The Thesis Committee for Melissa Faith Keller
Certifies that this is the approved version of the following Thesis:

The Effect of the Menstrual Cycle on Hemoglobin Mass

APPROVED BY
SUPERVISING COMMITTEE:

Sophie Lalande, Supervisor

Hirofumi Tanaka

The Effect of the Menstrual Cycle on Hemoglobin Mass

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Melissa Faith Keller

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Dedication

Dedicated to my parents, Brett and Patricia, for your endless love and encouragement. Your support as I pursued my dreams has been essential in my educational journey. I love you both with all my heart.

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Thank you to my advisor, mentor, and friend, Dr. Sophie Lalande. The hours of support, conversation, debate, and attention you gave me were unparalleled. Your consistency to help me develop as a scientist and as a person has left a lasting impression.

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Most importantly, I'd like to acknowledge and give all the credit to my Lord and Savior, Jesus Christ.

Abstract

The Effect of the Menstrual Cycle on Hemoglobin Mass

Melissa Faith Keller, M. S. Kin.

The University of Texas at Austin, 2019

Supervisor: Sophie Lalande

The impact of the menstrual cycle on oxygen-carrying capacity remains equivocal. Previous studies reported either reductions or no significant changes in hemoglobin concentration during the follicular phase when compared to the luteal phase of the menstrual cycle. Changes in plasma volume associated with fluctuating estrogen and progesterone levels likely contribute to the variations in hemoglobin concentration observed throughout the menstrual cycle. Thus, measures of hemoglobin concentration do not accurately represent the oxygen-carrying capacity of the blood. Hemoglobin mass represents a more direct measure of oxygen-carrying capacity. However, the impact of menstrual blood loss on hemoglobin mass remains unknown. **PURPOSE:** To determine the effect of the menstrual cycle on hemoglobin mass in pre-menopausal women. **METHODS:** Twenty-one women (age: 23 ± 6 years, height: 167 ± 7 cm, weight: 66 ± 13 kg) with a regular menstrual cycle using (n = 9) and not using hormonal contraceptives participated in the study. Hemoglobin mass was assessed using the carbon monoxide rebreathing technique on three separate occasions. Visits for women using hormonal contraceptives were scheduled in the early follicular phase (3-5 days post-onset of menses),

late follicular phase (14 days post-onset of menses), and mid-to-late luteal phase (10 days after the late follicular visit). Visits for women not using hormonal contraceptives were scheduled in the early follicular phase (3-5 days post-onset of menses), late follicular phase (1-2 days post-surge of luteinizing hormone in urine), and mid-to-late luteal phase (10 days after the late follicular visit). **RESULTS:** No differences were observed in hemoglobin concentration across phases of the menstrual cycle (early follicular: 12.9 ± 1.3 g/dl, late follicular: 12.7 ± 0.9 g/dl, mid-to-late luteal: 12.8 ± 0.9 g/dl, p = 0.08). Likewise, hemoglobin mass did not significantly differ between menstrual cycle phases (early follicular: 606 ± 73 g, late follicular: 602 ± 73 g, mid-to-late luteal: 606 ± 68 g, p = 0.90). Hemoglobin mass for women using hormonal contraceptive tended to be higher than non-users across the menstrual cycle (early follicular: 629 ± 53 g vs. 590 ± 83 g, late follicular: 635 ± 58 g vs. 577 ± 75 g, mid-to-late luteal: 626 ± 61 vs. 592 ± 71 g, respectively, p = 0.12). **CONCLUSION:** The menstrual cycle has no significant impact on hemoglobin mass or oxygen-carrying capacity in eumenorrheic women. The use of hormonal contraceptives may improve oxygen-carrying capacity.

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LITERATURE REVIEW

The menstrual cycle consists of physiological endometrial shedding and blood loss from endometrial arteries (Raven et al., 2013). It is divided into two phases, the follicular and luteal phase, and lasts on average 28 days (Javaid et al., 2007). The follicular phase, also known as the proliferative or pre-ovulatory phase, begins with menses and ends with ovulation. The luteal phase, also known as the secretory or post-ovulatory phase, follows a surge in gonadotropic hormones which stimulates the release of progesterone and estrogen from the corpus luteum and ovarian follicles, respectively (Marsh & Jenkins, 2002). Estrogen peaks just prior to ovulation and progesterone peaks in the mid-luteal phase (Stachenfeld, 2008) (Figure 1). Both remain significantly elevated during the luteal phase compared to the follicular phase (Chapman et al, 1997). The decline in estrogen and progesterone concentration mark the end of the luteal phase and stimulates the shedding of the endometrium for the next cycle (Fraser, 1999; Javaid et al., 2007). The mean average of blood loss during menstruation ranges from 25-40 ml (Hallberg et al., 1966a; Fraser et al., 2001).

