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SUBSTRATE OXIDATION AT REST AND DURING EXERCISE: EFFECTS OF MENSTRUAL CYCLE PHASE AND DIET COMPOSITION

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The estrogen hormones have been shown to be highly glycogenic as well as lipolytic in nature. It is unknown whether the metabolic actions of estrogens impact upon energy metabolism during exercise. The composition of prior diet, however, does affect exercise energy metabolism. This study examined the influence of menstrual cycle phase (mid-follicular [FP; low estrogen] vs. mid-luteal [LP; high estrogen]) and diet composition on the rate of substrate oxidation for carbohydrate (CHO) and lipid at rest and during various intensities of physical exercise. Nine subjects completed an experimental session under four different menstrual cycle-diet conditions: 1) FP following a 3-day high CHO diet [75% total caloric intake], 2) FP following a 3-day low CHO diet [35% total caloric intake], 3) LP following a 3-day high CHO diet, and 4) LP following a 3-day low CHO diet. In each of the experimental sessions substrate oxidation was determined at rest and during cycle ergometer exercise at intensities of 30, 50, and 70% VO_{2max}, respectively. Statistically significant ($p<0.05$) interaction effects on substrate oxidation due to the menstrual cycle phase and diet conditions were found at rest and during 30% — 50% exercise. In general, CHO oxidation was lowest and lipid oxidation highest in the LP under a low CHO diet condition.

Key words: *estrogen, diet, exercise, hormones, females*

INTRODUCTION

Dietary carbohydrates (CHO) are stored in the body as glycogen and are subsequently utilized as an energy source (1). Glycogen is considered the key energy substrate during physical exercise, therefore considerable research has been conducted to determine if the type of diet a athlete consumes will affect exercise performance. Generally, the research indicates that an enhanced dietary CHO consumption will increase the hepatic and muscle glycogen stores (1, 2); and these enhanced stores will increase exercise capacity (1, 3—6).

The influence of estrogens on dietary macronutrient metabolism has been looked at numerous times in the animal model (i.e., primarily the rat). This

research strongly supports the concept that high levels of estrogens (and to some extent progesterone) are highly glycogenic (7, 8) as well as lipolytic in nature (9—11). In women, the menstrual cycle is characterized by large estrogen and progesterone fluctuations; and, the cycle may be divided into two primary ovarian phases — the follicular and luteal. In general, during the follicular phase estrogen and progesterone levels are low, while both hormone levels are high in the luteal phase (with progesterone levels increasing and peaking about midway through the phase and then decreasing) (12). The hormonal fluctuations of the menstrual cycle provide a human model by which to study the *in vivo* effects of estrogen on CHO and lipid metabolism. Little research, however, has looked at diet and a women's exercise energy metabolism because of the complication the hormonal fluctuation of the menstrual cycle presents. The intent of the present study was to address this short coming in the research literature. Our purpose was to examine the influence of menstrual cycle phase (mid-follicular [low estrogen] vs. mid-luteal [high estrogen]) and diet composition on the rate of substrate oxidation for CHO and lipid at rest and during various intensities of physical exercise.

MATERIAL AND METHODS

Subjects

The subjects of this study were nine moderately trained, healthy females between the ages of 18 and 34. All subjects signed an informed consent prior to participating in the study. Their menstrual cycles were of a eumenorrheic status for the six months prior to the study, and their daily oral temperatures were monitored for at least 3 months prior to the study beginning to ensure they were ovulatory. The physical characteristics as well as the menstrual cycle traits of the subjects appear in *Table 1.*

Table 1.

Physical characteristics (X±SD)				
Age (yr)	Weight (kg)	Height (cm)	Body Fat * (%)	VO_{2max} (L/min)
25.3 ±4.0	68.7 ±9.6	173.0 ±4.1	23.2 ±8.1	2.70 ±0.45
Menstrual cycle characteristics (X±SD)				
Total Cycle Length (d)	Follicular Phase Length (d)		Luteal Phase Length (d)	
28.4 ±2.4	16.6 ±3.4		11.8 ±2.7	

* skinfold assessment (see reference (13)

