitum* access to food and fluid to offset water loss. The ability to regulate TBW is due to an interplay between neuroendocrine and renal responses to blood volume and tonicity changes. Further socio-behavioral factors such as water seeking behavior or removal from thermal

conditions add to TBW regulation. When fluids are inadequately consumed, water retention is dependent on hormonal release (Sawka et al., 2005).

Arginine vasopressin (AVP), also known as antidiuretic hormone, is vital to fluid balance. AVP is produced in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus and secreted by the posterior pituitary gland. AVP is released in response to feedback from osmolality (osmoreceptors) and blood pressure changes (baroreceptors) in order to maintain homeostasis (Bankir, 2013; Baylis & Robertson, 1980; Koshimizu et al., 2012; Robertson, 1984; Robertson et al., 1976; Share, 1996). AVP release occurs at a plasma osmolality of ~280 mOsm/kg (Robertson, 1984; Robertson et al., 1976) and for every 1% increase in plasma osmolality a 1.8 pg/mL increase in AVP is induced (Baylis & Robertson, 1980).

When fluid losses are greater than gains, resulting in a hypohydrated state, plasma osmolality increases and AVP is released. At a slightly higher plasma osmolality (~ 290-300 mOsm/l) (Baylis & Robertson, 1980; Thornton, 2010), osmoreceptors in the brain increase thirst (Thornton, 2010). Magnocellular neurons in the hypothalamus (PVN and SON) then stimulate the release of AVP (Thornton, 2010). Similarly, as blood volume decreases, the reduction in blood pressure triggers baroreceptors that lead to AVP release. The action of AVP is widespread and, in some cases, its actions are not clearly understood. However, one of the most important actions is water conservation at the kidney. AVP acts on vasopressin receptor 2 (V2) in the renal tubules and collecting duct, which stimulate the action of aquaporin channels to reabsorb water into the vasculature, producing a concentrated urine (Johnson et al., 2016; Koshimizu et al., 2012). The action of AVP on vasopressin receptor 1 alpha (V1a) located in the walls of the vasculature also cause increases in blood pressure, subsequently increasing cardiovascular

stability (Koshimizu et al., 2012). AVP has many other non-fluid regulatory actions such as influencing pain perception, cognitive function, bone development and breakdown and the aging process (Mavani et al., 2015), thus it has also been termed a survival hormone (Johnson et al., 2016; Koshimizu et al., 2012).

Other hormones such as angiotensin and aldosterone are responsible for fluid balance and act to concentrate urine and conserve fluid (Gellai et al., 1979). As renal perfusion decreases, the juxtaglomerular apparatus detects these changes and releases renin. (Gellai et al., 1979). Renin acts to convert angiotensinogen to angiotensin I, a biologically inert hormone (Sparks et al., 2014). Angiotensin I is then converted to angiotensin II when angiotensin converting enzyme (ACE) is abundant, which directly induces blood pressure increases through actions on smooth muscle of the vasculature (Sparks et al., 2014). Angiotensin II stimulates the release of aldosterone from the adrenal glomerulosa and thirst centers in the brain (Sparks et al., 2014; Thornton, 2010). Aldosterone increases blood pressure through actions in the vasculature and stimulates the reabsorption of Na^+ and excretion of K^+ from the kidney. This aids in fluid preservation as the increased sodium retention allows for greater water movement into the vasculature as a result of increased osmolality (Thornton, 2010). Concomitantly, the renin-angiotensin-aldosterone system (RAAS) helps maintain fluid balance through thirst stimulation to increase water seeking behaviors, which are essential to proper hydration (Thornton, 2010). AVP, angiotensin I and II, and aldosterone are primary hormones needed to maintain fluid balance. However, the interplay between these hormones and other circulating hormones must be considered to fully conceptualized hormonal influence on fluid retention and excretion.

Female Hormones and Fluid Balance

Why Sex Differences Matter

It has become standard practice to only test females during the early follicular phase of the menstrual cycle when including them in hydration and fluid balance studies. During the early follicular phase, estrogen and progesterone are at their lowest concentrations (Constantini et al., 2005; Sims & Heather, 2018; Stachenfeld, DiPietro, et al., 1999; Stachenfeld & Taylor, 2014). The theory is lower concentrations of circulating female hormones reveal outcomes similar to males allowing for a homogenous sample size. However, resting plasma AVP is greater in males than in females during the follicular phase of the menstrual cycle and males exhibit greater AVP sensitivity to hypertonic saline infusion (Stachenfeld, Keefe, et al., 2001). Females have greater water turnover compared to men in both phases of the menstrual cycle in response to a water bolus (Claybaugh et al., 2000) suggesting influences of biological sex. Testing females during the early follicular phase, may create a false assumption that females are, in fact, physiologically like males. However, only testing female participants during the first four days of the menstrual cycle (early follicular) only provides insight to physiological responses for <15% of the menstrual cycle (Sims & Heather, 2018). Differences within phases of the menstrual cycle are greater than those between sexes when comparing the osmotic threshold for AVP release (Stachenfeld, Splenser, et al., 2001). Stachenfeld at al. (2001). demonstrated these differences in within-sex and between-sex responses to a 3% NaCl infusion. Researchers found a lower osmotic threshold for AVP release in the midluteal phase, when estrogen is higher than the follicular phase, but no differences between sexes were present. These findings suggest that a better understanding of both between-sex differences and within-sex differences in fluid balance may be warranted for better hydration and overall health recommendations.

The Menstrual Cycle and Fluid Balance

The primary sex hormones produced by the ovaries are estrogen and progesterone. These ovarian hormones, along with hypothalamic hormones, influence the female reproductive system and various other tissues (Constantini et al., 2005). Estrogen and progesterone peak and fall to create what is known as the menstrual cycle. The menstrual cycle lasts ~28-32 days and consists of three phases: the follicular phase (onset of bleeding to ovulation), the ovulatory phase (~day 14), and the luteal phase (~days 14-28). Each of these phases consists of varying hormone concentrations. Further, they are associated with different responses to fluid loading and retention, as well as mood states and sleep (Baker & Driver, 2007; Charkoudian & Stachenfeld, 2016; De Souza et al., 1989; Giersch et al., 2019; Sims & Heather, 2018; Stachenfeld, 2008; Stachenfeld, DiPietro, et al., 1999; Stachenfeld & Taylor, 2014) During the follicular phase, specifically the early follicular phase (days 1-4) of the menstrual cycle, estrogen and progesterone are at their lowest concentrations. The middle of the cycle (ovulatory phase) is characterized by peaks of estrogen, luteinizing hormone (LH) and follicular stimulating hormone (FSH). The ovulatory phase is ~24 hours in length and although demonstrates surges in both LH and FSH these hormones are not known to influence fluid balance. The luteal phase is characterized by higher circulating levels of both estrogen and progesterone.

Hormones responsible for regulating TBW and Na^+ balance also vary over the course of the menstrual cycle. During the midluteal phase, plasma aldosterone concentration and plasma renin activity are greater at rest and during exercise when compared to the follicular phase. Resting plasma AVP concentration is directly related to plasma estrogen levels, as in the late follicular phase and midluteal phase (De Souza et al., 1989) when estrogen levels peak. Resting hematocrit, plasma estrogen, and plasma progesterone are greater in the luteal phase than in the

follicular phase. However, plasma osmolality is lower in the luteal phase due to the downward shifting of osmotic control (Stachenfeld, DiPietro, et al., 1999).

Estrogen and Progesterone Interaction with AVP on Fluid Balance

Both estrogen and progesterone influence renal and neural systems responsible for fluid balance. Receptors for sex hormones are found in nonreproductive tissues involved in fluid balance such as the kidney tubules, cardiovascular system and the hypothalamus (Sims et al., 2008a; Stachenfeld, 2008) suggesting that sex hormones influence fluid balance systemically. Fluid balance is further influenced by thirst, sodium appetite or satiety, renal fluid and sodium regulation, all of which fluctuate throughout the menstrual cycle (Sims et al., 2008a). The renal and neural systems are reactive to fluid deficits in the extracellular space due to sodium concentrations, tonicity, or plasma osmolality. These systems are far more sensitive to osmolar changes versus volume loss whereas a 2-3% change in plasma osmolality is needed to induce thirst or an increase in AVP concentrations, whereas a 10% loss in plasma volume is required before inducing an increase in AVP or thirst (Stachenfeld, 2008).

It is difficult to isolate the individual effects of estrogen and progesterone as both hormones are elevated or decreased simultaneously during the menstrual cycle. Stachenfeld et al., (2008) performed a series of studies using either a gonadotropin-releasing hormone (GnRH) agonist or antagonist. This method suppressed estrogens, progesterone, and gonadotropins to pharmacologically oophorectomize female subjects for a short time (Stachenfeld, Keefe, et al., 2001; Stachenfeld & Keefe, 2002; Stachenfeld & Taylor, 2004, 2005a). In studies that used a GnRH analog (leuprolide acetate), subjects were administered the drug continuously over two weeks. Leuprolide acetate down regulates the hypothalamic-pituitary-ovarian axis so that plasma concentrations of both estrogen and progesterone are undetectable within 14 days (Stachenfeld,

Keefe, et al., 2001; Stachenfeld & Keefe, 2002, 2002). Other studies used a GnRH antagonist (ganirelix acetate) which acts by competitively blocking the GnRH receptors on the pituitary gonadotroph and therefore, induces a suppression of gonadotropin release and subsequently leads to a decrease of both estrogen and progesterone to postmenopausal levels (Stachenfeld, 2008; Stachenfeld et al., 2003; Stachenfeld & Taylor, 2005a). After the hormone suppression phase in these studies, participants were dosed with estrogen only (estradiol), progesterone only or combined hormones to better understand the individual or combined effects of sex hormones. This allowed for within- and between-participant comparisons of osmotic regulation of AVP, thirst, sodium and overall fluid regulation (Stachenfeld, 2008). Reproductive hormones (either estrogen or progesterone) were given to participants post-GnRH suppression. After hormone administration, a hypertonic saline infusion (3% NaCl) was administered. Estrogen administration shifted the osmotic threshold for the release of AVP to a lower plasma osmolality independently of progesterone. High levels of circulating estrogen increased the sensitivity for a change in plasma osmolality needed to induce AVP release from the anterior pituitary (Stachenfeld & Keefe, 2002; Stachenfeld & Taylor, 2004, 2005a). It is likely that the estradiol related shift in osmotic regulation of AVP occurs via the central nervous system. Estrogens cross the blood brain barrier and may influence AVP release at the hypothalamic level (Stachenfeld, 2008). Estrogen lowering of the osmotic threshold for AVP release is likely due to a downward shift of the osmoregulatory set point rather than a change in the fluid regulation. The greater concentration of plasma AVP did not seem to contribute to fluid retention. There were small increases in overall fluid retention during estradiol administration, renal free water clearance was unaffected. This suggests that estrogens may alter renal sensitivity to AVP or affect AVP action in the kidney (Stachenfeld, 2008).

In further utilization of the GnRHa protocol, Stachenfeld at al., (2003) tested the hypothesis that estradiol administration would attenuate the urine concentrating response when compared to GnRHa administration without estradiol. When participants were infused with synthetic AVP (pitressin), there was no estradiol effect on AVP-mediated renal concentrating response. In the physiological range where AVP had the greatest renal concentrating effect, urine osmolality was significantly decreased during estradiol treatment suggesting that greater circulating estrogen attenuates the renal concentrating response (Stachenfeld et al., 2003).