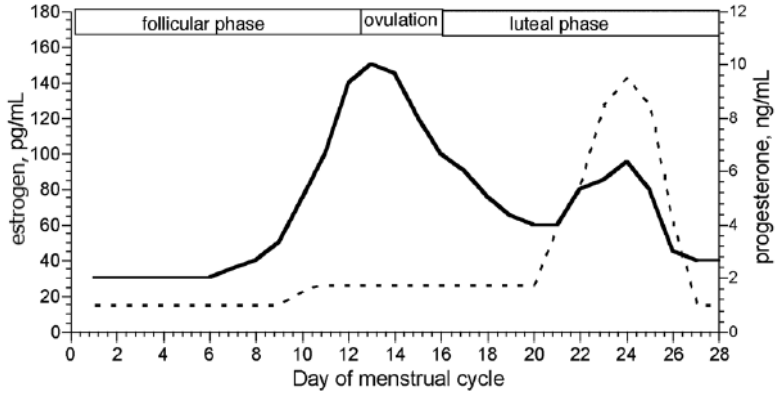

Figure 1. Natural fluctuations in estrogen and progesterone over the course of a 28-day cycle

IMPACT OF ESTROGEN AND PROGESTERONE ON FLUID REGULATION

In addition to being markers of menstrual cycle phases, estrogen and progesterone are also involved in fluid regulation. Estrogens increase plasma volume by increasing plasma protein capillary permeability (Tollan et al., 1992). 17-β-estradiol, the prominent estrogen involved in the reproductive system, contributes to fluid regulation by stimulating the liver to synthesize angiotensinogen, the substrate for the hormone renin (Gaebelein & Senay, 1982). Angiotensin I is then catalyzed from renin and converted to angiotensin II via angiotensin converting enzyme (Skeggs et al., 1954) and stimulates the release of aldosterone from the adrenal glands to promote sodium retention to increase water resorption (Aguilera & Catt, 1978). Similarly, progesterone stimulates the production of adrenal aldosterone (Szmuilowicz et al., 2006) to increase water retention. However, progesterone also acts as a natriuretic, promoting sodium diuresis (Laidlaw et al., 1962) and consequently fluid loss (Marsh & Jenkins, 2002).

Stephenson & Kolka (1988) reported a reduced resting plasma volume in the luteal phase compared to the follicular phase and attributed this to the increase in aldosterone concentration. However, the study sample size was small (n = 5) and plasma volume was calculated from an estimated blood volume and measured hematocrit values. Chapman et al. (1997) using a modified carbon monoxide rebreathing method found no significant changes in plasma volume between follicular and luteal phases. Using the Evans blue dye dilution technique, Stachenfeld et al. (2000) also reported that resting plasma volume does not change between luteal and follicular phases where plasma volume was calculated as the product of the volume and concentration of the injected dye divided by post-mixing plasma concentration. Despite the complex interactions that estrogen and progesterone

have on fluid regulation, it is generally reported that plasma volume does not changed across the luteal phase.

IMPACT OF THE MENSTRUAL CYCLE ON OXYGEN-CARRYING CAPACITY

Hemoglobin concentration

Hemoglobin concentration, the amount of hemoglobin in a volume of blood, is commonly used as a clinical marker of oxygen-carrying capacity. The hemoglobin molecule, with its four subunit polypeptide chains and corresponding heme groups (McArdle et al., 2015), functions as the oxygen-binding site on red blood cells (Hoppe-Seyler, 1892). The impact of the menstrual blood loss on hemoglobin concentration and oxygen-carrying capacity remains equivocal. Previous studies have measured variations in hemoglobin concentration between follicular and luteal phases. Vellar (1974) observed a trend towards a higher luteal hemoglobin concentration compared to the follicular phase (13.43 g/100 ml vs 13.06 g/100 ml), possibly due to an increase in progesterone and its natriuretic effect during the luteal phase. These results were confirmed by later studies which reported significantly less hemoconcentration during the follicular phase compared to the luteal phase (Kim et al., 1993; Javaid et al., 2007).

It is important to note that the stability of hemoglobin concentration depends on water movement between intra and extracellular fluid spaces. Indeed, plasma volume fluctuates with physical activity, hydration status and posture (Berkow, 2013). Postural

transitions from a supine to a standing position reduce plasma volume as the hydrostatic pressure gradient causes plasma to leak into the interstitial spaces resulting in greater hemoglobin concentration (Gaebelein & Senay, 1982). Therefore, because hemoglobin concentration depends on plasma volume levels (Janse de Jonge, 2003), hemoglobin concentration is not the most consistent measure of the amount of hemoglobin in the blood.

