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Metabolic Responses to Exercise

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**Menstrual Phase Effects on Fat and Carbohydrate Oxidation During Prolonged Exercise in Active Females**

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<sup>2</sup>Department of Human Performance Studies, University of Alabama, Tuscaloosa, AL; <sup>3</sup>Department of Physical Education, Tennessee Wesleyan College, Athens, TN; <sup>4</sup>College of Nursing, University of Alabama, Tuscaloosa, AL.

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CANDI D. ASHLEY, PHILIP BISHOP, JOE F. SMITH, PAUL RENEAU AND CINDY PERKINS. **Menstrual Phase Effects On Fat And Carbohydrate Oxidation During Prolonged Exercise In Active Females.** JEPonline, 3(4):67-73, 2000. The purpose of this study was to examine between-phase effects of resting levels of estradiol (E<sub>2</sub>) on fat and carbohydrate oxidation during a 60 minute submaximal exercise bout. Ten physically active females performed two 60-minute treadmill runs at an intensity of 70% of maximal aerobic capacity (VO<sub>2</sub>max) once each in the follicular phase (FP) and luteal phase (LP) of the menstrual cycle. Resting levels of E<sub>2</sub> were assessed prior to exercise. Participants also completed four-day food and activity diaries. Data analysis revealed a significant between-phase difference (p>0.05) in E<sub>2</sub> and respiratory exchange ratio (RER) between the FP and LP runs. Further, there was no relationship between E<sub>2</sub> and RER in either the FP or LP. However, there were significant correlations between FP RER and average protein intake (r=0.68; p<0.05). In conclusion, our results suggest that there was a between-phase difference in RER concomitant with a between-phase difference in resting E<sub>2</sub> levels. However, it appears that the difference in fat oxidation is not related to differences in E<sub>2</sub> between the FP and LP of the menstrual cycle.

Key words: estrogen, substrate oxidation

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**INTRODUCTION**

It has been postulated that estradiol (E<sub>2</sub>) may have enhance fat metabolism (1). A number of studies have examined the relationship between menstrual cycle phase and substrate metabolism at rest and have found higher resting levels of free fatty acids in the blood (2), and a lower respiratory exchange ratio (RER) in the luteal phase (LP) when E<sub>2</sub> is elevated (2).

During exercise, there appears to be a tendency toward greater endurance and decreased lactate concentration (3) as well as enhanced fat metabolism in the LP (4). However, the results of Hackney et al. (5) suggest a greater fat oxidation and utilization during ovulation than during the mid-LP. Further, most researchers have found no significant menstrual phase or menstrual status effect on fat metabolism during exercise (6,7,8).

Based on the aforementioned studies, the influence of E<sub>2</sub> on fat metabolism has not been fully established. There are several possible reasons for the discrepancies in prior research. A number of researchers have examined menstrual effects of metabolism in healthy participants with "normal menstrual cycles" (5,7,9), while other researchers have examined between-phase differences in substrate metabolism in eumenorrhic participants (4,6,9,10). Researchers have also utilized participants of varied training levels. There appears to be a direct relationship between training and menstrual dysfunction (11,12,13). Highly trained females may not

experience characteristic fluctuations of hypothalamic-pituitary-ovarian hormones over the menstrual cycle. Further, several authors employed an incremental exercise protocol (3,4,8) or had participants perform multiple submaximal exercise bouts in one testing session (8,13) which might affect substrate availability in subsequent exercise bouts. In addition, nutritional status and diet may have an effect on substrate utilization during exercise, however, most researchers did not specify or control the nutritional status of participants (2,3,4). Berend et al. (15) reported that a carbohydrate-rich diet, which is common among athletes, appears to negate between-phase differences in the lactate response to exercise. Therefore, the primary purpose of this study was to examine the relationship between resting levels of  $E_2$  and fat metabolism during a prolonged submaximal exercise bout in physically active females.

## **METHODS**

### **Participants**

Ten physically active females with regular menstrual cycles, 26-33 days in length served as participants. All participants were nulliparous and were not taking oral contraceptives presently or in the past six months. Any volunteer who was diabetic, was pregnant, or had reason to believe she might have been pregnant was excluded from the study. Prior to data collection, procedures were approved by the university Institutional Review Board for the protection of human subjects. All participants provided written informed consent.

