CARDIOVASCULAR DRIFT AND MAXIMAL OXYGEN UPTAKE DURING HEAT STRESS IN WOMEN

by

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ABSTRACT

During prolonged constant-rate exercise, heart rate and stroke volume progressively increase and decrease, respectively, characterizing cardiovascular (CV) drift. CV drift is greater when driven by hyperthermia and generally results in proportional decreases in maximal oxygen uptake ($\dot{V}O_{2max}$). Less is known about CV drift and decrements in $\dot{V}O_{2max}$ in women because nearly all studies on this topic focused on men. This dissertation determined the effects of hormonal status, fitness level, and sudomotor function on CV drift and $\dot{V}O_{2max}$ in women. In 3 separate studies, CV drift was measured during 45 min of cycling in 35 °C, immediately followed by measurement of $\dot{V}O_{2max}$. $\dot{V}O_{2max}$ also was measured after 15 min in a separate trial to assess changes in $\dot{V}O_{2max}$ over the same time interval that CV drift occurred. Study 1 compared follicular (FP) and luteal phases (LP) of the menstrual cycle during exercise at 60% $\dot{V}O_{2max}$. Resting and exercise core temperatures (T_{re}) were higher in LP, but increases during exercise (ΔT_{re}) were similar to FP, so the CV drift/$\dot{V}O_{2max}$ relationship was not modulated by phase. Study 2 compared high-fit (HI) and low-fit (LO) women during exercise at 60% $\dot{V}O_{2max}$ (REL) and 500 W of metabolic heat production (FIXED). During REL, heat production and ΔT_{re} were significantly greater in HI versus LO, as were magnitudes of CV drift and decrements in $\dot{V}O_{2max}$. During FIXED, heat production, ΔT_{re}, CV drift, and $\dot{V}O_{2max}$ were similar between groups. Study 3 compared women to men during exercise at 500 W of metabolic heat production. For women, sweating plateaued and accelerated ΔT_{re} compared to men, but differences in CV drift and $\dot{V}O_{2max}$ were not statistically discernible between sexes. In conclusion, the relationship between CV drift and $\dot{V}O_{2max}$ during heat stress does not change

across the menstrual cycle and is not affected by fitness level, independent of metabolic heat production. The relationship is similar between men and women during 45 min of exercise at the same, relatively high load.

DEDICATION

Dedicated to Mom. Thank you for everything you did to help get me here.

LIST OF ABBREVIATIONS AND SYMBOLS

Δ	difference, change
$\dot{}$	rate
15FIXED	15 min of exercise at a fixed metabolic heat production of 500 W (CHAPTER 3)
15FP	15 min of exercise in the follicular phase of the menstrual cycle (CHAPTER 2)
15LP	15 min of exercise in the luteal phase of the menstrual cycle (CHAPTER 2)
15MIN	15 min of exercise at a fixed rate of metabolic heat production of 500 W (CHAPTER 4)
15REL	15 min of exercise at a relative intensity of 60% (CHAPTER 3)
45FIXED	45 min of exercise at a fixed metabolic heat production of 500 W (CHAPTER 3)
45FP	45 min of exercise in the follicular phase of the menstrual cycle (CHAPTER 2)
45LP	45 min of exercise in the luteal phase of the menstrual cycle (CHAPTER 2)
45MIN	45 min of exercise at a fixed rate of metabolic heat production of 500 W (CHAPTER 4)
45REL	45 min of exercise at a relative intensity of 60% (CHAPTER 3)
$(a-\bar{v})O_2$	arteriovenous oxygen difference
ANOVA	analysis of variance
BLA	blood lactate
BP	blood pressure

BSA	body surface area
CV drift/$\dot{V}O_{2max}$	relationship between cardiovascular drift and maximal oxygen uptake
C	convective heat transfer
CON	control data
CV	cardiovascular
DBP	diastolic blood pressure
DIW	deionized water
E	evaporative heat transfer
ec	caloric equivalent per liter of oxygen for oxidation of carbohydrates
ef	caloric equivalent per liter of oxygen for oxidation of fat
ELISA	enzyme-linked immunosorbent assay
FP	follicular phase
GXT	graded exercise test
Hb	hemoglobin
Hct	hematocrit
HI	high-fit (CHAPTER 3)
HR	heart rate
K	conductive heat transfer
kg	kilogram
LO	low-fit (CHAPTER 3)
LP	luteal phase
LSR	local sweat rate

$M - W$	metabolic heat production
M	metabolic rate
MAP	mean arterial pressure
OC	oral contraceptive
PP	pulse pressure
PV	plasma volume
$\dot{Q}$	cardiac output
R	radiant heat exchange
RER	respiratory exchange ratio
RH	relative humidity
RPE	rating of perceived exertion
S	storage of body heat
SBP	systolic blood pressure
SD	standard deviation
SkBF	skin blood flow
SPSS	Statistical Package for Social Sciences
S_t	fraction of systole
STPD	standard temperature and pressure, dry
SV	stroke volume
T_{arm}	arm temperature
$\overline{T}_b$	mean body temperature
T_c	core temperature
T_{chest}	chest temperature

T_{leg}	leg temperature
T_{re}	rectal temperature
$\overline{T}_{sk}$	mean skin temperature
TPR	total peripheral resistance
T_{thigh}	thigh temperature
USG	urine specific gravity
$\dot{V}CO_2$	carbon dioxide production
$\dot{V}_E$	minute ventilation
$\dot{V}O_2$	oxygen uptake
$\dot{V}O_{2max}$	maximal oxygen uptake
$\dot{V}O_{2peak}$	peak oxygen uptake
W	absolute rate of metabolic heat production
W	work rate (mechanical power)

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CHAPTER 1

INTRODUCTION

Cardiovascular (CV) drift is a well-known phenomenon that occurs over time during prolonged, constant-rate, submaximal intensity exercise (12). Heart rate (HR) increases progressively, and stroke volume (SV) decreases progressively, whereas cardiac output ($\dot{Q}$) remains relatively constant (12). The magnitude of CV drift is greater during heat stress and has been shown to negatively affect maximal oxygen uptake ($\dot{V}O_{2max}$) (3, 4, 8-13). Several factors have been shown to affect the relationship between CV drift and $\dot{V}O_{2max}$, including hyperthermia (12), hydration status (3), body cooling (10), exercise mode (13), exercise intensity (11), and ambient temperature (4). Despite these studies, full understanding of the relationship between CV drift and a decrement in $\dot{V}O_{2max}$ during heat stress remains incomplete. Furthering knowledge regarding the relationship between CV drift and a decrement in $\dot{V}O_{2max}$ during exercise in the heat is important in understanding the limitations to work capacity in the heat as well as in designing optimal training programs for endurance events held in hot temperatures. As such, further research in this area is warranted.

Recent studies have provided clarity to past disparities in the literature regarding the physiological responses of women during exercise in the heat. However, gaps in the literature remain. For example, to what extent menstrual cycle phase may influence the relationship between CV drift and decrements in $\dot{V}O_{2max}$ is unknown. Reproductive hormones fluctuate across the menstrual cycle in young women and contribute to a transient increase in core body temperature (T_c) during the luteal phase of the cycle (1, 5). Compared to the follicular phase of

the cycle, resting T_c and the onset threshold for thermoeffector responses (i.e., sweating and cutaneous vasodilation) are higher (6, 7). Because of greater thermal strain during the luteal phase of the menstrual cycle, women may experience a greater magnitude of CV drift and accompanying decrement in $\dot{V}O_{2max}$ during exercise in the heat compared to the follicular phase, but this has not been tested.

In addition to menstrual cycle, fitness level may modulate the relationship between CV drift and decrements in $\dot{V}O_{2max}$ during exercise in hot conditions. Compared to women with lower cardiorespiratory fitness, women with higher cardiorespiratory fitness would be expected to generate a higher rate of metabolic heat production, which would be expected to result in higher heat storage and greater thermal strain, and thereby greater CV strain (i.e., CV drift), at a given percentage of $\dot{V}O_{2max}$. This may result in a greater decrement in $\dot{V}O_{2max}$. In contrast, if women of varying cardiorespiratory fitness exercise at the same rate of metabolic heat production, heat storage, and thermal strain may be comparable, so CV drift and decrements in $\dot{V}O_{2max}$ may be comparable as well.

Lastly, biological sex may modulate the relationship between CV drift and decrements in $\dot{V}O_{2max}$ during exercise heat stress. For instance, women experience lower evaporative heat loss and sweat rate responses—and thereby greater thermal strain—than men during high-intensity exercise (2); however, most of these studies have been conducted in a temperate environment, so whether similar responses are observed in a hot environment remains unknown. If hyperthermia is greater in women during exercise at high requirements for heat loss, greater magnitudes of CV drift and decrements in $\dot{V}O_{2max}$ would be expected compared to men, but this has not been tested.

This dissertation is comprised of 3 studies that investigate the relationship between CV drift and $\dot{V}O_{2max}$ during heat stress in women. The overall aim was to determine if the

relationship between CV drift and $\dot{V}O_{2max}$ is affected by menstrual cycle phase, cardiorespiratory fitness level, and biological sex.

Purposes and Hypotheses

Study 1. The purpose of Study 1 was to test the hypothesis that a greater magnitude of CV drift, and accompanying decrement in $\dot{V}O_{2max}$, occurs during exercise performed in the luteal phase of the menstrual cycle compared to the follicular phase.

Study 2. The purpose of Study 2 was to test the hypothesis that CV drift and associated decrements in $\dot{V}O_{2max}$ would be greater in high-fit individuals compared to low-fit individuals during exercise prescribed at a relative metabolic intensity, but that magnitudes of CV drift and decreases in $\dot{V}O_{2max}$ would be similar between groups during exercise at the same rate of metabolic heat production.

Study 3. The purpose of Study 3 was to test the hypothesis that women would experience a greater magnitude of CV drift and accompanying decrement in $\dot{V}O_{2max}$ than men during exercise performed at a rate of metabolic heat production known to result in sudomotor sex differences.

Significance of the Dissertation

Women are underrepresented in research studies involving thermoregulatory and cardiovascular responses to exercise during heat stress. This is particularly evident for studies investigating the relationship between CV drift and $\dot{V}O_{2max}$, where the majority have used male participants. Although literature concerning exercise and the menstrual cycle is well developed, Study 1 expands on previous findings because it focuses on thermoregulatory and cardiovascular responses specific to the CV drift/$\dot{V}O_{2max}$ relationship. Study 2 contributes by furthering our understanding of the influence of relative and absolute metabolic intensity during exercise on the

CV drift/$\dot{V}O_{2max}$ relationship, and the importance of controlling for heat production between independent groups, particularly for research involving cardiovascular and temperature measures. Study 3 is unique because it is the first to investigate the effect of sex differences on the relationship between CV drift and $\dot{V}O_{2max}$ during heat stress. Collectively, these studies offer new insights into the extent to which cardiovascular and thermoregulatory responses to exercise as well as work capacity in hot conditions can be modulated by high levels of hyperthermia. Furthermore, these findings help distinguish whether modifiers of hyperthermia are related to physiological variables, e.g., menstrual cycle phase and lower sudomotor activity, or related to external influences like exercise intensity and rate of metabolic heat production, or both.

The practical significance of this dissertation relates to individuals with occupations involving prolonged physical activity interspersed with periods of vigorous work during heat stress, such as firefighting and construction or industrial work. These workers are required to perform the same work-related tasks, regardless of fitness level, physical characteristics, or sex. Therefore, knowing more about factors that limit work capacity in the heat could help improve strategies for preventing heat-related injuries, and contribute to the development of new countermeasures, which could optimize occupational performance.

CHAPTER 2

INFLUENCE OF MENSTRUAL CYCLE PHASE ON CARDIOVASCULAR DRIFT AND MAXIMAL OXYGEN UPTAKE DURING HEAT STRESS

ABSTRACT

Cardiovascular (CV) drift is related to reduced maximal oxygen uptake ($\dot{V}O_{2max}$) during heat stress. High levels of hyperthermia typically result in the most substantial magnitude of CV drift and accompanying decrement in $\dot{V}O_{2max}$. Elevated core temperature during the luteal phase of the menstrual cycle may lead to increased hyperthermia during exercise, and thus, modulate the relationship between CV drift and $\dot{V}O_{2max}$. To test the hypothesis that CV drift and the accompanying decrement in $\dot{V}O_{2max}$ would be greater in the luteal phase (LP) versus the follicular phase (FP), CV drift and $\dot{V}O_{2max}$ were assessed in 7 women (mean ± SD; age = 24 ± 5 y) during each phase of the menstrual cycle. Participants performed a control graded exercise test (GXT) in 22 °C to determine $\dot{V}O_{2max}$ and then one 15-min and one 45-min trial in both FP and LP. Each of the 4 experimental trials were performed at 60% $\dot{V}O_{2max}$ in 35 °C and immediately followed by a GXT to measure $\dot{V}O_{2max}$. CV drift was measured between 15 and 45 min during the 45-min trials. Rectal temperature (T_{re}) at rest and during exercise was higher in the LP ($P < 0.05$), but the change in T_{re} (ΔT_{re}) from rest was similar to FP during exercise (all time points $P > 0.05$). The increase in heart rate between 15 and 45 min (9%, $P < 0.001$) was not modulated by menstrual cycle phase ($P = 0.78$). Stroke volume decreased proportionately more in the LP (18%) compared to the FP (11%; $P = 0.02$), but absolute values at 45 min (59 ± 6 mL vs. 60 ± 8 mL for LP and FP, respectively) were not different ($P = 0.57$). $\dot{V}O_{2max}$ decreased over time (P =

0.002), but menstrual cycle phases were not different (16% and 13% for LP and FP, respectively, P = 0.33). Comparable levels of thermal and cardiovascular strain between phases of the menstrual cycle resulted in comparable decrements in $\dot{V}O_{2max}$ during exercise in the heat.

INTRODUCTION

Cardiovascular drift (CV drift), the progressive increase in heart rate (HR) and decrease in stroke volume (SV) over time during prolonged, constant-rate, moderate-intensity exercise, is related to reduced maximal oxygen uptake ($\dot{V}O_{2max}$) (11, 21, 47-52). The reduction in $\dot{V}O_{2max}$ associated with CV drift is 1) proportional to the magnitude of increase in HR and decrease in SV and 2) greater during heat stress (48). Furthermore, for a given level of CV drift, that resulting from thermal strain results in greater decrements in $\dot{V}O_{2max}$ than that resulting from dehydration (11, 48, 51). As such, conditions resulting in higher levels of hyperthermia and concomitant CV drift may result in greater reductions in $\dot{V}O_{2max}$ than conditions resulting in lower levels of hyperthermia.

For women, greater levels of hyperthermia would be expected to occur during the luteal phase of the menstrual cycle. In most women, resting core body temperature (T_c) is approximately 0.5 °C higher during the luteal phase (days 16–28) than during the follicular phase (days 1–15) (6, 13, 17, 30, 37), and the T_c threshold for thermoregulatory effector responses (i.e., sweating and cutaneous vasodilation) is shifted to a higher level (9, 40). As such, phase-related differences in T_c during exercise have been reported. Kolka & Stephenson (19) found that higher resting T_c in the luteal phase of the menstrual cycle vs. the follicular phase was sustained during passive (i.e., 3 h duration) and active (i.e., ~ 9 min of cycling at 80% $\dot{V}O_{2peak}$) uncompensable heat stress (50 °C, protocol I). T_c differences were also maintained during 35 min of cycling at 85% $\dot{V}O_{2peak}$ in a compensable environment [35 °C; protocol II (18)]. Similar results for T_c

responses during prolonged cycled have been observed in a temperate environment [24 °C (42)]. Pivarnik et al. (30) also reported a greater exercise core temperature during 60 min of constant-rate submaximal cycling in a temperate environment [22 °C; 60% relative humidity (RH)] during the luteal phase compared to the follicular phase, which was accompanied by higher heart rates (HR) (~ 10 beats·min^{-1}).

Consistent with Pivarnik et al. (30), other studies have shown higher HRs during the luteal phase than the follicular phase when exercise was performed in a temperate environment (13, 41), as well as a hot/humid environment (15). However, none of these studies measured SV, so an analysis of CV drift was not possible. Furthermore, only 1 study investigating effects of CV drift on $\dot{V}O_{2max}$ has tested women (52), but it did not involve heat stress, and it did not assess effects of the menstrual cycle. If SV responses follow a similar pattern as HR responses during the luteal phase, when core temperature is also higher, then a greater magnitude of CV drift—accompanied by a greater decrement in $\dot{V}O_{2max}$—would be expected in the luteal phase compared to the follicular phase. Therefore, the purpose of this study was to test the hypothesis that a greater magnitude of CV drift, and accompanying decrement in $\dot{V}O_{2max}$, would occur during exercise performed in the luteal phase of the menstrual cycle compared to the follicular phase.

METHODS

Research Design

Participants completed a total of 5 exercise trials performed on a cycle ergometer. The first trial (22 °C) was always a control trial (CON) consisting of a graded exercise test (GXT) to determine $\dot{V}O_{2max}$. Experimental trials (35 °C, ~ 40% RH, no fan airflow) included a 15- and 45-min trial during the follicular phase of the menstrual cycle and a 15- and 45-min trial in the luteal

phase of the menstrual cycle (4 experimental trials in total; 2 trials for both phases of the menstrual cycle). The 4 experimental trials were performed at a moderate intensity (60% $\dot{V}O_{2max}$), and they were each immediately followed by a GXT to measure $\dot{V}O_{2max}$. Counterbalanced treatment orders were randomly assigned for the 2 experimental trials within both phases, i.e., the 15- and 45-min trials were randomized in the follicular phase, and the 15- and 45-min trials were randomized in the luteal phase. The purpose of the separate 15- and 45-min trials was to measure CV drift and $\dot{V}O_{2max}$ during the same time interval that CV drift occurred. Trial 2 was scheduled by convenience according to self-reported menstrual cycles, followed 1–2 days later by Trial 3 in the same phase. Menstrual cycles were then track so that Trial 4 could be scheduled to occur when participants were in the later phase of their menstrual, and Trial 5 was administered 1–2 days later (just like Trial 3 in the previous phase).

Participants

Seven healthy women from the university community volunteered to participate in this study. A power analysis revealed 7 subjects were necessary to detect a moderate effect of menstrual cycle phase on CV drift, assuming power ~ 0.8 and the correlation among the repeated measurements of CV drift variables is 0.9 (29). Participants were recreationally trained in either cycling (~ 44 km·wk^{-1}), running (~ 46 km·wk^{-1}), or both, or some other type of aerobic exercise/physical activity during the 3 months before testing as confirmed by a physical activity history questionnaire. Participants were required to be asymptomatic and eumenorrheic, i.e., an average of 28 days for their menstrual cycle (24); without any known cardiovascular, metabolic, or renal disease; and able to engage in vigorous physical activity in the heat as determined by a medical history questionnaire. Physical characteristics of the participants (mean ± SD) were age

= 24 ± 5 y, mass = 58.3 ± 9.3 kg, height = 164.7 ± 5.8 cm, body mass index (BMI) = 21.5 ± 3.1 $kg \cdot m^{-2}$, and percent body fat = 23.9 ± 5.7%.

Three women were taking oral contraceptives (OC) for contraceptive purposes only. These participants were on a monophasic contraceptive regimen, with 21 days of consumption of a combined dosage of ethinyl estradiol and a synthetic progestin followed by a 7-day withdrawal (placebo) phase. One woman used Sprintec (0.035 mg ethinyl estradiol/0.25 mg norgestimate), another used Previfem (0.035 mg ethinyl estradiol/0.25 mg norgestimate), and the last used Apri 28 (0.030 mg ethinyl estradiol/0.15 mg desogestrel). One woman had a copper (non-hormonal) intrauterine device, and the remaining 4 subjects had not used OC in the past year. The study was approved by the university's institutional review board (IRB protocol # 16-009-ME-R3), and written informed consent was obtained before testing.

Procedures

Control $\dot{V}O_{2max}$ Trial

The purpose of the first visit was to measure maximal oxygen uptake and to familiarize participants with the indirect Fick CO_2-rebreathing technique to noninvasively determine cardiac output ($\dot{Q}$). Participants reported to the laboratory after a 2-h fast, well hydrated [urine specific gravity (USG) ≤ 1.020] and well rested. They were instructed to abstain from alcohol, caffeine, strenuous exercise, and all medications (excluding OC use) the day before and the day of testing. Adherence to pre-test procedures was confirmed via a 24-h history questionnaire. Height and body mass were measured while wearing shorts, socks, sports bra, and a loose-fitting tank top. Next, body fat percentage was estimated using the sum of 3 skinfolds (14) and then resting blood pressure (BP) was measured in the seated position (1).

Maximal oxygen uptake was measured during a graded exercise test (GXT) performed on a cycle ergometer in an environmental chamber maintained at 22 °C, 40% RH. Participants cycled at an initial stage of 100 W, with the intention of inducing exhaustion within 8–12 min. Every 2 minutes, power output was increased by 25 *W* until participants could no longer maintain the workload at a cadence > 40 revolutions·min^{-1}. Heart rate (HR) was recorded continuously, and rating of perceived exertion (RPE) was obtained during the last 10 s of each 2-min stage. Three minutes post-test, a 2-mL venous blood sample was drawn for the measurement of blood lactate.

To ensure a plateau in $\dot{V}O_2$ occurred, participants were asked to perform a follow-up test after a 20-min rest period. The participants cycled until exhaustion at a workload equivalent to the last workload performed during the initial GXT (if < 1 min was completed during the last stage of the GXT) or at a workload 25 *W* greater than the last workload achieved in the initial GXT (if ≥ 1 min was completed during the last stage of the GXT). To determine a plateau in $\dot{V}O_2$, the expected increase in $\dot{V}O_2$ per watt was calculated using the slope of the relationship between power output and $\dot{V}O_2$ from the initial GXT (31). A plateau in $\dot{V}O_2$ was established for all 7 participants.

After another 20-min rest period, participants cycled for an additional 10–15 min while the workload corresponding to 60% $\dot{V}O_{2max}$ was determined, and they were familiarized with the indirect Fick CO_2-rebreathing technique to determine $\dot{Q}$. After stopping exercise, participants had their BP measured again. Before departing the laboratory, they were instructed how to document the start of a menstrual cycle. This information was used to track the menstrual cycle to schedule testing within follicular and luteal phases.

Experimental Trials

Four experimental trials were completed on separate days in which participants cycled for either 15 or 45 min at 60% $\dot{V}O_{2max}$. Two trials were completed during the follicular phase (15FP; 45FP) and 2 during the luteal phase (15LP; 45LP), each followed immediately by the measurement of $\dot{V}O_{2max}$ and a 3-min posttest venipuncture for assessment of blood lactate. During the 45-min trials, participants cycled at 60% $\dot{V}O_{2max}$ for 45 min with CV drift measured between 15 and 45 min. The purpose of the separate 15-min trials was to measure $\dot{V}O_{2max}$ during the same time interval that CV drift occurred. Experimental trials were counterbalanced within cycle phases, performed in the late afternoon to minimize effects of circadian rhythm on HR and core temperature, and were separated by at least 24 h but no more than 3 days within a cycle phase. Women with normal (ovulatory) menstrual cycles completed experimental trials during the early follicular phase (days 2–5 after menstruation had begun) when progesterone concentrations are relatively low, and mid-luteal phase (days 19–22) when progesterone levels are elevated (43). To schedule experimental trials in the proper phase, these women were asked to report the first and last day of menses in their previous menstrual cycle. Women using OC followed a 28-d regimen and were tested during the placebo pill week (days 2–5) for FP trials and third week of hormone pills (days 19–22) for LP trials because these days most closely reflect hormone levels during the ovulatory menstrual cycle (7).

For each of the experimental trials, participants reported to the laboratory after following the same pre-test procedures as CON, confirmed via 24-h history questionnaire. They were provided a clothing ensemble consisting of spandex cycling shorts (25.4-cm inseam) and a mesh polyester tank top (JiffyShirts.com). Nude body mass and BP were measured, and then

participants provided a urine sample to assess hydration status and inserted a rectal temperature probe 10 cm past the anal sphincter.

For the 45-min trials, a flexible venous catheter was inserted into an antecubital vein, and a 2-mL venous blood sample was collected. After the catheter was secured for exercise, participants then entered an environmental chamber maintained at 35 °C and 40% RH and remained seated in an upright position for 30 min while instrumentation was applied. A laser-Doppler probe and sweat rate capsule were affixed to the mid- and distal-posterior surface of the forearm, respectively, and skin temperature probes were secured to the chest, deltoid, thigh, and mid-calf. After resting measures were taken, participants began cycling at 60% of their predetermined $\dot{V}O_{2max}$ for 45 min. Between 8 and 18 min and between 35 and 45 min, BP, RPE, skin blood flow (SkBF) with the arm stable for 30 s, HR, $\dot{V}O_2$, and $\dot{V}CO_2$ were measured; a blood sample was obtained; and 2–3 trials of CO_2 rebreathing to estimate $\dot{Q}$ were performed. Measurements were performed in the same order for each participant and time point. CV drift was assessed as the differences in HR and SV between 15 and 45 min.

