CHAPTER 2

Effect of menstrual phase on the acetate correction factor used in metabolic tracer studies

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Effect of Menstrual Phase on the Acetate Correction Factor Used in Metabolic Tracer Studies

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Mots-clés: *isotopes du carbone, femmes, hormones ovariennes, exercice physique, oxydation de substrats*

Abstract/Résumé

The acetate correction factor is used to account for retention of carbon label in exchange reactions of the tricarboxylic acid cycle in studies estimating free fatty acid oxidation with carbon-labeled tracers. Previous evidence indicates that substrate utilisation and metabolic rate vary across the menstrual cycle, which may alter the correction factor. We therefore derived the acetate correction factor for each of three menstrual phases (early follicular [EF], late follicular [LF], and midluteal [ML] phase) from the fractional recovery of $^{13}CO_2$ from a constant infusion of sodium-[1-^{13}C]acetate during 90 min of submaximal exercise (60% $\dot{V}O_2$max) in sedentary eumenorrhoeic women. There was no difference in the correction factor between the EF and LF or the LF and ML phases, but the correction factor derived in the ML phase was significantly lower than in the EF phase ($p < 0.05$). Neither energy expenditure nor whole body substrate utilisation during exercise varied significantly between menstrual phases and therefore cannot explain the observed difference in the correction factor. The lower correction factor in the ML phase, compared to the EF phase, would result in only a small increase of ~6% in the calculated plasma free fatty acid oxidation rate.

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Dans les études évaluant l'oxydation des acides gras libres à l'aide de traceurs du carbone marqué, l'acétate est un facteur de correction utilisé pour prendre en compte la rétention de carbone marqué dans les réactions de substitution du cycle de l'acide citrique. D'après des études antérieures, l'utilisation de substrats et l'activité métabolique varient au cours du cycle menstruel, ce qui peut modifier le facteur de correction. Dans cette étude, nous définissons donc le facteur de correction pour chacune des trois phases du cycle menstruel (folliculaire initiale [EF], folliculaire terminale [LF], et mi-lutéale [ML]) en analysant la portion récupérée de $^{13}CO_2$ au cours d'une infusion constante d'acétate-[1-^{13}C] de sodium lors d'un exercice sous-maximal (60% $\dot{V}O_2max$) d'une durée de 90 min accompli par des femmes euménorrhéiques et sédentaires. Le facteur de correction ne diffère pas entre EF et LF, et entre LF et ML, mais il est significativement plus faible ($p < 0,05$) en ML qu'en EF. La dépense énergétique et l'utilisation de substrats par l'organisme pendant l'exercice ne varient pas significativement d'une phase à l'autre; la modification observée du facteur de correction demeure donc inexpliquée. En admettant un facteur de correction plus faible durant ML comparativement à EF, on n'observerait qu'une légère augmentation du taux d'oxydation calculé (~6%) des acides gras plasmatiques.

Introduction

The dynamics of fat utilisation in women during exercise during the various menstrual phases has not been extensively studied, yet the availability of stable isotope-labeled tracers allows for the design of comprehensive studies of metabolism in women. With such studies, however, there is a methodological problem due to the incomplete recovery of carbon-labeled free fatty acid tracers from cellular oxidation due to variable retention of the label in the products of exchange reactions of the tricarboxcylic acid (TCA) cycle, i.e., glutamate/glutamine, aspartate, and pyruvate/lactate (Sidossis et al., 1995b). The extent of label retention is determined by the so-called acetate correction factor, which must be derived under the conditions of each experimental intervention and subsequently applied to the calculation of plasma free fatty acid (FFA) oxidation (Sidossis et al., 1995a). The correction factor is based on a validated assumption that all infused acetate is converted to acetyl CoA and enters the TCA cycle as if it were derived from the β oxidation of FFA (Sidossis et al., 1995b).