Red blood cell volume and hemoglobin mass

Only a handful of studies have investigated the impact of the menstrual cycle phases on red blood cells. Chapman et al. (1997) found that red blood cell mass did not change throughout menstrual cycle phases. However, as stated by the authors, red blood cell mass was calculated as total blood volume multiplied by hematocrit and is therefore still susceptible to changes in plasma volume. Later, Belza et al. (2005) considered red blood cell distribution width, a measure of the range of variation in red blood cell volume, between menstrual cycle phases and found no significant changes as well as a low day-to-day coefficient of variation of 2.4 % across the menstrual cycle.

Hemoglobin mass represents the absolute value of circulating hemoglobin content and therefore uninfluenced by changes in plasma volume. Measures of hemoglobin mass using the carbon monoxide rebreathing method are highly reliable (Schmidt & Prommer, 2005) and thus represent a direct and accurate measure of oxygen-carrying capacity (Schmidt & Prommer, 2010). To our knowledge, no previous studies have directly investigated the impact of the menstrual cycle on hemoglobin mass. In 10 female athletes across a 10-month training season, the maximal intra-individual oscillations in hemoglobin

mass was 8.5 % and the average variation in hemoglobin mass for the group was 3.3 % (Garvican et al., 2010). Furthermore, Prommer et al. (2008) determined that intra-individual oscillations for hemoglobin mass were approximately 7 % in four trained females over the course of one full year. However, neither study reported on the menstrual blood loss that these female athletes may or may not have experienced. The contribution of the menstrual cycle on hemoglobin mass remains to be determined

RELATION BETWEEN HEMOGLOBIN MASS AND MAXIMAL OXYGEN CONSUMPTION

Oxygen consumption (VO_2) represents the rate at which the body utilizes oxygen (McArdle et al., 2015) as defined by the Fick equation:

$$VO_2 = Cardiac\ output \times arteriovenous\ oxygen\ difference$$

Where cardiac output is the product of heart rate (HR) and stroke volume (SV), the volume of blood pumped with each heart beat. Arterio-venous oxygen difference is defined as arterial oxygen content (CaO_2) minus venous oxygen content (CvO_2). Thus, the Fick equation can be broken down further into the following:

$$VO_2 = (SV \times HR) \times (CaO_2 - CvO_2)$$

CaO_2 can be expanded further into the following variables:

$$CaO_2 = [Hb]\left({}^{g}/_{dl}\right) \times 1.34\left({}^{ml\ O_2}/_{g\ Hb}\right) SaO_2(\%) + 0.0032 \times PO_2(torr)$$

Where [Hb] is hemoglobin concentration, the constant 1.34 reflects the amount of O_2 capable of binding to each gram of hemoglobin, SaO_2 represents the percentage of oxygen saturation of arterial blood, and PO_2 is the partial pressure of oxygen.

At sea level, where the partial pressure of oxygen does not change and arterial oxygen saturation is maintained, hemoglobin concentration primarily determines arterial oxygen content. Higher levels of hemoglobin concentration would therefore represent a greater oxygen-carrying capacity and consequently a greater oxygen consumption. While the relation between hemoglobin concentration and maximal oxygen consumption is relatively weak (Figure 2A), a very strong relation between hemoglobin mass and maximal oxygen consumption exists (Figure 2B). Indeed, a 1 g increase in hemoglobin mass equates to a 4 ml/min increase in maximal oxygen consumption (Schmidt & Prommer, 2010).

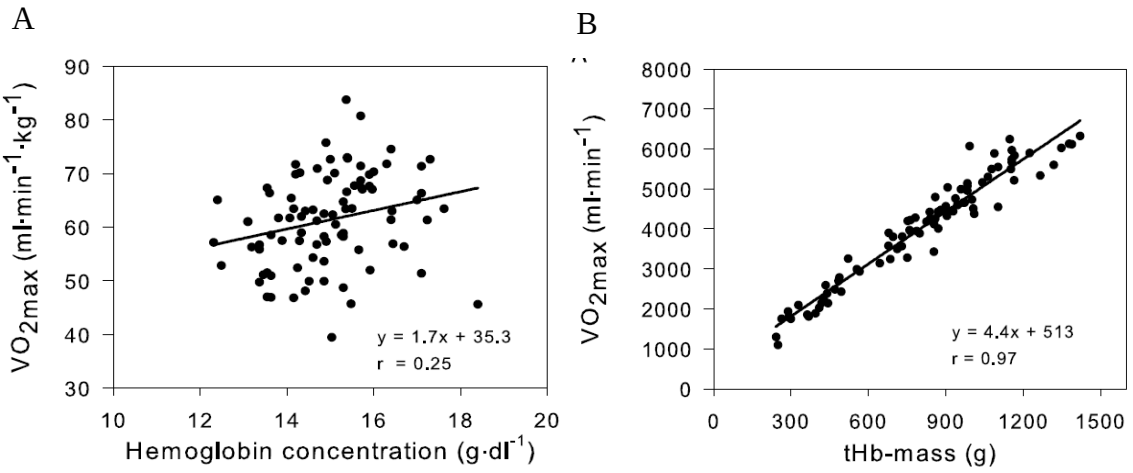

Figure 2. Relation between maximal oxygen consumption and A) hemoglobin concentration and B) hemoglobin mass (tHb-mass).

