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Systematic Review

Influence of Oestradiol Fluctuations in the Menstrual Cycle on Respiratory Exchange Ratio at Different Exercise Intensities: A Systematic Review, Meta-Analysis and Pooled-Data Analysis

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Abstract: Background: Oestradiol has been implicated as a factor in substrate utilisation in male and mouse studies but the effect of acute changes during the menstrual cycle is yet to be fully understood. **Objective:** To determine the role of oestradiol in respiratory exchange ratio (RER) during exercise at various intensities. Methods: This systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. From inception to November 2023, four online databases (Cochrane, SPORTDiscus, MEDline and Web of Science) were searched for relevant articles. Studies that reported a resting oestradiol measurement in naturally menstruating women with exercise at a percentage of maximal aerobic capacity (%$V\dot{O}_{2max}$) were included. Mean and standard deviation for oestradiol, RER and exercise intensity were extracted and study quality assessed using a modified Downs and Black checklist. Risk of bias was assessed using I_2 measure of heterogeneity and Egger's regression test, assessment of bias from methodological quality was identified by sensitivity analysis. Eligible datasets were extracted for pairwise comparisons within a meta-analysis and correlation between change in oestradiol and change in RER. Data were also pooled to produce a mean and standard deviation for RER for menstrual stage and for low and high oestradiol groups. **Results:** Twenty-four articles were identified, over 50% were identified as high quality. Sixteen articles included datasets eligible for meta-analysis. Eleven articles utilised a submaximal constant-load exercise intensity, finding a standardised mean difference of − 0.09 ([CI: −0.35–0.17], $p = 0.5$) suggesting no effect of menstrual phase on constant-load exercise RER. In six articles using incremental exercise tests to exhaustion, a standardised mean difference of 0.60 ([CI 0.00–1.19], $p = 0.05$) was identified towards a higher maximal RER attained in follicular compared to luteal phase. There was no correlation ($R = -0.26$, $p = 0.2$) between change in oestradiol and change in RER between phases. All 24 articles, totalling 650 participants, were included in pooled analysis. When grouped by menstrual cycle phase or when grouped by oestradiol levels, RER was higher in the follicular phase than the luteal phase at low and high constant load exercise intensities. **Discussion:** Findings from the pooled-analysis and meta-analysis suggest that there may be menstrual cycle phase differences in RER that are intensity dependent. These differences may be related to sex hormone levels, but this was not supported by evidence of correlation between differences in RER and differences in oestradiol. At present, it remains best practice to assess performance in the same menstrual cycle phase if seeking to assess change from baseline.

Keywords: sex hormones; metabolism; oestrogen

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1. Introduction

Substrate metabolism is reflected in a respiratory exchange ratio (RER), with an RER of 1 indicating a reliance on carbohydrate and below 1 implying mixed fuelling and/or predominant reliance on fats [1]. Fat is primarily utilised as the substrate for energy metabolism, and at rest, adults typically have an RER below 0.8 [2]. With increasing exercise duration, fat oxidation progressively increases until, at intensities above ~60% maximal aerobic capacity ($V\dot{O}_{2\,max}$), fat oxidation is restricted as fatty acid availability and delivery is limited and becomes insufficient to meet the energy requirements of the exercise [3]. In turn, circulating blood glucose and stored glycogen become the primary energy substrate. The efficient shifting between substrates, based on the demands of exercise, is referred to as metabolic flexibility. Compared to males, females demonstrate a delayed shift, and subsequently later onset of glycogen depletion, meaning females exhibit greater fat oxidation per unit of fat-free mass than males [4–6]. These sex differences in substrate utilisation are not evident until puberty, implying that sex hormones have a significant impact [7]. Moreover, changes in female hormones over the menstrual cycle and over the lifespan [8] may contribute to reduced metabolic flexibility.

The utility of an RER above 1.0 for the quantification of substrate utilisation is limited. Yet maximal RER can facilitate understanding of the contribution of non-oxidative energy systems to adenosine triphosphate (ATP) production. Since RER results from the rate of carbon dioxide production ($V\dot{C}O_2$) against the rate of oxygen consumed ($V\dot{O}_2$), maximum values reflect the rate of systemic metabolic acidosis buffering, which causes the rate of carbon dioxide production to increase disproportionately to the rate of oxygen consumption [9,10]. Consequently, maximal RER is considered to be a determinant of performance during high-intensity exercise that is underpinned by anaerobic power and capacity [11]. The data demonstrating reduced $V\dot{C}O_2$ during maximal exercise in the follicular phase, along with maintained oxygen delivery, indicates potential shifts in energy system contributions to ATP provision towards greater reliance on anaerobic glycolysis. In this case, it may be that buffering of metabolic acidosis occurs at a greater rate in the follicular phase than in the luteal phase [12]. This might be related to the effects of sex hormones on oxidative respiration, as oestrogen and progesterone have been reported to regulate mitochondrial function and enhance mitochondrial respiration in the brain [13] and skeletal muscle [14]. Therefore, when these hormone levels are lower during the follicular phase, there might be a greater reliance on anaerobic ATP production due to reduced efficiency of mitochondrial respiration [13].

The impact of oestradiol on substrate metabolism and on exercise performance determinants throughout the menstrual cycle are debated [15,16], and the effect of chronic oestradiol deficiency, such as in menopause, is unclear. Due to the small number of studies in this field, pooling the results for a larger analysis may support a more comprehensive understanding of the role of oestradiol in substrate utilisation. When blood glucose concentrations are high, the use of glucose as a fuel is prioritized; this glucose homeostasis is reported to be partly mediated by oestrogen whereby estrogen acts to enhance uptake into the muscles and liver [17,18]. Current findings suggest that oestradiol acts to increase the availability of fatty acids and limit blood glucose concentrations in multiple ways: stimulating the use of triglycerides and fatty acids as a primary energy substrate and limiting storage of excess as fat by increasing lipolysis, the conversion of triacylglycerols to fatty acids and glycerol [19]. This increased reliance on fat oxidation delays the onset of glycogen oxidation, termed glycogen sparing, and is thought to be beneficial in prolonged exercise and has been demonstrated in rats with oestradiol supplementation [20]. The proposed mechanism describes oestradiol as exerting effects across the central nervous system, pancreatic β cells, skeletal muscle, liver and white adipose tissue [21,22] via nuclear oestrogen receptors α (ERα) and β (ERβ) surface oestrogen receptors.

Current research explores the impact of menstrual cycle phase on RER [23] but not the direct effect of oestradiol in this relationship in women. Instead, research has assessed the impact of oestradiol supplementation in men, where an increased reliance on lipid

metabolism [24], increased plasma-free fatty acid availability [25], increased muscle triacylglycerol content [25] and a lower RER has been observed [24,25]. Findings have also confirmed an increase in muscle glycogen storage at rest and increased glycogen sparing in exercise during the luteal phase [16]. Therefore, it could be hypothesised that women with higher oestradiol concentrations, usually occurring in the late follicular and luteal phases of the menstrual cycle, would demonstrate acute increases in fat oxidation, and lower RER values during exercise. Current literature exploring the impact of menstrual phase and oestradiol on RER in naturally menstruating women is conflicting and spans multiple exercise intensities. This review allows synthesis and assessment of conflicting findings, for the first time exclusively using data from studies that include hormone concentrations to allow the assessment of differences in maximal RER attained between menstrual cycle phases. Subsequently the objective of this review was to evaluate the impact of both menstrual cycle phase and oestradiol levels on RER with consideration for substrate utilisation.

2. Results

2.1. Literature Search

There were 24 studies identified as eligible for analysis; these studies are detailed in the appendix. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram for literature search, screening and selection is presented below in Figure 1. Notably, three papers were excluded from the database search due to use of salivary or urinary oestradiol [26,27], which has previously been shown to poorly correlate with blood oestradiol [28].