During exercise the acetate correction factor is dependent on energy expenditure adjusted for fat free mass and the respiratory exchange ratio (RER) (Schrauwen et al., 2000). Therefore the correction factor should ideally be derived concurrently with the rate of free fatty oxidation by administering a free fatty acid and acetate tracer labeled with different isotopes of carbon. However, when studying fat metabolism in women, the correction factor will have to be determined in an independent trial from the main experimental intervention, as carbon-13, being the only stable isotope of carbon, would be the isotope of choice for both the estimation of plasma FFA oxidation and the correction factor. Thus any factor that may alter energy expenditure and the RER between the trials when the correction factor is derived and the trials determining fat oxidation will need to be considered in designing an experimental trial.

Most studies have found basal metabolic rate to be higher in the luteal phase (LP) of the menstrual cycle compared to the follicular phase (FP) (Matsuo et al.,

1999; Melanson et al., 1996). Furthermore, one study has reported total energy expenditure at a given exercise intensity to be greater in the LP compared to the FP (Hessemer and Brück, 1985); however, others have found no difference in energy expenditure during exercise between menstrual phases (Dombovy et al., 1987; Hackney et al., 1991; Jurkowski et al., 1981). Nonetheless, a higher energy expenditure would result in a greater rate of TCA cycling without a corresponding increase in the rate of exchange reactions, leading to less label retention with greater acetate recovery (Sidossis et al., 1995a, 1995b).

Moreover, whole body substrate utilisation based on RER may vary according to menstrual phase (Dombovy et al., 1987; Hackney et al., 1991; Zderic et al., 2001), although these findings are not consistently observed (De Souza et al., 1990; Kanaley et al., 1992). Nonetheless, RER has been reported to be lower during rest in the midluteal (ML) phase (Nicklas et al., 1989) and during exercise in the ML (Dombovy et al., 1987, Zderic et al., 2001) or late follicular (LF) phase (Hackney et al., 1991) when compared to the midfollicular (MF) phase. Lower RER suggests greater whole body fat utilisation that appears to be coincident with high estrogen levels (D'Eon et al., 2002). Progesterone antagonizes this effect of estrogen; however, a high enough ratio of estrogen to progesterone will negate the negative effect of progesterone (D'Eon et al., 2002; Hatta et al., 1988).

Estrogen acts to increase fat use directly by increasing the activity of key enzymes involved in fatty acid transport and oxidation (Campbell and Febbraio, 2001) and by increasing FFA availability (Ellis et al., 1994). Estrogen promotes carbohydrate sparing by suppressing glycogenolysis (D'Eon et al., 2002; Hackney, 1999) and generally whole body glucose flux (Ruby et al., 1997). Based on previous animal and human studies, D'Eon et al. (2002) suggest that an elevated estrogen concentration causes tissue responses to favor the lipolytic vs. the glycogenolytic action of epinephrine.

Schrauwen et al. (2000) explained how a change in RER would influence the acetate correction factor. That is, a high respiratory quotient, or greater carbohydrate oxidation, would increase acetate recovery as carbohydrate oxidation produces 21.8 kJ per litre of CO_2 whereas fat produces 28.9 kJ per litre of CO_2. Therefore, for a given amount of energy expenditure, less CO_2 is liberated when energy is derived from fat oxidation vs. glucose oxidation. A greater carbohydrate oxidation rate thus implies a smaller chance of carbon label being retained in the TCA cycle, and consequently a higher acetate recovery (Schrauwen et al., 2000).

Therefore we proposed that menstrual phase could affect the acetate correction factor, thus the aim of this study was to compare the acetate correction factor in young eumenorrhoeic women during prolonged moderate exercise during three menstrual phases: early follicular (EF), late follicular (LF), and midluteal (ML).