While Stachenfeld et al., (2008) chose to isolate hormone expression to better understand the individual responses attributed to both estrogen and progesterone, it is equally important to understand hormone responses for applicability purposes. Although dehydration increases plasma osmolality, it also decreases TBW, plasma volume and blood pressure (Stachenfeld, DiPietro, et al., 1999). Using a hypertonic saline infusion creates a larger increase in plasma volume, (16-20 mOsmol/kg H_2O) allowing for the effects of estrogen to be dramatized and therefore, better determined (Stachenfeld, 2008). Both methods are useful tools, but using a hypertonic saline infusion lacks real world applicability as it pertains to female physiology in sport or work environments where dehydration may be prevalent. Therefore, researchers have investigated exercise induced dehydration and fluid restoration post-exercise (Rodriguez-Giustiniani & Galloway, 2019)

Considering, hormonal differences between the late follicular and midluteal phases on fluid may impact fluid restoration balance due to the increase in estrogen during the midluteal phase. Rodriguez-Giustiniani and Galloway (2019), studied eumenorrheic participants that dehydrated via fluid restriction to 2% body mass loss. Participants completed an exercise trial in the heat and following exercise dehydration, participants rehydrated with the equivalent of 100%

body mass loss with a commercially available sports drink. Menstrual cycle phase did not affect urine volume excreted, net fluid balance, sodium balance, urine osmolality or thirst intensity (Rodriguez-Giustiniani & Galloway, 2019). Although this finding contradicts previous findings that water retention increases during the luteal phase (Spruce et al., 1985; Stachenfeld, 2008; Vokes et al., 1988), this study was the first to investigate acute rehydration post-exercise in the heat in eumenorrheic females.

Sodium and Fluid Retention. The RAAS maintains blood volume and blood pressure by regulating electrolyte levels and fluid balance (Komukai et al., 2010). Sex hormones seem to govern RAAS as evident by the positive cardiovascular and renal health outcomes in premenopausal women (Komukai et al., 2010). Estrogen-only therapy increases fluid retention via RAAS by increasing plasma renin levels but, when combined with progesterone, this effect is negated (Oelkers, 1996). Fluid dynamics are shifted as estrogen enhances vasodilation and lowers the set point of plasma osmolality (Oelkers, 1996; Sims et al., 2008a; Stachenfeld, Keefe, et al., 2001). Yet, progesterone competes with the same receptor that aldosterone binds to, potentially leading to natriuresis. Stachenfeld et al., (1999) found that there was little change in free water clearance during combined (estrogen and progesterone) administration similar to the naturally occurring luteal phase and, although AVP concentration is greater in the midluteal phase, the luteal phase is not associated with water retention (Calzone et al., 2001; Stachenfeld, Silva, et al., 1999).

Overall changes in water and fluid retention are present during hormone administration. These changes were of small magnitude and it is expected that the small amount of water retention associated with estradiol administration may be due to an increase in sodium retention rather than AVP mechanisms (Stachenfeld, Silva, et al., 1999; Stachenfeld & Keefe, 2002).

Plasma renin activity was greater at baseline with estradiol administration, yet the greater plasma renin activity did not continue with saline infusion nor was there an effect on aldosterone suggesting that the increased Na+ retention associated with elevated estrogen is independent of RAAS. There is conflicting evidence because others have reported that estradiol administration may influence RAAS by enhancing angiotensin synthesis, inhibiting angiotensin-converting enzyme activity and augmenting plasma and tissue levels of renin (Kuroski de Bold, 1999; Oelkers, 1996; Stachenfeld, 2008). It is likely that the pathways through which hormones have been delivered for research purposes (oral vs. transdermal) may have an effect on whether or not RAAS is activated (Komukai et al., 2010). It is plausible that oral contraceptive use and therefore, oral delivery of exogenous hormones, may result in a greater influence in RAAS activation due to metabolism of oral contraceptives in the liver (Stachenfeld, 2008).

Proper sodium and electrolyte balance is important for maintenance of blood and plasma volume (Sims et al., 2008a). Sims et al, (2008) investigated fluid balance, renal-sodium sensitivity, and responses to a sodium concentrated beverage during hormonal extremes of the menstrual cycle in eumenorrheic females and OC users. In a normal menstruating group, participants were tested during the early follicular phase of the menstrual cycle (low hormone) and the midluteal phase (high hormone). In the OC group, participants were tested during the third week of a triphasic medication (high hormone) and during last week or placebo pill dosing (low hormone). Participants consumed a sodium concentrated beverage (164 mmol/L) in the amount of 10mL/kg body mass over the course of one hour, without an exercise component. Plasma volume expansion was significantly different between menstrual cycle phase but not between groups. The low hormone phases in both groups had greater plasma volume expansion. AVP increased with sodium loading in the high hormone phase of both groups. Plasma

osmolality was significantly different between phases. The AVP response in the high hormone phases in both natural menstruating women and oral contraceptive users correlated with a lower plasma osmolality. This study supported previous findings of decreased plasma osmolality with elevated AVP when high hormones are present (Stachenfeld, 2008), regardless of pill use. Water clearance was similar between groups with a lower water clearance in the high hormone phase of both groups. High hormone phases also had higher urine osmolality and attenuated urine flow volume. Sims et al. (2008) hypothesized it may be due to a resetting of osmoregulatory mechanisms and the downward shift of plasma osmolality (Sims et al., 2008a). Further indicating that fluid balance is maintained similarly between phases of the menstrual cycle but, during high hormone phases, the cascade of events to induce fluid conservation begins at a lower plasma osmolality (~281 mOsm/L vs. ~285 mOsm/L) (Sims et al., 2008a).

Oral Contraceptives and Fluid Balance

Oral contraceptives (OCs) are a group of exogenous steroidal sex hormones most commonly prescribed for pregnancy prevention. They are effective in inhibiting ovulation by suppressing luteinizing hormone secretion from the pituitary and thickening cervical mucous, preventing endometrial development. While there are several methods of hormonal contraceptives- combined OCs containing both estrogen and progesterone are the most commonly prescribed and studied in young, athletic populations (Sims & Heather, 2018). Oral contraceptives create synthetic hormonal control by promoting a negative feedback loop that turns off the hypothalamic-pituitary-gonadal axis preventing endogenous estrogen or progesterone release (Sims & Heather, 2018). Therefore, circulating exogenous sex hormone concentrations are set by the daily dose of prescribed medication. The synthetic hormones mimic endogenous hormones but vary greatly in the pathway of entry into the bloodstream. The

circulating hormone concentrations and molecular structure and bioactivity may act on receptors differently than naturally occurring steroids (Sims & Heather, 2018).

Long term OC use may cause adaptation in the fluid balance system. OCs exhibit 3-10 times the bioactivity of endogenous estrogen in combined (estrogen and progesterone OCs) (Sims & Heather, 2018; Stachenfeld, Silva, et al., 1999), but the progestin component of OCs do not compete with the same receptor as aldosterone, unlike endogenous progesterone. Therefore, ethinyl estradiol (synthetic estrogen) may induce fluid expansion to a greater degree than endogenous hormones. Circulating angiotensin is synthesized primarily by the liver and orally administered estradiol is metabolized by the liver. Increases in plasma angiotensin and aldosterone may be a consequence of oral estradiol administration (Stachenfeld, 2008).

To determine estrogen effects from OC use, Stachenfeld et al., (1999) administered OCs (combined progesterone and estrogen OCs and progesterone only OCs) to young women and evaluated responses to exercise dehydration and a rehydration period. They found that OCs containing estrogen (combined hormone OCs) increased osmotically induced AVP and thirst during dehydration and *ad libitum* rehydration even though there were no changes in water retention (Stachenfeld, Silva, et al., 1999). Confirming the hypothesis that estrogen is responsible for shifting the plasma osmotic threshold to a lower set point.

Future Considerations in Fluid Balance Research

Estrogen and progesterone affect body fluid and sodium regulation. Primarily, they act by shifting the set point for osmotic regulation and AVP release in multiple conditions including exercise induced dehydration, oral fluid loading and osmotic stimulation via hypertonic saline infusions (Stachenfeld, 2008). The difference in osmoregulation seems has minimal effects on TBW and sodium retention, but changes the body water distribution within the extracellular

space (Stachenfeld, Keefe, et al., 2001). In future studies, the influence of sex hormones will be important considerations in understanding the regulation of water and sodium (Sims & Heather, 2018; Stachenfeld, 2008). Influences may be particularly important in individuals with differing conditions such as, susceptibility to hyponatremia, pregnancy, pre-menarche, or post-menopause. Fluid and electrolyte balance are critical for normal cellular function and maintenance of both blood and plasma volume. The interactions of female sex hormones and the fluid regulatory system is crucial. OCs will continue to evolve as pharmacological technology advances and new methods of hormonal contraceptives emerge; therefore, it is important to continue research into female endogenous and exogenous hormones and their effects on TBW and fluid balance. Further research is needed to make best practice recommendations and positively contribute to women's health.

Exercise in the Heat and Importance of Fluid Balance

Exercising in the heat requires cardiovascular and thermoregulatory adjustments to maintain both core body temperature and mean arterial pressure (González-Alonso et al., 2000; Montain et al., 1995; Montain & Coyle, 1992; Sawka et al., 2001; Wendt et al., 2007). At the onset of exercise, feed-forward signals from central command indicate an increase in heart rate and blood pressure to maintain blood flow to the brain and working musculature. Vasoconstriction in the cutaneous vasculature aids in the maintenance of central venous pressure. As exercise continues, especially in the heat, blood flow to the periphery and sweating increase as core temperature and skin temperature rise to cool the body (Nadel, 1979; Nadel, Bullard, & Stolwijk, 1971; Rowell, 1974; Sawka et al., 2011). The redistribution of blood causes a subsequent reduction in central blood volume. To maintain cardiac output (Q), heart rate and cardiac contractility are increased (Rowell, 1974). As intensity increases during exercise in the

heat the competition for blood flow between the cutaneous and skeletal muscle vasculature induces greater cardiovascular strain. As a result, performance is attenuated, particularly during endurance events (Cheuvront, Roberts, & Montain, 2007; Sawka et al., 2011).

There is substantial impairment in stroke volume due to blood volume reductions when exercise is combined with dehydration (González-Alonso et al., 2000). Q is reduced compared to a euhydrated state (González-Alonso et al., 1997). The attenuation in Q results in a reduction in muscle blood flow leading to sacrificed performance (Casa et al., 2010; González-Alonso et al., 1998). Dehydration impairs skin blood flow and sweating (González-Alonso et al., 2000). Therefore, there is a decreased ability to dissipate heat, resulting in elevated core temperatures during exercise compared to a euhydrated state (Casa et al., 2010; Cheuvront & Kenefick, 2014; González-Alonso, Mora-Rodríguez, Below, & Coyle, 1995; González-Alonso et al., 1997).