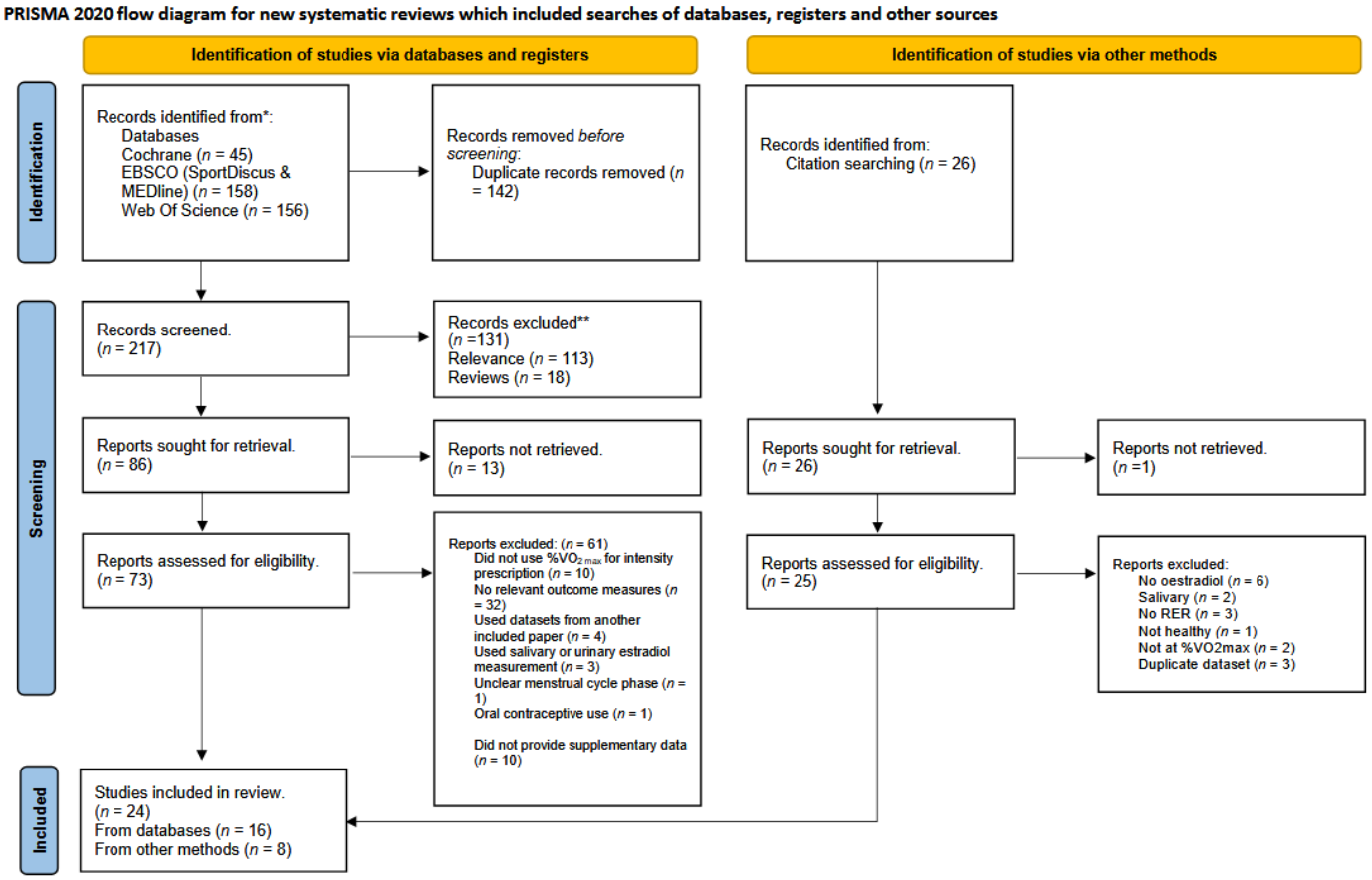


Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram for literature search, screening and selection.

2.2. Study Characteristics and Quality Assessment

Study characteristics are presented in Table 1. Studies spanned 25% to 100% $V\dot{O}_{2max}$ across three modalities including treadmill, cycle ergometer and row ergometer [29–52]. Twenty-two were in active women whilst two were in sedentary women [45,48]. The primary source of bias was failure to use blood hormone concentrations for accurate menstrual cycle phase allocation despite the collection of blood samples. Several studies used basal body temperature, a technique with a high risk of user error [53]. The luteal phase was rarely assessed with consideration for phases such as early, mid- or late luteal, whilst the follicular phase was often split into early and late without required precision or understanding of varying cycle lengths, most often relying on days since menses. Quality assessment was completed using the adapted Downs and Black checklist [54] which resulted in the assessment of 8% of studies as very low quality, 8% as low, 25% as moderate and 58% as high (Figure 2). Three studies conducted a power analysis to determine sufficient power [35,40,43]. All studies except one [44] accounted for at least one of the following: prior nutritional intake, prior exercise and/or time of day.

Table 1. Description of included studies, menstrual cycle confirmation methods, and eligible number of participants included in this analysis. LH refers to luteinizing hormone surge, $V\dot{O}_{2max}$ refers to aerobic capacity, RER refers to respiratory exchange ratio, and yrs refers to years.

Author	Eligible Sample (*n*)	Phase and Number (*n*)	Menstrual Cycle Verification	Exercise Type	Population	Standardised Mean Difference in RER [CI]
Ashley et al., 2000 [29]	10	Follicular (n = 10) Luteal (n = 10)	- Menstrual cycle history - Basal body temperature changes **Early to mid-follicular:** 4–7 days after the onset of menses **Luteal:** 7–12 days after ovulation	60 min at 70% $V\dot{O}_{2max}$ (treadmill)	Physically active regularly and naturally menstruating women (23 ± 5 yrs)	0.64 [−0.26–1.54]
Beidleman et al., 2002 [30]	8	Follicular (n = 8) Luteal (n = 8)	- Blood progesterone analysis **Early follicular:** 3–6 days after onset of menses **Midluteal:** 6–9 days after ovulation.	70% $V\dot{O}_{2max}$ until exhaustion (no average time specified) (cycle ergometer)	Healthy physically active naturally and regularly menstruating women (33 ± 3 yrs)	−0.47 [−1.47–0.52]
Bemben et al., 1992 [31]	8	Luteal (n = 8)	- Basal body temperature changes - Blood progesterone analysis **Luteal:** 3–10 days after ovulation	50% of $V\dot{O}_{2max}$ for 90 min (cycle ergometer)	Healthy moderately active naturally menstruating or oral contraceptive users (26 ± 1 yrs)	No comparator
Braun et al., 2000 [32]	15	Follicular (n = 15) Luteal (n = 15)	- Menstrual cycle history - Urinary LH surge **Follicular:** day 1 **Luteal:** day after ovulation	50% of $V\dot{O}_{2max}$ (cycle ergometer)	Healthy active women with regular menstrual cycles (22 ± 1 yrs)	0.00 [−0.72–0.72]
Casazza et al., 2002 [33]	8	Early follicular (n = 8) Midluteal (n = 8)	- Urinary LH surge **Early follicular:** 4–8 days after onset of menses **Midluteal:** 17–25 after ovulation.	Incremental test to exhaustion (cycle ergometer)	Healthy physically active naturally and regularly menstruating women (26 ± 2 yrs)	−0.66 [−1.67–0.35]
De Souza et al., 1990 [34]	8	Follicular (n = 8) Luteal (n = 8)	- Urinary LH surge **Follicular:** days 2–4 after onset of menses **Luteal:** 2–8 days after ovulation.	$V\dot{O}_{2max}$ and 80% for 40 min (treadmill)	Healthy naturally regularly menstruating trained women (running over 35 miles per week) (29 ± 4 yrs)	0.33 [0.66–1.32] 0.57 [−0.43–1.57]