For the 15-min trials, HR, T_{re}, RPE, and metabolic measures were taken at 15 min. Upon completion of 15 min of exercise during 15FP and 15LP and of 45 min of exercise during 45FP and 45LP, participants immediately transitioned to a GXT without stopping. Power output was increased 25 *W* above that which corresponded to 60% $\dot{V}O_{2max}$ and every 2 min thereafter until the participant reached volitional exhaustion. $\dot{V}O_2$ and related metabolic measures were recorded continuously and averaged over 1 min (27). A 3-min post-test blood sample was drawn, nude body mass was measured, and BP was measured again.

Measurements/Instrumentation

Height was measured using a stadiometer (SECA 213, Seca Ltd., Hamburg, Germany). Body mass was measured using a digital scale (Tanita BWB-800, Tanita Corp., Tokyo, Japan). All exercise took place on an electronically braked cycle ergometer (Velotron Pro, Quarq Technology, Inc., Spearfish, SD, USA). $\dot{V}O_2$ and related gas exchange measures were determined by open-circuit spirometry. Expired gas was analyzed for $\dot{V}O_2$ and $\dot{V}CO_2$ using a Parvo Medics TrueOne 2400 Metabolic Measurement System (Parvo Medics, Inc., Salt Lake City, UT, USA). Gas analyzers were calibrated using gases of known concentrations, and the flowmeter was calibrated using a 3-L syringe. RPE was assessed using the Borg 6–20 scale (3, 4).

Blood Measures

Blood samples were collected into Vacutainer tubes containing EDTA (BD Vacutainer, Becton, Dickinson and Co., Franklin Lakes, NJ, USA). Lactate was measured in duplicate using a benchtop analyzer (YSI 2300 STAT Plus, Yellow Spring Instruments, OH, USA), hemoglobin (Hb) was measured in duplicate using a HemoPoint H2 Hemoglobin Meter (EKF Diagnostics, Inc., Boerne, TX, USA) and hematocrit (Hct) was measured in triplicate using a micro-capillary reader (Model 3201, International Equipment Co., Boston, MA, USA) after samples were centrifuged (Autocrit Ultra 3 Microhematocrit Centrifuge, model 420575, Becton, Dickinson and Co., Franklin Lakes, NJ, USA). Plasma volume change (ΔPV) from baseline was estimated from measures of Hb and Hct using the Dill-Costill equation (10).

Collection, Extraction and Assay of Progesterone

Resting blood samples from the 45-min trials were kept cool in a laboratory refrigerator (4 °C) for the duration of the trials (2.5 ± 0.5 h) and then centrifuged (Model 5418, Eppendorf,

Hauppauge, NY, USA) for 5 min at 10,000 × *g*. Then, red blood cells were discarded, and plasma was stored at -80 °C until assays were performed. For each sample, 100 µL of plasma was pipetted directly into 20 mL of deionized water (DIW) in individual 18 × 150 mm borosilicate culture tubes. Samples were then slowly passed, under vacuum, through Hypersep C18 columns (3 cc, 500 mg bed weight, Thermo Fisher Scientific, Inc., Waltham, MA, USA) fitted to a 24-port manifold using Tygon 2275 formulation tubing. Columns were primed before use with 2 consecutive washes of 2 mL of methanol followed by 2 consecutive washes with 2 mL of DIW. Progesterone was eluted from the columns with 3 consecutive washes with 2 mL of methanol. Ultrapure nitrogen gas was used to evaporate the solvent in a water bath (37 °C). The remaining hormone residue was then re-suspended in 50 µL of ethanol, vortexed, and mixed with 450 µL enzyme-linked immunosorbent assay (ELISA) buffer supplied by the manufacturer (www.caymanchem.com). ELISA kits were used to quantify progesterone concentration according to the manufacturer's procedures. Samples were run in duplicate, and the kits were validated by determining parallelism of the kit standard curve with serial dilutions of hormone extract from the female participants. Briefly, 80 µL was taken from each of the 7 follicular phase samples and combined into a pool; 80 µL also were taken from each of the 7 luteal phase samples and combined into a pool. The pools were serially diluted from 1:1 to 1:128 and assayed in duplicate. Both serial dilutions were parallel to the standard curve (comparison of slopes test (53); follicular: $t_{12} = 0.12$, $P = 0.90$; luteal: $t_{12} = -0.05$, $P = 0.96$), indicating no matrix effects. The serial dilutions also identified 1:8 as the appropriate dilution for samples taken from females in the follicular phase, and 1:16 as the appropriate dilution for samples taken from females in the luteal phase. Dilutions were achieved by mixing 150 µL of 1:1 sample with 150 µL of ELISA

buffer. Pooled samples were run in duplicate at the beginning and end of the ELISA plate; the intra-assay coefficient of variation was 7.41%. All assays were performed at the host institution.

Body Temperatures

Rectal temperature (T_{re}) was measured using a flexible thermistor (MEAS 401, Measurement Specialties, Andover, MN, USA). Skin temperature was measured using thermistors (Thermistor Transducer, TSD202B, Biopac Systems, Inc., Goleta, CA, USA) integrated with wireless amplifiers (BioNomadix Wireless SKT Transmitter, Biopac Systems, Inc., Goleta, CA, USA) set to a sampling frequency of 1000 Hz. Mean skin temperature ($\overline{T}_{sk}$) was calculated according to the formula of Ramanathan (32):

$$\overline{T}_{sk} = 0.3(T_{chest} + T_{arm}) + 0.2(T_{thigh} + T_{leg}),$$

where T_{chest}, T_{arm}, T_{thigh}, and T_{leg} are the local temperatures of the chest, deltoid, thigh, and calf, respectively. Mean body temperature ($\overline{T}_b$) was calculated with the following formula (11, 12, 28):

$$\overline{T}_b = 0.9(T_{re}) + 0.1(\overline{T}_{sk}).$$

Temperature measures were recorded continuously throughout the exercise bout using a computerized data acquisition system (MP150, Biopac Systems, Inc., Goleta, CA, USA) and analyzed using data analysis software (Acq*Knowledge* 4.2, Biopac Systems, Inc., Goleta, CA, USA).

Heat Loss Responses

Local sweat rate (LSR) was measured and recorded continuously using capacitance hygrometry. This technique involved placing a small plastic capsule (3.976 cm^2) over the distal-posterior surface of the forearm with compressed nitrogen gas flowing (0.3 $L \cdot min^{-1}$) through it over the surface of the skin. The humidity of the effluent air was measured with a humidity

sensor (HMT333, Vaisala, Helsinki, FI) and used to calculate LSR. Values for LSR were calculated as 30-s averages obtained at baseline, 15 and 45 min, and post $\dot{V}O_{2max}$ test and expressed in $mg \cdot cm^{-2} \cdot min^{-1}$.

Laser-Doppler flowmetry (moorVMS-LDF2, Moor Instruments Inc., Wilmington, DE, USA) was used to measure red blood cell flux and provide an index of SkBF. Fiber optic flow probes (model VP12) fitted to probe adapters (model SHP1) were secured to the mid-posterior forearm using surgical tape.

Cardiovascular Measures

Resting BP was measured using an automated monitor (BPM-100, BpTRU Medical Devices, Coquitlam, BC, CA). HR was recorded continuously during all 5 trials using a HR monitor (RS800CX, Polar Electro, Woodbury, NY, USA). $\dot{Q}$ was measured using the indirect Fick CO_2-rebreathing technique (16) using the Parvo Medics system. This technique involves measuring $\dot{V}CO_2$, end-tidal CO_2 concentrations, and the equilibrium CO_2 concentration after rebreathing in succession (51). SV was calculated by dividing $\dot{Q}$ by HR, and mean arterial pressure [(MAP) mm Hg] was estimated using the equation proposed by Moran et al. (24):

$$MAP = DBP + S_t(PP),$$

where DBP = diastolic blood pressure, PP = pulse pressure, and S_t = the fraction of systole from the heart cycle, calculated as (25):

$$S_t = 0.01e^{[4.14-(40.74/HR)]},$$

where HR = heart rate. Total peripheral resistance [(TPR) $dyn \cdot s^{-1} \cdot cm^{-5}$] was calculated by dividing MAP by $\dot{Q}$.

Data Analysis

A one-way repeated measures analysis of variance (ANOVA) was used to test the significance of mean differences (± SD) in $\dot{V}O_{2max}$ among the control GXT and GXTs after the 4 experimental trials. For cardiovascular, temperature, hematological, and metabolic measures, a two-way repeated measures ANOVA was conducted (menstrual cycle phase × time). When appropriate, pairwise comparisons incorporating a Bonferroni correction factor were performed to determine individual differences between treatments and time points. All hypothesis tests used an α level of 0.05 and were performed using SPSS v. 23.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Plasma progesterone levels before the 45-min trials are presented in Table 2.1 for each subject. On average, concentrations in the follicular phase were lower than the luteal in women with normal ovulatory menstrual cycles (1.8 ± 0.7 $ng \cdot mL^{-1}$ and 7.3 ± 1.4 $ng \cdot mL^{-1}$ respectively, P = 0.006), while concentrations were maintained at a constant level for women taking OCs (1.9 ± 0.8 $ng \cdot nL^{-1}$ and 1.8 ± 0.9 $ng \cdot mL^{-1}$ for the follicular and luteal phase, respectively, P = 0.23). Resting blood pressure was similar between phases before the 45-min trials (SBP = 105 ± 9 mm Hg, DBP = 68 ± 13 mm Hg for FP and SBP = 104 ± 14 mm Hg, DBP = 68 ± 8 mm Hg for LP, P > 0.05 for both). As expected, baseline T_{re} was higher in the LP for both trials (T_{re}: FP15 = 37.1 ± 0.3 °C, LP15 = 37.4 ± 0.4 °C, P = 0.01; FP45 = 37.1 ± 0.3 °C, LP45 = 37.4 ± 0.3 °C, P = 0.003), but baseline T_{re} at the start of the 15- and 45-min trials within both respective phases of the menstrual cycle was not different (all P > 0.05).

Pre-exercise nude body mass (58.0 ± 9.2 kg and 58.2 ± 9.0 kg for FP15 and FP45, respectively; 58.9 ± 9.4 and 58.9 ± 9.5 kg for 15LP and 45LP, respectively) and urine specific gravity [(USG) = 1.010 ± 0.006 and 1.003 ± 0.002 for FP15 and FP45, respectively; 1.011 ±

0.005 and 1.004 ± 0.004 for 15LP and 45LP, respectively] did not differ (all P > 0.05) across phases of the menstrual cycle, suggesting women began each experimental trial in a similar state of hydration. Body mass decreased 1.0 ± 0.3 and 1.1 ± 0.4 kg from pre- to post-exercise for 45FP and 45LP, respectively (P < 0.001), but phases were not different (P = 0.37 for interaction). Percent dehydration (-1.8 ± 0.8% and -1.9 ± 0.8% for FP and LP, respectively; P = 0.51) and whole-body sweat rate (1.2 ± 0.4 $L \cdot h^{-1}$ and 1.3 ± 0.5 $L \cdot h^{-1}$ for FP and LP, respectively; P = 0.27), calculated from the change in body mass pre- to post-exercise after correcting for blood drawn and respiratory water loss during the 45-min trials, were similar between phases.

Cardiovascular and Metabolic Responses to Submaximal Exercise

Cardiovascular (CV) and gas exchange measures during 45 min of submaximal cycling are shown in Table 2.2. As intended, submaximal $\dot{V}O_2$ was not different between cycle phases (P = 0.39) and remained constant over time (P = 0.16). Because relative metabolic intensity was held constant, blood lactate was stable over time—around 2 $mmol \cdot L^{-1}$—for both phases. There was a main effect for time for HR and SV (Figure 2.1; P < 0.001 for both phases). Menstrual cycle phase did not differentially affect HR (FP = +13 $bts \cdot min^{-1}$, +9% increase between 15 and 45 min and LP = +14 $bts \cdot min^{-1}$, +9%, P = 0.78). However, the decrease in SV was ~ 60% larger in the LP (-13 $mL \cdot beat^{-1}$, -18%) compared to the FP (-8 $mL \cdot beat^{-1}$, -11%; P = 0.02), which evidently caused the drop in $\dot{Q}$ over time (Table 2.2; P = 0.003), whereas levels were maintained over time in the FP (P = 0.16). The decrease in PV from rest after the first 15 min of exercise was greater in the LP, but by 45 min the decrease was not different between phases. Lastly, participants perceived the exercise as being more strenuous over time (Table 2.2 RPE), but to the same extent regardless of menstrual cycle phase.

Thermoregulatory Responses to Submaximal Exercise

Temperature measures and thermoeffector responses during 45 min of submaximal cycling are shown in Table 2.2. Like baseline resting, T_{re} was 0.20 ± 0.16 °C greater in the LP relative to the FP after 30 min of seated rest in the heat that preceded the 45-min trials (P = 0.02), but there were no differences between baseline resting and exercise baseline T_{re} within either phase (both P > 0.05). The LP-FP difference in T_{re} observed at rest remained statistically significant throughout 45 min of exercise (15-min = +0.24 ± 0.12 °C, P = 0.002; 45-min = +0.22 ± 0.20, P = 0.03), while $\overline{T}_{sk}$ increased from baseline after 15 min (both P < 0.01) but remained relatively constant between 15 and 45 min (P = 0.42) across both phases of the menstrual cycle (P = 0.71). T_{re} increased over time during both 45FP and 45LP (P < 0.001 for main effect of time), but phases were not different (P = 0.77 for interaction) (Figure 2.2). $\overline{T}_{b}$ followed a similar pattern. Given these results, the lack of phase-related differences for sweat rate and skin blood flow responses is not surprising.

Responses to Maximal Exercise

GXTs performed after the 15-min trials in both phases of the menstrual cycle were similar in duration to the control test; thus, there were no differences in maximal power output among these trials, and participants reported similar RPE values (Table 2.3; P > 0.05 for test duration, power output, and RPE). Maximal responses during GXTs in 15FP and 15LP did not differ from responses during CON for $\dot{V}O_{2max}$, minute ventilation, HR, O_2 pulse, or blood lactate levels (all P > 0.05 Table 2.3). However, values for respiratory exchange ratio at maximum were lower in the 15-min trials compared to CON (Table 2.3; P < 0.05 for both phases) but were not affected by menstrual cycle phase (P = 0.42).

The change in $\dot{V}O_{2max}$ was essentially proportional to the magnitude of CV drift that occurred in both phases (Figure 2.1), where reductions from values at 15 min were 13% and 16% for 45FP and 45LP, respectively (main effect of time, P = 0.001). However, menstrual cycle phase did not affect the observed reduction in $\dot{V}O_{2max}$ (main effect of group, P = 0.97). Maximal workload, test duration, and O_2 pulse were lower after 45 min of submaximal cycling compared to 15 min (all $P < 0.05$ main effect of time), but again, these reductions were not different between FP and LP. The LP-FP difference in maximal T_{re} (0.25 ± 0.22 °C, P = 0.03) was comparable to the differences during submaximal exercise; however, the change in T_{re} from rest after the 45-min GXT (Figure 2.2) and thermoeffector responses were not different between phases of the menstrual cycle.

Table 2.1. *Plasma progesterone levels before the 45-min trials for follicular and luteal phases of the menstrual cycle.*

	Progesterone Concentration ($ng \cdot mL^{-1}$)	
Subject	Follicular	Luteal
1	1.7	8.4
2	1.9	5.2
3	2.7	8.1
4	1.0	7.3
5 §	2.6	2.6
6 §	2.0	1.9
7 §	1.0	0.8

N = 7 women.§ Denotes oral contraceptive use.

Table 2.2. *Responses to submaximal exercise during follicular and luteal phases of the menstrual cycle.*

	Follicular Phase		Luteal Phase	
Variable	15-min	45-min	15-min	45-min
$\dot{V}O_2$ ($L \cdot min^{-1}$)	1.4 ± 0.2	1.5 ± 0.3	1.5 ± 0.3	1.5 ± 0.2
$\dot{V}O_2$ (% control $\dot{V}O_{2max}$)	58.3 ± 4.1	59.5 ± 3.7	60.0 ± 4.4	62.5 ± 8.7
$\dot{Q}$ ($L \cdot min^{-1}$)	10.1 ± 1.2	9.7 ± 1.4	10.7 ± 1.2	9.6 ± 0.9*
SV ($mL \cdot beat^{-1}$)	68 ± 10	60 ± 8*	72 ± 8	59 ± 6*
HR ($beats \cdot min^{-1}$) **	149 ± 13	162 ± 16	150 ± 11	164 ± 9
O_2 pulse ($mL \cdot beat^{-1}$)	9.7 ± 1.6	9.1 ± 1.5*	9.9 ± 1.5	9.1 ± 1.4*
BLA ($mmol \cdot L^{-1}$)	2.1 ± 0.9	2.1 ± 0.7	2.2 ± 0.9	1.9 ± 0.7
MAP (mm Hg)	92 ± 13	95 ± 14	97 ± 10	97 ± 12
TPR ($dyn \cdot s^{-1} \cdot cm^{-5}$) **	733.7 ± 102.7	786.3 ± 78.2	725.3 ± 43.3	817.5 ± 118.3
LSR ($mg \cdot cm^{-2} \cdot min^{-1}$) **	0.55 ± 0.14	0.75 ± 0.17	0.56 ± 0.12	0.72 ± 0.11
ΔSkBF from rest (%)	373 ± 169	638 ± 489	353 ± 142	474 ± 246
ΔPV from rest (%)	-7 ± 7	-11 ± 5	-12 ± 5	-12 ± 5
T_{re} (°C) ** ‡	37.5 ± 0.3	38.1 ± 0.5	37.7 ± 0.3	38.3 ± 0.5
$\overline{T}_{sk}$ (°C)	35.9 ± 0.5	36.1 ± 0.5	36.0 ± 0.5	36.0 ± 0.5
$\overline{T}_b$ (°C) ** ‡	37.3 ± 0.3	37.9 ± 0.4	37.5 ± 0.3	38.1 ± 0.4
RPE **	11 ± 2	13 ± 1	10 ± 2	13 ± 2

$\dot{V}O_2$, oxygen uptake; $\dot{Q}$, cardiac output; SV, stroke volume; HR, heart rate; BLA, blood lactate; MAP, mean arterial pressure; TPR, total peripheral resistance; LSR, local sweat rate; SkBF, skin blood flow; PV, plasma volume; $\overline{T}_{sk}$, mean skin temperature; $\overline{T}_b$, mean body temperature; T_{re}, rectal temperature; RPE, rating of perceived exertion. ** $P < 0.05$ main effect of time; ‡ $P < 0.05$ main effect of phase; * $P < 0.05$ versus 15-min value within the same phase; † $P < 0.05$ versus value at the same time point in the follicular phase.

TABLE 2.3. *Responses to maximal exercise in follicular and luteal phases of the menstrual cycle.*

		Follicular Phase		Luteal Phase	
Variable	Control	15-min	45-min	15-min	45-min
$\dot{V}O_2$ ($L \cdot min^{-1}$) **	2.5 ± 0.4	2.5 ± 0.5	2.2 ± 0.4§	2.5 ± 0.5	2.1 ± 0.5§
$\dot{V}O_2$ ($mL \cdot kg \cdot min^{-1}$) **	43.6 ± 9.4	43.8 ± 8.5	37.8 ± 6.9§	44.0 ± 10.5	36.9 ± 8.6§
$\dot{V}_E$, (STPD, $L \cdot min^{-1}$)	79.1 ± 9.4	76.5 ± 17.5	75.0 ± 12.5	78.7 ± 9.2	73.2 ± 15.4
RER	1.12 ± 0.6	0.98 ± 0.04§	0.95 ± 0.09§	0.98 ± 0.03§	0.96 ± 0.07§
HR ($beats \cdot min^{-1}$)	187 ± 9	187 ± 8	188 ± 9	189 ± 8	186 ± 8
O_2 pulse ($mL \cdot beat^{-1}$) **	13.4 ± 2.4	13.5 ± 2.5	11.7 ± 0.5	13.5 ± 2.6	11.5 ± 2.4§
BLA ($mmol \cdot L^{-1}$) **	9.0 ± 3.1	9.0 ± 0.6	7.2 ± 1.4	8.2 ± 1.5	7.0 ± 1.7
LSR ($mg \cdot cm^{-2} \cdot min^{-1}$)	—	—	0.77 ± 0.17	—	0.75 ± 0.12
ΔSkBF from rest (%)	—	—	766 ± 688	—	540 ± 273
ΔPV from rest (%)	—	—	-16 ± 6	—	-15 ± 6
T_{re} (°C) **‡	—	37.8 ± 0.5	38.4 ± 0.5	38.1 ± 0.4	38.6 ± 0.5
$\overline{T}_{sk}$ (°C)	—	—	36.0 ± 0.6	—	35.8 ± 1.0
$\overline{T}_b$ (°C)	—	—	38.1 ± 0.5	—	38.3 ± 0.5†
RPE	19 ± 1	19 ± 2	19 ± 1	19 ± 1	19 ± 1
Test duration (min) **	12.2 ± 3.6	9.0 ± 1.6	6.8 ± 1.0§	10.1 ± 2.1	6.8 ± 2.4§
Power output (*W*) **	219 ± 38	216 ± 51	186 ± 40§	224 ± 49	191 ± 50
Δ Body Mass (%) **	—	-0.9 ± 0.4	-1.8 ± 0.8	-0.9 ± 0.4	-1.9 ± 0.8

$\dot{V}O_2$, oxygen uptake; $\dot{V}_E$, minute ventilation; RER, respiratory exchange ratio; HR, heart rate; BLA, blood lactate; LSR, local sweat rate; SkBF, skin blood flow; PV, plasma volume; $\overline{T}_{sk}$, mean skin temperature; $\overline{T}_b$, mean body temperature; T_{re}, rectal temperature; RPE, rating of perceived exertion. § $P < 0.05$ versus control; for experimental trials ** $P < 0.05$ main effect of time, ‡ $P < 0.05$ main effect of phase, † $P < 0.05$ versus value at the same time point in the follicular phase.

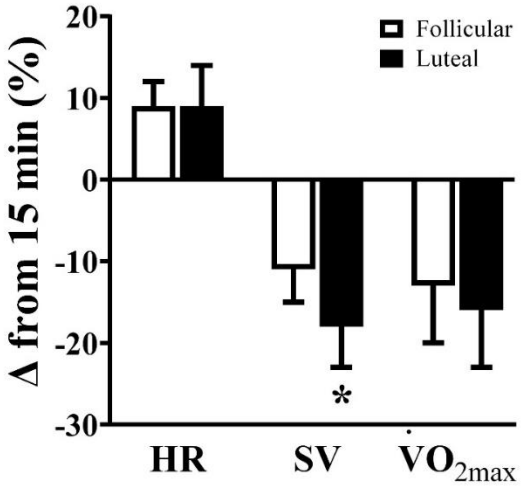

Figure 2.1 Changes in mean (± SD) heart rate (HR), stroke volume (SV), and maximal oxygen uptake ($\dot{V}O_{2max}$) between 15 and 45 min of submaximal cycling (60% $\dot{V}O_{2max}$) in the follicular and luteal phases of the menstrual cycle. * Significantly different from follicular phase ($P < 0.05$).

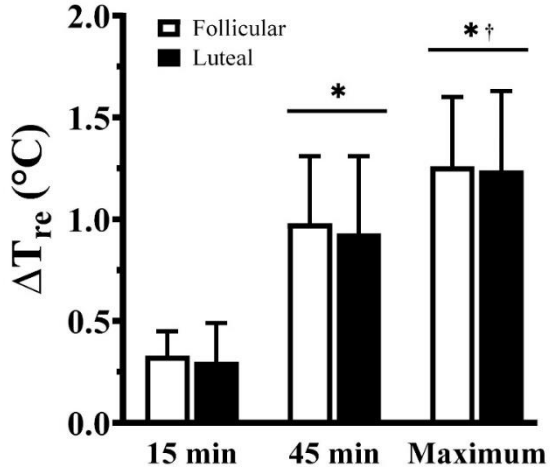

Figure 2.2 Mean (± SD) change in rectal temperature (ΔT_{re}) from baseline at 15 min, 45 min, and after maximal exercise after 45 min of submaximal cycling (60% $\dot{V}O_{2max}$) in the follicular and luteal phases of the menstrual cycle. No significant between-phase differences were observed ($P > 0.05$). * Main effect of time; significantly different from 15-min value ($P < 0.05$). † Main effect of time; significantly different from 45-min value ($P < 0.05$).

DISCUSSION

The purpose of this study was to determine whether a greater magnitude of CV drift and accompanying decrement in $\dot{V}O_{2max}$ would occur in the luteal phase of the menstrual cycle—when resting core temperature is elevated—compared to the follicular phase. The main finding was that, despite higher core temperature at rest and during 45 min of submaximal cycling in the heat, coupled with an ~ 60% greater decline in SV during the LP (but no difference in absolute SV in mL), menstrual cycle phase did not affect the relationship between CV drift and $\dot{V}O_{2max}$. HR increased by a similar magnitude, and $\dot{V}O_{2max}$ decreased by a similar magnitude in both phases. The magnitude of CV drift from 15 to 45 min was essentially proportional to the decrement in $\dot{V}O_{2max}$, which further supports the notion that reductions in work capacity after prolonged, constant-rate, moderate-intensity exercise in the heat are at least partially related to CV drift.

