EVALUATION OF HORMONAL CONTRACEPTION EFFECTS ON STRENGTH AND RECOVERY ACROSS THE HORMONE CYCLE

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ABSTRACT

Hannah E. Cabré: Evaluation of hormonal contraception effects on strength and recovery across the hormone cycle
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Few investigations have evaluated the effects of hormonal contraception on exercise performance. The biphasic response of endogenous hormones in eumenorrheic (EUM) females is altered with the delivery of exogenous hormones, with oral contraceptives (OC) and intrauterine devices (IUD) most commonly used. Hormonal alterations may have undesirable consequences on muscle strength and power performance, fatigability, and recovery. The purpose of the study was to evaluate the effects of OC and IUD use, compared to a EUM cycle, on maximal strength, power, fatigability, and recovery between menstrual cycle (MC) phases. Sixty healthy, active women who were monophasic OC users (n=21), had a hormonal-IUD (H-IUD; n=20), or had regular naturally occurring menstrual cycles or were using a non-hormonal IUD (EUM; n=19) were evaluated in the in the follicular phase/placebo pill (FP) or in the luteal phase/active pill (LP). Strength was assessed from upper and lower body one repetition max (1RM) and peak force from isometric dynamometry. Power was assessed from counter movement jump and reactive strength index. Peak power (PP), average power (AP), time to PP, and fatigue index were measured with a repeated sprint ability test (RSA). Blood lactate, vessel diameter, and blood flow were measured prior to and immediately post-RSA. Leg press 1RM was significantly different across the MC between groups ($p=0.027$), with higher leg press 1RM in the LP for the OC group (mean difference[MD: ΔLP-FP]±standard error [SE]: Δ7.3±4.5 kg; $p=0.045$) compared to the IUD group (Δ-8.8±4.6 kg; $p=0.045$). The results showed no significant changes across the MC for other study outcomes ($p>0.05$). Despite no significance, the H-IUD group (Δ320.3±260.3 W) and EUM group (Δ24.0±13.3 W) demonstrated greater changes in AP and PP, respectively, in the LP while the OC exhibited greater AP in the FP (Δ-

248.2±254.0 W). All groups demonstrated greater blood flow in the FP (OC:Δ-133.4±10.3 mL/min; H-IUD:Δ-128.6±10.6 mL/min; EUM:Δ-137.3±10.8 mL/min) after exercise. Collectively, OC and H-IUD users have similar performance and recovery across the MC, suggesting that exercise training does not need to be modified for phase or group. It appears that OC and H-IUD users can be equally included in research with EUM women.

To women in science.

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LIST OF ABBREVIATIONS

ADP	Air displacement plethysmography
AP	Average Power
BIA	Bioelectrical impedance analysis
BIS	Bioelectrical impedance spectroscopy
BF	Blood flow
BMI	Body mass index
BV	Total body volume
CMJ	Countermovement jump
CNS	Central nervous system
DXA	Dual-energy x-ray absorptiometry
DJ	Drop jump
ECF	Extracellular fluid
EI	Echo intensity
EUM	Eumenorrheic
FFM	Fat free mass
FI	Fatigue index
FM	Fat mass
FP	Follicular phase
FSH	Follicle-stimulating hormone

4C	Four compartment model
H-IUD	Hormonal intrauterine device
HC	Hormonal Contraception
ICF	Intracellular fluid
IUD	Intrauterine device
LM	Lean mass
LH	Luteinizing Hormone
LP	Luteal phase
mCSA	Muscle cross sectional area
Mo	Total body bone mineral density
MVC	Maximal voluntary Contraction
OC	Monophasic oral contraception
1RM	One repetition maximum
%BF	Percent body fat
PF	Peak Force
PP	Peak power
PQCT	Peripheral quantitative computed tomography
REE	Resting energy expenditure
RER	Respiratory exchange ratio
RMR	Resting metabolic rate

RSA	Repeated sprint ability
RSI	Reactive strength index
TBW	Total body water
tPP	Time to peak power
US	Ultrasound

CHAPTER I: INTRODUCTION

Over the past decade, female participation in exercise and sport rose from 14.9% in 2003 to 18% in 2019 according to the Bureau of Labor Statistics.[1] Despite the exponential increase in participation of females in exercise and sport, the sex data-gap in exercise physiology research is wide.[1] In a recent review of 5,261 exercise and sport science publications, only 6% of the studies had female-only participants compared to 31% of studies having male-only participants.[1] Most of the current exercise and sport recommendations for exercise training and nutrition have been derived from research studies utilizing male participants.[2] However, these recommendations based on male physiology neglect the anatomical, physiological, and endocrinological differences that exist between sexes, specifically in regards to the menstrual cycle, as well as the influence of hormonal contraception.

Male and female physiology is similar until puberty where sex hormones diverge leading to sex-based differences in metabolism,[3] fatigability,[4] recovery,[4] and body composition.[5] Female endogenous (estrogen and progesterone) and exogenous (ethinyl estradiol and progestin) sex hormones fluctuate in a cyclical pattern throughout the menstrual and hormonal contraception cycles, respectively. Estrogen receptors are widely distributed in skeletal muscle and mitochondria suggesting variations in estrogen and progesterone concentrations across the hormone phases may influence skeletal muscle performance and recovery after exercise.[6] As such, female skeletal muscle may be more efficient at resynthesizing ATP from oxidative phosphorylation during high-intensity exercise, which has particular implications for decreased fatiguability and increased exercise recovery.[4] Research examining the anabolic functions related to estrogen suggests increased concentrations of estrogen, coupled with increased muscle protein synthesis via exercise stimulation, can improve skeletal muscle performance. In contrast, progesterone,

which is high in the later phases of the menstrual cycle, may elicit catabolic effects.[7–9] Furthermore, estrogen has been purported to have a positive effect on voluntary activation during muscle contractions, while progesterone appears to have neuroinhibitory properties.[10] Despite these hormone counteractions, several studies have demonstrated no significant differences in muscle strength between the low hormone follicular phase (FP), and the high hormone luteal phase (LP), [8,11,12] although the data are conflicting.[13,14] The impact of exogenous hormones from hormonal contraception is even less understood, with existing data suggesting HC use does not significantly impact muscle strength.[15–18] Research in healthy, active females understanding the influences of various hormonal profiles on strength outcomes is limited.

More than 60% of US adult women use some form of HC with oral contraception (OC; 12.6%) being the most common form followed by intrauterine devices (IUD: 7.9%).[19,20] The exogenous sex hormones provided from OC use suppress endogenous estrogen and progesterone, which may have undesirable consequences for optimizing recovery and muscle strength performance.[21] Alternatively, IUD administers progestin locally having minimal influence on endogenous hormone production, mimicking a EUM menstrual cycle. Although intended to prevent pregnancy, HC is commonly prescribed to therapeutically reduce menstrual cycle symptoms, treat amenorrhea, and for maintenance of bone density [21,22] Previous research has shown conflicting findings on the directional effects of OCs on exercise outcomes such as muscle function,[13,23] anaerobic capacity,[24] and power-based tests.[23,25] Females utilizing monophasic OC, which provides consistent concentrations of ethinyl estradiol and progestin, demonstrated larger reductions in recovery compared to eumenorrheic (EUM) or progestin-only pill users regardless of cycle phase possibly due to greater inflammatory responses associated with monophasic OC use.[26,27] In female collegiate soccer players utilizing OC, stress and inflammatory biomarker levels were elevated throughout a competitive season compared to non-OC users resulting in less favorable body composition changes.[28] Currently, there are no previous studies providing an assessment of metabolism or exercise performance in females utilizing IUD.[29] Given the likelihood that IUD use will continue to increase, investigations into the impact of IUD on performance and recovery are warranted.

While many animal studies have demonstrated support for the potential of estrogen to support improved recovery and skeletal muscle maintenance,[30] research in humans has been much less clear.[31] The fluctuations of estrogen across the menstrual cycle and the addition of exogenous hormones may influence performance and the rate of recovery, yet the current state of the literature does not warrant general guidance on the impact of a normal eumenorrheic menstrual cycle or use of exogenous manipulation. The primary aim of the proposed study was to assess the effects of chronic use (≥ 6 months) of OC, IUD, compared to a EUM on strength, recovery, body composition, and muscle characteristics. A secondary aim was to evaluate whether there are differences in outcomes between the hormone phases (low hormone phase vs. high hormone phase) among healthy adult women.

Specific Aims

Specific Aim 1: To evaluate the effects of female monophasic OC vs. IUD vs. no hormonal contraception (EUM) on maximal upper and lower body strength (1 repetition max and isometric dynamometry) and power (reactive strength index and counter movement jump) between hormone phases (low hormone phase vs. high hormone phase).

Hypothesis 1: It was hypothesized that 1 repetition max bench press and leg press values, upper and lower body peak force, drop jump force, and countermovement jump height would be similar between monophasic IUD vs. EUM but both groups would have greater strength and power than OC.

Hypothesis 2: It was hypothesized that there would be no differences in 1 repetition max bench press and leg press values, upper and lower body peak force, drop jump force, and countermovement jump height between hormone phases.

Specific Aim 2: To assess the effects of female monophasic OC vs. IUD vs. EUM on recovery measured as peak power, fatigue index, workload decrements, blood lactate, and blood flow following a 10 × 6

second sprint protocol on a cycle ergometer between hormone phases (low hormone phase vs. high hormone phase).

Hypothesis 3: It was hypothesized that recovery outcomes would be similar between monophasic IUD vs. EUM but both groups would have greater recovery than OC.

Hypothesis 4: It was hypothesized that there would be a greater decrease in peak power, fatigue index, workload decrements, and blood lactate clearance in the high hormone phase.

Hypothesis 5: It was hypothesized that there would be no differences in blood flow between hormone phases due to estrogen's influence on vasodilation.

Specific Aim 3: To determine the effects of female monophasic OC vs. IUD vs. EUM on resting total body water, intracellular and extracellular fluid, body composition measures (including lean mass, fat mass, and percent body fat), muscle characteristics (including muscle cross sectional area and echo intensity), and metabolic outcomes (including respiratory exchange ratio and resting energy expenditure) between hormone phases (low hormone phase vs. high hormone phase).

Hypothesis 6: It was hypothesized that there would be no differences in fluid balance, body composition, muscle characteristics, and metabolic outcomes between monophasic OC vs. IUD vs. EUM.

Hypothesis 7: It was hypothesized that intracellular fluid and resting energy expenditure would be elevated during the high hormone phase.

Exploratory Specific Aim: To determine the effects of female monophasic OC vs. IUD vs. EUM protein turnover between hormone phases (low hormone phase vs. high hormone phase) in a subsample.

Hypothesis 8: It was hypothesized that protein turnover outcomes would be similar between monophasic IUD vs. EUM but both groups would have less protein turnover compared to OC.

Hypothesis 9: It was hypothesized that protein turnover, measured by [15N]alanine would be increased during the high hormone phase.

Delimitations

1. Women between the ages of 18-40 years.

2. Recreationally active to active: participated in moderate to vigorous-intensity exercise at least 3 days a week but were not participating in more than 200 minutes of vigorous exercise and/or more than 4 days per week of resistance training.
3. Normal weight to obese status: body mass index (BMI) of 20.0-35.0 kg/m^2
4. Generally healthy, non-smokers, who were apparently free from disease, reported no current or history of metabolic disease (cardiovascular disease, Type 1 or Type 2 diabetes), musculoskeletal disorders, disease that could result in significant changes in total body water (i.e. renal disease) or weight status (i.e. thyroid abnormalities), polycystic ovarian syndrome (PCOS), or medical or surgical events, such as reconstruction surgery, that could significantly influence study outcomes or prevent safe participation in exercise.
5. Was not currently taking medications or inconsistently taking medications that could influence study outcomes (i.e. stimulants, insulin, thyroid medications).
6. Had not experienced a musculoskeletal injury within the past three months.
7. For women who were not using hormonal contraception: eumenorrheic, reported consistent menstruation for three months prior to enrollment, and were not pregnant or planning on becoming pregnant.
8. Women using hormonal contraception had been utilizing the same method for a minimum of 6 months.
9. Was not currently nursing or had a child within the previous 6 months prior to enrollment.
10. Had not undergone a full or partial hysterectomy.
11. Did not have a self-identified or clinically diagnosed eating disorder.
12. Weight stable: had not lost or gained greater than eight pounds within two months prior to the enrollment.

13. Was willing and/or able to comply with the study protocol, including abstaining from caffeine, tobacco, alcohol, and physical activity before testing days and recording temperature daily.

Limitations

1. One repetition max testing assessed muscle strength, but may not have directly measure muscle activation, which can be a limiting factor in exercise performance.
2. Drop jumps served as a measure of leg extensor muscle function and reactive strength but do not provide a direct measure of muscle contraction.
3. The repeated sprint ability test served as an indirect measure of muscular fatigue, but does not directly measure depletion and repletion of ATP and intramuscular accumulation of metabolic by-products.
4. Measurements during the follicular/low hormone phase and the luteal/high hormone phase informed possible difference between cycle phase, but does not directly inform every change across the menstrual/hormone cycle which may occur.
5. Results may not be transferable to other hormonal contraception types such as triphasic oral contraception, implants, patches, injections, etc.
6. Results may not translate to older (>40 years) and younger (<18 years) females.

Assumptions

Theoretical

1. Subjects accurately report health and exercise history information.
2. Subjects adhered to pre-testing guidelines and provided accurate daily temperature reports (if applicable).
3. Subjects maintained normal daily activity throughout the intervention.

Statistical

1. The population from which the sample was taken was normally distributed.
2. The sample variability was equal.

3. There was no time-order effect.

Significance of Study

In kinesiology research, only 6% of studies are conducted in female-only cohorts.[1] The lack of female-only studies is often attributed to the complexity of hormonal fluctuations throughout the menstrual cycle or the perceived barrier of various hormonal contraception types. Results of this study will provide an understanding of how OCs and IUDs influence strength and recovery outcomes between hormone cycle phases, providing the opportunity to expand inclusion criteria for females in exercise science research. The addition of IUD users in the present study is unique as no previous studies have provided an assessment of exercise performance in females utilizing copper IUDs or hormonal IUDs.[29] Results from this study will provide a basis for understanding the physiological impact of OCs and IUDs on muscular adaptations thereby informing future research regarding performance considerations such as nutrient timing, exercise prescription and recovery recommendations, and dietary supplements. Understanding potential performance differences between hormonal contraception types and hormone cycle phases could have significant implications for improving strength, recovery, and other performance outcomes in female populations.

CHAPTER II: REVIEW OF LITERATURE

Introduction

In sport and exercise science research conducted from 2014 to 2020, only 6% of studies were conducted exclusively with female participants, demonstrating a large gap in female-specific research.[1] Considering the growing number of females participating in exercise and sports, accurately assessing female physiology is imperative. It is well established that female physiology differs from males largely due to the biphasic responses of estrogen and progesterone across the menstrual cycle. Yet most performance and recovery recommendations have been derived from studies utilizing male participants, then generalized to females.[32] It is naïve to assume that all research in males can be directly applied to females, particularly when acknowledging the diversity in ovarian hormone profiles between regularly menstruating eumenorrheic (EUM) females and those utilizing hormonal contraception (HC).

The impact of exogenous hormonal manipulation on exercise physiology cannot go unresolved; over 60% of US adult women and 49.5% of female athletes utilize some form of HC with oral contraceptive (OC) pills and intrauterine devices (IUD) being the most common forms utilized [19,33]. However, many previous research studies have not included HC users or have failed to accurately define reproductive status. OCs introduce varying concentrations of circulating exogenous estrogen and progesterone, which may moderate physiological adaptations to exercise differently than endogenous sex hormones. The use of IUDs may be more favorable than Ocs in the context of optimizing female performance, as they do not suppress endogenous hormone production, therefore resulting in a physiological response similar to a normal menstrual cycle. Currently, there are no previous studies providing an assessment of metabolism or exercise performance in females utilizing IUD.[29] Thus, this review has no data regarding IUD users amplifying the need of female HC specific research. The

omission of HC users in research ignores the complexity and generalizability of various female hormone profiles and fails to close the gap in understanding the impact of sex hormone perturbations across natural and exogenous hormonal manipulation.

Female Sex Hormone Profiles

Male and female physiology is similar until puberty where sex hormones diverge. Once the transition through puberty is complete, females typically experience a circamensal rhythm, termed the menstrual cycle, that is characterized by predictable fluctuations in four female sex hormones, estrogen, progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH).[32] A regular menstrual cycle can range from 23-38 days, and females who experience monthly menstruation are considered EUM until they reach menopause (average age 51 years).[32] The menstrual cycle consists of two main phases: the follicular phase (FP) and the luteal phase (LP) which are separated by ovulation. During the FP (days 0- ~13), estrogen levels begin low (menstruation), rise then peak at the periovulatory phase. During the LP (days ~16- 28), estrogen level rises again, and progesterone peaks until the pre-menstrual phases where estrogen and progesterone levels fall if pregnancy does not occur (Figure 1).[34]

Hormonal contraceptives provide exogenous female sex hormones to systematically control the concentrations of endogenous sex hormones by acting on the hypothalamus and anterior pituitary glands leading to the suppression of gonadotropin-releasing hormone, FSH, and LH, thus reducing endogenous estrogens and progesterone levels.[15] In both nonathletes and athletes, HC are commonly prescribed for cycle regulation, contraception, treatment of amenorrhea, and for maintenance of bone density.[21,22] There are various delivery methods for HCs including OC, implant, injection, transdermal patch, vaginal ring, and IUD, with OC and IUD being the most commonly prescribed HC in the United States.[20] Furthermore, HC can be classified by type: combined, with an estrogenic and progestin component, or progestin-only. The type and concentration of estrogen and progestin varies between different HC type and may influence physiological responses to exercise.

The OC pill is usually prescribed in the form of a combined estrogen and progesterone tablet that is consumed daily. The combined pill can be described as a monophasic, biphasic, or triphasic dose with each providing varying release of hormones across a 28-day period.[21] Monophasic OC are the most common OC pill prescribed and contain low to standard does of ethinyloestradiol and progestins that are delivered at a constant dose over 21-days with a 7-day withdrawal period (Figure 1). The second most common OC pill prescribed is a triphasic pill in which the dose of estrogen and progesterone change three times in a 28-day period.[21] This form is thought to mimic the "natural" menstrual cycle more closely and has a slightly lower dose of hormones overall. However, the main drawback of this form is difficulty with cycle timing due to the variations in synthetic hormone concentrations throughout the doses.[21] The biphasic form is the least popular form and offers no particular advantage over the other two forms. With the biphasic pill, the progesterone level is increased halfway through the pill cycle, while the level of progesterone stays consistent.[21] Of the various forms, monophasic OC allows for easy manipulation of the menstrual cycle for treatment of dysmenorrhea, the timing of menstruation for training and competitions for active females, and effective contraception.[21,35]

Unlike an OC which must be taken daily, IUDs are long-acting reversible contraceptives placed directly into the uterus and do not require patient intervention for multiple years. There are two forms of IUD, a copper IUD that does not contain hormones, and progestin containing IUDs. Copper IUDs have less therapeutic benefits than hormonal IUDs but do not provide any exogenous hormones unaltering the female sex hormonal profile. There are two types of progestin IUDs: one containing 52 mg of Levonorgestrel (progestin) which can last for five years and the other containing 13.5 mg Levonorgestrel which lasts for three years.[36] Both hormonal IUDs administer the progestin locally, which does not systemically suppress endogenous hormone production.[37] The hormonal IUDs act by thickening the cervical mucus causing endometrial decidualization and glandular atrophy, which inhibits the binding of sperm to the ovum and in partial inhibition of ovulation.[36] The therapeutic benefits of hormonal IUD include possible loss of menstruation aiding in dysmenorrhea and blood loss. The use of IUDs may be

more favorable than Ocs in the context of optimizing female performance, as they mimic a EUM cycle, therefore resulting in a physiological response similar to a normal menstrual cycle.

Muscular Strength and Power

Adequate levels of muscular strength and power are needed for physical functioning, improved quality of life, reduced risk of injuries, and optimal performance in athletes.[38,39] Females typically have a greater proportional area of type I muscle fibers, thereby incurring greater capillarization, mitochondrial respiratory capacity, and fatigue resistance compared to males.[40] This may be indicative of muscular strength and power in females. The contractile apparatus of the skeletal muscle motor pathway, composed of actin, myosin, and other proteins, is ultimately responsible for force generation and human movement.[41] Muscle contractions are initiated by the central nervous system (CNS), and muscle activation is a limiting factor to exercise performance with changes in neurotransmitter concentrations and motoneurons influencing the ability to drive the muscle.[19,42] Previous data suggest that while voluntary activation does not differ between sexes,[4,40] the fluctuations of estrogen and progesterone across the EUM menstrual cycle can affect CNS function due to their ability to cross the blood-brain barrier.[43] Specifically, voluntary activation appears to be highest when estrogen levels peak and lowest when progesterone levels peak, suggesting neuromuscular function and fatigability varies across the menstrual cycle;[10] yet there is a lack of data supporting exercise-induced central fatigue differs when exercise is performed in different phases of the menstrual cycle.[10]

Muscular power is a major factor for evaluating injury risk and sports performance, especially in sports including high-speed moments.[44] The mechanical power of jump movements depends on neuromuscular coordination and restitution of elastic energy stored in muscles and tendons. Previous research has demonstrated significant variations in knee joint laxity, neuromuscular coordination, and postural control during the menstrual cycle.[24,25,45] Estrogen receptors are widely distributed in skeletal muscle, tendons, and mitochondria, so variations in estrogen concentrations, naturally or via exogenous influence, may impact skeletal muscle performance and repair.[6] Given the fluctuations of sex hormones

across the menstrual cycle, skeletal muscle performance, and recovery after exercise may differ in response to variations in estrogen and progesterone levels. Several authors have concluded that there are no significant differences in muscle strength during different phases of the menstrual cycle,[11,12,15–17,25] although there are conflicting data.[13,23,46] In the limited current body of literature on strength and recovery related to the menstrual cycle, studies often neglect to account for differences between hormone phases or external (e.g. hormonal contraceptives) perturbations to the menstrual cycle. To date, there is minimal research investigating EUM menstrual cycle-induced changes in strength, with the impact of OC or IUD use on strength is even less understood.

Maximal Strength and the Hormone Cycle

To assess muscular strength, one-repetition maximum (1RM) tests are a common field-based test.[39,44] The 1RM is defined as the maximal weight that can be lifted at once while maintaining the correct lifting technique, and it allows for assessing strength in multi-joint exercises.[44] While 1RM tests are considered the "gold standard" for assessing dynamic strength, most commonly used muscle strength tests in previous studies have included a handgrip test and isokinetic testing for knee flexors and extensors. There is limited research evaluating the effect of varied hormonal profiles on 1RM strength. Romero-Moraleda et al.[12] sought to investigate the fluctuations of female sex hormones across the EUM menstrual cycle on muscle performance using the Smith machine half-squat exercise in 13 female triathletes. Results demonstrated no significant differences in 1RM during the early FP (mean ± standard deviation: 97.0 ± 23.2 kg), late FP (98.5 ± 18.1 kg), and mid-luteal phase (98.1 ± 22.2 kg) suggesting there are no systematic variations in muscle performance during the different EUM menstrual cycle phases.[12] Another study examining the effects of HC use on 1RM leg press demonstrated similar strength between HC users (114 ± 15 kg) compared to EUM females (118 ± 18 kg).[15] A similar trend was observed between HC users and EUM for 1RM bench press values (43 ± 8 kg and 41 ± 10 kg, respectively).[16] However, these two studies did not control for the phase of the hormone cycle participants were tested in, or the different types of HC used, limiting the understanding of hormonal influences on

1RM strength. The limited amount of research regarding maximal strength output in different hormonal profiles highlights the necessity of understanding the differences between HC types and hormone phases on maximal strength to accurately prescribe training and nutrition recommendations to active females across the lifespan.

Isometric Dynamometer and the Hormone Cycle

In laboratory-based settings, muscular strength is most commonly assessed with force measurements from static or dynamic muscle contractions. Static muscle contractions are muscle contractions where the force is exerted on a stationary object and involved joints do not move during the contraction.[47] Specifically, isometric contractions using a dynamometer are the most widely used method of measuring static muscle strength.[47] Several authors have concluded that there are no significant differences in muscle strength during different phases of the menstrual cycle,[11,15,17,48] although there are conflicting data.[13,46] Early research evaluating the isometric strength of the quadriceps across the EUM menstrual cycle found that there was a peak in strength around the time of ovulation.[46] This observation was supported by another study reporting an 11% increase in quadriceps maximum voluntary isometric force in the EUM ovulation period compared to early FP or LP.[13] However, females using OC in the study demonstrated no changes in quadriceps maximum voluntary isometric force suggesting that a peak in estrogen may have contributed to the observed changes in isometric strength. Since these early investigations, research regarding isometric strength across the menstrual cycle has been equivocal, possibly due to the various methods used to estimate the phase of the EUM menstrual cycle and the different definitions of reproductive status. When comparing HC users to EUM females, isometric leg extension force was similar between groups (HC: 2,840 ± 405 N and EUM: 2,680 ± 631 N, respectively) suggesting exogenous hormones may not influence isometric strength.[15] However, this study did not account for the hormone phase or types of HC. Furthermore, when OC users were compared to EUM females, there was no significant differences in the maximal voluntary force of the quadriceps between groups despite a significant increase in the concentration of progesterone and estrogen in the LP for EUM

and active pill for OC compared to FP and withdrawal.[17] Research regarding isometric strength in different hormone phases and with different HC types remains limited.

