CHAPTER 7

Conclusions and Future Directions

7.1 CONCLUSION

The work presented in this thesis constitutes a number of novel contributions to the field of menstrual phase comparative physiology. The main contributions as new findings include:

7.1.1 Menstrual cycle and metabolism

The two metabolic studies presented in this thesis (Chapter 2 and 3) identified multiple effects of the ovarian hormones, oestrogen and progesterone, on metabolism during submaximal exercise.

Firstly, the acetate correction factor (or fractional recovery of $^{13}CO_2$ from a labelled acetate infusion) was found to be significantly lower during submaximal exercise in the ML phase than EF phase (Chapter 2). Although the difference appears to be only a small marginal change, if not considered in calculations of plasma FFA oxidation it will reduce the sensitivity of comparisons between the EF and ML phase and may result in differences being overlooked in this type of study.

Furthermore the physiological reason for the lower acetate correction factor in the ML versus EF phase was not obvious. In the subjects studied, metabolic rate and RER (at the fixed exercise intensity), which were the variables suspected to vary between phases and so impact the correction factor, were similar between these menstrual phases. We speculated that an increase in protein metabolism, known to occur during the ML phase (Lamont et al. 1987), could account for the small but significant reduction in the acetate correction factor in this menstrual phase, via changes in the rate of the alpha ketoglutarate-glutamate transamination reaction. Interestingly, in the subsequent FFA kinetics study (Chapter 3), we measured urinary nitrogen excretion and thus provided an estimate of protein use during submaximal exercise. The novel observation was made that protein utilisation in exercise correlated with the ovarian hormone concentrations,

such that oestrogen decreased protein use and progesterone increased protein use during exercise. The E/P ratio of the subjects participating in the acetate correction factor study (Chapter 2) ranged from 9.9 to 15.5 in their ML phase. An E/P ratio in this range is fairly low and according to the linear regression of change in protein use versus log E/P in Chapter 3 (Figure 8c), this would correspond with a positive protein use from EF to ML phase and supports the suggestion of increased protein use in the ML phase contributing to the lower acetate correction factor or greater label retention.

In the second metabolic study (Chapter 3) we demonstrated that FFA kinetics and availability is influenced by the ovarian hormones in eumenorrhoeic women during submaximal exercise. This has previously been suggested by animal studies (Ellis et al. 1994, Hatta et al. 1988) but has not previously been verified in eumenorrhoeic women. Rather previous menstrual phase comparisons have yielded no difference in FFA kinetics between follicular and luteal phases (Horton et al 2006; Jacobs et al. 2005). However, based on our findings (Chapter 3), inter-subject variability is an important determinant. That is, this study is the first to identify relations between FFA metabolic variables and the ovarian hormones (Chapter 3):

1. A high oestrogen concentration appears to favour lipoysis (based on near significant correlation between the change in glycerol concentration from EF to LF/ML/LL phase and oestrogen concentration).

2. Oestrogen increases the release and uptake of plasma FFA while progesterone acts antagonistically, as change in palmitate Ra/Rd over EF phase values correlated best with the E/P ratio. Thus the luteal phase will only be favourably characterised by an increase in FFA Ra/Rd during endurance exercise when the E/P ratio is sufficiently high.

3. The strong correlation between the change in plasma FFA concentration during exercise from EF to LF/ML/LL phase and oestrogen concentration confirms oestrogen's tendency to increase FFA availability during prolonged exercise.

Thus inter-subject and day to day variations in the E/P ratio will determine the FFA metabolic response to endurance exercise and this can explain the non significant findings of previous studies of EF verse ML phase comparisons.

The number of menstrual phase comparative studies using FFA tracers has been limited, possibly due to the complicating factor of having to complex the FFA tracer to a carrier in order to hold it in solution for parenteral delivery. As described in Chapter 4, the use of 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) as a carrier for the intravenous delivery of FFA tracers is an attractive alternative to the routinely used carrier, human serum albumin (HSA). Results presented in Chapter 4 provide evidence for its suitability as a valid alternative to HSA as a carrier, as HP-β-CD does not appear to alter the in vivo behaviour of the FFA tracer. However, the protocol for complexing the tracer to HP-β-CD remains to be perfected, to make this a routine technique. While a 1:3 molecular ratio is effective, a high incubating temperature throughout the complexing procedure is critical. Once a refined mixing protocol is developed, the use of HP-β-CD as a carrier for parenteral delivery of FFA tracers may encourage more work in the field of FFA kinetics and exercise in women as HP-β-CD does not carry the associated risks of HSA.