Female Hormones and Thermoregulation

Like fluid balance and hydration research, few studies in thermoregulatory physiology have included females due to perceived sex hormone differences. (Hutchins et al., 2021; Kolka & Stephenson, 1997b). In previous studies the results are inconsistent with the impact of menstrual cycle phase on thermoregulation (Giersch et al., 2020). It is likely that sex steroids affect thermoregulation by changing the overall regulated hypothalamic temperature, or thermoregulatory "set-point" (Stachenfeld et al., 2000) and animal studies have shown that estrogen and progesterone act on sex steroid binding neurons in the preoptic, anterior hypothalamus (Silva & Boulant, 1986). Progesterone decreases the firing rate of preoptic warm sensitive neurons and increases the firing rate of preoptic cold sensitive neurons (Kolka & Stephenson, 1997a). Concomitantly, progesterone is highest during the luteal phase of the menstrual cycle and is associated with an increase in core body temperature of 0.3-0.8°C at rest

(Baker et al., 2020; Charkoudian & Johnson, 1999; Charkoudian & Stachenfeld, 2016; Constantini et al., 2005; Kolka & Stephenson, 1989; Pivarnik et al., 1992; Rogers & Baker, 1996). Estrogen may modify temperature increases associated with progesterone even though the mechanism by which body temperature is affected has not been established (Stachenfeld et al., 2000). During the midluteal phase, both estrogen and progesterone are elevated, yet, progesterone dominates the effects of estrogen on overall body temperature (Charkoudian et al., 2017). Regardless of mechanism, temperature thresholds for sweating and vasodilation during exercise and resting core body temperature are greater in the midluteal phase, when progesterone concentrations are greater, than in the follicular phase (Charkoudian & Stachenfeld, 2016; Kolka & Stephenson, 1997a, 1997b, 1997b). Observations support an upward (rightward) shift in a thermoregulatory set-point and higher regulated set body temperature during the luteal phase (Giersch et al., 2020). It is important to note that this set-point shift does not happen if ovulation does not occur. Meaning that without ovulation, a rise in progesterone and estrogen responsible for the set-point shift, would not be present (Kolka & Stephenson, 1997a; Stachenfeld et al., 2000). Theoretically, the rightward shift in a thermoregulatory set-point could affect female's ability to thermoregulate and effectively cool during work in the heat during the luteal phase of her menstrual cycle (Pivarnik et al., 1992). An already elevated core temperature could make a person more susceptible to uncompensable heat stress. The potential for heat illness and performance decrements would be observed. An attenuation in sweat sensitivity or an overall decrease in total sweat losses would increase risk of heat illness. Several studies have investigated the thermoregulatory effects between the midluteal and follicular phases of the menstrual cycle.

In both resting (Kolka & Stephenson, 1989) and exercise conditions (Kolka & Stephenson, 1997b), the threshold for skin vasodilation and the onset of sweating occur at a higher esophageal temperature during the midluteal phase when compared to the early follicular phase. Observations are evident in non-acclimatized but regularly exercising females. In one study, the average esophageal temperature for the onset of sweating at the forearm was 36.91°C during the follicular phase and 37.45°C during the luteal phase (Kolka & Stephenson, 1989). Interestingly, some studies have found no difference in sweat rates, overall sweat loss (Carpenter & Nunneley, 1988; Haslag & Hertzman, 1965; Pivarnik et al., 1992), or mean weighted skin temperature between the midluteal and early follicular phases (Kuwahara et al., 2005; Lei et al., 2017; Pivarnik et al., 1992). Kuwahara et al. (2005) showed that cutaneous blood flow and sweat rate were significantly less in the midluteal phase than in the mid-follicular phase, but this was only demonstrated in an untrained group of participants, and there were no differences in trained females.

Skin temperature is important for heat loss and, when skin temperature is greater than the environmental temperature, it allows for heat loss due to an increased temperature gradient. The increased gradient allows for a greater evaporative capacity and more efficient cooling via sweat loss (Wendt et al., 2007). During the midluteal phase, the greater threshold in vasodilation decreases skin blood flow (Carpenter & Nunneley, 1988; Charkoudian & Stachenfeld, 2016).

During the midluteal phase, females experience increased core temperatures, hotter skin temperatures and decreases in sweat rate. During exercise in the heat, heart rate will increase to maintain CO and therefore, heart rate will be elevated during the midluteal phase when compared to the early follicular phase (Pivarnik et al., 1992). Pivarnik et al. (1992) demonstrated that during 60 minutes of exercise at 65-70% VO_2peak, heart rate was 10 bpm lower during the

follicular phase than the midluteal phase. However, rather than environmental conditions, intensity and duration were used as a heat load (Pivarnik et al., 1992).

Oral Contraceptives and Thermoregulation

According to the National Center for Health Statistics data from the 2015-2017 National Survey of Family Growth, 64.9% of the 72.2 million women aged 15-49 in the U.S were using contraception (Daniels, 2015). Of the reversible methods of contraception, the most common was the use of oral contraceptive pills (12.6%) within the previously mentioned age range (Daniels, 2015). Stachenfeld et al. (Stachenfeld, 2018) further reported that > 90% of European and U.S. females are either currently taking or have previously taken oral contraceptives. In athletes, the prevalence of females taking combined, monophasic oral contraceptives is 50% (Lei et al., 2019). Oral contraceptive use follows the same patterns as the natural menstrual cycle by creating a pseudo-cycle of high and low hormone concentrations. In thermoregulatory investigations, OC use has been compared to normal menstruating females but exogenous hormones are not the same as endogenous hormones (Pivarnik et al., 1992; Sunderland & Nevill, 2003; Tenaglia et al., 1999). It is possible exogenous estrogen may attenuate the thermoregulatory effects of progestin (synthetic progesterone) due to estrogens effect on thermosensitive neurons in the central nervous system (Stachenfeld et al., 2000).

In oral contraceptive users, baseline core temperature is significantly greater during the midluteal phase due to the higher concentration of progesterone.(Lei et al., 2019; Rogers & Baker, 1996; Tenaglia et al., 1999). Rogers and Baker (1996) found that core temperature was 0.31°C higher during OC use (high hormone) when compared to the placebo week (low hormone) in long-term OC users. The elevated core temperature in OC users is similar to the elevated core temperature of eumenorrheic females in the midluteal phase (Tenaglia et al., 1999).

Core temperature remains elevated during moderate exercise in both untrained and trained participants who take OCs (Lei et al., 2019; Rogers & Baker, 1996). Long-term OC users may have an attenuated sweat response (onset of sweating and thermosensitivity) during exercise in the heat (Kuwahara et al., 2005; Lei et al., 2019) whereas, the onset of sweating occurs at a core temperature of 37.8°C (0.6°C) during active pill dosing versus 37.48 (0.4°C) the placebo week. (Rogers & Baker, 1996). Findings support the thermoregulatory set point shift is present with oral contraceptive use (Charkoudian & Johnson, 1999; Lei et al., 2019; Stachenfeld et al., 2000).

Environment, Hydration and Training Status

Heat loss is affected by menstrual cycle phase and environmental factors. Even more so, by the combination of phase and environment. The largest determinant in work capacity is evaporative capacity or humidity and heat (Lei et al., 2017). Work capacity determined by mean power output is similar between menstrual cycle phases but greater in dry conditions than humid conditions (T. Lei et al., 2019a). In both OC users and eumenorrheic females, there is a thermoregulatory vs. performance trade off that ensures thermoregulation is not impaired with the sacrifice of attenuated performance (Lei et al., 2019). In hot environments, core temperature is elevated throughout aerobic exercise independently of menstrual cycle phase. In hot, humid environments the rate of rise of core temperature is 19% greater during the luteal phase and exercise time to exhaustion is significantly longer during the follicular phase (5.7%) in eumenorrheic women (Janse De Jonge et al., 2012). The rate of perceived exertion (RPE) is also greater during the luteal phase (Janse De Jonge et al., 2012; Lei et al., 2017; Pivarnik et al., 1992). Time to exhaustion and core temperature do not differ between phases during exercise in a temperate environment (Janse De Jonge et al., 2012; Pivarnik et al., 1992.).

A greater training status may alleviate the greater core temperatures associated with high hormone phases. Kuwahara et al. (2005), found that menstrual cycle phase had no effect on heat loss responses in trained participants. Esophageal temperatures of trained participants are lower during the midluteal phase when compared to an untrained cohort. However, the temperature differences are not evident during the follicular phase between untrained and trained participants (Kuwahara et al., 2005) Heat loss responses were improved by long-term physical training suggesting that a greater training status may attenuate heat storage during the midluteal phase (Kuwahara et al., 2005). However, it should be noted that basal body temperature was used to determine menstrual cycle phase, a method shown to not be as reliable as plasma samples when used as a one-time measure (Giersch et al., 2020).

The rate of rise of core temperature in dehydrated women is greater than in dehydrated men during the first stages of exercise (Giersch et al., 2021). Therefore, women may be more affected in the early stages of exercise in the heat when dehydrated than men. During moderate intensity exercise (50% VO_2peak for 90 minutes) in hot conditions (30 ± 2°C, 50 ± 5% RH) , rectal temperature is greater in the high progesterone phase than in the low progesterone phase. Without water consumption, rectal temperature is greater by 0.4°C during the high progesterone phase. With water consumption, rectal temperature during the high progesterone phase is greater than the low progesterone phase by 0.2°C (Hashimoto et al., 2016). Heart rate is significantly greater without water consumption during the high progesterone phase (Hashimoto et al., 2016) Water consumption may useful for suppressing the increase in body temperature associated with greater progesterone as is in the midluteal phase (Hashimoto et al., 2016) and may attenuate the increased core temperature associated in the luteal phase (Garcia et al., 2006). Despite differences in pre- and during- exercise hydration status, post-exercise rehydration does not seem

to be affected by menstrual cycle phase (Rodriguez-Giustiniani & Galloway, 2019). Although menstrual cycle phase may be responsible for some physiological differences during exercise in the heat (higher resting core temperature) it may be more likely that hydration status and environmental factors may have a greater impact on physiological outcomes.

Importance of Including Females in Research

Few studies have investigated thermoregulatory responses across the entirety of the menstrual cycle including the low hormone concentrations of the early follicular phase, the peak in estrogen in the late follicular phase and the midluteal phase where both estrogen and progesterone concentrations are concomitantly increased. The menstrual cycle phase is often controlled for during the follicular phase, when circulating sex hormones are at their lowest concentration. In theory, this allows for comparisons between sex when males are included in the research design. However, it is inappropriate as it fails to investigate the changing hormone profile over the female menstrual cycle as well as those taking oral contraceptives. An increase in heart rate or RPE could affect sport or work performance (Constantini et al., 2005) especially in hot and humid environmental conditions. In some military settings, heat intolerance is defined as a peak rectal temperature of 38.5 °C, a peak heart rate > 150bpm or the inability to reach equilibrium at these values. Using this criterion, 67% of female participants are classified as "heat intolerant" compared to 33% of males (Druyan et al., 2012). Although females do not exhibit symptoms of heat stress indicating that the criteria for determining heat intolerance in females should be reconsidered (Druyan et al., 2012). Furthermore, there may be practical recommendations for endurance athletes to align competition with menstrual cycle phase especially in hot, humid conditions (Janse De Jonge et al., 2012; Sunderland & Nevill, 2003). Training responses may be dependent on RPE and heart rate and may vary with menstrual cycle

phase (Janse De Jonge et al., 2012). A better understanding of female thermoregulation may lead to advantages in sport and work performance, establish better criteria for determining heat intolerance and potentially lead to better safety guidelines as more females play sports, join the military or occupational workforce.

