Endocrine responses to acute and chronic high-altitude exposure (4,300 meters): modulating effects of caloric restriction

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Energy requirements while energy intake declines. Consequently, HA sojourners are often forced to adapt to the hypoxic effects of altitude and the metabolic effects of energy imbalance concurrently. Although hypobaric hypoxic chambers have eliminated stresses such as cold, limited food availability, and rugged terrain, many subjects at simulated altitude still show progressive weight loss during prolonged investigations when caloric consumption is not carefully controlled (40, 51, 56).

Altitude-induced weight loss can be minimized by matching dietary intake with energy requirements (5, 6, 30). The stabilization in body weight achieved from such studies allows assessment of the unique physiological effects of altitude independent of any confounding effects from negative energy balance. However, to our knowledge, no investigation has directly compared altitude-induced neuroendocrine changes with or without energy imbalance or examined how energy sufficiency at altitude alters the endocrine acclimatization response. Furthermore, although adipocytokines, such as leptin and adiponectin, have been highlighted as major factors in the regulation of appetite and metabolism at sea level (SL) in recent years, their roles in preserving energy homeostasis at altitude are not well understood.

Altitude exposure is known to stimulate neuroendocrine systems as part of the acute hypoxic and chronic adaptive acclimatization process (2). In contrast, prolonged caloric restriction (CR) tends to blunt some of the same neuroendocrine pathways that are stimulated at altitude as the body attempts to reduce basal energy expenditure (44). In this report, we describe the hormonal and metabolic responses to HA as measured in three groups of subjects over the course of a 21-day dietary intervention. The goals of the investigation were to 1) determine the physiological response to altitude independent of CR using a weight-stable group at Pikes Peak, CO (4,300 meters), 2) compare the altitude-induced hormonal response of an adequately fed group with that of a CR group, and 3) compare endocrine and metabolic patterns in a CR group at altitude with those of a CR group at SL. We hypothesized that effects of altitude and CR would oppose each other to create a more moderate response pattern in the CR subjects compared with the fully fed subjects at Pikes Peak. This study was part of a larger investigation designed to determine the interactive

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effects of altitude and CR on work performance and metabolism.

METHODS

Subjects

Twenty-nine nonsmoking, normal-weight, active men between the ages of 18 and 35 yr volunteered to participate in the study. For inclusion, subjects were required to have maintained a stable body weight for the previous 6 mo, to be in good health without chronic illnesses, and to have been born and to have resided at an altitude 2,000 meters for the previous three years. Subjects were recruited from advertisements and fliers placed in local newspapers in and around the Palo Alto/San Jose, CA, area. The protocol was approved by the Administrative Panels for the Protection of Human Subjects at Stanford University and the U.S. Army Research Institute for Environmental Medicine (USARIEM). Each participant gave written consent before screening.

Screening

Upon admission to the Clinical Studies Unit (CSU), participants completed a medical history questionnaire and underwent a physical examination, a resting 12-lead electrocardiogram, routine blood and urine analyses, and a nutrition assessment. Each participant then completed a continuous, progressive exercise test to volitional exhaustion on a cycle ergometer (model 800; Sensormedics, Yorba Linda, CA) for the determination of peak oxygen consumption (VO2peak) using an on-line Truemax system (Parvomedics Consentius Technologies, Sandy, UT). For participation in the study, subjects were required to have a VO2peak >40 ml·kg⁻¹·min⁻¹.

General Study Design

Three different groups of subjects were studied during a 21-day intervention period. All subjects completed a body weight stabilization period and baseline measurements at SL during the spring months in the CSU at the Veterans Affairs Palo Alto Health Care System. One group completed the entire intervention at the CSU in Palo Alto. This group was fed a hypocaloric diet (HYPO) that was 40% deficient in calories needed to maintain body weight. Two other groups traveled to the USARIEM facility on the summit of Pikes Peak (4,300 meters) in Colorado during the summer months for 21 days of HA exposure. Subjects traveled by air directly from SL in California to Colorado Springs, CO (1,840 m). Subjects remained overnight on oxygen, and were expected to finish all the food provided and eat nothing else. The food items consisted of whole foods (e.g., pasta with red sauce, bread, cheese, apples, etc.) that were adjusted in quantity according to subject needs. The diets for all subjects provided 100% of the recommended daily allowances for nutrients. A multivitamin and mineral supplement were also provided daily.