Methods

SUBJECTS

Young, healthy, eumenorrhoeic women ($N = 5$) were recruited. All were selected on the basis that they were sedentary, i.e., did not participate in any sport or follow a regular training program and exercised less than 2 hours per week. Sedentary subjects were selected for this study since exercise training alters the metabolic

response to exercise (Friedlander et al., 1998), and we wanted specifically to elucidate the effects of the menstrual cycle on the correction factor without any additional effects that may result from training. Therefore we recruited subjects who rarely or never exercise. Their average age, height, mass, and body mass index (BMI) was 24 ± 1 years, 55 ± 9.5 kg, 158.1 ± 6.7 cm, and 22.1 ± 2.3 $kg \cdot m^{-2}$, respectively. All gave written consent to participate in the study after they were informed about the purpose and possible risks. Ethical clearance for the study protocol was obtained from the Committee for Research on Human Subjects at the University of the Witwatersrand.

PRELIMINARY SCREENING

Basal body temperature should increase by an average of 0.3 °C post-ovulation due to elevated circulating progesterone levels. Therefore, all subjects recorded their oral temperature shortly after awakening each morning for one complete menstrual cycle using a digital thermometer accurate to 0.1 °C (Vital sign VS-10, Soar Corp., Nagoya, Japan). Day 1 of the menstrual cycle corresponds with the first day of menstruation. The day of ovulation was predicted by measuring urine luteinising hormone (LH) concentration using a home ovulation test (Clearplan, Unipath Ltd., Bedford, England).

$\dot{V}O_2$max was determined in the follicular phase (Days 2–9) on a stationary bicycle ergometer (Medifette, Medical fitness equipment, Maarn, Netherlands) using a discontinuous incremental protocol. Subjects exercised for 3-min bouts at an exercise intensity that began at 80 Watts and increased in increments of 20 W, interspersed by recovery periods of approximately 10–20 min. They were allowed to rest for as long as they felt it necessary and until their heart rate dropped below 100 $beats \cdot min^{-1}$. Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) rates were measured using open-circuit spirometry (Oxycon-4, Mijnhardt, Bunnik, Netherlands). The metabolic analyser was zeroed using nitrogen gas and was calibrated using room air and a known gas mixture (5% CO_2 and 16% O_2). $\dot{V}O_2$max was considered to have been attained when $\dot{V}O_2$ varied by less then 1.5 $ml \cdot kg^{-1} \cdot min^{-1}$ between successive workloads.

We chose a discontinuous over a continuous protocol because sedentary persons who are not accustomed to exhaustive exercise tend to find a discontinuous protocol more tolerable, whereas they often fatigue prematurely, i.e., before reaching a plateau in $\dot{V}O_2$, when using a continuous protocol that produces only a $\dot{V}O_2$peak value. No significant difference in $\dot{V}O_2$max has been reported when comparing continuous and discontinuous protocols (Duncan et al., 1997).

EXPERIMENTAL PROTOCOL

The acetate correction factor was derived during 90 min of exercise at 60% $\dot{V}O_2$max during three phases of the menstrual cycle: early follicular, 2 to 7 days after the onset of menses; late follicular, 2 days before ovulation to the day of ovulation; and midluteal, 4 to 10 days after ovulation. A resting blood sample was taken on the day of each trial so that serum progesterone and estrogen concentration could be measured (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA), thereby confirming the woman's menstrual phase. All samples from each subject were

analysed in the same assay, and intra-assay coefficient of variation was 5.3% and 4.7% for estrogen and progesterone, respectively. The accepted range of ovarian hormones for each menstrual phase included 37–220 pmol·L^{-1} and <3 nmol·L^{-1} for the EF phase; 360–1377 pmol·L^{-1} and <5 nmol·L^{-1} for the LF phase; and 220–955 pmol·L^{-1} and >10 nmol·L^{-1} for the ML phase for estrogen and progesterone, respectively (Coat-A-Count). Each subject performed all three trials at the same time of day. The order of trials was randomised with respect to menstrual phase. The women completed the three trials over two to three menstrual cycles.

For 48 hours before the first trial, each subject kept a record of all her meals and was asked to follow the same diet before the subsequent trials. Two hours before each trial, subjects consumed a packed meal (2,062 kJ) consisting of a 175-ml yogurt, 175-ml orange juice, and 30-g granola bar providing a total of 74 g of carbohydrate, 14 g of fat, and 12 g of protein. Preparation for the experimental session from the time of the subject's arrival to the beginning of exercise took approximately 1 hr, and therefore subjects were approximately 3 hrs postabsorptive when they began the exercise.