IMPACT OF HORMONAL CONTRACEPTIVES ON HEMATOLOGICAL VARIABLES

Hormonal contraceptives decrease the volume of blood lost during menses (Larsson et al., 1992). Hormonal contraceptive use has also been linked with significantly higher serum ferritin levels (Frassineii-Gunderson et al., 1985; Milman et al., 1992), a carrier for iron (Li et al., 2010), likely as a result of the lower blood loss. Indeed, both current and former users of hormonal contraception have higher serum ferritin levels and greater iron stores than those who never used hormonal contraception (Milman et al., 1992). Low serum ferritin levels have been linked with lower hemoglobin concentration (Milman et al., 1992; Åsberg et al., 2013). Furthermore, plasma iron levels have been tightly, positively correlated with erythropoiesis (Hillman et al., 1974). Indeed, once released from the marrow, maturing red blood cells bind iron to function as hemoglobin for the erythrocytic lifecycle (Adamson, 1994). Although hormonal contraceptives increase serum ferritin and hemoglobin levels (Lei et al., 1998), the specific mechanisms remain unclear. It has been hypothesized that contraceptives aide gut iron absorption, resulting in higher serum ferritin levels (Margen & King, 1975). The effect of hormonal contraceptives on hemoglobin mass remains unknown. However, since serum iron levels correlate positively to erythropoiesis, it can be hypothesized that hemoglobin mass would be higher among hormonal contraceptive users.

MANUSCRIPT

INTRODUCTION

Oxygen transport is achieved through the binding of oxygen to hemoglobin in the blood. Hemoglobin concentration, reported in grams per deciliter, serves as a clinical marker of oxygen-carrying capacity. However, hemoglobin concentration varies with fluctuations in plasma volume induced by hydration status and posture during blood sampling (Berkow, 2013), training status of the individual (Goodman et al., 2005), and time of day (Janse de Jonge, 2003). Moreover, hemoglobin concentration correlates only to a modest extent with red blood cell mass (Murphy, 2014) and maximal oxygen consumption (Schmidt & Prommer, 2010). On the other hand, hemoglobin mass, reported in grams, directly represents the oxygen-carrying capacity of the blood and strongly correlates with maximal oxygen consumption (Schmidt & Prommer, 2010).

The menstrual cycle consists of the follicular phase, which starts with menstruation and ends with ovulation, and the luteal phase. On average, menstrual blood loss ranges between 25 to 40 ml (Hallberg et al., 1966a; Hallberg et al., 1966b; Baldwin et al., 1961; Fraser et al., 2001). The effect of the menstrual cycle, specifically the monthly blood loss, on oxygen-carrying capacity remains equivocal. Hemoglobin concentration has been observed to be lower during menstruation and the follicular phase (Javaid et al., 2007; Garvican et al., 2010) in comparison to the luteal phase. Yet, others (Belza et al., 2005; Lebrun et al., 1995) found no significant changes in hemoglobin concentration across phases of the menstrual cycle. Changes in plasma volume due to varying hormone levels possibly contribute to the equivocal findings in hemoglobin concentration during the

follicular phase in comparison to luteal phase. The natriuretic effect of progesterone results in loss of sodium and water from plasma that potentially contributes to the increase in hemoglobin concentration observed in the luteal phase (Laidlaw et al., 1962). These findings further underline the fact that measures of hemoglobin concentration do not precisely estimate red blood cell mass or oxygen-carrying capacity across the menstrual cycle (Murphy, 2014). Therefore, the purpose of this study was to determine the effect of the menstrual cycle on hemoglobin mass in women with regular menstrual cycles. We hypothesized that hemoglobin mass would not change across the phases of the menstrual cycle.

METHODS

Twenty-one healthy, non-smoking women (age: 23 ± 6 years, height: 167 ± 7 cm) with regular menstrual cycles and blood loss participated in the study. Hematological variables were determined on three occasions throughout one menstrual cycle. Visits to the Clinical Exercise Physiology Laboratory were scheduled in the early follicular phase (Visit 1: 3-5 days following the onset of menses), late follicular phase (Visit 2: 1-2 days following the surge of luteinizing hormone in urine), and mid-to-late luteal phase (Visit 3: 9-10 days after Visit 2) of the menstrual cycle. Eight days following the onset of menses, women started to self-administer ovulation tests to detect luteinizing hormone in urine. Tests were administered daily until positive for ovulation. Nine out of 21 women used hormonal contraceptives (43%). Visits for women using hormonal contraceptives were scheduled in the early follicular phase (Visit 1: 3-5 days following the onset of menses), late follicular phase (Visit 2: 14 days following the onset of menses), and mid-to-late luteal phase (Visit

3: 9-10 days after Visit 2) of the menstrual cycle. On each visit, measures of blood pressure and heart rate were obtained following 5 minutes of supine rest.

