Hormonal and metabolic responses to exercise across time of day and menstrual cycle phase


Health Sciences, Bethesda 20814; and 3Clinical Neuroendocrinology Branch, National Institute of Mental Health, Bethesda, Maryland 20892

Galliven, E. A., A. Singh, D. Michelson, S. Bina, P. W. Gold, and P. A. Deuster. Hormonal and metabolic responses to exercise across time of day and menstrual cycle phase. J. Appl. Physiol. 83(6): 1822–1831, 1997.—Two studies, each utilizing short-term treadmill exercise of a different intensity, assessed the metabolic and hormonal responses of women to exercise in the morning (AM) and late afternoon (PM). In study 1, plasma concentrations of growth hormone, arginine vasopressin, catecholamines, adrenocorticotropic hormone, cortisol, lactate, and glucose were measured before, during, and after high-intensity exercise (90% maximal O\textsubscript{2} uptake) in the AM and PM. In study 2, plasma concentrations of adrenocorticotropic hormone, cortisol, lactate, and glucose were measured before, during, and after moderate-intensity exercise (70% maximal O\textsubscript{2} uptake) in the AM and PM in the follicular (days 3–9), midcycle (days 10–16), and luteal (days 18–26) phases of the menstrual cycle. The results of studies 1 and 2 revealed no significant diurnal differences in the magnitude of responses for any measured variable. In addition, study 2 revealed a significant time-by-phase interaction for glucose (P = 0.014). However, net integrated responses were similar across cycle phases. These data suggest that metabolic and hormonal responses to short-term, high-intensity exercise can be assessed with equal reliability in the AM and PM and that there are subtle differences in blood glucose responses to moderate-intensity exercise across menstrual cycle phase.

adrenocorticotropic hormone; circadian variation; cortisol; estrogen; eumenorrheic; glucose; running

METHODS

All women were eumenorrheic according to self-reports of regular menstrual cycles and normal menses. No subject with a history of menstrual irregularity or other gynecological problems was admitted to the study. All women were nonsmokers, medication free, and had not been on oral contraceptives for at least 6 mo. Before participation, each subject had a physical examination and an electrocardiogram. All subjects had normal results from laboratory screening tests including routine chemistries, thyroid function tests, urinalysis, and
complete blood counts. Subjects were asked to refrain from caffeine and alcohol for 16 h before studies, from strenuous activity for 24 h, and from food for at least 6 h before testing. Resting hemoglobin (Hb) and hematocrit (Hct) determinations before all exercise tests were within the normal range for each subject. Electrocardiograms and heart rate were monitored continuously throughout exercise testing. Both studies were approved by the Institutional Review Board of the Uniformed Services University of the Health Sciences. Participants were informed of the risks of the study and gave written informed consent before participation.

Study 1: High-Intensity Exercise

Nine healthy female subjects [6 Caucasian, 2 African-American, 1 Asian; age 29 ± 1 (SE) yr] participated in this study. Subjects were of low to moderate fitness. Subject characteristics are shown in Table 1.

Experimental procedure. Each subject reported to the laboratory on three occasions. During the first visit, each subject underwent a progressive maximal aerobic treadmill test to determine VO\textsubscript{2max} by using a modification of the procedure described by Kyle et al. (22). Two follow-up exercise test sessions were scheduled on separate occasions: one between 0700 and 0800, after an overnight fast, and one between 1500 and 1600, after a 6-h fast. The order of testing was randomized, and each test was separated by at least 1 wk. Exercise testing was conducted on a motorized treadmill (Q55 Quinton Instruments, Bothell, WA). O\textsubscript{2} uptake (VO\textsubscript{2}) and CO\textsubscript{2} production were determined with a metabolic measurement cart (2900c Sensor Medics, Yorba Linda, CA).

