Catecholamine response during 12 days of high-altitude exposure (4,300 m) in women

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Mazzeo, Robert S., Avon Child, Gail E. Butterfield, Jacinda T. Mawson, Stacy Zamudio, and Lorna G. Moore. Catecholamine response during 12 days of high-altitude exposure (4,300 m) in women. J. Appl. Physiol. 84(4): 1151–1157, 1998.—We have previously demonstrated that acclimatization to high altitude elicits increased sympathetic nerve activity in men. The purpose of this investigation was to determine 1) whether women respond in a similar manner as found previously in men and 2) the extent to which menstrual cycle phase influences this response. Sixteen eumenorrheic women (age, 23.6 ± 1.2 yr; weight, 56.2 ± 4.3 kg) were studied at sea level and during 12 days of high-altitude exposure (4,300 m) in either their follicular (F; n = 11) or luteal (L; n = 5) phase. Twenty-four-hour urine samples were collected at sea level and during each day at altitude. Catecholamines were determined by high-performance liquid chromatography with electrochemical detection. Compared with sea-level values, urinary norepinephrine excretion increased significantly during altitude exposure, peaking on days 4–6. Thereafter, levels remained constant throughout the duration of altitude exposure. The magnitude of this increase was similar between the F (138%) and L (93%) phase. Urinary epinephrine levels were elevated on day 2 of altitude exposure compared with sea-level values for both F and L subjects (93%). Thereafter, urinary epinephrine excretion returned to sea-level values, and no differences were found between F and L subjects. Plasma catecholamine content was consistent with urinary values and supports the concept of an elevation in sympathetic activity over time at altitude. Mean and diastolic blood pressure as well as heart rate adjustments to high altitude correlated significantly with urinary norepinephrine excretion rates. It was concluded that 1) urinary and plasma catecholamine responses to 12 days of high-altitude exposure in women are similar to those previously documented to occur for men; 2) whereas no differences in catecholamine levels were observed between F- and L-phase assignments, for a given urinary norepinephrine excretion rate, blood pressure and heart rates were lower for F vs. L subjects; and 3) several cardiovascular adaptations associated with high-altitude exposure correlated with 24-h urinary norepinephrine excretion rates and thus sympathetic nerve activity.

These responses are significant in that the sympathoadrenal system plays a critical role in regulating a number of key physiological (heart rate, stroke volume, vascular resistance, blood pressure) and metabolic (substrate utilization) functions necessary to adjust to the stress imposed by acute and prolonged exposure to high altitude (9, 11, 16, 19). However, to date, little is known with regard to how women adapt and/or respond to both acute and chronic high-altitude exposure. Specifically, it was the purpose of this study to determine whether women demonstrated similar sympathoadrenal adjustments to those previously found in men and the extent to which these responses contributed to the cardiovascular adaptations associated with acclimatization to high altitude. Furthermore, because sympathoadrenal responses can be influenced by menstrual cycle phase (5, 20, 21) and because cycle phase can be influenced by sympathoadrenal responses (1), it was a second purpose of this study to examine the interaction between cycle phase (luteal or follicular) and sympathoadrenal responses at altitude.

METHODS

Subjects. Sixteen healthy, nonsmoking, eumenorrheic sea-level residents (age, 23.6 ± 1.2 yr; weight 56.2 ± 4.3 kg) volunteered to participate in the study. All subjects read and signed an informed consent approved by the Human Subjects Committees from the University of Colorado Health Sciences Center, Stanford University, and the US Army Research Institute of Environmental Medicine.

Protocol. Sea-level measurements were conducted at the Palo Alto Veterans Affairs Health Care System (752 mmHg). Each subject, on admission to the study, kept a menstrual cycle diary, noting the date and duration of menstruation, the date of a luteinizing hormone (LH) surge, and the duration of the cycle. On the basis of a 3-mo history documented by diary or by information provided from the subject on cycle length, each subject began testing for her LH surge by using an ovulation predictor kit (OvuQuick, Becton-Dickinson, Rutherford, NJ) at least 4 days before the estimated time of the LH surge.
Subjects began their studies at sea level and at high altitude the day after menses began, or the day after an LH surge was detected, and this was designated day 1 of the study. Ovarian steroid hormones were measured on days 3, 10, and 12 at sea level during each cycle study (1 during follicular and 1 during the luteal phase). Ovarian steroid hormones were measured on days 3, 6, 9, 10, and 11 at high altitude.