Hematological variables

Hemoglobin mass, red blood cell volume, plasma volume and total blood volume were determined using the optimized carbon monoxide rebreathing method (Schmidt & Prommer, 2005; Lalande et al., 2012) and calculated according to the equations of Burge & Skinner (1995). Briefly, baseline levels for carboxyhemoglobin, hematocrit, and hemoglobin levels were first determined from a pre-rebreathing venous blood draw (ABL FLEX CO-OX, Radiometer, CA, USA). A bolus of carbon monoxide was calculated for each participant based on body surface area and hemoglobin levels (Burge & Skinner, 1995). This bolus of carbon monoxide was added to a closed-circuit rebreathing system containing room air. Participants rebreathed on this system for a period of 2 minutes. A second venous blood draw was obtained 10 minutes after the start of the rebreathing maneuver. Hemoglobin mass was calculated based on the change in carboxyhemoglobin levels induced by the carbon monoxide rebreathing.

Estradiol and progesterone concentrations

In order to confirm menstrual phase in women not using hormonal contraceptives, a blood sample was obtained from the blood draw prior to carbon monoxide rebreathing and stored at -80 °C for later analysis. Once all samples were collected, concentrations of

serum estradiol and progesterone were determined using Milliplex® MAP Kit multi-species hormone magnetic bead panel and BioVendor® enzyme-linked immunosorbent assays, respectively.

Statistical analysis

A repeated measures analysis of variance was used to evaluate the effect of different phases of the menstrual cycle (early follicular, late follicular, and mid-to-late luteal) on all variables. A two-way repeated measures analysis of variance was used to evaluate the effect of group (women using hormonal contraceptive vs. women not using hormonal contraceptive) and phases of the menstrual cycle (early follicular, late follicular, and mid-to-late luteal). When appropriate, post hoc analyses were performed using Tukey's test. Significance was set at $p \leq 0.05$. All values reported as means ± standard deviation.

RESULTS

For women not using hormonal contraceptive, Visit 1 took place 3 ± 1 days following the onset of menses, Visit 2 took place 1 ± 1 days following a positive ovulation test, and Visit 3 took place 9 ± 1 days following Visit 2. For women using hormonal contraceptive, Visit 1 took place 3 ± 1 days following onset of menses, Visit 2 took place 14 ± 3 days following menses, and Visit 3 took place 9 ± 2 days following Visit 3. Participants' characteristics are presented in Table 1.

Table 1. Participants' characteristics

	EF	LF	ML	Phase
Wt (kg)	65.7 ± 13.0	66.0 ± 12.8	66.1 ± 12.9	0.09
SBP (mmHg)	112 ± 9	110 ± 10	110 ± 9	0.12
DBP (mmHg)	70 ± 8	68 ± 7	69 ± 9	0.27
HR (bpm)	62 ± 8	67 ± 11	67 ± 8	0.04
Hb (g/dl)	12.9 ± 1.3	12.7 ± 0.9	12.8 ± 0.9	0.12
Hct (%)	39.8 ± 3.8	39.0 ± 2.6	39.3 ± 2.8	0.13
BV indexed (ml/kg)	80.1 ± 11.1	80.5 ± 10.1	80.9 ± 13.7	0.93
Hb mass indexed (g/kg)	9.4 ± 1.3	9.3 ± 1.2	9.4 ± 1.5	0.79

Wt: weight; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; Hb: hemoglobin; Hct: hematocrit; BV: blood volume. EF: early follicular; LF: late follicular; ML: mid-to-late luteal.

There was no effect of menstrual cycle phase on weight, systolic blood pressure or diastolic blood pressure. The menstrual cycle had a significant effect on heart rate, with a lower heart rate being observed in the early follicular phase in comparison to the mid-to-late luteal phase. No differences in hemoglobin concentration, hematocrit, blood volume indexed to weight or hemoglobin mass indexed to weight were observed across phases of the menstrual cycle (Table 1). Hemoglobin mass did not differ between menstrual cycle phases (main effect for phase of $p = 0.90$) (Figure 3). Similarly, there was no difference in red blood cell volume, plasma volume or total blood volume (main effect for phase of $p = 0.89$, $p = 0.78$, $p = 0.90$, respectively) (Figure 4).