For exercise test sessions, each subject reported to the laboratory 1 h before the initiation of exercise, weighed herself, and drank 5 ml water/kg body weight to ensure uniform hydration. An indwelling catheter was then placed in an antecubital vein at least 20 min before the first basal blood sample (i.e., time -40 min relative to the start of exercise, time0). Samples were collected before exercise (-20 and -10 min), midway through exercise (10 min), and immediately after exercise (20 min); subjects were kept in a standing position. Four postexercise blood samples were taken (30, 40, 60, and 80 min after the start of exercise) with the subject in a semirecumbent position. The total treadmill exercise lasted 20 min followed by a 5-min cool-down period. During the initial 5 min, each subject exercised at an intensity equivalent to 50% of her VO\textsubscript{2max} as determined from her VO\textsubscript{2max} test. During this time, the treadmill grade was maintained constant at 5% while the speed was adjusted to produce 50% VO\textsubscript{2max}. For the next 5 min, the treadmill was set at a 10% grade, and the speed was adjusted to elicit 70% VO\textsubscript{2max}. Then there was a 2-min break for blood sampling, after which each subject resumed exercise at an intensity of 70% VO\textsubscript{2max} for another 5 min. For the last 5 min of exercise the speed was adjusted to produce a relative intensity of 90% of each subject's VO\textsubscript{2max}, the grade was set at 10%. A 5-min cool-down period (3 miles/h, 2% grade) followed each test. The speeds and grades of the treadmill were identical during the AM and PM tests for each subject to ensure identical workloads across test sessions.

Blood samples were collected into a syringe and dispensed into chilled EDTA tubes (1.6 mg EDTA/ml blood) for hormonal analyses and into room-temperature EDTA tubes for determination of Hb and Hct. Additional blood was aliquoted into chilled heparinized tubes (15 IU heparin/ml blood) containing sodium fluoride (1 mg fluoride) for lactate and glucose measurements and into chilled tubes containing ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid and glutathione [90 mg ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid/ml deionized water with 90 mg glutathione, pH 8.2–8.4] for catecholamine determination. Plasma for hormones and catecholamines was separated by centrifugation within 30 min and stored at -50°C until assayed. Plasma samples for lactate and glucose were refrigerated and assayed within 24 h.

Biochemical analyses. Lactate and glucose concentrations were determined in duplicate (model 27YSI analyzer; Yellow Springs Instruments, Yellow Springs, OH). Hb and Hct were determined in duplicate by the cyanomethemoglobin and microcapillary methods, respectively. Plasma cortisol was measured by using a commercial radioimmunoassay (RIA) kit (Diagnostic Systems Laboratory, Webster, TX). Plasma ACTH and growth hormone (GH) were determined by using a commercial immunoradiometric kit (Nichols Institutes Diagnostics, San Juan Capistrano, CA). Detection limits of the assays were 8.3 nM for cortisol, 0.22 pM for ACTH, and 0.02 μg/l for GH. The intra-assay coefficients of variation (CV) for ACTH, cortisol, and GH were <8, 6, and 5%, respectively. The interassay CV for ACTH was <15 and <10% for both cortisol and GH. Samples from all tests for one individual were assayed together. Plasma arginine vasopressin (AVP) was extracted and assayed by RIA as previously described by Rittmaster et al. (28). The recovery with use of this procedure was >90%. All samples were included in one assay; the intra-assay CV for AVP was 7%. Catecholamines were extracted and measured by high-performance liquid chromatography using a modification of the procedure described by Hunter et al. (17). All catecholamine samples were measured in one assay; the percent recovery for the extraction was 60 ± 5%. The intra-assay CV was 17.0% for norepinephrine (NE) and 20.4% for epinephrine (Epi).

Study 2: Moderate-Intensity Exercise

Eight healthy female subjects (6 Caucasian, 2 African-American; age 31 ± 1 yr) participated in this study. Subjects were of low to moderate fitness and of normal body fat ([BF]: 21 ± 0.6%; range 16–25%). BF was determined from skinfold thickness at four sites (triceps, suprailliac, abdomen, and thigh) on the right side of the body by using calipers, and percent BF was calculated by using an equation from Jackson et al. (19). Subject characteristics are shown in Table 1.

Experimental procedure. All subjects reported to the laboratory on seven occasions. During the first visit, each subject underwent a progressive maximal aerobic treadmill test and used the same procedure described for study 1. Each subject then underwent six identical submaximal treadmill tests at a relative intensity of 70% of her previously determined VO\textsubscript{2max}.