Gaps in Literature and Methodological Considerations

Several studies have sought to identify the effect of menstrual cycle on thermoregulatory effects (Giersch et al., 2020; Lei et al., 2019; Lei et al., 2017; Notley et al., 2019). Few studies have strong methodologies. At the very least, studies have failed to measure the same main outcome variables for comparison or have found conflicting evidence. Poor methodological considerations have led to an unclear consensus on thermoregulatory response across the menstrual cycle. Giersch et al. (2020) aimed to summarize findings in a meta-analysis of nine studies (sample size determined by hormone concentrations verified by plasma sample). During the luteal phase, eumenorrheic female participants had a greater initial internal temperature compared to the follicular phase, but differences were absent during and post-exercise (Giersch et al., 2020). Further, there were no differences in mean skin temperature, sweat rate or exercise heart rate between phases in eumenorrheic females (Giersch et al., 2020). Because of these small but significant findings in resting conditions, researchers should explore thermoregulatory responses in exercise under differing environmental conditions. Especially, conditions that induce uncompensable heat stress such as, heat and humidity should be considered. The effects of exogenous hormones should be explored concomitantly with exercise in heat and humidity.

Study Design

Both Elliot-Sale et al. (2021) and Heather and Sims (Sims & Heather, 2018) have set forth best practice methodologies of studying females in exercise physiology. Stating the need

for hormone concentration status validation via blood draw, OCP use in study design, and applicable considerations.

Control of menstrual cycle. More often than not, menstrual cycle status or the hormonal profile of the participant is often implied rather than confirmed. (Elliott-Sale, 2021.; Giersch et al., 2020) It is necessary for the literature to become consistent in the terminology used to describe participants and the inclusion and exclusion criteria used to define participant eligibility (Elliott-Sale, 2021). In research questions that involve the effect of ovarian steroids, for instance, the effect of high progesterone levels (midluteal phase) on hydration or thermoregulation the quantification of sex steroid concentration should be reported via blood analysis (Elliott-Sale, 2021) especially, due to the lack of a rightward thermoregulatory shift without ovulation (Giersch et al., 2020; Stachenfeld et al., 2000).

Oral contraceptives. Endogenous and exogenous hormones are not the same, which adds complexity to study design (Stachenfeld, 2018). In research design, it should be considered that OC taking days (active pills) and OC-free days (inactive, placebo, sham pills) that the endogenous concentration of estrogen and progesterone rises during the OC-free days and the exogenous hormones increases during the active OC dosing. While taking a combined monophasic OC, ethinyl estradiol increased twofold from day 1 of active pill dosing to day 21 and progestin increases threefold from day 1 of active OC to days 8-11 and then maintains that level of concentration (Elliott-Sale, 2021). Therefore, it is important to establish a study design that takes the concentration levels into consideration if concentrations affect main outcome variables. It is possible comparing the active OC dose with the inactive dose is not a best practice because the inactive dose is more reflective of a transient hormone phase due to varying half

lives (Sims & Heather, 2018). However, with a large portion of the female population taking OCs this research is still applicable to better understand physiological outcomes.

Hydration

In several of the aforementioned studies, hydration status was not controlled for or quantified during exercise (Janse De Jonge et al., 2012; Kolka & Stephenson, 1989, 1997b; Kuwahara et al., 2005; Pivarnik et al., 1992.). It is well known dehydration is deleterious to performance and thermoregulatory effects (Bardis et al., 2013; José et al., 1997; Montain & Coyle, 1992). These studies have become more indicative of a model for dehydration and exercise in the heat rather than thermoregulatory differences. Further complicating the assessment of sex hormones and thermoregulation. Matched conditions help to compare phases of the menstrual cycle but do not negate the outcomes that may be affected by dehydration (lack of heat dissipation, increased heart rate, sweat rate, total sweat loss). Females may be more susceptible to the effects of dehydration prior to exercise in the heat (Giersch et al., 2021). Therefore, it is important to control for hydration status to isolate the effects of sex hormones on thermoregulation.

Summary

Overall, there is evidence that female sex-hormones affect both fluid balance and thermoregulation. Estrogen and progesterone may have individual effects on physiological mechanisms but when both concentrations are high, as seen in the post-ovulatory, midluteal phase, these hormones may act concomitantly and in balance with one another. OCs are taken by many females and should be part of ever- evolving research. Guidelines and practices should consider significant differences that may found regarding sex and sex hormones these differences

for best outcomes. If significant differences are not present, the exclusion of female participants in exercise physiology studies is unwarranted.

Key Takeaways

- Menstrual cycle phase and related hormone fluctuations affect neural and hormonal systems that influence fluid regulatory systems
- Estrogen shifts osmotic threshold for AVP release to the left, meaning that AVP is released at a lower osmolality when estrogen concentration is greater
- Progesterone shifts thermoregulatory control to the right, causing core body temperature to be greater at rest
- Oral contraceptives continue to evolve and therefore, researchers should continue to investigate exogenous hormone influences on both fluid balance and thermoregulation.

III. Methods

Eleven healthy, aerobically trained (VO_{2peak} > 45 ml/kg/min), female long term (> 3 months, Elliott-Sale, 2021) oral contraceptive users were recruited from the University and surrounding areas to participate in this crossover, counterbalanced design study. All procedures were approved by the University Institutional Review Board and written informed consent was obtained from all individuals prior to study participation and data collection. Participants completed a total of five visits including one pre-test to establish VO_2peak and familiarization of the exercise protocol, two experimental days (one on active pill dosing of oral contraception and one on her non-active pill dosing), and two quick visits to drop off urine containers post-experiment. Trials were scheduled so that the participant was on her dosing for at least 48-h prior to data collection. Trials were scheduled at least 7 days apart to avoid acclimatization. One trial was scheduled during the third week of active pill dosing, and the other during the placebo dosing. Exclusionary criteria included previous heat exhaustion or heat stroke within the past three years, current musculoskeletal injury, hypertension where vigorous exercise is contraindicated, diagnosed sickle cell trait, use of medications that may alter thermoregulation or fluid balance, and use of tobacco or vaping. All participants were asked to refrain from alcohol use for 24-h, caffeine for 12-h, and vigorous exercise for 24-h prior to each trial. Body composition was assessed via dual energy x-ray absorptiometry (DXA, Lunar Prodigy, General Electric, Madison, WI, USA).

Familiarization Day

During the familiarization visit, participants signed an informed consent form and complete a medical history questionnaire. Baseline demographic information was collected (height, weight, body surface area) and body composition was assessed via DXA. Participants

underwent a graded exercise test on a cycle ergometer to assess peak oxygen uptake (VO_{2peak}) and, due to the linear relationship between VO_2 and work rate, to establish exercise intensities for subsequent trials (55% of max wattage attained). VO_{2peak} was assessed via a graded exercise test (GXT) on a Velotron Dynafit Pro cycle electronically braked cycle ergometer (Racer Mate, Seattle, WA). Prior to testing, seat and handlebar preferences were established for each individual and recorded for use in all future testing sessions. Participants were instrumented with a Polar heart rate monitor (FT1/T31, Polar Inc, Lake Success, NY, USA). Subjects warmed up at 100 W for 2 minutes at a self-selected cadence. Upon completion of the warm-up, the resistance increased by 25 W in 2-minute intervals until the participant could no longer maintain 60 revolutions per minute (RPM) on the ergometer or reached volitional fatigue. VO_{2peak} was measured using breath-by-breath analysis and analyzed via open-circuit spirometry (PARVO Medics, Sandy, UT). The two highest consecutive 15-second VO_{2peak} values recorded were used as the peak measurement provided it meets at least two of the following criteria: a) a plateau in heart rate or heart rate is within 10% of the age predicted maximum, b) a plateau in VO_{2peak} (an increase of no more than 150 ml·min ^{1}), and/or c) an RER value > 1.15. Following their VO_{2peak} assessment, participants were familiarized with the heat chamber and experimental protocol. During this time, participants were also informed on the perceptual scales for rating of perceived exertion (RPE) (Borg, 1970), thermal sensation (Toner, Drolet, & Pandolf, 1986), and perceived thirst (Engell et al., 1987)

Experimental Days

Participants reported to the Exercise Science Research Center after having a light meal 2 hours prior (matched between trials). Upon arrival, participants completed a 24-h history questionnaire, provided a spot urine sample, and completed a nude body mass. The spot urine

sample was analyzed for urine specific gravity (USG, refractometer, model Master-SUR,NM, Atago Co Ltd, Tokyo, Japan) and osmolality (freezing point depression, Model 3250, Advanced Instruments Inc., Norwood, MA) to confirm hydration status. Participants could begin their trial if they were euhydrated confirmed by USG (< 1.020). If participant's USG was >1.020, 500mL of water was provided prior to trial start. A blood draw via venous venipuncture was taken for later analysis of copeptin and aldosterone concentration and quantification of plasma volume change. Participants were provided a rectal thermometer (RET-1, Physitemp Instruments Inc, Clifton, NJ, USA) to insert ~15 cm past the anal sphincter to assess rectal temperature (T_{rec}). Participants were instrumented with electrodes and an automated blood pressure cuff (Tango+, Suntech, Medical Inc., Morrisville, NC, USA) and skin temperature thermochrons (iButton, Maxim Integrated, San Jose, CA, USA) to assess four-site mean weighted skin temperature (T_{sk}) (Ramanathan, 1964). Sweat patches (Tegaderm+Pad, 3M, St. Paul, MN, USA) to assess sweat electrolytes were applied to the dorsal forearm. Participant attire consisted of running shorts or cycling bibs, sports bra, socks, and shoes (matched between trials). Participants transitioned to an environmental chamber (30°C, 55% relative humidity) and completed a 10-minute up-right seated acclimation period. Physiological and perceptual measures were assessed at the end of the ten minutes. Body mass was obtained, and the participant mounted the cycle ergometer. The cycle ergometer was positioned on a scale to measure changes in body mass due to sweat loss. Once comfortable on the ergometer, the participants began 90 minutes of exercise at 55% of the wattage attained during the final stage of the VO_2peak test. $T_{rec,}$ heart rate, blood pressure and perceptual measurements were recorded every 15-minutes. Body mass change was measured continuously and recorded every 15-minutes and subsequently used to provide water (warmed to 38°C) to replace sweat losses. Water was given to the participant to match 50% of sweat loss at

each time point. Participants finished drinking the water over the subsequent 15-minutes. Metabolic data including oxygen consumption (VO_2 and the respiratory exchange ratio (RER), wattage and cadence was collected at 30-minutes, 60-minutes, 75-minutes and at the cessation of exercise (Hashimoto et al., 2016) to ensure work intensity was kept constant. Following exercise, participants completed a self-selected cool down for 2-3 minutes while remaining on the cycle ergometer. They got off the cycle ergometer and moved to a seated position where they recovered until 30-minutes post-exercise. The participants then exited the chamber, removed instrumentation, and provided a final nude body mass, urine sample and blood sample. Participants were provided 125% of sweat lost in fluids and a recovery beverage (P:26g/CHO:6g/F: 4.5g; CorePower, fairlife LLC, Chicago, IL, USA) and water to consume within 60 minutes but were asked to refrain from any other fluid or food intake. They were provided urine jug containers to collect their urine over the following three hours post-fluid consumption and asked to record thirst, sense of urgency, and time of urination on the container. Participants provided a forced urine sample at 3-hours post-trial in a separate urine container. Participants brought urine collection jugs back to the laboratory later that same day or the following morning. Urine samples were used to assess 3-hour USG, volume, and urine osmolality.