7.1.2 Effects of the menstrual cycle on exercise performance

7.1.2.1 As a consequence of alterations to FFA metabolism:

The oestrogen-induced increase in FFA availability identified in the FFA kinetics study (Chapter 3) is expected to have major implications for endurance performance. According to the well established model, a shift from carbohydrate to lipid metabolism during exercise results in glycogen sparing and better endurance performance.

Therefore, an increase in FFA availability, whether it results in an increase in plasma FFA oxidation or intramuscular stores, or both, should benefit endurance especially ultra-endurance events. Thus a high oestrogen concentration on it's own in the LF phase, or a high E/P ratio in the ML phase, can be speculated to have these positive affects.

7.1.2.2 As a consequence of alterations to ventilation:

The ventilatory response to submaximal exercise may differ from the EF to ML phase, but the significance of the change depends on the oestrogen and progesterone concentration in the ML phase (Chapter 5). This is based on our novel finding of significant correlations between the change in ventilatory parameters from EF to ML phase during submaximal exercise and the oestrogen and progesterone concentration, with both oestrogen and progesterone acting to augment ventilatory drive. Thus again inter-subject variability in the ovarian hormone concentration is also an important determinant of ventilatory response to submaximal exercise in the ML phase.

In the subjects studied (Chapter 5), we found respiratory rate to be persistently higher throughout prolonged exercise in the ML compared to EF phase. However, the greater respiratory rate in the ML phase had no significant impact on metabolic demand and therefore is not expected to exacerbate fatigue or impede endurance performance.

While the main focus of Chapter 6 was the direct measure of exercise performance between menstrual phases, a ventilatory parameter (the ventilatory equivalent (V_E-Eq)) was recorded and compared between menstrual phases. As reported in Chapter 6, we found no significant difference in the ventilatory equivalent, averaged over the duration of the time trial, between menstrual phases. In further agreement, when changes in the ventilatory equivalent between menstrual phases in Chapter 6 was regressed against the ovarian hormone parameters, no relations where identified (data not shown). We chose to use the ventialtory equivalent, which expresses minute ventilation relative to metabolic rate (VO_2), because the exercise intensity was not fixed but rather dictated by

the subject as is the principle of time trial methodology. Thus the sensitivity of the comparison is reduced, as we compared an average over the total time trial duration, where variability in minute ventilation and oxygen consumption may have varied considerably throughout the session. However, in Chapter 5, where subjects exercised at a fixed submaximal workload, no significant correlation was found between changes in the ventilatory equivalent between menstrual phases and ovarian hormones, despite the significant correlations that where identified with minute ventilation and respiratory rate. The ventilatory equivalent, however, may not be the most sensitive variable with which to identify differences in exercising ventilation between menstrual phases, as oxygen consumption may be influenced by menstrual phase such that higher rates could be expected in the ML phase as reported by some (Hessemer et al. 1985; Williams and Karenbuhl 1997). However, as previously mentioned, in Chapter 5 we did not find any significant difference in oxygen consumption during submaximal exercise between menstrual phases. Nonetheless, increases in minute ventilation in the ML phase will be diluted by even small changes in VO_2 when reported as the ventilatory equivalent and thereby conceal possible differences in ventilatory responses between menstrual phases.

Based on the findings reported in Chapter 5 we came to the conclusion that the metabolic changes associated with the varying ovarian hormone concentrations corresponding to various menstrual phases appear to have greater relevance for athletic performance than the ventilatory changes.

7.1.2.3 Based on actual measures of exercise performance:

The “exercise performance promoting” properties of high oestrogen concentrations (due to metabolic benefits) are often concealed in the ML phase by concomitant increases in progesterone concentration. In Chapter 6, oestrogen’s positive influence on exercise performance was suggested by the near significant trend for best cycling time trial performance in the LF phase associated with the pre-ovulatory surge in oestrogen that is

uninhibited by any antagonistic influences of progesterone. Following this finding of a change in exercise performance in non fasted subjects between the EF and LF phase, we do not agree with those who suggest that a positive nutritional status will negate the influence of the ovarian hormones on altering exercise performance (Campbell et al. 2001). Rather we suggest, as do others (D'Eon et al. 2002; Hatta et al. 1988) that the ratio of E/P is the major determinant of the overall impact of menstrual phase on metabolism and hence exercise performance, as oestrogen promotes and progesterone antagonises the metabolic systems that facilitate aerobic exercise.