Blood for analysis of ovarian steroid hormones was obtained by venipuncture, allowed to clot for 30 min, and then centrifuged at 3,000 revolutions/min for 10 min. The serum was immediately frozen. All samples were analyzed at the same time, 1 mo after the completion of the altitude studies. Serum aliquots were assayed for progesterone and estradiol by the General Clinical Research Center Laboratory at the University of Colorado Health Sciences Center. Estradiol and progesterone concentrations were measured by using a Diagnostic Products Coat-A-Count radioimmunoassay.

Sea-level urinary catecholamines were determined during the middle and late stages of both the follicular and luteal phases of the menstrual cycle (averaged over 6 days). Approximately 1 mo after sea-level studies, subjects were initially assigned to arrive at altitude at the beginning of either their follicular (n = 8) or luteal (n = 8) phase as described above. Altitude studies were performed as the subjects resided at the summit of Pikes Peak, CO (462 mmHg) for 12 days. On the basis of both urinary and plasma hormone measurements made during the subjects' 12-day stay at altitude, it was determined that three women originally assigned to the luteal phase had crossed over into the follicular phase. Thus data were analyzed as 11 women in the follicular and 5 in the luteal phase. Subjects then resided at the summit for 12 days. Energy intake as well as physical activity were regulated both at sea level as well as at altitude to avoid weight and exercise fluctuations as previously described (2).

Catecholamine measurements. Twenty-four-hour urine samples were collected at sea level and during each of the 12 days while the subjects were at 4,300 m. After determination of the 24-h sample volume, a 10-ml aliquot was mixed with sulfuric acid (5 mM) to control for catechol oxidation and was stored at −20°C until analyzed (on dry ice) to Boulder, CO, at which time the samples were stored at −70°C until analysis.

Resting blood samples were collected by venipuncture during both cycle phases while the subjects were at sea level and on days 4 and 11 at altitude. These samples were mixed with reduced glutathione and centrifuged, and the plasma was stored in liquid nitrogen until transfer to the Boulder laboratory for storage at −70°C.

Urinary and plasma catecholamine levels were determined by means of high-performance liquid chromatography (HPLC) (model 1330 pump and model 1340 electrochemical detector, Bio-Rad) with electrochemical detection as previously described (11). Dihydroxybenzylamine (Sigma Chemical) was used as the internal standard. Catecholamines were absorbed onto acid-washed alumina with 1.5 M tris(hydroxymethyl)aminomethane buffer at pH 8.6 in 2% EDTA. The alumina was then washed 2× with 3 ml of distilled water. The catecholamines were extracted with 100 µl of 0.1 N perchloric acid with 10 min of shaking and final centrifugation at 12,000 g. One hundred milliliters of eluate were then injected into the HPLC column (reverse phase, Bio-Sil ODS-5S, BioRad) and eluted with mobile phase (6.8 g sodium acetate-anhydrous, 1.0 g sodium heptane sulfonate, 60 ml acetonitrile, and 1.0 g Na2EDTA in 1 liter pH adjusted to 4.8). The flow rate was set at 1.1 ml/min at 2,000 lb./in.² at 0.65 V. The chromatogram was integrated on a Shimadzu integration system (model C-R3A).

Cardiovascular measurements. Resting systolic and diastolic blood pressures were determined in the morning while subjects remained supine for measurements at sea level and on days 2–12 at altitude by an Accutrac II ambulatory blood pressure monitor (Ambumedix Systems, Atlanta, GA). This instrument was calibrated as recommended by the National High Blood Pressure Education Program Coordinating Committee. Mean arterial pressure was determined as follows: mean arterial pressure (mmHg) = 0.33(systolic pressure – diastolic pressure) + diastolic pressure. Resting heart rates were determined at the same time by using a pulse oximeter.

Statistics. All values reported are means ± SE. Differences across all testing (altitude) conditions as well as differences between follicular and luteal groups were determined by a repeated measures two-way analysis of variance with significance set at P < 0.05. Tukey post hoc comparisons were used to identify significant differences among means. Pearson-product correlations were used to assess the relationship between urinary norepinephrine excretion and mean and diastolic blood pressure as well as resting heart rates.

