THE IMPACT OF SEX HORMONE PROFILE ON OXYGEN UPTAKE KINETICS IN FEMALE MIDDLE-DISTANCE RUNNERS.

by

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The phase II time constant of the VO_2 kinetics response ($\tau\dot{V}O_2$) represents the exponential increase in oxygen consumption by the skeletal muscle, which is a key determinant of performance in middle-distance runners. Current data is inconsistent regarding whether $\tau\dot{V}O_2$ is influenced by hormone profile. We aimed to determine if sex hormone profile affects $\tau\dot{V}O_2$ in highly trained female middle-distance runners. We hypothesized that a high hormone profile would result in higher $\tau\dot{V}O_2$ and a decreased time to exhaustion (TTE) relative to a low hormone profile. Twelve highly trained female runners participated in four study visits. Visit 1 included a graded exercise test to determine maximal oxygen consumption and lactate turn point. A familiarization trial was followed by two experimental visits scheduled on either a high or low hormone profile day. The speed was within the severe domain, with the goal of participants reaching fatigue in four minutes. During the experimental visits, venous blood samples were collected to determine serum concentrations of sex hormones, including estrogen and progesterone. Paired t-tests were used to assess differences in $\tau\dot{V}O_2$ between hormone profiles and TTE duration. The relationship between changes in $\tau\dot{V}O_2$ and TTE was evaluated using a Pearson correlation. No $\tau\dot{V}O_2$ differences were found when compared to hormone profile ($P=0.599$, low: 29.6 ± 5.88 sec, high: 30.2 ± 5.84sec). There was no association between $\tau\dot{V}O$ and TTE ($r = 0.13$, $P = 0.69$). The Phase II time constant of the $\dot{V}O_2$ kinetics response during severe domain middle-distance running does not appear to be significantly influenced by sex hormone profile, although considerable responses were variable within individuals. Furthermore, $\tau\dot{V}O_2$ does not appear to be a determinant of performance.

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Introduction

Women were only officially allowed to compete in Olympic track and field for the first time at the 1928 Amsterdam Games. At the 2024 Olympic Games in Paris, male and female athletes were represented in equal numbers for the first time (Olympics.com). Over the last 100 years, research on female-specific physiology has remained underrepresented. Previous research has excluded female participants to avoid accounting for the complexity of female sex hormonal fluctuations. As a consequence, whether the menstrual cycle influences performance remains an ongoing question in both science and sport. To understand how these physiological differences may influence performance, it is important to examine the fundamental mechanisms of exercise responses.

From puberty through menopause, women undergo hormonal fluctuations associated with the menstrual cycle. The primary female sex hormones, estrogen and progesterone, fluctuate in cyclic patterns to regulate the menstrual cycle. During the follicular phase, estrogen levels steadily rise and peak just before ovulation, while progesterone levels remain low. After ovulation, the luteal phase begins, characterized by elevated progesterone levels alongside a second, smaller estrogen peak. If pregnancy does not occur, both hormone levels decline, triggering menstruation and the start of a new cycle (Reed and Carr, 2000). These hormones have wide-ranging physiological effects beyond reproduction and may influence exercise performance. Previous studies have shown that the hormones influence several systems, including cardiovascular function, the central nervous system, substrate metabolism, fluid balance, and thermoregulation. All key processes contribute to one's ability to exercise and maximize performance. Around the world, exercise physiologists and coaches are using evidence-based approaches to customize training, helping athletes optimize performance and

peak during competition. However, as of 2022, only 16% of performance research focused exclusively on females, and just 14% of those studies attempted to define menstrual status (Smith et al., 2022). In the absence of data on how menstruating females respond to exercise, coaches are likely to continue basing training on male physiology, potentially disadvantaging the female athletes and limiting their true performance potential. The menstrual cycle is a naturally occurring process that supports bone health and immune function in females and, therefore, should not be ignored; instead, research should account for it when designing studies and interpreting results. In response to this gap, the present study examines running performance across different menstrual cycle hormonal profiles, aiming to provide more foundational knowledge to support female athletes.

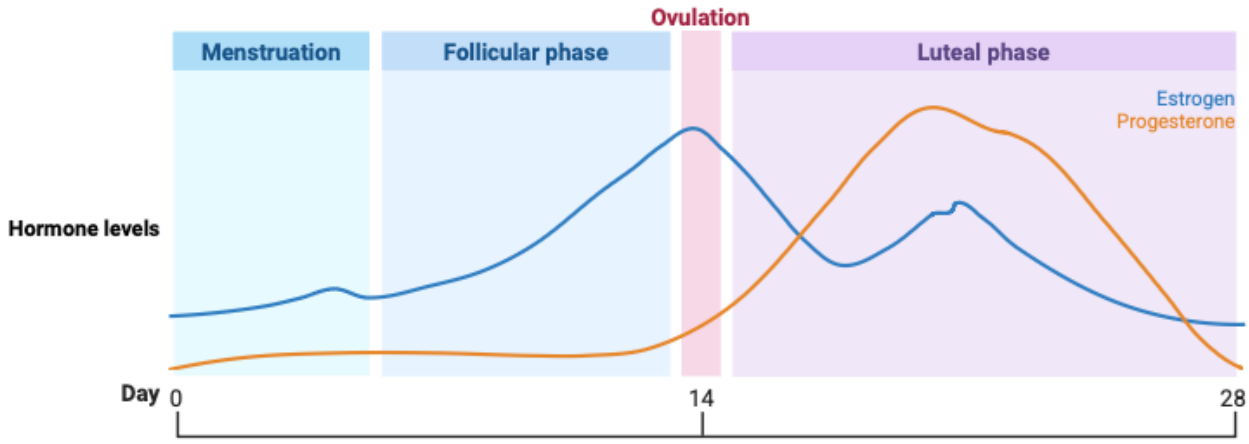


Figure 1. Estrogen and progesterone levels across the phases of the menstrual cycle.

The follicular phase is characterized by high estrogen and low progesterone, while the luteal phase is characterized by high progesterone and slightly lower estrogen.

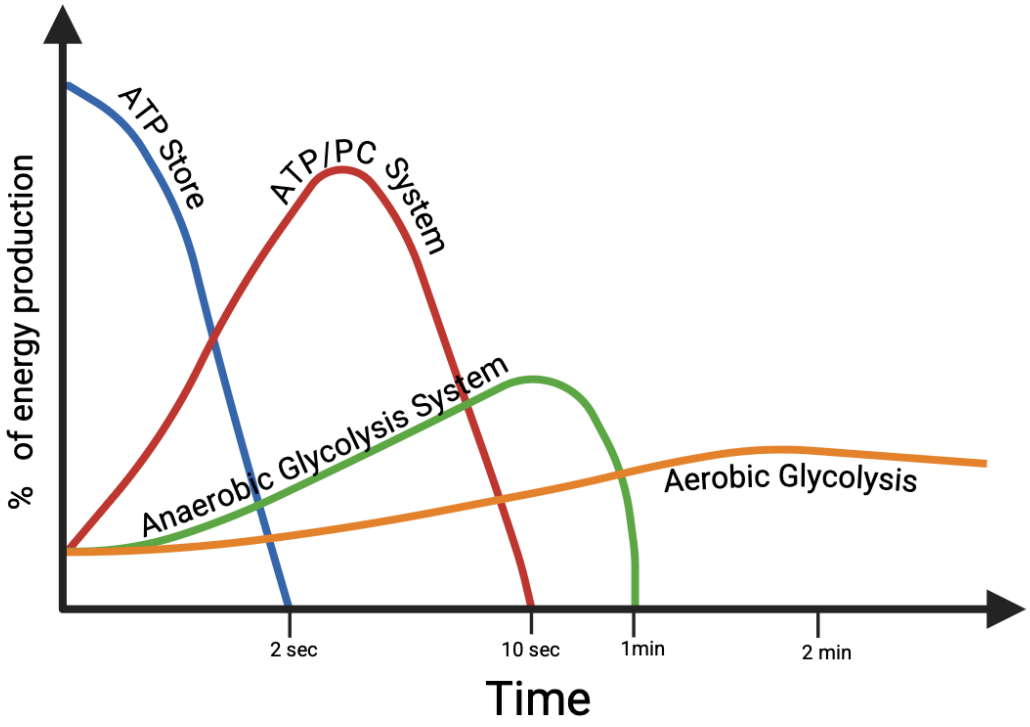


Figure 2. Energy pathways during severe exercise

Within the first few minutes of exercise, anaerobic pathways predominate until oxygen demands are met and aerobic glycolysis increases.