Muscular Power and the Hormone Cycle

Muscle power is a major factor for evaluating injury risk and sports performance, especially in sports including high-speed moments.[44] Drop jumps or maximal vertical jumps can be used to assess reactive strength and power. Of the available research assessing muscular power in EUM females and HC users, there appears to be no difference between groups or hormone phases on muscular power.[23–25] When evaluating the effect of the hormone cycle phase on maximal jump height in OC users compared to EUM, there were no differences between groups or between hormone cycles for maximal jump height.[24] Another study observing monophasic OC use on a 45 cm drop jump reported lower muscular power occurring during the withdrawal phase compared to the active phase in trained athletes.[23] Considering variations in estrogen levels may influence knee joint laxity and neuromuscular coordination, a thorough evaluation of the hormone cycle phase and various hormone profiles on muscular power is needed.[24,25,45]

Recovery in Females

For exercise performance, it is essential to be able to perform and recover rapidly from high-intensity bouts of exercise. Recovery is a critical period relative to the adaptation process during training regimes and is associated with an adequate balance between training load and recovery which is essential for exercise adaptations.[49] The relationship between fitness (a positive outcome of training) and fatigue (a negative result of training) is important for exercise performance and adequate recovery. Higher levels of fatigue can predispose individuals to injury or overreaching. Importantly, there are sex-based differences regarding fatigue, with evidence demonstrating that female skeletal muscle is more resistant to fatigue elicited by equivalent dosages of high-intensity exercise compared to males.[40] Trained females have exhibited faster oxygen uptake kinetics during moderate-intensity exercise and smaller decreases in ATP concentrations after all-out- exercise compared to males.[50,51] As such, female skeletal muscle may be more efficient at resynthesizing ATP from oxidative phosphorylation during high-intensity exercise,

which has particular implications for decreased fatiguability and increased exercise recovery.[4] Therefore, it is possible that females require less rest between intense interval exertions for adequate metabolic recovery.

Effective recovery strategies have demonstrated increased blood flow to the recovering muscles may improve oxygen and nutrient delivery, and therefore resynthesis of phosphocreatine and glycogen.[52,53] The perfusive and hemodynamic properties of female skeletal have indicated improved oxygen and metabolite removal during and after exercise aiding in reduced fatigue during exercise and increased recovery post-exercise.[40] Elevated estrogen levels in females have been shown to promote vasodilation by stimulating nitric oxide synthesis and decreasing the production of vasoconstrictor agents.[54] This increased vasodilatory responses paired with a higher density of capillaries per unit of skeletal muscle provides greater blood flow to exercising skeletal muscle. This possibly decreases the metabolic perturbations that induce fatigue.[4,50,55,56] Increased blood flow may aid in transporting lactate and other metabolic by-products away from the active muscle to removal sites possibly increasing blood lactate threshold during exercise and expediating lactate clearance post-exercise.[57] However, there is limited research characterizing blood lactate levels or clearance in both EUM females and those using HC.

Furthermore, estrogen may act to diminish skeletal muscle damage and inflammatory responses after exercise by exerting a protective effect on muscle membranes via direct receptor-mediated mechanisms.[30,31] This reduction of muscle membrane disruption may also be important for muscle repair and regeneration. Estrogen has also demonstrated antioxidant-like characteristics inhibiting inflammatory responses post-exercise, accelerating faster recovery.[30,31] A recent review on the menstrual cycle effects on exercise-induced fatigability found 15 studies with a statistical difference between the FP and LP in EUM menstrual cycles, with eight studies demonstrating greater fatigue resistance in the FP, and seven studies demonstrating greater fatigue resistance in the LP.[58] The inconsistencies across studies may be a consequence of the differences in methodologies and the techniques used to define the menstrual cycle

phase (e.g., serum concentration vs. reported day of menses). Therefore, an evaluation of the impact of changes in estrogen concentration across the menstrual cycle, or with exogenous use, is important for characterizing recovery and fatigue in females.

Recovery and The Menstrual Cycle

Due to estrogen's role in reduced muscle damage and inflammation after exercise, there may be an enhanced recovery response in the FP when estrogen peaks. However, the current body of literature examining recovery differences between EUM menstrual cycle phases provides equivocal results. Recovery is often measured by evaluating the concentration of inflammatory markers, such as creatine kinase (CK) and interleukin-6 (IL-6), which are products of the muscle cell myoblasts and satellite cells' response to muscle injury. Early work reported that resting IL-6 levels are lowest in the LP when progesterone levels are elevated, and highest in the FP during normal menstruation when estrogen and progesterone are low.[59] Recent work by Hackney, Kallamen, and Ağgön 2019 confirmed this observation in EUM active females, reporting elevated IL-6 values in the mid-FP at rest, immediately after running for 90 minutes at 70% VO_{2max} on a treadmill, and 24-hrs post-exercise (mean difference ± standard deviation: 1.4 ± 1.9 pg/mL, 24.9 ± 13.2 pg/mL, and 10.3 ± 7.1 pg/mL, respectively) compared to the mid-LP (1.2 ± 0.5 pg/mL, 13.5 ± 6.2 pg/mL, and 5.0 ± 3.0 pg/mL, respectively).[60] In contrast, another study in well-trained EUM females following an eccentric exercise demonstrated a significant interaction for IL-6 indicating a possible inflammatory response in the mid-LP compared to mid-FP.[61] However, the participants were well trained and may not have received enough muscle damage to fully observe recovery measures. Other work in EUM females has demonstrated no differences in blood markers of recovery between menstrual cycle phases.[62,63] Additionally, there have been limited studies utilizing other recovery outcomes between menstrual cycle phases.

Recovery and Hormonal Contraception

Considering OC use downregulates the cyclic hormone activity of the hypothalamic-pituitary-gonadal axis, recovery outcomes in OC users may differ from EUM females. Some research has shown

exogenous hormones of OC may modulate the recovery metrics, such as respiratory rate, sleep, and heart rate variability, in a different pattern than the EUM menstrual cycle, with reduced indices of adaptation to stress across pill types (progestin-only and combined) and phases.[64] One study in healthy, active females (EUM and OC users) demonstrated decreased recovery as measured by heart rate reserve, across the menstrual cycle from the mid-FP to the mid-LP in EUM females.[64] Females utilizing combined OC in the same study demonstrated larger reductions in recovery with every unit of increased strain compared to EUM or progestin-only pill users regardless of cycle phase.[64] The authors suggested recovery in OC users may have been attenuated due to greater inflammatory responses associated with OC use,[26,27] particularly due to increases in free circulating cortisol in the blood often observed with combined OC.[65] In female collegiate soccer players utilizing OC, stress, inflammatory, and cortisol levels were elevated throughout a competitive season compared to EUM females suggesting OC use may be pro-inflammatory leading to decreased recovery.[28] When observing workload and power decrements during repeated sprint ability test, another marker of recovery, in the withdrawal week and active week of monophasic OC, team sport athletes demonstrated equivocal workload and power decrements during the withdrawal phase compared to the active phase suggesting exercise recovery may not be negatively influenced by OC use.[23] However, the study did not utilize a EUM group to compare values to. As such, with conflicting findings, it is important to understand the implications HCs have on training adaptations and recovery.

Body Composition and the Menstrual Cycle

Body composition assessment is essential for optimizing performance, recovery, and overall health. However, little is known about the influence of female sex hormones, both endogenous and exogenous, on the body composition assessment across the hormone cycle phase. Most previous investigations on the influence of the EUM menstrual cycle or HC on body composition outcomes have utilized only 2-compartment (2C; i.e. skinfolds, bioelectrical impedance analysis [BIA], air displacement plethysmography [ADP]) and 3- compartment (3C; i.e. dual-energy x-ray absorptiometry [DXA]) models. Early research utilizing skinfolds in both EUM and women utilizing OC to assess percent body fat (%BF)

in the early-FP (day 1 of menstruation), ovulation (day 14), and LP (day 21) found no differences in %BF estimations across cycle phase.[66] Recent research using four different BIA devices at four time-points (menstruation, late-FP, early-LP, and late-LP) reported no significant differences in body mass, %BF, or fat-free mass (FFM) between the time-points for any device.[67] However while this study included women utilizing HC, separate statistical analyses were not conducted for the impact of exogenous hormones on study results. Another study in healthy EUM females and those utilizing HC assessed body composition using DXA, ADP, and BIA during menstruation and late-FP.[68] There were no significant differences in %BF or body mass estimates taken between the two-time points in either group suggesting body composition may not change in the FP and/or HC use may not influence body composition. However, testing was not performed during the LP limiting the understanding of changes in body composition across the hormone cycle. When comparing changes in body composition estimates over the EUM menstrual cycle (early FP, late-FP, and mid-luteal) inactive EUM females using DXA, standardized brightness-mode ultrasound (US), and skinfolds, there were no differences in %BF between cycle phases for any of the measurement devices.[69] Taken together, it does not appear that %BF measures change across menstrual cycle phases. However, the mixed methodologies and lack of division between EUM and HC users limit the understanding of changes in female sex hormones on %BF, and other compartments of the body that may be more sensitive to hormonal alterations (i.e. fluid, lean mass, etc). Additionally, body composition research evaluating changes in lean mass (LM), a compartment largely influenced by total body water, is lacking.

Utilization of a more-detailed methodology that accounts for multiple body compartments, such as a 4-compartment (4C) model across the hormone cycle is warranted. As sex hormones influence many physiological aspects, the validity of body composition assessments that rely on assumptions, especially in 2C and 3C models, may be violated by shifts induced by hormonal fluctuations (i.e. changes in body water, fat free body density, body volume, etc.). Due to the 4C model accounting for total body water (TBW), body volume, and bone mineral density, the 4C model may be more sensitive to compartmental

changes induced by the menstrual cycle and hormonal fluctuations from both endogenous and exogenous sources. There is limited research assessing body composition differences with the 4C model across hormone phases. One study in EUM females found 4C model measures of FM, FFM, and %BF were similar between early-FP and late-FP.[70] However, during early-FP, body volume and body mass were significantly greater than in late-FP, which is similar to other reports.[67,68,71] We are unaware of any studies utilizing the 4C model to evaluate body composition changes between the FP and LP or use of a 4C model to understand the impact of HC.

Female Sex Hormones and Total Body Water

Fluctuations in body mass throughout the menstrual cycle have often been attributed to shifts in fluid retention between hormone cycle phases due to the impact of ovarian hormones on body water compartments even at the transcapillary level.[72–74] Three studies utilizing bioelectrical impedance devices to assess TBW have reported no impact of the hormone cycle phases on TBW or other body composition measures both in EUM and HC users,[67,75,76] while other studies using similar methodologies have reported significant shifts in FFM and TBW [77–80]. These inconsistent results may be due to varying pre-assessment guidelines that failed to control for hydration status, exercise restrictions, fasting protocols, and HC use/type, which highlights the need for a thorough evaluation of TBW in EUM females and HC users.

The variations in female sex hormones impact fluid distribution, with two studies that demonstrated changes in the extracellular fluid (ECF) [68] and intracellular fluid (ICF) [81]. Elevated estrogen has been shown to increase retention of extracellular fluid in plasma, while elevated levels of both estrogen and progesterone generally favor retention of fluid within the interstitial space.[71] This observation was confirmed with recent data comparing LM measurements from early FP, where estrogen is low, to mid-FP, where estrogen peaks, in EUM females.[70] The results demonstrated extracellular fluid was significantly lower in the mid-FP compared to early FP suggesting elevations in estrogen may influence measures of total body water and hydration status.[70] Fluid distribution changes throughout a

EUM cycle, and possibly with HC use, may alter body composition measurements and have implications for recovery, such as hydration and thermoregulation, throughout the hormone phases.

Female Sex Hormones and Muscle Size and Quality

Determining the impact of endogenous and exogenous hormones on muscle size (muscle cross sectional area [mCSA]) and quality (echo intensity [EI]) is important for performance, recovery, and metabolism among females. While it is well known that estrogen has several metabolic effects on skeletal muscle, more recent research has demonstrated the role of estrogen in muscle mass maintenance.[82] In animal models, 24-weeks of estrogen deficiency resulted in a 10% decrease in strength that corresponded with an 18% decrease in mCSA demonstrating estrogen may support greater force generation and skeletal muscle quality.[83] In humans, much of the estrogen work has been completed in relation to menopause, where females experience a severe decrease in endogenous estrogen and progesterone levels. In a cross-sectional analysis of 840 postmenopausal women (259 on hormone replacement therapy and 581 controls), females utilizing hormone replacement therapy demonstrated greater mCSA and grip strength than non-users.[84] Currently, research characterizing muscle size and quality between hormone cycle phases is lacking. One study observing mCSA of the rectus femoris, vastus intermedius, and vastus lateralis measured via ultrasound in EUM active females demonstrated no differences between measurement values.[8] However, after a three-month resistance training protocol, there were significant increases in mCSA that were not phase dependent. Another study characterizing mCSA using a peripheral quantitative computed tomography (pQCT) in EUM and OC users demonstrated no significant differences in mCSA or muscle quality between early-FP, late-FP, or mid-LP in either group.[85] However, this study did not report the activity level of the participants, which may be a confounding factor. Gaining insight into the impact of the various hormonal profiles on muscle size and quality will allow for a deeper understanding of the role of endogenous and exogenous estrogen in skeletal muscle.

Metabolic Outcomes in Females

The fluctuations of female hormones across a EUM menstrual cycle facilitate variations in energy expenditure and macronutrient metabolism.[32] Substantial evidence suggests that estrogen is a master regulator of bioenergetics in females.[86] The impact of EUM menstrual cycle phase on metabolism and performance has been well evaluated.[32,87–90] Most previous research demonstrates a decrease in fat oxidation, protein oxidation, and glycogen storage with an increase in carbohydrate oxidation in the FP.[32] During the LP, there is an increase in fat oxidation, protein oxidation, and glycogen storage with a decrease in carbohydrate oxidation.[32] Implications of these phase-based differences in metabolism have not been fully evaluated in HC users, with no previous research in IUD users specifically.

Resting Energy Expenditure

At rest, EUM females exhibit heightened fat oxidation, as indicated by decreased respiratory exchange ratio (RER), and 2.5–11.5% higher resting energy expenditure (REE) during the luteal phase of the menstrual cycle when ovarian hormones peak.[91,92] One study evaluating REE in EUM females during the mid-LP (elevated estrogen and progesterone), the early-FP (low estrogen), and after 6 days of estrogen suppression, demonstrated the changes in REE paralleled the changes in estrogen.[93] Resting energy expenditure was the highest in the mid-LP, lower (−29 kcal/d) in the early-FP, and further reduced (−42 kcal/d) after estrogen suppression.[93] These results suggest elevated estrogen supports an increased energy expenditure in females and that REE may vary across the EUM menstrual cycle. Currently, only six studies have investigated the effect of varied OC types on REE with inconsistent results. When normal EUM women were compared to OC users, five studies showed no difference in REE or basal metabolic rate [94–98] and one study showed an increase in RER for OC users.[99] Due to the heterogeneity of the few available studies (i.e., study population, study design, OC formulation and dose), the influence of HC use on REE is unclear.

Protein Synthesis and Female Sex Hormones

Skeletal muscle is critical for sport performance, injury risk reduction, and long-term athlete health. Protein turnover, the rate of muscle protein synthesis and breakdown, determines the anabolic or catabolic state of skeletal muscle.[100] Recent evidence suggests estrogen reduces protein oxidation while progesterone, which peaks in the LP of the menstrual cycle, may elicit catabolic effects.[34,101] Protein turnover research is mostly conducted in association with the menopause transition. A recent cross-sectional study observing protein turnover from [^{15}N]alanine isotope demonstrated a significant reduction in whole-body protein balance from pre- to perimenopause, suggesting that with alterations in estrogen concentrations, protein turnover may be blunted.[102] This reduction in protein turnover, which may parallel estrogen concentrations, was consistent with reductions in lean mass, mCSA, and leg strength. Of the limited data evaluating protein turnover across the menstrual cycle in healthy EUM females, studies with small sample sizes have consistently found phenylalanine, lysine, and leucine oxidation are greater in the LP compared to FP.[103–105] This suggests elevated progesterone may increase the oxidative disposal of amino acids,[32] possibly facilitating a global catabolic environment in the LP. Reports on amino acid flux and net balance are less consistent, highlighting the need for further evaluation into protein turnover in various hormonal profiles. Understanding protein turnover response to the fluctuating hormone concentrations across the menstrual cycle is essential for protein and recovery recommendations in female athletes. To date, we are not aware of any data evaluating the effects of various HC phases or IUD use on protein turnover.

Conclusion

There are key physiological differences between the males and females, primarily driven by differences in sex hormone concentrations. To optimize female strength performance and recovery, the unique responses of female physiology, both naturally and under exogenous hormonal manipulation must be considered. The fluctuations of estrogen and progesterone across the menstrual cycle in EUM females are known to influence substrate utilization, fluid distribution, and muscle composition. The exogenous

sex hormones provided from OC use suppress endogenous estrogen and progesterone, which may have undesirable consequences for optimizing recovery and muscle strength performance. Alternatively, IUD administers progestin locally having minimal influence on endogenous hormone production, mimicking a EUM menstrual cycle, possibly leading to similar performance outcomes as observed in EUM females. The impact of exogenous hormonal manipulation on strength and recovery cannot go unresolved considering a large percentage of female utilize HC; furthermore, we are aware of no other studies that have evaluated exercise performance in participants using IUD. Future research should focus on inclusion of HC use which may expand female inclusion criteria in scientific research, while also potentially highlighting an optimal testing window within the menstrual cycle for future studies to minimize confounding effects.

CHAPTER III: METHODOLOGY

Experimental Design

Using a cross-sectional cohort study design monophasic oral contraceptives (OC) users, intrauterine devices (IUD) users, and eumenorrheic (EUM) women, users were recruited through flyers, email, word of mouth, and social media. Each cohort was followed prospectively across the hormone cycle; two visits occurred during the low hormone phase (days 0-9: EUM/IUD) or placebo week (OC), and two visits occurred in the high hormone phase (between two days after ovulation and five days before the next predicted period; EUM/IUD) or active pill (OC) (Figure 2). Participants began the study protocol in random order of hormone phase (i.e., beginning in the FP or LP) to limit the influence of time effects and testing on study outcomes. Participants were tested over one hormone cycle (n= 50), but due to scheduling conflicts, data collection also occurred over two (n=7) or three (n=3) hormone cycles. Participants in cohorts were matched based on relative lower body (mean difference ± standard deviation: 0.2 ± 0.2 kg) and upper body (0.01 ± 0.05 kg) strength to ensure a normal distribution of relative strength in each group as our primary outcomes were one repetition max (1RM) strength. Relative strength was assessed by normalizing the total weight lifted (kg) during the 1RM testing to the participant's body mass (kg).

Prior to enrollment, all participants completed a phone screening for inclusion/exclusion criteria. Those determined to be eligible based on the phone screening were read a verbal consent form. If the participant verbally agreed to be in the study, written informed consent was obtained, and the participants completed a health history questionnaire to confirm inclusion/exclusion criteria. Participants were asked to arrive to testing sessions following an 8 hour fast, consuming no food, caffeine, or alcohol and were also asked to abstain from physical activity for 48 hours prior to testing. Participants provided a urine

sample to confirm negative pregnancy status via urine human chorionic gonadotropin concentration and to obtain hydration status via urine specific gravity. Measurements of strength, body composition, muscle characteristics, and metabolic rate were completed at visits 1 and 3, and explosive strength and recovery measures were completed 24 hours later at visits 2 and 4.

Subjects

An original 227 women expressed interest and were sent initial information about the study. Of those who initially expressed interest, 14 declined, 42 were excluded for not meeting inclusion criteria, 84 did not respond to the initial contact, 16 met initial criteria but were lost to follow up, resulting in 71 individuals who met initial inclusion criteria and verbally agreed to be in the study. Prior to providing written consent, four women were excluded for reasons related to lack of time (N=3) and musculoskeletal injury (N=1). This resulted in 67 women who provided written consent and were scheduled for visits. Five women withdrew from the study prior to completing any visits for reasons related to incorrectly reporting oral contraception type (N=3), lack of time (N=1), and musculoskeletal injury (N=1). This resulted in 62 women completing at least one visit. Two women failed to complete the study for reasons related to musculoskeletal injury (N=1) and withdraw for personal reasons (N=1) and were excluded from the final analysis. Full CONSORT information is reported in figure 3.

Sixty healthy, active females between 18 and 40 years participated in the current study (Table 1). Recruitment was planned for a 2:2:1 enrollment with a goal of 25 women using monophasic OC, 25 women using intrauterine devices (IUD), and 15 women having eumenorrheic menstrual cycles. Groups for the final analysis were stratified as follows: monophasic OC users (n=21), hormonal-IUD (H-IUD; n=20) users, or were using a non-hormonal IUD or had regular naturally occurring menstrual cycles (EUM; n=19). Women using monophasic OC, or those who had an H-IUD were required to utilize the same hormonal contraception form for at least six months prior to enrollment (Table 2). Eumenorrheic women and women with non-hormonal IUDs were required to have consistent menstruation for at least three months prior to enrollment. The EUM group was utilized as a comparator for the two hormonal

contraception groups. Power was calculated based on effect sizes from previously published data evaluating maximal isokinetic/isometric muscle strength[9,15,16,25,106] (average effect size= 0.36) and 1 repetition maximum testing[15,16] (average effect size= 0.49) between an oral contraceptive group and a non-oral contraceptive group. With an α of 0.05, power of 0.8, three groups, two measures, an estimated correlation of 0.5 among repeated measures, a nonsphericity correction ϵ of 1, and a 15% drop-out rate, the study would be sufficiently powered with an average of sixty participants. This sample size was feasible based on prior work in our laboratory. Additionally, data for the university campus health reported there were 646 IUD placed and 1,657 prescriptions for OC over the last year, suggesting there was a wide range of qualifying individuals available on campus.

Potential participants were excluded if they were currently pregnant, planning to become pregnant, were currently nursing, or had a child in the previous six months. Age inclusion criteria was 18-40 years old, and a body mass index (BMI) criterion of 20.0-35.0 kg/m^2. A larger BMI range was used as BMI categories may not be accurate in active participants or as women age.[107] Participants had to be healthy, non-smokers, and participated in moderate to vigorous intensity exercise at least 3 days a week, but no more than 200 minutes of vigorous exercise and/or more than 4 days per week of resistance training. Individuals were excluded from participation if they: 1) had current and/or history of metabolic disease (cardiovascular disease, Type 1 or Type 2 diabetes), musculoskeletal disorders, disease that may result in significant changes in total body water (i.e. renal disease) or weight status (i.e. thyroid abnormalities), polycystic ovarian syndrome, or medical or surgical events, such as reconstruction surgery, that could have significantly influence study outcomes or prevent safe participation in exercise; 2) were currently taking medications or inconsistently taking medications that could have influence study outcomes (i.e. stimulants, insulin, thyroid medications); 3) experienced a musculoskeletal injury within the past three months; 4) undergone a full or partial hysterectomy; 5) had a self-identified or clinically diagnosed eating disorder; 6) had lost or gained greater than 3.6 kg within two months prior to the enrollment; 7) was not willing and/or able to comply with the study protocol, including recording

temperature daily and/or abstaining from caffeine, tobacco, alcohol, and physical activity before testing days. A health history questionnaire was used to confirm inclusion/exclusion criteria. All methodology was approved by the University's Institutional Review Board, and all participants provided verbal and written informed consent prior to participation.

Menstrual Cycle Tracking

Upon enrollment participants, participants in the EUM or H-IUD groups who were not already tracking their menstrual cycles were asked to begin tracking their menstrual symptoms and basal body temperature with an app/website (FertilityFriend) that was accessible to both the researcher and participant to plan ideal visit times. Body temperature along with symptoms (e.g. dizziness, lower back pain, mood changes/anxiety, etc.) was recorded daily to further inform cycle length and to determine visit scheduling (i.e. FP vs LP). Participants who were EUM or were using an H-IUD were asked to utilize ovulation tests between days 12-16 (Femometer, Princeton, NJ) according to manufacture guidelines at their predicted ovulation dates to confirm ovulation date and hormone cycle phase.

Salivary Hormone

Estrogen and progesterone concentrations were determined using a 2.5 mL saliva sample taken at visits 1 and visit 3. Estrogen and progesterone levels were determined using ELISA assays (Salivary 17 β-Estradiol Enzyme Immunoassay Kit, Salimetrics, LLC, State College, PA, USA; Salivary Progesterone (P4) Enzyme Immunoassay Kit, Salimetrics, LLC, State College, PA, USA). Participants were asked to avoid brushing their teeth for 45 minutes prior to and undergoing dental work for 48 hours prior to sample collection to avoid blood contamination. All samples were immediately frozen at -20°C until analysis using established enzymatic assays.

Specific Aim 1 Procedures

One Repetition Max (1RM) Testing

Participants completed a five-minute warm-up on a treadmill at a 1% grade and 5.6 kilometer per hour pace. To determine 1RM (kg) for leg press and bench press, each participant performed a set of 8-10

repetitions, with a weight that is approximately 50% of the anticipated 1RM. Participants then rested for 2 minutes, after which a set of 4-6 repetitions was completed with a load of 80% of the predicted 1RM.[108] After another 2-minute rest period, the weight was increased to an estimated 1RM load, and the participants attempted a single repetition with the weight. After the completion of each successful 1RM attempt, the weight was increased until failure was reached, with 2-3 minutes of rest between 1RM attempts. 1RM was determined for leg press first, followed by 1RM for bench press. A systematic review on test-retest reliability for 1RM tests demonstrated a median intraclass correlation coefficient (ICC) of 0.97 and median coefficient of variation (CV) of 4.2%.[39]

Isometric Dynamometer

Lower Body Isometric force

Lower body peak and rapid force variables were examined with a calibrated commercial dynamometer (HUMAC Norm, Computer Sports Medicine Inc., Stoughton, MA) during an isometric maximal voluntary contraction (MVC) on the dominant leg. Force signal was sampled at either at 600 Hz or 2000 Hz and low-pass filtered at 50 Hz using a zero-phase-shift, fourth-order Butterworth filter. Participants were seated in the dynamometer chair with straps over the shoulders, hips, and non-dominant thigh to prevent extraneous movements during the leg extension. The participants were also asked to place their arms across their chest during testing. The dominant knee joint of the participants was fixed at 60-degrees below full extension, as verified with a goniometer. The padded arm of the dynamometer was positioned approximately three centimeters above the lateral malleolus. All chair adjustments were recorded and replicated for each testing session.