Our findings confirm an elevation in resting core temperature (T_c) in the LP of the menstrual cycle (8, 13, 18, 30, 40) that was maintained during heat stress (19, 20, 30, 43). Furthermore, the core temperature increases we observed are similar to those of Notley et al. (28) in which women cycled at 70% $\dot{V}O_{2peak}$ in a hot environment (40 °C, 15% RH) for 30 min. Women were tested in the early- and late-follicular and mid-luteal phases of the menstrual cycle. The change from baseline T_c (early-follicular ~ 1.5 °C, late-follicular ~ 1.4 °C; mid-luteal ~ 1.3 °C) at the end of the exercise bout was comparable to ΔT_{re} from rest after maximal exercise in the present study both for 45FP (+1.3 °C) and 45LP (+1.2 °C). Our results and those of Notley et al. (28) for T_c are similar to findings in previous studies conducted in hot ambient conditions (15, 36, 43), but differ from Pivarnik et al. (30) where exercise was conducted in a temperate environment (22 °C, 60% RH). Women in this study cycled at 65% $\dot{V}O_{2peak}$ for 60 min in mid-

follicular and mid-luteal phases of the menstrual cycle. T_{re} plateaued after ~ 30 min of exercise in the follicular phase but continued to increase during exercise in the luteal phase, suggesting a lower thermal sensitivity of sweating, skin blood flow, or both in the luteal phase. However, thermal sensitivities were not measured by Pivarnik et al. (30) and other studies conducted in a temperate environment have reported no between-phase differences for ΔT_{re} (15). Therefore, whether the environment modulates temperature effects related to the menstrual cycle remains inconclusive.

Importantly, the observed statistical differences in T_{re} may not be indicative of physiologically meaningful differences in body heat content during 45 min of exercise in the heat. Similar levels of body heat storage may explain why the women in this study experienced a similar magnitude of CV strain (similar absolute HR and SV responses at 45 min) across menstrual cycle phases, because differences in CV strain would not be expected without comparable differences in thermal strain (17, 39, 40, 45).

The results of this study are comparable to previous studies investigating the relationship between CV drift and $\dot{V}O_{2max}$ during cycling exercise when relative intensity, duration, and environmental conditions were similar (21, 49-51). For instance, based on studies included in a review by Wingo (48), the magnitude of CV drift may be on the order of a 10–15% increase and decrease in HR and SV, respectively, coupled with proportional decreases in $\dot{V}O_{2max}$, under conditions like those used in the current study.

While comparable to other studies in this topic area, the current study extends prior findings by adding 2 unique aspects. The first unique contribution to this body of literature is that this study is the first to investigate the effect of menstrual cycle on the relationship between CV drift and $\dot{V}O_{2max}$. Wingo et al. (52) previously investigated this relationship in women, but

menstrual cycle phase was not controlled, and participants exercised in a temperate environment. The second unique contribution of this study is that, of studies investigating the CV drift/$\dot{V}O_{2max}$ relationship [studies detailed in Wingo review (48)], it is the first to show a larger decrease in SV (expressed as % change) in one condition versus another, but with an ensuing similar SV, expressed as $mL \cdot beat^{-1}$ (Table 2.2).

While not statistically significant, the higher absolute SV at 15 min in 45LP may have been physiologically meaningful, but the underlying mechanisms are unclear given similar power output, HR, and $\dot{V}O_2$ between phases. Estrogen was not measured in this study, but presumably higher levels in the blood during the luteal phase may have played a role. Estrogen affects fluid regulation (35) and increases resting plasma volume (41) and blood volume during the luteal phase when levels are high (26, 38). Blood volume expansion results in increased SV and $\dot{Q}$ at rest and during exercise. Although changes in plasma volume were similar between phases in the present study, plasma volume in the luteal phase could have started at a higher level than the follicular phase. If present in the current study, it is reasonable to expect that these estrogenic effects contributed to the initially higher absolute SV during exercise in the luteal phase compared to SV in the follicular phase when estrogen levels, and thereby plasma and whole blood volume, would have been presumably lower. For example, Stachenfeld et al. (36) reported higher SV and $\dot{Q}$ values in the luteal phase at rest and during 40 min of cycling at 60% $\dot{V}O_{2peak}$ in a hot environment (35 °C). Similar to our results, SV and $\dot{Q}$ at 15 min were ~14 $mL \cdot beat^{-1}$ and ~ 0.9 $L \cdot min^{-1}$ greater, respectively, during the luteal phase. Contrary to our results at 45 min, subsequent measurements for SV by Stachenfeld et al. (36) remained greater in the luteal phase, which is likely because these measures were taken within 10 min of the first. If SV

was measured after another 20 min of exercise in Stachenfeld et al. (36), then it may have been similar to levels obtained in the current study at 45 min.

Our findings for similar SV values at 45 min across menstrual cycle phases have implications for further characterizing the relationship between CV drift and $\dot{V}O_{2max}$. The SV resulting from CV drift appears to modulate subsequent reductions in $\dot{V}O_{2max}$ to a greater extent than does the magnitude (% change) of CV drift per se. Thus, SV levels in mL may explain why $\dot{V}O_{2max}$ decreased by a similar amount across 45-min trials in the present study. Since the max test durations and peak power outputs achieved were similar between trials (Table 2.3), the metabolic demand of the exercise would presumably be comparable; thus, we speculate that SV increased by a similar magnitude from the values at 45 min during the GXTs in 45FP and 45LP. Maximal HR was similar in both 45-min trials (Table 2.3), so similar increases in SV from those at 45 min would have resulted in comparable peak SV and peak $\dot{Q}$. Given previous findings suggesting that peak arteriovenous oxygen difference [$(a-\bar{v})O_2$] would not be expected to be different between 45-min trials under the conditions of this study (12, 34, 46), equivalent SV values during the GXT for 45FP and 45LP likely explains the equivalent $\dot{V}O_{2max}$ observed under these conditions.

Our results for $\dot{V}O_{2max}$ are like those of Janse De Jonge et al. (15). In their study, women completed 60 min of cycling exercise at 60% $\dot{V}O_{2max}$ in the heat (32 °C, 60% RH), followed by a GXT to volitional exhaustion in follicular and luteal phases of the menstrual cycle. Although exhaustion was reached in less time in the luteal phase, results for $\dot{V}O_{2max}$ were similar to the follicular phase. Importantly, the change in T_c from rest to 45 min was similar between phases (~ 1.4 °C), as was the increase in HR between 15 and 45 min (FP ~ 17 beats·min^{-1}, LP ~ 16

beats·min^{-1}) (15). SV was not measured, but the trends may have been like those in present study based on results for T_c, HR, and $\dot{V}O_{2max}$.

A potential limitation of the present study is that some participants were using oral contraceptives (OC), which may have impacted results. However, chronic use of synthetic hormones does not modulate the endogenous thermoregulatory rhythm of the menstrual cycle (8, 22, 43). Moreover, adjustments in exercise T_c and the onset of thermoeffector responses during the "high hormone" or "quasi-luteal" phase of combination OC use are comparable to the luteal phase of the normal (ovulatory) menstrual cycle (8, 17, 33, 36) with little variability among different types of combination OCs (2, 8). While studies comparing endogenous progesterone and synthetic progestogens have been essential to addressing variation in hormonal effects on systemic physiological function and the advancement and comprehensive understanding of female physiology, there is little evidence to support meaningful differences with respect to exercise performance and work capacity (5, 22, 23, 43, 44). Furthermore, a separate analysis of change in HR, SV, $\dot{V}O_{2max}$, and T_{re} during the 45-min trials between OC users and non-users revealed no differences in either phase of the menstrual cycle. Thus, we do not consider the inclusion of both normally menstruating women and women taking combination OCs for the 28-d cycle to have confounded results in the present study.

CONCLUSION

In summary, the magnitude of CV drift and decrement in $\dot{V}O_{2max}$ that occur during prolonged submaximal cycling in the heat were not affected by the phase of the menstrual cycle. Higher resting and exercise T_{re} at the levels observed in this study in the luteal phase may not lead to greater CV strain compared to the follicular phase if body heat storage is similar. Future research should determine if a bout of exercise longer than 45 min in the heat would induce a

higher level of thermal strain in the luteal phase and elevate CV strain/CV drift during exercise in the heat, thereby resulting in greater decrements in $\dot{V}O_{2max}$. Given the results of this study, controlling for menstrual cycle phase is likely unnecessary for studies investigating work capacity during exercise in the heat, at least for a duration like that used in the present study. Lastly, these data add further support to the notion that a given magnitude of CV drift (combination of increased HR and decreased SV) is accompanied by a proportional reduction in $\dot{V}O_{2max}$ during prolonged exercise in the heat.

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CHAPTER 3

CARDIOVASCULAR DRIFT AND MAXIMAL OXYGEN UPTAKE DURING HEAT STRESS IN WOMEN WITH DIFFERENT FITNESS LEVELS

ABSTRACT

Previous studies have demonstrated that cardiovascular (CV) drift is directionally and proportionally related to reductions in maximal oxygen uptake ($\dot{V}O_{2max}$) after a bout of constant-rate, moderate intensity, aerobic exercise. Under conditions that cause hyperthermia, responses that characterize CV drift (i.e., progressive increases in HR and decreases in SV manifesting after approximately 10 min), are markedly greater compared to lower heat stress situations, and $\dot{V}O_{2max}$ is reduced more in consequence. At a given relative metabolic intensity (%$\dot{V}O_{2max}$), individuals with high cardiorespiratory fitness likely experience greater CV drift and decrements in $\dot{V}O_{2max}$ because of greater metabolic heat production resulting from exercising at a higher absolute intensity compared to individuals with lower cardiorespiratory fitness. It follows, then, that the CV drift/$\dot{V}O_{2max}$ relationship would be similar between high- and low-fit individuals exercising at the same rate of metabolic heat production, but this has not been empirically tested. The purpose of this study was to test the hypothesis that CV drift and associated decrements in $\dot{V}O_{2max}$ would be greater in high-fit individuals compared to low-fit individuals during exercise at the same %$\dot{V}O_{2max}$ but they would be comparable during exercise at the same rate of metabolic heat production. To test these hypotheses, 6 high-fit women [(HI); mean ± SD $\dot{V}O_{2max}$ = 49.4 ± 4.7 $mL \cdot kg^{-1} \cdot min^{-1}$] and 6 low-fit women [(LO) $\dot{V}O_{2max}$ = 34.7 ± 3.3 $mL \cdot kg^{-1} \cdot min^{-1}$] matched for body size (63.8 ± 4.7 kg and 65.2 ± 8.2 kg for HI and LO respectively, P = 0.73)

cycled for 45 min on 2 occasions in 35 °C at either 60% $\dot{V}O_{2max}$ (45REL) or 500 W of metabolic heat production (45FIXED). CV drift was measured between 15 and 45 min, and $\dot{V}O_{2max}$ was measured immediately at the end of exercise. On separate days, $\dot{V}O_{2max}$ was measured after 15 min of cycling at both intensities (15REL and 15FIXED) so that changes in $\dot{V}O_{2max}$ could be assessed in the same 15 to 45-min time interval. For REL trials, HI exercised at a higher rate of heat production than LO (496 ± 51 W and 364 ± 44 W, respectively, P = 0.001), which resulted in greater end-exercise rectal temperatures (T_{re}) (38.7 ± 0.4 °C and 38.2 ± 0.1 °C for HI and LO, respectively, P < 0.001). Accordingly, HR in HI increased more than LO between 15 and 45 min (10% and 6% for HI and LO, respectively; P = 0.03), and decreases in SV were greater (16% and 8% for HI and LO, respectively; P = 0.001). Thus, reductions in $\dot{V}O_{2max}$ for HI (16%) were also greater than LO (5%; P = 0.02). Despite LO exercising at a higher relative intensity than HI during FIXED trials (P = 0.001), T_{re} responses were similar and there were no differences between groups for CV drift (P > 0.05 for changes in HR and SV) or decreases in $\dot{V}O_{2max}$ (P = 0.52). Based on these results, we conclude fitness level does not modulate the CV drift/$\dot{V}O_{2max}$ relationship, independent of metabolic heat production. These results support previous findings showing the magnitude of CV drift is proportional to subsequent reductions in $\dot{V}O_{2max}$.

INTRODUCTION

Fitness level has been shown to influence core temperature (T_c) (37), sweating (22) and cutaneous blood flow (14) during prolonged, submaximal exercise. Exercise physiologists in the past have prescribed exercise using a fixed percentage of maximal oxygen uptake ($\dot{V}O_{2max}$) to control for fitness level, with the expectation that participants would be exercising at the same relative intensity, and thus, experience the same relative stimulus. In doing so, unbiased physiological comparisons between independent experimental groups could be made. However,

at a given percentage of $\dot{V}O_{2max}$, individuals of higher fitness levels exercise at a higher absolute metabolic intensity which results in a greater rate of metabolic heat production [denoted as (M – W) in the heat balance equation: $S = (M - W) - (R + C + K + E)$, where S = rate of heat storage; M = metabolic energy equivalent; W = external workload; R, C, and K = rates of heat loss/gain via radiation, convection, and conduction, respectively; and E = rate of evaporative heat loss (34). Unless the higher rate of metabolic heat production is matched with greater increases in heat loss (i.e., increased sweating and cutaneous vasodilation), then increased heat storage—and accompanying larger increases in core body temperature—will ensue.

Jay et al. (30) compared body temperature and heat loss responses in individuals with largely different peak oxygen uptake ($\dot{V}O_{2peak}$) values (~ 60 vs. ~ 40 $mL \cdot kg^{-1} \cdot min^{-1}$) during 60 min of cycling at 60% $\dot{V}O_{2peak}$ in a temperate environment [~ 25 °C, 26% relative humidity (RH)]. The rate of metabolic heat production (834 vs. 600 W) and associated submaximal workloads (176 *W* vs. 115 *W*) were greater in the high-fit group, resulting in higher whole-body sweat loss and sweat rate, and a greater increase in core body temperature (1.4 vs. 0.9 °C) (30). These differences were not observed when exercise was administered using a fixed rate of metabolic heat production (540 W), despite participants in the low-fit group cycling at a greater percentage of their $\dot{V}O_{2peak}$ (~ 58 vs. 40%). These results provide evidence that metabolic heat production, rather than relative metabolic intensity per se (%$\dot{V}O_{2max}$), mediates thermoregulatory effector—and importantly, core body temperature—responses during exercise.

Considering these findings, the rate of metabolic heat production may be an important consideration regarding the assessment of cardiovascular responses during exercise since such responses are influenced by increases in T_c (7, 9). For example, heart rate (HR) rises, and stroke volume (SV) falls progressively overtime during constant-rate, moderate-intensity aerobic

exercise—a condition known as cardiovascular (CV) drift—with greater effects occurring in hot environments with resultant hyperthermia (35, 52, 56). Furthermore, the magnitude of CV drift is accompanied by proportional decrements in $\dot{V}O_{2max}$ (21, 35, 53-57). Exercise performed at a higher rate of metabolic heat production results in greater hyperthermia in uncompensable environments where the rate of heat production exceeds that of heat loss and a steady-state T_c cannot be achieved for a given activity (47). As such, aerobically fitter individuals would be expected to experience a greater magnitude of CV drift and accompanying decrement in $\dot{V}O_{2max}$ at given relative metabolic intensity and resulting level of heat production, but this has not been tested. Additionally, it remains unknown if high-fit and low-fit individuals will experience a similar magnitude of CV drift and accompanying decrement in $\dot{V}O_{2max}$ at a fixed rate of metabolic heat production. Accordingly, the primary aims of this study were to determine if fitness level modulates the magnitude of CV drift and accompanying decrement in $\dot{V}O_{2max}$ in a hot environment at 1) a given relative metabolic intensity (%$\dot{V}O_{2max}$) and 2) a fixed absolute rate of metabolic heat production (W). We hypothesized that when participants were matched for biophysical and biographical characteristics, the magnitude of CV drift and decrement in $\dot{V}O_{2max}$ in high-fit individuals would be greater than low-fit individuals at 60% $\dot{V}O_{2max}$ but that groups would experience similar magnitudes of CV drift and decrements in $\dot{V}O_{2max}$ when exercise intensity was normalized to a fixed rate of metabolic heat production equal to 500 W.

METHODS

Participants

Two groups of 6 women between the ages of 18 and 40 y participated in this study. Sample sizes for high- (HI) and low-fit (LO) groups were determined using an a priori power analysis performed with G*Power 3.1.7 (13). The power analysis revealed a minimum of 6

participants per group would be sufficient to detect a moderate (27) within-between interaction effect [fitness level (HI, LO) × time (15 min, 45 min)] for $\dot{V}O_{2max}$, assuming power (1-ß) = 0.8, the correlation among repeated measures = 0.9, and $\alpha = 0.05$.

Participants provided written informed consent before each of the 5 trials, and the study was approved by the university's institutional review board prior to testing. Participants were required to be non-smoking; eumenorrheic (35); free of any signs/symptoms of or known cardiovascular, metabolic, or renal disease; not sedentary; and able to engage in vigorous physical activity in the heat, as determined by a medical history questionnaire (2). In the 3 months prior to participating in this study, women in the HI group ($\dot{V}O_{2max}$ = ~ 49 $mL \cdot kg^{-1} \cdot min^{-1}$) were running ~ 50 $km \cdot wk^{-1}$ and/or cycling ~ 58 $km \cdot wk^{-1}$, while women in the LO group ($\dot{V}O_{2max}$ ~ 35 $mL \cdot kg^{-1} \cdot min^{-1}$) were running ~ 15 $km \cdot wk^{-1}$ and did not regularly engage in cycling exercise as confirmed by a physical activity history questionnaire. To control for the effect of body size between groups and hormonal milieu throughout the menstrual cycle, high- and low-fit women were matched in pairs for body mass and performed experimental trials within days 1-10 after the onset of menses for normal (ovulatory) menstrual cycles or during the withdrawal (placebo) pill phase of oral contraceptive (OC) use (20). Five women were taking OCs for contraceptive purpose only (HI, n = 3; LO, n = 2). HI were using monophasic OCs (Previfem; Apri 28; Orsythia) that provided a 21-d hormone regimen of 20–35 mcg ethinyl estradiol and a synthetic progesterone, followed by a 7-d placebo pill regimen. For LO, 1 participant used a monophasic OC (Sprintec) similar to types used in HI, and the other used a triphasic OC (Tri-Legest Fe) that provided increasing doses of ethinyl estradiol every 7 d (Week 1, 20 mcg; Week 2, 30 mcg; Week 3, 35 mcg) and a constant dose of synthetic progesterone for 21 d, followed by 7 d of a

placebo pill. Venous blood samples obtained before the 45-min trials were analyzed for plasma progesterone concentration to verify hormonal status.

Research Design

Women in HI (n = 6) and LO (n = 6) groups completed a total of 5 exercise trials on an electronically braked ergometer. The first trial (CON) was always a graded exercise test (GXT) to determine $\dot{V}O_{2max}$ (22 °C). The remaining 4 experimental trials were performed on separate days in counterbalanced order, with the orders randomly assigned. Trials consisted of a 15-min trial (15REL) and a 45-min trial (45REL) performed at a relative percentage of $\dot{V}O_{2max}$ (60%) and a 15-min trial (15FIXED) and a 45-min trial (45FIXED) performed at a fixed rate of metabolic heat production (500 W) in a hot environment (35 °C; 40% RH). Each experimental trial was immediately followed by a GXT to measure $\dot{V}O_{2max}$. CV drift was measured between 15 and 45 min during the 45-min trials. The separate 15-min trials were necessary to capture $\dot{V}O_{2max}$ during the same time interval that CV drift occurred.

Procedures

Control $\dot{V}O_{2max}$

Prior to the first day of testing, participants were instructed to report to the laboratory after a 2-h fast; well rested and well hydrated [urine specific gravity (USG) ≤ 1.020] (8, 33); having refrained from ingesting alcohol, non-prescription drugs, and caffeine on the day of testing; and having refrained from strenuous exercise during the prior 24 h, all confirmed via 24-h history questionnaire. After completing physical activity, medical history, and informed consent forms, participants disclosed the last day of previous menses. This information was used to track the menstrual cycle and schedule experimental trials to take place in the targeted phase. Participants were given a pair of spandex cycling shorts (25.4-cm inseam) and a mesh polyester

tank top (JiffyShirts.com) to wear for each of the 5 trials. After providing a urine sample to measure USG and donning the clothing ensemble, height, and body mass were measured, and body fat percentage was estimated using the sum of 3 skinfolds for women (29). Measurements for height and body mass were used to estimate body surface area (BSA) using the equation of Dubois and Dubois (12). Resting heart rate (HR) and blood pressure (BP) were measured before exercise (1).

Maximal oxygen uptake ($\dot{V}O_{2max}$) was measured during a graded exercise test (GXT) performed on an electronically braked ergometer in a temperate environment (22 °C, 40% RH). Participants cycled at an initial stage of 100 *W*, and power output was increased 25 *W* every 2 minutes until participants could no longer maintain the workload at a cadence > 40 revolutions·min^{-1}. Heart rate, oxygen uptake ($\dot{V}O_2$), and other related gas exchange measures were recorded continuously during the GXT, and rating of perceived exertion (RPE) was obtained during the last 10 s of each 2-min stage using the Borg 6–20 scale (4, 5). Three minutes posttest, a 2-mL venous blood sample was drawn to measure blood lactate concentration using a benchtop analyzer. After a 20-min rest period, participants performed a follow-up test to ensure a plateau in $\dot{V}O_2$ occurred. The follow-up test began at a workload equivalent to the last workload performed during the initial GXT (if < 1 min was completed during the last stage of the GXT) or at a workload 25 *W* greater than the last workload achieved in the initial GXT (if ≥ 1 min was completed during the last stage of the GXT). Participants cycled at this intensity until exhaustion. The slope of the relationship between power output and $\dot{V}O_2$ from the initial GXT was used to calculate the expected increase in $\dot{V}O_2$ and determine a plateau (42). A plateau in $\dot{V}O_2$ was established for all 12 participants.

Participants were given another 20 min of rest and then asked to cycle for an additional 20–30 min while workloads corresponding to 500 W of metabolic heat production (FIXED) and then 60% $\dot{V}O_{2max}$ (REL) were determined. Procedures outlined in Cramer and Jay (10) were followed to prescribe exercise intensities that would elicit a fixed rate of metabolic heat production of 500 W. In brief, metabolic heat production (in W) was calculated from the external workload (*W*) and the rate of metabolic energy expenditure (*M*) estimated from values averaged over 1 min for $\dot{V}O_2$ and respiratory exchange ratio (RER) using the equation of Nishi (40):

$$\text{M (watts)} = \dot{V}O_2 \frac{\left(\frac{\text{RER - 0.7}}{0.3}\text{ec}\right) + \left(\frac{\text{1 - RER}}{0.3}\right)\text{ef}}{60} \times 1000,$$

where $\dot{V}O_2$ is in L/min, ec is the caloric equivalent per liter of oxygen for the oxidation of carbohydrates (21.13 kJ), and ef is the caloric equivalent per liter of oxygen for the oxidation of fat (19.62 kJ). Participants were familiarized with the indirect Fick CO_2-rebreathing technique to determine cardiac output ($\dot{Q}$) while workloads for both FIXED and REL trials were verified. Before departing, BP was measured again, and participants were instructed when to return to the laboratory.

Experimental Trials

For the 4 experimental trials, HI and LO groups cycled for 15 and 45 min at 60% $\dot{V}O_{2max}$ (15REL, 45REL) and a target rate of metabolic heat production of 500 W (15FIXED, 45FIXED), immediately followed by a GXT to measure $\dot{V}O_{2max}$. Approximately 5 weeks were required to complete the 4 experimental trials in the targeted phase of the menstrual cycle, and thus, properly control for the effect of hormone status. Sessions within an intensity category (i.e., 60% $\dot{V}O_{2max}$ vs. 500 W of metabolic heat production) were performed in the mid- to late-afternoon and separated by at least 24 h but no more than 3 days.

Participants were reminded 24 h before each trial to report to the laboratory well hydrated, well rested, and at least 2 h postprandial, confirmed via 24-h history questionnaire. Upon arrival, they reconsented to study procedures, had resting BP measured, provided a urine sample to measure USG and confirm hydration status, and had nude body weight measured. Participants then donned the required clothing ensemble, inserted a rectal temperature probe 10 cm past the anal sphincter, and were equipped with the same HR monitor used in CON.

For the 45-min trials, a flexible venous catheter was inserted into an antecubital vein, and a 2-mL venous blood sample was collected and immediately placed in a refrigerator kept at 4 °C. This sample was prepared and stored within 2.5 ± 0.5 h and assayed for progesterone concentration to confirm that participants were tested in the targeted phase of the menstrual cycle or OC use (hormone analysis procedures in following section). Another 2-mL blood sample was then taken to measure baseline concentrations of lactate and hemoglobin as well as hematocrit. After the catheter was secured for exercise, participants entered an environmental chamber maintained at 35 °C, 40% RH with no fan airflow. During instrumentation, participants sat in an upright position for 30 min while a laser-Doppler probe and sweat rate capsule were affixed to the posterior forearm and skin temperature probes to 4 different sites on the body.

