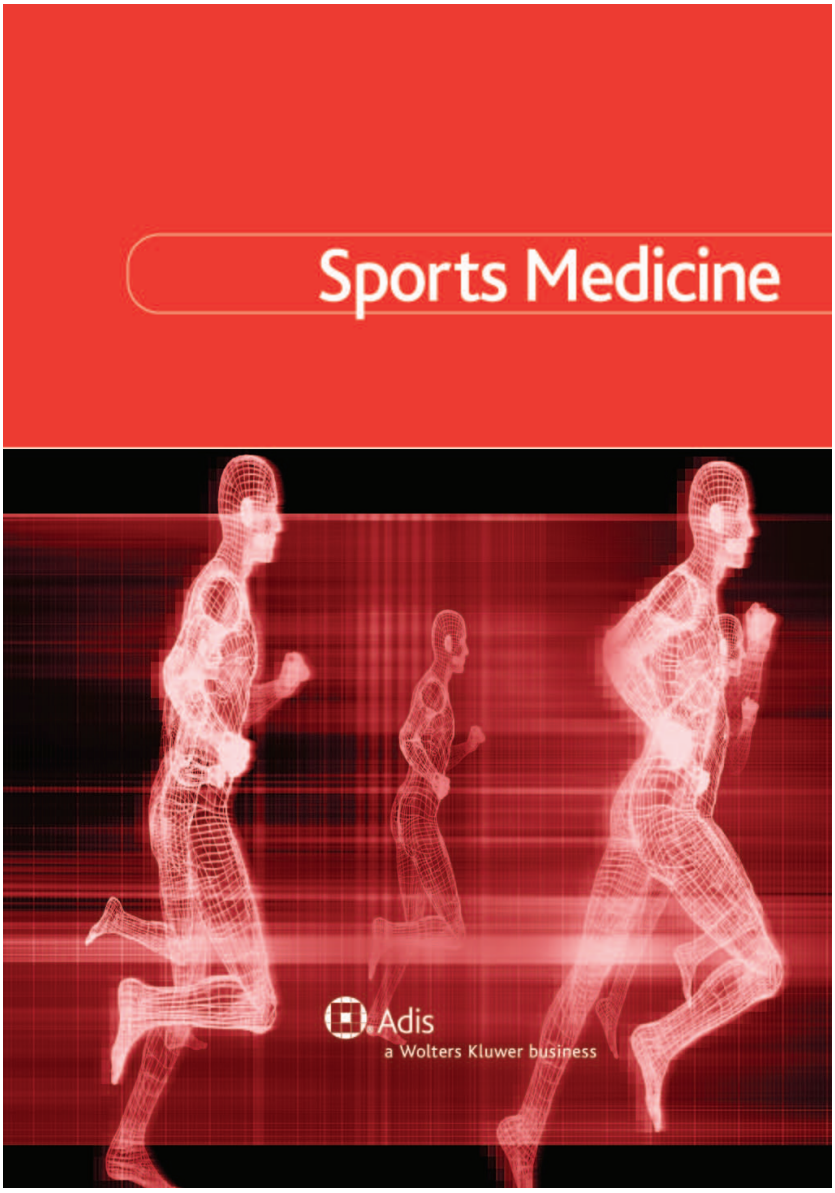

REVIEW ARTICLE

Sports Med 2010; 40 (3): 207-227
0112-1642/10/0003-0207/$49.95/0

The Effect of the Menstrual Cycle on Exercise Metabolism

Implications for Exercise Performance in Eumenorrhoeic Women

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Abstract

The female hormones, oestrogen and progesterone, fluctuate predictably across the menstrual cycle in naturally cycling eumenorrhoeic women. Other than reproductive function, these hormones influence many other physiological systems, and their action during exercise may have implications for exercise performance. Although a number of studies have found exercise performance – and in particular, endurance performance – to vary between menstrual phases, there is an equal number of such studies reporting no differences. However, a comparison of the increase in the oestrogen concentration (E) relative to progesterone concentration (P) as the E/P ratio (pmol/nmol) in the luteal phase in these studies reveals that endurance performance may only be improved in the mid-luteal phase compared with the early

follicular phase when the E/P ratio is high in the mid-luteal phase. Furthermore, the late follicular phase, characterized by the pre-ovulatory surge in oestrogen and suppressed progesterone concentrations, tends to promote improved performance in a cycling time trial and future studies should include this menstrual phase. Menstrual phase variations in endurance performance may largely be a consequence of changes to exercise metabolism stimulated by the fluctuations in ovarian hormone concentrations. The literature suggests that oestrogen may promote endurance performance by altering carbohydrate, fat and protein metabolism, with progesterone often appearing to act antagonistically. Details of the ovarian hormone influences on the metabolism of these macronutrients are no longer only limited to evidence from animal research and indirect calorimetry but have been verified by substrate kinetics determined with stable tracer methodology in eumenorrhoeic women. This review thoroughly examines the metabolic perturbations induced by the ovarian hormones and, by detailed comparison, proposes reasons for many of the inconsistent reports in menstrual phase comparative research. Often the magnitude of increase in the ovarian hormones between menstrual phases and the E/P ratio appear to be important factors determining an effect on metabolism. However, energy demand and nutritional status may be confounding variables, particularly in carbohydrate metabolism. The review specifically considers how changes in metabolic responses due to the ovarian hormones may influence exercise performance. For example, oestrogen promotes glucose availability and uptake into type I muscle fibres providing the fuel of choice during short duration exercise; an action that can be inhibited by progesterone. A high oestrogen concentration in the luteal phase augments muscle glycogen storage capacity compared with the low oestrogen environment of the early follicular phase. However, following a carbo-loading diet will super-compensate muscle glycogen stores in the early follicular phase to values attained in the luteal phase. Oestrogen concentrations of the luteal phase reduce reliance on muscle glycogen during exercise and although not as yet supported by human tracer studies, oestrogen increases free fatty acid availability and oxidative capacity in exercise, favouring endurance performance. Evidence of oestrogen's stimulation of 5′-AMP-activated protein kinase may explain many of the metabolic actions of oestrogen. However, both oestrogen and progesterone suppress gluconeogenic output during exercise and this may compromise performance in the latter stages of ultra-long events if energy replacement supplements are inadequate. Moreover, supplementing energy intake during exercise with protein may be more relevant when progesterone concentration is elevated compared with menstrual phases favouring a higher relative oestrogen concentration, as progesterone promotes protein catabolism while oestrogen suppresses protein catabolism. Furthermore, prospective research ideas for furthering the understanding of the impact of the menstrual cycle on metabolism and exercise performance are highlighted.

In many fields of physiology, sex is considered to be a variable that should be 'controlled for'. Therefore, men and women are expected to respond differently to various interventions or conditions. Often, sample groups are restricted to including only men, possibly because male physiology remains relatively consistent from day to day. Conversely, women between the ages of