The importance of considering the E/P ratio appears to be relevant for endurance performance (more than 60 min) as demonstrated in Table 2 of the introduction (Chapter 1, section 1.2.4.2) where a high E/P ratio in the luteal phase was associated with a better endurance capacity in the luteal phase than in the follicular phase, and a low E/P ratio in the luteal phase was associated with no difference in endurance capacity between the follicular and luteal phase; with emphasis on the fact that these studies measured endurance capacity and not performance. However, in our performance study (Chapter 6) in which the average exercise intensity was moderate to high (roughly 75-85%VO_2max) and lasting less than 60 min (30-55 min), we found no difference between the EF and ML phase despite a relatively high average E/P ratio (21±10.8, range 5.6-40.8) in the ML phase. Thus it is possible that where exercise demands are met mainly from carbohydrate catabolism, the presence of progesterone (which in these subjects was fairly high on average- 40±22 nmol/L) will counter the benefits of oestrogen (regardless of the E/P ratio) to increase the supply of plasma glucose via actions on hepatic glycogenolysis and contraction-stimulated glucose uptake. This has been demonstrated in the rat model where ovarectomy reduced contraction-stimulated glucose uptake, which could be restored with oestrogen therapy (Hansen et al.1996). The presence of progesterone, however, not only inhibits oestrogen's potential to restore contraction-stimulated glucose uptake but also reduces GLUT 4 transporter content (Campbell and Febbraio 2002). The effect on GLUT4 transporters is exclusive to progesterone as

oestrogen does not influence GLUT 4 transporter content (Campbell and Febbraio 2002, Hansen et al. 1996), thus further demonstrating how the presence of progesterone in the luteal phase, regardless of the E/P ratio, could antagonise any benefits afforded by the presence of oestrogen when activity is mainly dependent on glucose metabolism. Therefore in summary, the LF phase is the best time for optimal aerobic performance of moderate to high intensity and most likely prolonged submaximal endurance. However the presence of progesterone in the ML phase, will negate the benefits of oestrogen in this phase, and thus moderate to high intensity exercise performance will be unaffected by the ovarian hormones in the ML compared to the EF phase. We do maintain however, that for longer endurance events a high E/P ratio in the ML phase may well favour a better performance compared to EF phase, as for longer events fat metabolism will become increasingly important and E/P ratio determines FFA availability in the ML phase as previously discussed.

Overall the work presented in this thesis provides convincing evidence for the importance of considering the inter-individual variability in the absolute concentration of the ovarian hormones and E/P ratio when investigating menstrual phase comparative physiology.

7.2 FUTURE DIRECTIONS

1. Is FFA kinetics greater in the LF phase when oestrogen increases without progesterone?

The main observational findings of the work presented in Chapter 3 suggest that FFA Ra/Rd could be most pronounced in the LF phase when oestrogen increases without progesterone. Further research should be conducted to prove or disprove this.

2. Is the greater plasma FFA Ra and Rd with increased oestrogen concentration observed in Chapter 3 related to greater plasma FFA oxidation, or intramuscular lipid storage?

Although plasma FFA oxidation rate has been quantified during exercise during EF and ML phases in one recent study (Jacobs et al. 2005), the E/P ratio should be considered during the ML phase and the LF phase (with the pre-ovulatory surge in oestrogen) should be included in the comparison. Furthermore, based on our finding of a lower acetate correction factor in the ML versus the EF phase (Chapter 2), it is suggested that the acetate correction factor applied to the calculation of plasma FFA oxidation rate be menstrual phase specific to increase the sensitivity of the comparison. The previous report of increased activity of the enzymes that catalyse beta oxidation in the presence of elevated oestrogen concentration, which is reversed when both oestrogen and progesterone increase concomitantly (Campbell and Febbraio 2001) provide evidence for an increased potential for FFA oxidation in the presence of oestrogen. Furthermore the increased FFA availability and suggestion of increased lipolytic activity associated with high oestrogen or a high E/P ratio in Chapter 3 could support the increase in cellular FFA oxidative potential by increasing the supply of substrate. Thus we hypothesize that oestrogen alone increases plasma FFA oxidation rate during submaximal exercise. However, the preliminary finding of oestrogen increasing FFA uptake could also alter intramuscular triacylglycerol storage and use and this too should be quantified across menstrual phases in future studies. Once FFA oxidation has been estimated using carbon FFA tracers, the percentage of FFA Rd (or FFA uptake) that is oxidized can be calculated and will provide insight into the extent of intramuscular triacylglycerol storage.

3. If plasma FFA oxidation rate is greater with high oestrogen concentrations, is it a consequence of increased beta oxidative capacity as suggested by animal studies?