Table 1. *Cont.*

Author	Eligible Sample (*n*)	Phase and Number (*n*)	Menstrual Cycle Verification	Exercise Type	Population	Standardised Mean Difference in RER [CI]
Dean et al., 2003 [35]	10	Early-follicular (n = 10) Mid-follicular (n = 10) Mid-luteal (n = 10)	- Basal body temperature changes - Blood hormone analysis **Early follicular:** days 2 to 6 after onset of menses **Mid-follicular:** day 7 ± 11 after onset of menses. **Mid-luteal:** day 19 to 23 following onset of menses	Incremental test to exhaustion (cycle ergometer)	Naturally menstruating active women (30–60 min aerobic exercise 3–6 days a week) (28 ± 5 yrs)	0.95 [0.09–1.98]
Devries et al., 2006 [36]	7	Follicular (n = 7) Luteal (n = 7)	- Menstrual cycle history - Urinary LH surge - Blood hormone analysis **Mid-follicular:** 8–10 days after the onset of menses **Luteal:** day 19–21 after the onset of menses	90-min at 65% $V\dot{O}_{2max}$ (cycle ergometer)	Healthy recreationally active naturally menstruating or oral contraceptive users (22 ± 2 yrs)	0.06 [−0.70–0.45]
Galliven et al., 1997 [37]	8	Follicular (n = 8) Midluteal (n = 8)	- Blood hormone analysis **Follicular:** 3–9 days after onset of menses **Midluteal:** 18–26 days after onset of menses	70% $V\dot{O}_{2max}$ for 15 min (treadmill)	Healthy, low to moderate fitness, and regularly menstruating women (29 ± 1 yrs)	0.48 [−0.46–1.41]
Horton et al., 2002 [38]	11	Early-follicular (n = 10) Mid-follicular (n = 11) Mid-luteal (n = 10)	- Menstrual cycle history and symptoms - Urinary LH surge **Early follicular:** days 1 to 4 after onset of menses. **Mid-Follicular:** days 8–11 after onset of menses **Luteal days:** 19–23 after onset of menses	90 min at 50% $V\dot{O}_{2max}$ (cycle ergometer)	Naturally regularly menstruating active (over 90 min per week of aerobic exercise) women (29 ± 5 yrs)	0.96 [0.03–1.88]
Horton et al., 2006 [39]	10	Luteal (n = 10)	- Blood hormone analysis **Luteal:** 19–23 days after onset of menses	50% $V\dot{O}_{2max}$ for 90 min (cycle ergometer)	Healthy physically active and regularly menstruating women (34 ± 6 yrs)	No comparator
Isacco et al., 2015 [40]	10	Midluteal (n = 10)	- Blood hormone analysis **Mid-luteal:** 18–22 days after onset of menses	Incremental test to exhaustion (cycle ergometer)	Healthy physically active regularly menstruating women (23 ± 4 yrs)	No comparator
Kanaley et al., 1992 [41]	13	Follicular (n = 7) Late follicular (n = 7) Mid-luteal(n = 7)	- Basal body temperature changes - Urinary LH surge **Early follicular:** days 3–5 after onset of menses **Late follicular**: days 14–16 after onset of menses **Mid-luteal phases:** days 22–25 after onset of menses	90-min at 60% $V\dot{O}_{2max}$ (cycle ergometer)	Naturally menstruating athletes (running over 35 miles per week) (25 ± 1 yrs)	No standard deviation
Kraemer et al., 2013 [42]	5	Follicular (n = 5) Luteal (n = 5)	- Menstrual cycle history and symptoms **Early follicular:** days 3–7 after onset of menses **Mid-luteal:** days 20–22 following onset of menses	90-min at 60% $V\dot{O}_{2max}$ (cycle ergometer)	Healthy naturally menstruating women able to complete 90 min of aerobic exercise (24 ± 5 yrs)	No standard deviation

Table 1. *Cont.*

Author	Eligible Sample (*n*)	Phase and Number (*n*)	Menstrual Cycle Verification	Exercise Type	Population	Standardised Mean Difference in RER [CI]
Lebrun et al., 1995 [43]	16	Follicular (n = 16) Luteal (n = 16)	- Menstrual cycle history and symptoms - Basal body temperature changes - Blood progesterone analysis **Early follicular:** days 3–8 after onset of menses **Mid-luteal:** days 4–9 after ovulation	Incremental test to exhaustion (treadmill)	Healthy naturally regularly menstruating trained women ($V\dot{O}_{2max}$ over 50 mL/kg/min) (28 ± 4 yrs)	1.95 [1.11–2.79]
Lebrun et al., 2003 [44]	14	Follicular (n = 7) Luteal (n = 7) Follicular (n = 7) Luteal (n = 7)	- Blood progesterone analysis **Early follicular:** days 3–8 after onset of menses **Mid-luteal:** days 4–9 after ovulation.	Incremental test to exhaustion (treadmill)	Healthy trained women (V˙O2 max over 50 mL/kg/min) naturally regularly menstruating followed by oral contraceptive or placebo (18–40 yrs)	No standard deviation
Oosthuyse et al., 2005 [45]	13	**Untrained:** Early follicular (n = 8) Late follicular(n = 7) Mid-luteal(n = 7) **Trained:** Early follicular (n = 5) Late follicular (n = 4) Mid-luteal (n = 4)	- Basal body temperature changes - Urinary LH surge - Blood hormone analysis. **Early follicular:** 2–7 days after the onset of menses **Late follicular:** 2 days before a positive LH result to the day of a positive LH reading **Mid-luteal:** 4–10 days following ovulation.	Time trials of ~41 min at approximately 70–75% $V\dot{O}_{2max}$ (cycle ergometer)	Healthy naturally & regularly menstruating untrained & trained (5.4 ± 2.3 h aerobic per week) (Untrained: 23 ± 3 yrs Trained: 24 ± 3 yrs)	00.32 [−0.71–1.33] 0.30 [−1.03–1.62]
Rael et al., 2021a [46]	21	Follicular (n = 21) Luteal (n = 21)	- Menstrual cycle history and symptoms - Urinary LH surge - Blood hormone analysis **Follicular:** 2–5 days after onset of menses **Luteal:** within 3 days of ovulation.	8 bouts at 3 min at 85% of $V\dot{O}_{2max}$ (treadmill)	Healthy naturally regularly menstruating women with 3–12 h of endurance training a week (31 ± 7 yrs)	−0.18 [−0.78–0.43]
Rael et al., 2021b [47]	47	Early follicular (n = 47)	- Blood hormone analysis **Follicular:** 2–5 days after onset of menses	Incremental test to exhaustion (treadmill)	Healthy physically active and regularly menstruating women (33 ± 5 yrs)	No comparator
Redman et al., 2003 [48]	14	Follicular (n = 14) Luteal (n = 14)	- Menstrual cycle recording - Urinary LH surge - Blood progesterone analysis **Early to mid-follicular:** 5–7 days after onset of menses **Mid-luteal:** days 21–23	Incremental test to exhaustion (cycle ergometer) & 25% and 75% of $V\dot{O}_{2max}$ for 20 min (cycle ergometer)	Healthy naturally and regularly menstruating sedentary women (21 ± 4 yrs)	No standard deviation
Smekal et al., 2007 [49]	19	Follicular (n = 19) Midluteal (n = 19)	- Menstrual cycle history - Basal body temperature changes **Follicular:** 8–10 days after onset of menses **Midluteal:** 23–27 days after onset of menses	Incremental test to exhaustion (cycle ergometer)	Healthy physically active naturally and regularly menstruating women (27 ± 2 yrs)	−0.03 [−0.66–0.61]