Procedures

Prior to the study beginning, the subjects performed a 3-day dietary recall, with at least one weekend day included, during which they ate their normal diet. This was evaluated by a registered dietitian to determine daily caloric intake and macro-nutrient content. From these basal records, experimental diets were subsequently established. The daily caloric intake of the experimental diets was held constant ($\sim$2450 kcal $\cdot$ d^{-1}), while carbohydrate ingestion was altered to meet desired condition level of 35% (low CHO) and 75% (high CHO) of the daily caloric intake. The level of dietary fat consumed was also altered to make up the remaining necessary caloric intake while dietary protein content was held constant at approximately 15% of daily caloric intake. All meals were prepared for the subjects at the metabolic kitchen of the General Clinical Research Center of the University. Meal consumption and compliance were monitored and controlled throughout the study.

Each subject completed an experimental session under four different menstrual cycle-diet conditions: 1) mid-follicular following a 3-day high CHO diet, 2) mid-follicular following a 3-day low CHO diet, 3) mid-luteal following a 3-day high CHO diet, and 4) mid-luteal following a 3-day low CHO diet. The mid-points in each phase of the cycle were determined from the daily oral temperature records obtained for the three months prior to the study beginning.

Initially each subject performed a graded exercise test to volitional fatigue on a cycle ergometer to determine maximal oxygen uptake (VO_{2max}). This protocol has been described in detail elsewhere (13). Results of the VO_{2max} testing were utilized to determine the submaximal workloads incorporated in the experimental sessions (discussed below).

Each of the experimental sessions began with the subject reporting to the laboratory at approximately 05:30 h to first perform a basal metabolic rate test. This began with them lying supine and resting undisturbed for 30 minutes. A resting blood specimen was then obtained via a veni-puncture procedure. Next, respiratory gases were collected for 20 minutes and analyzed for oxygen uptake (VO_2) and carbon dioxide production (VCO_2) by standard laboratory procedures (14, 15). These values were used to calculate the respiratory exchange ratios (RER) which were subsequently used with standard indirect calorimetry equations to calculate CHO and lipid oxidation rates (14).

Following the basal metabolic rate test, the subject began the exercise portion of the experimental session (06:00 h). This protocol was on a cycle ergometer and consisted of 3 exercises stages at 30, 50, and 70% VO_{2max} respectively. Each exercise stage was 6 minutes in duration, separated by a 6 minute rest period. Throughout each exercise stage, steady-state VO_2, VCO_2, and RER values were monitored so that CHO and lipid oxidation rates could be calculated as with the basal metabolic test. It should be noted that all calorimetry calculations were conducted using the non-protein respiratory exchange ratio (15). For each experimental session the subjects reported to the laboratory after having abstained from physical exercise for 24 h and 12 h post-prandial.

Resting blood specimens were placed on ice until centrifugation at 3,000 $\times$ g for 15 minutes. Separated sera was stored frozen (-20°C) until later analysis. The sera was analyzed for the levels of estrogen and progesterone using commercially available radioimmunoassay procedures (DPC Inc., Los Angles, CA).

Statistics

Statistically, the primary dependent variables analyzed was CHO and lipid oxidation rates (g $\cdot$ min^{-1}). The data were analyzed with a 2 $\times$ 2 repeated measures analysis of variance. The post hoc test applied following the ANOVA was the Fisher LSD procedure. Statistical significance was set at a probability level of 0.05.

RESULTS

Analysis of the blood samples indicated that each of the subjects were found to be in the appropriate menstrual cycle phase and hormonal status (see *Table 2*).

Table 2.

Resting hormonal values (X ± SE)				
	Low CHO		High CHO	
	FP*	LP	FP	LP
Estrogen ($pg \cdot mL^{-1}$)	45 ±61	276 ±180	52 ±74	295 ±150
Progesterone ($ng \cdot mL^{-1}$)	22 ±30	903 ±51	31 ±46	1056 ±598

* FP = follicular phase, LP = luteal phase

The variables VO_2, VCO_2, and RER significantly changed from rest throughout exercise. These were comparable across the treatment conditions and not significantly different (although variable), and of a magnitude that compared to previous reports (14, 15). These data are not present here.