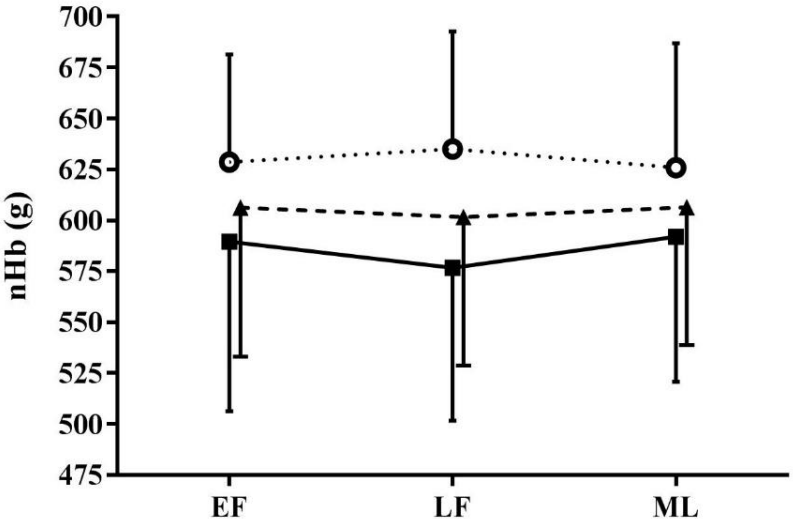

Figure 3. Hemoglobin mass (nHb) across the menstrual cycle for all participants (filled triangles), participants using hormonal contraceptives (open circles), and participants not using hormonal contraceptives (filled squares). EF: early follicular; LF: late follicular; ML: mid-to-late luteal.

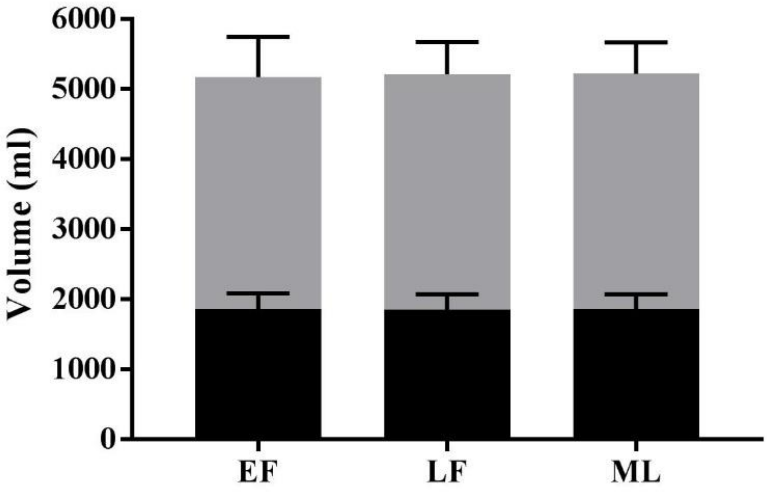

Figure 4. Red blood cell volume (black bar), plasma volume (gray bar) and total blood volume (maximal value) across phases of the menstrual cycle. EF: early follicular; LF: late follicular; ML: mid-to-late luteal.

The use of hormonal contraception did not induce any change in hematological variables across all three visits (Table 2). No differences were observed between groups for weight, systolic and diastolic blood pressure, and heart rate. There were no differences in hemoglobin mass between women using hormonal contraceptives vs. women not using hormonal contraceptives (main effect for group of $p = 0.12$) (Figure 3.) As expected, participants not using hormonal contraceptives had a peak in serum estradiol levels during the late follicular phase (early follicular: 42 ± 50 pg/ml, late follicular: 188 ± 203 pg/ml, mid-to-late luteal: 87 ± 119 pg/ml, $p = 0.05$) and a peak in serum progesterone levels during the mid-to-late luteal phase (early follicular: 0.4 ± 0.1 ng/ml, late follicular: 0.5 ± 0.2 ng/ml, mid-to-late luteal: 2.5 ± 2.7 ng/ml, $p < 0.01$), confirming testing took place in different phases of the menstrual cycle.

Table 2. Characteristics for women using hormonal contraceptives and women not using hormonal contraceptives