Table 1. *Cont.*

Author	Eligible Sample (*n*)	Phase and Number (*n*)	Menstrual Cycle Verification	Exercise Type	Population	Standardised Mean Difference in RER [CI]
Suh et al., 2003 [50]	12	Follicular ($n = 7$) Luteal ($n = 5$)	- Urinary LH surge **Follicular:** Days 3–9 following onset of menses **Luteal:** 18–24 days following onset of menses.	45 and 65% $V \cdot O_{2max}$ for 60 min (cycle ergometer)	Healthy naturally regularly menstruating moderately active women (2–6 h exercise per week) (FP: 25 ± 1 yrs LP: 26 ± 2 yrs)	0.00 [−1.15–1.15] 0.00 [−1.15–1.15]
Vaiksaar et al., 2010 [51]	11	Follicular ($n = 11$) Luteal ($n = 11$)	- Blood hormone analysis **Follicular:** 7–11 days after onset of menses **Midluteal:** 18–22 days after onset of menses	70% $V \cdot O_{2max}$ for 60 min (rowing ergometer)	Healthy physically active and regularly menstruating women (18 ± 2 yrs)	−0.70 [−1.56–0.16]
Vaiksaar et al., 2011 [52]	15	Competitive follicular ($n = 8$) Competitive luteal ($n = 8$) Recreational follicular ($n = 7$) Recreational luteal ($n = 7$)	- Menstrual cycle history - Blood hormone analysis **Follicular:** 5–11 days after onset of menses **Midluteal:** 18–22 days after onset of menses	Incremental test to exhaustion (rowing ergometer)	Healthy recreational or competitive and regularly menstruating women (Competitive: 19 ± 2 yrs Recreational: (18 ± 1 yrs)	−0.27 [−1.25–0.71] 0.08 [−0.97–1.13]

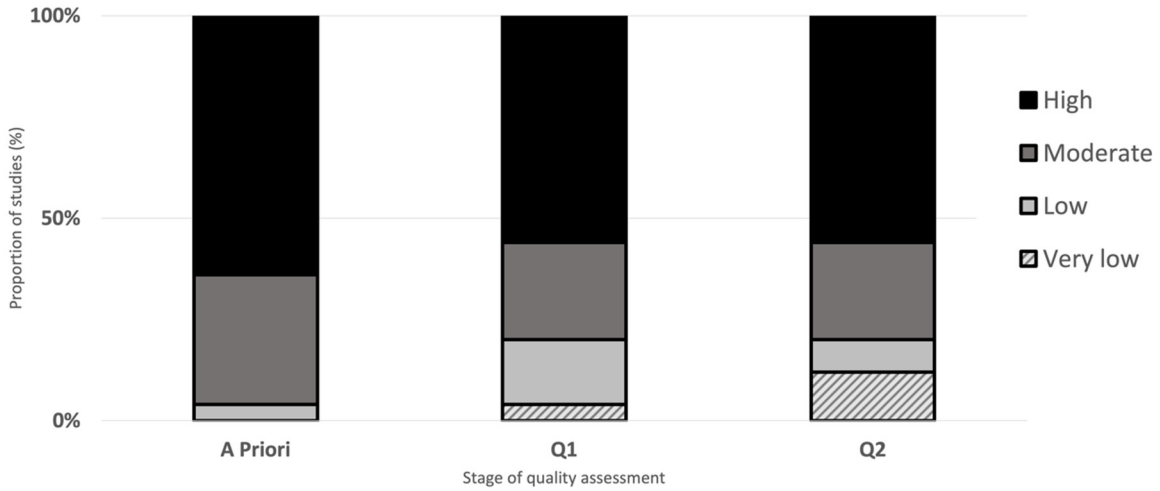
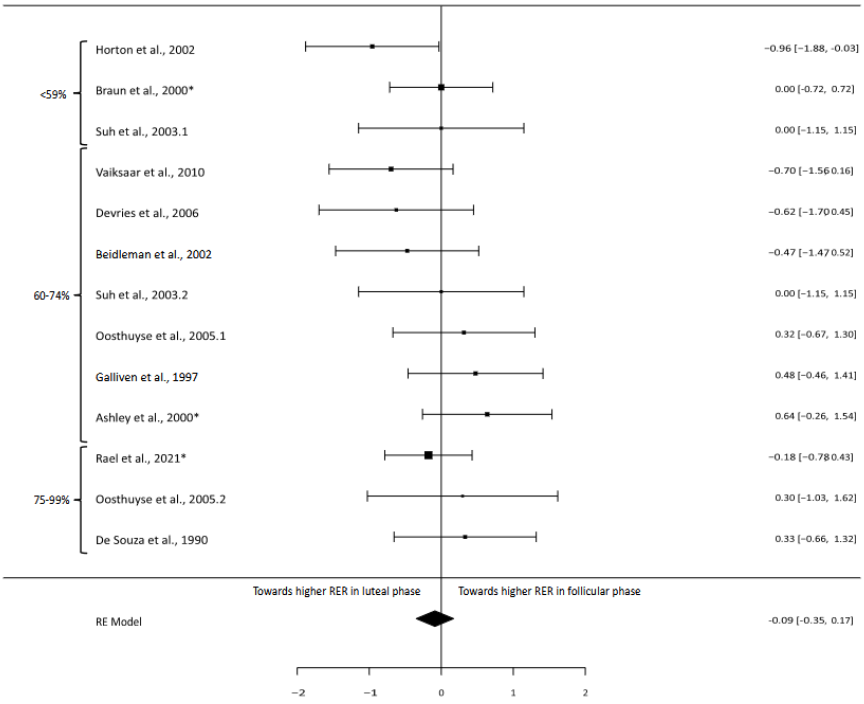


Figure 2. Quality of studies included in review and analysis ($n = 24$).

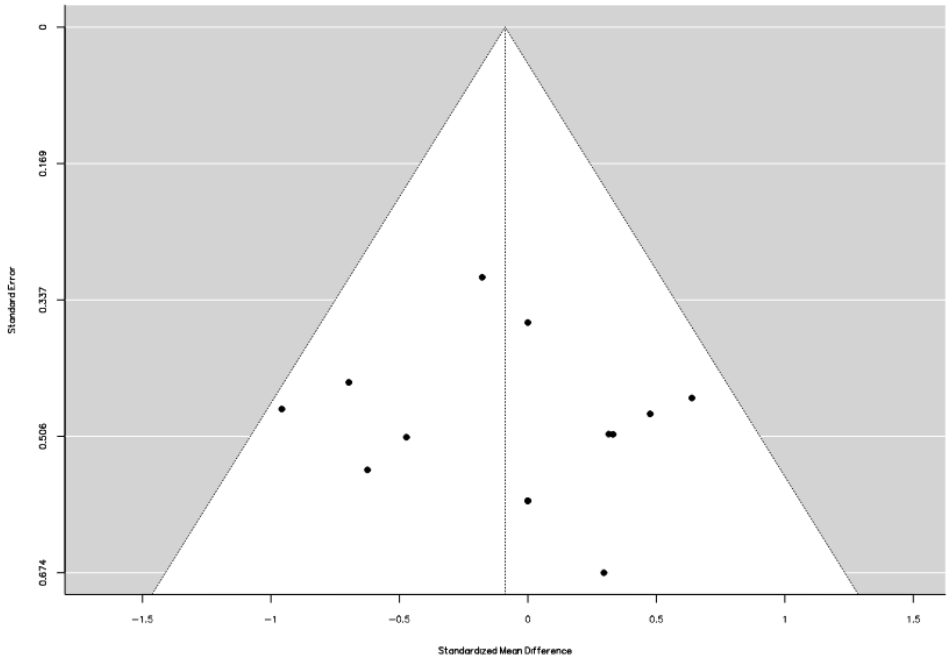
A sensitivity analysis was conducted whereby low-quality studies were removed, as these were most likely to incorrectly verify phase, or sedentary groups were removed, as fitness level has a significant influence on RER [45,48]. The full dataset for extraction and quality assessment details is available in Tables S1 and S2.

2.3. Pairwise Meta-Analysis of Phase Differences

Of the 24 articles identified, 16 were eligible for meta-analysis due to the provision of data from the early-mid follicular phase compared to the early-mid luteal phase. Where studies had produced comparisons between the menstrual cycle phases, these were included as paired datasets. Where studies included multiple paired datasets, these are numbered. Studies were included if means and standard deviation for RER for both early to mid-follicular and mid-luteal phases of the menstrual cycle had been provided. Therefore, the total of number of participants for low, moderate and high intensity constant load exercise were 36 [32,38,50], 56 [29,30,36,37,45,50,51] and 20 respectively [34,45,46] (Figure 3). For incremental exercise, 76 [33–35,44,49,51] participants were included (Figure 4).