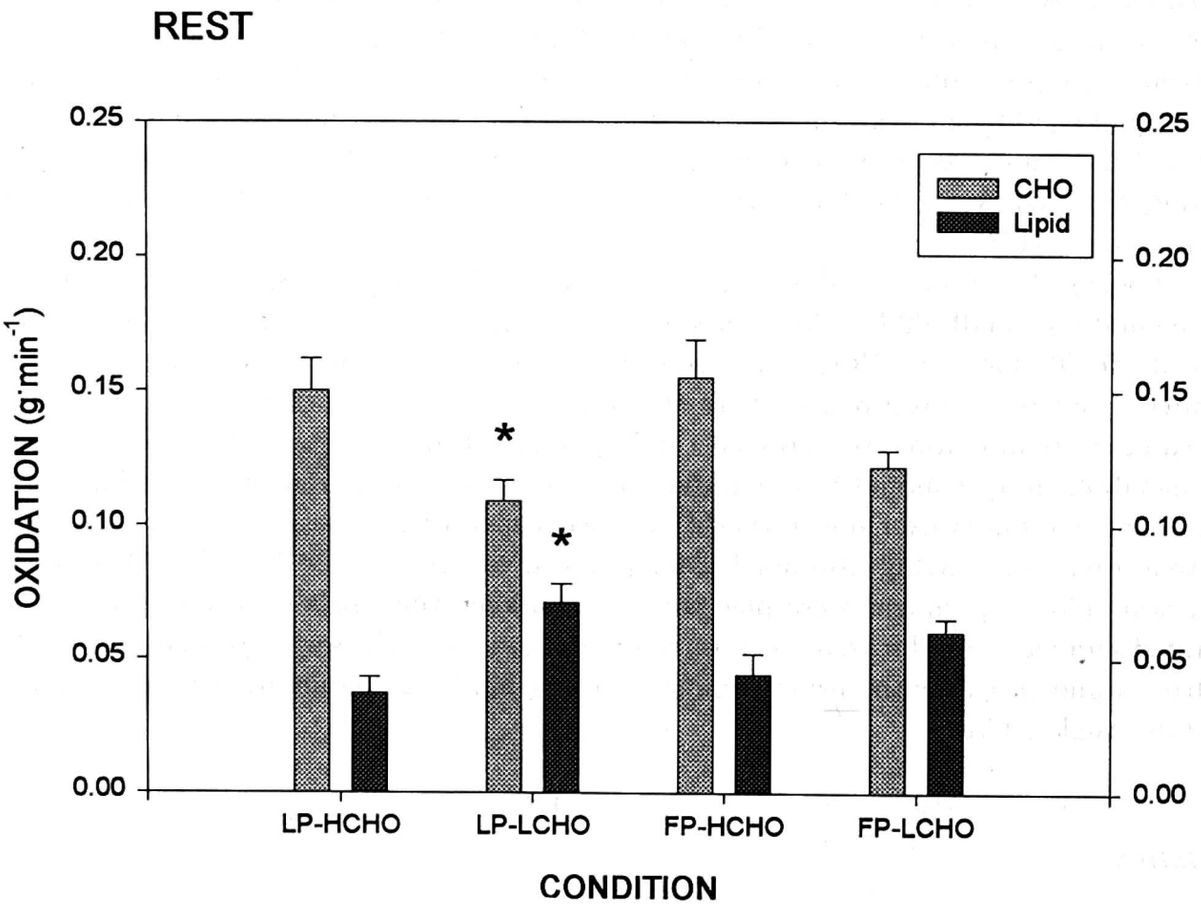

Fig. 1. Carbohydrate (CHO) and lipid oxidation rates at rest. The *denotes that the mean(±SE) values are significantly ($p < 0.05$) different from all other condition means observed for the CHO and lipid, respectively (LP-HCHO = luteal phase high carbohydrate diet; LP-LCHO = luteal phase low carbohydrate diet; FP-HCHO = follicular phase high carbohydrate diet; FP-LCHO = follicular phase low carbohydrate diet).

Statistically significant ($p<0.05$) effects on substrate oxidation due to the menstrual cycle phase and diet conditions studied were found with the data (see *Figs 1—4*). At rest, CHO oxidation was significantly ($p<0.05$) less during the low CHO diet in both the luteal phase (LP) and follicular phase (FP) of the cycle than in the high CHO diet. Furthermore, the lowest level of CHO oxidation was observed in the LP low CHO condition. Conversely, lipid oxidation was greatest during the LP low CHO condition, but none of the other conditions responses were significantly different from one another.