After resting measures were taken, participants began cycling for 45 min at either 60% of control $\dot{V}O_{2max}$ or the predetermined workload required to elicit a rate of heat production of 500 W. Metabolic data for $\dot{V}O_2$ and RER were monitored closely throughout the 45-min trials at 5, 10, 25, and 35 min to assure participants maintained the prescribed exercise intensity. If necessary, adjustments up or down in workload were made in 10- or 15-*W*. These 4 time points were used to determine the average rate of metabolic heat production over the submaximal

exercise portion of the 45-min trial (Table 3.2). A cadence of 70–80 revolutions·min^{-1} was maintained during submaximal cycling in all experimental trials.

Systolic (SBP) and diastolic (DBP) blood pressure, RPE, skin blood flow (SkBF), $\dot{V}O_2$ and $\dot{V}CO_2$, HR, and 2–3 trials of CO_2 rebreathing to estimate $\dot{Q}$ were measured and a 2-mL blood sample was obtained, in that order, between 8 and 18 min and between 35 and 45 min. CV drift was characterized by the differences in HR and SV values between 15- and 45-min time points.

Pre-exercise procedures for the 15-min trials were identical to those for the 45-min trials. However, instrumentation was limited to HR and T_{re}, which were recorded continuously throughout exercise. RPE and metabolic data were measured at 5 and 13 min.

Upon completion of each 15-min and 45-min trial, participants immediately began a GXT without interrupting exercise. Power output was increased 25 *W* above the workloads that corresponded to 60% $\dot{V}O_{2max}$ or 500 W of metabolic heat production and then every 2 min thereafter until volitional exhaustion. $\dot{V}O_2$ and related metabolic measures were recorded continuously and averaged over 1 min (39). Three to 5 min after ending the GXT, a 2-mL blood sample was drawn, then participants were de-instrumented and exited the environmental chamber. Next, they measured nude body mass, and resting/seated BP was measured before discharge.

Measurements/Instrumentation

Height was measured using a stadiometer (SECA 213, Seca Ltd., Hamburg, Germany), and body mass was measured using a digital scale (Tanita BWB-800, Tanita Corp., Tokyo, Japan). All exercise took place on an electronically braked cycle ergometer (Velotron Pro, Quarq Technology, Inc., Spearfish, SD, USA). $\dot{V}O_2$ and related gas exchange measures were determined by open-circuit spirometry. Expired gas was analyzed for $\dot{V}O_2$ and $\dot{V}CO_2$ using a

Parvo Medics TrueOne 2400 Metabolic Measurement System (Parvo Medics, Inc., Salt Lake City, UT, USA). Standardized gas concentrations were used to calibrate the gas analyzers, and the flowmeter was calibrated using a 3-L syringe. RPE was assessed using the Borg 6–20 scale (4, 5).

Blood Measures

Blood samples were collected into Vacutainer® tubes containing EDTA (BD Vacutainer, Becton, Dickinson and Co., Franklin Lakes, NJ, USA). Lactate was measured in duplicate using a benchtop analyzer (YSI 2300 STAT Plus, Yellow Spring, OH, USA), hemoglobin (Hb) was measured in duplicate using a HemoPoint H2 Hemoglobin Meter (EKF Diagnostics, Inc., Boerne, TX, USA) and hematocrit (Hct) was measured in triplicate using a micro-capillary reader (Model 3201, International Equipment Co., Boston, MA, USA) after samples were centrifuged (Autocrit Ultra 3 Microhematocrit Centrifuge, model 420575, Becton, Dickinson and Co., Franklin Lakes, NJ, USA). Plasma volume change (ΔPV) from baseline was estimated from measures of Hb and Hct using the Dill-Costill equation (11).

Collection, Extraction and Assay of Progesterone

Resting blood samples from the 45-min trials were centrifuged (Model 5418, Eppendorf, Hauppauge, NY, USA) for 5 min at 10,000 × *g*, red blood cells were discarded, and plasma was stored at -80 °C until assays were performed. For each sample, 100 µL of plasma was pipetted directly into 20 mL of deionized water (DIW) in individual 18 × 150 mm borosilicate culture tubes. Samples then were slowly passed, under vacuum, through Hypersep C18 columns (3 cc, 500 mg bed weight, Thermo Fisher Scientific, Inc., Waltham, MA, USA) fitted to a 24-port manifold using Tygon 2275 formulation tubing. Columns were primed before use with 2 consecutive washes of 2 mL of methanol followed by 2 consecutive washes with 2 mL of DIW.

Progesterone was eluted from the columns with 3 consecutive washes with 2 mL of methanol. Ultrapure nitrogen gas was used to evaporate the solvent in a water bath (37 °C). The remaining hormone residue was then re-suspended in 50 µL of ethanol, vortexed, and mixed with 450 µL of enzyme-linked immunosorbent assay (ELISA) buffer supplied by the manufacturer (www.caymanchem.com). ELISA kits were used to quantify progesterone concentration according to the manufacturer's procedures. Samples were run in duplicate, and the kits were validated by determining parallelism of the kit standard curve with serial dilutions of hormone extract from the 7 women that participated in this study. Briefly, 80 µL was taken from each sample and combined into a pool that was serially diluted from 1:1 to 1:128 and then assayed in duplicate. The serial dilution was parallel to the standard curve [comparison of slopes test (58) t_{12} = 0.22, P = 0.83], indicating no matrix effects, and identified 1:8 as the appropriate dilution for samples. Dilutions were achieved by mixing 150 µL of 1:1 sample with 150 µL of ELISA buffer. Pooled samples were run in duplicate at the beginning and end of the ELISA plate; the intra-assay coefficient of variation was 7.41%. All assays were performed at the host institution.

Body Temperatures

Rectal temperature (T_{re}) was measured using a thermistor probe (MEAS 401, Measurement Specialties, Andover, MN, USA). Skin temperature was measured using thermistor probes (Thermistor Transducer, TSD202B, Biopac Systems, Inc., Goleta, CA, USA) integrated with wireless amplifiers (BioNomadix Wireless SKT Transmitter, Biopac Systems, Inc., Goleta, CA, USA) set to a sampling frequency of 1000 Hz. Mueller sports tape (Mueller Sports Medicine Inc., Prairie du Sac, WI, USA) was used to secure 4 probes to sites on the body

specified in the formula of Ramanathan (43) for calculating mean skin temperature ($\overline{T}_{sk}$):

$$\overline{T}_{sk} = 0.3(T_{chest} + T_{arm}) + 0.2(T_{thigh} + T_{leg}),$$

where T_{chest}, T_{arm}, T_{thigh}, and T_{leg} are the local temperatures of the chest, deltoid, thigh, and calf, respectively, on the same side of the body. Mean body temperature ($\overline{T}_b$) was calculated with the following formula (11, 12, 28):

$$\overline{T}_b = 0.9(T_{re}) + 0.1(\overline{T}_{sk}).$$

Temperature measures were recorded continuously throughout exercise using a data acquisition system (MP150, Biopac Systems, Inc., Goleta, CA, USA).

Heat Loss Responses

Local sweat rate (LSR) was measured and recorded continuously using capacitance hygrometry. This technique involved placing a small plastic capsule (3.976 cm^2) with compressed nitrogen gas flowing through it over the skin on the distal-posterior surface of the forearm. The humidity of the effluent air was measured with a humidity sensor (HMT333, Vaisala, Helsinki, FI), and combined with the flow rate of nitrogen gas through the capsule (0.3 $L \cdot min^{-1}$) was used to calculate LSR. Values for LSR were 30-s averages obtained at baseline, 15 and 45 min, and post $\dot{V}O_{2max}$ test. Whole-body sweat rate was calculated from the change in body mass adjusted for blood and respiratory water losses.

Laser-Doppler flowmetry (moorVMS-LDF2, Moor Instruments Inc., Wilmington, DE, USA) was used to measure red blood cell flux and provide an index of SkBF. Fiber optic flow probes (model VP12) fitted to probe adapters (model SHP1) were affixed to the mid-posterior surface of the forearm using Mueller sports tape for adapters and surgical tape to secure the probes for exercise.

Cardiovascular Measures

Resting BP was measured using an automated monitor (BPM-100, BpTRU Medical Devices, Coquitlam, BC, CA). BP during exercise was measured using auscultation of the brachial artery. The formula proposed by Moran et al. (37) was used to estimate mean arterial pressure [(MAP) mm Hg] during exercise:

$$MAP = DBP + S_t(PP),$$

where DBP = diastolic blood pressure, PP = pulse pressure, and S_t = the fraction of systole from the heart cycle, calculated as (38):

$$S_t = 0.01e^{[4.14-(40.74/HR)]},$$

where HR = heart rate. HR was recorded continuously during exercise using a wireless HR monitor (RS800CX, Polar Electro, Woodbury, NY). $\dot{Q}$ was measured using the indirect Fick CO_2-rebreathing technique (31) with the Parvo Medics system. This technique involves measuring $\dot{V}CO_2$, end-tidal CO_2 concentrations, and the equilibrium CO_2 concentration after rebreathing in succession (56). SV was calculated by dividing $\dot{Q}$ by HR, and total peripheral resistance [(TPR) $dyn \cdot s^{-1} \cdot cm^{-5}$] was calculated by dividing MAP by $\dot{Q}$.

Data Analysis

Mean (± SD) data were generated on the indicated outcome measures. A one-way repeated-measures analysis of variance (ANOVA) was used to test the significance of mean differences for $\dot{V}O_{2max}$ across CON and experimental trials. For metabolic, cardiovascular, temperature and hematological measures at 15 and 45 min during the 45-min trials, a mixed model ANOVA with a between-groups factor (fitness level; HI vs. LO) and within-groups factor (time; 15 vs. 45 min) was used. T-tests using a Bonferroni-adjusted α level were used to

determine individual differences for significant omnibus tests. All hypothesis tests used an α level 0.05 and were performed using SPSS v 23.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS

There were no differences in pre-exercise body mass between groups across trials (P = 0.67 for group × trial interaction). For HI, pre-exercise USG (1.009 ± 0.006 and 1.006 ± 0.007, for 15REL and 45REL, respectively; 1.006 ± 0.003 and 1.006 ± 0.006, for 15FIXED and 45FIXED, respectively) did not differ (all P > 0.05) across exercise treatments. Values were also similar (all P > 0.05) across treatments for LO (1.006 ± 0.004 and 1.005 ± 0.004 for 15REL and 45REL, respectively; 1.004 ± 0.003 and 1.005 ± 0.003, for 15FIXED and 45FIXED, respectively). There were no differences between groups across trials for either treatment (group × time interaction P = 0.63 and P = 0.95 for REL and FIXED, respectively). These data taken together suggest participants were in a similar state of hydration before each trial. Body mass decreased from pre- to post-exercise in the 45-min trials (both P < 0.001), but the decrement in HI was larger than that in LO in both 45REL (~ 1.1 kg and ~ 0.6 kg for HI and LO, respectively, P = 0.006; Table 3.5) and 45FIXED (~ 1.1 kg vs. ~ 0.8 kg for HI and LO, respectively, P = 0.04), as was whole-body sweat rate (1.29 ± 0.27 $L \cdot h^{-1}$ and 0.67 ± 0.31 $L \cdot h^{-1}$ in 45REL for HI and LO, respectively, P = 0.004; 1.33 ± 0.28 $L \cdot h^{-1}$ and 0.93 ± 0.32 $L \cdot h^{-1}$ in 45FIXED for HI and LO, respectively, P = 0.04). PV decreased from rest 6–12% over 45 min for 45REL, as well as 45FIXED, and was not modulated by fitness level. The decrement was similar during 45FIXED; however, the percent change after 45 min in LO was larger than that after 15 min (P = 0.01).

Participant Characteristics

Descriptive characteristics for HI and LO groups are presented in Table 3.1. Participants were successfully match-paired so that groups were similar in age (P = 0.07), body mass (P =

0.73), body fat percentage (P = 0.35), and body surface area (P = 0.57). As planned, $\dot{V}O_{2max}$ for HI was 35% higher than LO (P < 0.001 both for absolute $L \cdot min^{-1}$ and for values relative to body mass, Table 3.1). Resting HR across trials was on average 5 $beats \cdot min^{-1}$ lower in HI (P = 0.41), while resting Hb concentration (45REL, 13.6 ± 1.0 $g \cdot dL^{-1}$ and 12.8 ± 1.5 $g \cdot dL^{-1}$ for HI and LO respectively, P = 0.29; 45FIXED, 13.7 ± 1.0 $g \cdot dL^{-1}$ and 12.8 ± 1.5 $g \cdot dL^{-1}$ for HI and LO respectively, P = 0.25) and Hct (45REL, 38.2 ± 2.3% and 36.9 ± 3.2% for HI and LO respectively, P = 0.46; 45FIXED, 38.4 ± 1.8% and 37.0 ± 3.0% for HI and LO respectively, P = 0.35) were not statistically different from LO. There were no differences in MAP at rest between groups for 45REL (88 ± 14 mm Hg and 80 ± 5 mm Hg for HI and LO, respectively, P = 0.25) or 45FIXED (85 ± 13 mm Hg and 80 ± 6 mm Hg for HI and LO, respectively, P = 0.41). Progesterone levels before the 45-min trials were similar between groups across trials (P = 0.83 for group × time interaction; Plasma P_4 Table 3.1).

Heat Production and Markers of Exercise Intensity

Mean values for heat production and relative exercise intensity and associated workloads are reported in Table 3.2. As intended, HI and LO groups maintained a similar rate of heat production for the FIXED trials (P = 0.06), which resulted in a significantly higher relative exercise intensity in LO compared to HI (P = 0.001). The external workload required to elicit the prescribed heat load of 500 W in HI was higher than in LO (P = 0.02), which was somewhat unexpected but could be attributed to a lower mechanical efficiency during cycling exercise in LO (20.4 ± 1.4% and 18.6 ± 1.2% for HI and LO, respectively, P = 0.04). Groups successfully maintained a similar relative exercise intensity during both REL trials (P = 0.93). However, HI cycled at a greater external workload (P < 0.001) and thus higher rate of metabolic heat production (P = 0.001) than LO. Blood lactate increased 25% over time in HI during 45REL (P =

0.04), while levels increased over time across both groups during 45FIXED (Table 3.4; main effect of time, P = 0.003).

Cardiovascular, Metabolic, and Perceptual Responses during Submaximal Exercise

CV and gas exchange measures during 45 min of cycling at 60% $\dot{V}O_{2max}$ are shown in Table 3.3 and at 500 W of metabolic heat production in Table 3.4. $\dot{V}O_2$ was constant over time in both groups for 45REL (P = 0.90) and 45FIXED (P = 0.94) but was 33% higher in HI compared to LO in 45REL (P < 0.001 for main effect of group). Values also were higher in HI for 45FIXED (P = 0.006 for main effect of group), but by a smaller margin (11%).

60% $\dot{V}O_{2max}$ (45REL)

Figure 3.1 (REL panel, A and B) illustrates the magnitude of CV drift during 45REL for HI and LO. HR increased over time in both groups (P < 0.001 for HI and P = 0.005 for LO), but the drift was 50% greater in HI compared to LO (P = 0.03 for change score). Likewise, SV decreased over time in both groups (P < 0.001 for HI and P = 0.01 for LO), but the decrease was twice as large in HI (16%) compared to LO [(8%); P = 0.001 for change score]. SV for HI was greater compared to LO at 15 min (P = 0.02), but because of the greater decrement in that group, values at 45 min were not different between groups (P = 0.29). The decrease in SV was proportionally greater than the increase in HR in HI, so $\dot{Q}$ decreased over time (P = 0.03), but it was maintained in LO. MAP was unchanged, but TPR increased 8% over time (main effect of time, P = 0.002) and was higher in LO (main effect of group, P = 0.001). RPE increased about 2 units over time (P < 0.001), but because they cycled at the same relative intensity, there were no between-group differences (P = 0.68).

500 W of Metabolic Heat Production (45FIXED)

In 45FIXED, the magnitude of CV drift was not different between HI and LO (Figure 3.1 FIXED panel, A and B). HR increased on average 15–17 bts·min^{-1} (9–11%) across groups ($P < 0.001$ for main effect of time; Table 3.4), and SV decreased about 10 mL·beat^{-1} [(14–16%) main effect of time, $P < 0.001$) although values for HI were about 10 mL·beat^{-1} higher on average than LO (main effect of group, $P = 0.048$). Like the REL condition, HR increased proportionately less than SV decreased, so $\dot{Q}$ decreased 7% over time ($P = 0.003$), but unlike the REL condition groups were not different ($P = 0.36$). MAP remained fairly stable over time. There was a significant interaction effect for RPE ($P = 0.002$). Both groups perceived exercise at 45 min to be harder than exercise at 15 min (HI, $P = 0.02$; LO, $P = 0.001$), and while values were not different between groups at 15 min ($P = 0.49$), LO reported a greater RPE than HI at 45 min ($P = 0.007$), likely because of the higher relative intensity of exercise in that group (Table 3.2).

Thermoregulatory Responses during Submaximal Exercise

60% $\dot{V}O_{2max}$ (45REL)

For 45REL, resting T_{re} (HI = 37.3 ± 0.4 °C, LO = 37.4 ± 0.1 °C, $P = 0.51$), $\overline{T}_{sk}$ (HI = 34.6 ± 0.6 °C, LO = 34.9 ± 0.8 °C, $P = 0.51$), and $\overline{T}_b$ (HI = 37.2 ± 0.7 °C, LO = 37.1 ± 0.1 °C, $P = 0.73$) were not different between groups. T_{re} and $\overline{T}_b$ rose over time in both groups ($P < 0.001$ for HI and LO), but the rate of increase after 15 min was greater in HI compared to LO, so while there were no differences between groups at 15 min (T_{re}, $P = 0.60$; $\overline{T}_b$, $P = 0.84$ Table 3.3), values at 45 min for HI were 0.5 °C higher than LO on average (T_{re}, $P = 0.03$; $\overline{T}_b$, $P = 0.02$ Table 3.3). $\overline{T}_{sk}$ remained stable over time ($P > 0.05$). Local sweat rate (LSR) increased in both groups from 15 min (main effect of time, $P < 0.001$), but values for HI were greater than LO throughout the 45-min exercise bout (main effect of group, $P = 0.01$), which was likely an effect of working

at a higher rate of heat production. ΔSkBF from rest increased across groups (main effect of time, P = 0.03) by about the same magnitude over time (main effect for group, P = 0.59).

500 W of Metabolic Heat Production (45FIXED)

Like 45REL, during 45FIXED, resting T_{re} (HI = 37.2 ± 0.4 °C, LO = 37.4 ± 0.3 °C, P = 0.35), $\overline{T}_{sk}$ (HI = 34.1 ± 1.6 °C, LO = 34.9 ± 0.6 °C, P = 0.33), and $\overline{T}_{b}$ (HI = 36.9 ± 0.4 °C, LO = 37.1 ± 0.3 °C, P = 0.30) were not different between groups. Working at a fixed rate of heat production resulted in comparable increases in T_{re} from rest after 15 (HI = 0.42 ± 0.10 °C; LO = 0.41 ± 0.19 °C, P = 0.92) and 45 min (HI = 1.30 ± 0.25 °C; LO = 1.24 ± 0.35 °C, P = 0.89) and between 15 and 45 min (HI = 0.85 ± 0.25; LO = 0.83 ± 0.19 °C, P = 0.90). Accordingly, requirements for heat loss, and thereby reliance on mechanisms of heat dissipation (LSR and SkBF) were not different between groups (both P > 0.05) but increased over time (main effect of time, P = 0.002 and P = 0.005 for LSR and ΔSkBF, respectively).

Responses to Maximal Exercise

Maximal Responses during Control (CON) Trial

In addition to higher $\dot{V}O_{2max}$ (Table 3.1), O_2 pulse (16.4 ± 0.9 mL·beat^{-1} vs. 11.7 ± 1.9 mL·beat^{-1}, P < 0.001) and power output (263 ± 30 W vs. 213 ± 21 W, P = 0.007) were greater during CON, and GXTs were longer (14.3 ± 3.6 min vs. 9.5 ± 2.4 min, P = 0.02) in HI vs. LO, respectively. There were no differences between HI and LO for maximal HR (191 ± 7 beats·min^{-1} and 193 ± 6 beats·min^{-1}, respectively, P = 0.48), minute ventilation (95.4 ± 10.3 L·min^{-1} and 81.0 ± 19.3 L·min^{-1}, respectively, P = 0.14), RER (1.08 ± 0.06 and 1.14 ± 0.04, respectively, P = 0.06), blood lactate levels (7.2 ± 1.0 mmol·L^{-1} and 9.2 ± 2.6 mmol·L^{-1}, respectively, P = 0.10), or RPE (19 ± 1 and 19 ± 0, respectively, P = 0.73) . For HI, the maximal responses for CON were not different from those reached in either 15-min trial (Table 3.5, all P > 0.05). The GXTs for

both 15-min trials were shorter in duration compared to CON (P = 0.03 for 15REL and P = 0.02 for 15FIXED), but maximal workloads were within ~ 10 *W* across trials (main effect for time, P = 0.68). For LO, RER at maximum in 15REL was lower than CON (P = 0.01), but all other variables did not differ across trials (main effect for time, P > 0.05 for all).

Maximal Exercise during REL and FIXED Trials

Responses to maximal exercise after 15 and 45 min of submaximal exercise are shown in Table 3.5 for both conditions. Similar to CON, $\dot{V}O_{2max}$, O_2 pulse, and maximal power output were greater in HI vs. LO after 15REL and 15FIXED. The greater magnitude of CV drift between 15 and 45 min in 45REL in HI corresponded to a 16% decrease (P = 0.01) in $\dot{V}O_{2max}$ compared to 5% (P = 0.002) in LO (Figure 3.1 REL panel, C), which also resulted in no group differences for $\dot{V}O_{2max}$ (P = 0.21) or maximal power output after 45 min (P = 0.81). Conversely, the comparable magnitude of CV drift in HI and LO during 45FIXED corresponded to proportional decreases in $\dot{V}O_{2max}$ (Figure 3.1 FIXED panel, C; HI = 14%, LO = 13%; P = 0.14 for interaction effect), although the higher $\dot{V}O_{2max}$ values were maintained in HI across time points (main effect of group, P = 0.02). The higher $\dot{V}O_{2max}$ values in HI were accompanied by higher maximal power output (P = 0.01 for main effect of group). Maximal blood lactate in HI after 15REL was higher than that after 45REL (P = 0.02), while levels in LO after 15FIXED were higher than that after 45FIXED (P = 0.02).

T_{re} after the GXT in the 45-min trials was higher compared to the 15-min trials for both treatments and groups (P < 0.05), but values for HI were about 0.4 °C greater on average after 45REL compared to LO (P = 0.03; Table 3.5). $\overline{T}_b$ followed a similar trend. Maximal values for $\dot{V}_E$, $\overline{T}_{sk}$, RPE, test duration, ΔPV from rest, and ΔSkBF from rest were not different between groups (all P > 0.05) at the end of 45REL, but RER at maximum was lower than maximal values

for 15REL across groups (main effect of time, P = 0.02) and collectively higher in LO compared to HI (main effect of group, P = 0.009). LSR was about 0.3 $mg \cdot cm^{-2} \cdot min^{-1}$ greater (P = 0.04) in HI vs. LO after 45REL, but groups were not different after 45FIXED (P = 0.12).

Maximal values for $\dot{V}_E$, RER, T_{re}, $\overline{T}_{sk}$, $\overline{T}_b$, blood lactate, ΔPV from rest, ΔSkBF from rest, RPE, and test duration were not different between groups after 45FIXED (all P > 0.05), while HR was higher (main effect for group, P = 0.03) and O_2 pulse (main effect for group, P = 0.001) was lower in LO vs. HI.

TABLE 3.1. *Mean ± SD descriptive characteristics for high (HI) and low (LO) cardiorespiratory fitness groups*.

	HI	LO
Age (y)	25 ± 5	21 ± 2
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	49.4 ± 4.7	34.7 ± 3.3*
$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	3.13 ± 0.13	2.26 ± 0.32*
Body Mass (kg)	63.8 ± 4.7	65.2 ± 8.2
Body Fat (%)	24.7 ± 5.5	27.3 ± 3.2
BSA (m^2)	1.71 ± 0.05	1.75 ± 0.14
Plasma P_4 ($ng \cdot mL^{-1}$)		
45REL	1.4 ± 0.6	1.8 ± 0.8
45FIXED	1.3 ± 0.7	1.6 ± 0.9

$\dot{V}O_{2max}$, maximal oxygen uptake; BSA, body surface area; P_4, plasma progesterone concentrations before the 45-min trials. * P < 0.05 versus HI.