Subjects were tested over the course of two menstrual cycles during the 1) follicular phase between days 3–9 after the start of menses; 2) midphase between days 10–16; and 3) luteal phase between days 18–26. Menstrual cycle phase was

| Table 1. Descriptive characteristics of women in study 1 and study 2 |
|-----------------|-----------------|-----------------|
|                | VO\textsubscript{2max}, m\textsuperscript{L}/min \textsuperscript{-1} |
| Study 1 (n = 7) | Mean ± SE       | Range           |
| Mean ± SE      | 29 ± 1          | 64.6 ± 3.3      | 165 ± 3          |
| Range          | 23–36           | 52.5–82.0       | 157–185          |
| Study 2 (n = 8) | Mean ± SE      | Range           |
| Mean ± SE      | 31 ± 1          | 58.0 ± 1.2      | 162 ± 2          |
| Range          | 24–37           | 51.9–66.0       | 155–173          |

VO\textsubscript{2max}, maximum O\textsubscript{2} uptake; n = no. of women.
verified from estrogen and progesterone samples taken 20 min before each submaximal-exercise test. Testing across one menstrual cycle occurred in the AM (subjects reported to the lab between 0700 and 0800). Testing through the other phase occurred in the PM (subjects reported to the lab between 1500 and 1600). The order of AM and PM testing cycles was randomized. The exercise test session has already been described for study 1. In contrast with study 1, subjects remained standing for all recovery blood draws in study 2. The treadmill exercise test employed here was the same as described in study 1, except for the last 5 of the 20 min, at which time subjects in study 2 continued exercising at 70% \( \dot{V}O_2 \)max instead of achieving 90% \( \dot{V}O_2 \)max. A 5-min cool-down period (3 miles/h, 2% grade) again followed each test. Respiratory exchange ratios (RER) were continuously calculated from the \( \dot{V}O_2 \) and \( \dot{CO}_2 \) production data collected during the exercise test.

RER at 70% \( \dot{V}O_2 \)max for the data analysis was taken as the average of the last 2 min of exercise. The blood collection procedures for lactate, glucose, ACTH, cortisol, Hb, and Hct determinations have been described above. For estrogen and progesterone determinations, blood was collected in tubes without an anticoagulant, allowed to clot, and centrifuged for the removal of serum. The serum was stored as described above.

Biochemical analyses. Lactate, glucose, ACTH, cortisol, Hb, and Hct determinations were made by using the same procedure described in study 1. Detection limits of the assays were 8.3 nM for cortisol and 0.22 pM for ACTH. The intra-assay CV values for ACTH and cortisol were <8 and 6%, respectively. The interassay CV for ACTH and cortisol were <15% and <10%, respectively. All samples from one individual were included in the same assay. Estrogen and progesterone were determined by using a commercial RIA kit (Diagnostic Products, Los Angeles, CA). Detection limits of the assays were 29 pM for estrogen and 0.1 nM for progesterone. Each sex hormone was measured in one assay. The intra-assay CV for either estrogen or progesterone was <7%.

**Statistical Analyses**

A Statistical Analysis System software program (SAS Institute, Cary, NC) was used for all data analyses. Data in text, Tables 1 and 2, and Figs. 1–7 are presented as the mean \( \pm SE \). Differences across time of day and menstrual cycle phase were evaluated by using repeated-measures analysis of variance. If significant effects were noted, Duncan’s multiple-range test was used to find differences across phase. Paired t-tests were performed to detect baseline differences across time of day. Significance was set at the 0.05 level. Net integrated area under the curve (AUC) was calculated by the trapezoidal method after subtraction of the baseline. AUC across time of day was analyzed by using paired t-tests. Pearson’s correlation coefficient was used to test relationships among variables.