For exercise at 30% VO_{2max}, CHO oxidation was significantly less in the LP under the low CHO diet than in all other conditions. The other phase and diet conditions, however, were not different from one another. Lipid oxidation was significantly greater in the LP low CHO condition, and none of the other three conditions differed from one another.

For exercise at 50% VO_{2max}, diet seemed to be of a more significant influence on physiological outcomes than did menstrual cycle phase. In this condition the LP high CHO and FP high CHO condition values were significantly greater than the LP low CHO and FP low CHO values (though, none of the similar diet condition values differed significantly). The similar

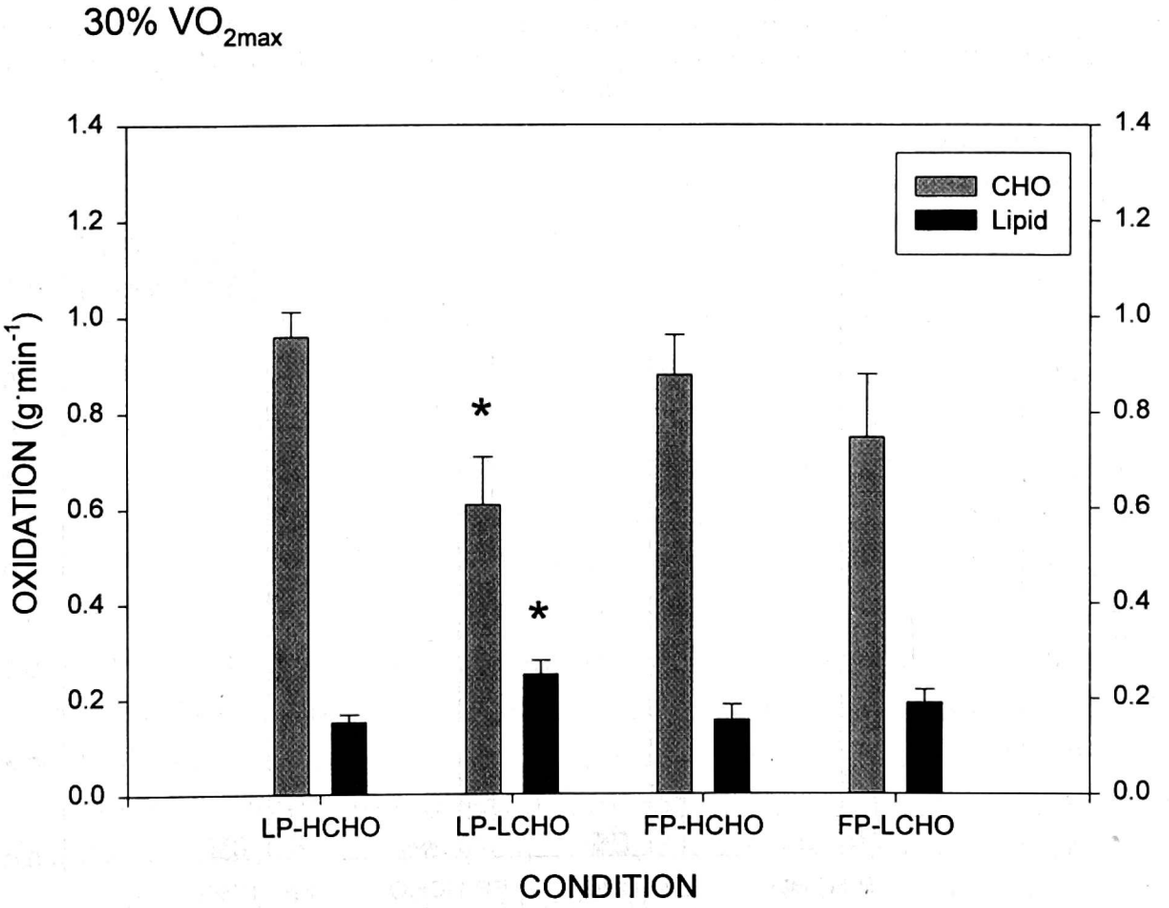

Fig. 2. Carbohydrate (CHO) and lipid oxidation rates during exercise at 30% of VO_{2max}. The*denotes that the mean($\pm$SE) values are significantly ($p<0.05$) different from all other condition means observed for the CHO and lipid, respectively (LP-HCHO = luteal phase high carbohydrate diet; LP-LCHO = luteal phase low carbohydrate diet; FP-HCHO = follicular phase high carbohydrate diet; FP-LCHO = follicular phase low carbohydrate diet).