TABLE 3.2. *Mean ± SD absolute rate of metabolic heat production, percentage of $\dot{V}O_{2max}$, and external workload for high (HI) and low (LO) cardiorespiratory fitness groups during REL and FIXED 45-min trials.*

	45REL		45FIXED	
	HI	LO	HI	LO
External Workload (*W*)	129 ± 12	85 ± 11*	130 ± 9	112 ± 12*
Rate of Metabolic Heat Production				
W	496 ± 51	364 ± 44*	509 ± 16	476 ± 34
$W \cdot kg^{-1}$	7.9 ± 1.2	5.6 ± 0.7*	8.0 ± 0.7	7.3 ± 0.8
$W \cdot m^{-2}$	290 ± 36	208 ± 23*	296 ± 15	273 ± 24
% Control $\dot{V}O_{2max}$	58.8 ± 3.4	58.6 ± 2.8	59.8 ± 2.2	75.4 ± 8.5*

W, watts; $W \cdot kg^{-1}$, watts per unit mass; $W \cdot m^{-2}$, watts per unit surface area; $\dot{V}O_{2max}$, maximal oxygen uptake. 45REL, exercise intensity set at 60% $\dot{V}O_{2max}$; 45FIXED, exercise intensity set at a fixed rate of metabolic heat production of 500 W. * P < 0.05 versus value in HI.

TABLE 3.3. *Mean ± SD responses to submaximal exercise at 60% $\dot{V}O_{2max}$.*

	HI		LO	
Variable	15-min	45-min	15-min	45-min
$\dot{V}O_2$ (L·min^{-1}) ‡	1.8 ± 0.2	1.8 ± 0.2	1.3 ± 0.2	1.3 ± 0.2
$\dot{Q}$ (L·min^{-1}) **‡	11.1 ± 0.7	10.2 ± 0.9	8.7 ± 0.8	8.4 ± 1.0
SV (mL·beat^{-1})	70 ± 7	59 ± 7*	58 ± 7†	53 ± 9*
HR (beats·min^{-1})	160 ± 12	175 ± 8*	150 ± 14	160 ± 16*
O_2 pulse (mL·beat^{-1}) **‡	11.6 ± 1.5	10.5 ± 1.1	8.8 ± 1.4	8.4 ± 1.4
BLA (mmol·L^{-1})	1.6 ± 0.7	2.0 ± 0.7*	2.2 ± 1.0	2.1 ± 1.0
MAP (mm Hg)	102 ± 5	106 ± 8	100 ± 7	103 ± 7
TPR (dyn·s^{-1}·cm^{-5}) **‡	736.7 ± 41.9	836.5 ± 78.7	931.6 ± 77.5	978.9 ± 79.9
LSR (mg·cm^{-2}·min^{-1}) **‡	0.68 ± 0.10	0.91 ± 0.23	0.44 ± 0.15	0.56 ± 0.17
ΔSkBF from rest (%) **	240 ± 106	315 ± 135	201 ± 154	262 ± 179
ΔPV from rest (%)	-9 ± 5	-10 ± 5	-6 ± 7	-12 ± 5
T_{re} (°C)	37.6 ± 0.5	38.7 ± 0.4*	37.7 ± 0.1	38.2 ± 0.1†
$\overline{T}_{sk}$ (°C)	35.6 ± 0.6	35.8 ± 0.7	35.3 ± 0.7	35.4 ± 0.5
$\overline{T}_b$ (°C)	37.4 ± 0.4	38.4 ± 0.4*	37.4 ± 0.1	37.9 ± 0.1†
RPE **	12 ± 1	14 ± 1	11 ± 1	13 ± 2

HI, high cardiorespiratory fitness group; LO, low cardiorespiratory fitness group; $\dot{V}O_2$, oxygen uptake; $\dot{Q}$, cardiac output; SV, stroke volume; HR, heart rate; BLA, blood lactate; MAP, mean arterial pressure; TPR, total peripheral resistance; LSR, local sweat rate; SkBF, skin blood flow; PV, plasma volume; T_{re}, rectal temperature; $\overline{T}_{sk}$, mean skin temperature; $\overline{T}_b$, mean body temperature; RPE, rating of perceived exertion. ** $P < 0.05$ for time main effect; ‡ $P < 0.05$ for group main effect; * $P < 0.05$ versus 15-min value within group; † $P < 0.05$ versus value at the same time point in HI.

TABLE 3.4. *Mean ± SD responses to submaximal exercise at 500 W of metabolic heat production.*

	HI		LO	
Variable	15-min	45-min	15-min	45-min
$\dot{V}O_2$ ($L \cdot min^{-1}$) ‡	1.9 ± 0.1	1.9 ± 0.1	1.7 ± 0.1	1.7 ± 0.1
$\dot{Q}$ ($L \cdot min^{-1}$) **	11.1 ± 0.7	10.6 ± 1.0	10.2 ± 1.7	9.3 ± 1.7
SV ($mL \cdot beat^{-1}$) **‡	72 ± 6	61 ± 6	61 ± 11	51 ± 10
HR ($beats \cdot min^{-1}$) **	155 ± 8	172 ± 6	167 ± 14	181 ± 10
O_2 pulse ($mL \cdot beat^{-1}$) **‡	12.0 ± 1.0	10.9 ± 0.7	10.2 ± 1.2	9.1 ± 1.3
BLA ($mmol \cdot L^{-1}$) **	1.7 ± 0.7	1.9 ± 0.5	2.5 ± 1.4	2.9 ± 1.6
MAP (mm Hg)	104 ± 7	107 ± 9	105 ± 9	99 ± 7
TPR ($dyn \cdot s^{-1} \cdot cm^{-5}$) **	744.4 ± 26.1	808.0 ± 49.1	841.0 ± 111.4	878.4 ± 134.3
LSR ($mg \cdot cm^{-2} \cdot min^{-1}$) **	0.66 ± 0.12	0.87 ± 0.26	0.56 ± 0.15	0.64 ± 0.18
ΔSkBF from rest (%) **	361 ± 312	449 ± 305	282 ± 125	354 ± 129
ΔPV from rest (%) **	-6 ± 3	-7 ± 4	-9 ± 5	-12 ± 6
T_{re} (°C) **	37.6 ± 0.5	38.5 ± 0.5	37.8 ± 0.3	38.6 ± 0.4
$\bar{T}_{sk}$ (°C)	35.6 ± 0.6	35.6 ± 0.8	35.4 ± 0.4	35.6 ± 0.6
$\bar{T}_b$ (°C) **	37.4 ± 0.4	38.2 ± 0.5	37.6 ± 0.3	38.3 ± 0.4
RPE	12 ± 2	13 ± 2*	13 ± 1	17 ± 1*†

$\dot{V}O_2$, oxygen uptake; $\dot{Q}$, cardiac output; SV, stroke volume; HR, heart rate; BLA, blood lactate; MAP, mean arterial pressure; TPR, total peripheral resistance; LSR, local sweat rate; SkBF, skin blood flow; ΔPV, change in plasma volume; T_{re}, rectal temperature; $\bar{T}_{sk}$, mean skin temperature; $\bar{T}_b$, mean body temperature; RPE, rating of perceived exertion. ** $P < 0.05$ for time main effect; ‡ $P < 0.05$ for group main effect; * $P < 0.05$ versus 15-min value within group; † $P < 0.05$ versus value at the same time point in HI.

TABLE 3.5. *Mean ± SD responses to a maximal graded exercise test after 15 and 45 min of cycling at 60% $\dot{V}O_{2max}$ and 500 W of metabolic heat production.*

	REL				FIXED			
	HI		LO		HI		LO	
Variable	15 min	45 min	15 min	45 min	15 min	45 min	15 min	45 min
$\dot{V}O_2$ ($L \cdot min^{-1}$) [c d]	3.1 ± 0.1	2.5 ± 0.3[*]	2.4 ± 0.4[†]	2.2 ± 0.4	3.1 ± 0.2	2.6 ± 0.2	2.4 ± 0.4	2.1 ± 0.3
$\dot{V}O_2$ [c d] ($mL \cdot kg \cdot min^{-1}$)	47.3 ± 4.9	40.3 ± 6.1[*]	35.9 ± 4.2[†]	34.2 ± 5.8	48.1 ± 4.5	41.4 ± 6.0	36.0 ± 3.9	31.7 ± 3.1
$\dot{V}_E$, STPD ($L \cdot min^{-1}$) [c]	87.8 ± 14.2	74.5 ± 6.1	79.7 ± 16.9	79.7 ± 10.3	87.3 ± 10.7	77.5 ± 8.9	81.0 ± 14.8	70.3 ± 18.5
RER [a b c]	1.00 ± 0.05	0.95 ± 0.04	1.06 ± 0.03	1.01 ± 0.06	1.00 ± 0.05	0.96 ± 0.07	1.04 ± 0.06	0.95 ± 0.07
HR ($beats \cdot min^{-1}$)	190 ± 6	190 ± 6	194 ± 5	196 ± 5	191 ± 6	190 ± 5	196 ± 4	198 ± 4[†]
O_2 pulse [c d] ($mL \cdot beat^{-1}$)	16.0 ± 1.1	13.4 ± 1.7[*]	12.2 ± 1.9[†]	11.4 ± 2.3[*]	16.1 ± 1.1	13.8 ± 1.3	12.1 ± 2.0	10.5 ± 1.5
BLA ($mmol \cdot L^{-1}$)	7.7 ± 2.1	5.0 ± 0.8[*]	7.8 ± 1.4	7.8 ± 2.1	6.9 ± 1.1	5.7 ± 1.8	8.5 ± 2.3	5.5 ± 1.4[*]
LSR ($mg \cdot cm^{-2} \cdot min^{-1}$)	—	0.92 ± 0.26	—	0.60 ± 0.19[†]	—	0.90 ± 0.28	—	0.66 ± 0.18
ΔSkBF from rest (%)	—	320 ± 183	—	369 ± 123	—	589 ± 480	—	530 ± 200
ΔPV from rest (%)	—	-12 ± 3	—	-19 ± 7	—	-11 ± 3	—	-13 ± 7
T_{re} (°C) [c]	38.0 ± 0.5	38.9 ± 0.4[*]	37.9 ± 0.2	38.5 ± 0.2[*†]	38.0 ± 0.5	38.7 ± 0.4	38.0 ± 0.3	38.8 ± 0.4
$\overline{T}_{sk}$ (°C)	—	35.7 ± 0.7	—	35.2 ± 0.5	—	35.4 ± 0.9	—	35.5 ± 0.7
$\overline{T}_b$ (°C)	—	38.6 ± 0.3	—	38.1 ± 0.2[†]	—	38.4 ± 0.4	—	38.5 ± 0.4
RPE	19 ± 1	20 ± 1	19 ± 1	20 ± 1	20 ± 1	20 ± 1	19 ± 2	20 ± 0
Test duration (min) [c d]	9.3 ± 1.6	5.2 ± 2.3[*]	8.1 ± 1.0	6.8 ± 1.7	8.8 ± 1.4	6.0 ± 1.5	6.7 ± 2.0	4.4 ± 1.2
Power output (*W*) [c d]	253 ± 21	198 ± 24[*]	202 ± 47[†]	194 ± 36	254 ± 23	208 ± 17	199 ± 33	171 ± 31
Decrease in body mass (%) [a b c d]	-1.2 ± 0.5	-1.7 ± 0.5	-0.6 ± 0.3	-0.9 ± 0.5	-1.1 ± 0.3	-1.8 ± 0.4	-0.6 ± 0.2	-1.2 ± 0.5

REL, 15- and 45-min trials performed at 60% $\dot{V}O_{2max}$ (left panel); FIXED, 15- and 45-min trials performed at 500 W of metabolic heat production (right panel); $\dot{V}O_2$, oxygen uptake; $\dot{V}_E$, minute ventilation; RER, respiratory exchange ratio; HR, heart rate; BLA, blood lactate; PV, plasma volume; T_{re}, rectal temperature; $\overline{T}_{sk}$, mean skin temperature; $\overline{T}_b$, mean body temperature; RPE, rating of perceived exertion. [a] $P < 0.05$ for time main effect in the REL condition; [b] $P < 0.05$ for group main effect in the REL condition; [c] $P < 0.05$ for time main effect in the FIXED condition; [d] $P < 0.05$ for group main effect in the FIXED condition; [*] $P < 0.05$ versus 15-min value in the same group and condition; [†] $P < 0.05$ versus value at the same time point in HI in the same condition.

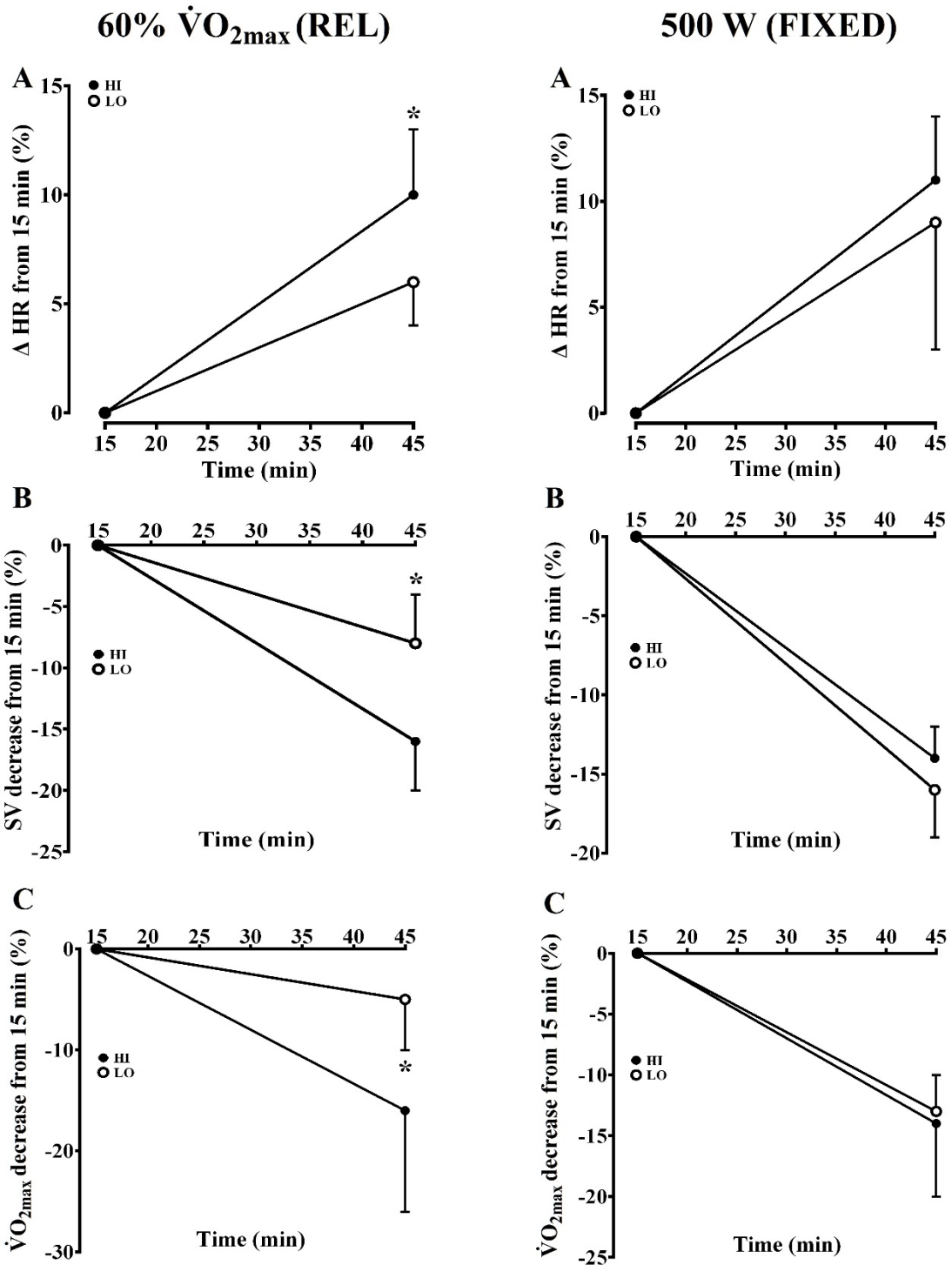

Figure 3.1 Changes in mean (± SD) heart rate (HR, A), stroke volume (SV, B), and maximal oxygen uptake ($\dot{V}O_{2max}$, C) between 15 and 45 min of cycling at 60%$\dot{V}O_{2max}$ (REL, left panel) and 500 W of metabolic heat production (FIXED, right panel). * $P < 0.05$ between HI and LO in the same condition.

DISCUSSION

This study is the first to investigate the independent effect of fitness level on the relationship between cardiovascular (CV) drift and maximal oxygen uptake ($\dot{V}O_{2max}$) during heat stress. Furthermore, the authors are not aware of any other studies that have evaluated the relationship between CV drift and $\dot{V}O_{2max}$ using a fixed rate of metabolic heat production to prescribe exercise intensity. The primary finding of this study was that CV drift and associated reductions in $\dot{V}O_{2max}$ were greater in high-fit individuals compared to low-fit individuals exercising at the same relative percentage of $\dot{V}O_{2max}$; however, fitness level did not affect this relationship during exercise performed at the same rate of metabolic heat production. These findings are consistent with our results for observed levels of hyperthermia, where T_{re} was higher in HI compared to LO during exercise at 60% $\dot{V}O_{2max}$ but comparable between groups during exercise performed at 500 W of metabolic heat production. Importantly, the results of this study not only confirm our hypothesis but also confirm previous findings that the magnitude of CV drift is proportional to the proceeding magnitude of decrement in $\dot{V}O_{2max}$. Additionally, our results expand this body of literature by showing that this relationship applies during prolonged, submaximal exercise, regardless of fitness level.

Comparisons with Past Findings on Cardiovascular Drift and $\dot{V}O_{2max}$

Across group and condition, HR increased ~ 9%, SV decreased ~ 14%, and $\dot{V}O_{2max}$ fell ~ 12% during 45 min of cycling in the heat. The proportional decrease in $\dot{V}O_{2max}$ with the magnitude of CV drift is comparable to previous studies that reported smaller magnitudes of CV drift and accompanying smaller decrements in $\dot{V}O_{2max}$ during prolonged (45–60 min) cycling using similar methodologies (21, 35). In Ganio et al. [(21), no fluid condition] changes in HR (+6%) and SV (-5%) were minimal, and $\dot{V}O_{2max}$ was essentially unchanged between 15 and 60

min of cycling. Exercise duration was longer, but environmental conditions were less severe (30 °C) and likely explain the smaller drift and effect on $\dot{V}O_{2max}$ compared to that which would be expected in 35 °C. Lafrenz et al. (35) reported a similar magnitude of CV drift (11% increase in HR and decrease in SV) and decrement in $\dot{V}O_{2max}$ (-15%) during 45 min of cycling at 60% $\dot{V}O_{2max}$ in 35 °C (40% RH). Other studies reported larger magnitudes of CV drift and decreased $\dot{V}O_{2max}$ (53, 54, 56), despite using the same environmental conditions and methodologies. The larger CV drift and accompanying decrements in $\dot{V}O_{2max}$ in those studies (53, 54, 56) are likely attributable to exercise being performed at a higher rate of metabolic heat production given the male subjects had higher levels of aerobic fitness and exercised at 60% $\dot{V}O_{2max}$. Our findings may best be compared with the women studied by Wingo et al. (57). CV drift and $\dot{V}O_{2max}$ were measured between 15 and 45 min of cycling at 60% $\dot{V}O_{2max}$ in 9 women (age = 23 ± 2 y, weight = 63 ± 9 kg, height = 166 ± 5 cm, percent body fat = 21 ± 5%). On average, aerobic fitness was ~ 43 $mL \cdot kg^{-1} \cdot min^{-1}$ (~ 2.7 $L \cdot min^{-1}$), and submaximal exercise intensities were similar to 45REL of the current study. Yet, changes in HR and SV reported in this study were smaller (+5% vs. +8% for HR and -7% vs. -12% for SV) compared to the changes across groups for 45REL and were therefore accompanied by a proportionally smaller decrement in $\dot{V}O_{2max}$ (-7% vs. -11%, respectively). Because ambient conditions in this study were 22 °C (44% RH), discrepancies between results for CV drift and $\dot{V}O_{2max}$ were likely because of differences in the level of hyperthermia experienced by participants, given that T_{re} in the present study was 0.4 °C greater than participants in Wingo et al. (57), both at 45 min and at maximum during a GXT. This same rationale may explain the results for exercise at 60% $\dot{V}O_{2max}$ in the current study, where the differences in T_{re} between HI and LO (0.5 °C and 0.4 °C at 45 min and maximum, respectively) corresponded with greater magnitudes of CV drift and reductions in $\dot{V}O_{2max}$.

Comparison with Studies on T_c Responses between Groups with Different Fitness Levels

Our results for T_{re} are consistent with previous findings that between-group differences in T_c changes during exercise are independent of fitness level and relative metabolic intensity (30). For example, Jay et al. (30) observed a greater change in T_{re} (ΔT_{re}) in high-fit (HF) men (~ 1.4 °C) compared to low-fit (LF) men (~ 0.9 °C) during 60 min of cycling at 60% $\dot{V}O_{2peak}$. External workloads required to achieve 60% $\dot{V}O_{2peak}$ were higher in HF vs. LF and resulted in greater heat production during exercise; there were no differences in $\overline{T}_{sk}$, but whole body and local sweat rate in HF were significantly greater than LF. It is noteworthy that ΔT_{re} was chosen for comparison between groups because resting T_{re} was significantly lower (~ 0.2 °C) in HF, which has been reported in other studies (28, 50), but was not observed in the present sample. Therefore, during 45REL in the present study, results reported for absolute T_{re} mirrored results for ΔT_{re}, which are comparable to findings of Jay et al. (30) in that ΔT_{re} was ~ 0.6 °C greater in HI vs. LO after 45 min and after the GXT (HI = 1.4 and 1.6 °C, respectively; LO = 0.8 and 1.1 °C, respectively).

Jay et al. (30) also administered the same exercise bout using a fixed rate of metabolic heat production (540 W) and found that ΔT_{re} was ~ 0.9 °C in both groups after 60 min of exercise. This change corresponds to our results for ΔT_{re} in 45FIXED, where HI and LO experienced similar changes from rest at 45 min (~ 1.3 °C) and after the GXT (~ 1.5 °C). The larger ΔT_{re} in the current study is likely explained by hotter ambient conditions than that of Jay et al. (~ 25 °C, 26% RH) (30). Therefore, the present findings expand upon previous findings in temperate conditions by showing similar effects during exercise in the heat. Cramer et al. (10) also showed similar T_c responses between groups of moderately different fitness levels (~ 53 vs. 45 $mL \cdot kg^{-1} \cdot min^{-1}$) during 60 min of cycling at a fixed rate of metabolic heat production (9 $W \cdot kg^{-1}$) in 35 °C; however, this study did not assess CV drift and $\dot{V}O_{2max}$. For the present study, unlike

45REL, magnitudes of CV drift and decreased $\dot{V}O_{2max}$ were similar, as was ΔT_{re}, between groups during exercise at the same rate of metabolic heat production and absolute $\dot{V}O_2$ in 45FIXED, despite relative exercise intensity for LO being 16 percentage points greater than HI (Table 3.2). Importantly, these results show that fitness level did not independently modulate the CV drift/$\dot{V}O_{2max}$ relationship, and furthermore, support the notion that hyperthermia is the most influential modifier of the CV drift/$\dot{V}O_{2max}$ relationship (52, 55).

Physiological Mechanisms Related to the Relationship between CV drift and $\dot{V}O_{2max}$

It is difficult to explain how reductions in $\dot{V}O_{2max}$ are mechanistically associated with CV drift without measuring each component of the Fick equation at maximum. Existing literature (23, 46, 48) and findings from previous studies on this relationship (53, 54, 56, 57) identify the progressive fall in SV as likely primarily responsible for decreasing $\dot{V}O_{2max}$ like that reported in the present study. High $\overline{T}_{sk}$ concomitant with high T_c is thought to reduce $\dot{V}O_{2max}$ because of cardiovascular impairments limiting performance or work capacity after prolonged exercise that are exacerbated by heat stress (7). As such, SV probably decreased because of progressively increasing T_{re} and $\overline{T}_{sk}$ during exercise. High $\overline{T}_{sk}$ increases SkBF, resulting in peripheral displacement of blood volume, consequently lowering central venous pressure and reducing ventricular filling (3, 23, 45). High T_c increases HR intrinsically (32), via augmented sympathetic activity (25), or both, which also decreases ventricular filling time, and thereby, SV (15, 24). Maximal HR was achieved in each GXT, indicating maximal cardiovascular capacity was reached, and maximal arteriovenous oxygen difference was unlikely to decrease in under the conditions of this study (23, 44, 51). Therefore, decreased maximal SV—regardless of the cause of the decrease—is the most plausible explanation for reductions in $\dot{V}O_{2max}$ observed across groups and conditions in the present study.