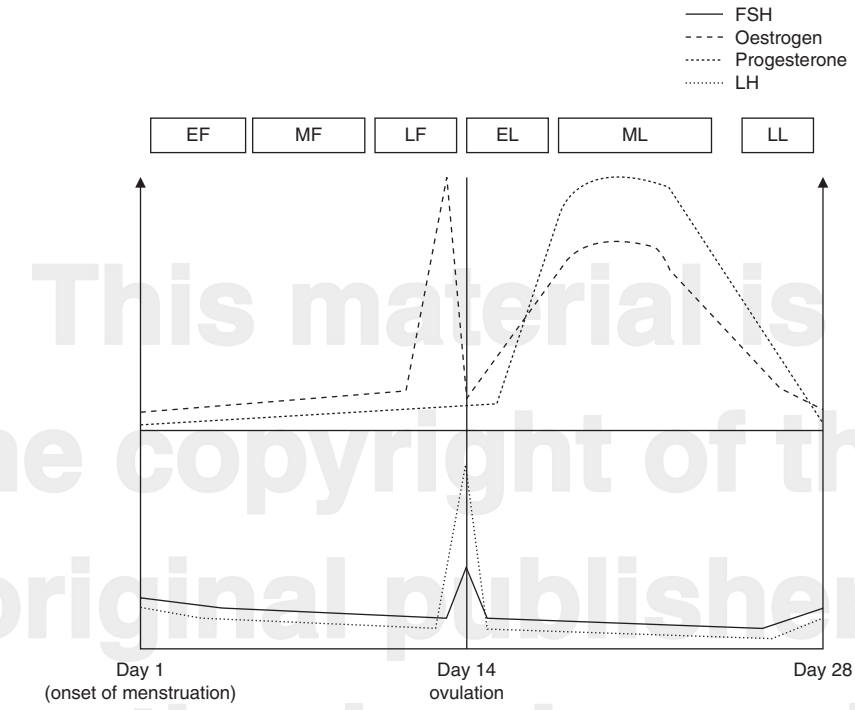

Fig. 1. Diagrammatic representation of the cyclical changes in the female sex hormones that characterize the various menstrual phases. 17β-Oestradiol is the primary oestrogen secreted, but may be metabolized further to form oestrone and oestriol, which are less potent oestrogens.[2] The luteinizing hormone (LH) surge commences 36 hours before ovulation occurs.[3] **EF** = early follicular; **EL** = early luteal; **FSH** = follicle-stimulating hormone; **LF** = late follicular; **LL** = late luteal; **MF** = mid follicular; **ML** = mid luteal.

approximately 13 and 50 years experience a circamensal rhythm termed the menstrual cycle, where the ovarian hormones fluctuate predictably over, on average, 23–38 days.[1] The ovarian hormones, oestrogen and progesterone, are secreted from the ovaries and to a lesser extent from the adrenal glands in women.[2] Although these hormones primarily function to support reproduction, they have been reported to influence other physiological systems. For this reason, studies have been conducted to compare established responses in men with the response in women. However, in these studies women are studied mostly only during the early stages of their menstrual cycle when the ovarian hormones are considered to be at their lowest, so as to avoid the 'moving target' scenario.

Women, however, function and compete in sporting events at all stages of the menstrual cycle. Therefore, some researchers have endeavoured to compare physiological responses in women between identified phases of the menstrual cycle, corresponding to accepted concentration ranges for the ovarian hormones (figure 1). The menstrual cycle is broadly divided into two phases – the follicular phase (FP) and the luteal phase (LP) – which are separated by ovulation. The system involved in the regulation thereof is termed the hypothalamic-pituitary-ovarian axis, and is thoroughly reviewed by Reilly[1] and Birch.[4]

Unfortunately, this field of research is plagued with many inconsistent findings, but it is possible that most inconsistencies can be solved by a closer examination of the hormone interactions.

Part one of this review considers studies that have compared exercise performance between menstrual phases. Part two presents a detailed review of the effects of the ovarian hormones (as they naturally occur during the various menstrual

phases) on substrate metabolism during exercise at submaximal intensities. Although the ovarian hormones are known to influence other physiological systems (such as the respiratory, thermoregulatory, and cardiovascular systems, and even muscle satellite cell activation), these fall outside the scope of the current review.

1. Effects of the Ovarian Hormones on Exercise Performance

1.1 Short Duration or Maximal Exercise Intensities

The maximum oxygen consumption ($\dot{V}O_{2max}$) and time to exhaustion in maximal ramp tests are mostly unchanged by menstrual phase.[5-14] However, there is one report of a 2% lower $\dot{V}O_{2max}$ in the mid-luteal (ML) phase compared with the early follicular (EF) phase[14] and another in which a 13% decrease in $\dot{V}O_{2max}$ after 4 months of oral contraceptive use was reported.[10] Conversely, altitude dwellers tended to have a higher $\dot{V}O_{2max}$ ($p = 0.06$) in the LP compared with the FP,[15] possibly due to the increased respiratory drive in the LP, which may facilitate a slightly higher oxygen saturation.[9] However, such findings are not found in women who are acutely exposed to altitude.[9] Nonetheless, it may be worthwhile to consider the potential for the progesterone-induced increase in respiratory drive in the LP to benefit maximal exercise at high altitudes in well-acclimatized athletic eumenorrhoeic women.

Conversely, others have reported that the higher respiratory drive in the LP jeopardizes maximal exercise performance in non-athletes due to the increased sensation of dyspnoea,[12] although the increased respiratory drive did not influence maximal performance in athletes.[12] In this regard, exercise-induced bronchoconstriction in asthmatic athletes is more severe during the ML phase compared with the mid-follicular (MF) phase following an incremental ramp test to exhaustion.[16] However, a consistently higher respiratory rate throughout 90 minutes of submaximal exercise in the ML phase compared with the EF phase has been found to not increase metabolic demand, and therefore should not influence rate of fatigue.[17]

It appears fairly consistent from curve-fitting methods that the exercise intensity that induces the point of inflection corresponding to either the lactate or ventilatory thresholds remains unchanged by menstrual phase.[7,11-13,18] However, one study has found that the ventilatory threshold occurs at a higher percentage of $\dot{V}O_{2max}$ in the EF phase compared with the late follicular (LF) and ML phases.[6] Moreover, Forsyth et al.[18] found that the intensity corresponding to 4 mmol/L lactate threshold was higher in the LP than FP. Similarly, others,[5,19-21] but not all,[6-8,22-25] have reported lower blood lactate concentrations during exercise in the LP compared with FP, thus suggesting the potential for a decreased blood lactate accumulation during exercise and hence, by implication, lower anaerobic glycolysis in the LP.

Performances in all-out sprints and in measures of muscle strength have been found to be best during menstruation.[26-28] However, others have found no differences in a Wingate performance test between menstruation and LP[29] or in 10-second sprints between MF and ML phases.[30]

In summary, menstrual phase has been found only occasionally to influence maximal aerobic or anaerobic performances. However, various physiological changes (such as respiratory drive) associated with the ovarian hormones, other than simply alterations to metabolism, may influence exercise at such high intensities.

1.2 Submaximal Exercise Intensities

1.2.1 Time to Exhaustion

Low dose oestrogen supplementation to ovariectomized rats has been shown to improve time to exhaustion in a prolonged submaximal treadmill run by 20% compared with sham injected rats.[31] Endurance time continued to improve with increasing oestrogen dose, resulting in up to a 42% improvement when oestrogen was increased within physiological concentrations compared with sham-injected controls,[31] and 50% improvements with a supraphysiological dose of oestrogen.[31] These massive improvements in endurance capacity coincided with glycogen-sparing

Table I. Relative changes in the ovarian hormones between the follicular (FP) and luteal phase (LP) in relation to submaximal endurance performance

Magnitude of increase in oestrogen in LP above FP	E/P in LP	Result	Reference
Time to exhaustion at submaximal intensity			
2.28-fold	12.3	NS; EF vs ML	33
2.87-fold	8	NS; EF vs ML	9
2-fold	21.3	$p < 0.02$; ML > MF	5
3.85-fold	18	$p < 0.07$; ML > MF	23
Time trial performance			
2.3-fold	5.5	$p < 0.05$ MF faster than ML without CHO supplement	22
2.5-fold	6	NS; MF vs ML with CHO supplement	22
1.4-fold	9.7	NS; MF vs ML following a normal or CHO-loading diet	34
4-fold	18.5	NS; EF vs ML but tendency for LF faster than EF ($p = 0.027$)	35

CHO = carbohydrate; **EF** = early follicular; **E/P** = oestrogen to progesterone ratio; **LF** = late follicular phase; **MF** = mid-follicular phase; **ML** = mid-luteal phase; **NS** = not significant.

in the red and white vastus muscle, myocardium and liver.[31]