Blood Analysis

Blood was collected pre-exercise trial and post-exercise trial. Serum collected at each time point clotted at room temperature followed by centrifugation at 1000g and 4°C for 15 minutes. Serum was used to assess osmolality via freezing point depression and electrolytes (ion-selective electrode, EasyElectrolyte, Medica Corporation, Bedford, MA, USA) in duplicate. Plasma collected at each time point was used to assess hemoglobin (Hb) in duplicate

(HemoCueHb 201+, HemoCue, Angelholm, Sweden) and hematocrit read in triplicate using microcapillary tubes with a Micro-Capillary Reader (International Equipment Company, Needham Heights, MA).

Statistical Analysis

All statistical analyses were completed using jamovi 2.2.5. A repeated measures multivariate analysis of variance was used to assess $T_{rec,}$ heart rate, blood pressure, and mean weighted skin temperature. Normality was assessed via Shapiro-Wilks's test and outliers were be removed if necessary. Statistical analyses were completed with and without outliers removed to identify the impacts on outcomes. If the outliers did not impact the findings, the outliers were removed, and results reported. Analyses requiring outlier removal (i.e., decreasing sample size) is noted in the results. When sphericity was violated, Hyunh-Feldt adjustments were used in the omnibus test. Post-hoc analyses involved pairwise comparisons with an appropriate Bonferroni corrected alpha to identify significant time point differences. Data that failed normality (muscle pain and change in T_{re}) was treated as normal due to skewness < 2. Alpha of 0.05 was set *a priori* to determine significance at the omnibus level. Based on a 0.80 power calculation using core temperature as the primary outcome variable with a correlation between time points of 0.5, a 2-standard deviation effect size, β of 0.20, and α of 0.05, it was determined 8 participants would be sufficient to complete this study. An increased experiment-wise type I error rate is acknowledged due to the multivariate and univariate analyses conducted.

IV. Results

Descriptive information for participants is displayed in Table 1. There were no significant differences in kcal (ACT: 1849 ± 783 kcal; PLA: 2128 ± 621 kcal, t_{10} = -0.919, P = 0.380), carbohydrate (ACT: 206 ± 100 g; PLA: 212 ± 58g , t_{10} = -.217, P = 0.833) protein (ACT: 78 ± 39

g, PLA:100 ± 35 g, t_{10} = -1.975, P = 0.077) or fat intake (ACT: 83 ± 50 g ; PLA: 100 ± 35 g , t_{10} = -0.943, P = 0.368) in the 24-h preceding trials. There were no significant differences in wet bulb globe temperature (WBGT) over time ($F_{1,10}$ = 2.275, P = 0.162), between trials ($F_{2.90,28.95}$ = 2.275, P = 0.162), or time x trial interaction ($F_{2.95,29.56}$ = 2.373, P = 0.091). By design, VO_2 was not significantly different over time ($F_{3,30}$ = 1.340, P = 0.280), between trials ($F_{1,10}$ = 0.163, P = 0.695), nor was there a time x trial interaction for VO_2 measures ($F_{3,30}$ = 1.825, P = 0.164, grand mean: ACT 32.6 ± 3.0 mL/kg/min; PLA 32.4 ± 3.0 mL/kg/min). Metabolic heat production was not different between trials (ACT: 9.37 ± 0.8 W/kg; PLA: 9.29 ± 0.74 W/kg, t10 = 0.508, P = 0.622) and participants cycled at 68 ± 6% of their VO_2 peak.

Hydration

There was a significant main effect for nude body mass (NBM) to decrease over time from pre-trial (ACT: 59.59 ± 5.88 kg, PLA: 59.46 ± 5.30 kg) to post-trial (ACT: 58.85 ± 5.81 kg vs. PLA: 58.85 ± 5.31 kg), regardless of trial ($F_{1,10}$ = 55.420, P < 0.001). NBM was not different between trials ($F_{1,10}$ = 0.042, P = 0.842) nor was there a time x trial interaction ($F_{1,10}$ = 1.190, P = 0.301).

Pre-trial spot USG (ACT: 1.011 ± .008; PLA: 1.011 ± .007) was not significantly different between trials ($F_{1,8}$ = 0.130, P = 0.728). Likewise, post-trial USG was similar between trials (ACT: 1.013 ± .005; PLA: 1.013 ± .004, $F_{1,8}$ = 0.084, P = 0.780). There was not a time x trial interaction ($F_{1,8}$ = 0.032, P = 0.864) for USG. Pre-trial urine osmolality (ACT: 384 ±299 mosm•L-1; PLA: 416 ± 387 mosm•L-1) was not different compared to post-trial (ACT: 413 ± 170 mosm•L-1; PLA: 411 ± 165 mosm•L-1, $F_{1,8}$ = 0.217, P = 0.654) and was not different between trials ($F_{1,8}$ = 0.008, P = 0.930) nor was there a trial x time interaction ($F_{1,8}$ = 0.047, P = 0.834).

Thermoregulation

T_{re} demonstrated a significant main effect difference for ACT to be greater than PLA, regardless of time point ($F_{1.55,15.53} = 74.019$, $P < 0.001$). There was also a significant main effect for T_{re} to increase over time, regardless of trial ($F_{1,10} = 24.064$, $P < 0.001$). There was not an interaction of time x trial ($F_{1.96,19.62} = 1.822$, $P = 0.189$) for T_{re}. There was no difference in overall change in temperature (baseline to maximum temperature) between trials (ACT: 1.3 ± 0.5ºC, PLA: 1.4 ± 0.4ºC, $t_{10} = -0.588$, $P = 0.570$). Data for T_{re} throughout trials is presented in Figure 1A.

Regardless of trial, T_{sk} significantly increased over time ($F_{6,24} = 2.595$, $P = 0.044$) but there was no difference between trials ($F_{1,4} = 0.902$, $P = 0.396$). There was no time x trial interaction for T_{sk} ($F_{6,24} = 0.856$, $P = 0.540$). Baseline T_{sk} was significantly lower than all other time points ($P = 0.002$). There were no other time point differences with T_{sk} ($P \geq 0.877$).

Total sweat loss was not different between trials (ACT: 1145 ± 189 mL; PLA: 1041 ± 275 mL, $t_{10} = 2.064$, $P. = 0.066$; Figure 2) nor was sweat rate (ACT: 764 ± 126 mL/hr; PLA: 694 ± 183 mL/hr, $t_{10} = 2.064$, $P = 0.066$). There was no difference in percentage body mass loss between trials (ACT: 1.2 ± 0.5%, PLA: 1.1 ± 0.6%, $t_{10} = 0.834$, $P = 0.424$).

Perceptual Measures

Perceptual measures are shown in Figure 4, A-D. RPE demonstrated a main effect for increasing over time, regardless of trial ($F_{2.115,21.153} = 106.286$, $P < 0.001$). There was a significantly greater RPE during the ACT trial ($F_{1,10} = 11.250$, $P = 0.007$), regardless of time point. There was not a significant time x trial interaction for RPE ($F_{2.716,27.162} = 1.894$, $P = 0.159$). Thermal sensation demonstrated a main effect for increasing over time, regardless of trial ($F_{2.32,23.23} = 22.669$, $P < 0.001$). There was no significant difference between ACT and PLA trials

($F_{1,10} = 0.676$, $P = 0.430$) nor was there a time x trial interaction ($F_{3.96,39.56} = 0.555$, $P = 0.694$) with thermal sensation. Thirst increased over time, regardless of trial ($F_{2.62,26.23} = 29.045$, $P < 0.001$). There were no significant thirst differences between ACT and PLA trials ($F_{1,10} = 1.861$, $P = 0.202$) nor was there a time x trial interaction ($F_{4.57,45.67} = 1.659$, $P = 0.169$). Muscle pain increased over time, regardless of trial ($F_{1.53,15.30} = 13.716$, $P < 0.001$). There was no significant difference between ACT and PLA trials ($F_{1,10} = 0.2.185$, P = 0.170) nor was there a time x trial interaction ($F_{3.0,30.03} = 0.663$, $P = 0.581$) for muscle pain.

Cardiovascular measures

Heart rate increased over time, regardless of trial ($F_{1.572,14.146} = 483.74$, $P < 0.001$) but there was no difference between trials ($F_{1,9} = 2.146$, $P = 0.176$). There was no time x trial interaction for HR ($F_{3.275,29.47} = 1.344$, $P = 0.254$). Data for HR throughout trials is presented in Figure 3A.

Mean arterial pressure increased over time, regardless of trial ($F_{6,24} = 3.378$, $P = 0.015$), but there was no difference in MAP between trials ($F_{1,4} = 0.763$, $P = 0.432$). There was not an interaction effect for MAP ($F_{6,24} = 0.765$, $P = 0.605$). Data for MAP throughout trials are presented in Figure 3B.

Blood Analysis

Blood markers of hydration are presented in Table 2. There was no time x trial interaction for plasma osmolality ($F_{1,8} = 0.917$, $P = 0.363$). There was not an interaction effect of time x trial for plasma sodium ($F_{1,8} = 1.073$, $P = 0.331$), plasma potassium ($F_{1,8} = 3.087$, $P = 0.117$), or plasma chloride ($F_{1,8} = 0.102$, $P = 0.758$). Hemoglobin and hematocrit demonstrated no time x trial interaction (Hemoglobin: $F_{1,9} = 0.514$, $P = 0.494$; Hematocrit $F_{1,9} = 0.028$, $P = 0.872$).

Post-Exercise Urinalysis

Post-exercise, there was no difference between trials in the amount of fluid consumed (ACT: 1007 ± 256 mL; PLA: 921 ± 448 mL, $t_{10} = 0.874$, $P = 0.403$). There was no difference in spot sample USG assessed 3-h post trial between trials ($t7 = -0.743$, $P = 0.487$). Likewise, 3-h spot sample urine osmolality was not different between trials ($t7 = -1.177$, $P = 0.287$). Urgency to void at 3-h post-trial was similar between trials ($t7 = -1.000$, $P = 0.351$). Perception of thirst was not different between trials at 3-h post-trial ($t7 = -0.3859$, $P = 0.711$). Urine frequency post-trial to 3-h post-trial was not different between trials ($t7 = -1.528$, $P = 0.170$). Post-exercise urinalysis is presented in Table 3.

When individual samples over the 3-h post trial were combined and assessed in total for USG, there were no differences between trials ($t6 = -1.774$, $P = 0.126$). Combined urine osmolality was not different between trials ($t5 = -0.614$, $P = 0.556$). Total urine volume immediately post-exercise to 3-h post-exercise was not different between trials ($t7 = 1.090$, $P = 0.307$) nor was there a difference between the percent of the bolus excreted by 3-h post trial ($t_9 = 0.648$, $P = 0.648$).

Table 1. *Participant Descriptive Characteristics*

n	Mass (kg)	Height (cm)	Age (y)	VO_2max (mL/kg/min)	Body Fat (%)	Relative Workload (W/kg)
11	58.7 ± 6.2	163 ± 5	25 ± 6	47.8 ± 4.7	28.3 ± 5.8	1.8 ± 0.2

Note: Mean ± SD.