At the start of each trial, height and body mass were recorded and a 20-G catheter was inserted into a forearm vein. A priming dose of 5.5 μmol·kg^{-1} $NaH^{13}CO_3^-$ (98% enriched, Isotec Inc., Miamisburg, OH) in 0.01N NaOH and 30 μmol.kg^{-1} Na^+[1-^{13}C]acetate (99% enriched, Isotec Inc.) in water was injected, followed by a continuous infusion of Na^+[1-^{13}C]acetate in 0.9% saline at a rate of 1 μmol·kg^{-1}·min^{-1}. All solutions were freshly prepared under a laminar flow hood not more than a week before the day of the trial and were stored at 2–8 °C. The tracers were weighed to 5 decimal places and made up to the correct volume using standard volumetric flasks. The solutions were sterilized by micropore filtration (0.22 μm) and passed a standard test (LAL, Gel clot test, sensitivity 0.06 EU) to ensure that they were endotoxin-free before use (Infectious control lab, SAIMR, Johannesburg, RSA). The $NaH^{13}CO_3^-$ and Na^+[1-^{13}C]acetate prime were administered to reduce the time required to equilibrate the respective pools into which the carbon-13 label passes before it appears in the subject's expired air.

The women exercised for 90 min at 60% $\dot{V}O_2$max on the same cycle ergometer as used for the $\dot{V}O_2$max tests. The start of the Na^+[1-^{13}C]acetate infusion coincided with the onset of exercise. Expired air samples were collected in 2-L urine bags at rest prior to infusion, and every 15 min during exercise after beginning the infusion. The CO_2 was extracted from the expired air samples by cryodistillation, and the isotopic ratio ($^{13}CO_2/^{12}CO_2$) of the samples was determined by isotope ratio mass spectrometry (MS20, Kratos, UK) and normalised according to the NBS-20 international standard. $\dot{V}O_2$ and $\dot{V}CO_2$ were recorded at rest and every 15 min throughout the 90 min of exercise using the same metabolic analyser as described in the $\dot{V}O_2$max protocol.

The $^{13}CO_2$ enrichment (ECO_2), measured as atom percent excess of the samples, was determined as follows:

$$ECO_2 = [(r_{sa} - r_{ref}) \times 100] / (r_{sa} + 1)\ (r_{ref} + 1)$$

Where r_{sa} and r_{ref} are the isotopic ratios of the sample and reference gas, respectively. The fractional recovery of $^{13}CO_2$, the acetate correction factor, was then calculated using the following equation (Sidossis et al., 1995a):

$$\text{Fractional recovery} = [(ECO_{2Sa} - ECO_{2Bgrd}) / 100 \times \dot{V}CO_2] / F$$

Where ECO_{2Sa} and ECO_{2Bgrd} represent $^{13}CO_2$ enrichment as atom percent excess of the test sample and background (0 min) sample, respectively. $\dot{V}CO_2$ represents carbon dioxide production rate in $\mu mol \cdot kg^{-1} \cdot min^{-1}$, and F represents the infusion rate in $\mu mol \cdot kg^{-1} \cdot min^{-1}$. Fractional recovery of $^{13}CO_2$ will be referred to hereafter as the acetate correction factor.

Energy expenditure (EE) for a given 15-min time interval (kJ) during exercise for each menstrual phase was calculated using the following equation (Wolfe, 1992):

$$EE = (16.49\ \dot{V}CO_2 / RER + 4.63\ \dot{V}CO_2) \times 15$$

Where $\dot{V}CO_2$ and $\dot{V}O_2$ are in $L \cdot min^{-1}$ and the sum of all 15-min time intervals was calculated to give total energy expenditure during the 90 min of exercise. Whole body rates of carbohydrate (CHO) and fat oxidation ($g \cdot min^{-1}$) were calculated from $\dot{V}CO_2$ and $\dot{V}O_2$ measurements using the following equations (Peronnet and Massicotte, 1991):

$$\text{CHO oxidation} = 4.585VCO_2 - 3.226\dot{V}O_2$$
$$\text{Fat oxidation} = 1.695VO_2 - 1.701\dot{V}CO_2$$

and was multiplied by 15 for each respective time interval between measurements and then summed together for total CHO and fat oxidised.