**RESULTS**

**Study 1: High-Intensity Exercise**

Physiological and metabolic responses. Percentage of \( \dot{V}O_2 \)max achieved, heart rates, and ratings of perceived exertion (RPE) across exercise intensities did not differ significantly between AM and PM tests (Table 2). Lactate and glucose responses to exercise across test sessions are presented in Fig. 1. As expected, plasma concentrations of lactate increased in an intensity-dependent manner and achieved an increase of \( \sim 10 \)-fold over basal levels at the 90% \( \dot{V}O_2 \)max intensity. No differences were noted in the pattern of change over time or AUC between test sessions. Similarly, the patterns of change and AUC for glucose were not significantly different between the AM and PM; peak glucose concentrations were noted at 10 min postexercise.

Hormonal responses. Plasma ACTH and cortisol responses to exercise in the AM and PM are shown in Fig. 2. Basal concentrations of ACTH were not significantly different in the AM (3.5 \( \pm \) 0.6 pM) and PM (2.6 \( \pm \) 0.4 pM). The patterns of ACTH responses to exercise in the AM and PM were similar, with peak concentrations achieved at the end of high-intensity exercise (time 20). The net integrated ACTH responses in the AM and PM were also similar. Although mean basal plasma cortisol levels (357.5 \( \pm \) 24.3 vs. 206.4 \( \pm \) 19.3 nM, AM vs. PM, respectively; \( P < 0.001 \)) were significantly higher in the AM compared with the PM, analysis of AUC revealed no significant differences in the net integrated response in the AM and PM.

Similar patterns of response were noted for both AVP and GH (Fig. 3). Peak levels were attained at the end of high-intensity exercise and returned to resting levels 20 min after exercise. The changes in AVP and GH over time in the AM and PM were not significantly different. Additionally, analysis of AUC for AVP and GH revealed no significant differences in the magnitude of response across test sessions.

Catecholamine responses to exercise in the AM and PM are shown in Fig. 4. Relative to basal levels, the concentration of NE and Epi increased significantly during exercise but returned to resting levels 20 min after terminating exercise. The pattern of change over time in the AM and PM was also similar for both catecholamines.

**Table 2. Physiological responses to selected exercise intensities applied in study 1 and study 2 in AM and PM tests and in study 2 in follicular, midcycle, and luteal phase tests**

<table>
<thead>
<tr>
<th>Condition</th>
<th>( % \dot{V}O_2 )max Achieved, %</th>
<th>Heart Rate, beats/min</th>
<th>Perceived Exertion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study 1: high-intensity exercise (n = 9)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>72.7 ( \pm ) 2.3</td>
<td>167 ( \pm ) 5</td>
<td>13 ( \pm ) 1</td>
</tr>
<tr>
<td>PM</td>
<td>71.3 ( \pm ) 2.2</td>
<td>167 ( \pm ) 4</td>
<td>13 ( \pm ) 1</td>
</tr>
<tr>
<td><strong>Study 2: moderate-intensity exercise (n = 8)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>70.6 ( \pm ) 2.2</td>
<td>169 ( \pm ) 2</td>
<td>14 ( \pm ) 1</td>
</tr>
<tr>
<td>PM</td>
<td>71.8 ( \pm ) 2.4</td>
<td>171 ( \pm ) 2</td>
<td>14 ( \pm ) 1</td>
</tr>
<tr>
<td>Follicular</td>
<td>71.4 ( \pm ) 1.6</td>
<td>172 ( \pm ) 3</td>
<td>14 ( \pm ) 1</td>
</tr>
<tr>
<td>Midcycle</td>
<td>72.1 ( \pm ) 3.0</td>
<td>170 ( \pm ) 3</td>
<td>14 ( \pm ) 1</td>
</tr>
<tr>
<td>Luteal</td>
<td>69.9 ( \pm ) 2.6</td>
<td>168 ( \pm ) 2</td>
<td>14 ( \pm ) 1</td>
</tr>
</tbody>
</table>
Study 2: Moderate-Intensity Exercise