Physiological Mechanisms Explaining Differences in CV drift and $\dot{V}O_{2max}$ between Groups with Different Fitness Levels

Magnitudes of CV drift and decrements in $\dot{V}O_{2max}$ were greater in HI during 45REL when the rate of metabolic heat production was higher than LO, but groups did not differ when heat production was normalized in 45FIXED. From the aforementioned mechanisms, we suspect that CV drift and associated decrements in $\dot{V}O_{2max}$ were greater in HI during 45REL because absolute and ΔT_{re} were greater than LO, given there were no group differences in $\overline{T}_{sk}$ and SkBF. Other studies have found that metabolic heat production per unit body mass and relative to BSA can influence T_c during exercise (10, 17, 30). Since participants in the present study were match-paired for body size, the level of heat production driving 33% greater ΔT_{re} in HI compared to LO during 45REL was evidently caused by the higher wattage requirement to achieve the prescribed intensity compared to LO. Workloads were not as different between HI and LO in 45FIXED during exercise at the same rate of metabolic heat production, so similar levels of absolute and ΔT_{re}, as well as $\overline{T}_{sk}$ and SkBF, were expected. The effect of normalized heat production, and thereby hyperthermia, on CV drift and $\dot{V}O_{2max}$ was less predictable, though, considering $\%\dot{V}O_{2max}$ for LO was 16 units higher than HI during the 45-min exercise bout. Despite considerable differences in relative exercise intensity, HI and LO experienced almost identical magnitudes of CV drift and reductions in $\dot{V}O_{2max}$. These findings support the hypothesis that the magnitude of CV drift and decrement in $\dot{V}O_{2max}$ during prolonged exercise heat stress is influenced by the level of hyperthermia and that CV drift is one way the effects of hyperthermia on $\dot{V}O_{2max}$ are mediated during aerobic exercise in the heat.

Perspectives and Study Implications

Our findings demonstrate that fitness level affects the CV drift/$\dot{V}O_{2max}$ relationship during exercise prescribed at a given percentage of $\dot{V}O_{2max}$, and thus, different rate of metabolic heat production between groups. By normalizing heat production, though, magnitudes of CV drift and consequent reductions in $\dot{V}O_{2max}$ did not differ between independent groups. Based on these results, when comparing CV responses and accompanying changes in $\dot{V}O_{2max}$, work capacity, or exercise performance between independent groups with varying aerobic fitness, prescribing exercise intensity based on %$\dot{V}O_{2max}$ is discouraged. In these circumstances, exercise intensity should be administered using a fixed rate of metabolic heat production, while also accounting for body size. If groups are similar in body mass and body surface area, then an absolute heat load such as that administered in the present study can be used. For groups of dissimilar body size, an absolute rate of heat production is not appropriate, and rather, prescriptions should be relative to body mass or surface area. It should be noted that %$\dot{V}O_{2max}$ is appropriate when prescribing exercise in studies employing within-subjects designs.

Differences in baseline $\dot{V}O_{2max}$ between the represented high- and low-fit samples were statistically significant but relatively modest concerning some previous studies (14, 26, 30). It is reasonable to suspect that CV differences during exercise prescribed at a given %$\dot{V}O_{2max}$ would be greater between groups with larger disparities in fitness level, which further supports standardizing exercise intensity to rate of metabolic heat production versus relative metabolic intensity, particularly when investigating CV and temperature responses to exercise heat stress in independent groups. Lastly, submaximal, prolonged aerobic exercise performed at a constant rate based on either relative/absolute metabolic intensity or rate of metabolic heat production—particularly in the heat—results in CV drift and reduced $\dot{V}O_{2max}$. For studies not assessing

$\dot{V}O_{2max}$ after exercise evoking CV drift, the magnitude of decrease in SV may be most reliable for estimating the ensuing degradation in aerobic capacity, given our findings showing that a given magnitude of drift downward in SV results in a directionally and proportionally similar magnitude of decrease in $\dot{V}O_{2max}$.

Considerations and Future Research

Findings of this study are only relevant to compensable heat stress situations, where the evaporative requirement for heat balance can be met via evaporation of sweat. Improving aerobic fitness can increase heat tolerance (6, 41, 49); thus, it is possible that CV drift and $\dot{V}O_{2max}$ would have been differentially affected in HI compared to LO during 45FIXED if the environment was physiologically uncompensable. Future studies should address whether the presented results are applicable to uncompensable environments, as this information would be practical for workers and laborers in hot conditions, such as firefighters, soldiers, and industrial and construction workers, who are frequently exposed to long periods of high heat stress while wearing protective clothing, all of which could precipitate CV drift. Furthermore, since men and women thermoregulate differently at high workloads and levels of heat production (16, 18-20), future studies should also investigate the physiological influence of sex on the relationship between CV drift and $\dot{V}O_{2max}$ during heat stress.

CONCLUSION

In conclusion, this study shows that aerobic fitness level influences the magnitude of CV drift and associated decrement in $\dot{V}O_{2max}$ during exercise at a relative percentage of $\dot{V}O_{2max}$, and thereby, different rate of metabolic heat production. However, when the rate of metabolic heat production is normalized, CV drift and reductions in $\dot{V}O_{2max}$ are similar between high- and low-fit groups. As such, modulations of the CV drift/$\dot{V}O_{2max}$ relationship are independent of an

individual's physiological capacity to perform at maximum, and rather, are driven by differences in heat production that influence hyperthermia during exercise.

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CHAPTER 4

SEX DIFFERENCES IN CARDIOVASCULAR DRIFT AND MAXIMAL OXYGEN UPTAKE DURING HEAT STRESS

ABSTRACT

The magnitude of cardiovascular (CV) drift during heat stress has been shown to be proportional to decrements in maximal oxygen uptake ($\dot{V}O_{2max}$), especially under conditions that drive hyperthermia and have a high requirement for heat loss to maintain heat balance. Under these conditions, women have a lower sweat production and evaporative heat loss than men, which could potentiate hyperthermia and thus result in a greater magnitude of CV drift and decrement in $\dot{V}O_{2max}$. The purpose of this study was to test the hypothesis that women would experience a greater magnitude of CV drift and accompanying decrement in $\dot{V}O_{2max}$ than men during exercise performed at a rate of metabolic heat production known to result in sudomotor sex differences. To test this, men (n = 7) and women (n = 7) matched for body mass (65.8 ± 7.5 kg and 66.5 ± 7.0 kg, respectively, P = 0.86) and body surface area (1.83 ± 0.09 m^2 and 1.77 ± 0.09 m^2, respectively, P = 0.26) cycled in 35 °C (40% RH) at a rate of metabolic heat production known to result in sudomotor sex differences (500 W) for 45 min, immediately followed by a graded exercise test (GXT) to measure $\dot{V}O_{2max}$. CV drift was measured between 15 and 45 min. To capture $\dot{V}O_{2max}$ during the same time interval that CV drift occurred, a 15-min bout of exercise at the same intensity and followed by a GXT to measure $\dot{V}O_{2max}$ was performed on a different day. Groups successfully maintained the exercise intensity of 500 W; however, baseline fitness level ($\dot{V}O_{2max}$) was lower in women compared to men (P = 0.01), so relative metabolic intensity

(%$\dot{V}O_{2max}$) during submaximal exercise was ~ 15 units higher in women compared to men (P = 0.002). LSR increased significantly over time for both groups (P = 0.001), but the increase after 15 min for men was twice that observed for women (+0.25 $mg \cdot cm^{-2} \cdot min^{-1}$, P = 0.21). Although there were no sex differences in sweating between 15 and 45 min, women experienced greater changes in rectal temperature (ΔT_{re}) from rest than men at 15 min (0.44 ± 0.16 °C and 0.22 ± 0.19 °C, respectively, P = 0.03), 45 min (1.37 ± 0.31 °C and 0.73 ± 0.23 °C, respectively, P = 0.001), and after the GXT at maximum (1.56 ± 0.27 °C and 1.10 ± 0.46 °C, respectively, P = 0.04). Greater heat storage in women possibly influenced heart rate (HR), where absolute values were higher than men at both time points (both P < 0.05). However, the change in HR between 15 and 45 min was not different between sexes (+12% and +11% for men and women, respectively, P = 0.83 for interaction), nor was the change in SV (-10% and -16% for men and women, respectively, P = 0.19 for interaction). Accordingly, reductions in $\dot{V}O_{2max}$ over time (main effect of time, P < 0.001) were also similar between groups (P = 0.22). It is likely that with longer exercise durations performed at 500 W of metabolic heat production, blunted sweating would become even more evident in women compared to men, thereby revealing statistically significant sex differences for magnitudes of CV drift and associated decrements in $\dot{V}O_{2max}$.

INTRODUCTION

Early studies investigating sex differences in thermoregulation during heat stress led to conclusions that women thermoregulate less effectively than men before acclimatization and more effectively than men after acclimatization (17, 36, 43, 51). Biophysical factors, such as body mass and body surface area (BSA) (3, 17, 25), constitute a vast proportion of the variability in thermoregulatory responses during exercise heat stress. Recent evidence suggests that past findings may have been confounded by sex differences in these biophysical parameters, rather

than actual physiological sex differences in thermoregulation. For this reason, subsequent studies have investigated physiological sex differences in temperature regulation while accounting for sex differences in body mass and BSA. Results from these studies show that men and women do not appear to differ in terms of central modulation of temperature regulation (onset of thermoeffector responses for sweating and cutaneous vasodilation) or thermosensitivities at lower exercise intensities and requirements for heat loss (6, 18, 19). However, at higher exercise intensities and requirements for heat loss (e.g., 500 W or 300 $W \cdot m^{-2}$ of metabolic heat production), women appear to have reduced sweating sensitivity—which has been attributed to a lower sweat output per gland (15)—and lower evaporative heat loss compared to men of comparable body size (18, 19). Importantly, these lower sudomotor responses in women result in higher core temperatures (T_c) than men regardless of whether heat production is prescribed at a fixed absolute rate (W) (19) or a rate normalized to BSA ($W \cdot m^{-2}$) (18).

High T_c amplifies cardiovascular responses during exercise in the heat, such as progressive increases in heart rate (HR) and decreases in stroke volume (SV) over time during constant-rate, moderate-intensity exercise, also known as cardiovascular drift (CV drift) (7, 33, 46). The magnitude of CV drift during heat stress is associated with proportional reductions in maximal oxygen uptake ($\dot{V}O_{2max}$), both of which could be greater in women during exercise that exceeds their sudomotor threshold compared to men (17, 19), but this has not been investigated. CV drift leads to higher relative exercise intensity and decreased aerobic exercise performance (7, 46-49), so answering this question has important implications for exercise prescription and experimental study design. Furthermore, findings related to this issue further our understanding of the influence (or lack thereof) that sex, as a biological variable, has on physiological responses to heat stress. Accordingly, the purpose of this study was to test the hypothesis that women

would experience a greater magnitude of CV drift and accompanying decrement in $\dot{V}O_{2max}$ than men during exercise performed at a rate of metabolic heat production known to result in sudomotor sex differences.

METHODS

Participants

Participant characteristics are presented in Table 4.1. Fourteen healthy, nonsmoking Caucasian men (n = 7) and women (n = 7) matched for age and body size participated in this study. All participants were students of the host university residing in the same geographical region at least 3 months before testing. Sample sizes for groups of men and women were determined using G*Power 3.1.7 (14). The power analysis revealed a minimum of 6 subjects per group would be sufficient to detect a moderate (27) within-between interaction effect (group × time) for $\dot{V}O_{2max}$, assuming power (1-ß) = 0.8, the correlation among repeated measures = 0.9, and $\alpha = 0.05$. Women were required to be eumenorrheic (34), and all participants were required to be asymptomatic and without cardiovascular, metabolic, or renal disease; not sedentary; and able to engage in vigorous physical activity in the heat, as determined by a medical history questionnaire (2). Men and women were participating in cycling (~ 67.1 and 66.4 $km \cdot wk^{-1}$, respectively) and/or running (~ 21.9 and 29.5 $km \cdot wk^{-1}$, respectively) during the previous 3 months as confirmed by a physical activity history questionnaire. Women performed experimental trials within the first 10 days after self-reported menses or during the withdrawal (placebo) phase if they were using oral contraception (OC). Three women were using a 21-d monophasic OC (30–35 µg ethinyl estradiol and 0.15–0.25 µg progestin) for contraceptive purpose only. Venous blood samples were obtained on the day of the 45-min trials to determine progesterone concentration and confirm menstrual cycle phase. Participants provided written

informed consent before each trial, and the study was approved by the university's institutional review board (IRB Protocol # 18-006-ME-R1) prior to testing.

Research Design

Men and women completed a total of 3 exercise trials on separate days. A control (CON) trial (22 °C) was always performed first to determine baseline $\dot{V}O_{2max}$ during a graded exercise test on an electronically braked ergometer. The 2 experimental trials were counterbalanced, assigned randomly to participants, and performed in 35 °C [40% relative humidity (RH)]. Participants cycled at the same rate of absolute metabolic heat production (500 W) for 15 min (15MIN) and 45 min (45MIN). Both trials were immediately followed by a GXT so that $\dot{V}O_{2max}$ could be measured over the same time as CV drift in the 45MIN trial (i.e., between 15 and 45 min).

Procedures

Control $\dot{V}O_{2max}$ Trial (CON)

Participants were instructed before each trial to avoid eating at least 2 h before reporting to the laboratory; to be well rested; well hydrated [urine specific gravity (USG) ≤ 1.020] (8, 32); to avoid alcohol consumption, non-prescription drug use, and caffeine ingestion on the day of testing; and to avoid strenuous exercise during the 24 h prior to the trial. All pre-test instructions were confirmed via 24-h history questionnaire upon arrival. Participants completed physical activity and medical history forms and signed an informed consent form. Women were asked to disclose the last day of previous menses so that menstrual cycles could be tracked, and experimental trials scheduled during the targeted menstrual phase. Participants provided a urine sample to measure USG and were given a pair of spandex cycling shorts (25.4-cm inseam) and a loose-fitting mesh polyester tank top (JiffyShirts.com) to wear for the 3 trials. After donning the

clothing ensemble, participants were equipped with a heart rate (HR) monitor strapped around the chest; height, body mass, and resting/seated blood pressure (BP) (1) were then measured. Body surface area (BSA, m^2) was estimated from values for height and weight using the equation of Dubois and Dubois (13). Body fat percentage was estimated using the sum of triceps, suprailiac, and thigh skinfolds for women and chest, abdominal, and thigh skinfolds for men (28).

Maximal oxygen uptake ($\dot{V}O_{2max}$) was measured during a graded exercise test (GXT) performed on an electronically braked ergometer in a temperate environment (22 °C, 40% RH). Participants cycled at an initial stage of 100 *W*, and power output was increased 25 *W* every 2 minutes until the workload could no longer be maintained at a cadence > 40 revolutions·min^{-1}. Heart rate, oxygen uptake ($\dot{V}O_2$), and related gas exchange measures were recorded continuously. Rating of perceived exertion (RPE) was obtained during the last 10 s of each 2-min stage using the Borg 6–20 scale (4, 5). Three min after test completion, a 2-mL venous blood sample was drawn to measure blood lactate concentration.

Participants were given a 20-min rest period before performing a follow-up test to ensure a plateau in $\dot{V}O_2$ occurred during the GXT. The follow-up test began at a workload equivalent to the last workload performed during the initial GXT (if < 1 min was completed during the last stage of the GXT) or at a workload 25 *W* greater than the last workload achieved in the initial GXT (if ≥ 1 min was completed during the last stage of the GXT). Participants cycled at this intensity until exhaustion. The slope of the relationship between power output and $\dot{V}O_2$ from the initial test was used to extrapolate the increase in $\dot{V}O_2$ and determine a plateau (39). A plateau in $\dot{V}O_2$ was established for all 14 participants using the follow-up procedure combined with the initial GXT.

After another 20 min of rest, participants were asked to cycle for an additional 20–30 min while workloads corresponding to 500 W of metabolic heat production were determined. Procedures outlined in Cramer and Jay (11) were followed to prescribe exercise intensities that would elicit the targeted heat production. In brief, rate of metabolic heat production (in W) was calculated from the external workload (*W*), and the rate of metabolic energy expenditure (*M*) estimated from $\dot{V}O_2$ and respiratory exchange ratio (RER) using the equation of Nishi (38):

$$\text{M (watts)} = \dot{V}O_2 \frac{\left(\frac{\text{RER - 0.7}}{0.3}\,\text{ec}\right) + \left(\frac{\text{1 - RER}}{0.3}\right)\text{ef}}{60} \times 1000,$$

where $\dot{V}O_2$ is in L/min, ec is the caloric equivalent per liter of oxygen for the oxidation of carbohydrates (21.13 kJ), and ef is the caloric equivalent per liter of oxygen for the oxidation of fat (19.62 kJ). Participants were familiarized with the indirect Fick CO_2-rebreathing technique to determine cardiac output ($\dot{Q}$) while adjustments up or down in workload were made in 10- or 15-W increments. BP was measured again before departing, and instructions were given to participants regarding their next scheduled visit to the laboratory.

Experimental Trials

Men and women cycled at the same constant rate of metabolic heat production equal to 500 W for 15 min (15MIN) and 45 min (45MIN), immediately followed by a GXT to measure $\dot{V}O_{2max}$. Trials for the men (including CON) were completed within 2 weeks, while trials for women were completed within 1 month, depending on information reported at the control trial regarding menstrual cycle phase. Adherence to pre-test guidelines (same as CON) was confirmed via 24-h history questionnaire and participants reconsented to study procedures. Resting BP, USG, and nude body weight were measured before participants inserted a rectal temperature

probe 10 cm past the anal sphincter, donned the required clothing ensemble, and were equipped with the same HR monitor and chest strap used in the control trial.

A flexible venous catheter was inserted into an antecubital vein before the 45-min trial. For women, a resting 2-mL venous blood sample was collected for hormone analysis purposes and immediately placed in a refrigerator kept at 4 °C for the remainder of the trial (2.5 ± 0.5 h). All participants provided a 2-mL blood sample for baseline measurements of lactate and hemoglobin concentrations and hematocrit. After the catheter was secured for exercise, participants entered an environmental chamber maintained at 35 °C, 40% RH with no fan airflow. During instrumentation, participants sat in an upright position for 30 min while a laser-Doppler probe and sweat rate capsule were affixed to the posterior surface of the forearm and skin temperature probes were secured at 4 different sites on the body.

After resting measures were taken, participants began cycling for 45 min at the required workload predetermined to elicit a rate of heat production of 500 W. All participants maintained a cycling cadence of 70–80 revolutions·min^{-1} in both experimental trials. Metabolic data for $\dot{V}O_2$ and RER were measured at 5, 10, 25, and 35 min to assure the prescribed heat production was maintained throughout; these 4 time points were used to determine the average rate of metabolic heat production over the submaximal exercise portion of the 45-min trial (Table 4.2). Adjustments to workload were made in the same manner as the control trial if needed.

Systolic BP (SBP) and diastolic BP (DBP), RPE, skin blood flow (SkBF), $\dot{V}O_2$ and $\dot{V}CO_2$, HR, and 2–3 trials of CO_2 rebreathing to estimate $\dot{Q}$ were measured and a 2-mL blood sample was obtained between 8 and 18 min and between 35 and 45 min and were performed in the same order for each participant and time point. Cardiovascular (CV) drift was characterized by the differences in HR and stroke volume (SV) values between 15- and 45-min time points.

Procedures for 15MIN were identical to those for 45MIN, but $\dot{Q}$ was not measured, and instrumentation was limited to HR and T_{re}, which were recorded continuously throughout exercise. RPE and metabolic data were measured at 5 and 13 min.

Upon completion of 15 min (15MIN) or 45 min (45MIN) of cycling, participants immediately began a GXT without stopping exercise. Power output was increased 25 *W* above the workloads that corresponded to 500 W of metabolic heat production and then every 2 min thereafter until volitional exhaustion. $\dot{V}O_2$ and related metabolic measures were recorded continuously and averaged over 1 min (37). A 2-mL blood sample was drawn 3–5 min after volitional exhaustion was achieved to measure blood variables at maximum. Participants were then de-instrumented and exited the environmental chamber and nude body mass and resting BP were measured before discharge.

Measurements/Instrumentation

Height was measured using a stadiometer (SECA 213, Seca Ltd., Hamburg, Germany), and body mass was measured using a digital scale (Tanita BWB-800, Tanita Corp., Tokyo, Japan). All exercise took place on an electronically braked cycle ergometer (Velotron Pro, Quarq Technology, Inc., Spearfish, SD, USA). $\dot{V}O_2$ and related gas exchange measures were determined by open-circuit spirometry using the Parvo Medics TrueOne 2400 Metabolic Measurement System (Parvo Medics, Inc., Salt Lake City, UT, USA) to analyze expired $\dot{V}O_2$ and $\dot{V}CO_2$. Gas analyzers were calibrated using standardized gas concentrations, and the flowmeter was calibrated using a 3-L syringe.

Blood Measures

All blood samples were drawn into Vacutainer tubes containing EDTA (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA). Lactate was measured in duplicate using a

benchtop analyzer (YSI 2300 STAT Plus, Yellow Spring, OH, USA), hemoglobin (Hb) was measured in duplicate using a HemoPoint H2 Hemoglobin Meter (EKF Diagnostics, Inc., Boerne, TX, USA) and hematocrit (Hct) was measured in triplicate using a micro-capillary reader (Model 3201, International Equipment Co., Boston, MA, USA) after samples were centrifuged (Autocrit Ultra 3 Microhematocrit Centrifuge, model 420575, Becton Dickinson, Franklin Lakes, NJ, USA). Plasma volume change (ΔPV) from baseline was estimated from measures of Hb and Hct using the Dill-Costill equation (12).

Collection, Extraction and Assay of Progesterone

Resting blood samples obtained from women before the 45-min trials were centrifuged (Model 5418, Eppendorf, Hauppauge, NY, USA) for 5 min at 10,000 × *g*, red blood cells were discarded, and plasma was stored at -80 °C until hormone assays were performed. For each sample, 100 µL of plasma was pipetted directly into 20 mL of deionized water (DIW) in individual 18 × 150 mm borosilicate culture tubes. Samples then were slowly passed, under vacuum, through Hypersep C18 columns (3 cc, 500 mg bed weight, Thermo Fisher Scientific, Inc., Waltham, MA, USA) fitted to a 24-port manifold using Tygon 2275 formulation tubing. Columns were primed before use with 2 consecutive washes of 2 mL of methanol followed by 2 consecutive washes with 2 mL of DIW. Progesterone was eluted from the columns with 3 consecutive washes with 2 mL of methanol. Ultrapure nitrogen gas was used to evaporate the solvent in a water bath (37 °C). The remaining hormone residue was then re-suspended in 50 µL of ethanol, vortexed, and mixed with 450 µL of enzyme-linked immunosorbent assay (ELISA) buffer supplied by the manufacturer (www.caymanchem.com). ELISA kits were used to quantify progesterone concentration according to the manufacturer's procedures. Samples were run in duplicate, and the kits were validated by determining parallelism of the kit standard curve with

serial dilutions of hormone extract from the 7 women that participated in this study. Briefly, 80 µL was taken from each sample and combined into a pool that was serially diluted from 1:1 to 1:128 and then assayed in duplicate. The serial dilution was parallel to the standard curve [comparison of slopes test (52) $t_{12} = 0.22$, $P = 0.83$], indicating no matrix effects, and identified 1:8 as the appropriate dilution for samples. Dilutions were achieved by mixing 150 µL of 1:1 sample with 150 µL of ELISA buffer. Pooled samples were run in duplicate at the beginning and end of the ELISA plate; the intra-assay coefficient of variation was 7.41%. All assays were performed at the host institution.

Body Temperatures

Rectal temperature (T_{re}) was measured using a thermistor (MEAS 401, Measurement Specialties, Andover, MN, USA). Skin temperature was measured using thermistors (TSD202B, Biopac Systems, Inc., Goleta, CA, USA) integrated with wireless amplifiers (BioNomadix Wireless SKT Transmitter, Biopac Systems, Inc., Goleta, CA, USA) set to a sampling frequency of 1000 Hz. Probes were affixed on the same side of the body with athletic tape (Mueller Sports Medicine Inc., Prairie du Sac, WI, USA) at sites per the formula of Ramanathan (40) for calculating mean skin temperature ($\overline{T}_{sk}$):

$$\overline{T}_{sk} = 0.3(T_{chest} + T_{arm}) + 0.2(T_{thigh} + T_{leg}),$$

where T_{chest}, T_{arm}, T_{thigh}, and T_{leg} are the local temperatures of the chest, deltoid, thigh, and calf, respectively. Mean body temperature ($\overline{T}_b$) was calculated with the following formula (10, 11, 27):

$$\overline{T}_b = 0.9(T_{re}) + 0.1(\overline{T}_{sk}).$$

Temperature measures were recorded continuously throughout exercise using a data acquisition system (MP150, Biopac Systems, Inc., Goleta, CA, USA).

Heat Loss Responses

Local sweat rate (LSR) was measured and recorded continuously using capacitance hygrometry. This technique involves passing compressed nitrogen gas over the skin through a small plastic capsule (3.976 cm^2) affixed to the distal-posterior surface of the forearm. The humidity of the effluent air was measured with a humidity sensor (HMT333, Vaisala, Helsinki, FI); the humidity of effluent air and the flow rate of nitrogen gas through the capsule (0.3 $L \cdot min^{-1}$) were used to calculate LSR. Values for LSR were 30-s averages obtained at baseline, 15 and 45 min, and post $\dot{V}O_{2max}$ test.