Intervention Diet

Energy deficit was created by decreasing the intake of fat and carbohydrate foods. However, the carbohydrate intake was kept above at least 3 g·kg⁻¹·day⁻¹ to minimize the effect of low carbohydrate intake on glycogen stores. To meet all the dietary criteria listed above, only subjects who normally consumed >2,700 kcal/day at SL were selected for participation. Adjustments were made as needed in accordance with daily weight measurements. All foods were pre-weighed, packaged, and given to the subjects throughout the study period. As in the stabilization period, diets for all subjects provided 100% of the recommended daily allowances for nutrients. A multivitamin and mineral supplement were also provided daily.

SL Group

Subjects in the HYPO group (n = 9) were fed the stabilization diet for 7 days (Baseline −7 to −1) before the intervention phase to attain energy, nitrogen, and body weight balance. Immediately after this stabilization period, subjects were fed a hypocaloric diet for 21 days based on a 40% reduction from their baseline calorie requirements. Participants in the HYPO group lived in the CSU throughout the 3-wk intervention. Subjects were allowed to leave the CSU to attend classes, work, etc., during the day but were expected to return when not actively engaged in outside activities. All daily food was provided, and subjects were required to eat both breakfast and dinner in the unit everyday. Participants were asked to continue with their normal activity patterns during the intervention period, and a daily schedule of activities was given to them based on their preintervention exercise logs.

Altitude Groups

Subjects in the two altitude groups (ADQ and DEF) also underwent a 7-day dietary stabilization period at SL. Subjects then underwent 5 days of SL baseline testing. Deployment to Pikes Peak for the 21-day intervention phase took place 1–2 mo after SL baseline testing. To minimize acute effects of diet on initial altitude measurements, subjects in the ADQ and DEF groups completed an additional 3-day dietary stabilization period immediately before the HA intervention. During the 21-day intervention, the ADQ group (n = 7) consumed the SL body weight stabilization diet plus an additional 200 kcal/day to account for the increases in basal metabolic rate (BMR) associated with acute altitude exposure (6). In contrast, the DEF group (n = 10) ate a diet deficient by ~40% of the calories (mean deficit = 1,300 kcal/day) from their SL body weight maintenance intake plus an additional 200 kcal/day to account for the anticipated altitude-induced BMR increase.

Participants in the ADQ and DEF groups resided at the USARIEM Laboratory for the entire 21-day intervention period. All food was provided for them daily and adjusted as needed. Activity level at SL was monitored through 24-h exercise logs. At altitude, these diaries were used to devise an exercise program mimicking SL activities as much as possible to prevent detraining and to balance energy expenditure under the two environmental conditions. The program included cycle ergometry, hiking, and weight lifting.

Body Composition

Fasting body weight was measured every other morning throughout the study. Skinfold measurements were made using calipers (Lange
Table 1. Baseline subject characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>ADQ (n = 7)</th>
<th>DEF (n = 10)</th>
<th>HYPO (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>21.3 ± 2.8</td>
<td>22.1 ± 2.8</td>
<td>23.1 ± 5.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>178.6 ± 5.1</td>
<td>178.9 ± 6.6</td>
<td>176.9 ± 7.8</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74.1 ± 6.9</td>
<td>79.6 ± 11.7</td>
<td>78.5 ± 6.1</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>9.2 ± 2.9*</td>
<td>13.6 ± 3.1</td>
<td>16.8 ± 4.8</td>
</tr>
<tr>
<td>LBM, kg</td>
<td>67.2 ± 5.7</td>
<td>68.5 ± 7.7</td>
<td>65.4 ± 6.1</td>
</tr>
<tr>
<td>VO₂ peak (mL/kg/min⁻¹)</td>
<td>52.6 ± 5.5</td>
<td>51.9 ± 6.6</td>
<td>45.2 ± 7.3†</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of subjects. ADQ, adequately fed; DEF, diet deficient in calories; HYPO, hypocaloric diet; LBM, lean body mass; VO₂ peak, peak oxygen consumption. P < 0.05, *significant from DEF and HYPO and †significant from DEF and ADQ.