Participants were instructed to push into the lever arm as fast and as hard as possible and to maintain maximal effort for approximately 3-4 seconds. Research assistants provided standardized oral encouragement, and real-time visual feedback of torque production was displayed on a computer monitor in front of the dynamometer. Following three submaximal “warm-up” contractions (50% - 75% of perceived maximum effort), three to five practice MVCs were conducted to ensure production of maximal

effort. Practice trials were continued until the torque measurements ceased to increase within 5% of the previous trial. The values of the two highest practice trial MVCs were averaged and used to provide visual feedback on a computer monitor in front of the dynamometer of 100% and 110% of the practice trials. Participants were instructed to preform three maximal effort MVCs by pushing into the lever arm as fast and as hard as possible and to maintain maximal effort for approximately 3-4 seconds. Each force output was examined to see if the participants remained relaxed (i.e., performed a countermovement) before the MVC and explosively produced force during the MVC (visual inspection of the force-time curve). If needed, an additional MVC was performed. Custom written software (LabView 21; National Instruments) was used to process all the signals offline. Three maximal effort MVCs were averaged and used in the final data analysis. For the knee extensor isometric strength, the ICC= 0.95 and standard error of the measure (SEM)= 38 N.[109]

Upper Body Isometric Force

Upper-body peak force (PF) was determined from an upper-body strength assessment utilizing an isometric upright row with a calibrated load cell (TSD121C, Hand Dynamometer, Biopac Systems Inc., Goleta, CA, USA) on a flat metal platform. The participants were instructed to stand on the flat metal platform and hold a metal bar connected to an adjustable chain. Participants were asked to grip the bar with a pronated grip, align their hands with their shoulders, their shoulders abducted, and elbows flexed. The chain was adjusted to one chain link below the level of the umbilicus and the chain length was keep consistent between visits. Following three submaximal warm-up contractions (50%–75% of perceived maximum effort), participants were asked to perform three isometric MVCs with a one-minute recovery period in between each muscle contraction. Participants were instructed to pull the bar sub-maximally to remove the slack in the chain immediately prior to the start of the MVC, while being given strong verbal encouragement to pull as hard and as fast as possible on the chain for 3–4 seconds once the baseline force was completely steady as confirmed by visual assessment. Each force output was examined to see if the participants remained relaxed (i.e., performed a countermovement) before the MVC and explosively

produced force during the MVC (visual inspection of the force-time curve). Three MVCs were performed with a one-minute recovery period in between contractions. The force signal was sampled at 2 kHz with a Biopac data acquisition system (MP150WSW, Biopac Systems Inc.) and stored on a laptop computer (ThinkPad T420; Lenovo). Custom written software (LabView 21; National Instruments) was used to process all the signals offline. If needed, an additional MVC was performed. For the upright row isometric strength, the ICC= 0.96 and SEM= 36.5 N.[110]

Drop Jump to Countermovement Jump

Reactive strength ability was assessed utilizing drop jump test with 30 cm box height positioned 50% of the participants height from the front edge of two floor-embedded force plates (odel 4060-NC, Bertec Corporation, Columbus, Ohio). All participants were outfitted with a rigid cluster of 3 retroreflective markers placed over the sacrum. Participants were instructed to jump forward off the box to a double leg landing with 1 foot on each force plate, followed by an immediate vertical jump for maximum height. Prior to the jump trials, participants were asked to stand still in the center of the force plates to collect baseline retroreflective marker height. A minimum of 3 practice trials were performed followed by 3 successful jump-landing trials. If a trial was unsuccessful, a subsequent trial was collected and utilized for analysis. Drop jump data were sampled at 1200 Hz and low-pass filtered at 10 Hz using a fourth-order recursive Butterworth filter. Jump height in meters and contact time in seconds was calculated using a custom MATLAB code (MATLAB R2021b, Mathworks, Inc., Natick MA.). Jump height was normalized to body height in meters then converted into centimeters. Reactive strength index (RSI; cm s−1) was calculated by dividing jump height (cm) by ground contact time (s). The best result based on RSI was used for analysis. In healthy male and female adults, the jump height for counter movement jump ICC= 0.99 and CV= 6.91% and RSI ICC= 0.97 and CV= 11.92%.[111]

Specific Aim 2 Procedures

Repeated Sprint Ability (RSA)

Participants completed an RSA test on a friction-loaded cycle ergometer (Monark 894E, Stockholm, Sweden). To warm-up, the participants cycled for two minutes at 50 rpm against a resistance of 0.5 kg, followed by two 30 second bouts of cycling at a resistance of 1.5 kg, keeping the cadence between 85-115 repetitions per minute (rpm). Each warm-up sprint was followed by a 60 second passive recovery. Once the warm-up was completed, the participants began the RSA test consisting of 10 six-second maximal sprints with 30 seconds of passive recovery between each sprint with a load applied of 65 g/kg of body mass. Peak power (PP; watts [W])), time to peak power (tPP; seconds [s]), average power (AP; W), and fatigue index (FI; %) were recorded from the manufacturer's software. Previous test–retest reliability from our lab for peak power is reported as an intraclass correlation coefficient (ICC)= 0.96 and standard error of measure (SEM)= 19.7 Watts, fatigue index ICC = 0.97 and SEM = 4.0%, average power ICC = 0.97 and SEM = 120.8 W, and time to peak power ICC=0.92 and SEM=678.3 seconds.[112]

Blood Lactate

Blood lactate was measured prior to the RSA test, half-way through (after finishing the 5th bout), within 30 seconds of completing the tenth bout, and after resting for 10-minutes post-exercise using the Lactate Pro portable blood lactate meter (Arkray, Kioto, Japan) on micro blood samples drawn from the tip of the index finger according to the manufacturer's instructions. Test–retest reliability from for Lactate Pro is reported as ICC= 0.99 and SEM= 5.4 mmol/L.[113,114] Blood lactate clearance was calculated from finger capillary concertation.[115]

Equation 3: Lactate clearance = $(\text{lactate}_{\text{delayed}} - \text{lactate}_{\text{initial}}) / \text{lactate}_{\text{delayed}} \times 100$ (expressed as percentage).

Blood Flow

Brightness-mode ultrasound (US;Logiq-e, GE Healthcare, Madison, WI) was used to assess vessel diameter and blood flow through the brachial artery. Measurements were taken prior to and immediately after the RSA. For all measures, the participants were lying down with their right arm

extended and positioned about 80 degrees away from the torso. For the prior to exercise measure, a blood pressure cuff was positioned on the arm and inflated to 120 mmHg for two minutes. The US was set to view continuous blood volume flow in the vascular, pulse wave, and colorflow setting. Transmission gel was applied to the US transducer probe (12LRS, 5-13 mhz), and the probe was held against the skin with sufficient pressure to obtain a clear image of the brachial artery without compressing its diameter. The US was used to record a minimum of four pulses using the pulse wave setting. Artery diameter and arterial blood flow (BF) was estimated using the measure function in the device's default software (Software version R8.0.7). Test-retest reliability using these methods for brachial artery diameter from our laboratory include ICC= 0.82, SEM= 0.03 cm and blood flow ICC = 0.86, SEM= 5.92 mL/min.[1,112]

Specific Aim 3 Procedures

Body Composition

To address our third aim and account for the role of lean mass in strength outcomes, body composition was assessed using the 4-compartment model as previously validated by Wang et al.,[116] was used to estimate fat mass (FM; kg), percent body fat (%fat), and fat-free mass (FFM; kg). Components of this equation include: 1) body volume, derived from a dual-energy x-ray absorptiometry total body scan (DXA); 2) total body water, measured using multi-frequency bioelectrical impedance spectroscopy (BIS); and 3) total body bone mineral density, calculated using total body bone mineral content, measured from the DXA.

Equation 1: FM (kg) = 2.748(BV) – 0.699(TBW) + 1.129(Mo) – 2.051(body mass; BM)

%BF = (FM/BM) × 100

FFM (kg) = BM – FM

Equation 2: Mo = BMC × 1.0436

where BV is body volume, TBW is total body water, Mo is total body bone mineral and BM is body mass. Test re-test reliability from our laboratory for the 4C model is reported as ICC= 0.995, 0.982, 0.996, SEM= 0.831 kg, 0.960%, 0.999 kg for FM, %BF, and LM, respectively.[14]

Dual-energy X-ray Absorptiometry (DXA)

For total body DXA scans, participants were positioned in a supine position in the center of the scanning table, with arms and legs inside the scanning parameter. Participants were asked to dorsiflex their feet and a Styrofoam pad was placed at the bottom of the feet and secured with a Velcro strap to ensure a standardized foot position. A thin Styrofoam pad was placed between each of the participants hands and hips. Participants were asked to straighten their arms fully and hold the Styrofoam pad in place to ensure standardized hand placement. Participants were asked to remove all metal, thick clothing, and heavy plastic which could interfere with the DXA scans. The participants' alphanumeric number, age, ethnicity, height and weight was entered into the computer prior to the scanning. Regions-of-interest from the default software (enCORE Software Version 16) was adjusted by a trained technician. Test-retest reliability for DXA measurements from a similar population our lab are as follows: ICC= 0.998, 0.995, 0.998 and SEM= 0.462 kg, 0.807%, 0.777kg for FM, %BF and LM, respectively.[117]

Bioelectrical Impedance Spectroscopy

A multi-frequency bioelectrical impedance device was used (SFB7 ImpediMed, Queensland, Australia) to measure TBW. Prior to testing, participant height, weight, age, and sex were entered into the device. Participants laid supine on a table with arms positioned apart from the torso and enough space between the legs to ensure no contact. Two electrodes were placed 5 cm apart on the dorsal side of the right wrist and hand, and two electrodes were placed 5 cm apart on the dorsal side of the right ankle and foot. Device software was automatically estimate TBW, intracellular fluid (ICF), and extracellular fluid (ECF). The average of two trials was used to represent TBW. BIS test-retest reliability from our laboratory included an ICC= 0.99 and SEM of 0.93 L.[118]

Air-displacement Plethysmography (BodPod)

The BodPod device was calibrated according to manufacturer guidelines. In a private room, participants were asked to wear a swim cap and tight-fitting clothing such as a bathing suit or compression shorts and sports bra, and to remove all metal including jewelry, watches and glasses prior to measurement to reduce isothermal air. Body mass was measured to the nearest 0.01 kg using the software's corresponding scale. Participants were asked to sit upright in the BodPod, breathe normally and to minimize movement. Body volume (BV) was measured by a minimum of two trials that are within 150 mL of each other.

Muscle Characteristics

Muscle cross sectional area (mCSA) and echo intensity (EI) of the quadricep vastus lateralis (VL) was determined from panoramic brightness-mode US scans of the thigh (GE LOGIQ-e, Software version R8.0.7, GE Healthcare, Madison, WI) with standardized settings (frequency: 10.0; gain: 56; depth: 6 cm). The same technician performed each scan while the participant laid supine with the dominant leg extended. At the midpoint between the femoral greater trochanter and the femur lateral epicondyle, a high-density foam pad was placed under the thigh to guide a wide-band linear array US transducer probe (GE: 12L-RS). The probe was held directly against the skin, and equal pressure was applied as the technician swept the probe across the skin surface from the lateral VL border to medial fascia separation. The technician reviewed the initial quality of the scan on the US monitor, and two acceptable images were be saved. Muscle cross-sectional area and EI were measured offline using Image-J software (National Institute of Health, Version 1.37). The saved images of the panoramic scans were calibrated by measuring the number of pixels within a known distance of 6 cm. To measure mCSA and EI, the same technician traced the outline of the VL for each participant's scan along the fascial border as close as possible to capture only the muscle. Echo intensity was determined in the standard histogram function, which used gray scale analysis of pixels. To account for the influence of subcutaneous fat thickness (SFT) on EI, a previously described correction factor for SFT was used;[119] SFT was determined by an average of three linear measures from the epidermal layer to the fascial border of the VL at the lateral, medial, and

distal points of the image. The following equation was used to determine EI: $y_2 = y_1 + (t \times cf)$, where y_1 = raw EI, t = SFT, cf = 40.5278, and y_2 = corrected EI. Test-retest reliability for EI and mCSA measurements taken from this laboratory was an ICC= 0.99 and 0.99, respectively, and a SEM of 1.5 a.u. and 0.744 cm^2, respectively.[120]

Peripheral Quantitative Computed Tomography (pQCT)

Two-dimensional images of the dominant thigh of each participant was obtained using a peripheral quantitative computed tomography (pQCT) scanner (Stratec XCT 3000 software v. 6.0, Pforzheim, Germany). To determine the scan position, femur length was measured manually from the greater trochanter to femur lateral epicondyle and the midpoint was marked on the participant's skin with a permanent marker. The participants were asked to lay down on a table with their dominant leg extend through the gantry, which was manually positioned above the midpoint mark. The dominant leg was supported by a custom-built support device between the pQCT gantry opening and table. The participants' foot was secured on the opposite side of the gantry with a Velcro strap placed over the metatarsals. Using a longitudinal scout view scan, scanning was taken at 50% femoral length at a scan speed of 15 mm/s. The cross-sectional image obtained from the pQCT was calculated by subtracting the bone, skin, and subcutaneous fat CSA from the total CSA, which would only leave the mCSA. This calculation was performed by the pQCT software (Stratec XCT 3000 software v. 6.00, Pforzheim, Germany). Test-retest reliability for muscle CSA measured with the pQCT ICC= 0.996 to 0.998 and SEM= 1.660–1.101 cm^2.[121]

Resting Metabolic Rate

Resting metabolic rate (RMR) and respiratory exchange ratio (RER) was evaluated using a ventilated canopy with indirect calorimetry. Before each measurement, the metabolic cart underwent flow calibration using a 3-liter syringe and gas calibration using a standard gas concentration. Participants were asked to lay in a supine position on a table and breath normally while a plastic bubble hood was placed over their head and shoulders. Respiratory gases, oxygen uptake, and carbon dioxide production, was

analyzed over 15 second intervals with a metabolic cart (TrueOne 2400, ParvoMedics, Inc., Sandy, UT) for 30 minutes. The percentage of carbon dioxide was maintained between 1.0 – 1.2%, with the first five minutes of the test discarded to allow for gases to normalize; RMR and RER was be averaged over the remaining 25 minutes of the test. Test-retest reliability from our lab produced a RMR ICC= 0.94 and SEM of 125.6 kcal/day; RER ICC= 0.83 and SEM= 0.03 arbitrary units (a.u.).[122]

Specific Aim 4 Procedures

Total Body Protein Turnover

Whole body protein turnover (g N/24hr) was determined by [^{15}N]alanine isotope tracer (98% enriched, Cambridge Isotope Lab, Andover, MA)[123] in which a subsample of participants were asked to ingest a 0.30 gram dose of [^{15}N]alanine mixed with water. For the 24hrs following ingestion, participants were asked to collect urine from all voids and keep a diet record of all food and drink consumed. Diet records were analyzed with a nutrient analysis program (Food Processor, Esha research) for protein intake (g) to account for dietary nitrogen intake. Isotopically labeled nitrogen from the urine samples was used to determine nitrogen flux.[124] Total body protein synthesis and breakdown was calculated from urine samples[125] and used to determine net protein balance and flux. Samples were analyzed at Metabolic Solutions, Inc. (Nashua, NH).

Statistical Analysis

Statistical Procedures

Separate one-way analyses of variance tests were used to evaluate descriptive characteristics and performance outcome differences between OC, H-IUD, and EUM. Separate univariate analysis of covariance (ANCOVA) tests were used to assess the change from FP to LP between groups (OC, H-IUD, and EUM) for leg press 1RM, RSI, and CMJ, using total leg LM measures as a covariate. Similarly, separate univariate ANCOVAs were used to assess the change from FP to LP between groups for bench press 1RM and upper body peak force (PF), using total arm LM measures as a covariate. Lastly, when

covarying for dominant leg LM, the change from FP to LP between groups for lower body PF was evaluated.

For aim 2, separate one-way analyses of variance tests were used to evaluate descriptive characteristics and fatigue and recovery outcome differences between OC, H-IUD, and EUM. Separate univariate analysis of variance (ANOVA) tests were used to assess the change from FP to LP between groups (OC, H-IUD, and EUM) for PP, tPP, AP, FI. Separate univariate ANOVAs were used to assess the change from FP to LP between groups (OC, H-IUD, and EUM) for the change in blood lactate concentrations half-way through (concentration after finishing the 5th bout- pre concentration), the change in blood lactate concentrations immediately post RSA (concentration within 30 seconds of completing the tenth bout- pre RSA concentration and post-halfway concentration), for %blood lactate clearance. Separate univariate ANOVAs were used to assess the change from FP to LP between groups (OC, H-IUD, and EUM) for vessel diameter and BF prior to and post-RSA.

For aim 3, separate one-way analyses of variance tests were used to evaluate descriptive characteristics and body composition and metabolic outcome differences between OC, H-IUD, and EUM. Separate univariate analysis of covariance (ANCOVA) tests were used to assess the change from FP to LP between groups (OC, H-IUD, and EUM) for total body water, extracellular fluid, and intracellular fluid, covarying for hydration. Separate univariate ANOVAs were used to assess the change from FP to LP between groups (OC, H-IUD, and EUM) for the 4 compartment measures of fat mass, percent body fat, and fat free mass. Separate univariate ANOVAs were used to assess the change from FP to LP between groups (OC, H-IUD, and EUM) for muscle characteristics (muscle cross sectional area and echo intensity).

All statistical computations were performed using SPSS (Version 27, IBM, Armonk, NY, USA), using an $\alpha = 0.05$ to determine statistical significance.

CHAPTER IV: MANUSCRIPT 1

EVALUATION OF HORMONAL CONTRACEPTIONS EFFECTS ON MAXIMAL STRENGTH AND POWER

Introduction

Since the enactment of title IX 50 years ago, female participation in sport and exercise has exponentially increased.[126] With females participating in exercise now more than ever, the potential impacts of the cyclical menstrual hormones (i.e., estrogen and progesterone) on strength are important to understand as adequate levels of muscular strength and power are needed for optimal exercise performance.[38,39,127] Uniquely, the biphasic responses of endogenous hormones in eumenorrheic (EUM) females can be altered with the delivery of exogenous hormones (ethinyl estradiol and progestin or levonorgestrel) in the form of monophasic oral contraceptives (OC) or intrauterine devices (IUD).[128] Despite the increase in awareness of the need for female specific research,[1] the impacts of exogenous hormone delivery, particularly IUDs, on strength performance outcomes are largely unknown.

Estrogen has been shown to be beneficial for increasing muscle mass and strength, and is associated with increasing voluntary activation during muscle contractions.[10,82] Estrogen receptors are widely distributed in skeletal muscle and mitochondria, so variations in estrogen concentrations across the hormone phases [low hormone follicular phase (FP) vs. high hormone luteal phase (LP)] may impact skeletal muscle performance.[6] Exogenous sex hormones provided from OC purposely suppress endogenous estrogen and progesterone to prevent ovulation;[129] however, lowering estrogen levels may have undesirable consequences on muscle strength performance.[21] Despite these hormone counteractions, several studies in EUM and OC women have demonstrated no significant differences in muscle strength

and power between the low hormone FP and the high hormone LP, [8,11,12] although the data are conflicting.[13,14] One study comparing healthy, physically active women using OC to those who had EUM menstrual cycles demonstrated similar improvements in leg press one repetition max (1RM) and dynamometry isometric leg peak force after 10 weeks of resistance training.[15] In contrast, a recent review evaluating the impact of OC on exercise performance across hormonal phases reported a slight decrease in isometric muscle contractions and maximal anaerobic performance compared to EUM women.[35] Additionally, one of the most common forms of contraception, IUDs, has not been explored with respect to exercise performance [29].

Collectively, there is a lack of consensus on the impact of OC use on exercise performance, particularly across different hormonal landscapes and with varied contraceptive types. Previous studies observing maximal strength and power in women have often failed to account for the different hormonal phases, and hormonal contraception type.[9,12,15,25,35] Therefore, the purpose of this study was to evaluate the effects of OC use and IUD use, compared to a EUM cycle, on maximal strength and power between menstrual cycle (MC) phases in active, healthy adult women. It was hypothesized that 1RM bench press and leg press, upper and lower body dynamometry peak force, drop jump force, and countermovement jump height would be similar between IUD vs. EUM, but both groups would have greater strength and power than OC.

Methods:

Subjects

Of an original 227 individuals who expressed initial interest, 67 women provided written consent and were scheduled for visits (Figure 2). Recruitment was planned for a 2:2:1 enrollment with a goal of 25 women using monophasic oral contraceptives (OC), 25 women using intrauterine devices (IUD), and 15 women having eumenorrheic menstrual cycles. Prior to the completion of the study, 7 women (5 OC users, 1 IUD user, and 1 EUM woman) withdrew from the study due to musculoskeletal injury (n=2),

meeting exclusion criteria in health history questionnaire (n=3) and personal reasons (n=2) and were excluded from the final analysis.

Therefore, 60 healthy, active females were included for final analysis; groups for the final analysis were stratified as follows: monophasic OC users (n=21), hormonal-IUD (H-IUD; n=20) user, or were using a non-hormonal IUD or had regular naturally occurring menstrual cycles (EUM; n=19) (Table 1). Women using monophasic OC, or those who had an H-IUD were required to utilize the same hormonal contraception form for at least six months prior to enrollment (Table 2). Eumenorrheic women and women with non-hormonal IUDs were required to have consistent menstruation for at least three months prior to enrollment. The EUM group was utilized as a comparator for the two hormonal contraception groups. Inclusion into the study required that all participants were healthy, non-smokers, between the age of 18-40 years, with a BMI between 18.5-35.0 kg/m^2, and participated in moderate to vigorous intensity exercise at least 3 days a week, but no more than 200 minutes of vigorous exercise and/or more than 4 days per week of resistance training. Participants were not currently pregnant, planning to become pregnant, currently nursing, or had a child in the previous six months. Participants were excluded from participation if they had any disease or medication use that could influence the study outcomes, had undergone a full or partial hysterectomy, had a self-identified or clinically diagnosed eating disorder, or had lost or gained greater than 3.6 kg within the two months prior to the enrollment. A health history questionnaire was used to confirm inclusion/exclusion criteria. All methodology was approved by the University's Institutional Review Board, and all participants provided verbal and written informed consent prior to participation.

Experimental Design

Using a cross-sectional cohort study design, monophasic OC users, H-IUD users, and EUM women were recruited through flyers, email, word of mouth, and social media. Each cohort was followed prospectively across the hormone cycle; one visit occurred during the low hormone phase (days 0-9:

EUM/H-IUD) or placebo week (OC), and one visits occurred in the high hormone phase (between two days after ovulation and five days before the next predicted period; EUM/H-IUD) or active pill (OC). Participants began the study protocol in random order of hormone phase (i.e., beginning in the FP or LP) to limit the influence of time effects and testing on study outcomes. Participants were tested over one hormone cycle (n= 50), but due to scheduling conflicts, data collection also occurred over two (n=7) or three (n=3) hormone cycles. Participants in cohorts were matched based on relative lower body strength (average mean difference ± standard deviation: 0.2 ± 0.1 kg) and upper body strength (0.01 ± 0.01 kg) to ensure a normal distribution of relative strength in each group. Relative strength was assessed by normalizing the total weight lifted (kg) during the 1RM testing to the participant's body mass (kg).

Participants were asked to arrive to testing sessions following an 8 hour fast, consuming no food, caffeine, or alcohol and were also asked to abstain from physical activity for 48 hours prior to testing. Participants provided a urine sample to confirm negative pregnancy status via urine human chorionic gonadotropin concentration (HCG). Measurements of body composition, maximal strength, peak force, and power occurred at one visit in each cycle phase (FP/placebo pill and LP/active pill) (Figure 3).

Procedures

Menstrual Cycle Tracking

Upon enrollment, participants in the EUM or H-IUD groups who were not already tracking their menstrual cycles were asked to begin tracking the days between their periods. If women on H-IUDs were not having consistent periods, they were asked to record their menstrual symptoms and basal body temperature upon waking with a secure app/website (FertilityFriend) that was accessible to both the researcher and participant to plan ideal visit times. Body temperature along with symptoms (e.g., dizziness, lower back pain, mood changes/anxiety, etc.) were recorded daily to inform cycle length and determine visit scheduling (i.e., FP vs LP). Participants who were EUM or were using an H-IUD were asked to utilize ovulation tests between days 12-16 (Femometer, Princeton, NJ) according to manufacture guidelines at their predicted ovulation dates to confirm ovulation date and hormone cycle phase.

Salivary Hormone

Estrogen and progesterone concentrations were determined using a 2.5 mL saliva sample taken during the FP/placebo pill and during the LP/active pill. Estrogen and progesterone levels were determined using ELISA assays (Salivary 17 β-Estradiol Enzyme Immunoassay Kit, Salimetrics, LLC, State College, PA, USA; Salivary Progesterone (P4) Enzyme Immunoassay Kit, Salimetrics, LLC, State College, PA, USA). Participants were asked to avoid brushing their teeth for 45 minutes prior to and undergoing dental work for 48 hours prior to sample collection to avoid blood contamination. All samples were immediately frozen at -20°C until analysis using established enzymatic assays.