Table 2. *Blood Markers of Hydration Assessed Pre- and Post-Exercise by Trial Type*

	Active Trial		Placebo Trial	
Measure	Pre-Exercise	Post-Exercise	Pre-Exercise	Post-Exercise
Plasma osmolality (mOsm/kg)	290 ± 4	293 ± 3	293 ± 4*	294 ± 4*
Serum [Na^+] (mEq/L)	141.0 ± 3.4	141.8 ± 3.9	141.2 ± 2.7	140.9 ± 2.6
Serum K^+, mEq/L	3.9 ± 0.4	4.4 ± 0.4#	3.9 ± 0.2	4.2 ± 0.4#
Serum Cl-, mEq/L	104.8 ± 3.4	104.5 ± 3.9	105.0 ± 2.1	104.4 ± 2.6
Hemoglobin ($g \cdot dL^{-1}$)	13.2 ± 0.7	13.4 ± 0.5	13.3 ± 0.7	13.2 ± 0.8
Hct, %	41.5 ± 2.9	41.6 ± 1.6	41.3 ± 2.1	41.7 ± 2.6
Plasma volume change (%)		-5.4 ± 1.5		-4.9 ± 2.5

*Note: Uosm=urine osmolality; USG=urine specific gravity; Sosm=serum osmolality; Serum [Na^+] =serum sodium; Hb=hemoglobin; Hct=hematocrit. *Significantly different from ACT trial, P = 0.036. #Significantly different from pre-trial, P < .001. Mean ± SD.*

Table 3. *Urinalysis and Perceptual Responses Assessed 3-h Post-Exercise*

Measure	Active	Placebo
3-h spot USG	1.012 ± .008	1.014 ± .005
3-h spot U_{osm} (mOsm/kg)	437 ± 289	553 ± 161
Urgency	2 ± 1	2 ± 1
Thirst	5 ± 2	5 ± 2
Frequency	3 ± 1	2 ± 1
Total USG	1.009 ± .003	1.013 ± .007
Total Uosm, mOsm/kg	307 ± 121	381 ± 289
Total urine volume (mL)	465 ± 200	363 ± 260
Percent of bolus excreted (%)	50.6 ± 24.8	46.1 ± 30.0

Note: Uosm=urine osmolality; USG=urine specific gravity. Mean ± SD.

Figure Legends

Figure 1. **A)** Rectal temperature during exercise in the heat comparing active and placebo phase of OCP use. There was a significant main effect difference between trials ($P < 0.001$) and over time ($P < 0.001$). There was not a time x trial interaction ($P = 0.189$). All time points were significantly less than subsequent time points, regardless of trial ($P \leq 0.013$). **B)** Demonstrates skin temperature changes throughout trials. Regardless of trial, T_{sk} significantly increased over time ($F_{6,24} = 2.595$, P=0.044) but there was no difference between trials ($F_{1,4} = .902$, $P = 0.396$). There was no interaction effect for time x trial for T_{sk}($F_{6,24} = 0.856$, $P = 0.540$). [a]Indicates baseline T_{sk} was significantly lower than all other time points ($P \leq 0.002$). Pairwise analysis showed no other time point differences in T_{sk}. Error bars represent SD.

Figure 2. Demonstrates total sweat losses throughout trials. Total sweat loss was not different between trials ($P = 0.066$). Lines within bars represent the mean whereas length of bars represent SD. Individual responses are represented by connect dots.

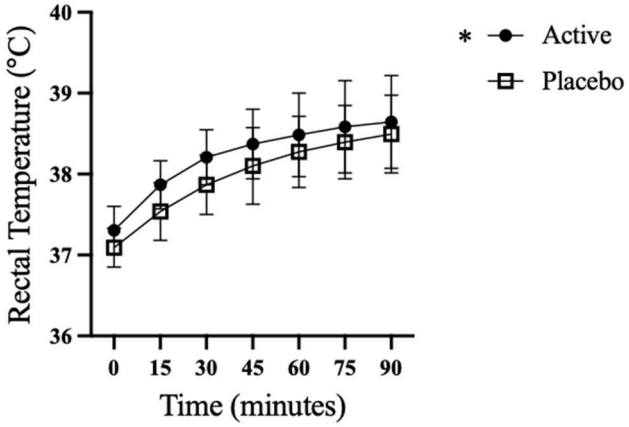
Figure 3. Cardiovascular responses during exercise in the heat comparing active and placebo phase of OCP use. **A)** There was a significant main effect for HR to increase over time regardless of trial ($P < 0.001$). There was no difference between trials ($P = 0.176$). There was not a time x trial interaction ($P = 0.254$). [a]Indicates a different heart rate than all other time points ($P < .001$). [b]Indicates a lower heart rate compared to 75 minutes and 90 minutes ($P \leq .002$). [c]Indicates a lower heart rate compared to 90 minutes ($P = 0.033$). **B.)** There was a significant main effect for MAP to increase over time, regardless of trial ($P = 0.015$). There was no difference between trials in MAP ($P = 0.432$) and there was not a time x trial interaction ($P = 0.605$). [a]Indicates a significantly different MAP compared to 60-minute time points ($P = 0.007$). Error bars represent SD.

Figure 4. Perceptual responses during exercise in the heat comparing active and placebo phase of OCP use. **A)** RPE demonstrated a main effect for increasing over time regardless of trial ($P < 0.001$) with all-time points significantly less than subsequent time points ($P \leq 0.005$). There was a significantly greater RPE during the ACT trial ($P = 0.007$). There was not a time x trial interaction for RPE. Pairwise comparisons demonstrated no difference between trials at each time point ($P \geq 0.232$). **B)** Thermal sensation increased, over time regardless of trial ($P < 0.001$). There was no significant difference between ACT and PLA trials ($P = 0.430$) nor was there a time x trial interaction ($P = 0.694$) for thermal sensation. [a]Indicates a significant lower thermal sensation than all other time points ($P \leq .027$). [b]Indicates a significantly lower thermal sensation than 60- 75- and 90-minutes ($P \leq .043$). [c]Indicates a significant difference from 75 and 90 minutes ($P \leq .034$). **C)** Thirst sensation demonstrated a significant main effect for increasing over time regardless of trial ($P < 0.001$). There was no significant difference between ACT and PLA trials trial ($P = 0.202$) nor was there a time x trial interaction ($P = 0.169$). [a]Indicates significantly lower perceived level of thirst when compared to all subsequent timepoints ($P \leq 0.002$). [b]Indicates significant lower perceived level of thirst when compared to 45-, 75-, and 90-minute timepoints ($P \leq 0.025$). [c]Indicates significantly lower perceived thirst when compared to 45- and 90- minutes ($P \leq 0.012$). [d]Indicates a significantly lower perceived level of thirst when compared to 90-minutes ($P \leq 0.012$). **D)** Perceived pain. Perceived pain demonstrated a main effect for increasing over time regardless of trial ($P < 0.001$). There was no significant difference between

ACT and PLA trials trial ($P = 0.170$) nor was there time x trial interaction ($P = 0.581$). [a]Indicates significantly lower perceived pain when compared to 60- and 75 minutes time points ($P \leq 0.049$). Error bars represent SD.

Figure 1.

A.


Rectal Temperature (°C)
40
39
38
37
36
0
15
30
45
60
75
90
Time (minutes)
*
Active
Placebo

B.

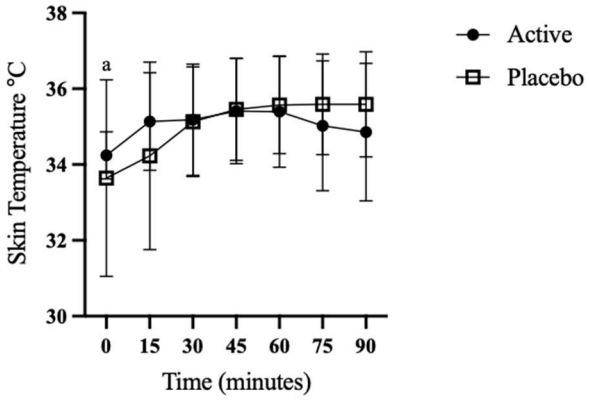

Skin Temperature °C
38
36
34
32
30
a
0
15
30
45
60
75
90
Time (minutes)
Active
Placebo

Figure 2.

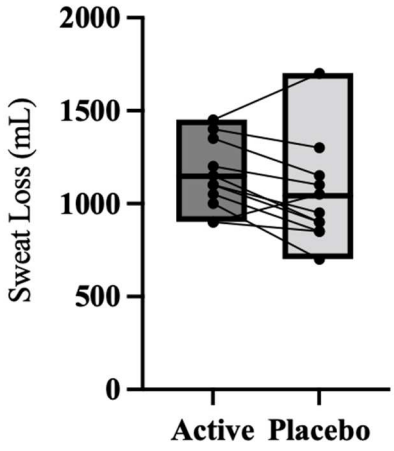


Figure 3.