Exercise requires the repeated contraction of skeletal muscles. As exercise continues, the body's demand for oxygen increases relative to rest to produce the energy required for muscle contraction. However, skeletal muscles can only store a limited amount of ATP; therefore, intracellular metabolic pathways must be activated to maintain a continuous supply of ATP (Baker et al., 2010). In the first 10 seconds of exercise, energy is primarily supplied by the small amount of ATP stored within the muscle, which is rapidly depleted and only sufficient for a few seconds of activity. Once these levels decrease, the body shifts to the phosphocreatine system, which rapidly breaks down high-energy phosphate compounds stored in the muscle to regenerate ATP. This system does not rely directly on oxygen. However, phosphocreatine stores are limited and can only sustain short durations of high-intensity exercise (Sahlin, 2014). Beyond this initial phase, the glycolytic system becomes the dominant energy pathway, producing ATP from

muscle glycogen. This process can occur with or without oxygen; however, in the absence of sufficient oxygen, lactate is produced as a byproduct. The accumulation of lactate may be a limiting factor in high-intensity running, contributing to the onset of fatigue. During these early stages of exercise, oxygen delivery does not immediately meet the increased metabolic demand, and the body relies on anaerobic pathways to compensate. This mismatch between oxygen supply and demand is known as the oxygen deficit, as shown in Figure 2. After several minutes of exercise, the slower and more complex aerobic system becomes increasingly dominant. In this process, mitochondria utilize carbohydrates and fats to produce a high volume of ATP (Gastin, 2001). As oxygen delivery increases, the gap between oxygen supply and demand narrows, reducing the oxygen deficit. The rate at which the oxygen consumption increases to meet the demands of intense exercise is referred to as the VO_2 Phase II component ($\tau\dot{V}O_2$).

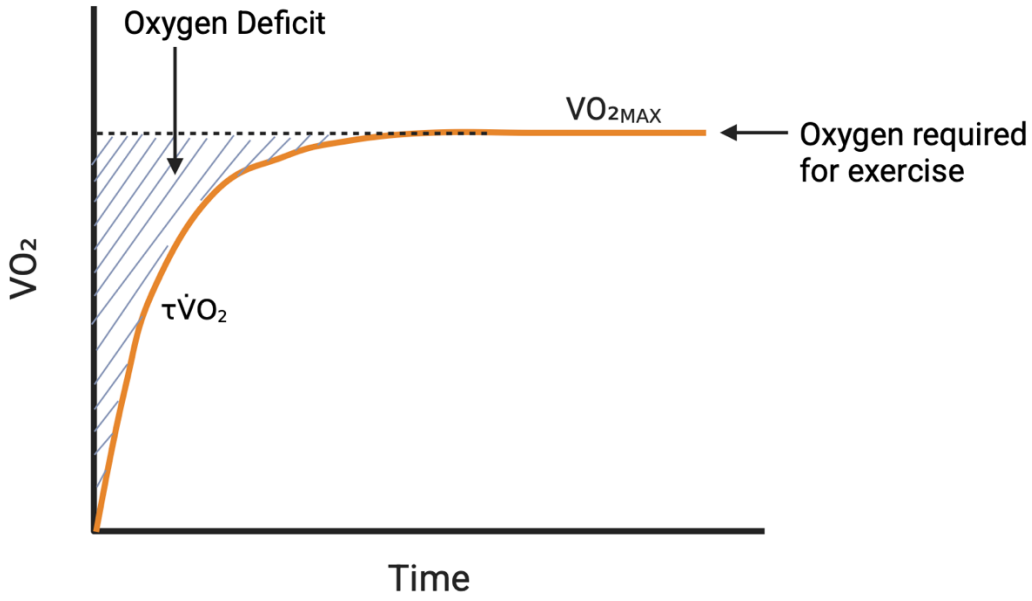


Figure 3. Example of oxygen uptake

The exponential rise in the Phase II time constant ($\tau\dot{V}O_2$) to $\dot{V}O_{2MAX}$ created an oxygen deficit, the area between the curve and the required oxygen.

Current literature remains conflicting regarding whether fluctuations in female sex hormones influence oxygen uptake kinetics. Many studies have examined these responses during moderate-intensity exercise (Gurd et al., 2007; Mattu et al., 2019; Freemas et al., 2021; Oosthuiyse and Bosch, 2009). For example, in 2007, Gurd et al. found no difference in $\tau\dot{V}O_2$ between menstrual cycle phases during moderate-intensity exercise. However, findings from moderate-intensity domains may not be representative of all exercise intensities. Distinct metabolic pathways are recruited during severe intensity exercise, which may be differently influenced by estrogen and progesterone. Examining VO2 kinetics in the severe domain may therefore be more performance relevant, as it better reflects the demand of athletic training and competition, where athletes operate at high levels of fatigue. Additionally, relatively few studies have specifically investigated τV̇O2, with many instead focusing on $\dot{V}O2_{MAX}$ (Mattu et al., 2019; Godbole et al., 2016). Some research has reported no significant differences in $\dot{V}O_{2MAX}$ across

menstrual cycle phases; however, $\dot{V}O_{2MAX}$ alone does not provide a complete representation of performance capacity (Mattu et al., 2019; Lara et al., 2020; Jans De Jonge, 2003). In contrast, analysis of $\dot{V}O_2$ kinetics offers greater insight into metabolic stability by reducing reliance on anaerobic energy systems and delaying fatigue (Goulding et al., 2021). One study investigating the influence of estrogen and progesterone during heavy-to-severe intensity cycling found that, when controlling for estrogen, progesterone showed a positive correlation with $\tau\dot{V}O_2$ (Schoeberlein et al., 2025). Specifically, higher progesterone concentrations were associated with a slower oxygen uptake response at exercise onset (Schoeberlein et al., 2025). Estrogen alone did not demonstrate a significant effect on oxygen uptake kinetics (Schoeberlein et al., 2025). However, these effects have yet to be examined using treadmill-based exercise, which may better represent running-specific performance. As far as current evidence suggests, no studies have directly linked oxygen uptake kinetics to female sex hormone profile, which could potentially have physiological implications for the oxygen deficit during severe-intensity exercise (Gurd et al., 2007; Taylor et al., 2024). The present study aimed to examine the relationship between $\tau\dot{V}O2$ and hormone profile using expired gases in females. Specifically, analyzing $\tau\dot{V}O2$ of time to exhaustion (TTE) trials on a low and high hormone profile day. Additionally, investigating the relationship between $\tau\dot{V}O2$ and fatigue progression during TTE trials.

Methods

Participants

Nineteen highly trained female middle-distance runners between aged 18 and 40 years were recruited for this study. Eligibility required the ability to complete 1500 meters in 5 minutes and 30 seconds or less. Three participants were excluded from analyses due to failure to complete the study protocol, injury, or non-fluctuating hormone levels, resulting in a final sample of sixteen participants. All participants provided written informed consent upon enrollment.