Total and Regional Body Composition

Body composition, specifically total body LM, dominate leg LM, and dominant arm LM were measured from a total body dual-energy x-ray absorptiometry scan (DXA; GE Lunar iDXA, GE Medical Systems Ultrasound & Primary Care Diagnostics, Madison, WI, USA). Participants were positioned in a supine position in the center of the scanning table, with arms and legs inside the scanning parameter. Participants were asked to dorsiflex their feet and a Styrofoam pad was placed at the bottom of the feet and secured with a Velcro strap to ensure a standardized foot position. A thin Styrofoam pad was placed between each of the participants hands and hips. Participants were asked to straighten their arms fully and hold the Styrofoam pad in place to ensure standardized hand placement. Participants were asked to remove all metal, thick clothing, and heavy plastic which could interfere with the DXA scans. The participants' alphanumeric number, age, ethnicity, height, and weight were entered into the computer prior to the scanning. Regions-of-interest from the default software (enCORE Software Version 16) was adjusted by a trained technician. Test-retest reliability for DXA measurements from a similar population our lab are: ICC= 0.998 and SEM=0.777 kg for LM.[117]

One Repetition Max (1RM) Testing

Participants completed a standardized five-minute warm-up on a treadmill at a 1% grade and 5.6 kilometer per hour pace. To determine 1RM (kg) for leg press and bench press, each participant

performed a set of 8-10 repetitions, with a weight that was approximately 50% of the anticipated 1RM. Participants then rested for 2 minutes, after which a set of 4-6 repetitions was completed with a load of 80% of the predicted 1RM.[108] After another 2-minute rest period, the weight was increased to an estimated 1RM load, and the participants attempted a single repetition with the weight. After the completion of each successful 1RM attempt, the weight was increased until failure was reached, with 2-3 minutes of rest between 1RM attempts. 1RM was determined for leg press first, followed by 1RM for bench press. A systematic review on test-retest reliability for 1RM tests demonstrated a median intraclass correlation coefficient (ICC) of 0.97 and median coefficient of variation (CV) of 4.2%.[39]

Isometric Dynamometer

Lower Body Isometric force

Lower body peak force was examined with a calibrated commercial dynamometer (HUMAC Norm, Computer Sports Medicine Inc., Stoughton, MA) during an isometric maximal voluntary contraction (MVC) on the dominant leg. Force signal was sampled at either at 600 Hz or 2000 Hz and low-pass filtered at 50 Hz using a zero-phase-shift, fourth-order Butterworth filter. The dominant knee joint of the participants was fixed at 60-degrees below full extension, as verified with a goniometer. The dynamometer arm was positioned approximately three centimeters above the lateral malleolus. The pelvis and torso were secured to the chair using adjustable straps, and the upper limbs were folded across the chest to isolate the contribution of the quadriceps musculature. Participants were instructed to push into the lever arm as fast and as hard as possible and to maintain maximal effort for approximately 3-4 seconds. Research assistants provided standardized oral encouragement, and real-time visual feedback of force production was displayed on a computer monitor in front of the dynamometer. Following three submaximal “warm-up” contractions (50% - 75% of perceived maximum effort), three to five practice MVCs were conducted to ensure production of maximal effort. Practice trials were continued until the force measurements ceased to increase within 5% of the previous trial. The values of the two highest practice trials were averaged and used to provide visual feedback on a computer monitor in front of the

dynamometer with lines at values that were at 100% and 110% of the highest practice trial. Participants were instructed to perform three maximal effort MVCs. Custom written software (LabView 21; National Instruments) was used to process all the signals offline. Three maximal effort MVCs were averaged and used in the final data analysis. For the knee extensor isometric strength, the ICC= 0.95 and standard error of the measure (SEM)= 38 N.[109]

Upper Body Isometric Force

Upper-body peak force was determined from an upper-body strength assessment utilizing an isometric upright row with a calibrated load cell (TSD121C, Hand Dynamometer, Biopac Systems Inc., Goleta, CA, USA) on a flat metal platform. The participants were instructed to stand on the flat metal platform and hold a metal bar connected to an adjustable chain. Participants were asked to grip the bar with a pronated grip, align their hands with their shoulders, their shoulders abducted, and elbows flexed. The chain was adjusted to one chain link below the level of the umbilicus and the chain length was kept consistent between visits. Following three submaximal "warm-up" contractions (50%–75% of perceived maximum effort), participants were asked to perform three isometric MVCs with a one-minute recovery period in between each muscle contraction. Participants were instructed to pull the bar sub-maximally to remove the slack in the chain immediately prior to the start of the MVC, while being given strong verbal encouragement to pull as hard and as fast as possible on the chain for 3–4 seconds once the baseline force was completely steady as confirmed by visual assessment. Three MVCs were performed with a one-minute recovery period in between contractions. The force signal was sampled at 2 kHz with a Biopac data acquisition system (MP150WSW, Biopac Systems Inc.) and stored on a laptop computer (ThinkPad T420; Lenovo). Custom written software (LabView 21; National Instruments) was used to process all the signals offline. For the upright row isometric strength, the ICC= 0.96 and SEM= 36.5 N.[110]

Drop Jump to Countermovement Jump

Reactive strength ability was assessed utilizing drop jump test with 30 cm box height positioned 50% of the participants height from the front edge of two floor-embedded force plates (odel 4060-NC,

Bertec Corporation, Columbus, Ohio). All participants were outfitted with a rigid cluster of 3 retroreflective markers placed over the sacrum. Participants were instructed to jump forward off the box to a double leg landing with 1 foot on each force plate, followed by an immediate vertical jump for maximum height. Prior to the jump trials, participants were asked to stand still in the center of the force plates to collect baseline retroreflective marker height. A minimum of 3 practice trials were performed followed by 3 successful jump-landing trials. If a trial was unsuccessful, a subsequent trial was collected and utilized for analysis. Drop jump data were sampled at 1200 Hz and low-pass filtered at 10 Hz using a fourth-order recursive Butterworth filter. Jump height in meters and contact time in seconds was calculated using a custom MATLAB code (MATLAB R2021b, Mathworks, Inc., Natick MA.). Jump height was normalized to body height in meters then converted into centimeters. Reactive strength index (RSI; cm s−1) was calculated by dividing jump height (cm) by ground contact time (s). The best result based on RSI was used for analysis. In healthy male and female adults, the jump height for counter movement jump ICC= 0.99 and CV= 6.91% and RSI ICC= 0.97 and CV= 11.92%.[111]

Statistical Analysis

Power was calculated based on effect sizes from previously published data evaluating maximal isokinetic/isometric muscle strength[9,15,16,25,106] (average effect size= 0.36) and 1 repetition maximum testing[15,16] (average effect size= 0.49) between an oral contraceptive group and a non-oral contraceptive group. With an α of 0.05, power of 0.8, three groups, two measures, an estimated correlation of 0.5 among repeated measures, a nonsphericity correction ϵ of 1, and a 15% drop-out rate, the study would be sufficiently powered with an average of sixty participants. Separate one-way analyses of variance tests were used to evaluate descriptive characteristics and performance outcome differences between OC, H-IUD, and EUM. Separate univariate analysis of covariance (ANCOVA) tests were used to assess the change from FP to LP between groups (OC, H-IUD, and EUM) for leg press 1RM, RSI, and CMJ, using total leg LM measures as a covariate. Similarly, separate univariate ANCOVAs were used to assess the change from FP to LP between groups for bench press 1RM and upper body peak force (PF), using total

arm LM measures as a covariate. Lastly, when covarying for dominant leg LM, the change from FP to LP between groups for lower body PF was evaluated. All statistical computations were performed using SPSS (Version 27, IBM, Armonk, NY, USA), using an $\alpha = 0.05$ to determine statistical significance.

Results:

When examining OC vs. H-IUD vs. EUM, there were no significant differences between groups age, body mass, BMI, or total and regional LM in the FP (p=0.121-0.644) or LP (p=0.121-0.761) (Table 3).

One Repetition Max Testing

When covaried for total leg LM, there was a significant difference for the change in leg press 1RM across the hormone phases between the groups (p=0.027), with a significantly higher leg press 1RM in the LP for the OC group (mean difference [MD; Δ LP-FP] ± standard error [SE]: Δ 7.3 ± 4.5 kg; p=0.045) compared to the H-IUD group (Δ -8.8 ± 4.6 kg; p=0.045). Although not significantly different, the EUM group (Δ 6.1 ± 4.7 kg; p=0.079) also demonstrated a higher leg press in the LP compared to the H-IUD group. Individual effects demonstrating the changes between each phase per group are presented in figure 4.

When covaried for total arm LM, there were no significant differences for changes in bench press 1RM across the hormone phases between the groups (p=0.392). All groups demonstrated similar bench press 1RM between phases (OC: Δ -0.9 ± 0.6 kg; H-IUD: Δ -0.2 ± 0.6 kg; EUM: Δ 0.4 ± 0.7 kg).

Isometric Dynamometry

When covaried for dominant leg LM, there were no significant differences for changes in lower body PF between groups (p=0.207). The OC group (Δ -5.8 ± 5.9 N) demonstrated greater PF in the FP,

while the H-IUD group (Δ 3.0 ± 6.1 N) and EUM group (Δ 9.6 ± 6.2 N) demonstrated greater PF in the LP.

When covaried for total arm LM, there were no significant differences for changes in upper body PF across the hormone phases between the groups (p=0.775). The OC group (Δ 9.7 ± 11.8 N) and H-IUD group (Δ 0.7± 11.9 N) reported higher upper body PF in the LP, while EUM group demonstrated higher PF in the FP (Δ -1.9 ± 12.3 N).

Power Outcomes

When covaried for total leg LM, there was no significant difference for changes in CMJ across the hormone phases between groups (p=0.402). The OC group (Δ 0.4 ± 1.5 cm) demonstrated higher jump height the LP, while the H-IUD group (Δ -0.7 ± 1.5 cm) and EUM group (Δ -2.5 ± 1.5 cm) demonstrated greater increases in the FP.

When covaried for total leg LM, there was no significant difference for changes in RSI across the hormone phases between groups (p=0.739). All groups demonstrated greater RSI values in the FP (OC: Δ -3.7 ± 4.0 cm/s; H-IUD: Δ -2.5 ± 4.1 cm/s; EUM: Δ -7.0 ± 4.1 cm/s).

Discussion:

Currently, only 6% of sport and exercise research studies have been conducted exclusively with female participants, with even fewer studies including women utilizing any form of OC.[1] Fluctuations of estrogen across the MC may mediate muscular performance;[127] muscular strength and power are imperative for optimal exercise performance and for injury prevention.[39] To date, we are unaware of any studies that have examined H-IUD, and just a few studies that have evaluated the impact of OCs on maximal strength and power.[35] The present study demonstrated significantly greater strength measured from leg press 1RM in the LP for OC (Δ 7.3 ± 4.5 kg) compared to lower strength in the LP in H-IUD (Δ -8.8 ± 4.6 kg). There were no differences in performance across the MC for bench press1RM, lower and

upper body PF, and power, when covarying for LM. OCs impacted leg press 1RM strength changes resulting in greater strength in the LP. The H-IUD group demonstrated greater 1RM strength and power outcomes in the FP. The EUM group resulted in greater CMJ and RSI in the FP, and greater leg press 1RM in the LP.

Maximal Strength

Collectively, previous data suggest that strength performance is decreased in the EUM early FP (days 1-5) where both estrogen and progesterone are low compared to the mid-FP (days 5-11) or the LP (days 17-26),[130] indicating that high levels of estrogen, with or without elevated progesterone, may be a driving factor in increased strength.[10,131] Yet there is conflicting information regarding the influence of the MC and OC on maximal muscular strength,[15,35] whether strength is greater in the LP or FP,[12,127] and if women should resistance train differently between MC phases.[130,132] The present study demonstrated MC phase-based differences in leg press 1RM between groups with the OC group (Δ 7.3 kg) demonstrating significantly greater strength in the LP compared to H-IUD reporting greater strength in the FP (Δ -8.8 kg). While our data support our hypothesis that overall maximal strength may be greater in H-IUD users (mean: 176.8 kg) than OC users (mean: 155.0 kg) due to H-IUDs not suppressing endogenous hormone production. The increase in strength during the LP for the OC group may be due to the elevated estrogen delivered during the active pill. Analysis of salivary hormones will provide greater insight into the role of hormonal concentrations on the maximal strength. Similar to previous research, the OC group (Δ 7.3 kg) in the present study did not vary significantly from the EUM group (Δ 6.1 kg) in total strength or in phase-based outcomes. One study examining OC use compared to EUM women demonstrated similar 1RM leg press strength between groups (OC: 114 ± 15 kg and EUM: 118 ± 18 kg, respectively),[15] indicating exogenous hormones may not influence maximal strength, or may provide consistent results across a cycle. However, this study and other research examining the impact of OC use on maximal strength has not reported the dosing/types of OCs utilized.[35,133] There are three types of OC pills, combined estrogen-progesterone, progesterone-only, and continuous or extended pills with varying

dosing amounts of the hormones provided,[129] all of which may impact performance outcomes differently. The present study utilized monophasic OC, which is the most common form prescribed.[129] It appears that monophasic OC users demonstrated variations in maximal lower body strength between cycle phases, even with slight variations in the dosing amounts and type of exogenous hormones utilized by study participants (table 2). Monophasic OCs provide consistent concentrations of estrogen and progesterone in the active pill (LP), and the withdrawal of the exogenous hormones during the placebo pill (FP) may mimic the early FP of a EUM cycle,[129] suggesting that the elevated estrogen levels in the OC LP may have led to the significant increases of leg press 1RM. The changes in maximal strength between cycle phases in each group surpassed the measurement error demonstrating these changes may be relevant for active women seeking to increase strength, particularly for hormonal contraceptive users. The leg press 1RM utilizes larger muscle groups, which may have more accurately represented muscle activation changes across the MC compared to the bench press 1RM which tests smaller muscle groups.[16] The present study demonstrated that all groups have similar non-significant changes in bench press 1RM (OC: Δ -0.9 kg; H-IUD: Δ -0.2; EUM: Δ 0.4 kg). Other data supports these findings with one study examining strength gains following a 12-week resistance training program in softball and water polo collegiate Division I student athletes using OC or EUM found that bench press 1RM (mean: 48 kg and 45 kg, respectively) and 10RM knee extension (mean: 27 kg and 28 kg, respectively) did not differ significantly between OC and EUM athletes.[16] Taken together, the delivery method of exogenous hormones may inversely impact lower body strength across the menstrual cycle.

Peak Force

Fluctuations in female sex hormones may alter force production, which is essential for maximal strength.[134] However, to date, there is a lack of consensus regarding the effects of the MC on maximal voluntary contractions and force production, which is the foundation for strength.[135] Muscular strength has been evaluated with various methodologies differing in participant inclusion (only EUM vs. EUM and OC) and force production outcomes. Existing studies have utilized isometric knee extension and handgrip

dynamometry,[15,16,25,48] isokinetic knee extension,[11,136] and barbell velocity[12] to evaluate group and phase differences with equivocal findings. In EUM and OC, it has been hypothesized that greater strength and peak force outcomes would be produced when progesterone remains low during the FP, with a reduction in strength resulting in the LP when progesterone is elevated.[134] While some studies have demonstrated increased PF in the FP compared to LP,[106,136–138] while others demonstrated no differences between MC phases.[11,12,16,25,139] It remains unclear whether force production varies across the MC, despite the expected influences of estrogen and progesterone, particularly when considering differences in hormonal profiles between EUM and OC/H-IUD. In the present study, the MC phases did not have an effect on lower body and upper body PF in the OC (Δ -5.8 N and Δ 9.7, respectively), H-IUD (Δ 3.0 N and Δ 0.7 N, respectively) and EUM (Δ 9.6 N and Δ -1.9 N, respectively) groups. Similar to our findings, one study observing the effects of the MC in OC (n=8) and EUM (n=9) active women demonstrated no significant differences in knee extensor strength (Δ 6.0 Nm and Δ 6.0 Nm, respectively) or handgrip strength (Δ 9.8 N and Δ 0.01 N, respectively) between the FP and LP or between groups.[140] Furthermore, the differences between MC phase in the present study for did not exceed the SEM for each measure, suggesting that inclusion of women in studies examining force production should not be limited to a specific MC cycle phase or only include EUM women.

Power

Across the MC, variations in knee joint laxity, neuromuscular coordination, and postural control have been reported.[45,136,139] These variations may influence the production of lower body power and the ability to quickly change from eccentric to concentric motions during exercise. When CMJ, a sensitive measure of lower body power, is performed as part of a drop jump exercise, RSI can be calculated by dividing jump height (cm) by ground contact time (s), thereby providing a comprehensive examination of lower body explosive capabilities.[111] Of the available research assessing neuromuscular characteristics in EUM females and OC users, there appears to be no difference between groups or hormone phases on muscular power [23–25] or jump height.[24] The present study supports these findings as there were no

significantly changes in CMJ (OC group: Δ 0.4 cm; H-IUD: Δ -0.7 cm; EUM: Δ -2.5 cm) across the MC. To our knowledge, this is the first study examining RSI across the MC in EUM, OC, and H-IUD. All groups demonstrated greater RSI values in the FP (OC: Δ -3.7 cm/s; H-IUD: Δ -2.5 cm/s; EUM: Δ -7.0 cm/s), despite randomization of testing. For EUM, CMJ and RSI measures were meaningful beyond the error of measurement, therefore, could be indicative of power being greater in the FP for all groups, although results were not significant. Taken together, muscle performance of the stretch-shortening cycle needed to produce power may be impacted by MC phase.[131]

Limitations

While the present study provided a comprehensive cross-sectional evaluation across the MC, there are limitations associated with cross-sectional study designs. Longitudinal studies may have more statistical power to detect group changes over time, however, women in the present study were required to have utilized the same contraceptive method for at least six months prior to enrollment with an average time of use of 2.7 years for OC users and 3.6 years for H-IUD users. Additionally, this study examined changes over one MC which may have prevented possible influences elicited from exercise training, however repeating the measurements over multiple MCs may aid in detecting longitudinal changes. While the present study utilized ovulation kits in EUM and H-IUD women to confirm cycle phase, it is possible some of the participants were tested when unpredictable hormone fluctuations were occurring. The present study collected salivary concentrations of estrogen and progesterone which is an acceptable method of hormonal analyses even though it is not as sensitive as analysis of hormonal concentrations from the blood; these data will be included prior to publication once the analyses are received by the research team. The present study had exercise inclusion criteria of no more than 200 minutes of vigorous exercise and/or more than 4 days per week of resistance training, with the aim of including active, but not highly active, women. While this allows for greater translatability to the general population, these results may not apply to women who are highly trained in strength and power exercises.

Conclusion

The current study provides evidence that maximal strength and power did not vary significantly between OC, H-IUD, and EUM women when evaluated in a cross-sectional view across the MC. It appeared that OC users had a greater leg press 1RM in the LP when compared to H-IUD users, possibly due to the variation of progesterone concentrations in the LP. This was the first study to our knowledge to include women using H-IUD, with results demonstrating little variation in strength outcomes across the MC and compared to OC and EUM. As the prevalence of H-IUDs continues to increase,[141] it is imperative that future research explores the physiological impacts of varying hormonal landscapes on strength and power outcomes to inform best research practices. Results from this study provide a basis for understanding the physiological impact of OCs and IUDs on muscular strength and power, thereby informing future research regarding training considerations across the MC. Future research should expand inclusion criteria for females in exercise science research as the present study demonstrated little variation in strength and power across the MC between OC, H-IUD, and EUM women.

CHAPTER V: MANUSCRIPT 2

THE MENSTRUAL CYCLE AND HORMONAL CONTRACEPTION EFFECTS ON FATIGABILITY AND RECOVERY

Introduction

Despite an increase in female participation in sport and exercise, only 6% of exercise and sport research has been conducted in female only participants.[1] Recovery from exercise is a particularly sparse area of research in females despite the known physiologic and metabolic differences between males and females, that would impact recovery.[40] The cyclical menstrual hormones (i.e., estrogen and progesterone) in eumenorrheic (EUM) women has been shown to impact fatigability during exercise and subsequent recovery adaptations.[10,52,53,58,60,64] Importantly, the EUM menstrual cycle can be altered with the delivery of exogenous hormones (ethinyl estradiol and progestin or levonorgestrel) in the form of monophasic oral contraceptives (OC) or intrauterine devices (IUD).[128] Over 10 million women in the United States (U.S.) use OC,[142] yet the impacts of exogenous hormone delivery, particularly IUDs, on fatigability and recovery is unclear.

Evidence suggests that estrogen and progesterone directly mediate fatigue through cardiorespiratory, metabolic, vascular, and neuromuscular impact.[40,55,86] For example, estrogen can stimulate the central nervous system, moderating muscle contractions and cardiovascular functions,[40] whereas progesterone increases glycogen depletion and subsequent lactate concentration.[143,144] Given these hormonal considerations, recovery rates may vary across the menstrual cycle phases or via exogenous hormone delivery.[145] Fatigability is often defined as an exercise-induced decrease in the

maximal force or power that the muscles can produce and is task dependent.[146] The current body of literature provides equivocal results in EUM and OC users with some studies demonstrating less muscular fatigability in the follicular phase (FP),[64,147,148] while others studies exhibit less fatigability in the luteal phase (LP).[10,17,60] The inconsistencies in study design, particularly with the fatiguing task, may contribute to the lack of clarity. Utilizing an intermittent high intensity exercise test, such as repeated sprint ability (RSA), may more closely mimic muscular movements commonly carried out in exercise outside of the laboratory.[149] One study observing workload decrements and peak power in the withdrawal week versus the active week of monophasic OC in team sport athletes demonstrated no differences in fatigue outcomes between phases.[23] This may suggest exercise recovery may vary within the OC hormone phases; however, the study did not utilize a EUM group to compare values to thereby limiting the understanding of the hormonal impacts on recovery.

Estrogen can mediate vasodilatory responses of the arteries, providing greater blood flow to exercising skeletal muscle.[54,55] Effective recovery strategies have demonstrated augmented blood flow may improve oxygen and nutrient delivery, [52,53] possibly decreasing metabolic perturbations that contribute to fatigue.[4,55] During exercise, increased blood flow may aid in transporting lactate and other metabolic by-products away from the active muscle,[52] thereby increasing blood lactate threshold and expediating lactate clearance post-exercise.[57] Considering these benefits of vasodilation, the fluctuations in estrogen across the hormone cycles may indicate recovery differences between cycle phases.[54] Additionally, as OC use downregulates the cyclic hormone activity of endogenous estrogen,[129] recovery outcomes in OC users may differ from EUM females. A recent review examining the impact of OCs and IUDs demonstrated that OC use decreased flow-mediated dilation, while IUDs had no effect.[150] In OC and EUM women, intense exercise appeared to blunt skin blood flow mechanisms in the OC, but did not appear to influence blood lactate concentrations between groups.[151] These results suggest OC use may impact exercise performance and recovery, particularly through changes in vascular responses. However,

few studies examine exercise and blood flow in EUM women and OC users, and one of the most common forms of contraception, IUDs, has not been explored with respect to recovery.[29]

Decreased fatigability and improved recovery are essential for optimal exercise performance and reduced injury risk.[49] Collectively, there is a lack of consensus on the impact of female sex hormones, both endogenous and exogenous, on exercise recovery.[10,23,58,152] With a high prevalence of women participating in exercise, along with high use of OC, the purpose of this study was to explore the impact of OC and IUD compared to EUM menstrual cycle on exercise fatigability and recovery across the FP and LP hormone cycle phases. It was hypothesized that there would be a larger decrease in peak power, fatigue index, workload decrements, and blood lactate clearance in the OC group compared to IUD and EUM, but that all groups would have decreased recovery in the high hormone phase.

Methods:

Subjects

Of an original 227 individuals who expressed initial interest, 67 women provided written consent and were scheduled for visits. Recruitment was planned for a 2:2:1 enrollment with a goal of 25 women using monophasic oral contraceptives (OC), 25 women using intrauterine devices (IUD), and 15 women having eumenorrheic (EUM) menstrual cycles. Prior to the completion of the study, 7 women (5 OC users, 1 IUD user, and 1 EUM woman) withdrew from the study and were excluded from the final analysis. Therefore, 60 healthy, active females who were using OC (n=21), had a hormonal IUD (H-IUD; n=24), or were using a non-hormonal IUD or had regular naturally occurring menstrual cycles (EUM; n=19) were included in the final analysis (Table 1). Women using monophasic OC, or those who had an IUD were required to utilize the same hormonal contraception form for at least six months prior to enrollment (Table 2). Eumenorrheic women and women with non-hormonal IUDs were required to have consistent menstruation for at least three months prior to enrollment. Prior to all testing, pregnancy was ruled out via urine human chorionic gonadotropin concentration (HCG). A health history questionnaire was used to confirm inclusion/exclusion criteria.

Experimental Design

Using a cross-sectional cohort study design, monophasic OC, H-IUD, and EUM users were followed prospectively across the hormone cycle; one visit occurred during the low hormone phase (days 0-9: EUM/H-IUD) or placebo week (OC), and one visits occurred in the high hormone phase (between two days after ovulation and five days before the next predicted period; EUM/H-IUD) or active pill (OC) (figure 2). Participants began the study protocol in random order of hormone phase (i.e., beginning in the FP or LP) to limit the influence of time effects and testing on study outcomes. Participants were tested over one hormone cycle (n= 50), but due to scheduling conflicts, data collection also occurred over two (n=7) or three (n=3) hormone cycles. Participants were asked to arrive to testing sessions following an 8 hour fast from caffeine and alcohol and were also asked to abstain from physical activity for 48 hours prior to testing. Measurements of repeated sprint ability, blood lactate, and blood flow occurred at one visit in each cycle phase (FP/placebo pill and LP/active pill). All methodology was approved by the University's Institutional Review Board, and all participants provided verbal and written informed consent prior to participation.

Procedures:

Menstrual Cycle Tracking

Upon enrollment, participants in the EUM or H-IUD groups who were not already tracking their menstrual cycles were asked to begin tracking the days between their periods. If women on H-IUDs were not having consistent periods, they were asked to record their menstrual symptoms and basal body temperature upon waking with a secure app/website (FertilityFriend) that was accessible to both the researcher and participant to plan ideal visit times. Participants who were EUM or were using a H- IUD were provided with ovulation tests (Femometer, Princeton, NJ) and were asked to test for ovulation around days 12-16 (to confirm ovulation date and hormone cycle phase.

Salivary Hormone

Estrogen and progesterone concentrations were determined using a 2.5 mL saliva sample taken during the FP and during the LP. Estrogen and progesterone levels were determined using ELISA assays (Salivary 17 β-Estradiol Enzyme Immunoassay Kit, Salimetrics, LLC, State College, PA, USA; Salivary Progesterone (P4) Enzyme Immunoassay Kit, Salimetrics, LLC, State College, PA, USA). Participants were asked to avoid brushing their teeth for 45 minutes prior to sample collection to avoid blood contamination. All samples were immediately frozen at -20°C until analysis using established enzymatic assays.