STATISTICAL ANALYSIS

Due to missing data points for Subjects 1 and 4, we used a paired *t*-test with the Bonferroni correction for multiple comparisons to compare the value for the correction factor averaged over the last 30 min of exercise between menstrual phases. The coefficient of variation (CV) was calculated as *SD*/mean × 100. A one-way repeated-measures ANOVA was used to compare energy expenditure and total carbohydrate and fat utilised during exercise between menstrual phases. All values are presented as mean ± 1 *SD*, and $p < 0.05$ was accepted as significant.

Results

AEROBIC CAPACITY AND MENSTRUAL CYCLE CHARACTERISTICS

As a group, the subjects' average $\dot{V}O_2max$ was 30.3 ± 1.7 $ml \cdot kg^{-1} \cdot min^{-1}$, with a CV of 5.6%. Thus there was very little interindividual variability in $\dot{V}O_2max$. The mean $\dot{V}O_2max$ value is typical of other published values for young sedentary women (Dombovy et al., 1987; Schoene et al., 1981). Average exercise intensity for all three trials was 59.0 ± 2.1% $\dot{V}O_2max$. As all subjects had a similar $\dot{V}O_2max$, there was little variation in the $\dot{V}O_2$ at which they exercised (17.9 ± 1.5; 15.7–19.9 $ml \cdot kg^{-1} \cdot min^{-1}$).

Resting serum estrogen and progesterone concentrations were within the expected range for each menstrual phase in all trials (Table 1) except for one subject. During the LF phase trial of this subject, the progesterone value exceeded the acceptable upper limit of 5 $nmol \cdot L^{-1}$. Hence her LF trial has been excluded from all data analysis.

Table 1 Resting Serum Concentration of Female Hormones

Menstrual phase	Estrogen (pmol/L)	Progesterone (nmol/L)
Early follicular	139 ± 61	1.7 ± 0.3
Late follicular	1271 ± 913	4.0 ± 1.3
Midluteal	770 ± 128	54.1 ± 19.7

Table 2 Carbon-13 Enrichment (atom % excess) of Expired Air Samples From Onset of Labeled Acetate Infusion During 3 Menstrual Phases

	Time (min)					
	15	30	45	60	75	90
Early follicular	0.079 ±0.015	0.077 ±0.009	0.081 ±0.005	0.086 ±0.004	0.090 ±0.007	0.087 ±0.006
Late follicular	0.069 ±0.005*	0.074 ±0.002#	0.079 ±0.003	0.080 ±0.005	0.085 ±0.001	0.084 ±0.004
Midluteal	0.071 ±0.013	0.072 ±0.012	0.074 ±0.009	0.080 ±0.006	0.082 ±0.005	0.085 ±0.004

Signif. different: *From 45, 60, 75, and 90-min LF, $p < 0.01$; #From 75-min LF, $p < 0.05$

ISOTOPIC EQUILIBRIUM

The carbon-13 enrichment of expired air achieved equilibrium rapidly during exercise in all menstrual phases (Table 2). No change was noted in breath enrichment at any time during the EF and ML trials, and steady state was attained by 45 min in the LF trials.

ACETATE CORRECTION FACTOR AND MENSTRUAL PHASE

The acetate correction factor (fractional recovery of $^{13}CO_2$) remained fairly constant over the 90 min of exercise during all menstrual phases. An average correction factor for each menstrual phase was calculated from the last 30 min of exercise (Table 3). While the correction factor did not differ significantly between the EF and LF phases or the LF and ML phases, it was significantly lower in the ML phase when compared to the EF phase (mean difference of 0.0175 ± 0.005 and $t = 7$, $p = 0.006$).