Experimental Design

Each participant completed four laboratory visits. Visit 1 consisted of a whole-body Dual-Energy X-ray Absorptiometry (DXA) scan to assess body composition, as well as maximal oxygen uptake (VO_{2MAX}) and lactate threshold testing. VO_{2MAX} was measured using a graded exercise test on a treadmill. Visit 2 functioned as a familiarization time trial session. Participants completed a self-selected warm-up followed by a TTE trial on the treadmill. Visits 3 and 4 were the experimental time to exhaustion (TTE) trials, conducted during distinct phases of the menstrual cycle to capture hormonal variation. At Visits 3 and 4, blood samples were collected to verify high and low hormone profiles. During visits 2 - 4 participants were blinded to speed, time, and distance, and remained connected to a safety harness throughout.

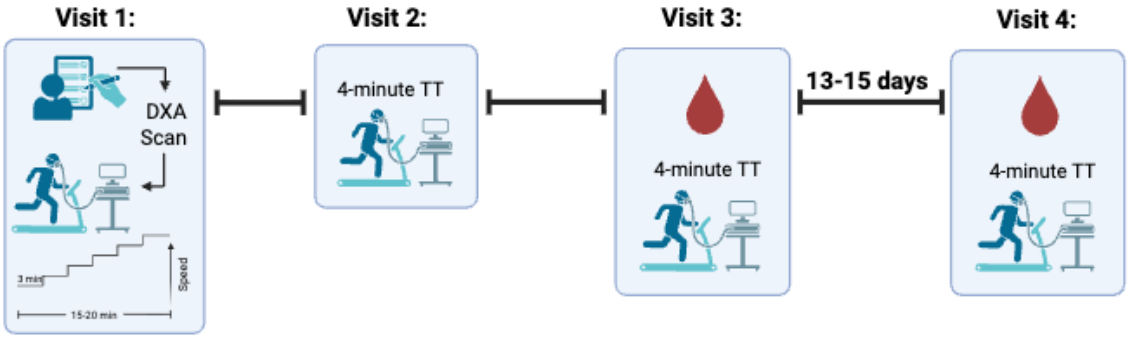


Figure 4. Schematic of experimental design

Visit 1 consisted of a DXA scan and a graded exercise test on a treadmill. Visit 2 acted as a familiarization TTE trial. Visits 3 and 4, scheduled on distinct hormone profiles, were experimental TTE trials. Both experimental trials included a venous blood draw. All TTE trials were set to a speed to calculate above critical velocity and elicited to cause fatigue in approximately 4 minutes.

Pre-Visit Requirements

For all four visits, participants were asked to abstain from alcohol, dietary supplements, vitamins, prescription drugs, and intense exercise in the 24 hours before each visit. Additionally, participants abstained from caffeine and food intake for at least two hours before each visit to control for the effects of stimulants and nutrition on performance.

Hormonal Assessment

To identify the hormone profile, venous blood samples were collected at the start of Visits 3 and 4. The samples were analyzed for serum progesterone and estrogen concentration using mass spectrometry within 7 days by QUEST Diagnostics. Hormone status was used to validate that upcoming visits were scheduled across distinct hormonal profiles of the menstrual cycle.

Maximal Oxygen Uptake (VO_{2MAX}) and Lactate Turn Point Assessment

Maximal oxygen uptake (VO_{2MAX}) and lactate turn point (LTP) were measured using a graded exercise test on a treadmill. Participants began with a self-selected 5-minute warm-up, after which the test began with a predetermined speed based on each participant's personal best 1500-meter time. Treadmill speed was increased by 1km/hr every 3 minutes until a speed of 15 km/hr was reached. At this point, the increments were reduced to 0.5km/hr every 3 minutes until exhaustion. Expired gases were collected using a mixing chamber (K5, COSMED) to measure total ventilation (VE), the volume of carbon dioxide produced (VCO_2), and the volume of oxygen consumed (VO_2), with values recorded at 10-second intervals. VO_{2MAX} was defined as the highest VO_2 over a 30-second average at the end of the test. Baseline VO_2 was determined from 60 seconds of standing before running, representing the cardiodynamic phase. Before the experiment, the mixing chamber system was calibrated for expired gas concentrations and respiratory gas concentrations. Blood lactate concentration was measured using a portable lactate analyzer at the end of each stage to construct an individual lactate curve, from which LTP was identified. Following the graded exercise test, critical velocity was determined using data derived from both the lactate curve and oxygen uptake response.

Time to Exhaustion Trial

During visits 2-4, participants completed a familiarization time trial. A predetermined treadmill speed was set based on the critical velocity and D' derived from the VO_{2MAX} and LTP assessment, calculated to cause fatigue at approximately 4 minutes. Visit 2 served as a familiarization trial to control learning effects and allow participants to become comfortable running on a treadmill. Participants completed a self-selected running warm-up and dynamic

stretches. Afterwards, they were attached to a safety harness. Before the trial, participants performed strides at speeds approximating the time trial speed and practiced mounting and dismounting the treadmill. Following the warm-up, participants straddled the treadmill for one minute to collect baseline VO_2 values. The treadmill was set to a 1% incline to replicate running outdoors. On command, participants mounted the treadmill and ran at the prescribed speed with the goal of continuing until fatigue. Participants were blinded to the treadmill speed, total elapsed time, and distance during the trial. The safety harness was set up to catch participants and instantly stop the treadmill upon fatigue. The VO_2 values were continuously recorded throughout the trial. Based on the outcome of the familiarization trial, treadmill speed was adjusted between

Determination of VO_2 Kinetics

VO_2 kinetics describe the dynamic adjustment of oxygen uptake at the onset of exercise and can be characterized by three distinct phases. Phase I reflects the immediate increase in pulmonary blood flow at the onset of exercise and is typically excluded from modelling, as it does not represent muscle oxygen consumption (Poole and Jones, 2012). Phase II, also known as the rapid onset component, begins approximately 15 to 20 seconds after exercise onset and represents the exponential rise in VO_2 driven by oxidative phosphorylation in working skeletal muscles. Phase III, known as the VO_2 slow component, is characterized by a continued rise in VO_2 above the initial increase and is observed primarily during heavy and severe intensity exercise.

VO_2 kinetics can be described using mathematical modeling techniques. Typically, a mono-exponential model is applied to the Phase II response, with the initial cardioynamic Phase I excluded from analysis. In cases where a VO_2 slow component is present, a bi-exponential

model is required to distinguish between the fundamental (Phase II) and slow components, using parameters such as amplitude, time delay, and time constant (τ). The time constant (τ) represents the time required for $\dot{V}O_2$ to reach 63% of its Phase II amplitude and is commonly used as an index of aerobic fitness and oxidative responsiveness. In the present study, $\dot{V}O_2$ kinetics were analyzed using a mono-exponential modelling approach, as the primary aim was to characterize the Phase II response. The onset of exercise was marked as time zero, and the initial cardiodynamic Phase I was excluded from data analysis. The remaining $\dot{V}O_2$ data values were fitted using the following equation:

$$VO_2(t) = VO_2\text{baseline} + A(1 - e^{(-\frac{t - TD}{\tau})})$$

where A represents amplitude, TD the time delay, and τ the time constant of the rapid-onset response (McNulty and Robergs, 2017). The τ value was determined for each participant and used as the primary measure to quantify the rate of increase in oxygen uptake.