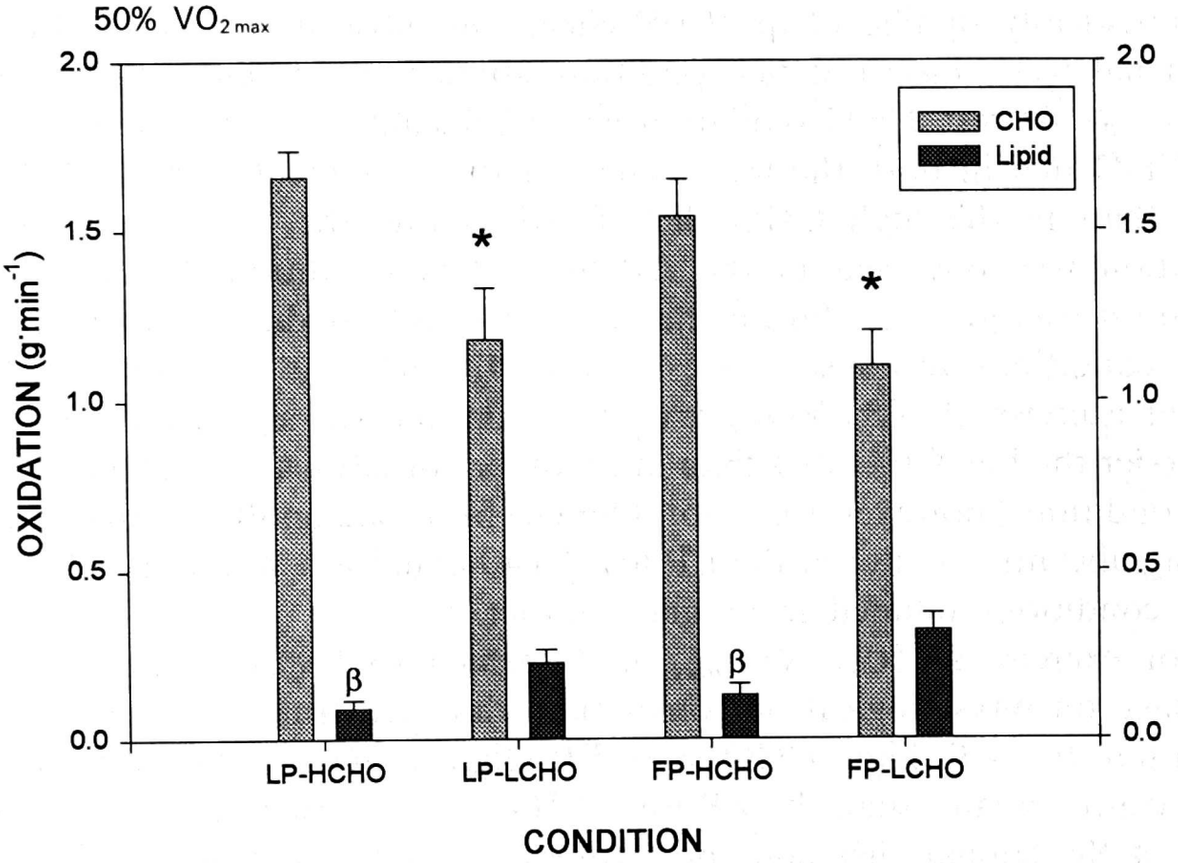

Fig. 3. Carbohydrate (CHO) and lipid oxidation rates during exercise at 50% of VO_{2max}. The*(and β) denotes that the mean(±SE) values are significantly ($p<0.05$) different from the other condition means observed for the CHO and lipid, respectively (LP-HCHO = luteal phase high carbohydrate diet; LP-LCHO = luteal phase low carbohydrate diet; FP-HCHO = follicular phase high carbohydrate diet; FP-LCHO = follicular phase low carbohydrate diet).