(**a**)



(**b**)

Figure 3. (**a**) Forest plot of meta-analysis comparison of respiratory exchange ratio (RER) across all exercise intensities between early-mid follicular and early to mid-luteal phases up to 99%$V\dot{O}_{2max}$. Squares indicate the weight of standardized mean difference and 95% confidence intervals [CI]. Negative effect sizes indicate a higher RER observed in the luteal phase, and positive effect sizes indicate a higher RER observed in the follicular phase. * denotes studies removed for sensitivity analysis. Brackets indicate exercise intensity as a %$V\dot{O}_{2max}$. (**b**) Funnel plot of studies comparing respiratory exchange ratio between menstrual cycle phases with standardised mean difference plotted against standard error (13 paired datasets) [29,30,32,34,36–38,45,46,50,51].

The analysis of 11 submaximal exercise studies provided a minimal mean effect size (ES = −0.09 [CI: −0.35–0.17], $p = 0.5$). There was also evidence of low heterogeneity between studies ($I^2 = 3.58\%$, $p = 0.4$), highlighting little variation between studies (Figure 4). Eggers regression test did not suggest a significant effect of publication bias (Egger = 0.37, $p = 0.71$).

Strict criteria for inclusion in the review, such as blood hormone measurement, did not eliminate low or very low-quality studies. A sensitivity analysis was conducted whereby low-quality studies were removed, of which there were three [29,32,46] Compared to the primary analysis, the I^2 was marginally increased to 14.8% and the mean effect size by −0.08. To exclude the impact of physical activity level, sedentary groups were removed [45], which did not alter heterogeneity meaningfully ($I^2 = 9.94\%$, $p = 0.32$) or size of effect (ES −0.14 [−0.42–0.15], $p = 0.38$).

When grouped to assess the role of exercise intensity, at intensities below 99% $V\dot{O}_{2max}$ the variance was reduced to suggest homogeneity at < 59%, 60–74%, 75–99% of VO_{2max} ($I^2 = 32.12\%$, $I^2 = 27.69\%$, $I^2 = 0\%$ respectively), implying that low-quality methodology was not the determinant of variance, but exercise intensity might be (Table 2). In moderate to high intensity (60–99% $V\dot{O}_{2max}$) the effect size was notably reduced, as was the heterogeneity suggesting lower exercise intensity is a factor in the variability. Of the 16 studies identified, six involved exercise at 100% $V\dot{O}_{2max.}$ There were no low-quality studies in the sample, but variance remained high. The homogeneity of the high intensity sample is likely related to the low number of studies included and can be seen in Table 2.

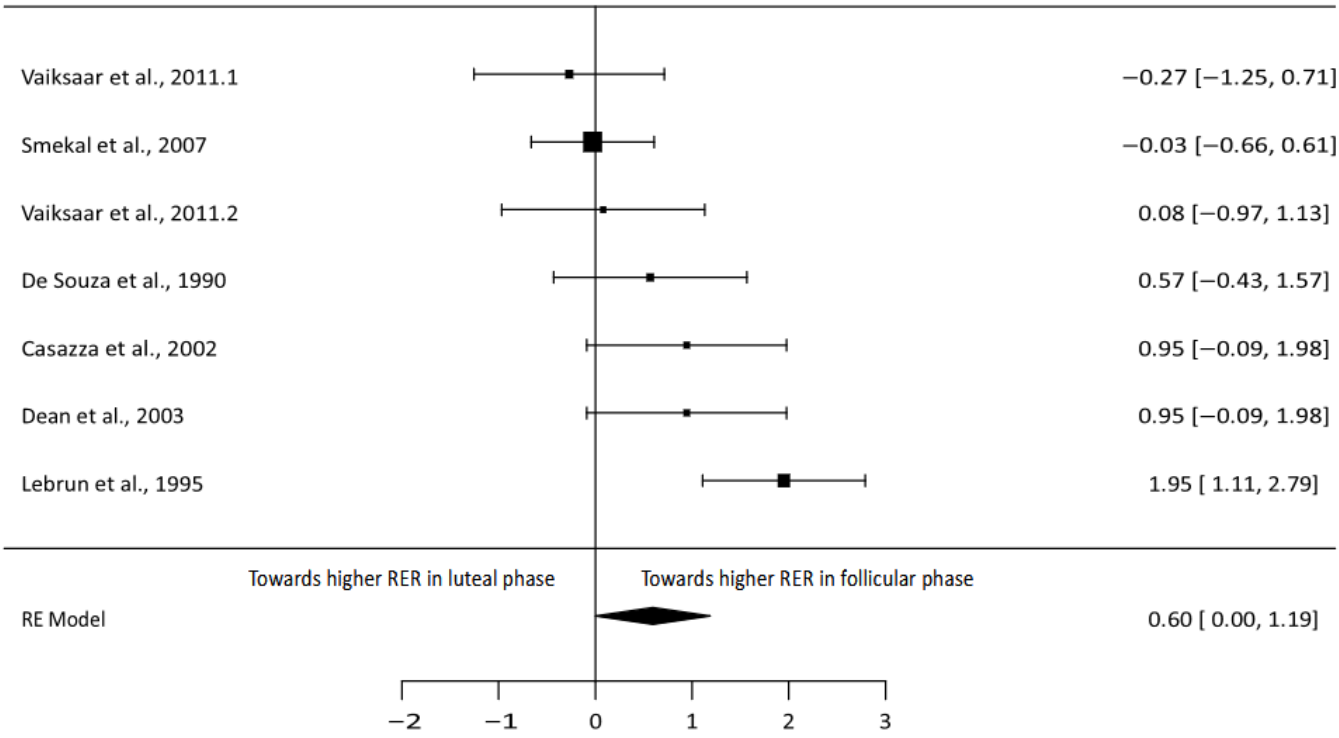


Figure 4. Forest plot of meta-analysis comparison of respiratory exchange ratio (RER) between early-mid follicular and early to mid-luteal phases at 100%$V\dot{O}_{2max}$. Squares indicate the weight of standardized mean difference and 95% confidence intervals [CI]. Negative effect sizes indicate a higher RER observed in the luteal phase, and positive effect sizes indicate higher RER observed in the follicular phase [33–35,43,49,52].

Table 2. Effect size, confidence intervals and I^2 for each intensity group, * indicates significance at $p < 0.05$ and ** at $p < 0.01$.

Intensity (%$\dot{V}O_{2max}$)	Standardised Mean Difference	Confidence Intervals	I^2
>59%	−0.31	−0.94–0.33	32.12%
60–74%	−0.05	−0.48–0.39	27.69%
75–99%	0.01	−0.48–0.49	0%
100%	0.60 *	0.00–1.19	65.65% **

Inclusive of datasets that did not provide standard deviations, 26 were identified for correlation analysis. When paired datasets were plotted based on change between phases (Δ = luteal phase – follicular phase) (Figure 5), there was no evidence of correlation between greater changes in oestradiol between menstrual cycle phases and a greater change in RER ($R = -0.26$, $p = 0.2$, $R^2 = 0.06$).