Blood Sample Collection and Analysis

Fasting blood samples were collected upon rising between 6:00 A.M. and 8:00 A.M. on days –3, 3, 5, 10, 19, and 21. Samples were immediately placed on ice, centrifuged for 10 min at 3,000 rpm, decanted, and frozen. Blood samples for the analysis of glucose concentration were collected in 8% perchloric acid. Plasma glucose concentration was determined by the Veterans Affairs Clinical Laboratories using a Beckman LX20 multichannel analyzer. Serum for the analysis of insulin, cortisol, testosterone, sex hormone-binding globulin (SHBG), lipid panel, erythropoietin (EPO), and adiponectin was collected in chilled tubes, and plasma for the analysis of leptin and thyroid hormones was collected in chilled tubes containing EDTA. All samples were stored at –80°C for future analysis using RIA kits (Diagnostic Systems Laboratories, Webster, TX). Adiponectin samples were analyzed using ELISA kits (Linco Research, St. Charles, MO). Twenty-four-hour urine samples were collected for the analysis of catecholamine levels at SL and during the intervention period on days 3, 10, and 21. Body weight and skinfold measurements were used to calculate lean body mass, fat mass, and body fat percentage.

Calculations

Homeostatic model assessment insulin resistance (HOMA-IR) was assessed from the HOMA calculation: [FI (µmol/l) × FG (mmol/l)]/22.5, where FI is fasting insulin and FG is fasting glucose (26). Free testosterone was calculated by dividing the total testosterone concentration by fasting SHBG concentration.

Statistics

One-way ANOVA was used for the analysis of subject baseline characteristics. Two-way ANOVAs (group × time) with repeated measures were used for analyzing hormone levels at all time points throughout the intervention. Post hoc analyses were performed where appropriate using the Fisher’s least-significant difference procedure. Statistical significance was accepted at P < 0.05. When significant baseline differences occurred, data were graphed and analyzed as a change from baseline. All values are presented as means ± SE unless otherwise noted.

RESULTS

Subject Characteristics, Diet, and Body Composition

Subject characteristics recorded during the baseline body weight stabilization period at SL (day –3) are presented in Table 1. Twenty-nine subjects were recruited to participate in this study, but three subjects withdrew from ADQ after baseline testing because of schedule conflicts, leaving n = 10, 7, and 9 for DEF, ADQ, and HYPO, respectively. There were no differences in age, height, weight, or lean body mass among groups. The mean percentage body fat was greater in HYPO and DEF compared with ADQ (P < 0.05). HYPO had lower VO₂ peak values than ADQ or DEF (P < 0.05).

Post hoc analyses of the diets from daily food checklists indicated that target caloric intakes were achieved for each group (Table 2). Both calorie-restricted groups showed a significant decrease from baseline values across all body composition measurements (Table 3). Changes in body composition were assessed from day 3 of the intervention as well as baseline to minimize the potential impact of altitude-induced fluid loss on weight measurements in the HA groups (22). Changes in weight, lean mass, and fat mass were negligible in the ADQ group when the standard fluid reduction phase (days 1 and 2) at altitude was omitted from the calculations (Table 3).

Glucose, Insulin, and HOMA-IR

There was no difference in fasting glucose concentration among the three groups at baseline. However, both ADQ and DEF groups experienced a significant increase in blood glucose concentration (9.3 and 5.1%, respectively, P < 0.05) upon acute exposure to altitude, whereas blood glucose fell (−5.5%, P < 0.05) in the HYPO group with the onset of CR (Fig. 1A). The disparate responses resulted in significantly different fasting glucose values between groups for days 3 and 5, but most differences between groups disappeared by the end of the intervention.

Baseline insulin values for DEF (12.4 ± 1.0 µU/ml) and ADQ (9.6 ± 1.3 µU/ml) were lower than the HYPO group (19.3 ± 3.2 µU/ml, P < 0.05). ADQ and DEF exhibited a transient increase in insulin concentration upon arrival at Pikes Peak (+68 and +23%, respectively), but only the rise in ADQ was significant from SL baseline (P < 0.05; Fig. 1B). The insulin level change in ADQ peaked on day 5 at 113% above baseline before returning to prealtitude values. Insulin in HYPO dropped significantly (−36% at day 3; P < 0.05) from baseline values and remained suppressed throughout the intervention (Fig. 1B).