RESULTS

Results from the Environmental Symptoms Questionnaire, which was administered twice daily to assess acute mountain sickness (AMS) scores, indicated that AMS scores were significantly greater than sea-level scores for days 1 and 2 at altitude (0.89 ± 0.14) only (12). Scores for the remaining days at altitude (0.22 ± 0.05) were not different from sea-level values. No statistical differences were found between menstrual cycle phases at any time.

Serum progesterone. Values for serum progesterone for all subjects across both sea-level and high-altitude conditions are shown in Table 1. Measurements were obtained serially over time within a given subject, and when values for progesterone reached or exceeded 2.5 ng/ml the subject was classified as being in the luteal phase. On the basis of this criterion, it was determined

Table 1. Resting serum progesterone values during days 3, 10, and 12 for both sea-level and altitude exposure across cycle phases

<table>
<thead>
<tr>
<th></th>
<th>Sea Level</th>
<th>Pikes Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3</td>
<td>Day 10</td>
</tr>
<tr>
<td>Follicular</td>
<td>11</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.3–1.1)</td>
</tr>
<tr>
<td>Luteal</td>
<td>5</td>
<td>3.6 ± 1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.5–6.9)</td>
</tr>
</tbody>
</table>

Values are means ± SE in ng/ml with ranges in parentheses; n, no. of subjects.
that at altitude 11 women were in the follicular phase and 5 women were in the luteal phase.

Urinary catecholamines. At sea level, 24-h urinary norepinephrine excretion did not differ between subjects when the samples were collected at midfollicular or midluteal phase (36.7 ± 2.6 vs. 41.8 ± 5.5 µg/day, respectively). After the subjects resided at 4,300 m for only 1 day, norepinephrine excretion rose significantly (53.1 ± 4.8 and 59.6 ± 5.6 µg/day for follicular and luteal, respectively). Urinary norepinephrine excretion continued to increase steadily during subsequent days at altitude, peaking at days 4–6 (Fig. 1A). Thereafter, excretion rates remained constant at this elevated level for the duration of the altitude residence. While the subjects were at altitude, no difference between follicular or luteal phase was observed ($P = 0.05$).

Table 2. Resting plasma norepinephrine and epinephrine levels at sea level and on days 4 and 11 of high-altitude exposure

<table>
<thead>
<tr>
<th></th>
<th>PG</th>
<th>Sea Level</th>
<th>Day 4</th>
<th>Day 11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Follicular</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>11</td>
<td>558 ± 27</td>
<td>609 ± 28</td>
<td>715 ± 35*</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>5</td>
<td>549 ± 26</td>
<td>618 ± 52</td>
<td>679 ± 56*</td>
</tr>
<tr>
<td><strong>Luteal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>11</td>
<td>92 ± 13</td>
<td>152 ± 24*</td>
<td>117 ± 36</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>5</td>
<td>83 ± 12</td>
<td>124 ± 26*</td>
<td>107 ± 33</td>
</tr>
</tbody>
</table>

Values are means ± SE in pg/ml; n, no. of subjects. *Significantly different from sea level, $P < 0.05$.

although a trend for greater excretion rates during the follicular phase was seen for the last days at 4,300 m.

Urinary epinephrine excretion rates also did not differ between groups when measured at sea level (8.7 ± 0.9 and 9.7 ± 1.6 µg/day for follicular and luteal, respectively). However, epinephrine excretion increased after 1 day at altitude, achieving the greatest values observed throughout the study (Fig. 1B). By day 6 epinephrine excretion rates had returned to sea-level values, demonstrating the opposite pattern to that found for norepinephrine excretion. Again, no differences in epinephrine excretion rates were observed between phase assignments during altitude exposure.

Plasma catecholamines. The time course for changes in plasma norepinephrine levels (Table 2) when measured at sea level and days 4 and 11 at altitude correlated with the changes in urinary excretion rates over time. Thus plasma norepinephrine levels were significantly greater on day 11 at altitude when compared with sea-level values. Day 4 norepinephrine levels were lower than those of day 11 but were not significantly different from sea-level values. Plasma epinephrine levels were significantly elevated above sea-level values on day 4 at altitude but were not significantly different from sea-level values by day 11. These findings are consistent with urinary epinephrine excretion rates observed. For both plasma norepinephrine and epinephrine, no differences were found between follicular- and luteal-phase assignments.