Laser-Doppler flowmetry (moorVMS-LDF2, Moor Instruments Inc., Wilmington, DE, USA) was used to measure red blood cell flux and provide an index of SkBF. Fiber optic flow probes (model VP12) fitted to probe adapters (model SHP1) were secured just proximal to the sweat rate capsule at the mid-posterior forearm using surgical tape.

Cardiovascular Measures

Resting BP was measured using an automated monitor (BPM-100, BpTRU Medical Devices, Coquitlam, BC, Canada). BP during exercise was measured using auscultation of the brachial artery. The formula proposed by Moran et al. (35) was used to estimate mean arterial pressure [(MAP) mm Hg]:

$$MAP = DBP + S_t(PP),$$

Where DBP = diastolic blood pressure, PP = pulse pressure, and S_t = the fraction of systole from the heart cycle calculated as (35):

$$S_t = 0.01e^{[4.14-(40.74/HR)]},$$

where HR = heart rate, which was recorded continuously during exercise using a wireless HR monitor (RS800CX, Polar Electro, Woodbury, NY, USA). The indirect Fick CO_2-rebreathing

technique was used for measurement of $\dot{Q}$ (30) using the Parvo Medics system. This technique involves measuring $\dot{V}CO_2$, end-tidal CO_2 concentrations, and the equilibrium CO_2 concentration after rebreathing in succession (49). SV was calculated by dividing $\dot{Q}$ by HR, and total peripheral resistance [(TPR) $dyn \cdot s^{-1} \cdot cm^{-5}$] was calculated by dividing MAP by $\dot{Q}$.

Data Analysis

Mean (± SD) data were generated on the indicated outcome measures. A one-way repeated-measures analysis of variance (ANOVA) was used to test the significance of mean differences for $\dot{V}O_{2max}$ across CON, 15MIN, and 45MIN trials. A two-way (sex × time) mixed model ANOVA—with a within groups repeated factor of time (15 vs. 45 min) and between groups factor of sex (men vs. women)—was used to test the significance of mean differences between men and women for $\dot{V}O_{2max}$, HR, SV, and other cardiovascular, temperature, and hematological measures taken at 15 and 45 min during the 45MIN trial. T-tests with a Bonferroni-adjusted α level were used to determine individual differences for significant omnibus tests. All hypothesis tests used an α level 0.05 and were performed using SPSS v 23.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Participant Characteristics

Men and women were successfully matched for age ($P = 0.43$), body mass ($P = 0.86$), and BSA ($P = 0.26$), although men had greater height ($P = 0.004$) and lower body fat percentage ($P < 0.001$, Table 4.1). Control $\dot{V}O_{2max}$ was higher in men, but groups did not differ when $\dot{V}O_{2max}$ was expressed as a function of fat-free mass ($P = 0.18$, Table 4.4).

Baseline and Resting Measures

Progesterone concentrations for the group of women were indicative of levels expected during the follicular phase (~ 10 days after menses) of the ovulatory menstrual cycles (1.5 ± 1.0 $ng \cdot mL^{-1}$) (41) and low-hormone (i.e., quasi-follicular) phase of OC use (0.8 ± 0.3 $ng \cdot mL^{-1}$). There were no sex differences in resting HR (75 ± 7 and 68 ± 10 $bts \cdot min^{-1}$ for men and women, respectively, P = 0.19) for 45MIN, and values within groups were comparable to resting measures in 15MIN (P > 0.05 for men and women). Expectedly, resting SBP before 45MIN was higher in men vs. women, but differences were not statistically discernible (116 ± 10 mmHg and 108 ± 13 mmHg for men and women, respectively, P = 0.22). DBP did not differ between sexes (71 ± 3 mmHg and 71 ± 13 mmHg for men and women, respectively, P = 0.98) measures. There were no sex differences in resting T_{re}, $\overline{T}_{sk}$, or $\overline{T}_b$ (T_{re} = 37.4 ± 0.2 and 37.4 ± 0.2 °C, P = 0.97; $\overline{T}_{sk}$ = 34.3 ± 1.5 and 33.6 ± 0.9 °C, P = 0.34; $\overline{T}_b$ = 37.2 ± 0.6 and 37.0 ± 0.2 °C, P = 0.33 for men and women, respectively). Consistent with body mass measured at control, nude body mass values before 15MIN (65.9 ± 6.8 and 66.6 ± 6.8 kg for men and women, respectively, P = 0.87) and 45MIN (65.7 ± 7.9 and 66.1 ± 6.8 kg for men and women, respectively, P = 0.91) were similar between men and women, and there were no differences across trials for either group (P > 0.05 for both). Urine specific gravity (USG) was also similar across trials for men (1.010 ± 0.005 and 1.006 ± 0.003 for 15- and 45-min trials, respectively, P = 0.16) and women (1.007 ± 0.006 and 1.005 ± 0.003 for 15- and 45-min trials, respectively, P = 0.22) and there were no differences between groups (P > 0.05 for both trials).

Body Mass Changes and Fluid Losses

Body mass decreased from pre- to post-exercise in 15MIN (-0.8 ± 0.2 kg and -0.6 ± 0.3 kg for men and women, respectively; P < 0.05 for both groups) and 45MIN (-1.2 ± 0.5 kg and -

0.9 ± 0.5 kg for men and women, respectively; $P < 0.05$ for both groups). Because changes in body mass were not different between groups, there were also no differences in post-exercise body mass between men and women for either trial ($P > 0.05$ for both trials). Likewise, there were no sex differences in percent dehydration (Δ body mass (%); Table 4.4) or whole body sweat rate (1.40 ± 0.55 L·h^{-1} and 1.06 ± 0.64 L·h^{-1} for men and women, respectively; $P = 0.32$), calculated from Δ body mass (corrected for blood and respiratory water loss) during the 45-min trials. The decrease in PV from rest over the 45-min exercise bouts was ~ 10% in both groups ($P > 0.05$).

Cardiovascular and Metabolic Responses to Cycling at a Fixed Rate of Absolute Heat Production

Table 4.2 shows the average workload, rate of metabolic heat production, and exercise intensity expressed as percentage of $\dot{V}O_{2max}$ for men and women in 45MIN. Per experimental design, groups exercised at the same rate of metabolic heat production, whether expressed in absolute W ($P = 0.18$), per unit body mass (W·kg^{-1}; $P = 0.52$), or relative to BSA (W·m^{-2}; $P = 0.98$). Men and women cycled at the same external workload to achieve the targeted heat production ($P = 0.84$). However, the associated workload required a higher percentage of $\dot{V}O_{2max}$ in women ($P = 0.002$), which likely caused the higher levels of blood lactate (BLA) compared to men, although differences were only significant at the 45-min time point ($P = 0.04$, Table 4.3).

Physiological responses for men and women during 45 min of cycling in the heat at 500 W of metabolic heat production are presented in Table 4.3. Because exercise was prescribed at a fixed absolute intensity, the oxygen demand to achieve the required metabolic heat production was the same for men and women. As such, there were no sex differences in $\dot{Q}$ ($P > 0.05$) or $\dot{V}O_2$ ($P > 0.05$) during the 45 min of cycling. However, the factors related to maintaining $\dot{Q}$ over time (HR and SV) appeared to differ between sexes. Figure 4.1 illustrates the drift in HR and SV

expressed as a percent change from 15 min in men and women. HR increased over time across both groups (main effect of time; $P < 0.001$) and was ~ 20 bts·min^{-1} higher in women across time points (main effect of group; $P = 0.02$). The magnitude of change from 15 to 45 min did not differ between groups (men = +16 bts·min^{-1}, +12% and women = +17 bts·min^{-1}, +11%, $P = 0.83$). Similarly, SV decreased over time (main effect of time; $P < 0.001$), and while absolute values (main effect of group, $P = 0.06$) and the magnitude of change in SV (men = -8 mL·beat^{-1}, -10% and women = -11 mL·beat^{-1}, -16%, $P = 0.21$) approached significance, values were not statistically different between sexes. O_2 pulse followed a similar trend as SV (main effect of time; $P < 0.001$), but values were higher in men compared to women (main effect of group; $P = 0.008$). MAP remained essentially constant over time ($P > 0.05$), while TPR increased ~ 6% over time across groups ($P = 0.02$). Finally, both groups perceived exercise to be more strenuous over time (main effect of time; $P < 0.001$), but ratings for women were ~ 2 points higher than men during the 45-min exercise bout (main effect of group, $P = 0.045$).

Temperature and Thermoeffector Responses

Temperature responses and measures for SkBF and LSR vs. mean body temperature ($\overline{T}_b$) for the 45-min trials are presented in Table 4.3 and Figures 4.2-4.4. For both men and women, T_{re} (Figure 4.2) was higher at 45 min compared to 15 min ($P = 0.001$ for men; $P < 0.001$ for women), as was $\overline{T}_b$. T_{re} was similar between groups at 15 min ($P = 0.12$) but 0.6 °C higher in women at 45 min ($P = 0.04$). $\overline{T}_{sk}$ increased from baseline after 15 min in men ($P = 0.02$) and remained stable for the duration of the exercise bout. In contrast, the increase in $\overline{T}_{sk}$ from baseline was less substantial in women ($P > 0.05$ at each time point) and not different from men during the 45 min of cycling. The change in T_{re} (ΔT_{re}) from rest was greater in women at 15 ($P = 0.03$) and 45 min ($P = 0.001$) (Figure 4.3), which may be indicative of reduced peripheral heat

exchange (i.e., decreased thermosensitivity) over time compared to men. The LSR data support this supposition (Figure 4.4); while not statistically different, the increase in sweating was twice as large in men (+0.25 $mg \cdot cm^{-2} \cdot min^{-1}$) compared to women (+0.11 $mg \cdot cm^{-2} \cdot min^{-1}$) between 15 and 45 min (main effect of time, P = 0.001), which may be practically meaningful. SkBF increased over 45 min in men and women (main effect of time; P = 0.002), but groups were not different (main effect of group, P = 0.21).

Responses to Maximal Exercise

Maximal responses during GXTs after 15 and 45 min of cycling in the heat are presented in Table 4.4 and Figure 4.1. $\dot{V}O_{2max}$ for men was higher than women in the control and 15MIN trials (both P < 0.05). Compared to $\dot{V}O_{2max}$ during the control or 15-min trial, $\dot{V}O_{2max}$ was lower after 45 min in both men and women (all P < 0.05). $\dot{V}O_{2max}$ decreased 10% and 18% from 15 min in men and women, respectively, but the magnitude of decrease was not different between groups (P = 0.22). Minute ventilation at maximum was less after 45 min compared to 15 min (main effect of time; P < 0.001) but group differences did not reach statistical significance (main effect of group, P = 0.051). RER for both sexes was lower after 45 min compared to control trials (P = 0.005 for men; P = 0.002 for women) and 15MIN (main effect of time; P < 0.001); there were no sex differences (all P > 0.05). HR reached maximal values during the GXTs after 15 and 45 min that were comparable to control values (P > 0.05). Maximal O_2 pulse in women was lower than men for both the 15MIN and 45MIN GXTs (main effect of group, P = 0.002), as well as control (P = 0.001), and 45-min GXT values were lower than values for the 15MIN GXT (main effect of time, P < 0.001) and control trials (P = 0.02 for men; P = 0.047 for women). Blood lactate was ~ 2–3 $mmol \cdot L^{-1}$ lower (main effect for time, P < 0.001) at maximum after 45 min compared to after 15 min in both men and women. Maximal LSR was greater in men

compared to women (P = 0.04), but ΔSkBF from rest to maximum was highly variable, and as a result, mean values did not differ (P = 0.12). However, SkBF continued to increase after 45 min leading up to the GXT in men (P = 0.03), but this progression was not evident in women (P = 0.19, Tables 4.3 and 4.4). T_{re} was ~ 0.8 °C higher (main effect for time, P < 0.001) at the end of the GXT after 45 min compared to 15 min in both sexes. Values for $\overline{T}_{sk}$ and $\overline{T}_{b}$ at maximum were not different between men and women (P = 0.80 and P = 0.12, respectively). Both groups perceived their effort to be at a similar level at maximum based on RPE (P = 0.40), despite achieving 12% lower power outputs (P = 0.001 for main effect of time) and exercising for about 3.5 min less after 45MIN compared to 15MIN. Although power output did not differ between groups for experimental trials (main effect for group, P = 0.15), men reached 16% higher levels than women during the control trial (P = 0.04).

TABLE 4.1. *Descriptive characteristics for men and women.*

	Men	Women
Age (y)	23 ± 3	25 ± 5
Body Mass (kg)	65.8 ± 7.5	66.5 ± 7.0
Height (cm) *	177 ± 4	170 ± 4
BSA (m^2)	1.83 ± 0.09	1.77 ± 0.09
Fat Mass (kg) *	8.2 ± 3.3	17.4 ± 4.5
FFM (kg) *	57.6 ± 4.3	49.2 ± 4.2
Body Fat (%) *	12.2 ± 3.2	26.6 ± 5.8

Values are mean ± SD. BSA, body surface area; FFA, fat-free mass. * P < 0.05 between men and women.

TABLE 4.2. *Mean ± SD external workload, rate of metabolic heat production and relative percentage of $\dot{V}O_{2max}$, for men and women during the 45-min trials.*

	Men	Women
External Workload (*W*)	121 ± 6	122 ± 17
Rate of Metabolic Heat Production		
W	514 ± 23	496 ± 24
$W \cdot kg^{-1}$	7.9 ± 0.9	7.6 ± 1.0
$W \cdot m^{-2}$	281 ± 17	281 ± 23
% of control $\dot{V}O_{2max}$	51.6 ± 4.3	66.3 ± 9.2*

Averages were calculated from values at 5 min, 10 min, 25 min, and 35 min during the 45-min trials. W, watts; $W \cdot kg^{-1}$, watts per unit mass; $W \cdot m^{-2}$, watts per unit surface area; $\dot{V}O_{2max}$, maximal oxygen uptake. * $P < 0.05$ vs. men.

TABLE 4.3. *Mean ± SD responses to 45 min of exercise at 500 W of metabolic heat production in men and women.*

	Men		Women	
Variable	15-min	45-min	15-min	45-min
$\dot{V}O_2$ ($L \cdot min^{-1}$)	1.9 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	1.8 ± 0.1
$\dot{V}O_2$ (% control $\dot{V}O_{2max}$) ‡	51.7 ± 4.6	51.5 ± 4.5	65.7 ± 10.4	66.8 ± 8.4
$\dot{Q}$ ($L \cdot min^{-1}$)	11.2 ± 1.8	11.3 ± 2.2	11.0 ± 1.0	10.3 ± 1.4
SV ($mL \cdot beat^{-1}$) **	83 ± 17	74 ± 17	70 ± 6	59 ± 7
HR ($beats \cdot min^{-1}$) **‡	138 ± 20	153 ± 21	159 ± 9	175 ± 5
O_2 pulse ($mL \cdot beat^{-1}$) **‡	13.8 ± 2.0	12.3 ± 1.5	11.3 ± 0.6	10.4 ± 0.8
BLA ($mmol \cdot L^{-1}$)	1.8 ± 1.0	1.7 ± 0.8	2.4 ± 0.7	2.7 ± 0.9†
MAP (mm Hg)	104 ± 6	108 ± 5	104 ± 7	104 ± 10
TPR ($dyn \cdot s^{-1} \cdot cm^{-5}$) **	756.6 ± 111.1	788.8 ± 158.4	759.1 ± 72.2	813.9 ± 79.5
LSR ($mg \cdot cm^{-2} \cdot min^{-1}$)	0.61 ± 0.08	0.86 ± 0.24	0.60 ± 0.09	0.71 ± 0.14
ΔSkBF from rest (%)	472 ± 401	713 ± 628	260 ± 129	392 ± 151
ΔPV from rest (%)	-8 ± 5	-10 ± 5	-8 ± 5	-10 ± 6
$\overline{T}_{sk}$ (°C)	35.4 ± 0.8	35.4 ± 1.0	35.4 ± 0.5	35.6 ± 0.7
$\overline{T}_b$ (°C) **	37.4 ± 0.3	38.0 ± 0.5	37.6 ± 0.2	38.5 ± 0.4
RPE **‡	11 ± 1	14 ± 1	13 ± 1	16 ± 2

$\dot{V}O_2$, oxygen uptake; $\dot{Q}$, cardiac output; SV, stroke volume; HR, heart rate; BLA, blood lactate; MAP, mean arterial pressure; TPR, total peripheral resistance; LSR, local sweat rate; SkBF, skin blood flow; PV, plasma volume; $\overline{T}_{sk}$, mean skin temperature; $\overline{T}_b$, mean body temperature; T_{re}, rectal temperature; RPE, rating of perceived exertion. ** $P < 0.05$ for main effect of time across men and women; ‡ $P < 0.05$ for main effect of group across both time points; * $P < 0.05$ versus 15-min value within the same group; † $P < 0.05$ versus value at the same time point in men.

TABLE 4.4. *Mean ± SD responses to maximal exercise.*

	Men			Women		
Variable	Control	15-min	45-min	Control	15-min	45-min
$\dot{V}O_2$ **‡ ($L \cdot min^{-1}$)	3.6 ± 0.3	3.3 ± 0.4	3.0 ± 0.4§	2.8 ± 0.5†	2.9 ± 0.3†	2.3 ± 0.3§
$\dot{V}O_2$ **‡ ($mL \cdot kg \cdot min^{-1}$)	56.1 ± 8.1	51.5 ± 8.9	45.3 ± 7.0§	42.4 ± 9.4†	43.8 ± 7.2	35.7 ± 6.1§
$\dot{V}O_2$ ** ($mL \cdot kgFFM \cdot min^{-1}$)	63.5 ± 7.1	57.3 ± 7.1	51.4 ± 7.2§	56.9 ± 10.2	58.9 ± 7.5	47.8 ± 6.9
$\dot{V}_E$, STPD **‡ ($L \cdot min^{-1}$)	111.4 ± 19.3	108.9 ± 16.4	89.6 ± 16.1§	91.9 ± 16.4	87.4 ± 12.7	78.3 ± 13.9
RER **	1.10 ± 0.05	1.05 ± 0.07	0.97 ± 0.05§	1.11 ± 0.06	1.01 ± 0.06	0.96 ± 0.08§
HR ($beats \cdot min^{-1}$)	187 ± 16	187 ± 15	188 ± 18	192 ± 6	192 ± 5	192 ± 5
O_2 pulse **‡ ($mL \cdot beat^{-1}$)	19.5 ± 1.5	17.7 ± 2.5	15.8 ± 2.5§	14.6 ± 2.4†	15.0 ± 1.8	12.2 ± 1.4§
BLA ** ($mmol \cdot L^{-1}$)	7.3 ± 1.6	7.2 ± 1.9	5.3 ± 1.8§	7.6 ± 1.6	8.3 ± 2.2	5.3 ± 1.4§
LSR ($mg \cdot cm^{-2} \cdot min^{-1}$)	—	—	0.95 ± 0.20	—	—	0.72 ± 0.15†
ΔSkBF from rest (%)	—	—	1037 ± 841	—	—	463 ± 184
ΔPV from rest (%)	—	—	13 ± 5	—	—	12 ± 7
$\overline{T}_{sk}$ (°C)	—	—	35.4 ± 1.0	—	—	35.5 ± 0.8
$\overline{T}_b$ (°C)	—	—	38.1 ± 0.6	—	—	38.6 ± 0.4
RPE	19 ± 1	19 ± 1	19 ± 1	19 ± 1	19 ± 1	20 ± 0
Test duration (min) **	10.5 ± 2.5	9.5 ± 1.8	6.2 ± 1.7§	11.2 ± 3.4	8.4 ± 1.6	4.8 ± 1.9§
Power output (W) **	277 ± 41	261 ± 45	225 ± 25§	236 ± 20†	239 ± 36	194 ± 31§
Δ Body mass (%)	—	-1.2 ± 0.2	-1.8 ± 0.7	–	-1.0 ± 0.6	-1.4 ± 0.9

$\dot{V}O_2$, oxygen uptake; FFM, fat-free mass; $\dot{V}_E$, minute ventilation; RER, respiratory exchange ratio; HR, heart rate; BLA, blood lactate; PV, plasma volume; $\overline{T}_{sk}$, mean skin temperature; $\overline{T}_b$, mean body temperature; T_{re}, rectal temperature; RPE, rating of perceived exertion. § $P < 0.05$ versus control; ** $P < 0.05$ for main effect of time across men and women for the experimental trials; ‡ $P < 0.05$ for main effect of group across both time points for the experimental trials; * $P < 0.05$ versus 15-min value within the same group; † $P < 0.05$ versus value at the same time point in men.

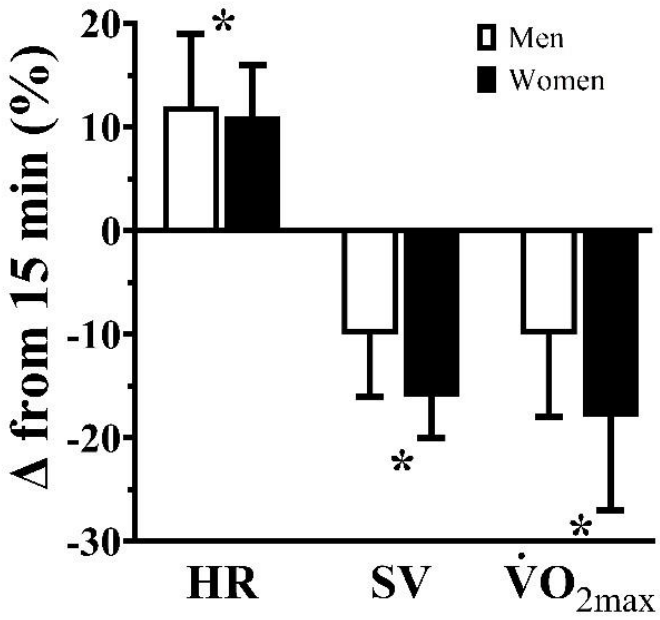

Figure 4.1 Changes in mean (± SD) heart rate (HR), stroke volume (SV), and maximal oxygen uptake ($\dot{V}O_{2max}$) between 15 and 45 min of cycling at 500 W of metabolic heat production in men and women. No significant differences between men and women for all change scores.* P < 0.05 for time main effect.

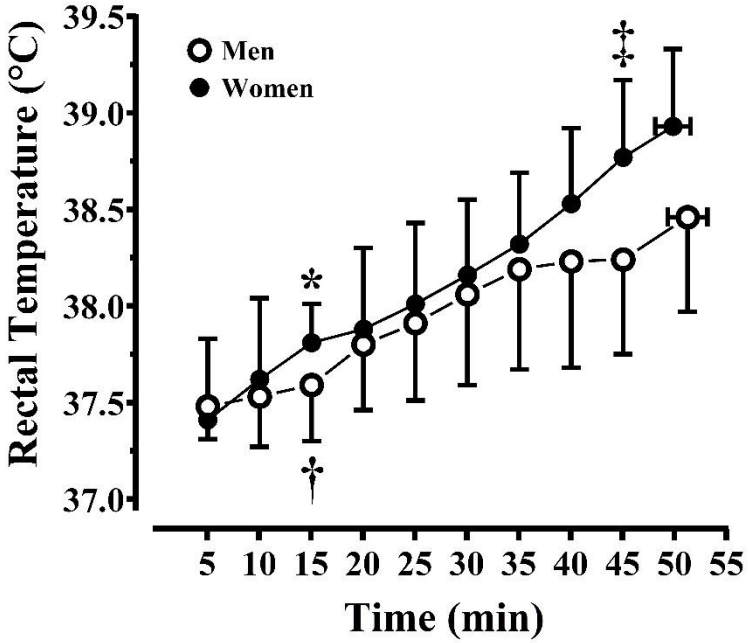

Figure 4.2 Mean (± SD) rectal temperature responses during 45 min of cycling at 500 W of metabolic heat production and at maximum after a GXT in men and women. * 15-min vs. 45-min value in women, P < 0.05; † 15-min vs. 45-min value in men, P < 0.05; ‡ vs. men at 45 min. There were no significant differences between men and women at maximum, P > 0.05.

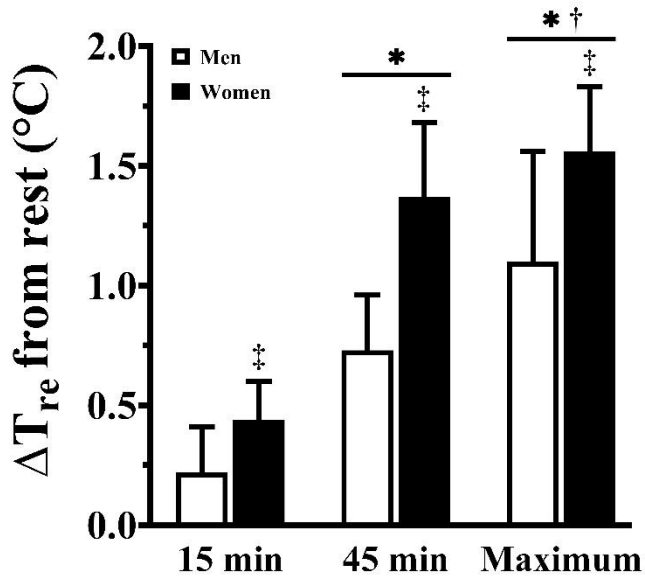

Figure 4.3 Mean (± SD) change in rectal temperature (ΔT_{re}) from baseline at 15 min, 45 min, and at maximum after 45 min of cycling at 500 W of metabolic heat production in men and women. ‡ $P < 0.05$ vs. men; * $P < 0.05$ for main effect of time vs. 15-min value; † $P < 0.05$ for main effect of time vs. 45-min value.