	No hormonal contraceptives (n=12)			Hormonal contraceptives (n=9)					
	EF	LF	ML	EF	LF	ML	Group	Phase	Group X Phase
Wt (kg)	66.6 ± 15.7	66.7 ± 15.6	67.0 ± 15.6	64.4 ± 9.0	64.9 ± 8.4	65.0 ± 8.8	0.73	0.09	0.76
BP (mmHg)	111 ± 9	106 ± 9	109 ± 10	115 ± 8	114 ± 10	112 ± 7	0.18	0.16	0.43
DBP (mmHg)	68 ± 8	66 ± 8	66 ± 8	72 ± 7	71 ± 6	74 ± 10	0.07	0.29	0.57
HR (bpm)	61 ± 8	63 ± 9	65 ± 7	64 ± 8	71 ± 11	70 ± 9	0.07	0.03	0.47
Hb (g/dl)	12.7 ± 1.2	12.6 ± 1.0	12.6 ± 0.98	13.2 ± 1.3	12.7 ± 0.7	13.0 ± 0.8	0.50	0.08	0.28
Hct (%)	39.1 ± 3.6	38.9 ± 3.0	38.9 ± 2.9	40.6 ± 4.0	39.1 ± 2.1	39.9 ± 2.6	0.50	0.08	0.27
VRBC (ml)	1810 ± 255	1771 ± 230	1820 ± 218	1929 ± 162	1949 ± 176	1924 ± 190	0.13	0.95	0.57
PV (ml)	3290 ± 538	3243 ± 446	3331 ± 423	3349 ± 642	3544 ± 441	3396 ± 500	0.48	0.68	0.27
BV (ml)	5099 ± 730	5014 ± 619	5151 ± 573	5278 ± 760	5494 ± 584	5320 ± 657	0.29	0.85	0.33
BV indexed (ml/kg)	78.5 ± 12.5	77.1 ± 11.1	79.5 ± 15.6	82.2 ± 9.1	85.1 ± 6.6	82.7 ± 11.2	0.29	0.92	0.43
Hb mass indexed (g/kg)	9.0 ± 1.3	8.8 ± 1.1	9.1 ± 1.5	9.9 ± 1.3	9.9 ± 1.0	9.8 ± 1.5	0.11	0.84	0.72

Wt: weight; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; Hb : hemoglobin; Hct: hematocrit; VRBC: red blood cell volume: PV: plasma volume; BV: blood volume. EF: early follicular; LF: late follicular; ML: mid-to-late luteal.

DISCUSSION

The purpose of this study was to determine the effect of the menstrual cycle on hemoglobin mass. No significant changes were observed in hemoglobin mass or red blood cell volume across phases of the menstrual cycle, indicating that the menstrual cycle does not impact oxygen-carrying capacity. Others (Chapman et al., 1997; Lebrun et al., 1995) have observed that mean red blood cell volume does not change between phases of the menstrual cycle. Although Chapman et al. (1997) used the carbon monoxide rebreathing method, red blood cell mass was calculated by multiplying total blood volume by hematocrit and may potentially be influenced by changes in plasma volume. Lebrun et al. (1995) analyzed blood samples by automated blood count to determine mean cell mass volume and found no significant changes between menstrual phases. On the other hand, Omorogiuwa and Igeleke (2014) found a significant reduction in red blood cell counts during menses (2 days after initial blood loss) compared to late follicular phase (day 12 of the menstrual cycle). However, they used two different groups of women for comparison between menses and the late follicular phase, and the groups' anthropometric characteristics were not provided. Garvican et al. (2010) observed that hemoglobin mass varied by 3.3 %, or 26 g, in competitive female athletes over the span of 2-10 months. Similarly, Gore et al. (1997) found no changes in hemoglobin mass over the course of 4 weeks in female rowers. These two latter studies did not control for different phases of the menstrual cycle and did not discuss whether the athletes were eumenorrheic, oligomenorrheic or amenorrhoeic. However, Garvican et al. (2010) did postulate that their observed oscillations in hemoglobin mass, although minimal, could be attributed to normal

menstrual blood loss. Overall, it appears that the blood lost during menses has no significant effect on oxygen-carrying capacity

Hemoglobin concentration variation

In the present study, hemoglobin concentration was not significantly different throughout menstrual phases, although its variation was greater than the variation in hemoglobin mass (2.1 % vs. 0.8%, respectively). Cyclical changes in estrogen and progesterone throughout the menstrual cycle contribute to fluctuations in hemoglobin concentration. The peak in estrogen during the late follicular phase produces a cascade of events ultimately resulting in plasma volume expansion (Oian et al., 1987; Tollan et al., 1992). On the other hand, the natriuretic effect of progesterone, peaking later in the luteal phase, causes fluid loss (Laidlaw et al., 1962; Oelkers, 1996). The resultant shifts in plasma volume likely explain the conflicting findings of either a lower hemoglobin concentration during the follicular phase (Javaid et al., 2007) or no significant changes in hemoglobin concentration across phases of the menstrual cycle (Belza et al., 2005; Lebrun et al., 1995).

Effect of the menstrual cycle on aerobic capacity

Numerous studies investigating the effect of the menstrual cycle on aerobic capacity have found no effect for menstrual cycle phase (Jurkowski et al., 1981; De Souza et al., 1990; Beidleman et al., 1999). Lebrun et al. (1995) found a significantly reduced absolute maximal aerobic capacity ($p = 0.04$) during the luteal phase compared to the