Table 2. Energy and macronutrient intake at SL and 4,300 meters HA

<table>
<thead>
<tr>
<th>Variable</th>
<th>kcal</th>
<th>CHO, g</th>
<th>Protein, g</th>
<th>Fat, g</th>
<th>Change in kcal, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL baseline</td>
<td>3,340</td>
<td>574</td>
<td>90</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>HA (day 1–4)</td>
<td>3,557</td>
<td>633</td>
<td>83</td>
<td>77</td>
<td>+1*</td>
</tr>
<tr>
<td>HA (day 18–21)</td>
<td>3,679</td>
<td>650</td>
<td>92</td>
<td>79</td>
<td>+4*</td>
</tr>
<tr>
<td>DEF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL baseline</td>
<td>3,530</td>
<td>589</td>
<td>100</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>HA (day 1–4)</td>
<td>2,190</td>
<td>348</td>
<td>87</td>
<td>50</td>
<td>−41*</td>
</tr>
<tr>
<td>HA (day 18–21)</td>
<td>2,186</td>
<td>348</td>
<td>86</td>
<td>50</td>
<td>−41*</td>
</tr>
<tr>
<td>HYPO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL baseline</td>
<td>3,245</td>
<td>555</td>
<td>92</td>
<td>73</td>
<td></td>
</tr>
</tbody>
</table>

SL sea level; HA, high altitude; CHO, carbohydrate. Protein set between 1.1 and 1.2 g/kg. *Change relative to SL diet +200 kcal to account for expected altitude-induced basal metabolic rate increase.
Figure 1C displays the change from baseline in insulin resistance (HOMA-IR) over time. IR increased temporarily in ADQ early in the acclimatization process but returned to SL values by day 10. HYPO exhibited a significant fall in HOMA-IR by day 3 that was maintained for the duration of the study. Subjects in DEF experienced no alteration in HOMA-IR values.

Other Hormones

Thyroid. No significant changes occurred in serum levels of thyroid-stimulating hormone (TSH) for either group that resided at the summit of Pikes Peak apart from a slight fluctuation in the DEF group at day 21 (Fig. 2A). However, at SL, CR

<table>
<thead>
<tr>
<th></th>
<th>Baseline to day 21</th>
<th>day 3 to day 21</th>
<th>Baseline to day 21</th>
<th>day 3 to day 21</th>
<th>Baseline to day 21</th>
<th>day 3 to day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Wt, kg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADQ</td>
<td>−0.8±0.7</td>
<td>−0.9±0.5</td>
<td>−1.4±0.5*</td>
<td>−0.1±0.4</td>
<td>+0.5±0.8</td>
<td>−0.8±0.2</td>
</tr>
<tr>
<td>DEF</td>
<td>−5.5±0.9*†</td>
<td>−4.0±0.3†‡</td>
<td>−2.9±0.7*</td>
<td>−1.2±0.3†‡</td>
<td>−2.9±0.5†</td>
<td>−2.8±0.4†‡</td>
</tr>
<tr>
<td>HYPO</td>
<td>−3.9±0.4*†‡</td>
<td>−2.9±0.3*†‡</td>
<td>−2.0±0.4*</td>
<td>−1.7±0.3*†‡</td>
<td>−1.7±0.4*†‡</td>
<td>−1.3±0.3*†‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, †significant change from baseline or day 3, ‡significant from HYPO, and §significant from ADQ.
led to a small but significant increase in TSH levels between days 5 and 10 of the intervention (HYPO, Fig. 2A).

Free thyroxine (fT4) levels changed in all three intervention groups (Fig. 2B). Subjects in DEF and ADQ exhibited a significant rise in serum fT4 values upon acute HA exposure (24.0 and 17.1%, respectively, \( P < 0.05 \)), and concentrations in both groups remained elevated for the 3 wk at 4,300 meters. In contrast, HYPO demonstrated an immediate suppression of fT4 after initiating the reduced calorie diet, and values remained low throughout the entire 21 days of CR (Fig. 2B).

Cortisol. In the DEF group, cortisol levels increased \( \sim 32\% \) (\( P < 0.05 \)) from baseline by day 3 at 4,300 meters, and concentrations remained elevated throughout the entire 21 days of CR (Fig. 3). Cortisol levels in ADQ rose more gradually to reach a peak on day 10. From day 10 to day 21, there was no difference in cortisol levels between ADQ and DEF, and both altitude groups had values significantly higher than baseline at the majority of the intervention measurement points. The serum cortisol concentrations in HYPO did not demonstrate any significant alterations throughout the 21-day period, but they were significantly lower than the HA groups from day 5 to 21.

Testosterone. Testosterone rose 30% in DEF and nearly 24% in the ADQ group within the first 48 h at 4,300 meters (\( P < 0.05 \); Fig. 4A). Testosterone in ADQ remained elevated, but concentrations in DEF started to decline after the initial peak on day 3. In contrast, HYPO exhibited a gradual decrease in testosterone concentration throughout the intervention that reached significance by day 19. No significant difference existed between calorie-restricted groups by the end of the intervention.