Repeated Sprint Ability (RSA)

Participants completed an RSA test on a friction-loaded cycle ergometer (Monark 894E, Stockholm, Sweden). To warm-up, the participants cycled for two minutes at 50 rpm against a resistance of 0.5 kg, followed by two 30 second bouts of cycling at a resistance of 1.5 kg, keeping the cadence between 85-115 repetitions per minute (rpm). Each warm-up sprint was followed by a 60 second passive recovery. Once the warm-up was completed, the participants began the RSA test consisting of 10 six-second maximal sprints with a load of 65 g/kg of body mass, interspersed with 30 seconds of passive recovery between each sprint. Peak power (PP; watts [W])), time to peak power (tPP; seconds [s]), average power (AP; W), and fatigue index (FI; %) were recorded from the manufacturer's software. Previous test–retest reliability from our lab for peak power is reported as an intraclass correlation coefficient (ICC)= 0.96 and standard error of measure (SEM)= 19.7 Watts, fatigue index ICC = 0.97 and SEM = 4.0%, average power ICC = 0.97 and SEM = 120.8 W, and time to peak power ICC=0.92 and SEM=678.3 seconds.[112]

Blood Lactate

Blood lactate clearance was calculated from finger capillary concertation.[115] A capillary finger prick to obtain a small blood sample occurred prior to the RSA test, half-way through (after finishing the

5th bout), within 30 seconds of completing the tenth bout, and after resting for 10-minutes post-exercise. The blood sample was analyzed using the Lactate Pro portable blood lactate meter (Arkray, Kioto, Japan) according to the manufacturer's instructions. Lactate clearance was then determined using the following equation: Lactate clearance = ($lactate_{initial} - lactate_{delayed}$) / $lactate_{initial} \times 100$ (expressed as percentage). Test–retest reliability from the Lactate Pro is reported as ICC= 0.99 and SEM= 5.4 mmol/L.[113,114]

Blood Flow

Brightness-mode ultrasound (US; Logiq-e, GE Healthcare, Madison, WI) was used to assess vessel diameter and blood flow through the brachial artery. Measurements were taken prior to and immediately after the RSA. For all measures, the participants were lying down with their right arm extended and positioned about 80 degrees away from the torso. At the resting baseline measure, a blood pressure cuff was positioned on the arm and inflated to 180 mmHg for two minutes. The US was set to view continuous blood volume flow in the vascular, pulse wave, and colorflow setting. Transmission gel was applied to the US transducer probe (12LRS, 5-13 mhz), and the probe was held against the skin with sufficient pressure to obtain a clear image of the brachial artery without compressing its diameter. The ultrasound was used to record a minimum of four pulses using the pulse wave setting. Artery vessel diameter and arterial blood flow (BF) was estimated using the measure function in the device's default software (Software version R8.0.7). Test-retest reliability using these methods from our laboratory for brachial artery diameter include ICC= 0.82, SEM= 0.027 cm and blood flow ICC = 0.86, SEM= 5.92 mL/min.[112]

Statistical Analysis

Power was calculated based on effect sizes from previously published data evaluating average power and peak power (average effect size= 0.9).[112,153] With an α of 0.05, power of 0.8, three groups, two measures, an estimated correlation of 0.5 among repeated measures, a nonsphericity correction ϵ of 1, and a 12% drop-out rate, the study would be sufficiently powered with an average of sixty two participants.

Separate one-way analyses of variance tests were used to evaluate descriptive characteristics and performance outcome differences between OC, H-IUD, and EUM. Separate univariate analysis of variance (ANOVA) tests were used to assess the change from FP to LP between groups (OC, H-IUD, and EUM) for PP, tPP, AP, FI. Separate univariate ANOVAs were used to assess the change from FP to LP between groups (OC, H-IUD, and EUM) for the change in blood lactate concentrations half-way through (concentration after finishing the 5th bout- pre concentration), the change in blood lactate concentrations immediately post RSA (concentration within 30 seconds of completing the tenth bout- pre RSA concentration and post-halfway concentration), for %blood lactate clearance. Separate univariate ANOVAs were used to assess the change from FP to LP between groups (OC, H-IUD, and EUM) for vessel diameter and BF prior to and post-RSA. All statistical computations were performed using SPSS (Version 26, IBM, Armonk, NY, USA), using an $\alpha = 0.05$ to determine statistical significance.

Results:

When examining OC vs. H-IUD vs. EUM, there were no significant between group demographic differences for age, body mass, or BMI in the FP (p=0.121-0.528) or LP (p=0.121-0.539) (Table 4).

Repeated Sprint Ability (RSA)

For PP, there were no significant differences across the MC between groups (p=0.256). The OC group (Δ -0.2 ± 16.6 W) and H-IUD group (Δ -4.9 ± 12.9 W) reported greater PP in the FP, while EUM group demonstrated greater PP in the LP (Δ 24.0 ± 13.3 W). Individual effects demonstrating the changes between each phase per group are presented in figure 5.

For tPP, there were no significant differences across the MC between groups (p=0.959). All groups demonstrated greater tPP values in the LP (OC: Δ 268.0 ± 136.5 s; H-IUD: Δ 222.4 ± 139.9 s; EUM: Δ 275.9 ± 143.5 s).

For AP, there were no significant differences across the MC between groups (p=0.293). The OC group (Δ -248.2 ± 254.0 W) demonstrated greater AP in the FP, while the H-IUD group (Δ 320.3 ± 260.3 W) and EUM group (Δ 113.9 ± 267.1 W) demonstrated greater AP in the LP.

For FI, there were no significant differences across the MC between groups (p=0.959). All groups demonstrated similar FI values between MC phases (OC: Δ 2.0 ± 3.6%; H-IUD: Δ -1.2 ± 3.7%; EUM: Δ 1.7 ± 3.8%).

Blood Lactate

For the change in blood lactate concentration half-way through compared to pre-RSA test, there were no significant differences across the MC between groups (p=0.553). All groups demonstrated similar changes in blood lactate between MC phases (OC: Δ -0.6 ± 0.5 mmol/L; H-IUD: Δ 0.02 ± 0.4 mmol/L; EUM: Δ -0.6 ± 0.6 mmol/L). Individual effects demonstrating the changes between each phase per group are presented in figure 6.

For change in blood lactate concentration immediately post compared to pre-RSA test, there were no significant differences across the MC between groups (p=0.137). All groups demonstrated similar changes in blood lactate between MC phases (OC: Δ -0.6 ± 0.4 mmol/L; H-IUD: Δ 0.4 ± 0.3 mmol/L; EUM: Δ -0.03 ± 0.4 mmol/L).

For blood lactate clearance, there were no significant differences across the MC between groups (p=0.503). The OC groups demonstrated greater blood lactate clearance in the FP (OC: Δ 3.9 ± 5.8%) while the H-IUD (Δ -5.5 ± 5.5%) group and EUM group (Δ -1.7 ± 6.9%) demonstrated greater blood lactate clearance in the LP.

Blood Flow

For vessel diameter pre-RSA, there were no significant differences across the MC between groups (p=0.196). All groups demonstrated similar changes in vessel diameter between MC phases (OC: Δ -0.01 ± 0.01 cm; H-IUD: Δ 0.01 ± 0.01cm; EUM: Δ -0.04 ± 0.01 cm).

For vessel diameter post-RSA, there were no significant differences across the MC between groups (p=0.428). All groups demonstrated similar changes in vessel diameter between MC phases (OC: Δ 0.00 ± 0.01 cm; H-IUD: Δ 0.01 ± 0.01 cm; EUM: Δ 0.01 ± 0.01 cm).

For blood flow pre-RSA, there were no significant differences across the MC between groups (p=0.958). All groups demonstrated greater blood flow in the LP (OC: Δ 1.8 ± 136.5 mL/min; H-IUD: Δ 0.09 ± 6.8 mL/min; EUM: Δ 2.9 ± 7.0 mL/min).

For blood flow post-RSA, there were no significant differences across the MC between groups (p=0.847). All groups demonstrated greater blood flow in the FP (OC: Δ -133.4 ± 10.3 mL/min; H-IUD: Δ -128.6 ± 10.6 mL/min; EUM: Δ -137.3 ± 10.8 mL/min).

Discussion:

Estrogen has been shown to have a positive influence on endothelial function during intense exercise, resulting in greater vasodilation around exercise, compared to men..[40] These vascular improvements may support greater fatigue resistance and removal of metabolic byproducts.[50] With OC use, and concomitant suppression of endogenous estrogen production,[129] greater fatigability and reduced recovery have been reported, although we are aware of only two studies in this area.[64,145] With the rising prevalence of H-IUD use, particularly among active women, there is an unmet need to understand the implications for exercise performance and recovery, with no data to date in this area. Collectively, results of the current study demonstrated minimal effects from the MC or OC/IUD use on exercise performance

and recovery in active women. There were no changes in tPP, AP, FI, and blood lactate, vessel diameter, or blood flow between MC phases in OC, H-IUD, and EUM women. Despite no significance, the H-IUD group (Δ 320.3 W) and EUM group (Δ 24.0 W) demonstrated greater changes in AP and PP, respectively, in the LP while the OC exhibited greater AP in the FP (Δ -248.2 W). While it appears fatigability differences between groups across the MC are minimal, OC users may experience less fatigability in the FP.

Fatigability Measures

It remains unclear whether fatigability varies across the MC, particularly when considering differences in hormonal profiles between EUM and OC/H-IUD. A recent review examining isometric and dynamic fatiguing tasks in EUM reported variable results with 17% of the studies reporting greater fatigue resistance during the FP and 15% in the LP.[58] The present study aligns with previous research demonstrating no significant differences in metrics of fatigability (PP, AP, tPP, or FI) across MC cycle phases. To our knowledge, this is the first study to examine the impact of OC and H-IUD on fatigability with results suggesting hormonal contraception has little to no impact on fatigue during intense exercise, when compared to EUM women. Interestingly, while not significant, the EUM change in PP between the phases (Δ 24.0 W) exceeded the error of the measure, and therefore could be indicative of greater power output in the LP. For AP, both the OC (Δ -248.2 W) and H-IUD (Δ 320.3 W) groups demonstrated changes in AP in the FP and LP, respectively. Taken together it appears that EUM and H-IUD demonstrated greater power output when estrogen levels were elevated in the LP, while the OC group exhibited greater fatigability in the LP, when all exogenous hormones were possibly elevated. This will be confirmed through analysis of salivary estrogen and progesterone. Previous research examining the effects of MC phase on high intensity sprinting in OC and EUM women have demonstrated minimal differences in PP (mean difference: 2 W) and AP (mean difference: 0.01 W), suggesting hormonal fluctuations may not influence anaerobic sprinting activity.[154] Uniquely, the present study evaluated FI and tPP, which few previous studies have examined.[23] The OC (Δ 2.0%) and EUM (Δ 1.7%)

demonstrated a greater FI in the LP, suggesting these groups were unable to maintain PP outputs once they were reached. The H-IUD group demonstrated greater FI in the FP (Δ -1.2%) aligning with the exhibited lower AP in the FP. Interestingly, all groups showed increased tPP in the LP demonstrating it took longer for participants to reach the peak output. Taken together, the OC and H-IUD groups may experience greater fatigability in the FP, which may be an important consideration in sports that require quick timing when exerting power, such as sprinting and most team sports.

Blood Lactate

Blood lactate concentrations throughout exercise and lactate clearance rate post exercise can represent the alterations in carbohydrate metabolism during exercise, thus these measures may be affected by MC phase.[155,156] In the present study, all groups demonstrated similar changes in blood lactate halfway through (OC: Δ -0.6 mmol/L; H-IUD: Δ 0.02 mmol/L; EUM: Δ -0.6 mmol/L) and immediately post exercise (OC: Δ -0.6 mmol/L; H-IUD: Δ 0.4 mmol/L; EUM: Δ -0.03 mmol/L) between MC phases indicating exercise capacity during intense exercise may not vary between groups or MC phases. When examining blood lactate concentrations prior to, during, immediately post, and 10-minutes post repeated intense sprints on a treadmill in EUM in the FP and LP, previous data reported a significant increase in blood lactate over time in both phases, with no differences between MC phases at each timepoint (prior: Δ 0.01 mmol/L; during: Δ -0.8 mmol/L; immediately post: Δ -0.03 mmol/L; 10-minutes post: Δ -0.4 mmol/L),[154] demonstrating similar results to the present study. Other studies examining blood lactate values in EUM women have also demonstrated equivocal lactate accumulation after intense exercise, supporting findings from the present study.[135,157–159] Furthermore, research examining blood lactate concentrations in EUM compared to OC users demonstrates no significant differences between groups across MC phases in rowing ergometry[160] and in intermittent exercise trials.[161] Similar to our results, these data show that blood lactate values immediately post exercise were slightly lower in the LP, which may be indicative of the favorable increases in fat oxidation,[89] possibly decreasing fatigability during high intensity exercise. With the equation utilized in the present study, a negative lactate clearance indicates a

decrease in lactate over time, while a positive lactate clearance indicates an increase of lactate over time. Lactate clearance rates were greater in the H-IUD (Δ -5.5%) and EUM (Δ -1.7%) in the LP. This supports our hypothesis that blood lactate clearance would be reduced in the OC group (Δ 3.9%) due to variations in exogenous hormones provided. While not significant, a 4% decrease in blood lactate clearance in the OC group may be meaningful, particularly in the context of recovery from intense exercise sessions.

Vessel Diameter and Blood Flow

A recent meta-analysis examining the impact of the MC on peripheral vascular function in premenopausal women found approximately 30 studies provided evidence that endothelial function increased in the LP.[162] Other research examining vascular function in EUM and OC women during exercise across the MC suggest there are minimal differences in vessel diameter and blood flow,[163] yet research is limited. In the present study, all groups demonstrated similar changes in vessel diameter at baseline (OC: Δ -0.01 cm; H-IUD: Δ 0.01; EUM: Δ -0.04 cm) and immediately post-exercise (OC: Δ 0.00 cm; H-IUD: Δ 0.01 cm; EUM: Δ 0.01 cm) between MC phases. Previous research has suggested that the ultrasound machine provides a reliable and feasible measure for evaluating vessel diameter and blood flow of the brachial artery.[164] Yet, there is minimal research examining vessel diameter across the MC in EUM and OC users. Similar to our findings, one study examining vessel diameter in OC and EUM women once each MC phase found no differences in vessel diameter between groups (Δ -0.01 mm and Δ -0.05 mm, respectively).[163] While this study did not include measures of exercise, the results suggest there may be minimal vessel diameter differences between groups across the MC. As vasodilation is task specific, it is possible that the exercise session (~10 minutes) was not long enough to elicit large changes in vessel diameter following the exercise. Interestingly, the blood flow measures in present study demonstrated opposite results to our hypotheses with all groups exhibiting slightly greater blood flow in the LP (OC: Δ 1.8 mL/min; H-IUD: Δ 0.09 mL/min; EUM: Δ 2.9 mL/min) at rest and greater blood flow in the FP (OC: Δ -133.4 mL/min; H-IUD: Δ -128.6 mL/min; EUM: Δ -137.3 mL/min) after exercise. The baseline findings are in line with the results from the recent meta-analysis suggesting increased

endothelial function in the LP.[162] Additionally, the study examining OC (Δ-0.04 mm) and EUM (Δ0.01mm) women at rest found virtually no difference between MC phases.[163] Furthermore, these results were not outside of the measurement error suggesting the blood flow changes at rest may not be different between MC phases. Following exercise, the blood flow values exceeded the measurement error indicating blood flow may be improved in the FP for all groups. This may be an adaptation mechanism to compensate for the observed lower PP and AP were the FP.

Limitations

The results of the present study evaluate the impact of female sex hormones on fatigability and recovery across the MC. It is possible there may have been some unpredictable hormonal fluctuations for women who were tested over two (n=7) or three (n=3) MCs. These data were not covaried for salivary estrogen and progesterone concentrations. However, the present study utilized ovulation kits to confirm cycle length and cycle phase, reducing the possibility of large variations in hormonal profiles. Salivary concentrations of estrogen and progesterone were taken and are being analyzed. While salivary concentrations are an acceptable method of hormone analysis, they are less sensitive than analysis of the hormone concentrations in the blood. Additionally, the findings in the present study apply to the RSA protocol, a highly anaerobic exercise capacity test. Results may be different in an endurance exercise protocol.

Conclusion

The current study provides evidence that there are little differences in fatigability and recovery between OC, H-IUD, and EUM women across the MC. It appears that there may be fatigability differences between H-IUD/EUM women compared to OC with OC experiencing less fatigability in the FP. Blood lactate clearance appeared to be increased in the LP for H-IUD and EUM women in the LP, while the OC group demonstrated a 4% decrease in lactate clearance in the LP suggesting OC women may need focus on recovery measures in the LP. Vessel diameter and blood flow were largely unchanged

with slight improvements in blood flow in the FP for all groups; yet this may be a compensatory mechanism for lower power production. Overall, these data suggest that inclusion of women in studies examining fatigability and recovery should not be limited to a specific MC cycle phase or only include EUM women. In recreationally active women, hormonal contraception and the MC likely have minimal impacts on anaerobic exercise performance, suggesting that exercise training does not need to be modified.

CHAPTER VI: AIM 3 SUMMARY OF RESULTS

Aim 3 Purpose:

The purpose was to determine the effects of female hormonal contraception use compared to EUM on resting total body water, intracellular and extracellular fluid, body composition measures (including lean mass, fat mass, and percent body fat), metabolic outcomes (including respiratory exchange ratio and resting energy expenditure), and muscle characteristics (including muscle cross sectional area and echo intensity) across the menstrual cycle.

Aim 3 Results:

When examining OC vs. H-IUD vs. EUM, there were no significant between group demographic differences for age, body mass, or BMI in the FP (p=0.121-0.528) or LP (p=0.121-0.539) (Table 5).

Total Body Water (TBW)

When covaried for hydration status, there were no significant differences for changes in TBW across the hormone phases between the groups (p=0.291). All groups demonstrated similar TBW between phases (OC: Δ 0.6 ± 0.3 L; H-IUD: Δ -0.1 ± 0.3 L; EUM: Δ -0.04 ± 0.3 L). Individual effects demonstrating the changes between each phase per group are presented in figure 6.

When covaried for hydration status, there were no significant differences for changes in extracellular fluid across the hormone phases between the groups (p=0.344). All groups demonstrated similar extracellular fluid between phases (OC: Δ 0.2 ± 0.1 L; H-IUD: Δ -0.03 ± 0.1 L; EUM: Δ 0.01 ± 0.1 L).

When covaried for hydration status, there were no significant differences for changes in intracellular fluid across the hormone phases between the groups (p=0.929). All groups demonstrated greater intracellular fluid in the FP (OC: Δ -0.2 ± 0.3 L; H-IUD: Δ -0.03 ± 0.3 L; EUM: Δ -0.2 ± 0.3 L).

4 Compartment Model

For fat mass, there were no significant differences across the MC between groups (p=0.447). The OC group demonstrated greater fat mass in the FP (OC: Δ -0.5 ± 0.4 kg) while the H-IUD (Δ 0.3 ± 0.5 kg) group and EUM group (Δ 0.1 ± 0.5 kg) demonstrated greater fat mass in the LP.

For percent body fat, there were no significant differences across the MC between groups (p=0.474). All groups demonstrated similar percent body fat between phases (OC: Δ -0.6 ± 0.7%; H-IUD: Δ 0.5 ± 0.7%; EUM: Δ 0.1 ± 0.7%).

For fat free mass, there were no significant differences across the MC between groups (p=0.580). All groups demonstrated similar fat free mass between phases (OC: Δ 0.4 ± 0.5 kg; H-IUD: Δ -0.3 ± 0.5 kg; EUM: Δ -0.1 ± 0.5 kg).

Resting Metabolic Rate

For respiratory quotient, there were no significant differences across the MC between groups (p=0.911). All groups demonstrated similar respiratory quotient between phases (OC: Δ 0.0 ± 0.01 a.u.; H-IUD: Δ -0.002 ± 0.01 a.u.; EUM: Δ 0.006 ± 0.01 a.u.).

For resting metabolic rate, there were no significant differences across the MC between groups (p=0.345). The OC and EUM groups demonstrated greater resting metabolic rate in the LP (OC: Δ 56.5 ± 40.1 kcal/day; EUM: Δ 16.2 ± 42.2 kcal/day) while the H-IUD (Δ -28.1 ± 41.1 kcal/day) group demonstrated resting metabolic in the FP.

Muscle Characteristics

For muscle cross sectional area, there were no significant differences across the MC between groups (p=0.769). All groups demonstrated greater muscle cross sectional area in the FP (OC: Δ -0.2 ± 0.5 cm^2; H-IUD: Δ -0.7 ± 0. 0.5 cm^2; EUM: Δ -0.6 ± 0.5 cm^2).

For echo intensity, there were no significant differences across the MC between groups (p=0.376). All groups demonstrated similar echo intensity between phases (OC: Δ -2.6 ± 2.6 a.u.; H-IUD: Δ 1.6 ± 2.6 a.u.; EUM: Δ -3.2 ± 2.7 a.u.).

Aim 3 Discussion:

The use of hormonal contraception does not appear to influence measures of body composition, metabolism, or muscle characteristics across the menstrual cycle. Our hypothesis that there would be no differences in fluid balance, body composition, muscle characteristics, and metabolic outcomes between monophasic OC vs. IUD vs. EUM was supported. However, our hypothesis that intracellular fluid and resting energy expenditure would be elevated during the high hormone phase for all groups was not fully supported. Intracellular fluid was elevated in the FP for all groups (OC: Δ -0.2 L; H-IUD: Δ -0.03 L; EUM: Δ -0.2 L). This aligns with previous work examining the influences of the menstrual cycle on fluid balance demonstrating that the rise of estrogen and progesterone levels in the LP decreases the ICF volume.[71] In the present study, resting metabolic rate varied slightly with the OC group (Δ 56.5 kcal/day) and EUM (Δ 16.2 kcal/day) demonstrating greater energy expenditure in the LP, while the H-IUD (Δ -28.1 kcal/day) exhibited greater energy expenditure in the FP. As H-IUDs provide localized progestin consistently throughout the menstrual cycle, it is possible that the expected slightly elevated levels of progesterone in the FP caused the shift in resting energy expenditure.[96] Interestingly, the respiratory quotient did not change between groups across cycle phases, suggesting the change in resting metabolic rate may be hormonally driven rather than as a result of substrate utilization.

While the 4-compartment model is the gold standard for body composition assessment,[165] there are currently no studies examining the influence of hormonal contraception or the different menstrual cycle phases on body composition measurements. Additionally, there is limited research examining if changes in hormonal profiles across the menstrual cycle influence muscle characteristics. The present study suggests that OC and H-IUD use does not influence body composition and muscle characteristic measurements across the menstrual cycle.

CHAPTER VII: CONCLUSIONS

Based on the current study results, it appears that OC and H-IUD users can be equally included in research with EUM women, and research focused on strength and body composition may not need to be limited to a specific phase of the menstrual cycle. For all outcomes, the OC and H-IUD groups did not differ significantly from the EUM group. Investigators should use caution when examining lower body 1RM strength, as OC users had a greater leg press 1RM (5% increase) in the LP when compared to H-IUD users. This may correspond to ~15-20 pound difference between menstrual cycle phases. These small changes may affect acute performance, but may be less relevant for overall training and testing. The significant difference in lower body 1RM between OC and H-IUD users may be due to the variation of progesterone concentrations in the LP, yet this should be evaluated further. Results from this study provide a basis for understanding the physiological impact of OCs and IUDs on maximal strength and power, thereby informing future research regarding training considerations and interventions across the menstrual cycle. These data suggest that cycle phase or hormonal contraception use may not be limiting considerations for testing points for exercise or nutrition interventions as there were no significant impacts on exercise and body composition outcomes.

The current study suggests that hormonal contraceptives may have minimal effects on fatigability during high intensity exercise and subsequent recovery. It appears that there may be fatigability differences between H-IUD/EUM women compared to OC, with OC experiencing less fatigability in the FP. Interestingly, vessel diameter and blood flow were largely unchanged between groups across the menstrual cycle with slight improvements in blood flow in the FP for all groups. This may be a compensatory mechanism for lower power production particularly as the exercise was high intensity anaerobic exercise. Results add to the growing body of evidence evaluating the influences of female sex

hormones on exercise recovery. In recreationally active women, hormonal contraception and the menstrual cycle likely have minimal impact on anaerobic exercise performance and recovery, suggesting that exercise training does not need to be modified across the menstrual cycle. Future research should evaluate if OC and H-IUD use impact adaptations to chronic exercise and nutrition interventions especially when considering the menstrual cycle phases.

TABLES

Table 1. Anthropometric and descriptive characteristics of study participants.

	Total Sample (n= 60)	**EUM (n=19)**	**OC (n=21)**	**H-IUD (n=20)**
Age (yrs)	26.5 ± 7.0	28.4 ± 7.3	24.0 ± 5.9	27.4 ± 7.5
Height (cm)	165.2 ± 6.3	166.2 ± 6.9	163.7 ± 6.3	165.5 ± 5.6
Weight (kg)	64.5 ± 8.4	65.0 ± 8.9	64.5 ± 8.9	66.7 ± 10.0
BMI (kg/m^2)	22.5 ± 5.9	23.0 ± 2.4	22.9 ± 6.0	24.0 ± 2.9
Race				
White	83%			
Black/African American	6%	-	-	-
Hispanic	3%			
Asian	1%			
Two or more races	2%			
Menstrual Cycle Length (days)	-	29.8 ± 5.7	-	29.6 ± 3.6
Length of Time for HC Use (yrs)	-	-	2.7 ± 1.4	3.6 ± 2.3

Data are presented at mean ± standard deviation (SD). EUM, eumenorrheic; OC, monophasic oral contraceptive users; H-IUD, hormonal intrauterine device users; HC, hormonal contraception.

Table 2. Types and doses of hormonal contraception used by participants.