However, time to exhaustion at submaximal exercise intensity is a measure of endurance capacity rather than a direct measure of exercise performance,[32] although it can provide an indication of an athlete's potential for endurance events. Such protocols do not have a high reproducibility, and studies have reported coefficients of variation as high as 30% when using these tests[32] thus reducing the statistical power of comparison between interventions, in this case between menstrual phases. Nonetheless, in humans, two studies have reported an effect of menstrual phase on endurance capacity. The first study found that following 40 minutes of submaximal cycling at low to moderate intensities, time to exhaustion at 90% of maximum power output was doubled in the ML phase compared with the MF phase.[5] This coincided with lower blood lactate levels in the ML phase.[5] The second study had a smaller sample size ($n = 6$); possibly because of this, the (on average) 10% longer time to exhaustion at 70% $\dot{V}O_{2max}$ in the ML phase compared with the MF phase did not quite reach significance ($p < 0.07$).[23] Conversely, two further studies also compared time to exhaustion at 70% $\dot{V}O_{2max}$ and did not find any difference between the EF and ML phase,[9,33] with or without carbohydrate supplements during exercise.[33]

While all these studies demonstrated a 2-fold or greater increase in oestrogen from the follicular to luteal phase, the oestrogen to progesterone concentration ratio (E/P; pmol/nmol) differed noticeably. Studies reporting a better performance in the LP had a higher E/P ratio, while the studies that found no change in endurance time had a lower E/P ratio (table I). This observation implies that the higher relative progesterone concentration in the latter studies impeded the metabolic benefits of oestrogen that may have been more prominent during the LP of the former studies.

However, 6 days of transdermal oestrogen supplementation in amenorrhoeic women failed to alter time to exhaustion at 85% $\dot{V}O_{2max}$ that was preceded by 90 minutes of submaximal running.[36] In this study though, the transdermal oestrogen supplement resulted in only modest increases in circulating oestrogen, to levels typically experienced in the early to mid-FP. Furthermore, while the duration of oestrogen exposure was sufficient to lower glucose kinetics, it may have been too short to produce certain other oestrogen effects, such as muscle glycogen-sparing during exercise.[37]

1.2.2 Time Trial Performance

Exercise protocols with a fixed endpoint – such as time to complete a given distance, or to expend a given amount of energy or distance

covered in a fixed time period etc. – are a good measure of exercise performance, having high-test-retest reproducibility as described by a low coefficient of variability (1–3%).[32,38]

Three studies have measured time trial performance between menstrual phases.[22,34,35] Campbell et al.[22] compared the time to expend a given amount of energy after completing a 2-hour submaximal session at 70% $\dot{V}O_{2max}$ in the MF and ML phase with and without carbohydrate supplements in overnight fasted subjects. They observed a 13% improvement in time trial performance in the MF phase without carbohydrate supplementation during exercise.[22] This better MF performance was associated with higher carbohydrate use and whole body rate of glucose appearance (hepatic glucose production) and rate of disappearance (or glucose uptake), suggesting a better capacity for carbohydrate use in the MF phase.[22]

An increased capacity for carbohydrate utilization is beneficial in short duration time trial events that take place at high intensities. This observation of better time trials in the MF phase[22] coincides well with another study from the same authors who found oestrogen to promote contraction-stimulated glucose uptake and hepatic glycogenolysis during exercise in ovariectomized rats during a short, high intensity run, while progesterone antagonized these responses.[39] The pre-exercise MF phase average oestrogen concentration was relatively high (360 pmol/L)[22] and hence oestrogen may have promoted glucose uptake into muscles during these trials. In addition, despite a 2.5-fold increase in oestrogen in the ML phase over the MF phase, the E/P ratio in the ML phase was comparatively low (5–6) (table I).[22] Thus, the relatively high progesterone concentration during the trials in the ML phase may have countered the benefits of an elevated oestrogen concentration and produced a worse performance. However, the use of carbohydrate supplements during exercise elevated the glucose rate of appearance, disappearance and plasma glucose use, providing sufficient fuel-of-choice to promote an optimal performance in a short duration, high intensity time trial, regardless of menstrual phase.[22]

McLay et al.[34] also compared cycling time trial performance (over 16 km) in the MF and ML phase after a lengthy submaximal exercise period (75 minutes). These authors found no difference in finishing time between menstrual phases when subjects participated following 3 days of either a normal mixed diet or a carbohydrate-loading diet. Unfortunately, the authors allowed the subjects to view their power output throughout the time trial, and this may have been a potential shortfall as subjects would have been able to consciously regulate their exercise intensity to match their previous time trial. Moreover, the group average oestrogen concentration increased by a meagre 1.4-fold more from the MF to ML phase, while the average progesterone concentration in the ML phase was substantial, resulting in an E/P ratio of only 9.7 (table I),[34] thus possibly partly explaining the lack of difference in performances.

A study was also performed in our laboratory to assess cycling time trial performance during the EF, LF and ML phase of the menstrual cycle.[35] The inclusion of the LF phase in this comparison is a novel contribution to the literature and is motivated by the many oestrogen-induced metabolic effects (see section 2) that should promote performance in such an event. In our subjects who participated in a non-fasted state, time trial performance was also not significantly different between the EF and ML phase.[35] However, we observed a strong tendency for better performance in the LF phase compared with the EF phase ($p = 0.027$), but this did not quite reach significance with Bonferroni correction applied for three multiple comparisons.[35] Nevertheless, the results suggest a positive influence of oestrogen on performance in such events. Conversely, the coincident increase of progesterone in the ML phase may have antagonized the benefits of an elevated oestrogen concentration despite a high E/P ratio (18.5) in the ML phase and average 4-fold increase in oestrogen. However, subjects in this study[35] and the study by McLay et al.[34] participated roughly 2 hours postprandially, which may have alleviated the metabolic demand that is thought necessary to potentiate ovarian hormone influences,

particularly regarding carbohydrate metabolism. Nonetheless, evidence of oestrogen's capacity to promote cycling time trial performance without progesterone antagonizm was still evident in the LF phase in the study by Oosthuyse et al.,[35] despite subjects exercising 2 hours postprandially.

In summary, only eight studies in total have considered menstrual phase variations in endurance exercise performance. Of the eight studies, four have reported menstrual cycle variations. Thus, the potential for naturally cycling ovarian hormones to alter performance can neither be excluded nor confirmed. However, strong evidence from animal research, specifically for oestrogen-induced promotion of better endurance capacity, and the various menstrual phase-associated metabolic perturbations discussed in part two of this review should provide motivation for further endurance performance studies. Future studies should consider the increase in oestrogen relative to progesterone in the LP and the absolute magnitude of increase in oestrogen between any two menstrual phases. Furthermore, all exercise trials to date have been limited to <2 hours in duration. The metabolic influences of oestrogen in promoting fat use and spare glycogen stores should best support performances in ultra-endurance events. Thus, future studies should investigate menstrual phase variations in ultra-endurance events and consider including the LF phase, which coincides with maximal increases in oestrogen independent of changes in progesterone.

2. Ovarian Hormones and Metabolism

Both oestrogen and progesterone are reported to alter metabolic responses. However, in this respect, progesterone displays largely anti-oestrogenic effects.[39-41] D'Eon et al.[42] have proposed that a metabolic response to changes in the ovarian hormones occurs only when the E/P ratio is sufficiently elevated and the magnitude of the increase in oestrogen from the EF to the phase of comparison such as LF or ML is at least in the order of 2-fold more. Nutritional status is also a determining variable, since most variations in metabolism between menstrual phases are reported when subjects participate in a study following an overnight fast, whereas a positive nutritional state may lessen the impact of the ovarian hormones.[22]

2.1 Carbohydrate Metabolism

2.1.1 Stable Isotopic Measures of Systemic Glucose Kinetics in Eumenorrhoeic Women

In a fasted state, glucose rate of appearance (Ra) is solely determined by endogenous glucose production, which is predominantly controlled by hepatic gluconeogenesis and glycogenolysis. Glucose rate of disappearance (Rd) is dependent on insulin-mediated glucose uptake and contraction-mediated glucose transport, with the latter predominating during exercise. Glucose Ra and Rd are naturally related to each other and primarily influenced by the rate of glucose utilization.[25]