Table 3 Acetate Correction Factor Averaged Over Last 30 Min of Exercise

Subject	Menstrual phase			Subjects' average
	Early follicular	Late follicular	Midluteal	
1	0.59 ± 0.03	—	0.58 ± 0.01	0.59 ± 0.01
2	0.56 ± 0.01	0.56 ± 0.00	0.54 ± 0.04	0.55 ± 0.02
3	0.53 ± 0.05	0.54 ± 0.02	0.51 ± 0.05	0.53 ± 0.02
4	0.60 ± 0.02	0.58 ± 0.02	—	0.59 ± 0.02
5	0.67 ± 0.04	0.60 ± 0.02	0.65 ± 0.04	0.64 ± 0.04
Group avg.	0.59 ± 0.05	0.57 ± 0.02	0.57 ± 0.06 #	0.58 ± 0.04

Note: Data from Subject 1's LF phase has been excluded due to progesterone concentration being out of range for this menstrual phase. Subject 4 does not have an ML phase datum point, due to contamination of the 60- to 90-min air samples for this phase.

Correction factor derived in ML phase was significantly lower than in EF phase, $p < 0.05$.

Although the LF and ML group averages were the same, 0.57 ± 0.02 and 0.57 ± 0.06, respectively, a significant difference was only identified between the EF and ML phase due to the repeated-measures design of the study that observed a consistent drop in the correction factor in all subjects from the EF to the ML phase (see Table 3). The average CV for the correction factor between menstrual phases was 3.1 ± 1.6% within subjects, and 7.4 ± 2.7% between subjects.

ENERGY EXPENDITURE AND WHOLE BODY SUBSTRATE UTILISATION

Total energy expenditure during exercise did not differ significantly between menstrual phases and was 1827 ± 413 kJ, 1918 ± 403 kJ, and 1864 ± 431 kJ for the EF, LF, and ML phases, respectively. Whole body substrate utilisation during exercise estimated from indirect calorimetry did not change across menstrual phases (Figure 1). Total carbohydrate oxidised was 66.9 ± 21.1 g, 62.2 ± 13.2 g, and 74.1 ± 22.9 g, while total fat utilised was 18.0 ± 4.5 g, 20.5 ± 10.7 g, and 17.8 ± 11.7 g for the EF, LF, and ML trials, respectively.

Discussion

The acetate correction factor determines the extent of carbon label retention in exchange reactions of the TCA cycle in studies that administer carbon tracers to quantify substrate oxidation (Sidossis et al., 1995a). The current study assessed the influence of the menstrual cycle on this correction factor. Interestingly, we have found that the acetate correction factor derived during submaximal exercise in eumenorrhoeic sedentary women is consistently lower in the ML phase than in the EF phase. However, there was no difference in the correction factor between the LF and EF phase or between the LF and ML phase.

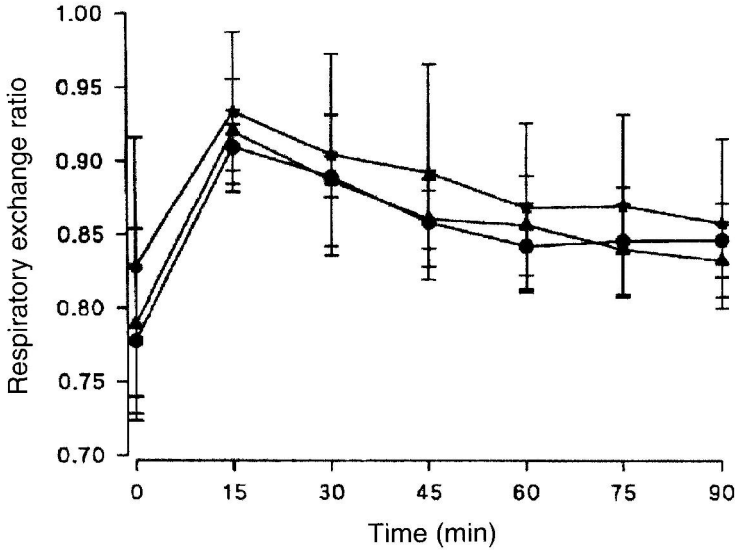

Figure 1. Respiratory exchange ratio at rest (0 min) and during 90 min of submaximal exercise during early follicular (circle), late follicular (triangle, and midluteal (star) phases of menstrual cycle. No significant difference noted between phases at any time point.