Statistical Analysis

All statistical analyses were conducted using an alpha level of 0.05 to evaluate statistical significance. To determine differences in hormone profile and time-trial performance associated with Phase II $\dot{V}O_2$ kinetic values, paired sample t-tests were conducted, as each participant served as their own control across Visits 3 and 4. This approach controlled for interindividual variability in fitness and hormone profiles. To examine the relationship between changes in Phase II $\dot{V}O_2$ kinetic values and total time-trial duration, Pearson's correlation was performed. Data are reported as means ± standard deviations.

Results

The sample population consisted of twelve highly trained female runners, with a mean age of 28.5 ± 4.67 years, body mass of 60.3 ± 7.13 kg, and height of 164.2 ± 4.89 cm. Participants' characteristics are displayed in Table 1 (means ± SD). All participants exhibited hormonal fluctuations, and contraceptive use was not an exclusion criterion. The mean VO_{2MAX} was 58.4 ± 6.03 ml/kg/min and is expressed relative to lean body mass (LBM). Mean VO_{2MAX} was calculated as the highest VO_{2MAX} recorded across the four visits. The average speed of Visits 3 and 4 TTE trials corresponded to a pace of 5:42 ± 0:30 min/mile. The mean TTE duration across both Visits 3 and 4 was 223.8 ± 39.8 seconds. Table 2 displays the mean venous hormone concentrations during the high and low hormone profiles. High hormone profile experimental visit mean concentrations were (E_2/P_4), and low hormone profile experimental visit mean concentrations were (E_2/P_4).

	N=12
Age (years)	28.5 ± 4.7
Height (cm)	164.2 ± 4.9
Weights (kg)	60.3 ± 7.1
Vo_{2MAX} (ml/kg/min)	58.4 ± 6.0
TTE speed (min/mile)	5:43 ± 0:25
TTE duration (sec)	244.2 ± 59.9

Table 1. Participants characteristics

Data presented as means ± SD for characteristics.

	N=12	
	Low	High
E_2 (pg/mL)	92.2 ± 56.2	125.8 ± 57.7
P_4 (ng/mL)	<0.1 ± 0	8.8 ± 4.4
FSH (mIU/mL)	7.3 ± 2.1	3.4 ± 1.2
LH (mIU/mL)	6.4 ± 2.8	3.7 ± 1.9

Table 2. Hormone concentrations

Calculated (means ± SD) $\tau\dot{V}O_2$ values from the monoexponential model associated with hormone profile are displayed in Table 3. A paired samples t-test was conducted to examine differences in VO_2 Phase II kinetics between high and low hormone profiles. Results indicated no statistical significance (P=0.599, low: 29.6 ± 5.88 sec, high: 30.2 ± 5.84 sec). When $\tau\dot{V}O_2$ was compared to the longest and shortest TTE duration, no differences were found (P=0.4339, shortest: 29.4 ± 6.44 sec, longest: 30.4v± 5.18 sec).

Hormone Profile			
	Low	**High**	**p-value**
$T\dot{V}O_2$ (sec)	29.6 ± 5.9	30.2 ± 5.8	0.60
TTE Duration			
	Short	**Long**	**p-value**
$T\dot{V}O_2$ (sec)	29.4 ± 6.4	30.4 ± 5.2	0.43

Table 3. Mean $\tau\dot{V}O_2$ relative to hormone profile and TTE duration

Data values presented as means ± SD for the Phase II time constant ($\tau\dot{V}O_2$). No statistical difference between low and high hormone profiles using paired t-test analysis (P=0.43). No statistical difference between shortest and longest TTE duration using paired t-test analysis (P=0.60).

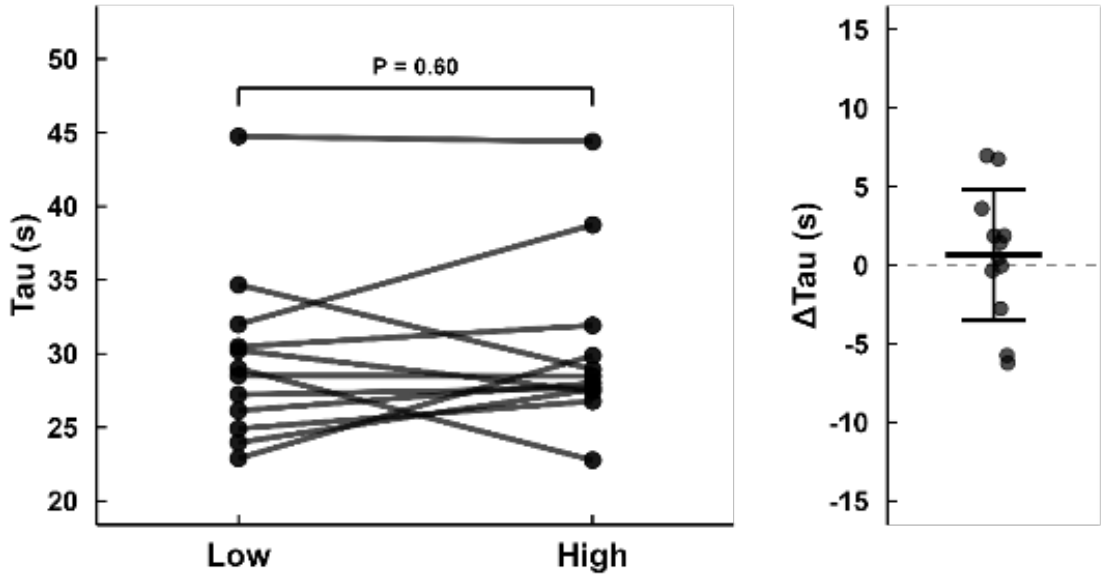


Figure 5. Effect of hormone profile on the Phase II oxygen uptake time constant (τ).

$\tau\dot{V}O_2$ was measured during visits scheduled at low and high hormone profile phases. No significant difference was observed between conditions (P=0.60). The left figure displays individual participant values connected by solid lines. The right figure displays the mean ± SD of the within-participant change (ΔTau), with individual data points overlaid.

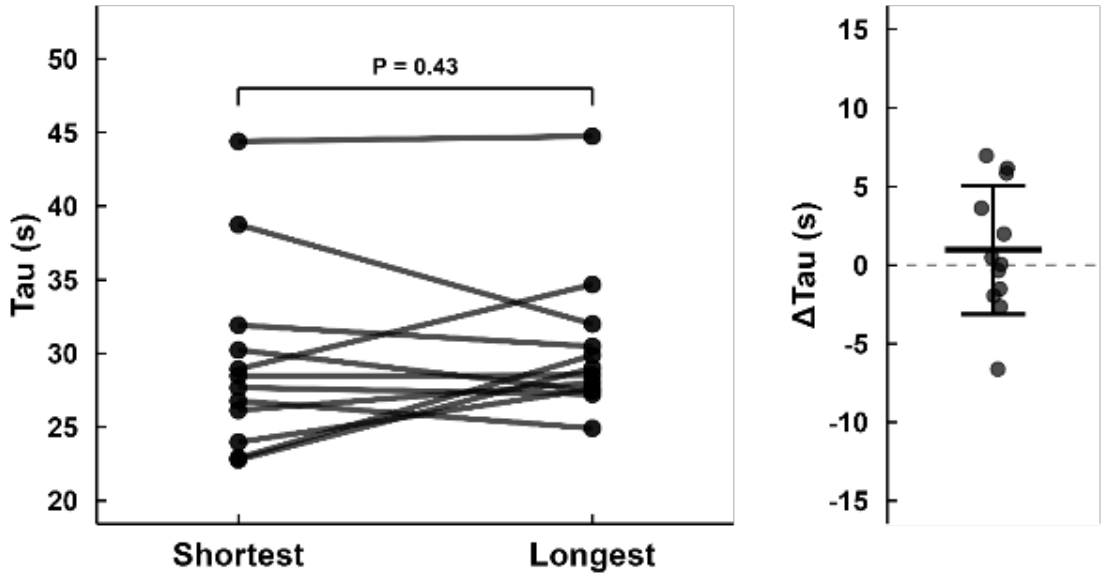


Figure 6. Effect of TTE trial duration on the Phase II oxygen uptake time constant (τ)

$\tau\dot{V}O_2$ was compared between each participant's shortest and longest exhaustion trial. No significant difference was observed between conditions (P=0.43). The left figure displays individual participant values connected by solid lines. The right figure shows the mean ± SD of the within-participant change (ΔTau), with individual data points overlaid.