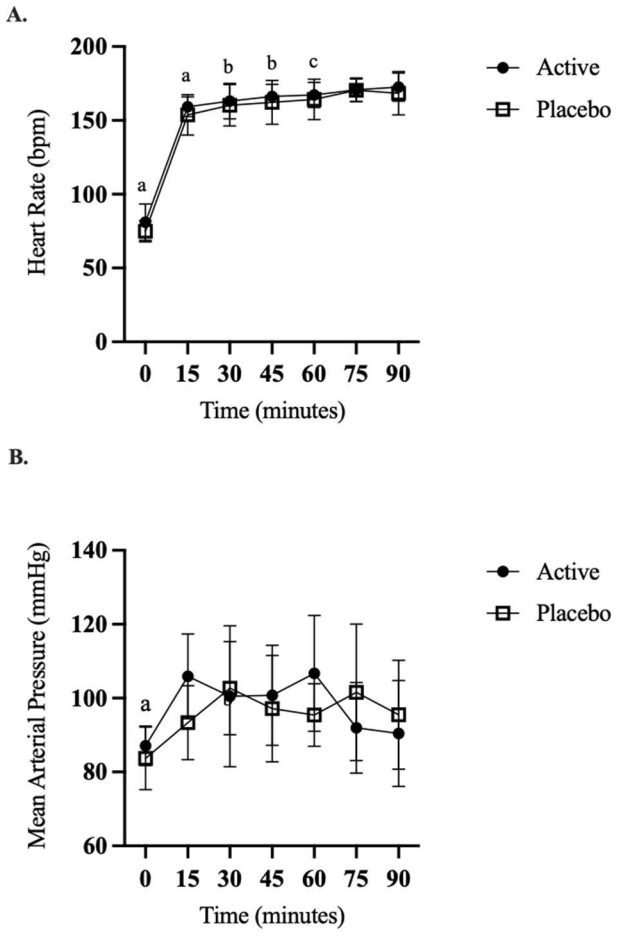
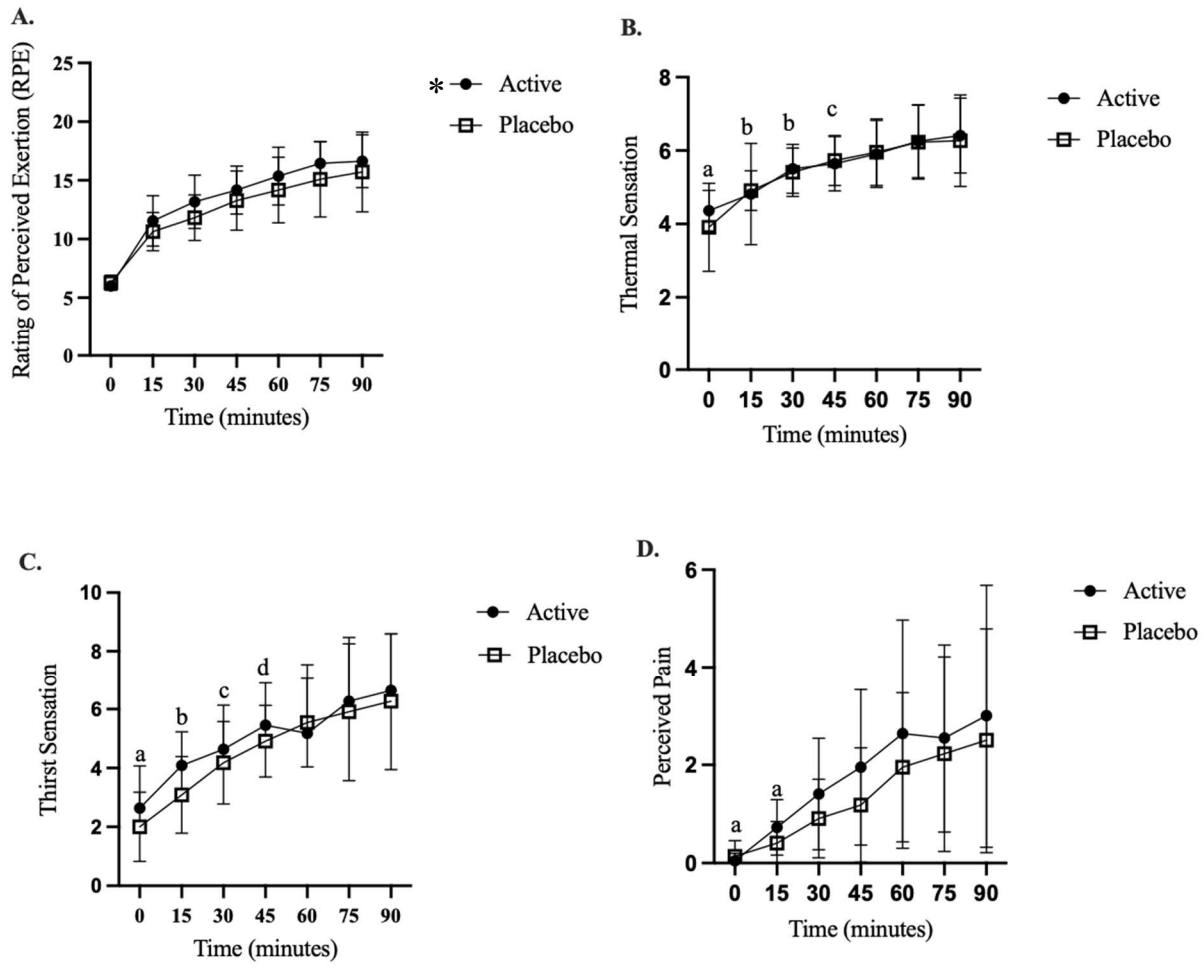

A.
Heart Rate (bpm)
200
150
100
50
0
a
a
b
b
c
0 15 30 45 60 75 90
Time (minutes)
Active
Placebo
B.
Mean Arterial Pressure (mmHg)
140
120
100
80
60
a
0 15 30 45 60 75 90
Time (minutes)
Active
Placebo

Figure 4.


A.
Rating of Perceived Exertion (RPE)
25
20
15
10
5
0
0 15 30 45 60 75 90
Time (minutes)
* Active
Placebo
B.
Thermal Sensation
8
6
4
2
0
a
b
b
c
0 15 30 45 60 75 90
Time (minutes)
Active
Placebo
C.
Thirst Sensation
10
8
6
4
2
0
a
b
c
d
0 15 30 45 60 75 90
Time (minutes)
Active
Placebo
D.
Perceived Pain
6
4
2
0
a
a
0 15 30 45 60 75 90
Time (minutes)
Active
Placebo

V. Discussion

The purpose of this investigation was to evaluate the effects of combined (estradiol and progestin) monophasic oral contraceptive pill (OCP) use on thermoregulation. We removed the impact of dehydration by replacing 50% of sweat loss throughout exercise. We found that T_{re} was significantly elevated during the ACT trial (third week of active pill dose). Other than a greater baseline temperature, there were no thermoregulatory differences between trials and T_{re} increases were not different between trials. Further, we sought to evaluate the effects of combined monophasic OCP use on acute rehydration post-exercise in the heat according recommended guidelines. We found no differences in fluid retention, thirst or fluid turnover between trials.

Thermoregulation

As hypothesized, participants maintained a higher core temperature during exercise in the heat when during ACT compared to PLA. Interestingly, there were no differences in T_{sk} or sweat loss between trials. Overall, we demonstrated that the ACT phase of OCP use caused a greater core temperature without a change to other thermoregulation variables. The change in T_{re} is likely due to the previously identified hypothalamic "set-point" shift (Charkoudian & Johnson, 1997; Charkoudian & Stachenfeld, 2016; Rogers & Baker, 1996; Stachenfeld et al., 2000).

The preoptic region of the anterior hypothalamus (POAH) is primarily responsible for body temperature control. Animal studies show that estrogen and progesterone act on sex steroid binding neurons in the POAH and that estrogen appears to augment heat dissipation responses (Silva & Boulant, 1986). The effect of progesterone on the hypothalamus is less clear (Charkoudian & Stachenfeld, 2016). In humans, female reproductive hormones change the

threshold temperature at which thermoregulatory responses (sweating and peripheral blood flow) begin.

Previous research has shown during the luteal phase of eumenorrheic women, when progesterone concentration is greatest, resting core body temperature is increased by 0.2-0.8°C when compared to the early follicular phase (Baker et al., 2020; Charkoudian & Johnson, 1999; Charkoudian & Stachenfeld, 2016; Constantini et al., 2005; Kolka & Stephenson, 1989; Pivarnik et al., n.d.; Rogers & Baker, 1996; Tenaglia et al., 1999). The increased T_{re} is likely due to the increased progesterone concentrations although estrogen is concomitantly increased (Charkoudian & Johnson, 1997; Rogers & Baker, 1996; Tenaglia et al., 1999). The set-point shift refers to the control of thermoregulatory physiology (onset of sweat) that is shifted to a higher resting core temperature during the luteal phase. This hypothalamic adjusted set-point is not unlike a fever, although mechanisms of action differ (Charkoudian & Stachenfeld, 2016). This thermoregulatory set-point shift is maintained with OCP use and is further exacerbated with exercise in hot and humid climates when thermoregulatory facilitation cannot compensate for heat gain (T. Lei et al., 2019b). Some research has suggested that the greater resting core temperature seen during ACT pill dosing is due to the residual effects of the menstrual cycle (Tenaglia et al., 1999). However, it is plausible that the exogenous progesterones are responsible for the set-point shift in OCP users (Charkoudian & Johnson, 1999; Charkoudian & Stachenfeld, 2016, 2014). Our data suggest that although both exogenous hormones are elevated during the third week of ACT pill dosing that the effects of progestins are stronger than those of estrogen.

Human thermoregulation can simply be explained via the heat balance equation; $S = W \pm C \pm K \pm R - E$. Whereas, ‘S’ represents heat storage, and ‘W’ represents heat produced via metabolic work. ‘C’ represents of a gain or loss of heat via convection (such as wind or fan use).

'K' represents heat loss or gain via conduction to surfaces that have direct contact with the skin. 'R' represents heat loss or gain via radiation (such as the sun). 'E' represents heat loss via evaporation. Evaporation is the primary driver of cooling and is responsible for 80-90% of heat loss by sweat. In a hot and humid environment, heat loss is decreased due to the impairment of sweat to evaporate from the skin. In an effort to dissipate heat, blood flow is directed to the periphery decreasing blood flow to the working muscles, which is detrimental to performance.

Laboratory investigations of OCP use and thermoregulation have reported conflicting evidence. Tenaglia et al, using similar phases as our study found that there was an elevated core temperature during ACT but that the differences compared to PLA were less pronounced after 75 minutes of exercise in a hot and humid climate (40ºC and 30% RH). Baseline T_{re} was 0.22 ºC greater during ACT, whereas our study had a similar 0.20 ºC increase during ACT. Also like our study, there were no differences in sweat loss or sweat rate and there were no differences in the ΔT_{re} between phases (Tenaglia et al., 1999). However, their study focused on light intensity exercise for long durations designed with intermittent bouts of treadmill walking between rest intervals to determine heat tolerance. Lei et al. demonstrated a higher core resting temperature during the quasi-mid-late luteal phase (days 10-20 of OCP use) that persisted through the first stage of exercise (12 minutes) at a fixed work rate of 125W (T. Lei et al., 2019b). Interestingly, the comparison of phases included a quasi-mid-late follicular (days 3-5 of OCP use) so that the higher core temperature was significant compared to the first week of ACT rather than PLA. In agreement with our findings, there was no difference in T_{sk}, although this comparison is not ideal when using different days of OCP use in testing. To our knowledge, our study is the first to demonstrate an elevated core temperature throughout 90 minutes of moderate-vigorous aerobic exercise.

In our study, we aimed to control variables in the heat balance equation to isolate effects of OCP phase on heat storage. Metabolic work was controlled and there were no differences between trials in heat production (ACT: 9.37 ± 0.8 W/kg; PLA: 9.29 ± 0.74 W/kg). A fan was used during trials but was matched between trials. This allowed a control for convection. Radiation and conduction were not quantified but all trials were conducted under the same conditions within our environmental chamber. Further, there were no differences in sweat loss between trials. Controlling for all other variables within the heat balance equation suggests that the defining outcome of our study was heat storage compared between trials. T_{re} was significantly greater during active pill dosing. Further, there was no differences in ΔT_{re} (ACT: 1.3 vs. PLA: 1.4 ºC), reaffirming the previously defined set point shift due to greater concentrations of progestins.

It should be noted that we compared the third week of active pill dosing when exogenous hormone concentration is highest. This does not account for the first two weeks of pill use and, therefore limits our findings to application across a month of OCP use. We maintained an acceptable level of dehydration (< 2%) throughout trials; therefore, we cannot ascertain any exacerbating effects of combined dehydration and exercise in the heat with OCP use. It should also be considered that VO_2peak tests were not all taken during the same OCP dosing phase. While we do not believe this would have changed our outcomes, there is a possibility that power output would have varied due to RPE, and work rate would have been established at a different % of VO_2. We used 55% of peak wattage achieved during our VO_2peak tests to determine workload. This method caused a greater percentage of max VO_2 to be achieved during trials. On average, our participants cycled at 68% ± 6% of VO_2peak however, this is intensity is quite applicable to trained individuals. Unfortunately, our intent to randomize trials was inhibited by

the feasibility of our timeline. Ten out of 11 participants completed the ACT trial first. Trials were separated by at least 7 days and conducted from November-March when the potential for acclimation is not likely.

Thermoregulation conclusions

Our results confirm previously reported increases of core temperature during ACT dosing of OCP use during exercise in the heat. We also demonstrated a greater RPE during ACT. We did not find any other differences between pill phases in measured thermoregulatory variables. Considering these results, we do not believe that exercising during ACT increases risk during exercise in the heat, but it may be pertinent for some individuals to note especially when performance outcomes are prioritized. One of our participants reached 40.08ºC during ACT in comparison to 39.77 ºC during PLA. Our study provided a fixed work intensity causing T_{re} to continually rise. It is possible that an attenuation in performance would be observed in self-paced exercise to avoid greater core temperatures (Lee et al., 2010). Contrarily, if work rate was held constant, this individual could be at risk for heat injury.