The lower correction factor in the ML phase would increase the estimation of plasma FFA oxidation rate by 6% compared to the EF phase. Thus the lower correction factor in the ML phase is not expected to cause a physiologically significant effect on plasma FFA oxidation rate. However, when comparing FFA oxidation rates between menstrual phases, it may be advisable to use a correction factor specifically derived for each menstrual phase, as some studies (Dombovy et al., 1987; Hackney et al., 1991; Zderic et al., 2001) have shown that whole body fat utilisation is augmented in the menstrual phases characterised by high ovarian hormones. Failure to apply menstrual phase-specific acetate correction factors in such a study may increase the chance of a type I error.

It is well known that the acetate correction factor is dependent on the duration of acetate infusion, and although isotopic equilibrium can be achieved over a relatively short period (approx. 90 min at rest and 60 min during exercise), when infusing for much longer periods (15 hrs at rest) a higher plateau is eventually achieved (Mittendorfer et al., 1998). Thus the first steady state is a "pseudo plateau" and would correspond to the isotopic equilibrium of the primary acetate pool, while the ultimate true plateau only occurs much later when the label retained in the TCA cycle-exchange reactions also establishes equilibrium within these secondary oxidisable pools. It is appropriate, however, to derive a correction factor based on the pseudo plateau, since the carbons from a free fatty acid tracer entering the TCA cycle as acetyl CoA would follow the same pattern (Mittendorfer et al., 1998).

Efforts to establish equilibrium by prolonged acetate infusion at rest prior to infusion, and measurements during exercise, will allow time for sufficient accumulation of label in the secondary oxidisable pools. This may lead to overestimation of acetate recovery during exercise, due to the appearance of labeled carbon in breath samples not only from the direct oxidation of acetate but also from the secondary oxidisable pools (Trimmer et al., 2001).

The correction factor, as previously described, is largely dependent on energy expenditure, percent body fat, and the RER (Schrauwen et al., 2000). However, unlike the reports of Hessemer and Brück (1985), we and others (Dombovy et al., 1987; Hackney et al., 1991; Jurkowski et al., 1981) have found energy expenditure during submaximal exercise to be unaltered by menstrual phase. In addition, in the current study we found whole body substrate utilisation based on the RER to be similar across menstrual phases during exercise, a finding supported by others (De Souza et al., 1990; Hessemer and Brück, 1985; Kanaley et al., 1992).

Most studies that report a difference in whole body substrate utilisation due to varying ovarian hormone concentrations have observed these responses in subjects who exercise after an overnight fast (Campbell et al., 2001; Zderic et al., 2001). For example, significant differences in RER between the follicular and luteal phases disappeared when subjects received a carbohydrate energy drink during exercise (Campbell et al., 2001). However, a few studies that employed a 3- to 4-hr postprandial period have found RER to vary significantly during exercise, depending on ovarian hormone concentrations (D'Eon et al., 2002; Dombovy et al., 1987). In addition, other studies conducted after an overnight fast have reported no difference in whole body substrate utilisation during exercise between menstrual phases (Horton et al., 2002; Kanaley et al., 1992). Therefore, although the preexercise meal in the current study most certainly would have influenced whole body substrate utilisation and may be partially responsible for the similar RER observed between menstrual phases, other factors must also be considered.