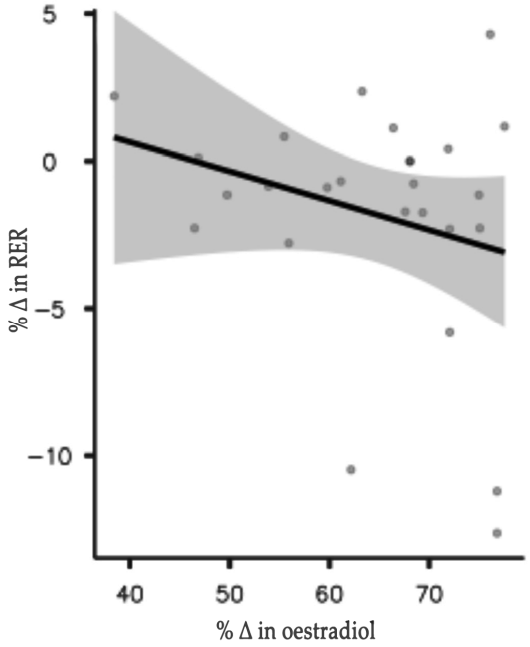


Figure 5. Correlation between percent change in oestradiol and percent change in respiratory exchange ratio ($n = 20$ studies).

2.4. Pooled-Analysis

When data from all 24 articles were pooled to assess this effect with the largest possible sample, differences in RER were highlighted between menstrual cycle phases and oestradiol groups at low and high-intensity exercise. The oestradiol ranges of the studies involved for menstrual cycle phases are included in Table 3.

Table 3. Oestradiol levels and ranges for each menstrual phase including the participant numbers (*n*) for each sample.

Menstrual Cycle Phase	Average Oestradiol (pmol/L)	Range (pmol/L)	*n*
Early to mid-follicular	154.4 ± 60.4	86–312	327
Late follicular	728.1 ± 494.9	662–1009	39
Luteal	432.4 ± 265.2	203–823	284

Significant differences in oestradiol were observed between all phases ($p < 0.001$; Table 3), with oestradiol significantly higher in the luteal and late follicular phase than in the early to mid-follicular phase. There were insufficient studies and participant numbers to include late follicular as a phase comparison group; thus, early to mid-follicular

phase participant groups were compared to early to mid-luteal groups across the four exercise intensities. However, late follicular groups were included in the oestradiol comparison groups.

There were significant differences in constant load RER between menstrual cycle phases ($p < 0.001$, Table 4(a)) and between the defined oestradiol groups ($p < 0.001$, Table 4(b)) indicating a higher RER in the follicular phase and low oestradiol group. Post-hoc comparisons evidenced significant differences in RER at low (0.03, $p < 0.001$) and high (0.04, $p < 0.001$) constant load exercise intensities.

Table 4. Grouped respiratory exchange ratio and oestradiol for each analysis (a) by reported phase (b) by high/low oestradiol groups inclusive of late follicular ($n = 39$, of which 22 were allocated to high oestradiol and 17 to low oestradiol). Presented as mean ± SD. p value indicates post-hoc multiple comparisons between groups at each exercise intensity. RER refers to respiratory exchange ratio, $\mathbf{V\dot{}O_{2\,max}}$ to aerobic capacity.

(a)		**Follicular (n = 327)**			**Luteal (n = 284)**				
	Intensity (%$V\dot{}O_{2\,max}$)	**RER**	**Oestradiol**	***n***	**RER**	**Oestradiol**	***n***	***p***	***Difference***
Constant load	Low (25–59%)	0.90 ± 0.01	149.12 ± 42.91	57	0.87 ± 0.01	435.68 ± 237.75	63	<0.001	0.03
	Moderate (60–74%)	0.89 ± 0.04	150.76 ± 38.78	72	0.89 ± 0.03	412.97 ± 267.66	69	0.99	0.00
	High (75–99%)	0.97 ± 0.04	129.06 ± 41.91	48	0.93 ± 0.06	483.34 ± 219.47	47	<0.001	0.04
Incremental	Max (100%)	1.18 ± 0.19	166.31 ± 76.72	15	1.16 ± 0.06	419.75 ± 119.63	105	0.22	0.02
(b)		**Low (n = 344)**			**High (n = 306)**				
	Intensity (%$V\dot{}O_{2\,max}$)	**RER**	**Oestradiol**	***n***	**RER**	**Oestradiol**	***n***	***p***	***Difference***
Constant load	Low (25–59%)	0.90 ± 0.01	149.12 ± 42.91	57	0.87 ± 0.01	435.68 ± 237.75	63	<0.001	0.03
	Moderate (60–74%)	0.89 ± 0.03	152.44 ± 62.29	89	0.88 ± 0.03	499.84 ± 284.03	59	0.23	0.01
	High (75–99%)	0.97 ± 0.04	129.06 ± 41.91	48	0.94 ± 0.05	588.33 ± 384.08	79	<0.001	0.03
Incremental	Max (100%)	1.18 ± 0.19	164.13 ± 75.30	15	1.16 ± 0.06	422.85 ± 129.94	105	0.30	0.02

3. Discussion

This work provides novel insight through combined data analysis of evidence examining the impact of oestradiol and menstrual cycle phase on RER, building on previous work seeking to elucidate the effect of the menstrual cycle on performance variables [23,55,56]. The impact of menstrual cycle phase on RER in women has been debated with a number of studies reporting no effect [30,33–35,37,38,40–46,49,50,52], some studies reporting a lower RER in the luteal phase [29,32,48] and others reporting a lower RER in the follicular phase [31,36,51]. In this review, at various intensities RER was demonstrated to be greater when oestradiol concentrations were low. This was evidenced at low and high submaximal exercise intensities in the pooled-analysis and at maximum in an incremental exercise test in the meta-analysis.

From the limited high-quality research published to date, this meta-analysis of 16 datasets demonstrated no effect of menstrual cycle phase on RER during constant load exercise at any submaximal intensity. From incremental exercise tests, a moderate effect towards higher maximal RER in the follicular phase compared to the luteal phase was evidenced. Subsequent results from the pooled data analysis revealed differences in RER during low and high-intensity constant load exercise between the early to mid-luteal phase and the early to mid-follicular phase, and between the low and high oestradiol group. This same effect was not evidenced in moderate constant load exercise or at maximum in an incremental exercise test. Subsequently, the effects of the menstrual cycle on exercise during submaximal exercise are minimal; however, the pooled-analysis does present differences of up to 0.04 in RER between phases. As illustrated by Goedeke et al. [2], changes of 0.02 to 0.04 between 0.70 and 0.94 RER reflect changes in fat oxidation of up to 13% which is likely to have significant implications for fuelling in extended duration events. Whilst the meta-analysis utilised 20 paired datasets from 14 studies to assess change between phases in smaller samples, the pooled-analysis of 24 studies provided a cross-sectional comparison between phases utilising larger samples. The use of these two methods allows a robust exploration of the data, and taken together, these analyses provide findings that the relationship between menstrual cycle phase and RER is intensity-dependent.

Exercise intensity did appear to have an impact on the size of effect of the menstrual cycle phase on RER in both meta-analyses and pooled-analyses. Results from pooled-analyses present that RER is higher in the follicular phase than the luteal phase at exercise intensities below 60% $V\dot{O}_{2max.}$ Since oestradiol enhances beta-oxidation of fatty acids and subsequently promotes fat oxidation [24], lower levels of oestradiol in the follicular phase may result in greater reliance on glucose metabolism at lower intensities of exercise. Both phases demonstrated similar RER at moderate intensities (60–74% $V\dot{O}_{2max}$), in agreement with findings from Hackney et al. [27], Zderic et al. [57] and D'Souza et al. [23], implying that during exercise requiring predominantly mixed fuelling, sex hormones exert limited impact. This lack of difference, likely due to metabolic requirements for mixed fuelling, is reflected in minimal differences in the pooled-analysis and no effect in the meta-analysis. At high intensities (75–99%), exercise involves mixed substrate utilisation with a greater contribution of glucose utilisation, but once again RER may be higher in the follicular phase which may indicate an earlier reliance on anaerobic metabolism due to the likelihood of reaching an RER of 1 earlier [58] and a more pronounced shift towards reliance on carbohydrates reflected by greater RER values [59]. These findings at low and high intensities contradict the effect evidenced in meta-analyses, where it appeared that there were no differences in RER or that RER was lower in the follicular phase than the luteal phase. Whilst using paired datasets in a meta-analysis allows the most robust assessment of the effect of menstrual cycle phase, the meta-analyses of low and high intensities included only three studies, with a total participant number of 36 and 20 respectively. This limits the possibility of drawing conclusions from these effect sizes due to the likelihood of high individual variability not being accounted for by such small samples. It is also likely these studies were underpowered as few provided a power calculation to justify sample size (Electronic Supplementary Material Table S1). Comparatively, in the pooled analyses, a greater number of participants could be utilised (Table 4) but the results must be considered with caution due to the possibility of a type 1 error.