### **Maximal aerobic capacity ( $VO_2\max$ )**

Participants first performed a maximal treadmill test to exhaustion on a Quinton Model 640 treadmill (Quinton Instruments Co., Seattle, Washington) at a constant speed (between 2.35 and 3.76 m/sec) with grade increasing 2% every 2 minutes. Expired gases were collected using a Rayfield Metabolic Measurement System (Waitsfield, Vermont) with an Ametek S-3A/I oxygen ( $O_2$ ) analyzer (Pittsburgh, Pennsylvania), SensorMedics LB2 carbon dioxide ( $CO_2$ ) analyzer (Anaheim, California), and a Rayfield Equipment dry gas meter. The metabolic measurement system was zeroed and calibrated with a gas of known  $O_2$  and  $CO_2$  concentration prior to each test. Expired gases were measured continuously and recorded each minute during the exercise test. Heart rate (HR) was monitored throughout the test using a Polar HR monitor. Rating of perceived exertion (RPE) using Borg's scale for perceived exertion was also monitored throughout the test. Attainment of  $VO_2\max$  was determined if any two of the following criteria were met: a) plateauing of  $VO_2$  despite an increase in workload (16), b)  $RER > 1.1$  (17) and c) HR within 10 beats of age predicted maximum heart rate.

### **Body fat**

Skinfold measurements of the triceps, suprailliac, and thigh were taken prior to the assessment of  $VO_2\max$ . Percent body fat was estimated using the generalized equations established by Jackson et al. (18).

### **Submaximal treadmill runs and estradiol collection/analysis**

Subjects performed two submaximal treadmill runs, once in the early to mid-follicular phase (FP) (4-7 days after the onset of menstruation) and once in the LP (7-12 days after ovulation). Menstrual phase was determined via menstrual history and participants' daily records of basal body temperature kept over a 2 to 3 month period prior to testing. A  $0.3^\circ C$  rise in basal body temperature was used as indication of ovulation (4,8,10). The order of testing was randomized with seven participants completing the first submaximal run in the LP, while eight participants completed the first submaximal run in the FP.

Due to potential effects on metabolism, participants were required to refrain from alcohol and caffeine consumption as well as any exercise for at least 24 hours prior to the submaximal tests, and reported to the Human Performance laboratory after a four-hour fast. Upon entering the laboratory, subjects were weighed and rested quietly for 30 minutes in a recumbent position.

After the initial rest period, venipuncture was used to collect blood from the superficial arm vein of each subject. Blood was immediately centrifuged and refrigerated. Radioimmunoassay (RIA) procedures to

determine plasma E<sub>2</sub> levels were performed by LabSouth, Inc. (Birmingham, AL) using Diagnostic Systems Laboratory (Webster, TX) Estradiol RIA Kit #4400. Assays were calibrated prior to each run of 12 to 15 samples at six levels (0, 20, 50, 250, 750, 1500, and 3000 pg/ml). In addition, two levels of controls ranging from 45 to 1800 pg/ml were run with each group of specimens. Sensitivity of the assays was 8 pg/ml at the 95% confidence limit. Intra- and inter-assay coefficients of variation were 10.7 and 8.9%, respectively.

After blood collection, subjects performed a 60-minute treadmill run at approximately 70% VO<sub>2</sub>max. Expired gases were collected continuously and HR was recorded every 5 minutes. The metabolic measurement system was calibrated prior to gas collection as well as every 20 minutes during the submaximal exercise bout. During the calibration procedures, participants were given the opportunity to drink water. The RER and VO<sub>2</sub> obtained during the submaximal treadmill run was used in conjunction with the table of the non-protein RQ established by Zuntz (as cited in 19) to determine total kcals expended, as well as fat and carbohydrate utilization (% of kcals from fat and carbohydrate) and oxidation (g of substrate/L O<sub>2</sub> consumed). Values for VO<sub>2</sub> and RER are the mean of the sixty-minute period.

### **Activity and food diaries**

Participants were requested to record all activity including sleep for four days (3 week days and 1 weekend day). Energy expenditure of daily physical activity was analyzed using the Compendium of Physical Activities (20). Each subject also kept a written record of all food and beverages consumed in four typical days (3 week days and 1 weekend day). This log included the day before each submaximal testing session. A registered dietitian provided specific instructions for recording food intake estimating portion. As the intra-subject macronutrient variability was minimal, nutrient intake was assessed as the mean of the four-day food diaries and was analyzed using the Dine System Software Package (Buffalo, NY).