In some military settings, heat intolerance is defined as a peak T_{re} of 38.5 °C, a peak HR < 150bpm or the inability to reach equilibrium at these values. Using this criterion, 67% of female participants are classified as "heat intolerant" compared to 33% of males (Druyan et al., 2012). As demonstrated in our study, females got significantly hotter during the ACT pill dosing although they did not exhibit symptoms of heat stress. While recognizing that this is a drastically different protocol, it may be beneficial that heat tolerance tests consider the variability of core temperature based on OCP phase.

Perceptual Measures

Participants reported a greater rating of perceived exertion (RPE) during the ACT pill dosing but there no differences in perceived thirst, perceived pain, or thermal sensation between trials. To our knowledge, there are no previous studies comparing perceptual data in OCP users. Therefore, we can only compare our findings to those in eumenorrheic females. For the purpose of comparison, we can assume that the greater hormone concentration in ACT is similar to that of the luteal phase. Janse De Jonge et al. (2012) investigated exercise performance over the menstrual cycle in a hot and humid environment and similar to our study found that participants reported a greater RPE during the luteal phase when compared to the early-follicular phase. Like our study, participants did not report a difference in thermal sensation between trials. Lei et al. (2017) investigated perceptual measures during different phases of the menstrual cycle in eumenorrheic females during self-paced exercise and found no differences in thermal or thirst sensation or RPE between phases of the menstrual cycle (T.-H. Lei et al., 2017). These findings are in agreement with Sunderland and Nevill (2003) whose participants also did not report differences in RPE between menstrual cycle phase while intermittently running in the heat. However, our data suggest that RPE is greater during the ACT phase of OCP use. Therefore, there may be practical recommendations for endurance athletes to align competition or training with pill dosing phase especially in hot, humid conditions as training and performance responses may be dependent on RPE (Janse De Jonge et al., 2012; Sunderland & Nevill, 2003).

Post-Exercise Fluid Balance

As hypothesized, there was no difference in fluid retention or fluid turnover post-exercise. This study used a bout of exercise in the heat (total ~90 minutes) to assess thermoregulatory variables. To make conclusions regarding thermoregulation, by design,

participants were prevented from becoming dehydrated (no more than 2% body mass loss) to avoid a combination of heat stress and hypohydration. Therefore, by using recommended sport guidelines for rehydration, the study provided an opportunity to assess fluid balance in OCP users post-exercise in the heat without concomitant effects of dehydration.

Sex differences in fluid balance, for instance sweating rates, are considered in hydration recommendations but do not address menstrual cycle phase or OCP use. Greater variability may exist within phases of the menstrual cycle verses differences between sex (Sims & Heather, 2018). Arginine vasopressin (AVP) has a lower osmotic threshold for stimulation when estrogen concentration is greater. Further, both endogenous and exogenous estrogens and progesterones promote water and sodium retention (Sims et al., 2008b; Stachenfeld & Keefe, 2002; Stachenfeld & Taylor, 2005b). However, our study did not find differences between phases of OCP use.

To our knowledge, this study is the first to investigate acute rehydration post-exercise in OCP users. Rodriguez-Guistiniani and Galloway previously investigated rehydration in eumenorrheic females and similarly dichotomized hormone profiles (mid luteal and early follicular) (Rodriguez-Giustiniani & Galloway, 2019). They reported no significant differences between menstrual cycle phase in urine volume or percent fluid retained after females were dehydrated to 2% body mass via fluid restriction and exercise in the heat. Their rehydration protocol included an amount of 100% body mass loss consumed in 4 equal boluses over thirty minutes. While their study consisted of eumenorrheic women and ours OCP users, both compared two different phases of greater and lesser hormone concentration. Neither their study nor ours identified differences in fluid balance post-exercise in the heat. In investigating eumenorrheic females vs. OCP users, it should be noted that the hormone profiles are not easily compared. OCP use turns off hypothalamic-pituitary-gonadal axis and provides an greater

concentration of exogenous hormones (Sims & Heather, 2018). However, previous research in our lab investigated the differences in OCP use in euhydrated, resting conditions. We found no differences in percent bolus excreted or volume turnover between OCP phases. Our study prevented participants from becoming dehydrated even when undergoing exercise in the heat. Our findings suggest that, in euhydrated scenarios, fluid balance may be tightly regulated to prevent influence of sex hormones.

Maintaining euhydration during exercise is difficult, especially when environmental conditions promote heavy sweating (i.e., in hot and humid conditions). Suboptimal hydration status may have implications to safety and performance. More so, poor hydration may also hinder recovery following exercise in the heat. Therefore, rehydration strategies have been recommended for physically active individuals (McDermott et al., 2017; Sawka, 2007). Those strategies include preventing dehydration greater than 2% of body mass loss during exercise (although detriments as small as 1% can affect performance) and replenishing fluid loss post-exercise. It is recommended that 125-150% of body weight loss should be consumed within 4-h post-exercise in recovery food and fluid (McDermott et al., 2017; Sawka et al., 2007, Sherriffs, 2004). We chose to provide recovery fluids equal to 125% body weight loss. This amount would be reasonable to achieve and applicable in a real-world scenario. Knowing that the exact amount of fluid needed to restore euhydration would depend on macronutrient content, we chose to provide a recovery beverage (P:26 g/CHO:6 g/F: 4.5 g) as well as water to facilitate appropriate rehydration. The additional consumption of electrolytes (sodium) and carbohydrates ideally compensated for the post-exercise diuresis and was matched between trials (McDermott et al., 2017).

As this study was part of a larger investigation of thermoregulation, there were limitations to this methodology. We provided participants with rehydration beverages to finish within one hour of completing exercise. Participants finished recovery fluids before leaving the laboratory, therefore, we relied on participants to complete the procedure and verbally confirm compliance at urine drop-off. The goal of this study was to assess differences in fluid handling post-exercise in the heat. Several analyses may be underpowered, increasing our risk of a Type 1 error. This is due to our small sample size and the use of paired samples t-tests. However, previous research in our lab (with a larger sample size) showed no differences in OCP phase when participants were euhydrated in resting conditions. Considering the design of the current study in that participants finished no greater than 2% dehydrated we believe that it would be unlikely to find significant differences due to tightly regulated physiological fluid volume control.

Fluid Balance Conclusions

In conclusion, the findings of this study apply to females who use OCPs and exercise or work in hot and humid environments. We demonstrate no significant impact of OCP phase to post-exercise fluid balance. These findings pertain to a maintenance of euhydration during exercise and proper recovery based on previously published guidelines for physically active individuals. Specifically, when maintaining no greater than 2% body mass loss during exercise and meeting proper recovery suggestions of 125% body mass loss in replenishing fluid is met. Although not the goal of our study, our rehydration protocol of 125% replacement ensured that participants maintained euhydration for 3-h post-trial, confirmed by a 3-h total USG of 1.012 ± 0.007 and a 3-h-spot USG of 1.013 ± 0.006 . If participants finished exercise hypohydrated, or failed to recover properly, there may have been differences in fluid handling due to estrogens

affinity with AVP (Stachenfeld, 2008; Stachenfeld & Keefe, 2002). However, we see no need for OCP users to follow rehydration guidelines other than those currently recommended. As such, to improve best outcomes, recovery fluids and prevention of exercise dehydration is necessary.

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UNIVERSITY OF ARKANSAS

To:	Whitley C Atkins
From:	Douglas J AdamsJustin R Chimka, Chair IRB Full Board
Date:	10/14/2021
Action:	**Approval**
Action Date:	10/14/2021
Protocol #:	2109354139
Study Title:	Examining the Effects of Oral Contraceptive Use on Thermoregulation
Expiration Date:	09/07/2022
Last Approval Date:	
Risk Level:	

The above-referenced protocol has been approved following Full Board Review by the IRB Committee that oversees research with human subjects.

If the research involves collaboration with another institution then the research cannot commence until the Committee receives written notification of approval from the collaborating institution's IRB.

It is the Principal Investigator's responsibility to obtain review and continued approval before the expiration date.

Protocols are approved for a maximum period of one year. You may not continue any research activity beyond the expiration date without Committee approval. Please submit continuation requests early enough to allow sufficient time for review. Failure to receive approval for continuation before the expiration date will result in the automatic suspension of the approval of this protocol. Information collected following suspension is unapproved research and cannot be reported or published as research data. If you do not wish continued approval, please notify the Committee of the study closure.

Adverse Events: Any serious or unexpected adverse event must be reported to the IRB Committee within 48 hours. All other adverse events should be reported within 10 working days.

Amendments: If you wish to change any aspect of this study, such as the procedures, the consent forms, study personnel, or number of participants, please submit an amendment to the IRB. All changes must be approved by the IRB Committee before they can be initiated.

You must maintain a research file for at least 3 years after completion of the study. This file should include all correspondence with the IRB Committee, original signed consent forms, and study data.

cc: Brendon P McDermott, Investigator
Aidan Fiol, Key Personnel
Nicholas P Greene, Key Personnel
Matthew S Ganio, Key Personnel
Juliet Tunberg, Key Personnel
Michaela K. Slosar, Key Personnel
Mary K. Smith, Key Personnel
Emily R. Nelson, Key Personnel
Jayla Verrett, Key Personnel
Abigail Chopelas, Key Personnel
Marissa A. Turner, Key Personnel
Justin A. Sturdevant, Key Personnel

Gabriella Joy Fernandez, Key Personnel
Sydney A. Key, Key Personnel
Brady Cross, Key Personnel
Savannah Campbell, Key Personnel
Kristin Michelle Garner, Key Personnel
Khenli Harp, Key Personnel
Brittany Michelle Martin, Key Personnel
Britton Shae Peters, Key Personnel
Elizabeth R. Pittman, Key Personnel
William W. Seifert, Key Personnel
Meghan Kathleen Underwood, Key Personnel
AnnaLee Grace Chitwood, Key Personnel
Deja Stanley, Key Personnel
Jayce S. Parmley, Key Personnel

UNIVERSITY OF ARKANSAS

To:	Whitley C Atkins
From:	Justin R Chimka, Chair IRB Expedited Review
Date:	11/09/2021
Action:	**Expedited Approval**
Action Date:	11/04/2021
Protocol #:	2109354139A001
Study Title:	Examining the Effects of Oral Contraceptive Use on Thermoregulation
Expiration Date:	09/07/2022
Last Approval Date:	11/04/2021

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Protocols are approved for a maximum period of one year. You may not continue any research activity beyond the expiration date without Committee approval. Please submit continuation requests early enough to allow sufficient time for review. Failure to receive approval for continuation before the expiration date will result in the automatic suspension of the approval of this protocol. Information collected following suspension is unapproved research and cannot be reported or published as research data. If you do not wish continued approval, please notify the Committee of the study closure.

Adverse Events: Any serious or unexpected adverse event must be reported to the IRB Committee within 48 hours. All other adverse events should be reported within 10 working days.

Amendments: If you wish to change any aspect of this study, such as the procedures, the consent forms, study personnel, or number of participants, please submit an amendment to the IRB. All changes must be approved by the IRB Committee before they can be initiated.

You must maintain a research file for at least 3 years after completion of the study. This file should include all correspondence with the IRB Committee, original signed consent forms, and study data.

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