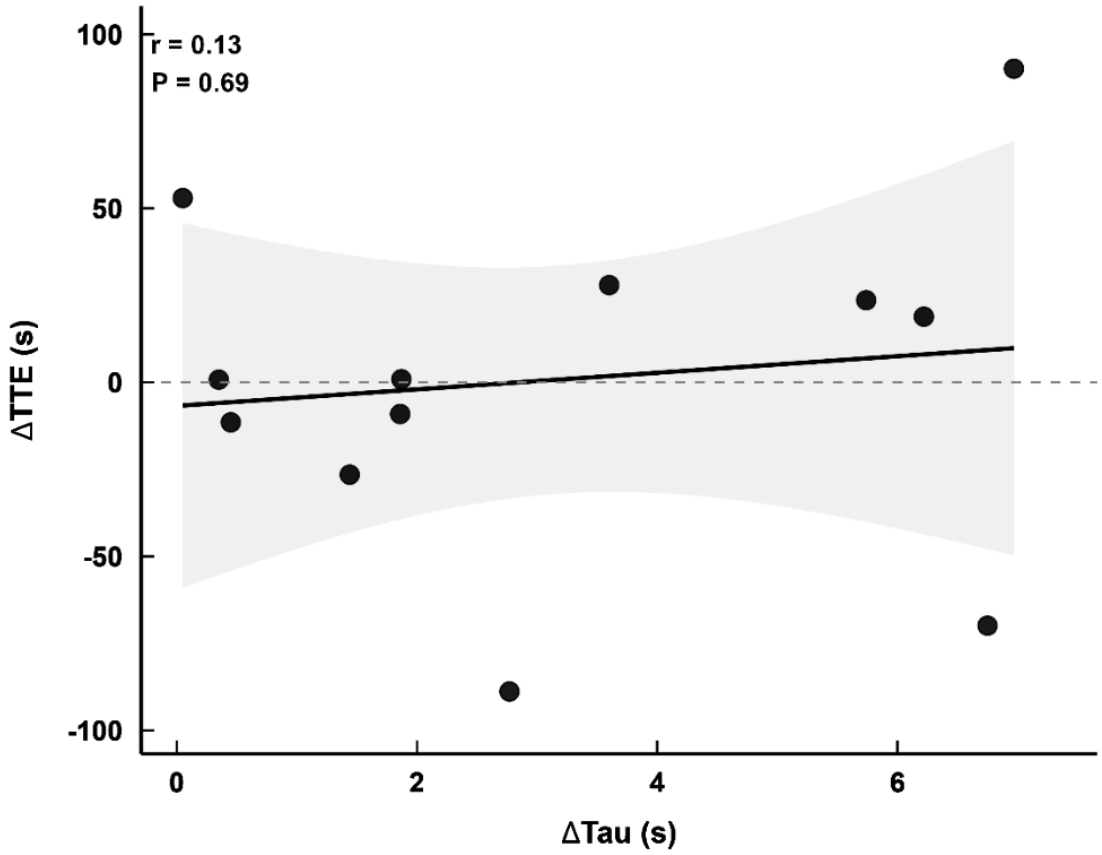


Figure 7. Correlation between the within-participant change in Phase II oxygen uptake time constant (ΔTau) and the change in TTE (ΔTTE).

The relationship between ΔTau and ΔTTE was not statistically significant (r = 0.13, P = 0.69), indicating that individual changes in oxidative kinetics did not predict changes in exercise tolerance. The solid line represents the line of best fit with the shaded region showing the 95% confidence interval.

Discussion

Main Findings and Hormone Identification

To the best of our knowledge, this is the first study to investigate the influence of female sex hormone profiles on the Phase II component of VO_2 kinetics. Our findings indicate that fluctuations in hormonal profiles do not elicit changes in $\tau\dot{V}O_2$ kinetics. Collectively, these results align with previous literature suggesting that the menstrual cycle has little to no effect on anaerobic performance (Shaharudin et al., 2011; Okudan et al., 2005).

Across a range of sports, including triathlons, rowing, football, skiing, and middle- and long-distance running, studies have reported no significant differences in Wingate anerobic power between the early follicular and mid-follicular phases, despite slightly elevated hormonal levels during the latter (Carmichael et al., 2021, Lara et al., 2020; Matsuda et al., 2023). However, a key limitation of this area of work is the frequent reliance on indirect methods to determine hormonal status, such as calendar-based tracking, basal body temperature, or urinary analysis, which are less accurate than direct measures. In contrast, the present study quantified hormone concentrations using venous blood sampling, providing a more accurate representation of participants' hormonal status. As such, previous findings may have been limited by the classification of hormonal status (Matsuda et al., 2023, Gurd et al., 2007; Redman et al., 2003), whereas we are confident in the validity of our measurements and their use in statistical analyses.

Exercise Intensity Domain

The influence of sex hormones on substrate metabolism becomes more apparent as exercise duration increases. Progesterone acts as a respiratory stimulant by increasing the sensitivity of the medullary respiratory center to carbon dioxide, leading to elevated ventilation,

reduced arterial carbon dioxide, and increased oxygen uptake. Progesterone has been associated with reduced insulin sensitivity, potentially impairing glucose uptake and increasing reliance on less efficient metabolic pathways (Williams and Krahenbuhl, 1997). Together, these effects could increase physiological strain for a given workload. In contrast, estrogen generally supports endurance performance by improving insulin sensitivity, enhancing glycogen storage in skeletal muscle, and promoting lipid oxidation while sparing carbohydrate availability (Oosthuyse and Bosch, 2010). This results in a greater capacity for sustained energy production during prolonged exercise. However, these hormonal influences are more relevant during long-duration, steady-state exercise. In the severe-intensity domain, where exercise lasts only a few minutes, energy production relies predominantly on phosphocreatine breakdown and aerobic glycolysis rather than fat oxidation or glycogen sparing (Oosthuyse and Bosch, 2010). Participants in the present study began exercise at high intensity immediately, thereby primarily engaging these anaerobic systems. If the exercise protocol had involved longer or lower intensity, hormonal differences in substrate utilization may have become more apparent.

Severe Exercise Masking Hormonal Influences

Overall, it may be likely that the physiological stress of severe intensity exercise overrules relatively small hormonal fluctuations. The severe exercise domain is characterized by intense metabolic responses, such as rapid depletion of phosphocreatine stores, increasing glycolysis rate, and progressive accumulation of fatiguing metabolites, all of which drive strong responses that mask the subtle effects of sex hormones (Baker et al., 2010). In this context, the extent of hormonal variation may be insufficient to produce a detectable shift in oxygen uptake kinetics. This interpretation is supported by evidence from other extreme physiological conditions. For example, in hypoxic environments such as altitude, sex related differences in

physiological responses are often attenuated, as the dominant hypoxic stress overrides progesterone-mediated ventilatory effects (Raberin et al., 2024). A similar mechanism may be occurring here, where the metabolic and cardiovascular demands of severe exercise mask subtle hormonal influences. It is therefore possible that hormonal fluctuations' effects on performance and oxygen uptake kinetics are more detectable at lower exercise intensities, where the physiological demand is less likely to override the relatively subtle influence of sex hormones.