A number of studies have found that the Ra and Rd of glucose during exercise is attenuated by either therapeutic increases in circulating oestrogen[36,37,42,43] or with the coincident rise in oestrogen and progesterone during the ML phase of the menstrual cycle versus the EF phase.[19,22,44] Therefore, the ovarian hormone-induced decrease in glucose kinetics is most likely an oestrogen-associated effect and is one that progesterone does not antagonize but may in fact potentiate.[42]

Glucose Ra was dependent on hepatic glucose production in all of the above studies, as the subjects did not receive any form of exogenous glucose during exercise and participated following an overnight fast (besides D'Eon et al.,[42] where differences in glucose kinetics did not quite reach significance). Therefore, the ovarian hormone-induced reduction in glucose kinetics noted in the above studies is supposedly due to the ability of oestrogen to hamper hepatic gluconeogenesis.[45] This hypothesis is supported by a study performed in carbohydrate-depleted women, where blood glucose was maintained during submaximal exercise in the MF phase but concentrations dropped progressively during exercise in the ML phase.[20]

However, Horton et al.[25] hypothesized that the effect of oestrogen on hepatic glucose output

Table II. Comparison of menstrual phase studies investigating plasma glucose kinetics during exercise with consideration of nutritional status and the absolute exercise intensity

Reference (year)	Menstrual phases	Training status $\dot{V}O_{2max}$ (mL/kg/min)	Exercise intensity (%$\dot{V}O_{2max}$)	Absolute $\dot{V}O_2$ (mL/kg/min)	Glucose Ra[a] (μmol/kg/min)	Significance
Overnight fasted studies						
Horton et al.[25] (2002)	EF vs MF vs ML	39.9 ± 5.8	50	20.2	20 vs 20 vs 18	NS
Zderic et al.[19] (2001)	EF vs ML	48.2 ± 1.1	42	20.2	20 vs 18	NS
			52	25.1	33.7 vs 28.8	$p < 0.05$
Campbell et al.[22] (2001)	EF vs ML	53.5 ± 0.9	69	36.8	33 vs 25	$p < 0.05$
Devries et al.[44] (2006)	MF vs ML (part OC)	39 ± 2	65	25.4	53 vs 49	$p = 0.03$
Ruby et al.[36] (1997)	Am; PL vs 72 h E vs 144 h E	45.5 ± 5.6	65	29.2	21.9 vs 18.9 vs 18.9	$p < 0.05$
Carter et al.[43] (2001)	M; PL vs E	53.3 ± 6.7	60	31.1	48 vs 42	$p < 0.05$
Devries et al.[37] (2005)	M; PL vs E	44 ± 2	65	28.6	65 vs 60	$p = 0.04$
Roughly 3-hours postprandial studies						
D'Eon et al.[42] (2002)	GnRH agonist vs E vs E + P	42.5 ± 8	54	23 (96 watts)	51 vs 45.6 vs 43.3	$(0.05 < p < 0.1)$ for E and E + P < GnRHa
Suh et al.[24] (2002)	EF vs ML	43.6 ± 2	45	20 (59 watts)	27.7 vs 28.3	NS
			65	29 (97 watts)	38.9 vs 40.6	NS
Exogenous glucose ingestion during exercise						
Campbell et al.[22] (2001)	EF vs ML	53.5 ± 0.9	70	37.8	46 vs 43	NS

a Some glucose Ra values are only approximations as estimated from figures.

Am = amenorrhoeic women; **E** = exogenous oestrogen supplements; **E + P** = exogenous oestrogen and progesterone supplements; **EF** = early follicular phase; **GnRHa** = gonadotropic-releasing hormone agonist that will suppress endogenous oestrogen and progesterone secretion; **M** = male subjects; **MF** = mid-follicular phase; **ML** = mid-luteal phase; **NS** = not significantly different; **OC** = oral contraceptive; **PL** = placebo; **Ra** = rate of appearance; **$\dot{V}O_2$** = oxygen uptake; **$\dot{V}O_{2max}$** = maximal oxygen uptake.

appears to become noticeable only when the exercise intensity is sufficient to increase the demands on glucose utilization to above a certain 'critical level'. At this 'critical level' the demand on endogenous glucose production is sufficiently elevated such that the effect of oestrogen suppression on gluconeogenesis is evident in a reduced glucose Ra. As illustrated by Horton et al.,[25] this is clearly supported by comparing the glucose kinetic results of Campbell et al.,[22] Horton et al.[25] and Zderic et al.[19] (table II).

When the subjects in the study by Zderic et al.[19] exercised at approximately 50% $\dot{V}O_{2max}$, glucose Ra was significantly lower in the ML phase versus the EF phase. However, no difference was noted between menstrual phases when these subjects exercised at only 42% $\dot{V}O_{2max}$.[19] Thus, when there was less of a demand on endogenous glucose production, and an increased reliance on lipid utilization, no menstrual phase effect was evident in glucose kinetics. In the study by Horton et al.,[25] the subjects were slightly less trained than those in the study by Zderic et al.[19] Therefore, when the subject groups of the two studies exercised at an equal intensity of 50% $\dot{V}O_{2max}$, the absolute workload was lower in the study by Horton et al.[25] Thus, the lower absolute workload demanded a lower total fuel utilization and hence lower glucose utilization with less demand on endogenous glucose production and consequently a lower glucose Ra. At this lower glucose Ra no difference was observed between the EF, MF and ML phases,[25] as was similarly observed in the study by Zderic et al.[19] at 42% $\dot{V}O_{2max}$. In contrast, well-trained subjects in the study by Campbell et al.[22] exercised at 70% $\dot{V}O_{2max}$. This higher intensity exercise increased the demand on endogenous glucose production above the 'critical level' and produced a noticeable difference in glucose Ra between the EF and ML phase. A study by Devries et al.[44] that included both eumenorrhoeic women and

women on oral contraceptives confirms these findings, with glucose Ra 6% lower in the LP compared with the FP when subjects exercised at 65% $\dot{V}O_{2max}$ following an overnight fast.

However, when subjects in the study by Campbell et al.[22] received an energy drink during exercise the difference in glucose Ra between menstrual phases was no longer significant, possibly because glucose Ra was now largely determined by exogenous glucose absorption (table II).[25] This theory can be extended to include subjects who exercise only a few hours postprandially (but do not receive glucose supplements during exercise).[24,42] Under these conditions it appears that the demands on glucose utilization need to exceed an even higher critical level before a difference in glucose kinetics between menstrual phases becomes evident (table II). It would be interesting to challenge this hypothesis with a study in which glucose kinetics parameters are measured in subjects exercising at high intensities of >70% $\dot{V}O_{2max}$ following a short postprandial period, in various menstrual phases.

Thus, in summary, glucose kinetics appears to be influenced by menstrual phase when the energy demand of exercise is sufficiently high to pressurise endogenous glucose production. However, it appears that the postprandial period is a major determinant of the level of the demand on endogenous glucose production that is necessary before the influence of menstrual phase becomes evident. This can be explained as oestrogen or oestrogen and progesterone impose a restriction on endogenous glucose production by suppressing gluconeogenesis.[45] However, when exercise follows a short postprandial period it would be expected that hepatic glycogenolysis would provide a greater proportional contribution to endogenous glucose production than from gluconeogenesis relative to a condition that imposes a longer fasting period. Finally, when exogenous glucose is provided throughout exercise the influence of menstrual phase on glucose kinetics is negligible, as this condition minimizes the demand on endogenous glucose production.[22] However, future studies should consider the influence of menstrual phase or ovarian hormone concentration on glucose kinetics during ultra-long events even with exogenous glucose supplements as, ultimately, gluconeogenic output will become increasingly relevant.