As suggested here, the effect of oestrogen on substrate utilisation has been reported to be exercise intensity and duration dependent, specifically achieving intensities high enough to elicit glucose utilisation [57] as at 90% $V\dot{O}_{2max,}$ lower carbohydrate oxidation is related to higher resting oestradiol concentrations [60]. This is echoed by the RER differences between phases (Table 4(a)) and between high and low oestradiol groups (Table 4(b)) at high intensities highlighted in this review, with higher oestradiol concentrations in the luteal phase equating to lower RER reflecting a lower carbohydrate dependence. If this is the case, there would be a potential improvement in the performance of longer-duration exercise in the luteal phase, due to increased lipid metabolism [55,61] resulting in a delayed onset of glycogen depletion, yet, this effect has not been seen in performance data [55,56], which authors suggest may relate to a number of confounding variables in addition to low methodological quality. Similarly, the difference in RER at intensities below 60% $V\dot{O}_{2max}$ does not seem to be reflected in a change in point of maximal fat oxidation [62], suggesting acute changes in oestrogen and progesterone do not alter metabolic flexibility meaningfully enough to have an impact on performance in either phase. It could be expected that when oestradiol is lower, this point of maximal fat oxidation would occur at lower intensities and therefore would be detrimental in long-duration events [63]. Yet, the point of maximal fat oxidation has been reported to be the same for mid-luteal, mid-follicular and late-follicular phases, between 47 and 54% of $V\dot{O}_{2max}$, and with no differences in fat oxidation between menstrual cycle phases in a graded exercise test [5]. This is at the lower end of the spectrum of intensity reported by Purdom et al. [64]. Compared to men, women have higher fat oxidation relative to fat-free mass under the age of 45 but not over 45 [5]. This change corresponds with the point at which oestrogen declines [65], and as such it may be expected that when oestradiol is acutely lower, women may have lower fat oxidation compared to high oestrogen phases. Based on Frandsen et al.'s [62] finding, this effect is not evidenced with acutely lower oestradiol during the menstrual cycle. Equally, findings ascertained from correlation analysis produced no correlation between change in oestradiol and change

in RER, suggesting that the magnitude of change in oestradiol between menstrual cycle phases does not equate to a larger change in RER. However, the effects were not supported by the meta-analysis, therefore should be interpreted with caution. Subsequently, further comparisons between menstrual cycle phases are required, utilising constant load exercise to assess the impact of menstrual phase on fat oxidation, as recent research has identified an association between the luteal phase and an increased reliance on fat [15].

Whilst the use of RER does not enable the assessment of substrate utilisation at maximal intensities, it does reflect the supply of ATP provided by non-oxidative energy systems [66]. There was a standardised mean difference in favour of higher maximal RER in incremental exercise in the follicular phase, with the analysis indicating a moderate effect size but paired with substantial heterogeneity. The greater RER reflects a greater maximal $V^{\cdot}CO_2$ in relation to $V^{\cdot}O_2$ and implies an altered metabolic response to maximal exercise. Elevated $V^{\cdot}CO_2$ at high intensities reflects an increased rate of hydrogen (H^+) buffering by bicarbonate ions (HCO_3^-), resulting in the formation of carbonic acid (H_2CO_3). Carbonic acid is ultimately expired by the pulmonary system as water (H_2O) and Carbon dioxide (CO_2) [67,68]. This phase effect has previously been noted; Takano and Kaneda [69] observed a reduction in arterial bicarbonate ion concentration in the luteal phase. During exercise, this would result in a lesser buffering capacity, and therefore, a lower maximal RER. This has implications in athletic populations as recent research has evidenced a large majority of athletes reaching $V^{\cdot}O_{2max}$ across various running distance events [70]. In fact, it has been reported that women spend a larger amount of training time in high-intensity zones than men [71]. Therefore, should the menstrual cycle have an effect on maximum RER as suggested by this meta-analysis, this implies an altered metabolic response to exercise during training and competition. In both untrained and trained women, a variety of different running workouts will also achieve intensities at or above 95%VO_{2max} such as tempo runs or maximal intervals [72,73]. Therefore, if the menstrual cycle is influential on maximal RER, future research studies may need to consider its impact on the outcome variables of interest and account for menstrual cycle phase. This difference in RER between phases is not reflected by a significant difference in pooled-analyses whereby the mean maximal RER in the follicular phase was 1.18 compared to 1.16 in the luteal phase (Table 4(a)). Therefore, whether this potential greater shift in anaerobic metabolism due to phase is present and of a magnitude to alter high-intensity exercise performance is unclear [56].

Aside from intensity, other influential factors in this relationship could be differing design methodologies, such as the inclusion of a variety of exercise modalities or differences in exercise duration, and participant characteristics such as training status. Training status has been highlighted to alter RER, with those who are more trained exhibiting a greater capacity to oxidise fat at higher intensities [64]. Due to the low number of studies for analysis this review included all participants regardless of activity levels, however the majority of the studies were in trained participants with only two studies involving sedentary participants [45,48]. In naturally menstruating women, Olenick et al. [74] suggested a greater effect of training status than menstrual cycle phase on substrate utilisation, whereby trained females exhibited greater fat oxidation during high-intensity intervals compared to low-activity females. The authors identified that high-activity females exhibited slightly higher fat oxidation during the luteal phase [74], although this was not significant. To confirm menstrual cycle phase, authors used urinary ovulation test strips, which limits the evaluation of the impact of hormonal profiles on substrate utilisation. Sensitivity analysis did not suggest that sedentary groups altered the effect size, but this only involved the removal of one study; more research is required to assess the difference in the effect of acute changes in hormones in the menstrual cycle on substrate utilisation between trained and untrained women.

3.1. Limitations Addressed

This review demonstrated that the impact of the menstrual cycle on RER during submaximal constant load exercise exists at low and high intensities, with a higher RER

in the follicular phase than the luteal phase. The lack of supporting findings from the meta-analysis are likely rooted in low study numbers resulting in studies with larger effect sizes biasing the effect. This review highlights a crucial issue in menstrual cycle research; whilst research value and rigour are provided by paired datasets that include adequate phase verification, these high-quality data sets are less common. Therefore, the pooled analysis in this study allows for the maximal involvement of eligible populations in studies and enables the investigator to account for the weighting of effect from small sample sizes. Variable research design and methodology within menstrual cycle research has been previously reported as problematic by McNulty et al. [56]. Often poor control of menstrual cycle phase, such as lack of consideration of cycle length and phase length [27] accounts for this variability. For example, there are considerable changes in oestradiol throughout the menstrual cycle, and the length of each phase is highly individual, which makes calendar counting alone unreliable. For example, days eight to 11 of the menstrual cycle are commonly referred to as late follicular [49,51,52] but if verified with blood hormone testing can be included as early to mid-follicular, hence the use of both phase and hormone analysis in the pooled-analyses. The studies included within this analysis resulted in a wide range of days included as follicular and luteal, due to the inclusion of blood hormone analysis in most studies. These menstrual cycle phase methodologies are likely to impact the relationship between RER and menstrual cycle phase or oestradiol, hence analysis by reported oestradiol concentrations (Table 4(b)) in addition to reported phase (Table 4(a)). Despite including only studies with hormone testing and being the first study to exclude for lack of blood sex hormone testing, there was considerable variability in oestradiol levels in the luteal phase, as shown by standard deviation, highlighting a greater need for more refined identification of the luteal phase into early, mid and late luteal due to the extreme variability of oestradiol in this phase.