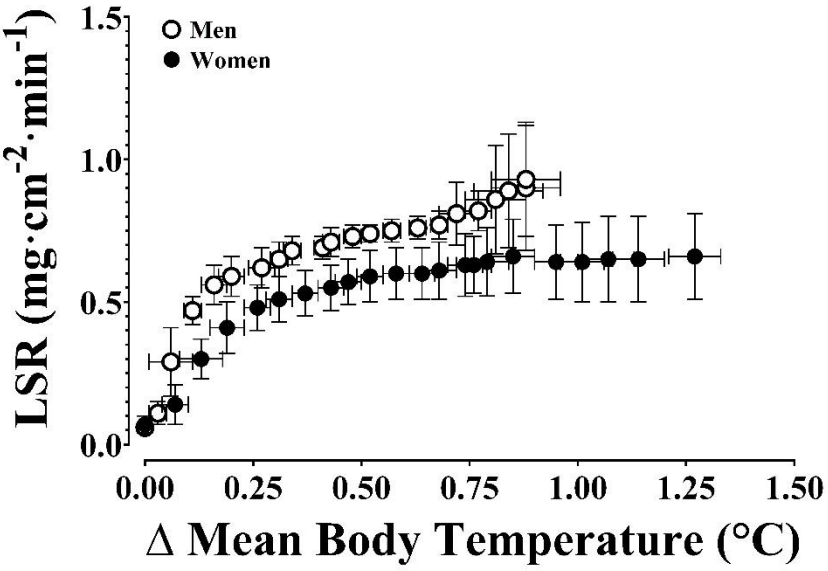

Figure 4.4 Mean (± SD) local sweat rate (LSR) vs. change (Δ) in mean body temperature from rest in men and women during 45 min of cycling at 500 W of metabolic heat production.

DISCUSSION

This study is the first to investigate the influence of sex, as a biological variable, on the relationship between cardiovascular (CV) drift and maximal oxygen uptake ($\dot{V}O_{2max}$) during prolonged exercise heat stress. Based on the statistical findings, our hypothesis was not supported in that the magnitude of CV drift and accompanying decrement in $\dot{V}O_{2max}$ was not different between men and women, despite the expectation that participants exercised at a rate of metabolic heat production known to result in sudomotor sex differences (18, 19). While women had a lower thermosensitivity for LSR—which resulted in greater increases in T_{re} from rest at each time point as well as higher T_{re} at 45 min—this did not translate into greater CV drift and concomitant decrements in $\dot{V}O_{2max}$.

The CV drift/$\dot{V}O_{2max}$ relationship observed in both groups agrees with previous findings, where the increase in HR and simultaneous fall in SV that manifest during prolonged moderate-intensity aerobic exercise are proportional to subsequent reductions in $\dot{V}O_{2max}$ (19, 32, 45, 46, 48-50). Therefore, this study further supports the notion that CV drift and decrements in $\dot{V}O_{2max}$ during exercise in the heat could be causally related.

For men in this study, magnitudes of CV drift and decreased $\dot{V}O_{2max}$ were relatively lower than those compiled from 5 previous studies (20, 33, 46, 47, 49) in a review by Wingo et al. (48), despite environmental and exercise conditions being similar. For example, one study (49) observed the same increase in HR but greater decreases in SV (16%) and $\dot{V}O_{2max}$ (19%) between 15 and 45 min of cycling at 60% $\dot{V}O_{2max}$ in 35 °C. On average, the men in the prior studies were more aerobically fit, and while rate of metabolic heat production was not reported, it was estimated to be around 740 W. Greater rates of metabolic heat production likely explain the greater changes in T_{re} in those studies (10, 16, 26, 29), which likely resulted in greater CV

strain (manifested as CV drift) (21, 23, 33) and accompanying decrements in $\dot{V}O_{2max}$ compared to the current study.

In contrast to men, CV drift and reductions in $\dot{V}O_{2max}$ for women in the present study were twice as large as those reported in the only other study of this sort that tested women (50). Discrepancies between the present study and the prior study are likely attributable to the fact that the prior study was not performed in the heat, which would be expected to impact the magnitude of CV drift and accompanying declines in $\dot{V}O_{2max}$ (33). Additionally, exercise in the prior study was performed at a lower absolute metabolic intensity ($\dot{V}O_2$)—and presumably rate of metabolic heat production—than the current study, which likely resulted in less thermal and CV strain, and ultimately, impact on $\dot{V}O_{2max}$.

It was intended that the prescribed heat load would exceed a level at which women can effectively meet the evaporative requirement for heat loss, leading to increased heat storage and a greater ΔT_{re} than men. This objective was essentially achieved in that T_{re} increased 0.4 °C more in women compared to men between 15 and 45 min during 45MIN, which resulted in higher values at 45 min. While not statistically different, values at maximum were 0.47 ± 0.17 °C higher in women. LSR was nearly equal between men and women at 15 min, which supports previous findings of no sex differences for the onset threshold for sweating (15, 17, 18). However, values for men were 20% higher than women at 45 min (Figure 4.4) and 28% higher after the GXT (Table 4.4). This effect was likely because of a smaller increase in sweating between 15 and 45 min in women compared to men, indicating that women had lower sweating responsiveness compared to men. The LSR increase per unit mean body temperature increase (slope, or thermosensitivity), was attenuated in women compared to men (Table 4.3). Based on quantitative assessments during exercise by Gagnon and Kenny (18, 19), as well as follow-up

dose-response experiments (15), it is likely LSR responses for women in the present study reflect blunted sudomotor thermosensitivity.

Apparently, the observed sex differences in sweating sensitivity, and accompanying higher thermal strain in women, were not substantial enough to drive differences in CV strain (indexed as CV drift) and accompanying decrements in $\dot{V}O_{2max}$. If exercise had been more prolonged, and thermosensitivities of men and women followed the same trend or differences became even more distinct, greater differences in heat storage and T_{re} may have occurred, resulting in augmented CV strain and greater CV drift in women compared to men. In this circumstance, greater decrements in $\dot{V}O_{2max}$ would be expected for women. We speculate the mechanisms explaining the observed decreases in $\dot{V}O_{2max}$ likely overlap with the same parameters that characterize CV drift, chiefly SV. An examination of the components of the Fick principle offers insight. HR values at maximum were not different across control and experimental treatments, suggesting that cardiovascular capacity had been attained. If arteriovenous oxygen difference [$(a-\bar{v})O_2$] was not reduced (22, 41, 44), reductions in SV during submaximal exercise that persisted during maximal exercise provide a plausible explanation.

PERSPECTIVES

Although decreases in SV were not statistically different between men and women, the 22% larger decrement in women was accompanied by a decrement in $\dot{V}O_{2max}$ that was twice as large as men, which may have been physiologically meaningful. Had exercise been protracted, the lower thermosensitivity of sweating in women may have resulted in greater heat storage, higher thermal strain, and ensuing greater CV strain (e.g., CV drift), which could have resulted in even greater differences in decrements in $\dot{V}O_{2max}$ between sexes. Future investigations should determine if these effects become more apparent during longer exercise bouts or during exercise

performed at a higher heat load than that prescribed in the present study. Additionally, previous research (18) has shown that thermoregulatory responses do not differ between men and women during exercise at lower levels of metabolic heat production. Further research is needed to determine if CV responses are more similar between sexes at a lower rate of metabolic heat production.

CONCLUSION

This study investigated the influence of sex differences on the relationship between CV drift and $\dot{V}O_{2max}$ during cycling exercise in the heat performed at a fixed absolute rate of metabolic heat production. Men and women experienced a magnitude of drift in HR and SV—and accompanying decrement in $\dot{V}O_{2max}$—between 15 and 45 min of exercise that was comparable. Although not statistically significant, women did experience larger decrements in SV and $\dot{V}O_{2max}$. Given the blunted sweating in women at the rate of metabolic heat production employed in this study, a longer duration of exercise or higher rate of metabolic heat production may have resulted in greater CV drift and ensuing decrements in $\dot{V}O_{2max}$ in women compared to men.

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CHAPTER 5

CONCLUSION

Cardiovascular (CV) drift manifests during constant-rate, submaximal, aerobic exercise after ~ 10 min. The progressive increase in heart rate (HR) and simultaneous fall in stroke volume (SV) after this time are associated with reductions in maximal oxygen uptake ($\dot{V}O_{2max}$) that are generally proportional to the magnitude of CV drift that occurred. Hyperthermia may have the greatest effect on this relationship, where higher levels of hyperthermia will typically result in greater magnitudes of CV drift, and thereby reductions in $\dot{V}O_{2max}$.

Most previous investigations on the relationship between CV drift and $\dot{V}O_{2max}$ included only male participants. The exclusion of women in these studies was likely because of lower fitness levels than men, or to avoid controlling for the menstrual cycle, and thereby confounding results with higher core temperatures in the luteal phase versus follicular phase. Further, men in these studies had high levels of aerobic fitness, and exercise was prescribed at a fixed relative metabolic intensity (%$\dot{V}O_{2max}$). Therefore, these studies provided little information on how CV drift and associated decreases in $\dot{V}O_{2max}$ may be influenced by core temperature and thermoregulatory differences associated with the menstrual cycle; differences in fitness level accompanied by differences in metabolic heat production among individuals during exercise at a given %$\dot{V}O_{2max}$; and sudomotor sex differences between men and women.

Women demonstrate decreased thermosensitivity for sweating during prolonged exercise at high requirements for heat loss, resulting in decreased evaporative heat loss and increased heat storage. In contrast, men have a greater maximal sweating capacity than women, and thus, can

perform the same exercise at a lower core body temperature. The interdependence between the thermoregulatory and cardiovascular systems suggests that HR and SV responses that characterize CV drift are affected more in women at high heat loads, which means $\dot{V}O_{2max}$ is reduced more in consequence compared to men.

The primary purpose of this research was to determine the extent to which hyperthermia resulting from hormonal fluctuations in the menstrual cycle, fitness level, and metabolic heat production, and sex—as a biological variable—influence the relationship between CV drift and $\dot{V}O_{2max}$ during prolonged exercise heat stress. Secondarily, this research was performed to include more women in studies investigating thermoregulatory and cardiovascular responses during exercise.

Cardiovascular drift and $\dot{V}O_{2max}$ were measured in 3 separate studies during 45 min of submaximal cycling exercise in a hot environment (35 °C). CV drift was assessed between 15 and 45 min during the 45-min trials, followed immediately by a graded exercise test (GXT) to measure $\dot{V}O_{2max}$. Separate 15-min trials were performed to determine the change in $\dot{V}O_{2max}$ during the same time interval that CV drift occurred. Only women were recruited to participate in Studies 1 and 2, while men were match-paired with women for body size in Study 3. All participants were considered recreationally active; however, to determine the effect of fitness level in Study 2, the low-fit group of women engaged in less physical activity but were not sedentary. Hormone status was verified in each study for women; however, this was particularly important for Study 1, where phase of the menstrual cycle—i.e., follicular phase (FP) vs. luteal phase (LP)—was manipulated to study its effect on CV drift and the resulting decrement in $\dot{V}O_{2max}$. Exercise was prescribed at 60% $\dot{V}O_{2max}$. Study 2 employed a within-between subject design to determine the impact of fitness level and metabolic heat production on CV drift and

$\dot{V}O_{2max}$. Four experimental trials were required in this study; 2 were performed at 60% $\dot{V}O_{2max}$ (REL), and 2 were performed at 500 W of metabolic heat production (FIXED). This level of metabolic heat production was also implemented in Study 3 in an effort to exceed a sudomotor sex-difference threshold in women and drive greater levels of hyperthermia than exhibited in men, which could reveal sex differences in the relationship between CV drift and $\dot{V}O_{2max}$.

For Study 1, T_{re} was ~ 0.3 °C higher at rest and during exercise in LP compared to FP. However, this may not be indicative of differences in heat storage, considering the change in T_{re} during exercise was not different between phases. Because levels of hyperthermia were similar, CV drift and $\dot{V}O_{2max}$ did not differ between phases of the menstrual cycle. Interestingly, the increase in HR was ~ 9% for both phases, but the decrement in SV was significantly greater in LP (16%) versus FP (11%); however, absolute SV was higher at 15 min in LP, so the greater proportional decrease resulted in absolute levels at 45 min that were similar to FP. As such, similar SV values between phases before the GXT explained the similar change in $\dot{V}O_{2max}$ (13% and 16% for FP and LP, respectively).

Findings for Study 2 satisfied both study hypotheses. For REL, CV drift and $\dot{V}O_{2max}$ were significantly greater in high-fit participants (HI) and accompanied by greater T_{re} during exercise compared to low-fit (LO). HR increased 50% more in HI, and the decrease in SV was twice that of LO (16% versus 8%, respectively). Differences in $\dot{V}O_{2max}$ reductions were even greater (16% and 5% in HI versus LO, respectively), and end-exercise T_{re} was different between groups (0.5 °C). These effects were likely driven by the greater rate of metabolic heat production in HI at 60% $\dot{V}O_{2max}$, given that fitness level did not modulate the CV drift/$\dot{V}O_{2max}$ relationship when metabolic heat production was normalized between HI and LO during FIXED trials, despite LO

exercising at a significantly higher relative metabolic intensity (+16 percentage points versus HI).

Sex differences in Study 3 did not reach statistical significance regarding CV drift and $\dot{V}O_{2max}$. Absolute HR was ~ 20 beats·min^{-1} higher in women at both time points, but the change between 15 and 45 min did not differ from men. The magnitudes of decrease in SV and $\dot{V}O_{2max}$ were 22% and 50%, respectively, greater in women compared to men, but these differences were not statistically different. Despite exercising at the same level of heat production as men, sweating responses plateaued after 15 min in women, resulting in significantly lower sweat rates and higher T_{re} at maximum.

The results presented in this dissertation support the following conclusions:

1. Elevated resting T_{re} in the luteal phase of the menstrual cycle does not affect the change in T_{re} during exercise, and thus, CV drift and $\dot{V}O_{2max}$ compared to the follicular phase of the menstrual cycle.

2. Fitness level does not modulate CV drift and $\dot{V}O_{2max}$ during heat stress, independent of metabolic heat production.

3. Sex differences in sweating did not affect CV drift and reductions in $\dot{V}O_{2max}$ during 45 min of exercise at the prescribed heat load but may become influential during longer bouts of exercise in the heat.

4. Across each study and condition, magnitudes of CV drift were proportional to associated decrements in $\dot{V}O_{2max}$, thereby supporting the notion that CV drift and decrements in $\dot{V}O_{2max}$ during exercise heat stress are causally related.

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APPENDIX A

STUDY 1 INSTITUTIONAL REVIEW BOARD CERTIFICATION

THE UNIVERSITY OF ALABAMA® | Office of the Vice President for Research & Economic Development
Institutional Review Board for the Protection of Human Subjects

July 14, 2016

Tori Stone
Department of Kinesiology
College of Education
The University of Alabama
Box 870314

Re: IRB Protocol # 16-009-ME
"Cardiovascular Drift: Menstrual Phase Dependent Fluctuations"

Ms. Stone:

The University of Alabama IRB has received the revisions requested by the full board on 4/21/16. The board has reviewed the revisions and your protocol is now approved for a one-year period. Please be advised that your protocol will expire one year from the date of approval, 4/14/16.

If your research will continue beyond this date, complete the Renewal Application Form. If you need to modify the study, please submit the Modification of An Approved Protocol Form. Changes in this study cannot be initiated without IRB approval, except when necessary to eliminate apparent immediate hazards to participants. When the study closes, please complete the Request for Study Closure Form.

Should you need to submit any further correspondence regarding this proposal, please include the assigned IRB application number. Please use reproductions of the IRB approved stamped consent/assent forms to obtain consent from your participants.

Good luck with your research.

Sincerely,

358 Rose Administration Building | Box 870127 | Tuscaloosa, AL 35487-0127
205.348.8461 | Fax 205.348.7189 | Toll Free 1.877.820.3066

THE UNIVERSITY OF ALABAMA | Office of the Vice President for Research & Economic Development
Office for Research Compliance

March 28, 2017

Tori Stone
Department of Kinesiology
College of Education
The University of Alabama
Box 870314

Re: IRB Protocol # 16-009-ME-R1
"Cardiovascular Drift: Menstrual Phase Dependent Fluctuations"

Ms. Stone:

The University of Alabama Medical IRB recently met to consider your renewal application. The IRB voted to approve your protocol for a period of one year.

Your application will expire on March 8, 2018. You will receive a notice of the expiration date 90 days in advance. If your research will continue beyond this date, complete the renewal portions of the FORM: IRB Renewal Application. If you need to modify the study, please submit FORM: Modification of An Approved Protocol. Changes in this study cannot be initiated without IRB approval, except when necessary to eliminate apparent immediate hazards to participants. When the study closes, please complete the FORM: Request for Study Closure.

Should you need to submit any further correspondence regarding this application, please include the above application number.

Good luck with your research.

Sincerely,

358 Rose Administration Building | Box 870127 | Tuscaloosa, AL 35487-0127
205-348-8461 | Fax 205-348-7189 | Toll Free 1-877-820-3066

THE UNIVERSITY OF ALABAMA®

Office of the Vice President for
Research & Economic Development
Office for Research Compliance

November 9, 2017

Tori Stone
Department of Kinesiology
College of Education
The University of Alabama
Box 870314

Re: IRB Protocol # 16-009-ME-R1-A
"Cardiovascular Drift: Menstrual Phase Dependent Fluctuations"

Ms. Stone:

The University of Alabama Medical Institutional Review Board has reviewed the revision to your previously approved full board protocol. The board has approved the change in your protocol.

Please remember that your protocol will expire on March 8, 2018.

Should you need to submit any further correspondence regarding this proposal, please include the assigned IRB application number. Changes in this study cannot be initiated without IRB approval, except when necessary to eliminate apparent immediate hazards to participants.

Good luck with your research.

Sincerely,

358 Rose Administration Building | Box 870127 | Tuscaloosa, AL 35487-0127
205-348-8461 | Fax 205-348-7189 | Toll Free 1-877-820-3066

THE UNIVERSITY OF ALABAMA® | *Office of the Vice President for* Research & Economic Development
Office for Research Compliance

March 8, 2018

Tori Stone
Department of Kinesiology
College of Education
The University of Alabama
Box 870314

Re: IRB Protocol # 16-009-ME-R2
"Cardiovascular Drift: Menstrual Phase Dependent Fluctuations"

Ms. Stone:

The University of Alabama Medical IRB recently met to consider your renewal application. The IRB voted to approve your protocol for a period of one year.

Your application will expire on February 7, 2019. You will receive a notice of the expiration date 90 days in advance. If your research will continue beyond this date, complete the renewal portions of the FORM: IRB Renewal Application. If you need to modify the study, please submit FORM: Modification of An Approved Protocol. Changes in this study cannot be initiated without IRB approval, except when necessary to eliminate apparent immediate hazards to participants. When the study closes, please complete the FORM: Request for Study Closure.

Should you need to submit any further correspondence regarding this application, please include the above application number.

Good luck with your research.

Sincerely,

358 Rose Administration Building | Box 870127 | Tuscaloosa, AL 35487-0127
205-348-8461 | Fax 205-348-7189 | Toll Free 1-877-820-3066

THE UNIVERSITY OF ALABAMA | *Office of the Vice President for* Research & Economic Development
Office for Research Compliance

April 4, 2019

Tori Stone
Department of Kinesiology
College of Education
The University of Alabama
Box 870314

Re: IRB Protocol # 16-009-ME-R3
"Cardiovascular Drift: Menstrual Phase Dependent Fluctuations"

Ms. Stone:

The University of Alabama Institutional Review Board has granted approval for your continuing review application. Your continuing review application has been given full board approval according to 45 CFR part 46.

The approval for your application will lapse on February 6, 2020. If your research will continue beyond this date, please submit a continuing review to the IRB as required by University policy before the lapse. Please note, any modifications made in research design, methodology, or procedures must be submitted to and approved by the IRB before implementation. Please submit a final report form when the study is complete.

Please use reproductions of the IRB approved informed consent form to obtain consent from your participants.

Good luck with your research.

Sincerely,

358 Rose Administration Building | Box 870127 | Tuscaloosa, AL 35487-0127
205-348-8461 | Fax 205-348-7189 | Toll Free 1-877-820-3066

APPENDIX B

STUDY 2 INSTITUTIONAL REVIEW BOARD CERTIFICATION

THE UNIVERSITY OF ALABAMA | *Office of the Vice President for* Research & Economic Development
Office for Research Compliance

July 12, 2018

Tori Stone
Department of Kinesiology
College of Education
The University of Alabama
Box 870314

Re: IRB Protocol # 18-007-ME
"Cardiovascular Drift and Maximal Oxygen Uptake During Heat Stress in Women with Different Fitness Levels"

Ms. Stone:

The University of Alabama Medical IRB has granted initial approval of the above application for a one-year period. Please be advised that your protocol will expire on June 13, 2019.

If your research will continue beyond this date, complete the Renewal Application Form. If you need to modify the study, please submit the Modification of An Approved Protocol Form. Changes in this study cannot be initiated without IRB approval, except when necessary to eliminate apparent immediate hazards to participants. When the study closes, please complete the Request for Study Closure Form.

Should you need to submit any further correspondence regarding this proposal, please include the assigned IRB application number. Please use reproductions of the IRB approved stamped consent forms to obtain consent from your participants.

Good luck with your research.

Sincerely,

358 Rose Administration Building | Box 870127 | Tuscaloosa, AL 35487-0127
205-348-8461 | Fax 205-348-7189 | Toll Free 1-877-820-3066

THE UNIVERSITY OF ALABAMA | *Office of the Vice President for* Research & Economic Development
Office for Research Compliance

June 6, 2019

Tori Stone
Department of Kinesiology
College of Education
The University of Alabama
Box 870314

Re: IRB Protocol # 18-007-ME-R1
"Cardiovascular Drift and Maximal Oxygen Uptake During Heat Stress in Women with Different Fitness Levels"

Ms. Stone:

The University of Alabama Institutional Review Board has granted approval for your continuing review application. Your continuing review application has been given full board approval according to 45 CFR part 46.

The approval for your application will lapse on June 5, 2020. If your research will continue beyond this date, please submit a continuing review to the IRB as required by University policy before the lapse. Please note, any modifications made in research design, methodology, or procedures must be submitted to and approved by the IRB before implementation. Please submit a final report form when the study is complete.

Please use reproductions of the IRB approved informed consent form to obtain consent from your participants.

Good luck with your research.

Sincerely,

358 Rose Administration Building | Box 870127 | Tuscaloosa, AL 35487-0127
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APPENDIX C

STUDY 3 INSTITUTIONAL REVIEW BOARD CERTIFICATION

THE UNIVERSITY OF ALABAMA® | *Office of the Vice President for* Research & Economic Development
Office for Research Compliance

July 12, 2018

Tori Stone
Department of Kinesiology
College of Education
The University of Alabama
Box 870314

Re: IRB Protocol # 18-006-ME
"Sex Differences in Cardiovascular Drift and Maximal Oxygen Uptake During Heat Stress"

Ms. Stone:

The University of Alabama Medical IRB has granted initial approval of the above application for a one-year period. Please be advised that your protocol will expire on June 13, 2019.

If your research will continue beyond this date, complete the Renewal Application Form. If you need to modify the study, please submit the Modification of An Approved Protocol Form. Changes in this study cannot be initiated without IRB approval, except when necessary to eliminate apparent immediate hazards to participants. When the study closes, please complete the Request for Study Closure Form.

Should you need to submit any further correspondence regarding this proposal, please include the assigned IRB application number. Please use reproductions of the IRB approved stamped consent forms to obtain consent from your participants.

Good luck with your research.

Sincerely,

358 Rose Administration Building | Box 870127 | Tuscaloosa, AL 35487-0127
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THE UNIVERSITY OF ALABAMA® | *Office of the Vice President for* Research & Economic Development
Office for Research Compliance

June 6, 2019

Tori Stone
Department of Kinesiology
College of Education
The University of Alabama
Box 870314

Re: IRB Protocol # 18-006-ME-R1
"Sex Differences in Cardiovascular Drift and Maximal Oxygen Uptake During Heat Stress"

Ms. Stone:

The University of Alabama Institutional Review Board has granted approval for your continuing review application. Your continuing review application has been given full board approval according to 45 CFR part 46.

The approval for your application will lapse on June 5, 2020. If your research will continue beyond this date, please submit a continuing review to the IRB as required by University policy before the lapse. Please note, any modifications made in research design, methodology, or procedures must be submitted to and approved by the IRB before implementation. Please submit a final report form when the study is complete.

Please use reproductions of the IRB approved informed consent form to obtain consent from your participants.

Good luck with your research.

Sincerely.

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