Societal and Practical Implications

Despite increasing research interest, the female menstrual cycle remains an under-discussed topic in sports science. Many athletes report discomfort or stigma when discussing the menstrual cycle, contributing to uncertainty around training adaptation based on hormonal fluctuations. One survey reported that only 8% of female athletes had ever been asked about their menstrual cycle by a coach, despite 54% indicting willingness to discuss it (Laske et al., 2024). This communication gap may lead athletes to self-adjust training without guidance, potentially affecting performance or increasing injury risk. The present findings contribute to addressing this gap by suggesting that the hormonal profile does not meaningfully influence the Phase II component of $\dot{V}O_2$ kinetics. This implies that training and competition schedules do not need to be modified based on cycle phase for this physiological domain. Given that race timing cannot be aligned with individual hormonal status, this provides practical reassurance that athletes can train and compete without concern for cycle-related impairments in this context. Furthermore, evidence that performance is largely unaffected may help reduce stigma surrounding female physiology in sport and counter misconceptions that female athletes are inherently more variable or fragile.

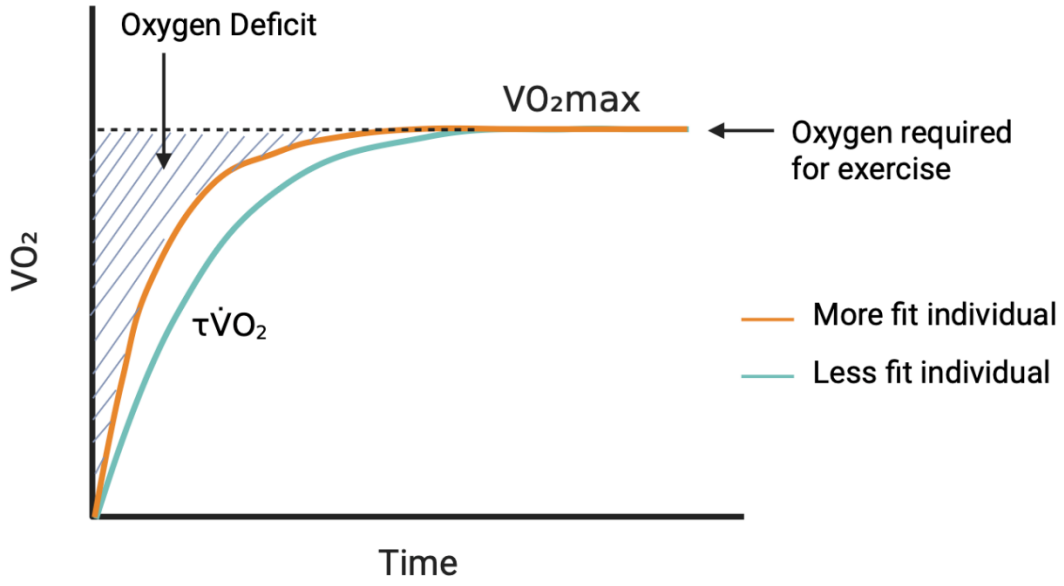


Figure 8. Oxygen deficit in trained vs untrained individuals

The exponential rise to VO_{2MAX} is steeper in more fit individuals, represented by $\tau\dot{V}O_2$, and creates a smaller oxygen deficit. Whereas less fit individuals take longer to reach VO_{2MAX}, creating a larger oxygen deficit.

VO_2 Kinetics and Training Relationship

In the present study, no evidence was found that we found $\tau\dot{V}O_2$ kinetics influence TTE performance. This contrasts with previous literature suggesting a relationship between oxygen uptake kinetics and performance outcomes (Jones et al., 2021). These discrepancies likely reflect differences in methodological approach and the interaction between multiple physiological systems. The oxygen deficit at the onset of exercise in the severe domain is primarily determined by $\tau\dot{V}O_2$. Faster kinetics reduce oxygen deficit by accelerating the transition to aerobic metabolism, thereby decreasing reliance on anaerobic glycolysis and phosphocreatine breakdown. A reduced oxygen deficit limits the accumulation of fatigue-associated metabolites, such as inorganic phosphate, hydrogen ions, and ADP. Training adaptations, including increased capillary density, improved vascular function, and enhanced mitochondrial capacity, are known to accelerate $\tau\dot{V}O_2$ kinetics (Mølmen et al., 2025). However, responses may vary depending on

the athlete's specialization. Endurance-trained individuals typically demonstrate faster oxygen uptake kinetics than sprint-trained or untrained individuals, potentially due to differences in phosphocreatine kinetics, mitochondrial sensitivity to creatine, or oxidative enzyme activity (Berger and Jones, 2007).

Previous research has examined similar physiological outcomes across the menstrual cycle. For instance, no significant differences in maximal accumulated oxygen deficit have been observed between follicular and mid-luteal phases during sprint cycling intervals (Shaharudin et al., 2011). However, interpretation of these findings may be limited by the characteristics of that cohort, who exhibited a relatively low VO_{2MAX} (34.92±4.85 mL·kg-1·min-1), which may not be representative of the trained population in the present study (Shaharudin et al., 2011). This distinction is relevant to the present findings, as highly trained individuals typically demonstrate faster $\tau\dot{V}O_2$ kinetics, which reduces reliance on anaerobic energy contribution and therefore decreases oxygen deficit accumulation during high-intensity exercise. One possible explanation for the absence of an observed relationship between $\tau\dot{V}O_2$ and TTE in the current study is that participants were not limited by peripheral metabolic factors but instead by central cardiovascular factors (Heinonen, 2025). Typically, in trained individuals, performance is limited by central cardiovascular delivery, specifically maximal cardiac output and stroke volume (Zając et al., 2015). Since the Phase II is driven by peripheral factors and reflects the muscle's ability to utilize oxygen, the skeletal muscles are already highly adapted and most likely are not what is hindering performance (Smith et al., 2023). In highly trained individuals, $\tau\dot{V}O_2$ may already be reaching the lowest possible values, leaving minimal room for hormonal fluctuations across the menstrual cycle to cause a detectable influence. This "ceiling effect", where further improvements in oxygen uptake kinetics are minimal and indistinguishable from random

variation in data, may explain why no hormonal influences on $\tau\dot{V}O_2$ were observed in the present study, in contrast to findings from less trained cohorts, where there is more room for improvements.

Supporting this hypothesis, the relatively homogeneous fitness level of the present cohort may have further limited the ability to detect meaningful relationships between $\tau\dot{V}O_2$ and performance. In highly trained populations, $\tau\dot{V}O_2$ values are already relatively fast (e.g., ~28.5 ± 8.5 s in trained runners vs. ~59.3 ± 12.0 s in untrained individuals) (Caputo et al., 2003), potentially reducing inter-variability and limiting the detectability of performance relationships. This homogeneity may reinforce the "ceiling effect" described above, where further improvements in $\tau\dot{V}O_2$ are negligible. Future research incorporating a broader range of fitness levels may help clarify whether $\tau\dot{V}O_2$ kinetics meaningfully contribute to performance variability.