Monophasic Oral Contraceptives (N=21)		
Hormones (progesterone/estrogen)	**Dose (mg/mcg)**	***N***
Norethindrone acetate/ethinyl estradiol	1.5/30	5
Norethindrone acetate/ethinyl estradiol	1/35	1
Norethindrone acetate/ethinyl estradiol	1 /20	7
Norethindrone acetate/ethinyl estradiol	1/10	1
Drospirenone/estetrol	3/14200	1
Drospirenone/ethinyl estradiol	3/20	1
Norgestimate/ethinyl estradiol	0.25/35	3
Desogestrel/ethinyl estradiol	0.15/30	1
Levonorgestrel/ethinyl estradiol	0.1/20	1
Hormonal Intrauterine Devices (N=20)		
Hormone (progestin)	**Dose (mg)**	***N***
Levonorgestrel	52	13
Levonorgestrel	19.5	6
Levonorgestrel	13.5	1

Mg, milligram; mcg, microgram

Table 3. Participant descriptive and performance outcomes between groups for total sample (n=60).

Descriptive Characteristics			
	OC (n=21)	**H-IUD (n=20)**	**EUM (n=19)**
Age (yrs)	24.0 ± 5.9	27.4 ± 7.5	28.4 ± 7.3
Height (cm)	163.7 ± 6.3	165.6 ± 5.6	166.3 ± 6.9
FP Weight (kg)	64.5 ± 8.7	66.7 ± 9.9	63.6 ± 8.1
LP Weight (kg)	64.5 ± 8.6	66.7 ± 10.0	63.6 ± 8.0
BMI (kg/m^2)	24.1 ± 2.9	24.4 ± 3.5	23.0 ±2.4
FP Total LM (kg)	42.3 ± 4.9	44.3 ± 5.2	43.6 ± 5.0
LP Total LM (kg)	42.3 ± 4.7	44.2 ± 5.2	43.7 ± 5.0
FP Leg LM (kg)	15.2 ± 2.1	15.8 ± 2.3	15.5 ± 1.8
LP Leg LM (kg)	15.2 ± 1.9	15.9 ± 2.0	15.6 ± 2.1
FP Arm LM (kg)	4.6 ± 0.7	4.8 ± 0.8	4.8 ± 0.7
LP Arm LM (kg)	4.6 ± 0.6	4.8 ± 0.8	4.8 ± 0.7
FP dLeg LM (kg)	7.6 ± 1.0	7.9 ± 1.1	7.8 ± 0.9
LP dLeg LM (kg)	7.7 ± 1.0	7.9 ± 1.0	7.8 ± 1.02
Performance Outcomes			
	OC (n=21)	**H-IUD (n=20)**	**EUM (n=19)**
FP LP1RM (kg)	151.2 ± 46.1*	181.2 ± 51.6*	161.6 ± 59.3
LP LP1RM (kg)	158.7 ± 45.7*	172.3 ± 50.5*	167.7 ± 63.2
FP BP1RM (kg)	37.6 ± 8.0	38.0 ± 9.1	35.6 ± 8.1
LP BP1RM (kg)	36.9 ± 8.3	37.7 ± 8.9	36.9 ± 7.8
FP Leg PF (N)	148.8 ± 39.3	148.3 ± 40.0	155.3 ± 31.5
LP Leg PF (N)	141 ± 35.7	152.4 ± 41.6	165.0 ± 40.0
FP Arm PF (N)	376.9 ± 87.3	355.6 ± 72.8	389.9 ± 64.7
LP Arm PF (N)	388.8 ± 91.1	355.5 ± 67.5	386.4 ± 94.2
FP RSI (cm/s)	47.6 ± 21.0	40.9 ± 10.8	52.2 ± 20.2
LP RSI (cm/s)	43.8 ± 14.3	38.4 ± 15.5	45.3 ± 22.7
FP CMJ (cm)	24.6 ± 3.7	25.2 ± 2.9	26.5 ± 5.0
LP CMJ (cm)	25.1 ± 3.0	24.5 ± 7.0	24.6 ± 5.8

OC=oral contraceptive; IUD= hormonal intrauterine device; EUM= eumenorrheic and non-hormonal IUD users; FP= follicular phase; LP= luteal phase; LM= lean mass; LP1RM= leg press one repetition max; BP1RM=bench press one repetition max; PF=peak force; RSI= reactive strength index; CMJ= countermovement jump; *indicates significance between OC and IUD as denoted from the ANCOVA

Table 4. Participant descriptive and fatigue and recovery outcomes between groups for total sample (n=60).

Descriptive Characteristics			
	OC (n=21)	**H-IUD (n=20)**	**EUM (n=19)**
Age (yrs)	24.0 ± 5.9	27.4 ± 7.5	28.4 ± 7.3
Height (cm)	163.7 ± 6.3	165.6 ± 5.6	166.3 ± 6.9
FP Weight (kg)	64.5 ± 8.7	66.7 ± 9.9	63.6 ± 8.1
LP Weight (kg)	64.5 ± 8.6	66.7 ± 10.0	63.6 ± 8.0
BMI (kg/m^2)	24.1 ± 2.9	24.4 ± 3.5	23.0 ±2.4
Fatigue and Recovery Outcomes			
	OC (n=21)	**H-IUD (n=20)**	**EUM (n=19)**
FP PP (W)	423.2 ± 109.7	475.3 ± 73.8	413.7 ± 84.5
LP PP (W)	422.9 ± 115.1	470.5 ± 105.2	437.7 ± 60.8
FP tPP (s)	1843.0 ± 578.1	1860.0 ± 646.8	1837.8 ± 548.4
LP tPP (s)	2111.0 ± 645.6	2082.5 ± 445.7	2113.7 ± 489.7
FP AP (W)	3278.1 ± 872.0	3137.7 ± 671.9	3089.4 ± 888.4
LP AP (W)	3029.9 ± 948.4	3457.9 ± 900.0	3203.3 ± 487.8
FP FI (%)	33.1 ± 14.8	34.1 ± 17.3	29.8 ± 10.5
LP FI (%)	35.1 ± 16.0	32.9 ± 18.3	31.5 ± 8.7
FP Pre-Blood Lactate (mmol/L)	1.4 ± 0.4	1.8 ± 0.8	1.5 ± 0.7
LP Pre-Blood Lactate (mmol/L)	1.8 ± 0.9	1.7 ± 0.8	2.0 ± 1.6
FP Half-Blood Lactate (mmol/L)	7.6 ± 2.4	7.3 ± 2.4	7.4 ± 2.0
LP Half-Blood Lactate (mmol/L)	7.4 ± 2.2	7.6 ± 1.8	7.0 ± 2.1
FP Post-Blood Lactate (mmol/L)	9.8 ± 2.4	10.4 ± 2.6	10.0 ± 2.0

LP Post-Blood Lactate (mmol/L)	9.6 ± 2.7	10.8 ± 3.4	10.5 ± 2.2
FP 10 min-Post-Blood Lactate (mmol/L)	8.5 ± 3.1	9.4 ± 3.1	9.0 ± 2.0
LP 10 min-Post-Blood Lactate (mmol/L)	8.3 ± 2.6	9.4 ± 2.5	9.0 ± 2.5
FP Blood Lactate Clearance (%)	10.8 ± 2.8	10.3 ± 3.0	10.4 ± 2.1
LP Blood Lactate Clearance (%)	11.4 ± 3.6	9.6 ± 2.1	10.0 ± 2.2
FP Resting Vessel Diameter (cm)	0.33 ± 0.04	0.30 ± 0.05	0.32 ± 0.05
LP Resting Vessel Diameter (cm)	0.32 ± 0.04	0.31 ± 0.05	0.31 ± 0.04
FP Post Vessel Diameter (cm)	0.31 ± 0.04	0.30 ± 0.06	0.30 ± 0.04
LP Post Vessel Diameter (cm)	0.32 ± 0.05	0.32 ± 0.04	0.31 ± 0.04
FP Resting BF (mL/min)	56.0 ± 29.3	59.0 ± 31.5	45.2 ± 22.4
LP Resting BF (mL/min)	57.8 ± 35.3	59.1 ± 32.2	48.1 ± 21.8
FP Post BF (mL/min)	65.8 ± 25.5	67.6 ± 33.8	61.0 ± 23.6
LP Post BF (mL/min)	67.6 ± 29.9	61.0 ± 29.9	76.3 ± 28.3

OC=oral contraceptive; H-IUD= hormonal intrauterine device; EUM= eumenorrheic and non-hormonal IUD users; FP= follicular phase; LP= luteal phase; PP= peak power; tPP= time to peak power; AP= average power; FI= fatigue index; BF= blood flow

Table 5. Participant descriptive, body composition, and metabolic outcomes between groups for total sample (n=60).

Descriptive Characteristics			
	OC (n=21)	**H-IUD (n=20)**	**EUM (n=19)**
Age (yrs)	24.0 ± 5.9	27.4 ± 7.5	28.4 ± 7.3
Height (cm)	163.7 ± 6.3	165.6 ± 5.6	166.3 ± 6.9
FP Weight (kg)	64.5 ± 8.7	66.7 ± 9.9	63.6 ± 8.1
LP Weight (kg)	64.5 ± 8.6	66.7 ± 10.0	63.6 ± 8.0
BMI (kg/m^2)	24.1 ± 2.9	24.4 ± 3.5	23.0 ±2.4
Body Composition and Metabolic Outcomes			
	OC (n=21)	**H-IUD (n=20)**	**EUM (n=19)**
FP TBW (L)	33.5 ± 4.5	34.8 ± 4.5	34.5 ± 4.0
LP TBW (L)	33.9 ± 4.0	34.8 ± 4.7	34.5 ± 4.1
FP ECF (L)	13.5 ± 1.5	14.2 ± 1.6	14.0 ± 1.6
LP ECF (L)	13.7 ± 1.4	14.2 ± 1.7	14.0 ± 1.6
FP ICF (L)	20.4 ± 3.3	20.6 ± 2.9	20.4 ± 2.5
LP ICF (L)	20.2 ± 2.7	20.6 ± 3.0	20.3 ± 2.4
FP 4C FM (kg)	18.8 ± 5.0	18.8 ± 6.3	17.3 ± 5.2
LP 4C FM (kg)	18.3 ± 5.2	19.1 ± 6.5	17.4 ± 6.1
FP 4C BF (%)	28.7 ± 4.9	27.7 ± 6.3	26.9 ± 6.5
LP 4C BF (%)	28.1 ± 5.8	28.2 ± 6.8	27.0 ± 7.6
FP 4C FFM (kg)	45.8 ± 5.2	48.0 ± 5.7	46.3 ± 5.7
LP 4C FFM (kg)	46.2 ± 5.8	47.6 ± 6.3	46.2 ± 5.9

FP RQ (a.u.)	0.8 ± 0.05	0.8 ± 0.06	0.8 ± 0.06
LP RQ (a.u.)	0.8 ± 0.06	0.8 ± 0.06	0.8 ± 0.07
FP RMR (kcal/day)	1525.6 ± 184.7	1547.0 ± 197.3	1423.1 ± 159.9
LP RMR (kcal/day)	1582.1 ± 189.4	1518.9 ± 252.3	1439.3 ± 188.5
FP mCSA (cm^2)	21.6 ± 5.1	22.2 ± 3.4	21.5 ± 5.4
LP mCSA (cm^2)	21.4 ± 5.4	21.5 ± 3.6	20.8 ± 5.1
FP EI (a.u.)	107.0 ± 18.6	108.7 ± 19.5	105.8 ± 17.3
LP EI (a.u.)	104.4 ± 17.3	110.3 ± 23.1	102.6 ± 17.9

OC=oral contraceptive; H-IUD= hormonal intrauterine device; EUM= eumenorrheic and non-hormonal IUD users; FP= follicular phase; LP= luteal phase; TBW= total body water; ECF=extracellular fluid; ICF= intracellular fluid; FM=fat mass; BF= percent body fat; FFM=fat free mass; RQ=respiratory quotient; RMR=resting metabolic rate; mCSA=muscle cross-sectional area; EI=echo intensity.

FIGURES

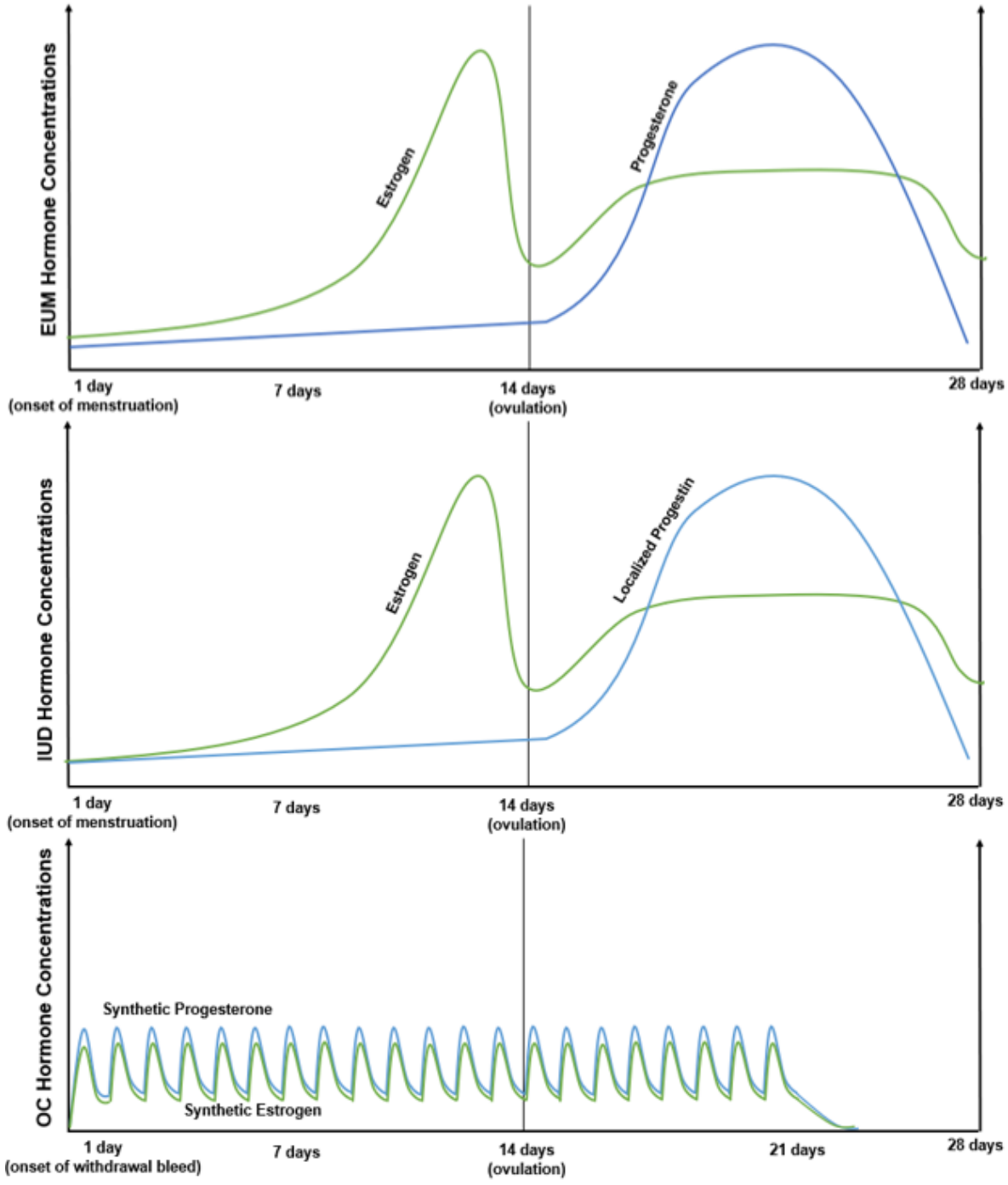

Figure 1. Various hormonal profiles of a eumenorrheic cycle, intrauterine device, and monophasic oral contraception. Adapted from a paper by Chidi-Ogbolu et al.[82]

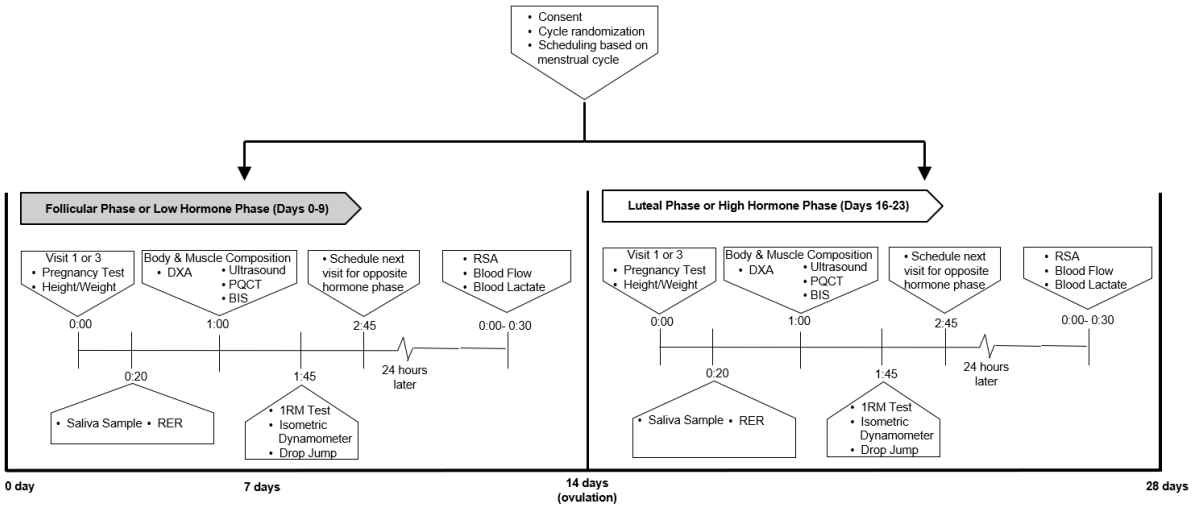

Figure 2. Study Design.

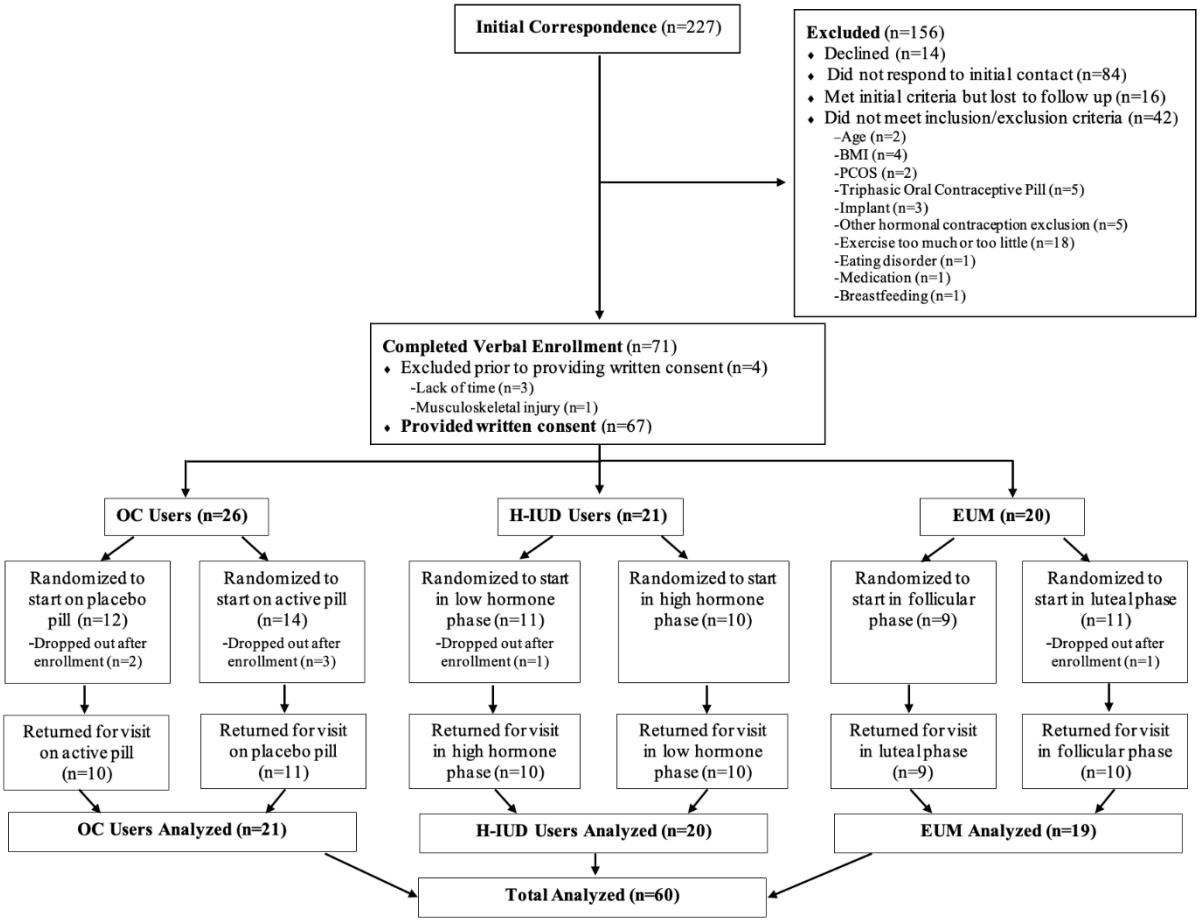

Figure 3. CONSORT diagram.

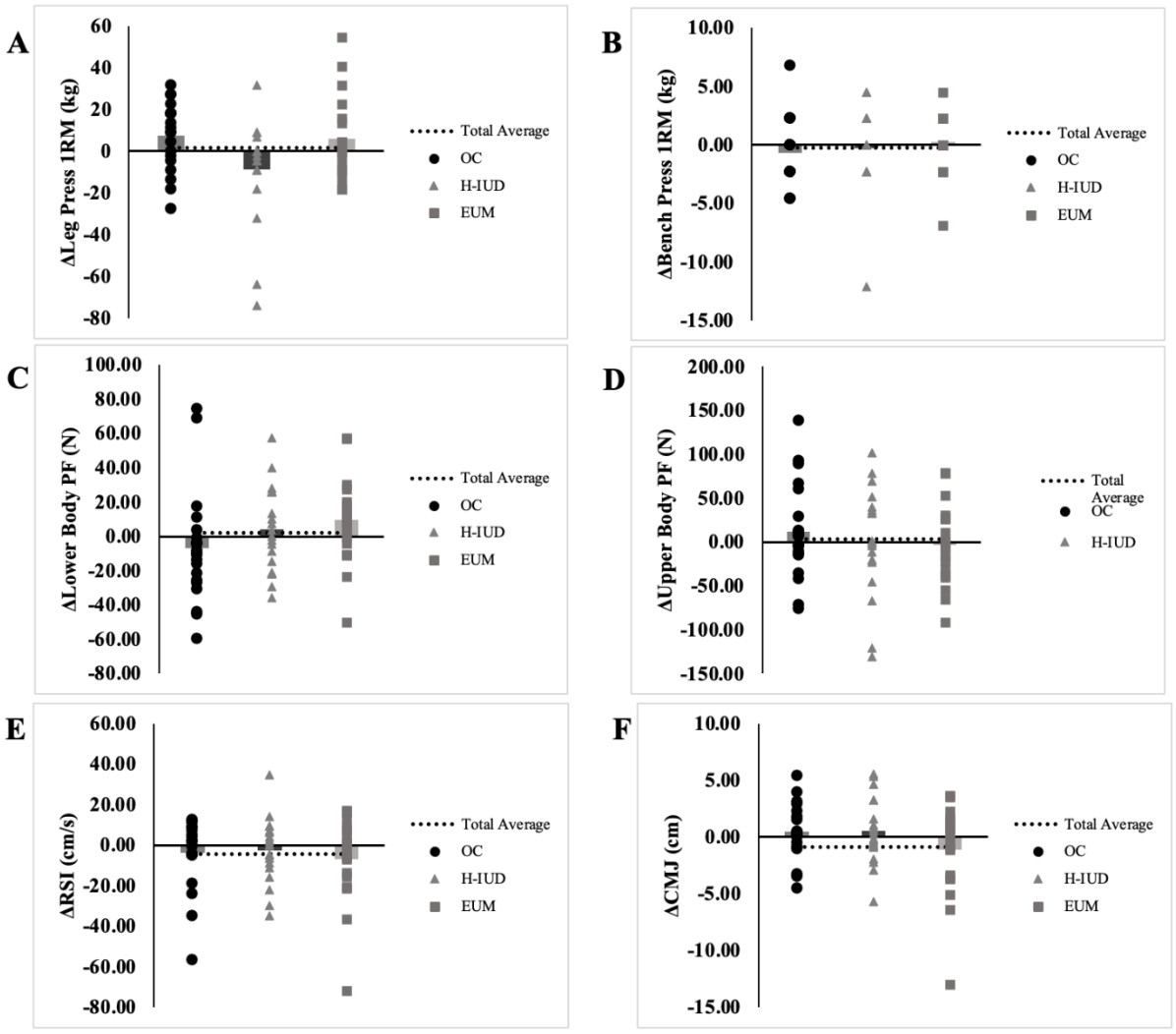

Figure 4 A-F. Individual data points for each group for strength and power outcomes. Bars represent group averages. OC= oral contraceptives; H-IUD= hormonal contraceptives; EUM= eumenorrheic women.