3.2. Implications for Practice

Notably, most research collects data in the early follicular phase and the mid-luteal phase, coinciding with points where both hormones are low or both hormones are high. To better evaluate the relationship, more research is required in the late follicular phase where oestradiol is highest, and progesterone is lowest. It is also important to stress the individual variability in peak oestradiol concentrations and therefore the use of relative change in oestradiol is potentially more informative than absolute change which may also mitigate some of the methodological challenges of identifying menstrual cycle phase accurately [15]. This could also be facilitated by use of the oestradiol to progesterone ratio to evaluate hormone influence on RER [15] as the limited impact of oestradiol on RER, despite previous findings in males and mouse models [17,24,25], indicates that an additional factor may mediate this relationship and this could be the oestradiol antagonist, progesterone. Therefore, assessment of the individual variability of this ratio in relation to RER may address the questions raised in this review. This review also highlights the importance and lack of blood hormone testing; many studies still rely on methods such as days counting, urinary luteinising hormone surges or salivary oestradiol over blood oestradiol, limiting the methodological quality of the research [75] and the investigation into how the individuality of hormone concentrations affects exercise physiology.

4. Materials and Methods

This systematic review and analysis were conducted and reported based on the PRISMA statement guidelines [76] and registered protocol (CRD42024524091).

4.1. Eligibility Criteria

The PICOS method was utilised to enable study formulation and search for the research question.

Population: Studies included recruited participants who were healthy women with no age, activity level or fitness restrictions. Participants were required to be naturally menstruating with no menstrual irregularities such as amenorrhea, or use of hormonal contraceptives.

Intervention: A specific intervention was not required; however, the study was required to have at least one group of healthy naturally menstruating women with measured resting blood oestradiol levels, performance of constant load exercise at a percentage of maximal oxygen consumption ($V\dot{O}_{2\,max}$) and reporting of a mean RER, or a maximal incremental exercise test reporting maximal RER. For studies that had used interval exercise the mean RER did not include resting RER. Studies were excluded if they used salivary or urinary oestradiol, did not have a sample of women, or used other measures of exercise intensity such as heart rate reserve or maximum heart rate.

Comparator: Studies must have included menstrual cycle stage and categorised phases at minimum as follicular or luteal. Studies must have also detailed the resting oestradiol concentrations of these groups. Classification of menstrual cycle was based on reporting in the original studies, which was as wide as follicular (days one to 16) and luteal (days 16 to 28), each study's method of assessment of menstrual cycle phase is detailed in Table 1 and reflected in quality assessment.

Outcome: Mean or maximal respiratory exchange ratio determined by measurement of expired gases via indirect calorimetry.

Study design: Cross-sectional, repeated measures, randomised controlled trials and cross-over studies were included. Reviews or meta-analyses, case studies, and conference abstracts were excluded.

4.2. Search Strategy

Four online databases were searched from inception to November 2023 to identify relevant articles (Cochrane, SPORTDiscus, MEDline and Web of Science). The following search terms were used in all databases: (oestrogen OR estrogen OR estradiol OR oestradiol) AND (substrate oxidation OR substrate utilisation OR substrate metabolism OR fat oxidation OR carbohydrate oxidation OR metabolic flexibility OR metabolic efficiency OR respiratory exchange ratio OR RER or Respiratory Quotient) AND exercise.

Selection of studies and data extraction were conducted manually by CR; records were excluded if the title did not demonstrate any relevance to the desired outcomes or study population, such as male participants or non-human populations. Citations of these eligible papers were verified for other studies which may be relevant. Where data were incomplete or presented in graphical format, supplementary data were sought by contacting authors. In instances where data were not provided, where possible, data were extracted using ImageJ software (V1.54, National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, Madison, WI, USA) [77]. The primary outcome variable was RER, with oestradiol and menstrual cycle phase defined as the influencing variables.

4.3. Quality Assessment, Data Synthesis and Analysis

The quality assessment of studies was completed by two reviewers (CR, LB), following independent assessments, any disputes were solved by re-assessment of the paper by both reviewers collaboratively. Quality assessment was completed using the modified Downs and Black checklist [54,75] which categorises studies as high, moderate, low or very low quality following three stages, using an a-priori rating followed by two question stages to assess the quality of menstrual cycle assessment.

Data extraction Data were extracted from studies whereby each group of participants and condition in a study was treated as independent. Mean RER for each exercise intensity, low (<59% $V\dot{O}_{2\,max}$), moderate (60–74% $V\dot{O}_{2\,max}$) and high (75–99% $V\dot{O}_{2\,max}$) was extracted. In maximal incremental exercise tests, maximal RER was extracted. These were taken alongside a resting oestradiol measurement. All oestradiol results were converted to pmol/L. Where data were extracted from figures for RER, the standard deviation

was not extractable, and therefore, was not included in the meta-analysis or pooled standard deviation.

Meta-analysis: A maximum likelihood random-effects meta-analysis model using continuous variables was performed as the primary analysis using eligible paired datasets due to the likelihood of high heterogeneity. Funnel plots were performed to assess individual and mean effect size and error. This was followed by the removal of studies identified for sensitivity analysis (The Jamovi project 2022, Jamovi, Version 2.3, https://www.jamovi.org, accessed 27 March 2024). Sub-group analyses were performed with different exercise intensities. Heterogeneity was assessed by I^2 whereby >30% was considered moderate heterogeneity and >50% to be substantial (Cochrane handbook, Version 6.4, 2023). Effect sizes were evaluated based on Cohen [78] with 0.2 as minimal, 0.5 as moderate and 0.8 as large. *n* indicates the number of studies included. Finally, correlation coefficients were interpreted as described by Schober et al. [79], <0.40 as weak, 0.40 to 0.69 as moderate, and >0.69 as strong.

Pooled-data analysis: Data were grouped into two menstrual cycle phases as reported by the authors (follicular or luteal) and then subsequently reallocated into two oestradiol levels (low or high). Grouping by oestradiol was based upon reference ranges with >275 pmol/L indicative of ovulation, and therefore, high oestradiol, while <275 pmol/L was categorised as low oestradiol [80]. These data were combined using the Cochrane Handbook formula for grouped mean and standard deviation (Cochrane Handbook, Version 6.4, 2023). Pooled standard deviation was completed inclusive and exclusive of missing values with minimal difference. Data were normally distributed, as determined by Shapiro-Wilk test for normality, and steady-state data were analysed using two-way analysis of variance (ANOVA) to assess the impact of both menstrual cycle phase or oestradiol level and the exercise intensity, where significance was present, Sidak's multiple comparisons test was utilised post hoc. For incremental exercise test data, an unpaired t-test was utilised (GraphPad Prism, 9.5.0, GraphPad Software, Boston, MA, USA).

5. Conclusions

This review evaluated the concept that in the follicular phase, when oestradiol is acutely low, women may exhibit a higher respiratory exchange ratio (RER) and a tendency toward greater carbohydrate oxidation, similar to findings in male and mouse studies. The data demonstrated that in exercise at constant load intensities below maximal, menstrual phase affects RER at low and high, but not moderate, intensities. There may also be a greater shift towards anaerobic metabolism in an incremental exercise test in the follicular phase compared to the luteal phase, evidenced by differences in RER at maximum, suggesting alterations in the metabolic response to maximal exercise that result in greater rates of carbon dioxide production. It is important to acknowledge the possible effects when measuring changes in cardiopulmonary function over time, for example, based on the present data, it appears important to match the hormonal status of participants when assessing cardiopulmonary responses to an intervention. More research should seek to elucidate the effects of the relationship between oestradiol and its protagonist, progesterone, on substrate utilisation and RER in women and this research should be of appropriate quality to confirm the impact of menstrual cycle phase on RER.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/physiologia4040033/s1. Supplementary Materials inclusive of quality assessment (Electronic Supplementary Material Table S1) and data extraction (Electronic Supplementary Material Table S2) are attached.

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