2.1.2 Indirect Estimation of Muscle Glycogen Use

Glucose metabolic tracer studies often make the assumption that 100% of glucose uptake (Rd) is oxidized and therefore glucose Rd approximates plasma glucose oxidation rate. The difference between total carbohydrate oxidation estimated by indirect calorimetry and glucose Rd provides an estimate of muscle glycogen use during exercise. However, this is a crude assumption, as the percentage of glucose Rd oxidized is probably closer to 60–90% and may vary depending on the study conditions; thus, the calculation underestimates glycogen use and should be considered as minimal muscle glycogen utilization.[46] In fact, when such indirect estimates of muscle glycogen use were compared with actual muscle biopsy measures, the values did not correlate.[44] Furthermore, while muscle biopsy data revealed menstrual phase differences between muscle glycogen use during exercise, no differences were evident when based on indirect tracer estimates in the same sample group.[44]

Possibly, for these reasons, some studies measuring glucose kinetics have not estimated muscle glycogen use,[24,25,43] which may explain why others have found no difference in estimated glycogen use between menstrual phases[22] or with oestrogen treatment.[24] Therefore, the results from indirect muscle glycogen estimations must be considered in light of the possible limitations of the method.

However, some studies that reported a lower glucose uptake (Rd) with elevated oestrogen[42] or oestrogen and progesterone[19] also reported lower estimated muscle glycogen use during exercise under these conditions compared with EF phase conditions. Interestingly, D'Eon et al.[42] found that pharmacologically elevated oestrogen plus progesterone resulted in greater estimated muscle glycogen use during exercise compared with a condition of suppressed ovarian hormones. Such a finding is contrary to reports from other authors whose muscle biopsy data suggest

muscle glycogen sparing in the LP, in which oestrogen and progesterone concentrations are elevated.[47] However, the rise in ovarian hormones in the study by D'Eon et al.[42] was not natural, and although during the oestrogen plus progesterone condition oestrogen was elevated to within normal LP levels, progesterone increased to around 151.4 nmol/L (47.6 ng/mL), which is higher than normal LP levels. Thus, the findings of D'Eon et al.[8] suggest that progesterone may antagonize glycogen sparing. The muscle glycogen sparing that has been reported to occur in the LP[19,47] must be largely due to the elevated oestrogen levels that occur during this phase and could possibly be more pronounced during the LF phase where oestrogen alone is elevated.

Furthermore, D'Eon et al.[42] described an interesting inverse correlation in which free fatty acid (FFA) concentration explained 50% of the variance in estimated muscle glycogen use, where FFA concentration was greater with oestrogen supplementation. This possibly infers that an oestrogen-induced increased FFA availability promoted glycogen sparing during exercise.[42] Therefore, the influence of oestrogen and progesterone on muscle glycogen utilization may depend on their influence on FFA availability or oxidation.

2.1.3 Muscle Glycogen Content Quantified from Muscle Biopsies

Estimation of muscle glycogen content by muscle biopsy in eumenorrhoeic women suggests that the hormone milieu in the LP promotes muscle glycogen storage[23,34,48] compared with the FP. Of particular note, given the current interest in multistage events, Nicklas et al.[23] reported greater muscle glycogen repletion following a period of induced glycogen depletion in the LP compared with the FP. However, a carbohydrate-loading diet increased muscle glycogen stores in the FP to the higher values previously attained in the LP when following a normal diet,[34] but the carbohydrate-loading diet failed to increase muscle glycogen stores further in the LP.[34] Thus, carbohydrate loading balances the capacity for muscle glycogen storage between the FP and LP.[34]

However, a recent study by Devries et al.[44] used a subject group comprising of part eumenorrhoeic women and part women using triphasic oral contraceptives and found no difference in resting muscle glycogen stores between the follicular and LP. Moreover, oestrogen supplementation in men resulted in a trend for lower resting muscle glycogen with a moderate oestrogen dose,[49] or significantly lower resting glycogen stores with a higher oestrogen dose compared with placebo treatment.[37] However, the oestrogen exposure period in these latter studies may have been too short to promote glycogen storage, or oestrogen supplementation may have decreased the calorie intake[50] of the men when compared with placebo treatment, because although their diet was self-controlled it was not rigorously regulated.[37] Nonetheless, in support of the latter findings, rat studies using male[51] or ovariectomized female rats[31,39] where the quantity of food-intake was controlled, reported no change in resting muscle glycogen stores following oestrogen and/or progesterone treatment. However, one such study did report an increase in liver glycogen stores with oestrogen supplements in ovariectomized rats compared with progesterone, combined progesterone and oestrogen, or placebo treatment,[39] thus demonstrating the potential for oestrogen to maximize glycogen stores. However, we cannot exclude the possibility of interspecies differences in carbohydrate metabolism.

Nevertheless, oestrogen has been shown to increase muscle glycogen synthase activity.[52] Moreover, oestrogen deficiency is associated with insulin resistance[53] and higher insulin concentrations.[42] Furthermore, intravenous oestrogen in postmenopausal women promoted insulin actions by increasing insulin-stimulated glucose uptake at rest during a hyperinsulinaemic clamp.[54] Other studies have also reported insulin sensitivity to be heightened in the presence of oestrogen[53,55] but often report no change in insulin responsiveness to a large glucose load.[53,55-57] Thus, we would expect increases in oestrogen concentration to promote glycogen storage. Moreover, the similar carbohydrate-loaded glycogen stores between menstrual phases could be

explained by the previously reported lack of an oestrogen effect on insulin responsiveness to a larger glucose load.

Conversely, increases in progesterone concentration are associated with a decrease in the insulin-responsive glucose transporter (GLUT4) content in insulin-sensitive tissue[39] and insulin resistance;[58-60] hence, progesterone most likely counters glycogen storage.

Given the controversy in the findings of resting muscle glycogen stores between studies from naturally cycling eumenorrhoeic women and oestrogen-supplemented studies, further studies are necessary to clarify whether the ovarian hormones alter resting muscle glycogen stores.

A number of studies have reported lower rates of glycogen use during exercise in the LP compared with the FP based on biopsy samples taken before and after 60 minutes of exercise at 70% $\dot{V}O_{2max}$.[44,47] In addition, one such study found that muscle glycogen use during exercise was negatively correlated with oestrogen concentration.[47] More specifically, Devries et al.[44] isolated muscle glycogen into proglycogen and macroglycogen fractions and found women in the FP used 30% more proglycogen and 16% more macroglycogen and together 24% more total muscle glycogen during exercise than when in their LP. However, their sample group included both eumenorrhoeic women and women using oral contraceptives.

Previously, the same group of researchers found no evidence of muscle glycogen sparing during endurance exercise in men receiving oestrogen supplements compared with placebo treatment.[37,49] However, the authors' speculate that the period of oestrogen treatment or sex differences in oestrogen receptor density may explain the lack of effect in the men.[44]

Animal research consistently finds that oestrogen treatment results in skeletal muscle glycogen sparing during exercise.[31,39,51] However, despite the greater pre-exercise liver glycogen in oestrogen-treated ovariectomized rats in the study by Campbell and Febbraio,[39] after 30 minutes of submaximal running at 0.35 m/sec, liver glycogen stores were no longer significantly different between ovarian hormone treatments. These results suggest a greater rate of hepatic glycogenolysis with oestrogen treatment. This is contrary to the finding of Kendrick et al.[31] where after 2 hours of submaximal running at the same intensity (0.37 m/sec) marked hepatic glycogen sparing was reported with oestrogen treatment in oophorectomized rats. The discrepancy in liver glycogen metabolism with oestrogen treatment may be related to the difference in exercise duration of the two studies. Therefore, the greater hepatic glycogen use with oestrogen treatment in the study by Campbell and Febbraio[39] may reflect initial responses to exercise that are characterized by an early-phase higher dependence on carbohydrate metabolism. Thus, the findings from this study reflect the capacity of oestrogen to increase glucose availability and, moreover, uptake into specifically type I muscle fibres during periods of demand.[39] In fact, two animal studies have found oestrogen to potentiate contraction-stimulated glucose uptake (50% increases have been reported with oestrogen replacement relative to oestrogen deficiency by ovariectomy).[39,55] Conversely, the findings of Kendrick et al.[31] depict metabolic preferences of prolonged exercise where the presence of increased oestrogen may promote a different response that includes liver glycogen sparing, which, in that study, coincided with substantial increases in endurance capacity.