Test sessions took place during the early follicular (day 6.1 ± 0.3; range, day 4–8), midcycle (day 13.6 ± 0.4; range, day 10–16), and luteal (day 21.1 ± 0.4; range, day 19–24) phases. Basal estradiol concentrations for each subject were in the expected range with respect to menstrual cycle phase (follicular: 200 ± 29 pM; midcycle: 489 ± 73 pM; luteal: 398 ± 55 pM). As expected, estradiol concentrations were significantly lower during the follicular phase compared with either the midcycle or luteal phases (P < 0.002). Basal progesterone levels were significantly higher in the luteal phase (30.2 ± 2.9 nM) compared with either follicular (1.1 ± 0.1 nM) or midcycle phases (6.8 ± 2.0 nM; P < 0.0001). Despite the expected higher group mean blood progesterone value in the luteal phase, one subject presented with low progesterone levels for both AM and PM tests (0.02 and 0.09 nM, respectively). Luteal-phase data from this subject were omitted from all analyses.

Diurnal comparisons. The patterns of change over time for ACTH and cortisol and their respective net integrated responses in the AM and PM are shown in Fig. 5. Basal concentrations of ACTH were not significantly different in the AM (3.7 ± 0.3 pM) and PM (3.3 ± 0.3 pM). The net integrated ACTH responses in the AM and PM were not significantly different (3.6 ± 0.9 vs. 6.5 ± 1.8 pM/80 min, AM vs. PM, respectively; P = 0.30). Although mean basal plasma cortisol levels (287.3 ± 30.4 vs. 186.4 ± 17.5 nM, AM vs. PM, respectively) were significantly higher in the AM compared with the PM (P < 0.001), analysis of AUC revealed no significant diurnal differences in the magnitude of responses (22.4 ± 38.8 vs. 141.0 ± 46.1 nM/80 min, AM vs. PM, respectively; P = 0.13). Similarly, the patterns of change in ACTH and cortisol were similar in
glucose responses were higher compared with both the midcycle and follicular phase immediately after exercise (time 20) and for the next 20 min thereafter (times 30 and 40; P < 0.05). There was a marginal main effect of phase for the net integrated glucose responses (P = 0.083).

RER values were not significantly different across menstrual cycle phase (follicular: 0.89 ± 0.02; midcycle: 0.88 ± 0.02; luteal: 0.88 ± 0.02).

Menstrual phase comparisons: hormonal responses. The patterns of change over time for plasma ACTH and cortisol and their respective net integrated responses across menstrual cycle phase are shown in Fig. 7. In all phases of the menstrual cycle, exercise induced a significant increase in plasma levels of ACTH and cortisol. Basal, exercise, and recovery concentrations of ACTH were similar across menstrual cycle phase, with peak concentrations achieved at the end of high-intensity exercise (time 20). The net integrated ACTH responses during each phase were also similar. Simi-

Fig. 3. Plasma arginine vasopressin (AVP; A) and growth hormone (GH; B) responses to exercise at 90% V\textsubscript{O\textsubscript{2}}\textsubscript{max} in AM (dashed line) and PM (solid line). Insets: corresponding net integrated AUC in AM and PM. Values are means ± SE.

the AM and PM (Fig. 5). Lactate and glucose determinations were not significantly different in the AM and PM (data not shown).

Menstrual phase comparisons: physiological and metabolic responses. Percentage of V\textsubscript{O\textsubscript{2}}\textsubscript{max} achieved, heart rates, and RPE across exercise intensities did not differ significantly between menstrual cycle phase tests (Table 2). In all cycle phases, exercise induced a significant increase in plasma lactate concentrations, achieving an increase of approximately fivefold over basal levels immediately after exercise (time 20, Fig. 6). The magnitudes of the lactate responses were similar across menstrual cycle phase. For all cycle phases, exercise induced a significant increase in glucose concentrations. In addition, there was a significant difference in the glucose responses across phases (P = 0.014). Glucose responses midway through exercise (time 10, Fig. 6) were higher in the luteal phase compared with the midcycle phase (P < 0.05). Moreover, luteal-phase glucose responses were higher compared with both the midcycle and follicular phase immediately after exercise (time 20) and for the next 20 min thereafter (times 30 and 40; P < 0.05). There was a marginal main effect of phase for the net integrated glucose responses (P = 0.083).

RER values were not significantly different across menstrual cycle phase (follicular: 0.89 ± 0.02; midcycle: 0.88 ± 0.02; luteal: 0.88 ± 0.02).