The magnitude of the increase in estrogen between menstrual phases, and the relative difference between estrogen and progesterone, have been suggested as being an important determinant of substrate utilisation (D'Eon et al., 2002). In addition, measurements of substrate utilisation based on RER recordings must be interpreted with caution, as various metabolic systems when stimulated will alter RER and lead to a false estimate of substrate utilisation. For example, estrogen administered to male rats in pharmacological doses stimulated net triacylglycerol synthesis during exercise in type I skeletal muscle fibres (Ellis et al., 1994). Triacylglycerol synthesis will elevate RER readings and thus cause carbohydrate utilisation to be overestimated. Therefore crude RER measurements could possibly conceal small changes in the ratio of carbohydrate to fat oxidised.

Moreover, our small sample size may have reduced the chances of detecting significant variances in whole body substrate utilisation. Nonetheless, convincing evidence suggests that estrogen promotes fat use during prolonged submaximal exercise (D'Eon et al., 2002; Hatta et al., 1988). However, these studies have emphasized that such estrogen-induced responses are dependent on the ratio of estrogen to progesterone in the ML phase, as progesterone is reported to have antilipolytic effects (D'Eon et al., 2002; Hatta et al., 1988). An increase in fat oxidation in the ML phase would have explained the decrease in acetate recovery (Schrauwen et al., 2000).

Alternatively, the decrease in the correction factor during the ML phase may be related to changes in protein metabolism during the menstrual cycle with concomitant exercise. Lamont et al. (1987) found total protein catabolism during and after exercise to be elevated during the ML phase vs. during the EF phase. The amino acids released from protein catabolism can be degraded to form ketoacids via transamination reactions and used as substrates in gluconeogenesis or oxidation (Wolfe, 1992). Most transamination reactions are catalized by either alanine or glutamate transaminase (i.e., the alpha ketoglutarate-glutamate exchange reaction) (Wolfe, 1992). In addition, the alpha ketoglutarate-glutamate transamination pathway is responsible for most of the label retention from a carbon-labeled acetate infusion (Schrauwen et al., 1998; Sidossis et al., 1995b). Hence the lower fractional recovery from a labeled acetate infusion in the ML phase in this current study could be due to an increased flux through the alpha ketoglutarate-glutamate exchange reaction as a direct result of the previously reported (Lamont et al., 1987) augmented protein catabolism that occurs during this menstrual phase.

The average acetate correction factor for each menstrual phase determined in the current study (0.57–0.59) is lower than would be estimated from a previously published model based on exercising $\dot{V}O_2$ relative to body mass (0.8) (Sidossis et al., 1995a). The discrepancy between the predicted acetate correction factor (Sidossis et al., 1995a) and the value we derived might be attributable to gender differences. The previous study (Sidossis et al., 1995a) used a mixed-ender group of predominately male subjects whereas our subject group was more homogenous and comprised only sedentary young women.

Men have a significantly greater acetate recovery during exercise than women, and this is related to differences in body composition and RER (Schrauwen et al., 2000). In addition, the training status of the subjects in the previous study may have been greater than for our group of sedentary women, as indicated by the difference in aerobic capacity of the two study groups: 43 $ml \cdot kg^{-1} \cdot min^{-1}$ in the previous study vs. 30 $ml \cdot kg^{-1} \cdot min^{-1}$ in the current study. Training might increase the ratio of TCA cycle enzymes to enzymes that make up subsidiary pathways involved in the exchange reactions, and thereby reduce label fixation at a given $\dot{V}O_2$ and increase acetate recovery. It must also be noted that the preexercise meal could have influenced acetate recovery by elevating RER, and therefore we must emphasize that the correction factor derived in our study is specific to the experimental conditions imposed on our subjects.

In summary, we have found that the acetate correction factor during moderate exercise is altered by menstrual phase. The lower correction factor during exercise in the ML phase compared to the EF phase would increase the estimation of plasma FFA oxidation by approximately 6%. Therefore, studies evaluating the effects of the menstrual cycle on rate of plasma FFA oxidation using carbon tracers can increase the sensitivity of their comparison by controlling for the variability in the acetate correction factor between menstrual phases.

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