2.1.4 Conclusion of the Influence of the Ovarian Hormones on Carbohydrate Metabolism

In summary, evidence from metabolic studies suggests that oestrogen and progesterone have various effects on carbohydrate metabolism. Oestrogen promotes insulin sensitivity and possibly greater glycogen storage, while progesterone promotes insulin resistance. Oestrogen promotes contraction-stimulated glucose uptake into type I muscle fibres during short duration exercise, which should be beneficial for performance in higher intensity aerobic exercise, while progesterone antagonizes this action.[39] Thus, the increase in oestrogen relative to progesterone may determine the influence of the ovarian hormones during the LP on insulin-stimulated and contraction-stimulated glucose uptake and so

variably influence glycogen storage and plasma glucose availability during exercise. However, whole body systemic glucose kinetics is reduced during prolonged exercise, with increases in oestrogen alone or in combination with progesterone. Such a decrease in whole body glucose kinetics is possibly due to suppression of hepatic gluconeogenic production when the exercise intensity is sufficiently intense to put pressure on the system. Gluconeogenic suppression may jeopardize exercise performance when glycogen stores are limited. However, muscle glycogen stores are spared during exercise in the LP or in rats with oestrogen supplementation and should promote better endurance.

Although researchers investigating menstrual phase comparisons have extensively studied carbohydrate metabolism during exercise, isotopic tracer measures of plasma glucose oxidation rate are still lacking.

2.2 Fat Metabolism

While occasionally indirect calorimetry measurements suggest greater whole body lipid use during exercise in the LF or ML phase compared with EF or MF phase,[11,19,22,61] this is not consistently reported.[24,25,44,62,63] However, when oestrogen supplements are administered independently of progesterone, the respiratory exchange ratio (RER) is lower during exercise compared with placebo in men[37] or compared with oestrogen and progesterone supplements together or gonadotropin-releasing hormone (GnRH) agonists (suppressed ovarian secretion) in women.[42]

2.2.1 Systemic Glycerol Kinetics as a Measure of Lipolytic Rate

Determination of glycerol Ra by tracer methodology is routinely used as an index of whole body lipolytic rate.[64] This is based on the assumption that following triacylglycerol hydrolysis in muscle and adipose tissue, glycerol must be released into the blood. Glycerol must be rephosphorylated by the enzyme glycerol kinase, present only in the liver and to a lesser degree in the kidneys before it can be reused in triacylglycerol re-esterification. Therefore, it is assumed that hepatic clearance of glycerol from the blood is the only significant route of irreversible loss of a glycerol tracer.[64] However, this assumption has been challenged, as some authors[65] have found that only half of the glycerol Ra is taken up by the splanchnic bed and therefore the periphery must be taking up the rest. Secondly, the findings of others[66] suggest that muscle may metabolize a significant amount of glycerol and therefore not all of the glycerol released by intramuscular triacylglycerol hydrolysis will appear in the bloodstream.

Nonetheless, glycerol kinetics have been compared at rest and during submaximal exercise in eumenorrhoeic women in their EF and ML phase and then after 4 months of oral contraceptive supplementation.[67] No significant difference was found between menstrual phases in a subsample (n = 5), but oral contraceptive use increased glycerol Ra during submaximal exercise (n = 8). Oral contraceptive use also resulted in higher cortisol concentration, which is presumably causative of the heightened lipolytic rate.[67] A further study has examined glycerol kinetics during moderate intensity exercise in eumenorrhoeic women and included the MF phase – which is associated with moderate increases in oestrogen independent of progesterone – in their comparison.[62] These authors also found no significant variation in glycerol kinetics between menstrual phases. However, the average increase in oestrogen in the MF and ML phases was modest (265 and 393 pmol/L, respectively).

Furthermore, exogenous oestrogen supplements given to amenorrhoeic women[36] or men[43] also failed to alter glycerol kinetics during submaximal exercise. However, the increase in oestrogen in the amenorrhoeic women was modest, approximating only FP levels. Conversely, the oral supplements in the men increased oestrogen to supraphysiological levels, and whether sex differences in lipolytic regulation[68,69] may have obscured an oestrogen effect on glycerol kinetics is indeterminate. In addition, the possibility of a sex difference in the response to oestrogen treatment cannot be excluded, as the receptor population of the endogenous sex hormones are

reportedly different between sexes.[70] Nonetheless, the limited evidence to date suggests that oestrogen and the ovarian milieu of the LP do not alter whole body systemic glycerol kinetics and, by inference, lipolytic rate during exercise.

Oestrogen's stimulation and progesterone's antagonizm of growth hormone response to exercise,[71] however, suggest oestrogen may stimulate lipolysis, but consideration of the E/P ratio in the LP would be prudent. Animal research presents convincing evidence to suggest that oestrogen can in fact increase lipolytic rate during exercise. For example, Beniot et al.[72] reported a heightened sensitivity to catecholamines in oestrogen-supplemented rats, with a corresponding increase in hormone-sensitive lipase activity. These authors suggest that oestrogen acts via its catechol-oestrogen derivative to potentiate the lipolytic action of adrenaline (epinephrine) by competing with catecholamines for catechol-O-methyltransferase.[72] In addition, Hansen et al.[73] demonstrated an increase in lipolysis and reduced fatty acid synthesis in isolated fat cells from oestrogen-treated rats, while progesterone had no effect compared with control/unsupplemented rats. Oestrogen supplementation in male rats has also been found to alter lipoprotein lipase (LPL) activity in a tissue-specific fashion.[74] While adipocyte LPL activity was reduced, muscle LPL activity was increased, promoting a redistribution of lipids from adipose to muscle tissue. Consequently, oestrogen supplementation not only elevated resting intramuscular lipid content but also promoted triacylglycerol esterification during submaximal exercise in the red vastus muscle, as triacylglycerol content was even greater post-exercise than at rest.[74] Therefore, whole body glycerol kinetics would not be able to elucidate oestrogen's tissue-specific action but instead presents the overall summated response. However, it must be considered that interspecies differences may occur in the regulation of lipid metabolism.[75]

Encouraged by the overwhelming evidence from animal studies, future studies should consider tissue-specific glycerol kinetics using methods such as arteriovenous balance during exercise in various menstrual phases including the LF phase, occurring approximately 2 days before ovulation and in which oestrogen peaks.

2.2.2 Plasma Free Fatty Acid Kinetics and Oxidation

FFA Ra provides an index of plasma FFA availability and measures the release of fatty acids that are primarily derived from the hydrolysis of adipose tissue triacylglycerol into plasma.[69] When used as a measure of lipolytic response, FFA Ra does not account for triacylglycerol re-esterification.[76] FFA Rd measures the rate of uptake into tissues and has been used as an estimate of plasma FFA oxidation rate;[77] however, this is a crude estimate as the actual proportion of FFA uptake that is oxidized can vary and has been reported to be as low as 50%.[78]

A number of studies have considered plasma FFA kinetics,[79-81] dietary FFA uptake into body stores[82] and plasma triacylglycerol kinetics[81] between menstrual phases,[79,81,82] and with and without oestrogen supplements in postmenopausal women[80] at rest. All studies reported no differences between menstrual phases or treatments. In fact, a similar FFA Ra at rest between menstrual phases is not surprising, as animal studies confirm that basal lipolysis is unchanged or even suppressed in the presence of oestrogen compared with oestrogen deficiency.[52,83] Conversely, oestrogen enhances catecholamine sensitivity as is noted by an upregulated lipolytic response to catecholamine stimulation with oestrogen treatment.[72,83]

More recently, plasma FFA kinetics and oxidation have been compared during submaximal exercise during various menstrual phases in eumenorrhoeic women.[62,63] Jacobs et al.[63] performed a longitudinal study comparing plasma FFA metabolic response in the EF and ML phases and then with subsequent oral contraceptive use. Unfortunately, their menstrual phase comparison was reduced to a sample size of five, which limited the statistical power of the comparison. Considering this limitation they reported no significant differences in the rates of whole body fat oxidation, plasma FFA oxidation, non-plasma FFA oxidation or plasma FFA

rate of appearance or disappearance or rate of re-esterification.[63] The average oestrogen concentration in the ML phase was a modest 311 pmol/L and the E/P ratio was fairly low, at 9. Furthermore, they failed to make use of the acetate correction factor in their calculation of plasma FFA oxidation, which is now established as necessary for more accurate estimates of plasma FFA oxidation rate.[84]

The acetate correction factor accounts for the proportion of tracer-derived carbon label that is retained in the products of secondary exchange reactions that occur with tricarboxylic acid cycle intermediates instead of being released as carbon dioxide.[84] Our laboratory has observed significant variability in the acetate correction factor between menstrual phases.[85] The correction factor was lower in the ML phase compared with the EF phase.[85] We speculate that this may be associated with increased protein catabolism during exercise in the ML phase, as reported by others.[86] That is, the increased flux through transamination pathways may result in a slightly greater proportion of FFA tracer-derived carbon label isotope being retained in the products of subsidiary reactions with tricarboxylic acid cycle intermediates. Thus, in order to further increase the sensitivity of the comparison of plasma FFA oxidation rate between menstrual phases, it would be necessary to simultaneously derive the acetate correction factor and plasma FFA oxidation for each menstrual phase.