Oestrogen-induced increase in beta oxidative enzyme activity and hence beta oxidative capacity as previously suggested by animal studies (Campbell and Febbraio 2001), should be verified in a human-model. This could be tested with the use of medium chain triacylglycerol (MCT) tracers, as MCT oxidation rate can provide an index of beta

oxidation. Normally, medium chain triaclyglycerols (MCT) form a negligible part of fat oxidation but as they are not limited by transport or uptake across the plasma membrane or into the mitochondria, they are directly oxidised by beta oxidation (Sidossis et al. 1996). However, MCT tracers must be administered by intravenous infusion rather then orally to limit its metabolism in the liver into ketones (Massicotte et al. 1992). Based on the animal study that measured beta oxidative enzyme activity (Campbell and Febbraio 2001), we would hypothesize that MCT oxidation rate, when compared between menstrual phases, would be the highest in the LF phase or when E/P ratio is high in the ML phase.

4. Can glycerol kinetics during submaximal exercise confirm the suggestion of oestrogen-induced increase in lipolysis, as supported by a positive relationship between the change in glycerol concentration from the EF phase to LF/ML/LL phase and oestrogen concentration?

Glycerol tracers have been used to indicate lipolytic response to oestrogen compared to placebo treatment in amenorrhoeic females (Ruby et al. 1997) and in men (Carter et al. 2001) during exercise. A recent study has considered EF versus ML phase comparisons (Cassaza et al. 2004). However, no difference was observed between treatments in the amenorrhoeic women or in the men, or between EF and ML phase in eumenorrhoeic women. These studies are limited by either too small an increase in oestrogen (Ruby et al. 1997) or use of males who may respond differently to women (Carter et al. 2001), or failure to consider inter-individual variability in the increase in ovarian hormones in the ML phase and hence E/P ratio (Cassaza et al . 2004). Following the strong suggestion of increased lipolytic response (based on changes in plasma glycerol concentration) to exercise with elevated oestrogen concentration and modulation of this response by progesterone in the ML phase in Chapter 3, further investigation using glycerol tracers is warranted in eumenorrhoeic women.

5. Amino acid kinetics and oxidation studies during submaximal exercise are necessary to confirm the negative relationship between oestrogen concentration and protein utilisation and in contrast the positive relationship between progesterone concentration and protein utilisation.

Previous studies (Lamont et al. 1987; Lariviere et al. 1994) suggest increased protein utilisation in the ML phase versus the EF phase. However, Chapter 3 provides evidence for the increased protein catabolism during exercise to be a progesterone effect, whereas oestrogen reduces protein use. Furthermore the decreased acetate correction factor in the ML versus EF phase (Chapter 2) may also be due to increased protein catabolism during exercise in the ML phase. Further studies should be conducted in which amino acid kinetics and utilisation during exercise is assessed across menstrual phases. Female athletes may need to be advised on altering their protein intake according to menstrual phase.

6. Is the tendency for best cycling time trial performance in the LF phase related to an increased capacity to utilise plasma glucose?

Further studies should be conducted to confirm the potential of high oestrogen concentration in the LF phase to promote endurance performance. Raised oestrogen concentration in the LF phase tended to optimise performance during a time trial at relatively high intensity (Chapter 6), where carbohydrate utilisation predominates as a primary energy source. Therefore it was speculated that oestrogen‘s ability to increase contraction-stimulated glucose uptake may promote high intensity endurance exercise by increasing the availability of the “fuel-of-choice” (i.e. carbohydrate). Studies should be conducted to compare the rate of plasma glucose oxidation at these moderate-to-high intensities during various menstrual phases. Previous studies have only considered glucose kinetics and indirect estimates of plasma glucose oxidation based on glucose Rd during moderate intensities and have not included the LF phase. The exercise intensity is

an important determinant of the metabolic affects of oestrogen because oestrogen appears to have the potential to promote both fat oxidation (Campbell and Febbraio 2001) and glucose utilisation (Campbell and Febbraio 2002) depending on the metabolic stimulus.

7. If the best performance in the LF phase is related to increased capacity to utilise plasma glucose, do these benefits persist with carbohydrate supplementation during the exercise session?

Future studies need to investigate whether the influence of menstrual phase (in particular the LF phase) on exercise performance still persists with carbohydrate supplementation during exercise, as most athletes consume energy drinks at periodic intervals throughout sports events. That is, the influence of oestrogen may be of less significance when plasma glucose levels are continually being replenished during exercise by exogenous supplements, instead of relying on endogenous systems to maintain these levels, as oestrogen may act partly by increasing the availability of hepatic glucose stores (Campbell and Febbraio 2002). While one study did consider carbohydrate supplementation during exercise on time trial performance in the follicular versus luteal phase (Campbell et al. 2001), the E/P ratio in the LP in that study was very low, thus suppressing the actions of oestrogen.