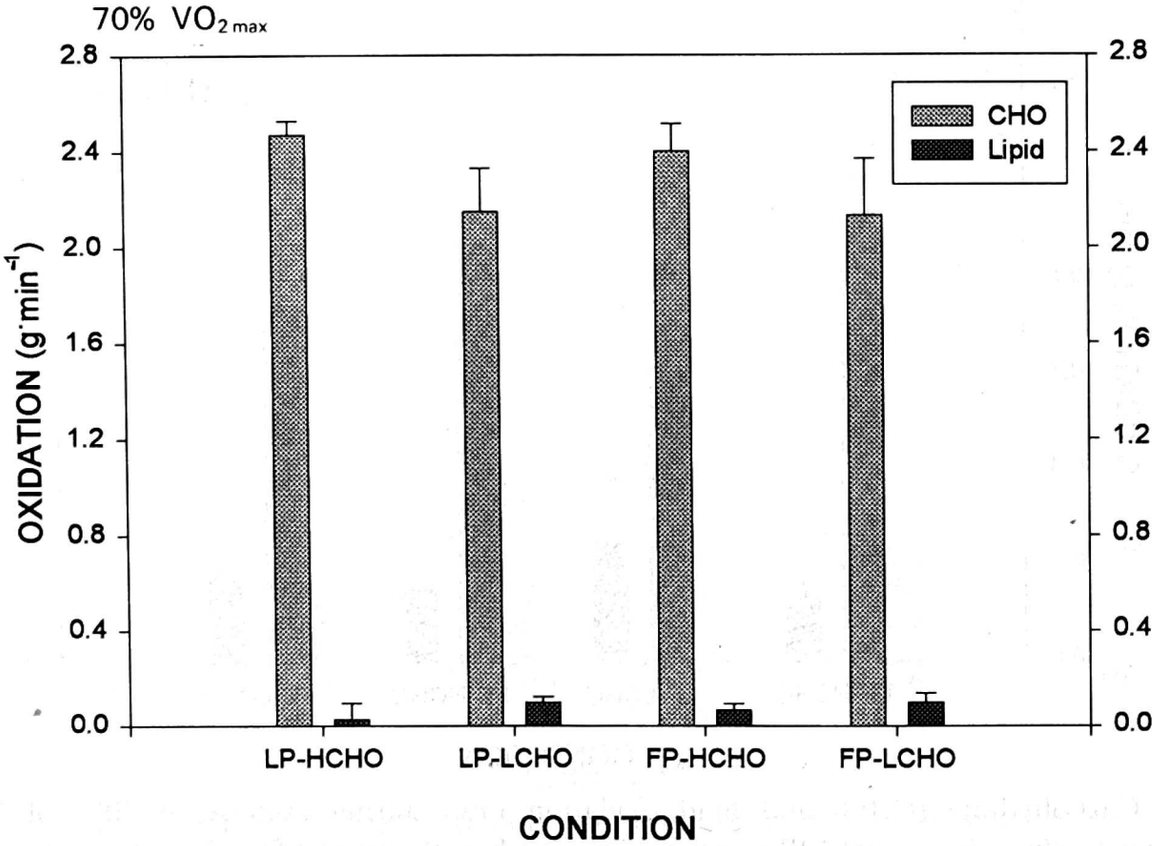

Fig. 4. Carbohydrate (CHO) and lipid oxidation rates during exercise at 70% of VO_{2max}. Mean(±SE) values are depicted (LP-HCHO = luteal phase high carbohydrate diet; LP-LCHO = luteal phase low carbohydrate diet; FP-HCHO = follicular phase high carbohydrate diet; FP-LCHO = follicular phase low carbohydrate diet).

finding occurred for lipid oxidation, but the effect was in an opposite direction (LP and FP low CHO values > LP and FP high CHO).

Finally, for the exercise at 70% VO_{2max} no significant differences were noted between CHO or lipid oxidation under any of the conditions studied.

DISCUSSION

The purpose of this study was to examine the influence of high and low CHO diets during the mid-follicular and mid-luteal phases of the menstrual cycle on substrate oxidation. In all cases, we were able to test the subjects in the desired menstrual cycle phase following the prescribed diet conditions. Thus we have confidence that our desired experimental conditions were obtained. Collectively, our data suggest that menstrual cycle phase and diet can have an influence on substrate oxidation.