Shortly following the study by Jacobs et al.,[63] a second study by Horton et al.[62] considered FFA kinetics during moderate exercise in the EF versus MF versus ML phases. The MF phase is characterized by a gradual increase in oestrogen concentration independently of progesterone. The authors found no variation in FFA Ra or Rd between menstrual phases. However, the average oestrogen concentration recorded in the MF phase of the study by Horton et al.[62] was moderate (264 pmol/L), and even the ML phase oestrogen value (393 pmol/L) was fairly modest for this menstrual phase, resulting in a low E/P ratio of 10.7. These authors, as with Jacobs et al.,[63] agreed that the magnitude of increase in oestrogen and the oestrogen increase relative to progesterone may be an important factor determining the impact of the ovarian hormones on fat metabolism. Horton et al.[62] went on to presume that variations may be noticeable in the LF or periovulatory period when oestrogen is elevated independently of progesterone.

Such speculations are based on compelling evidence from animal studies. Ovariectomy reduces the activity of key enzymes in fat metabolism, i.e. carnitine palmitoyl transferase-I (CPT-I) and beta-3-hydroxyacyl-CoA dehydrogenase (β–HAD).[40] Oestrogen restores the activity of these enzymes, while progesterone inhibits these positive actions when oestrogen is at physiological concentrations.[40] However, a supraphysiological concentration of oestrogen overrides the negative effects of progesterone.[40] Interestingly, the difference in β-HAD activity with oestrogen treatment was only evident in muscle sections composed primarily of type I fibres[40] and not in sections of predominantly type II fibres.[40,52] Nonetheless, this rat model demonstrates the ability of the ovarian hormones to alter the capacity for skeletal muscle to oxidize FFAs by directly impacting on the cellular metabolic pathways.

In an initial pilot study performed in our laboratory, we measured plasma palmitate Ra and Rd with a continuous infusion of 1-^{13}C palmitate during moderate intensity exercise over 90 minutes in eumenorrhoeic women (n = 5) who were 3-hours postabsorptive (Oosthuyse and Bosch, unpublished observations). The women all completed the trial twice, once in the EF phase and then again in either the LF (or periovulatory) phase or ML phase or late luteal (LL) phase. The intention was to obtain the full range of ovarian hormones and E/P ratios that may occur during a menstrual cycle. We found that plasma palmitate Ra was highly correlated with E/P ratio ($r = 0.85$; $p = 0.06$) and was significant between plasma palmitate Rd and E/P ratio ($r = 0.89$; $p = 0.04$). A trend for a relationship between palmitate Ra, Rd and oestrogen concentration was evident. However, due to the limited sample size, no definite conclusions can be drawn. Nonetheless, this pilot study provides motivation for further investigations of exercising FFA metabolism of this kind that consider oestrogen and progesterone

variability between subjects and from one day to the next even within a menstrual phase.

In summary, a first glance at the latest work covering plasma FFA kinetics and oxidation during exercise suggests no menstrual phase or ovarian hormone effect. All researchers in the field agree that considering interindividual and intraindividual variability in ovarian hormones and the relative ratio of E/P as they change even within a menstrual phase is imperative in convincingly identifying whether the fluctuations in oestrogen and progesterone, as occurs naturally across the menstrual cycle and specifically the LF phase, alter lipid metabolism during prolonged exercise.

2.2.3 Intramyocellular Stores and Use During Exercise

No study to date has considered the influence of the menstrual phase on intramyocellular lipid (IMCL) stores and use during exercise. Although an indirect estimation of IMCL use in exercise can be attained from isotopic tracer methods (by calculating the difference between whole body lipid oxidation and plasma FFA oxidation), it represents a rough estimate because it cannot differentiate IMCL oxidation from plasma triacylglycerol oxidation.[63]

A number of recent studies have investigated variability in IMCL stores and use during exercise between men and women.[87-94] A full discussion of the findings of these sex-comparative studies is beyond the scope of the current review. However, researchers should carefully consider these findings when designing studies to consider variations in IMCL stores and use during exercise between menstrual phases. In particular, such future studies should consider menstrual variations in IMCL use during ultra-long endurance exercise, as Devries et al.[89] reported a higher percentage association between IMCL droplets and mitochondria following 90 minutes of exercise in women compared with men, where no sex differences were found before exercise. Ovarian hormone effects on IMCL use may become more apparent during the latter stages of ultra-events.

2.2.4 5′-AMP-Activated Protein Kinase, a Key Regulator of Cellular Metabolism, is Influenced by Oestrogen

Recent advances in the metabolic regulatory actions of 5′-AMP-activated protein kinase (AMPK) suggest that it is the major cellular energy regulator driving metabolic processes to promote ATP production.[95,96] Increased AMPK activity corresponds with increased GLUT4 content, contraction-stimulated glucose uptake and increased cellular fatty acid uptake, although evidence for a dominant role over the regulation of fat oxidation is not yet conclusive.[95] Evidence of oestrogen affecting AMPK activity[83] draws together the many previously suspected, but often elusive, actions of oestrogen. Specifically, AMPK is known to increase translocation of fatty acid translocase (FAT/CD36) and plasma membrane-bound fatty acid-binding protein (FABPpm).[96] FABPpm is abundant and therefore not limiting FFA uptake, but an increase in FAT/CD36 will increase FFA transport and oxidation and thus may limit FFA uptake. Since oestrogen is thought to stimulate AMPK activity,[83] it could be speculated that oestrogen will increase FFA uptake when sufficiently elevated in eumenorrhoeic women. Although AMPK's role in enhancing fat oxidation has come under recent scrutiny,[97] AMPK is thought to inhibit the enzyme glycerol-3-phosphate acyltransferase, which initiates triacylglycerol synthesis, and acetyl-CoA carboxylase (ACC), which regulates the production of malonyl-CoA.[96] Malonyl-CoA is well known as a potent inhibitor of CPT-I and thus entry of long chain fatty acids (LCFAs) into mitochondria. Furthermore, AMPK is thought to increase the activity of malonyl-CoA decarboxylase, which breaks down malonyl-CoA to acetyl-CoA.[96] Thus, AMPK should promote entry of LCFA into beta oxidation.