Blood pressure responses. Mean arterial pressure increased on arrival of the subjects to altitude, peaking at days 3–4 and remaining elevated for the duration at 4,300 m (Fig. 2A). This finding was consistent for women in both the follicular and luteal phases with no differences observed between groups. The changes in mean arterial pressure over time at altitude followed a similar temporal pattern to that observed for urinary norepinephrine excretion (Fig. 2B). This relationship was consistent across phase assignments because both follicular ($r = 0.76$) and luteal ($r = 0.70$) correlations between mean arterial pressure and urinary norepinephrine excretion were similar ($P < 0.05$; Fig. 2B).

The elevation in mean arterial pressure at altitude was primarily due to a similar pattern of increase in diastolic blood pressure (Fig. 3A) because systolic blood
pressure was not found to change significantly while at altitude. Diastolic pressure peaked at days 3–4 at altitude and remained stable throughout the duration at 4,300 m. No differences between cycle phase assignments were observed. As with mean arterial pressure, a strong relationship ($P < 0.05$) was found between diastolic blood pressure and urinary norepinephrine excretion for both follicular- ($r = 0.75$) and luteal-phase ($r = 0.73$) assignments (Fig. 4B).

**DISCUSSION**

The major findings of the present investigation suggest that 1) the urinary and plasma catecholamine responses to both acute and chronic high-altitude exposure in women are similar to those previously documented to occur for men; 2) no significant differences in these sympathoadrenal responses were observed between follicular- and luteal-phase assignments across all altitude conditions; and 3) several cardiovascular adaptations associated with high-altitude exposure correlated with 24-h urinary norepinephrine excretion rates and thus sympathetic nerve activity.
We have previously documented the sympathoadrenal responses in men during 21 days of exposure to 4,300 m (9–11). In those studies, it was clearly demonstrated that sympathetic nerve activity increased steadily during the initial days at altitude compared with sea-level values. These conclusions were based on several lines of evidence, including increased urinary norepinephrine excretion rates, elevated plasma norepinephrine content, and increased net release of norepinephrine across the leg at rest. Those studies also demonstrated that measurement of 24-h urinary norepinephrine excretion is a valid indicator of sympathetic nerve activity both at sea level and during exposure to high altitude. It was determined that in men the increase in sympathetic activity reached a peak at days 6–7 at altitude. That observation is in close agreement with results from the present investigation on women. As shown in Fig. 1A, urinary norepinephrine excretion rose steadily during the initial days at altitude, reaching a plateau on days 4–6. This finding is consistent with the conclusion that sympathetic nerve activity is elevated on arrival of the subjects to high altitude and that this response is similar in both men and women.

As also shown in previous studies on men (10), urinary epinephrine excretion rates for the women in the present study demonstrated a different response than that observed for norepinephrine excretion. Epinephrine excretion rates increased on arrival of the subjects to altitude (day 2) but decreased thereafter, returning to sea-level values by day 6. Thus there appears to be a dissociation between the sympathetic response to high-altitude exposure with that of the adrenal medullary response such that sympathetic nerve activity is increasing over time at altitude, whereas adrenal medullary activity is declining toward sea-level values. This is also supported by the plasma epinephrine measurement (Table 1). The mechanisms for the epinephrine (and adrenal) response are likely related to the direct effects of hypoxia. Hypoxia has been shown to directly stimulate adrenal epinephrine release, resulting in increased arterial concentrations (6, 9, 11). The extent of this response is dependent on the degree and severity of the hypoxic stimulus. Thus, during acute hypoxia, in which arterial O₂ saturation is at its lowest levels, plasma and urinary epinephrine contents are highest. As arterial oxygenation improves with acclimatization, arterial O₂ saturation increases, whereas epinephrine content returns toward sea-level values. We have previously (9–11) documented this relationship in men at altitude, and this sympathoadrenal response appears to be similar in women.