### **Statistical analyses**

Between-phase differences on measures obtained in conjunction with the submaximal runs (weight, E<sub>2</sub>, VO<sub>2</sub>, RER, caloric expenditure, fat and carbohydrate utilization, and fat and carbohydrate oxidation) were examined using dependent t-tests. Differences in nutrient intake the day prior to each submaximal run was analyzed using dependent t-tests. A Dunn-Bonferoni follow-up test was used to control for the number of t-tests performed. Pearson correlation coefficients were generated to analyze the relationships between resting levels of E<sub>2</sub> and substrate oxidation during exercise for each menstrual cycle phase as well as between nutrient intake and RER. An a-priori alpha value of 0.05 was used for all statistical analyses.

## **RESULTS**

### **Subjects**

Subject descriptive data is shown in Table 1. All subjects completed the test protocol during one menstrual cycle. Further, all subjects had been running at least 40 km/wk or performing equivalent aerobic exercise for the past year. Most subjects' primary form of aerobic exercise was running, but swimming, biking, and aerobic dance were also performed. Resting FP E<sub>2</sub> levels were within the acceptable range of 10 to 60 pg/ml and were significantly less than LP E<sub>2</sub> levels ( $p < 0.05$ ) (Table 2). The participants in our study had a diet with appropriate percentages of calories from fat, carbohydrate, and protein (% of Kcals = 18, 66, and 15%, respectively). However, all participants with completed food and activity diaries ( $n=9$ ) exhibited a negative energy balance (EB=-982.00 Kcals). This is not surprising as other researchers have also reported a negative energy balance in physically active females (9, 21).

**Table 1:** Subject descriptive data.

<i>Variable</i>	<i>Mean±SD</i>
<i>Age (yrs)</i>	22.6±4.8
<i>Weight (kg)</i>	59.03±4.57
<i>Max VO<sub>2</sub> (ml/kg/min)</i>	49.50±3.37
<i>Max HR</i>	191.2±6.6
<i>Max RPE</i>	18.3±0.7
<i>Body Fat (%)</i>	20.18±3.42
<i>Daily energy expenditure (Kcals)</i>	2672.2±424.4
<i>Caloric intake</i>	1690.2±499.2
<i>Fat intake (Kcals)</i>	305.0±141.0
<i>Carbohydrate intake (Kcals)</i>	1098.9±314.5
<i>Protein intake (Kcals)</i>	243.4±114.7
<i>Quantity of training (Kcals/week)</i>	4462.4±2098.8

**Table 2:** Resting E<sub>2</sub> levels prior to the submaximal runs.

<i>Subject</i>	<i>FP E<sub>2</sub></i>	<i>LP E<sub>2</sub></i>
<i>1</i>	35	39
<i>2</i>	33	59
<i>3</i>	34	157
<i>4</i>	29	93
<i>5</i>	33	46
<i>6</i>	40	41
<i>7</i>	10	32
<i>8</i>	35	43
<i>9</i>	37	49
<i>10</i>	39	45
<i>Mean±SD</i>	32.3±8.4	60.4±37.9

### Between-phase differences in substrate oxidation

It has been postulated that menstrual cycle phase may have an effect on substrate oxidation during exercise. For our sample, dependent t-tests revealed significant between-phase differences in E<sub>2</sub>, RER, and fat oxidation during the submaximal runs ( $p < 0.05$ ) (Table 3). In addition, while there was no between-phase difference in calorie, protein, and carbohydrate intake the day prior to the submaximal runs, FP fat intake was less than LP fat intake ( $p = 0.05$ ) (Table 4).

**Table 3:** Metabolic responses during submaximal runs.

<i>Variables</i>	<i>Follicular Phase</i>	<i>Luteal Phase</i>
<i>Weight (kg)</i>	59.12±3.83	59.28±3.83
<i>Oxygen Consumption (L/min)</i>	1.99±0.16	1.99±0.13
<i>Respiratory Exchange Ratio</i>	0.90±0.03	0.88±0.03*
<i>Total Kcals expended</i>	588.3±49.3	583.0±41.2
<i>Fat utilization (Kcals)</i>	195.4±46.8	230.6±61.3*
<i>Carbohydrate utilization (Kcals)</i>	384.1±79.7	349.4±73.9

\* Values are significantly different ( $p < 0.05$ ).

**Table 4:** Nutrient intake the day prior to the submaximal runs.

	<i>Follicular Phase</i>	<i>Luteal Phase</i>
<i>Caloric intake</i>	1603.7±643.1	1769.7±508.5
<i>Fat intake (Kcals)</i>	183.3±86.2	381.1±171.7
<i>Carbohydrate intake (Kcals)</i>	1178.9±434.1	1040.6±293.9
<i>Protein intake (Kcals)</i>	241.6±163.0	310.3±137.2

\* Values are significantly different ( $p < 0.05$ ).