follicular phase in 16 regularly active female runners with normal menstrual cycles. However, this significance was lost when values were converted to relative maximal aerobic capacity ($p = 0.06$), with no change in weight between menstrual phases. A small but significant increase in core temperature of 0.5 °C occurs during the luteal phase (Schoene et al. 1981) which could reduce oxygen saturation and might explain the attenuated aerobic capacity reported by Lebrun et al. (1995). Moreover, the spike in progesterone in the luteal phase increases resting respiratory drive through central mechanisms (Schoene et al. 1981) which could further explain a reduced aerobic capacity. However, Schoene et al. (1981) was unable to link increases in respiratory drive to decrements in maximal exercise among 6 eumenorrheic athletes and 6 eumenorrheic non-athletes. Similarly, Jurkowski et al. (1981) found a mean elevated ventilatory response in the luteal phase compared to follicular phase ($p < 0.05$) in 9 females with normal menstrual cycles but there were no accompanying changes in aerobic capacity. Moreover, Beidleman et al. (1999) and De Souza et al. (1990) both reported no changes in ventilation during maximal aerobic capacity tests. Although there are some inconsistencies in the literature, phases of the menstrual cycle have no significant effect on relative maximal aerobic capacity.

Influence of hormonal contraception on hemoglobin mass

Although not statistically significant, participants using hormonal contraceptives tended to have a higher hemoglobin mass than those not using hormonal contraceptives (630 g vs. 586 g, respectively). According to Schmidt and Prommer (2010), a 1 g change in hemoglobin mass corresponds to a change of 4 ml/min in maximal oxygen consumption.

Thus, the observed maximal difference in hemoglobin mass of 58 g during the late follicular phase implies that women using hormonal contraceptives potentially have a 232 ml/min greater maximal oxygen consumption than women not using hormonal contraceptives. Despite this increase in oxygen-carrying capacity, several studies reported reductions in maximal aerobic capacity or peak aerobic capacity with the use of hormonal contraceptives. Indeed, maximal oxygen consumption was reported to be 4.7 % lower following 2 months (Lebrun et al., 2003), 11 % lower after 4 months (Casazza et al., 2002), reduced by 8 % after 6 months (Notelovitz et al., 1987), and 22 % after 12 months (Joyce et al., 2013) of oral contraceptive use. On the other hand, Rebolo et al. (2010) and Isacco et al. (2015) found no effect of hormonal contraceptive use on absolute or relative maximal aerobic capacity. It has been suggested that alternative mechanisms triggered by hormonal contraceptive use negatively impact aerobic capacity and that the magnitude of the impact has a high individual variability (Lebrun et al., 2003). Notelovitz et al. (1987) suggested one such mechanism was a reduced oxygen pulse, or the volume of oxygen consumed per heartbeat (12.1 vs. 11.2 ml/beat pre vs. post hormonal contraceptive use). Furthermore, Casazza et al. (2002) explained the reduced aerobic capacity based on the link between high exogenous estrogen and progesterone, found in hormonal contraceptive agents, with a blunted sympathetic nervous system activity and reduced catecholamine production. Less sympathetic nervous system activity would negatively impact the necessary redistribution of blood flow to working muscles by blunting vasoconstriction to inactive tissue and a lower catecholamine production would reduce hepatic and muscle glycogen mobilization during strenuous exercise. It is important to note that despite the lower maximal aerobic capacity, Lebrun et al. (2003) found no significant difference in aerobic performance at high intensities (> 90% maximal aerobic capacity) for hormonal contraceptive users.

Hormonal contraceptive use has been shown cause a 44% reduction in menstrual blood loss over a 3 - 6-month period (Larsson et al., 1992). Normal menstrual blood loss results in 16 mg of lost iron (Cook, 1990), which is eight times the normal daily iron loss of 0.5 - 1.0 mg (Green et al., 1968). Less blood loss in hormonal contraceptive users results in less iron lost each month. Iron stores are replenished through diet but also via the recycling of hemoglobin as iron bound to transferrin when the red blood cell has reached the end of its lifecycle (Adamson, 1994). Higher rates of erythropoiesis positively correlate with greater levels of iron bound to transferrin (Hillman et al., 1974). Thus, higher hemoglobin mass values in hormonal contraceptive users may be due to the combination of a reduced blood loss (Larsson et al., 1992) and improved iron conservation which helps support erythropoiesis (Hillman et al., 1974). Higher serum ferritin levels, markers of iron stores (Li et al., 2010), are associated with hormonal contraceptive use (Lei et al., 1998) and reflective of more efficient iron conservation likely contributing to the observed increase in hemoglobin mass in the present study.

Limitations

We did not control for hydration status or exercise prior to any visits. Hydration status and acute exercise greatly influence plasma volume (Berkow, 2013) but do not impact measures of hemoglobin mass since carbon monoxide molecules bind directly to the red blood cells. The order of the visits was not randomized, and the first visit always took place at the start of the menstrual cycle. However, familiarity or a lack of familiarity

with the carbon monoxide rebreathing technique does not affect measures of hemoglobin mass.

In conclusion, findings from the present study suggest that different phases of the menstrual cycle have no effect on hemoglobin mass and consequently oxygen-carrying capacity in eumenorrheic women. Our findings also suggest that oxygen-carrying capacity may be improved in women using hormonal contraception.

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