Menstrual phase comparisons: hormonal responses. The patterns of change over time for plasma ACTH and cortisol and their respective net integrated responses across menstrual cycle phase are shown in Fig. 7. In all phases of the menstrual cycle, exercise induced a significant increase in plasma levels of ACTH and cortisol. Basal, exercise, and recovery concentrations of ACTH were similar across menstrual cycle phase, with peak concentrations achieved at the end of high-intensity exercise (time 20). The net integrated ACTH responses during each phase were also similar. Simi-

Fig. 4. Plasma epinephrine (A) and norepinephrine (B) responses to exercise at 90% V\textsubscript{O\textsubscript{2}}\textsubscript{max} in AM (dashed line) and PM (solid line). Values are means ± SE.
larly, mean basal plasma cortisol levels were similar across menstrual cycle phase, as was the pattern of change over time. The *P* value for the net integrated cortisol responses across phases was 0.056.

No significant correlations between basal estrogen levels and peak responses or AUC were noted for any of the measured variables. In addition, there were no significant correlations between basal progesterone levels and peak responses or AUC for any of the measured variables. The ratio of estrogen to progesterone was not correlated with any of these variables.

There was a significant positive correlation between RER and cortisol levels immediately after exercise at time 20 (follicular: *r* = 0.68, *P* < 0.004; midcycle: *r* = 0.63, *P* < 0.02; luteal: *r* = 0.64, *P* < 0.02) for each cycle phase. In addition, there was a significant positive correlation between RER and cortisol levels at times 30 and 40 for each cycle phase (data not shown). No significant correlations were detected between RER and postexercise glucose concentrations.

**DISCUSSION**

The present data provide evidence that the magnitudes of metabolic and pituitary-adrenal responses to either high- or moderate-intensity exercise are not significantly different in the AM and PM, despite high cortisol levels in the AM compared with the PM. In addition, the magnitudes of GH, AVP, and catecholamine responses to high-intensity exercise were similar in the AM and PM (not assessed in study 2). The present data also provide evidence for subtle differences in blood glucose responses across phases of the menstrual cycle.

Fig. 5. Plasma ACTH (A) and cortisol (B) responses to exercise at 70% V\textsubscript{O\textsubscript{2}}\text{max} in AM (dashed lines) and PM (solid lines). Insets: corresponding net integrated AUC in AM and PM. Values are means ± SE. *P* < 0.001 compared with level in PM.

Fig. 6. Plasma lactate (A) and glucose (B) responses to exercise at 70% V\textsubscript{O\textsubscript{2}}\text{max} in follicular (FOL; ■, solid line), midcycle (MID; ▲, dotted line), and luteal (LUT; ●, dashed line) phases. Insets: corresponding net integrated AUC for menstrual cycle phase. Values are means ± SE. *P* < 0.05 compared with midcycle phase. **P* < 0.05 compared with other 2 phases of menstrual cycle.
Data on circadian rhythms in the present study are not consistent with several previous studies. Decreased adrenal responses in the AM compared with the PM have been reported in studies with other provocative tests of pituitary-adrenal function, including ovine CRH (oCRH) stimulation and insulin administration (10, 12, 18, 25, 31). Some investigators have reported no significant diurnal differences in the cortisol response to oCRH (33, 34); however, these studies involved fewer subjects and thus may have been more subject to a type 2 error. However, one prior study (5) suggested that diurnal differences in adrenocortical activation to exercise are not caused by the progressive decline in cortisol throughout the day but rather are caused by the timing of exercise relative to several major cortisol peaks associated with food intake.

The results of the present studies suggest that diurnal variations in cortisol do not significantly modulate acute hormonal responses to exercise at 90 and 70% VO2max. Our findings are consistent with those of Thuma et al. (32), who reported similar magnitudes of change in cortisol in response to 40 min of treadmill exercise at 70% VO2max in the AM and PM, after correcting for circadian baselines. The discrepancy between the exercise studies (Thuma et al. and the present study) and those using oCRH (10, 12, 18, 25, 31) may be caused by the fact that exercise is a central stimulus, whereas oCRH is a pituitary stimulus. It is possible that glucocorticoid negative feedback may operate differently for a central stimulus than for a pituitary stimulus, thereby making it possible to mount a stress response despite elevated cortisol levels.