Further analysis of the data revealed significant relationships between resting RER and nutrient intake, but not between resting  $E_2$  and RER obtained during the submaximal runs in either the FP or LP. For the FP run, RER was significantly related to mean protein intake ( $r=0.68$ ,  $p < 0.05$ ). LP carbohydrate oxidation was significantly related to carbohydrate intake the day prior to the LP submaximal run ( $r=0.77$ ,  $p < 0.05$ ).

## DISCUSSION

It has been postulated that menstrual cycle phase may have an effect on substrate oxidation during exercise. The primary purpose of this study was to examine between-phase differences in fat and carbohydrate oxidation and the relationship with resting  $E_2$  levels. The values for fat and carbohydrate oxidation were based on RER and  $VO_2$  values obtained during the one-hour run. In order to elicit an equivalent workload in both phases, care was taken to insure that each submaximal run was performed at the same relative intensity. The finding of no differences in  $VO_2$  between menstrual cycle phases is in agreement with other researchers who examined exercise response throughout the menstrual cycle (14). For our subjects, there was a between-phase difference in substrate oxidation measured via indirect calorimetry during a one-hour submaximal treadmill run which is in agreement with other researchers (2,8). Further analysis of our data did not reveal a relationship between resting  $E_2$  and RER obtained during either the FP or LP submaximal runs. However, several researchers propose that substrate availability, training, and diet may have a greater effect on substrate metabolism than  $E_2$  (1,15). As such, it seems logical to assume that greater fat intake prior to the LP run may have contributed to the greater LP fat oxidation. The effect of diet on substrate oxidation is further supported by the strong relationship between LP carbohydrate oxidation and carbohydrate intake the day prior to the LP run. The relationship between FP RER and mean protein intake may be due to the use of protein for energy. As all of our subjects exhibited a negative energy balance, they may have been consuming too few calories in the form of fat and carbohydrates for their daily physical activity. As such, protein may be used as a major energy source.

It has also been suggested that estrogen may exert its effects through alteration of gluconeogenic hormones which would directly affect fat oxidation. Estrogen tends to increase epinephrine and GH levels and decrease insulin levels, which would enhance the release of hormone sensitive lipase, the hormone which directly controls the release of free fatty acids. However, GH and epinephrine are influenced by factors other than estrogen such as diet and stress. As we did not measure these hormones, their effect on fat oxidation cannot be certain. Further, aerobic training brings about chronic adaptations that increase fat utilization. As such, training status may have an effect on substrate oxidation. However, there was not relationship between quantity of training and substrate oxidation in our subjects.

### Resting $E_2$ levels

Resting FP  $E_2$  levels were within the acceptable range of 10 to 60 pg/ml. Normal  $E_2$  levels during the LP range from 10 to 160 pg/ml, with standards for 7-14 days post-ovulation ranging from 10 to 110 pg/ml (22).

Although our  $E_2$  values are somewhat lower than those reported by some researchers for physically active females (7,12), they are similar to those reported by Chin and colleagues (23) for oligomenorrheic runners, as well as Loucks and Horvath (13) for eumenorrheic athletes. Defining  $E_2$  status based on two blood samples during the course of the menstrual cycle may not give an accurate representation of one's menstrual cycle as normal  $E_2$  levels have a wide range of 10 to 400 pg/ml and have a large variation over the course of a menstrual cycle (1). Substrate oxidation during prolonged exercise may be more influenced by  $E_2$  concentrations and fluctuations throughout the course of the menstrual cycle than to resting  $E_2$  levels observed once in each phase

of the menstrual cycle.

## CONCLUSION

Often females may appear to have a regular menstrual cycle evidenced by a 28 to 30 day cycle length, they may experience irregular hypothalamic-pituitary-ovarian hormone levels. To more accurately determine the effects of  $E_2$  on substrate metabolism during exercise, researchers should evaluate hormonal status of the participants. Further, as nutritional status seem to be related to menstrual phase effects of substrate metabolism, it would seem important to closely monitor these variables.

The issue of the effects of the menstrual cycle and associated hormones on substrate metabolism appears to be a multifactorial one. As such, the interrelationships between training volume, energy expended in daily physical activity, nutritional status, and hypothalamic-pituitary-ovarian hormone levels should be established in addressing this question.

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