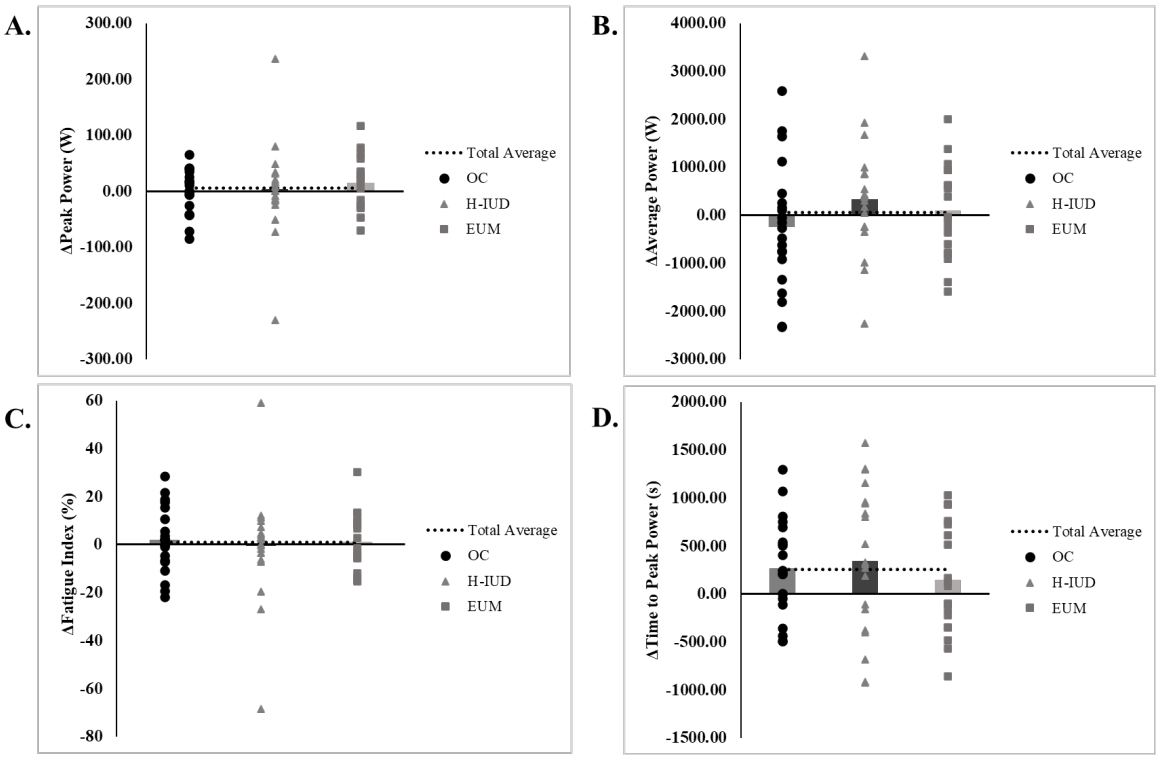

Figure 5 A-D. Individual data points for each group change score for fatigability outcomes. Bars represent group averages. OC= oral contraceptives; H-IUD= hormonal contraceptives; EUM= eumenorrheic women.

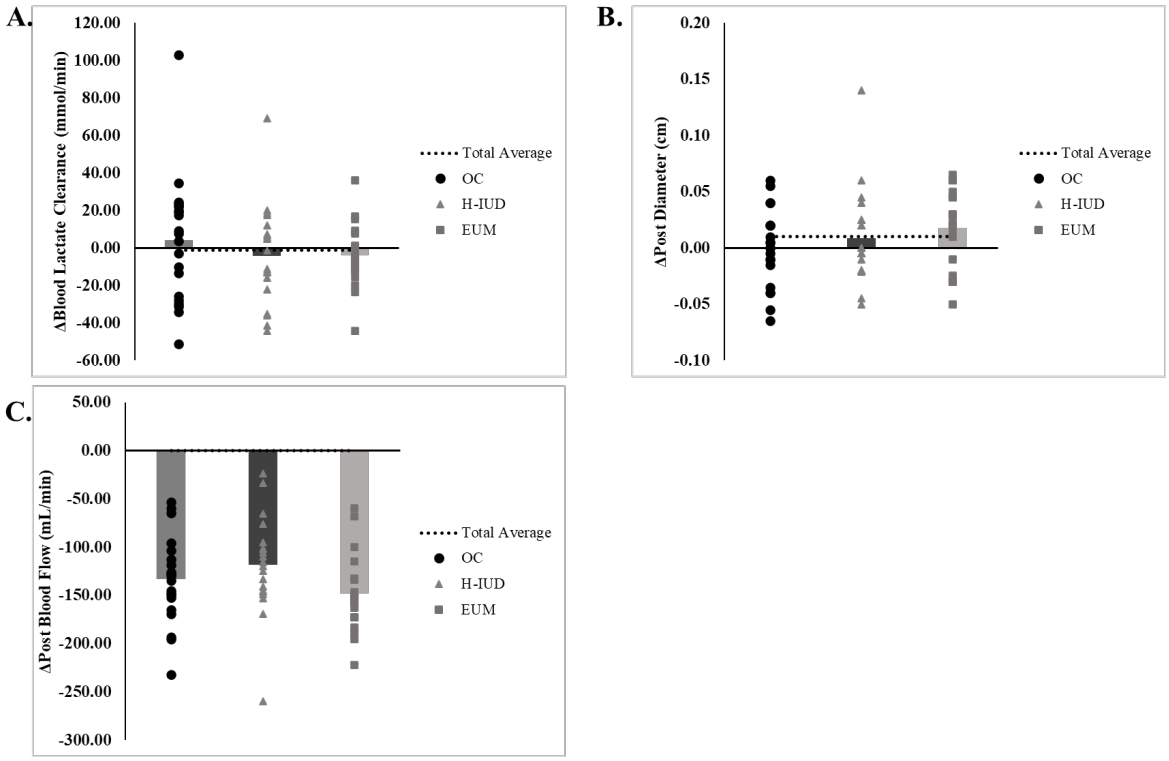

Figure 6 A-C. Individual data points for each group change score for recovery outcomes. Bars represent group averages. OC= oral contraceptives; H-IUD= hormonal contraceptives; EUM= eumenorrheic women.

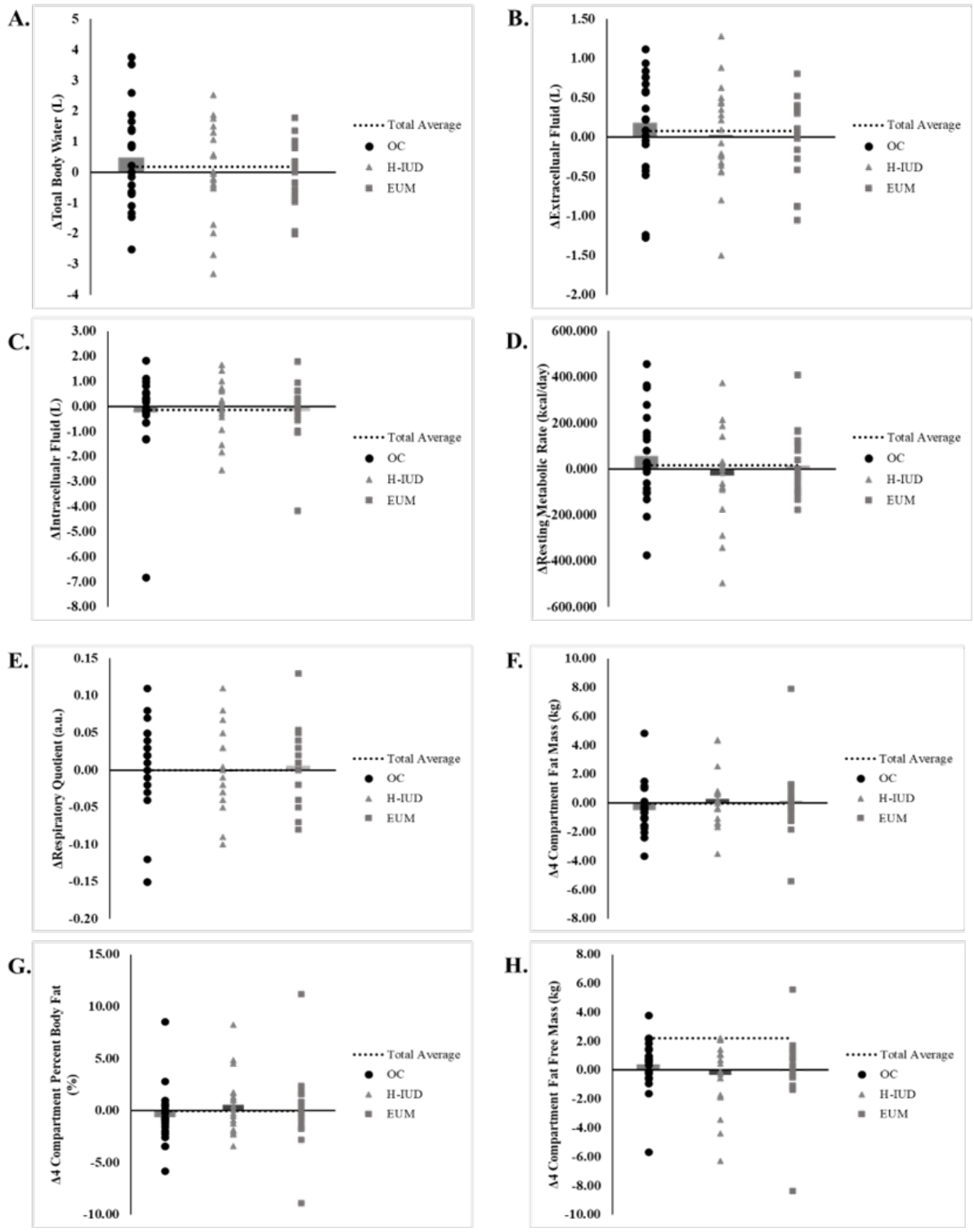

Figure 7 A- H. Individual data points for each group change score for body composition and metabolism outcomes. Bars represent group averages. OC= oral contraceptives; H-IUD= hormonal contraceptives; EUM= eumenorrheic women.

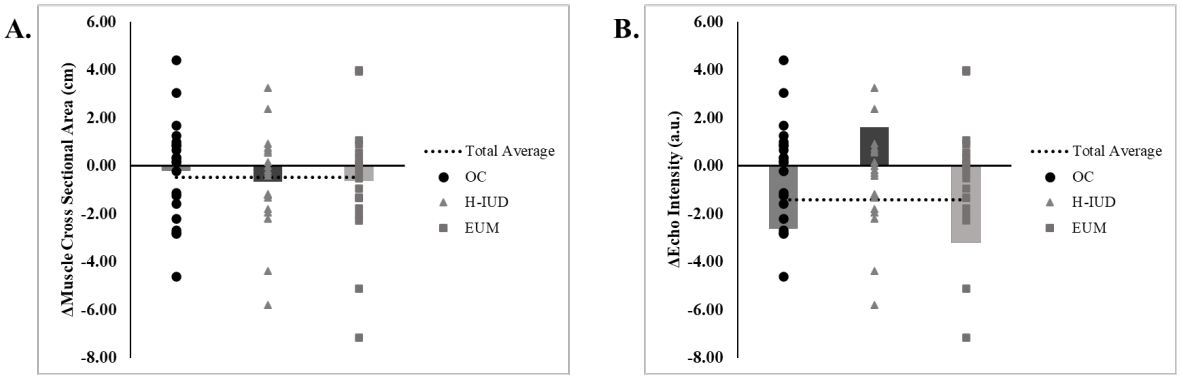

Figure 8 A-B. Individual data points for each group change score for muscle characteristics. Bars represent group averages. OC= oral contraceptives; H-IUD= hormonal contraceptives; EUM= eumenorrheic women.

APPENDIX 1: EXPLORATORY AIM

The exploratory aim of the originally proposed study included evaluation of protein turnover in a subsample to understand the impact of the menstrual cycle and use of hormonal contraception on protein breakdown. These samples were collected and sent to Metabolic Solutions for analysis. The processed data was recently received but has not been analyzed due to the quick timeline for the dissertation document. These data will be analyzed and results will be reported in a manuscript. Additionally, estrogen and progesterone samples were sent to the UNC Biobehavioral Laboratory to be analyzed. HEC and ASR have been in conversation with the laboratory manager and expect to receive the data in early April. Upon receipt, values will be analyzed and reported within manuscripts 1 and 2 prior to publication. Co-authors will be kept informed of progress of these analyses.

APPENDIX 2: RAW DATA

Table A.2. Participant descriptive and performance outcomes between groups for total sample

Descriptive Characteristics			
	OC (n=21)	**IUD (n=24)**	**EUM (n=15)**
Age (yrs)	24.0 ± 5.9	27.7 ± 7.0	28.2 ± 8.0
Height (cm)	163.7 ± 6.3	164.9 ± 5.7	167.5 ± 6.8
FP Weight (kg)	64.5 ± 8.7	64.9 ± 10.3	65.6 ± 6.9
LP Weight (kg)	64.5 ± 8.6	64.9 ± 10.4	65.6 ± 6.8
BMI (kg/m^2)	24.1 ± 2.9	23.8 ± 3.4	23.0 ± 2.4
FP Total LM (kg)	42.3 ± 4.9	43.4 ± 5.4	44.8 ± 5.4
LP Total LM (kg)	42.3 ± 4.7	43.3 ± 5.5	45.0 ± 4.3
FP Leg LM (kg)	15.2 ± 2.1	15.5 ± 2.3	15.5 ± 1.8
LP Leg LM (kg)	15.2 ± 1.9	15.9 ± 2.0	16.0 ± 1.4
FP Arm LM (kg)	4.6 ± 0.7	4.6 ± 0.8	5.0 ± 0.5
LP Arm LM (kg)	4.6 ± 0.6	4.6 ± 0.8	5.0 ± 0.5
FP dLeg LM (kg)	7.6 ± 1.0	7.7 ± 1.2	8.1 ± 0.7
LP dLeg LM (kg)	7.7 ± 1.0	7.8 ± 1.1	8.1 ± 0.9
Performance Outcomes			
	OC (n=21)	**IUD (n=24)**	**EUM (n=15)**
FP LP1RM (kg)	151.2 ± 46.1	168.2 ± 56.2	177.1 ± 56.2
LP LP1RM (kg)	158.7 ± 45.7	161.2 ± 50.5	184.2 ± 60.7
FP BP1RM (kg)	37.6 ± 8.0	36.9 ± 9.1	36.8 ± 8.1
LP BP1RM (kg)	36.9 ± 8.3	36.9 ± 8.3	36.6 ± 9.1
FP Leg PF (N)	148.8 ± 39.3	147.8 ± 36.9	157.9 ± 34.4
LP Leg PF (N)	141 ± 35.7	150.4 ± 39.5	171.5 ± 40.1
FP Arm PF (N)	376.9 ± 87.3	351.4 ± 68.4	405.6 ± 61.2
LP Arm PF (N)	388.8 ± 91.1	351.3 ± 63.6	401.4 ± 99.8
FP RSI (cm/s)	47.6 ± 21.0	43.0 ± 15.2	51.9 ± 25.6
LP RSI (cm/s)	43.8 ± 14.3	41.3 ± 19.9	42.6 ± 19.2
FP CMJ (cm)	24.6 ± 3.7	25.4 ± 2.8	26.6 ± 5.6
LP CMJ (cm)	25.1 ± 3.0	23.6 ± 7.8	24.6 ± 5.8

OC=oral contraceptive; IUD= hormonal and non-hormonal intrauterine device; EUM= eumenorrheic; FP= follicular phase; LP= luteal phase; LM= lean mass; LP1RM= leg press one repetition max; BP1RM=bench press one repetition max; PF=peak force; RSI= reactive strength index; CMV= countermovement jump. Summary of results per original recruitment goals. There were no significant differences reported between groups.

(n=60).

APPENDIX 3[1]: MANUSCRIPT[1]

Hormonal Contraception Prevalence and Perceived Side Effects in Active Adult U.S.A. Women

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Key words: Oral contraceptives, Intrauterine Devices, Exercise, Positive Side Effects, Negative Side Effects

[1] This manuscript was written with data that were collected in conjunction with the present dissertation. The manuscript is in review.

REFERENCES

1. Cowley, E. S., Olenick, A. A., McNulty, K. L. & Ross, E. Z. "Invisible Sportswomen": The Sex Data Gap in Sport and Exercise Science Research. *Women Sport Phys. Act. J.* **29**, 146–151 (2021).

2. Sims, S. T. & Heather, A. K. Myths and Methodologies: Reducing scientific design ambiguity in studies comparing sexes and/or menstrual cycle phases. *Exp. Physiol.* **103**, 1309–1317 (2018).

3. Tarnopolsky, M. A. Gender Differences in Substrate Metabolism During Endurance Exercise. *Can. J. Appl. Physiol.* **25**, 312–327 (2000).

4. Hunter, S. K. Sex differences in fatigability of dynamic contractions: Sex differences in skeletal muscle fatigue. *Exp. Physiol.* **101**, 250–255 (2016).

5. Bredella, M. A. Sex Differences in Body Composition. in *Sex and Gender Factors Affecting Metabolic Homeostasis, Diabetes and Obesity* (ed. Mauvais-Jarvis, F.) vol. 1043 9–27 (Springer International Publishing, 2017).

6. Wiik, A., Ekman, M., Johansson, O., Jansson, E. & Esbjörnsson, M. Expression of both oestrogen receptor alpha and beta in human skeletal muscle tissue. *Histochem. Cell Biol.* **131**, 181–189 (2009).

7. Davis, H. C. & Hackney, A. C. The Hypothalamic–Pituitary–Ovarian Axis and Oral Contraceptives: Regulation and Function. in *Sex Hormones, Exercise and Women* (ed. Hackney, A. C.) 1–17 (Springer International Publishing, 2017). doi:10.1007/978-3-319-44558-8_1.

8. Sung, E. *et al.* Effects of follicular versus luteal phase-based strength training in young women. *SpringerPlus* **3**, 668 (2014).

9. Wikström-Frisén, L., Boraxbekk, C.-J. & Henriksson-Larsén, K. Effects on power, strength and lean body mass of menstrual/oral contraceptive cycle based resistance training. *J. Sports Med. Phys. Fitness* **57**, 10 (2017).

10. Ansdell, P. *et al.* Menstrual cycle-associated modulations in neuromuscular function and fatigability of the knee extensors in eumenorrheic women. *J. Appl. Physiol.* **126**, 1701–1712 (2019).

11. Lebrun, C. M., McKenzie, D. C., Prior, J. C. & Taunton, J. E. Effects of menstrual cycle phase on athletic performance. *Med. Sci. Sports Exerc.* **27**, 437–444 (1995).

12. Romero-Moraleda, B. *et al.* The Influence of the Menstrual Cycle on Muscle Strength and Power Performance. *J. Hum. Kinet.* **68**, 123–133 (2019).

13. Sarwar, R., Niclos, B. B. & Rutherford, O. M. Changes in muscle strength, relaxation rate and fatiguability during the human menstrual cycle. *J. Physiol.* **493**, 267–272 (1996).

14. Phillips, S. K., Sanderson, A. G., Birch, K., Bruce, S. A. & Woledge, R. C. Changes in maximal voluntary force of human adductor pollicis muscle during the menstrual cycle. *J. Physiol.* **496**, 551–557 (1996).

15. Myllyaho, M. M. *et al.* Hormonal Contraceptive Use Does Not Affect Strength, Endurance, or Body Composition Adaptations to Combined Strength and Endurance Training in Women. *J. Strength Cond. Res.* **35**, 449–457 (2021).

16. Nichols, A. W., Hetzler, R. K., Villanueva, R. J., Stickley, C. D. & Kimura, I. F. Effects of Combination Oral Contraceptives on Strength Development in Women Athletes. *J. Strength Cond. Res.* **22**, 1625–1632 (2008).

17. Elliott, K. J. Does oral contraceptive use affect maximum force production in women? *Br. J. Sports Med.* **39**, 15–19 (2005).

18. Peters, C. & Burrows, M. Androgenicity of the progestin in oral contraceptives does not affect maximal leg strength. *Contraception* **74**, 487–491 (2006).

19. Kavanaugh, M. L. & Jerman, J. Contraceptive method use in the United States: trends and characteristics between 2008, 2012 and 2014. *Contraception* **97**, 14–21 (2018).

20. National Center for Health Statistics. *National Survey of Family Growth, 2017-2019*. https://www.cdc.gov/nchs/products/databriefs/db388.htm (2022).

21. Bennell, K., White, S. & Crossley, K. The oral contraceptive pill: a revolution for sportswomen? *Br. J. Sports Med.* **33**, 231–238 (1999).

22. Rickenlund, A. *et al.* Effects of Oral Contraceptives on Body Composition and Physical Performance in Female Athletes. *J. Clin. Endocrinol. Metab.* **89**, 4364–4370 (2004).

23. Rechichi, C. & Dawson, B. Effect of oral contraceptive cycle phase on performance in team sport players. *J. Sci. Med. Sport* **12**, 190–195 (2009).

24. Giacomoni, M., Bernard, T., Gavarry, O., Altare, S. & Falgairette, G. Influence of the menstrual cycle phase and menstrual symptoms on maximal anaerobic performance: *Med. Sci. Sports Exerc.* **32**, 486 (2000).

25. Ekenros, L., Hirschberg, A. L., Heijne, A. & Fridén, C. Oral Contraceptives Do Not Affect Muscle Strength and Hop Performance in Active Women. *Clin. J. Sport Med.* **23**, 202–207 (2013).

26. Cauci, S., Francescato, M. P. & Curcio, F. Combined Oral Contraceptives Increase High-Sensitivity C-Reactive Protein but Not Haptoglobin in Female Athletes. *Sports Med.* **47**, 175–185 (2017).

27. Dreon, D. M., Slavin, J. L. & Phinney, S. D. Oral contraceptive use and increased plasma concentration of C-reactive protein. *Life Sci.* **73**, 1245–1252 (2003).

28. Bozzini, B. N., McFadden, B. A., Elliott-Sale, K. J., Swinton, P. A. & Arent, S. M. Evaluating the effects of oral contraceptive use on biomarkers and body composition during a competitive season in collegiate female soccer players. *J. Appl. Physiol.* **130**, 1971–1982 (2021).

29. Henderson, Z & Scribbans, T. Intrauterine Contraception and Athletic Performance. *The Health & Fitness Journal of Canada* **13**, 37–45 (2020).

30. Tiidus, P. M. Influence of Estrogen on Skeletal Muscle Damage, Inflammation, and Repair: *Exerc. Sport Sci. Rev.* **31**, 40–44 (2003).

31. Enns, D. L. & Tiidus, P. M. The Influence of Estrogen on Skeletal Muscle: Sex Matters. *Sports Med.* **40**, 41–58 (2010).

32. Oosthuyse, T. & Bosch, A. N. The Effect of the Menstrual Cycle on Exercise Metabolism: Implications for Exercise Performance in Eumenorrhoeic Women. *Sports Med.* **40**, 207–227 (2010).

33. Martin, D., Sale, C., Cooper, S. B. & Elliott-Sale, K. J. Period Prevalence and Perceived Side Effects of Hormonal Contraceptive Use and the Menstrual Cycle in Elite Athletes. *Int. J. Sports Physiol. Perform.* **13**, 926–932 (2018).

34. Draper, C. F. *et al.* Menstrual cycle rhythmicity: metabolic patterns in healthy women. *Sci. Rep.* **8**, 14568 (2018).

35. Elliott-Sale, K. J. *et al.* The Effects of Oral Contraceptives on Exercise Performance in Women: A Systematic Review and Meta-analysis. *Sports Med. Auckl. NZ* **50**, 1785–1812 (2020).

36. Regidor, P.-A. The clinical relevance of progestogens in hormonal contraception: Present status and future developments. *Oncotarget* **9**, 34628–34638 (2018).

37. Xiao, B., Zeng, T., Wu, S., Sun, H. & Xiao, N. Effect of levonorgestrel-releasing intrauterine device on hormonal profile and menstrual pattern after long-term use. *Contraception* **51**, 359–365 (1995).

38. Dave, H., Shook, M. & Varacallo, M. *Anatomy, Skeletal Muscle*. https://www.ncbi.nlm.nih.gov/books/NBK537236/ (2021).

39. Grgic, J., Lazinica, B., Schoenfeld, B. J. & Pedisic, Z. Test-Retest Reliability of the One-Repetition Maximum (1RM) Strength Assessment: a Systematic Review. *Sports Med. - Open* **6**, 31 (2020).

40. Ansdell, P. *et al.* Physiological sex differences affect the integrative response to exercise: acute and chronic implications. *Exp. Physiol.* **105**, 2007–2021 (2020).

41. Dulhunty, A. Excitation-contraction coupling from the 1950s into the new millennium. *Clin. Exp. Pharmacol. Physiol.* **33**, 763–772 (2006).

42. Meeusen, R. & Roelands, B. Fatigue: Is it all neurochemistry? *Eur. J. Sport Sci.* **18**, 37–46 (2018).

43. Stoffel-Wagner, B. Neurosteroid metabolism in the human brain. *Eur. J. Endocrinol.* 669–679 (2001) doi:10.1530/eje.0.1450669.

44. Kraemer, W. *et al. Strength testing: development and evaluation of methodology.* (Human Kinetics, 2006).

45. Zazulak, B. T., Paterno, M., Myer, G. D., Romani, W. A. & Hewett, T. E. The Effects of the Menstrual Cycle on Anterior Knee Laxity: A Systematic Review. *Sports Med.* **36**, 847–862 (2006).

46. Phillips, S. K., Rook, K. M., Siddle, N. C., Bruce, S. A. & Woledge, R. C. Muscle weakness in women occurs at an earlier age than in men, but strength is preserved by hormone replacement therapy. *Clin. Sci. Lond. Engl. 1979* **84**, 95–98 (1993).

47. Fisher, A. L. Models of Sarcopenia. in *Handbook of Models for Human Aging* 977–991 (Elsevier, 2006). doi:10.1016/B978-012369391-4/50082-5.

48. Montgomery, M. M. & Shultz, S. J. Isometric Knee-Extension and Knee-Flexion Torque Production During Early Follicular and Postovulatory Phases in Recreationally Active Women. *J. Athl. Train.* **45**, 586–593 (2010).

49. Kellmann, M. *et al.* Recovery and Performance in Sport: Consensus Statement. *Int. J. Sports Physiol. Perform.* **13**, 240–245 (2018).

50. Beltrame, T., Villar, R. & Hughson, R. L. Sex differences in the oxygen delivery, extraction, and uptake during moderate-walking exercise transition. *Appl. Physiol. Nutr. Metab.* **42**, 994–1000 (2017).

51. Russ, D. W., Lanza, I. R., Rothman, D. & Kent-Braun, J. A. Sex differences in glycolysis during brief, intense isometric contractions. *Muscle Nerve* **32**, 647–655 (2005).