Salata et al. (29) provide compelling evidence that the apparent failure of high cortisol levels in the AM to significantly blunt pituitary responses to stress may actually reflect increased glucocorticoid restraint in combination with increased hypothalamic CRH drive in the AM. Using AVP stimulation, Salata et al. reported higher net integrated ACTH responses in the AM compared with the PM. This diurnal response in ACTH is opposite of that for oCRH administration, for which higher responses are reported in the PM when levels of cortisol are low. Salata et al. (29) speculated that perhaps an AVP stimulus might show greater potentiation of ACTH release in the AM when CRH levels are at a maximum compared with PM when CRH levels are presumably low. This possibility is supported by the finding that AVP by itself is a weak secretagogue for ACTH but markedly potentiates the effects of CRH (15). Consistent with this model, oCRH stimulation in the AM, when endogenous levels are high, should have little appreciable effect on ACTH release, thus accounting for the previously reported finding of reduced responses to oCRH in the AM.

Because the diurnal ACTH responses reported in the present study are more similar to those obtained by Salata et al. (29) for AVP and inconsistent with those obtained with oCRH, we speculate that AVP plays an important role in the acute pituitary-adrenal responses to exercise stress. Indeed, we have shown that short-term, high-intensity exercise is a potent stimulus for AVP release. Consistent with this model proposed by Salata et al., exercise performed in the AM, when endogenous CRH levels are high, may produce a relatively greater stimulation of ACTH release, which is partially blunted by higher ambient cortisol levels. Conversely, the same exercise stimulus in the PM, when CRH levels are low, may produce less ACTH secretion, which is partially offset by reduced glucocorticoid negative feedback. Our finding of nearly identical ACTH and cortisol responses to intense exercise in the context of differing cortisol levels suggests that these responses are determined, in part, by the degree of hypothalamic CRH drive in combination with the degree of glucocorticoid negative feedback at the time of the stress.

Also of particular interest is the finding that high-intensity exercise resulted in nearly identical net integrated ACTH and cortisol responses in the AM and PM.
(Fig. 2), whereas moderate-intensity exercise resulted in a trend towards decreased responses in the AM compared with the PM (Fig. 5). This trend raises the possibility of detecting diurnal differences with a less intense bout of exercise and/or with a greater number of subjects. It should be noted that the present studies were not designed to directly examine the effect of exercise intensity on the diurnal responsivity of the HPA axis to exercise. However, the two study populations were nearly identical (e.g., fitness level, age, weight, and race; see Table 1), and the experimental protocols were identical except for exercise intensity. General inspection of these data suggests that future research carefully examine diurnal variations in activation of the HPA axis as a function of exercise intensity. The ability of intense exercise (90% \( \dot{V}O_{2\text{max}} \)) to override negative feedback inhibition by cortisol may relate to the potency of the stressor to elicit an AVP response. The magnitude of the AVP responses to high-intensity exercise is typically greater than that observed for other challenge tests of the HPA axis, such as oCRH stimulation.

To our knowledge no studies have assessed whether there is a diurnal variation in stimulated AVP secretion. We measured basal and exercise-induced levels of plasma AVP in the AM and PM. Consistent with Altemus et al. (1), we found intense exercise to be a potent stimulus for AVP release in women, which resulted in a >35-fold increase over preexercise values. However, the patterns and magnitudes of change in AVP were essentially the same at both times of the day.

Our finding that exercise-induced GH release does not show a diurnal variation is in agreement with two studies of circadian rhythm that utilized insulin-induced hypoglycemia (18, 25). Another study reported significantly greater GH responses to insulin in the PM compared with the AM (31). However, these investigators measured PM GH levels at midnight, ~6 h later than determinations made in other studies. This raises the possibility that a small effect might be demonstrable at times of maximal quiescence.