These sympathoadrenal responses to high-altitude exposure in women likely contributed to a number of the cardiovascular adjustments observed in our subjects. The increase in urinary norepinephrine excretion over time at altitude (reflecting an elevation in whole body sympathetic nerve activity) correlated highly with the rise in mean arterial pressure (r = 0.74 for all subjects; Fig. 2). We have previously documented this temporal relationship in men during 21 days of residence at 4,300 m (9, 11, 19). In those studies, a significant correlation was found between mean arterial pressure and 24-h urinary norepinephrine excretion. Furthermore, those studies demonstrated that the elevation in sympathetic activity directly resulted in an increase in systemic vascular resistance contributing as an underlying mechanism for the rise in blood pressure. Results from the present investigation suggest that a similar mechanism is responsible for the increase in mean arterial pressure in women chronically exposed to high altitude. A heightened sympathetic response resulting in neural norepinephrine release is known to elicit vasoconstriction via the α-adrenergic receptors, thus producing an elevation in vascular resistance (3, 15, 17). The increase in mean arterial pressure found for the women is primarily related to the increase in diastolic pressure because...
systolic pressure was not significantly elevated over the 12 days at altitude. This observation is also consistent with that found for men such that, by day 8 of altitude, systolic blood pressure was not significantly different from sea-level values, whereas both mean arterial and diastolic pressures were increased in these male subjects (11, 19).

Heart rate responses for the women of this study increased initially in response to high-altitude exposure, peaking on days 2–5. Resting heart rates remained elevated for the duration of the altitude period. When compared with changes in urinary norepinephrine excretion rates, heart rates for both follicular (r = 0.81) and luteal (r = 0.78) subjects yielded high correlations (Fig. 4B). This relationship was stronger than that found for urinary epinephrine excretion rates, which had returned to sea-level values while heart rates were peaking (r = 0.33 and 0.40 for follicular and luteal phase, respectively). An attenuation in cardiac responsiveness to β-adrenergic stimulation has been reported to occur in men with chronic hypoxic exposure (7, 8, 14). We have previously shown that β-adrenergic-blocked male subjects demonstrated similar heart rate responses to prolonged exposure to 4,300 m as those of controls receiving a placebo (11, 19). Together, these results suggested that other mechanisms independent of β-mediated pathways, such as altered parasympathetic and/or α-adrenergic activity, contribute to the heart rate responses observed over time at altitude. Results from the present investigation are consistent with an α-adrenergic-mediated contribution to the heart rate pattern observed.

With regard to the urinary and plasma catecholamine responses observed both at sea level and during altitude exposure, no differences were found between follicular- and luteal-phase assignments. Although there have been few studies examining the relationship between menstrual cycle phase and sympathoadrenal activity, it has generally been reported that under sea-level conditions plasma norepinephrine levels are greater during the luteal phase (5, 20). However, no differences in plasma norepinephrine content or in urinary epinephrine excretion have been reported (20, 21). Because we did not measure plasma catecholamines on a daily basis both at sea level and during altitude exposure, it is not surprising that no statistical differences were found. However, we did measure urinary catecholamine excretion on each of the 12 days at altitude, and these results indicated that, in response to the stress imposed by both acute and chronic hypoxia, no differences in sympathoadrenal responses existed between the follicular and luteal phase. This finding was extended to the cardiovascular alterations known to be regulated by sympathoadrenal pathways. As such, no differences in the blood pressure or heart rate adaptations to altitude existed between phase assignments. However, examination of the relationship between urinary norepinephrine excretion rates with these cardiovascular variables over time at altitude suggested a relative insensitivity for subjects in their follicular compared with luteal phase. For a given urinary norepinephrine excretion rate, pressures and heart rates were lower for follicular vs. luteal subjects (Figs. 2A, 3A, 4A). Slopes for these relationships were significantly lower for follicular subjects (averaging 50% of that for luteal subjects). Although the mechanisms responsible for this observation are beyond the scope of the present study, this finding may reflect the interaction of hypoxia and ovarian hormones on adrenergic-receptor density, affinity and signal transduction. Further research in this area is warranted.

In summary, results from the present investigation suggest that sympathoadrenal responses during 12 days of high-altitude exposure in women are similar to those previously documented to occur for men. Although no differences in catecholamine levels were observed between follicular- and luteal-phase assignments, for a given urinary norepinephrine excretion rate, blood pressure and heart rates were lower for follicular vs. luteal subjects. Together, these sympathoadrenal responses likely contribute to several of the cardiovascular adaptations associated with high-altitude exposure. Future studies examining the role of α-adrenergic-mediated mechanisms to adaptations elicited by both acute and chronic high-altitude exposure are warranted.

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