52. Borne, R., Hausswirth, C. & Bieuzen, F. Relationship Between Blood Flow and Performance Recovery: A Randomized, Placebo-Controlled Study. *Int. J. Sports Physiol. Perform.* **12**, 152–160 (2017).

53. Malone, J. K., Coughlan, G. F., Crowe, L., Gissane, G. C. & Caulfield, B. The physiological effects of low-intensity neuromuscular electrical stimulation (NMES) on short-term recovery from supra-maximal exercise bouts in male triathletes. *Eur. J. Appl. Physiol.* **112**, 2421–2432 (2012).

54. Tostes, R. C., Nigro, D., Fortes, Z. B. & Carvalho, M. H. C. Effects of estrogen on the vascular system. *Braz. J. Med. Biol. Res.* **36**, 1143–1158 (2003).

55. Parker, B. A. *et al.* Sex differences in leg vasodilation during graded knee extensor exercise in young adults. *J. Appl. Physiol.* **103**, 1583–1591 (2007).

56. Roepstorff, C. *et al.* Higher skeletal muscle α_2 AMPK activation and lower energy charge and fat oxidation in men than in women during submaximal exercise: Sex difference in AMPK activation by exercise. *J. Physiol.* **574**, 125–138 (2006).

57. Korthuis, R. J. *Skeltal Muscle Circulation*. (Morgan & Claypool Life Sciences, 2011).

58. Pereira, H. M., Larson, R. D. & Bemben, D. A. Menstrual Cycle Effects on Exercise-Induced Fatigability. *Front. Physiol.* **11**, 517 (2020).

59. Angstwurm, M. W. A., Gärtner, R. & Ziegler-Heitbrock, H. W. L. Cyclic Plasma IL-6 Levels During Normal Menstrual Cycle. *Cytokine* **9**, 370–374 (1997).

60. Hackney, A. C., Kallman, A. L. & Ağgön, E. Female sex hormones and the recovery from exercise: Menstrual cycle phase affects responses. *Biomed. Hum. Kinet.* **11**, 87–89 (2019).

61. Romero-Parra, N. *et al.* Influence of the Menstrual Cycle on Blood Markers of Muscle Damage and Inflammation Following Eccentric Exercise. *Int. J. Environ. Res. Public. Health* **17**, 1618 (2020).

62. Markofski, M. M. & Braun, W. A. Influence of Menstrual Cycle on Indices of Contraction-Induced Muscle Damage. *J. Strength Cond. Res.* **28**, 2649–2656 (2014).

63. Chaffin, M. E. *et al.* Interleukin-6 and Delayed Onset Muscle Soreness Do Not Vary During the Menstrual Cycle. *Res. Q. Exerc. Sport* **82**, 693–701 (2011).

64. Sims, S. T., Ware, L. & Capodilupo, E. R. Patterns of endogenous and exogenous ovarian hormone modulation on recovery metrics across the menstrual cycle. *BMJ Open Sport Exerc. Med.* **7**, e001047 (2021).

65. Meulenberg, P. M. M., Ross, H. A., Swinkels, L. M. J. W. & Benraad, T. J. The effect of oral contraceptives on plasma-free and salivary cortisol and cortisone. *Clin. Chim. Acta* **165**, 379–385 (1987).

66. Ellard, C. O., Thompson, W. R. & Burks, S. Effects of the menstrual cycle on body composition. *Scand. J. Med. Sci. Sports* **1**, 147–150 (1991).

67. Cumberledge, E. A., Myers, C., Venditti, J. J., Dixon, C. B. & Andreacci, J. L. The Effect of the Menstrual Cycle on Body Composition Determined by Contact-Electrode Bioelectrical Impedance Analyzers. *Int. J. Exerc. Sci.* **11**, 625–632 (2018).

68. Hicks, C. S., McLester, C. N., Esmat, T. A. & McLester, J. R. A Comparison of Body Composition Across Two Phases of the Menstrual Cycle Utilizing Dual-Energy X-Ray Absorptiometry, Air Displacement Plethysmography, and Bioelectrical Impedance Analysis. *Int. J. Exerc. Sci.* **10**, 1235–1249 (2017).

69. Ong, J. N., Ducker, K. J., Furzer, B. J., Dymock, M. & Landers, G. J. Measures of body composition via Dual-energy X-ray absorptiometry, ultrasound and skinfolds are not impacted by the menstrual cycle in active eumenorrheic females. *J. Sci. Med. Sport* **25**, 115–121 (2022).

70. Gould, L. M. *et al.* Impact of Follicular Menstrual Phase on Body Composition Measures and Resting Metabolism. *Med. Sci. Sports Exerc.* **Publish Ahead of Print**, (2021).

71. Stachenfeld, N. S. & Taylor, H. S. Effects of estrogen and progesterone administration on extracellular fluid. *J. Appl. Physiol.* **96**, 1011–1018 (2004).

72. Stachenfeld, N. S. *et al.* Physiological variability of fluid-regulation hormones in young women. *J. Appl. Physiol.* **86**, 1092–1096 (1999).

73. Volpe, S. L., Poule, K. A. & Bland, E. G. Estimation of prepractice hydration status of National Collegiate Athletic Association Division I athletes. *J. Athl. Train.* **44**, 624–629 (2009).

74. Øian, P., Tollan, A., Fadnes, H. O., Noddeland, H. & Maltau, J. M. Transcapillary fluid dynamics during the menstrual cycle. *Am. J. Obstet. Gynecol.* **156**, 952–955 (1987).

75. Rael, B. *et al.* Body Composition Over the Menstrual and Oral Contraceptive Cycle in Trained Females. *Int. J. Sports Physiol. Perform.* **16**, 375–381 (2020).

76. McKee, J. E. & Cameron, N. Bioelectrical impedance changes during the menstrual cycle. *Am. J. Hum. Biol. Off. J. Hum. Biol. Counc.* **9**, 155–161 (1997).

77. Tomazo-Ravnik, T. & Jakopič, V. Changes in total body water and body fat in young women in the course of menstrual cycle. *Int. J. Anthropol.* **21**, 55–60 (2006).

78. Gleichauf, C. N. & Roe, D. A. The menstrual cycle's effect on the reliability of bioimpedance measurements for assessing body composition. *Am. J. Clin. Nutr.* **50**, 903–907 (1989).

79. Lusseveld, E. M., Peters, E. T. J. & Deurenberg, P. Multifrequency bioelectrical impedance as a measure of differences in body water distribution. *Ann. Nutr. Metab.* **37**, 44–51 (1993).

80. Mitchell, C. O., Rose, J., Familoni, B., Winters, S. & Ling, F. The use of multifrequency bioelectrical impedance analysis to estimate fluid volume changes as a function of the menstrual cycle. *Basic Life Sci.* **60**, 189–191 (1993).

81. Mitchell, C. O., Rose, J., Familoni, B., Winters, S. & Ling, F. The use of multifrequency bioelectrical impedance analysis to estimate fluid volume changes as a function of the menstrual cycle. *Basic Life Sci.* **60**, 189–191 (1993).

82. Chidi-Ogbolu, N. & Baar, K. Effect of Estrogen on Musculoskeletal Performance and Injury Risk. *Front. Physiol.* **9**, 1834 (2019).

83. Kitajima, Y. & Ono, Y. Estrogens maintain skeletal muscle and satellite cell functions. *J. Endocrinol.* **229**, 267–275 (2016).

84. Taaffe, D. R. *et al.* Estrogen Replacement, Muscle Composition, and Physical Function: The Health ABC Study. *Med. Sci. Sports Exerc.* **37**, 1741–1747 (2005).

85. Thompson, B. M., Hillebrandt, H. L., Sculley, D. V., Barba-Moreno, L. & Janse de Jonge, X. A. K. The acute effect of the menstrual cycle and oral contraceptive cycle on measures of body composition. *Eur. J. Appl. Physiol.* **121**, 3051–3059 (2021).

86. Van Pelt, R. E., Gavin, K. M. & Kohrt, W. M. Regulation of Body Composition and Bioenergetics by Estrogens. *Endocrinol. Metab. Clin. North Am.* **44**, 663–676 (2015).

87. Moore, D. R., Sygo, J. & Morton, J. P. Fuelling the female athlete: Carbohydrate and protein recommendations. *Eur. J. Sport Sci.* 1–13 (2021) doi:10.1080/17461391.2021.1922508.

88. Willett, H. N., Koltun, K. J. & Hackney, A. C. Influence of Menstrual Cycle Estradiol-β-17 Fluctuations on Energy Substrate Utilization-Oxidation during Aerobic, Endurance Exercise. *Int. J. Environ. Res. Public. Health* **18**, 7209 (2021).

89. Hackney, A. C., McCracken-Compton, M. A. & Ainsworth, B. Substrate Responses to Submaximal Exercise in the Midfollicular and Midluteal Phases of the Menstrual Cycle. *Int. J. Sport Nutr.* **4**, 299–308 (1994).

90. Wohlgemuth, K. J. *et al.* Sex differences and considerations for female specific nutritional strategies: a narrative review. *J. Int. Soc. Sports Nutr.* **18**, 27 (2021).

91. Isacco, L., Duché, P. & Boisseau, N. Influence of Hormonal Status on Substrate Utilization at Rest and during Exercise in the Female Population: *Sports Med.* **42**, 327–342 (2012).

92. Bisdee, J. T., James, W. P. T. & Shaw, M. A. Changes in energy expenditure during the menstrual cycle. *Br. J. Nutr.* **61**, 187–199 (1989).

93. Day, D. S., Gozansky, W. S., Van Pelt, R. E., Schwartz, R. S. & Kohrt, W. M. Sex hormone suppression reduces resting energy expenditure and {beta}-adrenergic support of resting energy expenditure. *J. Clin. Endocrinol. Metab.* **90**, 3312–3317 (2005).

94. Eck, L. H. *et al.* Differences in macronutrient selections in users and nonusers of an oral contraceptive. *Am. J. Clin. Nutr.* **65**, 419–424 (1997).

95. Diffey, B., Piers, L. S., Soares, M. J. & O'dea, K. The effect of oral contraceptive agents on the basal metabolic rate of young women. *Br. J. Nutr.* **77**, 853–862 (1997).

96. Napolitano, A. *et al.* Body composition and resting metabolic rate of perimenopausal women using continuous progestogen contraception. *Eur. J. Contracept. Reprod. Health Care* **21**, 168–175 (2016).

97. Duhita, M. R., Schutz, Y., Montani, J.-P., Dulloo, A. G. & Miles-Chan, J. L. Oral Contraceptive Pill Alters Acute Dietary Protein-Induced Thermogenesis in Young Women: Acute Protein-Induced Thermogenesis in Young Women. *Obesity* **25**, 1482–1485 (2017).

98. Jensen, M. D. & Levine, J. Effects of oral contraceptives on free fatty acid metabolism in women. *Metabolism* **47**, 280–284 (1998).

99. Kimm, S. Y. S. Effects of Race, Cigarette Smoking, and Use of Contraceptive Medications on Resting Energy Expenditure in Young Women. *Am. J. Epidemiol.* **154**, 718–724 (2001).

100. Church, D. D. *et al.* Essential Amino Acids and Protein Synthesis: Insights into Maximizing the Muscle and Whole-Body Response to Feeding. *Nutrients* **12**, 3717 (2020).

101. Kriengsinyos, W., Wykes, L. J., Goonewardene, L. A., Ball, R. O. & Pencharz, P. B. Phase of menstrual cycle affects lysine requirement in healthy women. *Am. J. Physiol.-Endocrinol. Metab.* **287**, E489–E496 (2004).

102. Gould, L. M. *et al.* Metabolic effects of menopause: a cross-sectional characterization of body composition and exercise metabolism. *Menopause* (In Press).

103. Miller, B. F. *et al.* No effect of menstrual cycle on myofibrillar and connective tissue protein synthesis in contracting skeletal muscle. *Am. J. Physiol.-Endocrinol. Metab.* **290**, E163–E168 (2006).

104. Hansen, M. *et al.* Effect of administration of oral contraceptives on the synthesis and breakdown of myofibrillar proteins in young women: Effect of OC on muscle protein turnover. *Scand. J. Med. Sci. Sports* **21**, 62–72 (2011).

105. Lariviere, F., Moussalli, R. & Garrel, D. R. Increased leucine flux and leucine oxidation during the luteal phase of the menstrual cycle in women. *Am. J. Physiol.-Endocrinol. Metab.* **267**, E422–E428 (1994).

106. Gordon, D. *et al.* The effects of menstrual cycle phase on the development of peak torque under isokinetic conditions. 8.

107. Gallagher, D. *et al.* How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? *Am. J. Epidemiol.* **143**, 228–239 (1996).

108. Haff, G. G. & N. T. Triplett. *Essentials of Strength Training and Conditioning, 4ed*. (2016).

109. Courel-Ibáñez, J., Hernández-Belmonte, A., Cava-Martínez, A. & Pallarés, J. G. Familiarization and Reliability of the Isometric Knee Extension Test for Rapid Force Production Assessment. *Appl. Sci.* **10**, 4499 (2020).

110. Trivisonno, A. *et al.* The Test-Retest Reliability of a Portable Upper-Body Isometric Strength Test in Probation Officers. *National Strength and Conditioning Association* (2018).

111. Montalvo, S., Gonzalez, M. P., Dietze-Hermosa, M. S., Eggleston, J. D. & Dorgo, S. Common Vertical Jump and Reactive Strength Index Measuring Devices: A Validity and Reliability Analysis. *J. Strength Cond. Res.* **35**, 1234–1243 (2021).

112. Roelofs, E. J., Smith-Ryan, A. E., Trexler, E. T., Hirsch, K. R. & Mock, M. G. Effects of pomegranate extract on blood flow and vessel diameter after high-intensity exercise in young, healthy adults. *Eur. J. Sport Sci.* **17**, 317–325 (2017).

113. Crotty, N. M., Boland, M., Mahony, N., Donne, B. & Fleming, N. Reliability and Validity of the Lactate Pro 2 Analyzer. *Meas. Phys. Educ. Exerc. Sci.* **25**, 202–211 (2021).

114. Arratibel-Imaz, I., Calleja-Gonzalez, J. & Terrados, N. Validity of blood lactate measurements between the two LactatePro versions. *Arch Med Deporte* **32**, 86–91.

115. Nguyen, H. B. *et al.* Early lactate clearance is associated with improved outcome in severe sepsis and septic shock*: *Crit. Care Med.* **32**, 1637–1642 (2004).

116. Wang, Z. M. *et al.* Six-compartment body composition model: inter-method comparisons of total body fat measurement. *Int. J. Obes. Relat. Metab. Disord. J. Int. Assoc. Study Obes.* **22**, 329–337 (1998).

117. Hirsch, K. R., Blue, M. N. M., Trexler, E. T., Ahuja, S. & Smith-Ryan, A. E. Provision of ready-to-drink protein following bariatric surgery: An evaluation of tolerability, body composition, and metabolic rate. *Clin. Nutr.* **40**, 2319–2327 (2021).

118. Smith-Ryan, A. E. *et al.* Validity and reliability of a 4-compartment body composition model using dual energy x-ray absorptiometry-derived body volume. *Clin. Nutr.* **36**, 825–830 (2017).

119. Young, H.-J., Jenkins, N. T., Zhao, Q. & Mccully, K. K. Measurement of intramuscular fat by muscle echo intensity: Muscle Echo Intensity and Fat. *Muscle Nerve* **52**, 963–971 (2015).

120. Cabre, H. E. *et al.* Exploring the "Athlete's Paradox": Division I Cross-Country Runners Demonstrate Similar Muscle Characteristics to Recreationally Trained Young Adults. *J. Strength Cond. Res.* **35**, 3213–3217 (2021).

121. Herda, A. A. *et al.* Muscle Performance, Size, and Safety Responses After Eight Weeks of Resistance Training and Protein Supplementation: A Randomized, Double-Blinded, Placebo-Controlled Clinical Trial. *J. Strength Cond. Res.* **27**, 3091–3100 (2013).

122. Smith-Ryan, A. E., Hirsch, K. R., Blue, M. N. M., Mock, M. G. & Trexler, E. T. High-Fat Breakfast Meal Replacement in Overweight and Obesity: Implications on Body Composition, Metabolic Markers, and Satiety. *Nutrients* **11**, E865 (2019).

123. Ferrando, A. A., Lane, H. W., Stuart, C. A., Davis-Street, J. & Wolfe, R. R. Prolonged bed rest decreases skeletal muscle and whole body protein synthesis. *Am. J. Physiol.-Endocrinol. Metab.* **270**, E627–E633 (1996).

124. Fern, E. B., Garlick, P. J. & Waterlow, J. C. The concept of the single body pool of metabolic nitrogen in determining the rate of whole-body nitrogen turnover. *Hum. Nutr. Clin. Nutr.* **39**, 85–99 (1985).

125. Stein, T. P. *et al.* Effect of reduced dietary intake on energy expenditure, protein turnover, and glucose cycling in man. *Metabolism* **40**, 478–483 (1991).

126. Senne, J. Examination of Gender Equity and Female Participation in Sport. *The Sport Journal* (2016).

127. Bambaeichi, E., Reilly, T., Cable, N. T. & Giacomoni, M. The Isolated and Combined Effects of Menstrual Cycle Phase and Time-of-Day on Muscle Strength of Eumenorrheic Females. *Chronobiol. Int.* **21**, 645–660 (2004).

128. Bansode, O. M., Sarao, M. S. & Cooper, D. B. Contraception. in *StatPearls* (StatPearls Publishing, 2022).

129. Cooper, D. B., Patel, P. & Mahdy, H. Oral Contraceptive Pills. in *StatPearls* (StatPearls Publishing, 2022).

130. McNulty, K. L. *et al.* The Effects of Menstrual Cycle Phase on Exercise Performance in Eumenorrheic Women: A Systematic Review and Meta-Analysis. *Sports Med.* **50**, 1813–1827 (2020).

131. Lowe, D. A., Baltgalvis, K. A. & Greising, S. M. Mechanisms Behind Estrogen's Beneficial Effect on Muscle Strength in Females. *Exerc. Sport Sci. Rev.* **38**, 61–67 (2010).

132. Kissow, J., Jacobsen, K. J., Gunnarsson, T. P., Jessen, S. & Hostrup, M. Effects of Follicular and Luteal Phase-Based Menstrual Cycle Resistance Training on Muscle Strength and Mass. *Sports Med.* (2022) doi:10.1007/s40279-022-01679-y.

133. Elliott-Sale, K. J. *et al.* Methodological Considerations for Studies in Sport and Exercise Science with Women as Participants: A Working Guide for Standards of Practice for Research on Women. *Sports Med.* **51**, 843–861 (2021).

134. Smith, M. J., Adams, L. F., Schmidt, P. J., Rubinow, D. R. & Wassermann, E. M. Effects of ovarian hormones on human cortical excitability. *Ann. Neurol.* **51**, 599–603 (2002).

135. Carmichael, M. A., Thomson, R. L., Moran, L. J. & Wycherley, T. P. The Impact of Menstrual Cycle Phase on Athletes' Performance: A Narrative Review. *Int. J. Environ. Res. Public. Health* **18**, 1667 (2021).

136. Ansdell, P. *et al.* Menstrual cycle-associated modulations in neuromuscular function and fatigability of the knee extensors in eumenorrheic women. *J. Appl. Physiol.* **126**, 1701–1712 (2019).

137. Graja, A. *et al.* Physical, Biochemical, and Neuromuscular Responses to Repeated Sprint Exercise in Eumenorrheic Female Handball Players: Effect of Menstrual Cycle Phases. *J. Strength Cond. Res.* **36**, 2268–2276 (2022).

138. Forouzandeh Shahraki, S., Minoonejad, H. & Moghadas Tabrizi, Y. Comparison of some intrinsic risk factors of shoulder injury in three phases of menstrual cycle in collegiate female athletes. *Phys. Ther. Sport* **43**, 195–203 (2020).

139. Hertel, J., Williams, N. I., Olmsted-Kramer, L. C., Leidy, H. J. & Putukian, M. Neuromuscular performance and knee laxity do not change across the menstrual cycle in female athletes. *Knee Surg. Sports Traumatol. Arthrosc.* **14**, 817–822 (2006).

140. Thompson, B. M., Drover, K. B., Stellmaker, R. J., Sculley, D. V. & Janse de Jonge, X. A. K. The Effect of the Menstrual Cycle and Oral Contraceptive Cycle on Muscle Performance and Perceptual Measures. *Int. J. Environ. Res. Public. Health* **18**, 10565 (2021).

141. King, L. A., Michels, K. A., Graubard, B. I. & Trabert, B. Trends in oral contraceptive and intrauterine device use among reproductive-aged women in the US from 1999 to 2017. *Cancer Causes Control* **32**, 587–595 (2021).

142. National Survey of Family Growth. *Current Contraceptive Status Among Women Aged 15–49: United States, 2017–2019*. https://www.cdc.gov/nchs/nsfg/nsfg_questionnaires.htm (2020).

143. Marsh, S. A. & Jenkins, D. G. Physiological Responses to the Menstrual Cycle: Implications for the Development of Heat Illness in Female Athletes. *Sports Med.* **32**, 601–614 (2002).

144. Vaiksaar, S. *et al.* No effect of menstrual cycle phase on fuel oxidation during exercise in rowers. *Eur. J. Appl. Physiol.* **111**, 1027–1034 (2011).

145. Elliott-Sale, K. J. *et al.* The Effects of Oral Contraceptives on Exercise Performance in Women: A Systematic Review and Meta-analysis. *Sports Med.* **50**, 1785–1812 (2020).

146. Enoka, R. M. & Duchateau, J. Muscle fatigue: what, why and how it influences muscle function: Muscle fatigue. *J. Physiol.* **586**, 11–23 (2008).

147. Tenan, M. S., Hackney, A. C. & Griffin, L. Maximal force and tremor changes across the menstrual cycle. *Eur. J. Appl. Physiol.* **116**, 153–160 (2016).

148. Petrofsky, J., Al Malty, A. & Suh, H. J. Isometric endurance, body and skin temperature and limb and skin blood flow during the menstrual cycle. *Med. Sci. Monit. Int. Med. J. Exp. Clin. Res.* **13**, CR111-117 (2007).

149. Kerhervé, H. A., Stewart, D. G., McLellan, C. & Lovell, D. Fatigue Indices and Perceived Exertion Highlight Ergometer Specificity for Repeated Sprint Ability Testing. *Front. Sports Act. Living* **2**, 45 (2020).

150. Williams, J. S. & MacDonald, M. J. Influence of hormonal contraceptives on peripheral vascular function and structure in premenopausal females: a review. *Am. J. Physiol.-Heart Circ. Physiol.* **320**, H77–H89 (2021).

151. Minahan, C., Melnikoff, M., Quinn, K. & Larsen, B. Response of women using oral contraception to exercise in the heat. *Eur. J. Appl. Physiol.* **117**, 1383–1391 (2017).

152. Hackney, A. C., Koltun, K. J. & Williett, H. N. Menstrual cycle hormonal changes: estradiol-β-17 and progesterone interactions on exercise fat oxidation. *Endocrine* (2022) doi:10.1007/s12020-022-02998-w.

153. Gordon, A. *et al.* The Effects of Creatine Monohydrate Loading on Exercise Recovery in Active Women Throughout the Menstrual Cycle. *European Journal of Sports Science* (In Review).

154. Tsampoukos, A., Peckham, E. A., James, R. & Nevill, M. E. Effect of menstrual cycle phase on sprinting performance. *Eur. J. Appl. Physiol.* **109**, 659–667 (2010).

155. Goodwin, M. L., Harris, J. E., Hernández, A. & Gladden, L. B. Blood Lactate Measurements and Analysis during Exercise: A Guide for Clinicians. *J. Diabetes Sci. Technol.* **1**, 558–569 (2007).

156. Devlin, J. *et al.* Blood lactate clearance after maximal exercise depends on active recovery intensity. *J. Sports Med. Phys. Fitness* **54**, 271–278 (2014).

157. Middleton, L. E. & Wenger, H. A. Effects of menstrual phase on performance and recovery in intense intermittent activity. *Eur. J. Appl. Physiol.* **96**, 53–58 (2006).

158. Lara, B., Gutiérrez Hellín, J., Ruíz-Moreno, C., Romero-Moraleda, B. & Del Coso, J. Acute caffeine intake increases performance in the 15-s Wingate test during the menstrual cycle. *Br. J. Clin. Pharmacol.* **86**, 745–752 (2020).

159. Štefanovský, M., Péterová, A., Vanderka, M. & Lengvarský, L. Influence of selected phases of the menstrual cycle on performance in Special judo fitness test and Wingate test. *Acta Gymnica* **46**, 136–142 (2016).

160. Vaiksaar, S. *et al.* No Effect of Menstrual Cycle Phase and Oral Contraceptive Use on Endurance Performance in Rowers. *J. Strength Cond. Res.* **25**, 1571–1578 (2011).

161. Lynch, N. J. & Nimmo, M. A. Effects of menstrual cycle phase and oral contraceptive use on intermittent exercise. *Eur. J. Appl. Physiol.* **78**, 565–572 (1998).

162. Williams, J. S., Dunford, E. C. & MacDonald, M. J. Impact of the menstrual cycle on peripheral vascular function in premenopausal women: systematic review and meta-analysis. *Am. J. Physiol.-Heart Circ. Physiol.* **319**, H1327–H1337 (2020).

163. Shenouda, N., Priest, S. E., Rizzuto, V. I. & MacDonald, M. J. Brachial artery endothelial function is stable across a menstrual and oral contraceptive pill cycle but lower in premenopausal women than in age-matched men. *Am. J. Physiol.-Heart Circ. Physiol.* **315**, H366–H374 (2018).

164. Harris, R. A., Nishiyama, S. K., Wray, D. W. & Richardson, R. S. Ultrasound Assessment of Flow-Mediated Dilation. *Hypertension* **55**, 1075–1085 (2010).

165. Smith-Ryan, A. E. *et al.* Validity and reliability of a 4-compartment body composition model using dual energy x-ray absorptiometry-derived body volume. *Clin. Nutr.* (2017) doi:10.1016/j.clnu.2016.05.006.