In summary, our findings do not support a diurnal variation in the magnitude of metabolic, pituitary-adrenal, or sympathetic responses to exercise at 90% \( \dot{V}O_{2\text{max}} \). From a methodological perspective, the present data suggest that time of day does not have a significant effect on pituitary-adrenal responses to high-intensity exercise. Thus, these responses can be assessed with equal reliability in the AM and PM. No definitive conclusions can be drawn for ACTH and cortisol responses to exercise <90% \( \dot{V}O_{2\text{max}} \) until diurnal differences in HPA axis responsivity, as a function of exercise intensity, are better characterized. However, it seems prudent to control the time of day for exercise at 70% \( \dot{V}O_{2\text{max}} \) to reduce variability of pituitary-adrenal responses.

Menstrual Phase Comparisons

The present study provides little evidence for significant differences in the hormonal and metabolic responses to short-term, moderate-intensity exercise across menstrual cycle phase. The patterns of responses for ACTH and cortisol were similar across menstrual cycle phase. Furthermore, analysis of AUC revealed only a marginal main effect for cortisol. Although the pattern of glucose responses over time revealed a main effect of phase, net integrated glucose responses were not significantly different across cycle phase. Although the effect of phase for glucose suggests a subtle metabolic difference across menstrual cycle phase, this is not supported by analysis of RER. Additionally, there were no significant correlations between basal levels of progesterone or estrogen and either glucose or cortisol responses to exercise.

The majority of prior research examining the effect of menstrual cycle phase on the metabolic and/or hormonal responses to exercise have excluded assessment of the midcycle phase. With the exception of a few reports (e.g., Ref. 23), most studies examining the follicular and luteal phases report no effect of menstrual cycle phase on the metabolic responses to exercise (2, 26). Metabolic data from the present study are consistent with those of Kanaley et al. (21), who examined all three phases of the menstrual cycle and reported no phase-related differences in either glucose responses or RER during exercise.

With regard to pituitary-adrenal responses to exercise, the present results are consistent with those of De Souza et al. (13). Using a similar exercise protocol and the same preexercise 6-h fast as the present study, De Souza et al. reported no difference in ACTH and cortisol responses to exercise when comparing the follicular and luteal phases of the menstrual cycle. Our finding that exercise-induced cortisol release does not vary according to menstrual cycle phase is also in agreement with Bonen et al. (2), who reported similar findings for subjects who fasted for 24 h. Kanaley et al. (20) also assessed cortisol responses to exercise across three phases of the menstrual cycle and reported no effect of cycle phase. However, their exercise protocol did not appear to activate the HPA axis. In fact, the endocrine responses they reported were similar to those noted for exercise at 50% \( \dot{V}O_{2\text{max}} \) (24). One study that has reported differential pituitary-adrenal responses to exercise as a function of menstrual phase was that of Lavoie et al. (23). They reported higher cortisol concentrations during the luteal compared with the follicular phase after 90 min of exercise. However, this finding may reflect the fact that subjects consumed a carbohydrate-poor diet 24 h before exercise (23). Thus controlling for exercise intensity and nutritional status is necessary when assessing pituitary-adrenal responses to exercise across the menstrual cycle.

In summary, most prior investigations report no effect of menstrual cycle phase on the metabolic (2, 21, 26) or hormonal (2, 13, 20) responses to exercise. The results of the present study demonstrate that, with the possible exception of glucose, the metabolic and responses to exercise are not significantly different in the follicular, midcycle, and luteal phases of the menstrual cycle. Future research should replicate the phase effect for glucose and extend the present research by examin-
ing glucose and cortisol, as well as AVP and NE, responses to exercise across all three phases of the menstrual cycle. Because glucose mobilization is stimulated by a number of hormonal responses to exercise, including AVP and NE, such a study could examine the possibility that glucose provides a more integrated and sensitive reflection of cyclic-related changes in metabolic responses to exercise.

The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Defense, Uniformed Services University of the Health Sciences, or the National Institutes of Health. Address for reprint requests: P. A. Deuster, Dept. of MIM, USUHS, 4301 Jones Bridge Rd., Bethesda, MD 20814-4799 (E-mail: PDEUSTER@USUHS.MIL).

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