The study by D'Eon et al.[83] demonstrates the role of oestrogen in regulating fat metabolism via genomic and non-genomic pathways. Oestrogen promotes leanness and decreases adipocyte size by decreasing fatty acid uptake into adipose tissue (via decreased expression of lipoprotein lipase [LPL]), decreasing lipogenesis (via decreased expression of ACC-1 and fatty acid synthase [FAS])

and increasing catecholamine stimulated lipolysis, but not basal lipolysis (via increased expression of the phosphoprotein, perilipin).[83] Oestrogen alters gene expression by binding to the oestrogen receptor response elements located in the promoters of target genes and thereby regulates liver X receptor α (LXRα) and sterol regulatory element-binding protein 1c (SREBP-1c), which regulate the transcriptional expression of ACC-1 and FAS.[83]

However, the genomic influence of oestrogen in muscle and liver differ from that of adipocytes.[83] In muscle and liver, oestrogen upregulates the transcription factor peroxisome proliferation activator receptor-δ (PPAR-δ), which leads to the increased expression of various enzymes (LPL, pyruvate dehydrogenase kinase, acyl-CoA oxidase, and uncoupling protein 2 and 3), which promotes energy dissipation and the oxidation of FFA.[83]

Moreover, oestrogen-treated ovariectomized mice displayed 5-fold increased AMPK activity in skeletal muscle compared with placebo-treated mice.[83] The AMPK response to oestrogen occurred rapidly in a time- and dose-dependent manner via binding to membrane-bound oestrogen receptors and thus stimulating increased fat uptake into mitochondria.[83] Interestingly, recent findings have shown that the expression of oestrogen receptors α and β in skeletal muscle increases with the level of endurance training,[98] and hence it is interesting to question whether a greater oestrogen-stimulated AMPK response could be expected in skeletal muscles of endurance-trained athletes. However, oestrogen's stimulation of AMPK is not aided by adipokines, as the study by D'Eon et al.[83] found leptin and adiponectin concentration to be lower in oestrogen-treated mice, possibly due to the smaller adipocyte size in the oestrogen-treated group and oestrogen's inhibition of SREBP-1c. Other studies have also found oestrogen to decrease adiponectin concentration in pregnancy.[99] However, one study showed large fluctuations in adiponectin in women across the menstrual cycle, but no association with the ovarian hormone profile was evident.[100]

D'Eon et al.[83] suggest that the 'oestrogen effect' is largely a result of oestrogen's manipulation of the following targets: SREBP-1c, PPAR-δ and AMPK. In fact, although oestrogen may act independently on SREBP-1c and PPAR-δ, cellular and transcriptional regulation afforded by AMPK[96] mimics many of the observations reported in the oestrogen-treated mice in the study by D'Eon et al.[83] Thus, it is tempting to propose that oestrogen's activation of AMPK is the major key to the metabolic perturbations of oestrogen. Further work in this area is suggested. Studies in eumenorrhoeic women should consider variations in the AMPK response to exercise across the menstrual cycle and to test for associations between oestrogen and/or progesterone concentration and changes in the AMPK response to exercise.

2.2.5 Conclusion of the Influence of the Ovarian Hormones on Fat Metabolism

Animal research presents strong evidence that oestrogen promotes lipolysis and increases plasma FFA availability during exercise, increases intramuscular lipid stores and increases cellular capacity for FFA oxidation. Recent evidence suggests that many of oestrogen's metabolic actions may occur through AMPK stimulation and activation of transcription factors. Such optimization of lipid metabolism with oestrogen would promote an ideal metabolic response for endurance exercise. However, isotopic tracer studies in resting or exercising eumenorrhoeic women have reported no differences in systemic glycerol or FFA kinetics. Future lipid metabolic studies should consider the magnitude of increase in oestrogen between menstrual phases and the increase in oestrogen relative to progesterone during the LP. Furthermore, studies focusing on tissue-specific metabolism in women may help to explain the divergent findings of animal and human research.

2.3 Influence of Ovarian Hormones on Protein Metabolism

Recent tracer studies have consistently found amino acid oxidation to be greater in the LP compared with the FP at rest.[101-103] Kriengsinyos et al.[101] observed the strongest correlation between measurements of phenylalanine oxidation and progesterone, suggesting that progesterone

has the greatest impact on amino acid catabolism in the LP. Furthermore, the greater amino acid oxidation in the LP coincided with greater dietary lysine requirement in the LP compared with the FP.[101] Reports on amino acid flux are less consistent. One study has reported greater flux in the LP compared with the FP at rest,[102] while others reported no differences.[101,103] Some have also found non-oxidative leucine disposal (NOLD) to be lower in the LP, suggesting less protein synthesis.[103] In a study in which ovarian hormone secretion was suppressed by administration of a gonadotropin releasing hormone agonist, it was found that leucine turnover and NOLD were attenuated compared with normally cycling women.[103] Thus, the ovarian hormones support maintenance of normal protein turnover in women, and as catabolism varies across menstrual phase, protein requirement varies coincidently.

Evidence of greater protein catabolism during exercise in the LP is provided by earlier studies. Bailey et al.,[33] found the concentration of various amino acids (i.e. alanine, glutamine, proline and isoleucine) to be lower in the ML phase compared with the EF phase at rest and during prolonged submaximal exercise in subjects exercising 3 hours postabsorptively. This suggests greater amino acid catabolism during exercise in the LP. However, the amino acid concentration difference between phases was smaller if a carbohydrate supplement was ingested during exercise compared with a placebo drink.[33] Lamont et al.,[86] also reported that protein catabolism was greater during the ML phase compared with the EF phase as measured by total urea nitrogen excretion over 4 days, including a 60-minute period of exercise at 70% $\dot{V}O_{2max}$. Total urinary urea nitrogen excretion over the exercise period was also greater in the ML phase compared with the EF phase.[86]

In contrast to the seemingly catabolic stimulation of protein metabolism in the LP, recent evidence suggests a positive influence of oestrogen in reducing protein oxidation.[104] For example, oral oestrogen supplementation in men reduced leucine oxidation by 16% at rest and during exercise compared with placebo treatment and so accounted for an increase in the protein balance (calculated as the difference between total protein synthesis and breakdown) by 8 mg of protein/kg/h at rest and 17 mg of protein/kg/h during exercise.[104]

A recent study examined the rate of myofibrillar and connective tissue protein synthesis following one-legged kicking exercise in two groups of eumenorrhoeic women.[105] One group participated in the FP and the other in the LP. No differences between phases were reported, although this study may have been limited by the statistical unpaired design.[105] Further studies regarding protein or amino acid kinetics and oxidation during exercise across the menstrual cycle are warranted.

In summary, the ovarian hormones have a noticeable influence on protein metabolism at rest and during exercise, which is often seen as increased catabolism in the LP. It appears that progesterone is responsible for the consistent finding of increased protein catabolism in the LP,[101] while oestrogen may reduce protein catabolism.[104] It would be interesting to investigate whether the E/P ratio in the LP is important in determining the extent of protein catabolism in this menstrual phase. Furthermore, studies in eumenorrhoeic women in the late follicular or pre-ovulatory phase to verify oestrogen's capacity to reduce protein oxidation would be valuable. Such studies conducted during exercise are necessary to determine the protein requirements of female athletes participating in endurance competition.

3. Conclusions

The potential of the ovarian hormones to impose major metabolic aberrations to carbohydrate, lipid and protein metabolism has been reported from both human and animal studies, and suggests repercussions for exercise performance in eumenorrhoeic women (as detailed in the summary following each subsection). However, the influences of the ovarian hormones on the various metabolic pathways appear highly complex and often tissue specific. Furthermore, oestrogen and progesterone have mostly opposing influences on the various systems, and responses

may be dependent on the concentrations of the respective ovarian hormones. Moreover, the extent of metabolic demand will also determine whether the ovarian hormone influences are physiologically significant. Consideration of these details is important for appreciating the effect on performance during different types of exercise.

Consequently, menstrual phase-associated changes to the various metabolic measurements are not consistently identified. By inference, this may explain the inconsistency in the reports of menstrual phase-associated changes to exercise performance. The findings of menstrual phase studies may be confounded by the high variability in the concentrations of ovarian hormones between subjects and from day to day within subjects during any particular menstrual phase. For this reason, investigating relations between metabolic and exercise performance parameters and the change in the ovarian hormone concentrations between the menstrual phases and/or the E/P ratio should increase the sensitivity of studies for identifying metabolic or performance changes caused by the naturally cycling ovarian hormones.

Acknowledgements

Studies cited in this review as unpublished findings were supported by grants from the University of the Witwatersrand Research Council, Medical Research Council of South Africa and the National Research Foundation. The authors have no conflicts of interest that are directly relevant to the content of this review.

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