Intensity Domain and the Role of the Slow Component

An important consideration in interpreting $\dot{V}O_2$ kinetics is the exercise intensity domain. During exercise above critical velocity, metabolic by-products progressively accumulate, and the $\dot{V}O_2$ slow component emerges. This slow component contributes substantially to fatigue development and is often considered a key determinant of exercise tolerance (Goulding et al., 2021). In this context, performance limitation may be more closely related to the magnitude of the $\dot{V}O_2$ slow component rather than the Phase II component. The present study was conducted within the severe-intensity domain, where both the Phase II component and potential slow component may both occur. It is therefore possible that previous studies demonstrating stronger associations between $\dot{V}O_2$ kinetics and performance were done at moderate or heavy intensities, where steady-state dynamics differ. While both $\dot{V}O_2$ kinetics and performance improve with

cardiovascular training adaptations, this does not necessarily indicate a direct causal relationship between the two variables. Instead, they may improve together as part of broader adaptation, rather than a simple "cause-and-effect" relationship (Inglis et al., 2025).

Methodological Considerations

This study utilized a mixing chamber technique to measure expired gases for the collection of $\dot{V}O_2$ data. While this method is widely accepted and provides reliable estimates of oxygen uptake, the use of 10-second averaged data may have limited the precision of $\tau\dot{V}O_2$ estimation. Previous literature indicates that $\tau\dot{V}O_2$ typically falls within a relatively short time frame (10 to 25 seconds), with one study reporting a mean of 12.1 ± 2.6 seconds in highly trained runners during incremental exercise (Jones et al., 2021). Given this short duration, 10-second averaging may yield only one or two data points within the critical window of the responses, making it difficult to accurately define the onset and shape of the exponential rise. In contrast, many previous studies have used breath-by-breath gas analysis, allowing for much higher collection frequency. For example, some investigations have collected data at millisecond sampling rates and subsequently filtered and interpolated values to 1-second intervals, enabling a more precise characterization of $\dot{V}O_2$ kinetics (Berger and Jones, 2007; Koppo et al., 2004; Carter et al., 2002). As such, future research examining changes in $\tau\dot{V}O_2$ should consider utilizing breath-by-breath data collection methods to improve measurement sensitivity and accuracy.

Additionally, the use of a monoexponential model to fit oxygen uptake kinetics may have inaccurately represented the body's response to severe intensity exercise. Exercise set above critical power is a compromise of fatigue-inducing metabolites that require a higher oxygen cost. As a result, $\dot{V}O_2$ slowly rises from steady state until it reaches $\dot{V}O_{2MAX}$ (Poole et al., 2016).

Therefore, a monoexponential model fails to capture this second, slow component. The use of a single monoexponential curve to capture the Phase II time constant (τ) in this study may have resulted in overestimations of the value. (McNulty et Robergs, 2017). Instead, future research should consider the use of a bi-exponential model to independently account for both the Phase II and the slow component (Carter et al., 2002).

Conclusion

In summary, these findings demonstrate that variations in hormonal profile had no significant effect on the Phase II component of the $\dot{V}O_2$ kinetics response during severe intensity in highly trained female athletes. The absence of changes in the time constant (τ) between two experimental visits suggests that estrogen and progesterone do not meaningfully influence the physiological system controlling oxygen uptake dynamics during exercise. This indicates that any hormone-driven alterations in substrate utilization or ventilatory response are likely minimal relative to the dominant physiological demands of severe-intensity exercise. Additionally, the lack of a relationship between the Phase II time constant and TTE duration suggests that $\tau\dot{V}O_2$ may not be a predictor of middle-distance running performance. Alternatively, this finding may reflect limitations in the data collection methodology, which may not have fully captured the rapid kinetics response. From a practical perspective, these results suggest that controlling for menstrual cycle phase may not be necessary when assessing Phase II $\dot{V}O_2$ kinetics in highly trained female athletes. Future research should explore $\dot{V}O_2$ kinetics across different exercise intensity domains using high-resolution, breath-by-breath data collection methods to determine whether the observed lack of association reflects methodological constraints or a potential ceiling effect in trained populations.

Bibliography

Baker, J. S., McCormick, M. C., & Robergs, R. A. (2010). Interaction among Skeletal Muscle Metabolic Energy Systems during Intense Exercise. *Journal of Nutrition and Metabolism*, *2010*, 905612. https://doi.org/10.1155/2010/905612

Berger, N. J. A., & Jones, A. M. (2007a). Pulmonary O2 uptake on-kinetics in sprint- and endurance-trained athletes. *Applied Physiology, Nutrition, and Metabolism = Physiologie Appliquee, Nutrition Et Metabolisme*, *32*(3), 383–393. https://doi.org/10.1139/H06-109

Caputo, F., Mello, M. T., & Denadai, B. S. (2003). Oxygen uptake kinetics and time to exhaustion in cycling and running: A comparison between trained and untrained subjects. *Archives of Physiology and Biochemistry*, *111*(5), 461–466. https://doi.org/10.3109/13813450312331342337

Carmichael, M. A., Thomson, R. L., Moran, L. J., & Wycherley, T. P. (2021). The Impact of Menstrual Cycle Phase on Athletes' Performance: A Narrative Review. *International Journal of Environmental Research and Public Health*, *18*(4), 1667. https://doi.org/10.3390/ijerph18041667

Carter, H., Pringle, J. S. M., Jones, A. M., & Doust, J. H. (2002a). Oxygen uptake kinetics during treadmill running across exercise intensity domains. *European Journal of Applied Physiology*, *86*(4), 347–354. https://doi.org/10.1007/s00421-001-0556-2

Freemas, J. A., Baranauskas, M. N., Constantini, K., Constantini, N., Greenshields, J. T., Mickleborough, T. D., Raglin, J. S., & Schlader, Z. J. (2021). Exercise Performance Is Impaired during the Midluteal Phase of the Menstrual Cycle. *Medicine and Science in Sports and Exercise*, *53*(2), 442–452. https://doi.org/10.1249/MSS.0000000000002464

Gastin, P. B. (2001). Energy system interaction and relative contribution during maximal exercise. *Sports Medicine*, *31*(10), 725–741. https://doi.org/10.2165/00007256-200131100-00003

Godbole, G., Joshi, A. R., & Vaidya, S. M. (2016). Effect of female sex hormones on cardiorespiratory parameters. *Journal of Family Medicine and Primary Care*, *5*(4), 822–824. https://doi.org/10.4103/2249-4863.201148

Goulding, R. P., Rossiter, H. B., Marwood, S., & Ferguson, C. (2021a). Bioenergetic Mechanisms Linking V˙O2 Kinetics and Exercise Tolerance. *Exercise and Sport Sciences Reviews*, *49*(4), 274–283. https://doi.org/10.1249/JES.0000000000000267

Gurd, B. J., Scheid, J., Paterson, D. H., & Kowalchuk, J. M. (2007a). O2 uptake and muscle deoxygenation kinetics during the transition to moderate-intensity exercise in different phases of the menstrual cycle in young adult females. *European Journal of Applied Physiology*, *101*(3), 321–330. https://doi.org/10.1007/s00421-007-0505-9

Heinonen, I. (2025). Cardiac output limits maximal oxygen consumption, but what limits maximal cardiac output? *Experimental Physiology*, *110*(5), 666–674. https://doi.org/10.1113/EP091594

Inglis, E. C., Rasica, L., Iannetta, D., Sales, K. M., Keir, D. A., MacInnis, M. J., & Murias, J. M. (2025). Exercise training-induced speeding of $\dot{V}O_2$ kinetics is not intensity domain-specific or correlated with indices of exercise performance. *European Journal of Applied Physiology*, *125*(5), 1297–1310. https://doi.org/10.1007/s00421-024-05674-1

Janse De Jonge, X. A. K. (2003). Effects of the Menstrual Cycle on Exercise Performance: *Sports Medicine*, *33*(11), 833–851. https://doi.org/10.2165/00007256-200333110-00004