The observed differences in substrate oxidation probably depend upon the effects of estrogen and progesterone exerting an influence on metabolic substrate availability. Hall-Jurkowski *et al.* (16) examined the effects of the menstrual cycle phases on exercise performance and cardio-respiratory variables. The cardiovascular responses to exercise did not vary with menstrual cycle phase, but the lactate responses to exercise were lower during the LP than FP. The authors attributed these findings to the effects of elevated estrogen and progesterone enhancing lipid metabolism and thereby decreasing carbohydrate metabolism (i.e., reduced lactate production). Dombovy *et al.* (17) examined the effects of the menstrual cycle phases on ventilatory measurements and exercise performance. They found lower maximal exercise RER during the luteal phase. They suggested that this finding was due to an increase in lipid oxidation and glycogen-sparing during the LP.

Lavoie and associates (18) looked at the effects of a 24-hour poor CHO diet on metabolic and hormonal responses during prolonged exercise during the FP and LP of the menstrual cycle. In that study the diet composition was as follows: about 54% fat, 39% protein, and 7% carbohydrate, and the exercise bout was 90 minutes of cycle ergometry at approximately 63% of leg VO_2 peak. The results indicated a significant decrease in blood glucose after 70 and 90 minutes of exercise in the LP as compared to the FP. The authors concluded that the liver glycogen stores of each female were most likely decreased due to the prior 24-hour poor carbohydrate diet, therefore allowing gluconeogenesis to make an important contribution during exercise. Furthermore, the significant decrease in blood glucose levels during the LP was attributed to the high levels of estrogen hormone present during the LP.

Nicklas *et al.* (19) also looked at diet, exercise, and the menstrual cycle. In this study a 3 day diet comprised of approximately 60% carbohydrates was

consumed by women after completing a "glycogen depletion" exercise bout (1.5 hours at 60% VO_{2max} followed by four 1-minute sprints at 100% VO_{2max}). Immediately following the 3 day diet the subjects were asked to exercise to exhaustion. This protocol was completed in both the FP and LP phases of the cycle. Furthermore, muscle biopsies were performed after the depletion exercise, prior to, and after the exercise to exhaustion. It was found that the total amount of repleted muscle glycogen during the 3 day diet was greater during the LP when compared to the FP. The RER during rest in the LP were significantly lower than during the FP, and during exercise RER values tended to be lower during the LP than the FP. Nicklas and associates suggested from these findings that muscle glycogen content are enhanced during the LP possibly due to a greater dependency upon lipid as a metabolic fuel at rest and during exercise. These latter findings are supported by many animal-based investigations in which elevated levels of estradiol and progesterone increase glycogen stores by facilitating lipolysis and thus sparing glycogen metabolism (20—23).

Not all of the existing research, however, agrees with the findings in the present study. DeSouza and associates (24) found no apparent influence on exercise metabolism (i.e., substrate oxidation) due to the menstrual cycle phase or menstrual status. Gamberale *et al.* (25) also found no difference in physiological responses (i.e., VO_2, VCO_2, substrate oxidation) during exercise due to the menstrual cycle phase. The discrepancies between these and the present study may be due in part to the following: variations in the diet control, intensity of exercise, duration of exercise, difference in exercise protocol (i.e., continuous vs. discontinuous) and the type of subjects (i.e., athletic vs. non-athletic).

In the present results, significant exercise differences only occurred at rest and low-moderate intensity exercise. The apparent influence of diet and menstrual cycle may not have been seen at the higher intensity due to the increasing "metabolic stress" of the exercise. That is, as the intensity of exercise increased there was a shift to greater CHO usage as an energy substrate. This shift in CHO usage was because of a necessity to provide ATP via glycolytic mechanisms (15). Thus, it appears that even the hormonal or dietary status of the subjects was unable to overcome this normal physiological drive.

In conclusion, the results of this study do support the theory that substrate oxidation does vary across the menstrual cycle. The variations seem to be affected by prior diet and exercise. These findings should be factors to consider when dealing with physiological research involving physical activity eumenorrheic women.

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