Jones, A. M., Kirby, B. S., Clark, I. E., Rice, H. M., Fulkerson, E., Wylie, L. J., Wilkerson, D. P., Vanhatalo, A., & Wilkins, B. W. (2021a). Physiological demands of running at 2-hour marathon race pace. *Journal of Applied Physiology*, *130*(2), 369–379. https://doi.org/10.1152/japplphysiol.00647.2020

Koppo, K., Bouckaert, J., & Jones, A. M. (2004). Effects of training status and exercise intensity on phase II VO2 kinetics. *Medicine and Science in Sports and Exercise*, *36*(2), 225–232. https://doi.org/10.1249/01.MSS.0000113473.48220.20

Lara, B., Gutiérrez Hellín, J., Ruíz-Moreno, C., Romero-Moraleda, B., & Del Coso, J. (2020). Acute caffeine intake increases performance in the 15-s Wingate test during the menstrual cycle. *British Journal of Clinical Pharmacology*, *86*(4), 745–752. https://doi.org/10.1111/bcp.14175

Laske, H., Konjer, M., & Meier, H. E. (2024). Menstruation and training – A quantitative study of (non-)communication about the menstrual cycle in German sports clubs. *International Journal of Sports Science & Coaching*, *19*(1), 129–140. https://doi.org/10.1177/17479541221143061

Matsuda, T., Takahashi, H., Nakamura, M., Ogata, H., Kanno, M., Ishikawa, A., & Sakamaki-Sunaga, M. (2023a). Influence of the Menstrual Cycle on Muscle Glycogen Repletion After Exhaustive Exercise in Eumenorrheic Women. *Journal of Strength and Conditioning Research*, *37*(4), e273–e279. https://doi.org/10.1519/JSC.0000000000004306

Mattu, A. T., Iannetta, D., Doyle-Baker, P. K., & Murias, J. M. (2019). Oxygen Uptake Kinetics During the Different Phases of the Menstrual and Oral Contraceptive Cycles: 2737 Board #3 May 31 1:00 PM - 3:00 PM. *Medicine & Science in Sports & Exercise*, *51*(6S), 763–764. https://doi.org/10.1249/01.mss.0000562777.62803.5a

McNulty, C. R., & Robergs, R. A. (2017a). New Methods for Processing and Quantifying VO2 Kinetics to Steady State: VO2 Onset Kinetics. *Frontiers in Physiology*, *8*, 740. https://doi.org/10.3389/fphys.2017.00740

Mølmen, K. S., Almquist, N. W., & Skattebo, Ø. (2025). Effects of Exercise Training on Mitochondrial and Capillary Growth in Human Skeletal Muscle: A Systematic Review and Meta-Regression. *Sports Medicine*, *55*(1), 115–144. https://doi.org/10.1007/s40279-024-02120-2

Okudan, N., Gökbel, H., Uçok, K., & Baltaci, A. (2005). Serum leptin concentration and anaerobic performance do not change during the menstrual cycle of young females. *Neuro Endocrinology Letters*, *26*(4), 297–300.

Oosthuiyse, T., & Bosch, A. (2009). Influence of menstrual phase on ventilatory response to submaximal exercise. *South African Journal of Sports Medicine*, *18*(2), 31. https://doi.org/10.17159/2413-3108/2006/v18i2a241

Poole, D. C., Burnley, M., Vanhatalo, A., Rossiter, H. B., & Jones, A. M. (2016). Critical Power: An Important Fatigue Threshold in Exercise Physiology. *Medicine and Science in Sports and Exercise*, *48*(11), 2320–2334. https://doi.org/10.1249/MSS.0000000000000939

Poole, D. C., & Jones, A. M. (2012). Oxygen uptake kinetics. *Comprehensive Physiology*, *2*(2), 933–996. https://doi.org/10.1002/cphy.c100072

Raberin, A., Burtscher, J., Citherlet, T., Manferdelli, G., Krumm, B., Bourdillon, N., Antero, J., Rasica, L., Malatesta, D., Brocherie, F., Burtscher, M., & Millet, G. P. (2024). Women at Altitude: Sex-Related Physiological Responses to Exercise in Hypoxia. *Sports Medicine*, *54*(2), 271–287. https://doi.org/10.1007/s40279-023-01954-6

Redman, L. M., Scroop, G. C., & Norman, R. J. (2003). Impact of menstrual cycle phase on the exercise status of young, sedentary women. *European Journal of Applied Physiology*, *90*(5–6), 505–513. https://doi.org/10.1007/s00421-003-0889-0

Reed, B. G., & Carr, B. R. (2000). The Normal Menstrual Cycle and the Control of Ovulation. In K. R. Feingold, R. A. Adler, S. F. Ahmed, B. Anawalt, M. R. Blackman, G. Chrousos, E. Corpas, W. W. de Herder, K. Dhatariya, K. Dungan, E. Hamilton, J. Hofland, S. Jan de Beur, S. Kalra, G. Kaltsas, N. Kapoor, M. Kim, C. Koch, P. Kopp, … D. P. Wilson (Eds.), *Endotext*. MDText.com, Inc. http://www.ncbi.nlm.nih.gov/books/NBK279054/

Sahlin, K. (2014). Muscle energetics during explosive activities and potential effects of nutrition and training. *Sports Medicine*, *44 Suppl 2*(Suppl 2), S167-173. https://doi.org/10.1007/s40279-014-0256-9

Schoeberlein, M. I., Hudgins, J. H., & Wilkins, B. W. (2025b). Impact of Fluctuating Sex Hormones on $\dot{V}O_2$ Kinetics during Heavy and Severe Domain Exercise. *Physiology*, *40*(S1), 0950. https://doi.org/10.1152/physiol.2025.40.S1.0950

Shaharudin, S., Ghosh, A. K., & Ismail, A. A. (2011a). Anaerobic capacity of physically active eumenorrheic females at mid-luteal and mid-follicular phases of ovarian cycle. *The Journal of Sports Medicine and Physical Fitness*, *51*(4), 576–582.

Smith, E. S., McKay, A. K. A., Kuikman, M., Ackerman, K. E., Harris, R., Elliott-Sale, K. J., Stellingwerff, T., & Burke, L. M. (2022). Auditing the Representation of Female Versus Male Athletes in Sports Science and Sports Medicine Research: Evidence-Based Performance Supplements. *Nutrients*, *14*(5), 953. https://doi.org/10.3390/nu14050953

Smith, J. A. B., Murach, K. A., Dyar, K. A., & Zierath, J. R. (2023). Exercise metabolism and adaptation in skeletal muscle. *Nature Reviews. Molecular Cell Biology*, *24*(9), 607–632. https://doi.org/10.1038/s41580-023-00606-x

Taylor, M. Y., Osborne, J. O., Topranin, V. D. M., Engseth, T. P., Solli, G. S., Valsdottir, D., Andersson, E., Øistuen, G. F., Flatby, I., Welde, B., Morseth, B., Haugen, T., Sandbakk, Ø., & Noordhof, D. A. (2024). Menstrual Cycle Phase Has No Influence on Performance-Determining Variables in Endurance-Trained Athletes: The FENDURA Project. *Medicine & Science in Sports & Exercise*, *56*(9), 1595–1605. https://doi.org/10.1249/MSS.0000000000003447

Williams, T. J., & Krahenbuhl, G. S. (1997). Menstrual cycle phase and running economy. *Medicine and Science in Sports and Exercise*, *29*(12), 1609–1618. https://doi.org/10.1097/00005768-199712000-00010

Zając, A., Chalimoniuk, M., Maszczyk, A., Gołaś, A., & Lngfort, J. (2015). Central and Peripheral Fatigue During Resistance Exercise—A Critical Review. *Journal of Human Kinetics*, *49*, 159–169. https://doi.org/10.1515/hukin-2015-0118