Both HA groups show an initial rise in free testosterone (calculated as total testosterone/SHBG) upon ascent to Pikes Peak, although only the increase in DEF was a significant change from SL baseline (Fig. 4B). Free testosterone remained elevated in ADQ, but concentrations gradually declined in DEF after the spike on day 3. Free testosterone also started decreasing in HYPO after day 3, and values in both calorie-restricted groups fell significantly lower than baseline by day 19.

EPO. EPO increased with HA exposure in both the DEF and ADQ groups (Table 4). However, EPO concentrations rose higher and remained elevated above baseline for longer in ADQ compared with DEF. In contrast, EPO levels in the HYPO group did not change throughout the intervention period at SL.

Adipocytokines

Adiponectin. There was no difference in baseline adiponectin levels between ADQ (9.43 ± 1.38 \( \mu g/ml \)) and HYPO (9.99 ± 1.94 \( \mu g/ml \)), but initial concentrations in DEF (7.06 ± 1.27 \( \mu g/ml \)) were lower (\( P < 0.05 \)). Levels of adiponectin remained constant in both groups that embarked to 4,300 meters (Fig. 5A). In contrast, subjects who remained at SL experienced a marked decline in adiponectin levels throughout the CR phase (Fig. 5A). HYPO subjects experienced a more dramatic change in adiponectin from baseline values than either HA group.

Leptin. Baseline leptin levels in the HYPO group were significantly higher (8.58 ± 2.98 ng/ml) compared with both the ADQ group (1.33 ± 0.24 ng/ml) and DEF group (2.26 ± 0.78 ng/ml; \( P < 0.05 \)). This discrepancy is likely because of the elevated percentage of body fat in the HYPO group at the start of the study in relation to the other groups. Leptin concentrations maintained a strong positive correlation with...
groups by ADQ and downward in DEF led to a separation between HA leptin concentration throughout the 21 days at 4,300 meters, and over the 21-day intervention period. Values are means \( \pm \) SE for each group at SL A and leptin levels (B) Fig. 5. Change in adiponectin (A) and leptin levels (B) for each group at SL and over the 21-day intervention period. Values are means \( \pm \) SE, \( P < 0.05 \), *significant from SL baseline (\( \ast \)), significant from ADQ (\( \dagger \)), and significant from HYPO (\( \ddagger \)).

Table 4. Erythropoietin levels at baseline and throughout the 21-day intervention period

<table>
<thead>
<tr>
<th>Group</th>
<th>SL Baseline</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 10</th>
<th>Day 19</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADQ</td>
<td>9.1 ± 0.6</td>
<td>7.9 ± 0.5</td>
<td>34.2 ± 14.8*</td>
<td>19.5 ± 4.8*</td>
<td>9.6 ± 0.6</td>
<td>8.9 ± 0.7</td>
<td>10.1 ± 0.8</td>
</tr>
<tr>
<td>DEF</td>
<td>11.0 ± 1.8</td>
<td>9.7 ± 1.9</td>
<td>28.5 ± 6.2*</td>
<td>15.9 ± 2.7</td>
<td>11.2 ± 2.0</td>
<td>9.2 ± 1.8</td>
<td>9.8 ± 2.0</td>
</tr>
<tr>
<td>HYPO</td>
<td>11.0 ± 1.9</td>
<td>9.5 ± 1.5</td>
<td>11.7 ± 2.0\†</td>
<td>11.5 ± 1.9</td>
<td>10.9 ± 1.8</td>
<td>11.3 ± 1.8</td>
<td>11.0 ± 2.3</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE. Units are \( \mu l/ml \). *significant from SL baseline and†significant from DEF and ADQ.

Neither DEF nor ADQ registered any significant change in leptin concentration throughout the 21 days at 4,300 meters, but the small changes from baseline in leptin levels upward in ADQ and downward in DEF led to a separation between HA groups by day 5 on Pikes Peak (Fig. 5B). CR at SL caused a 28% reduction of circulating leptin concentration in the HYPO group almost immediately (Fig. 5B). Leptin levels remained suppressed in HYPO group subjects throughout the 21-day CR period.

Urinary catecholamines. Urinary catecholamines were only determined in the HA groups. As shown in Fig. 6A, epinephrine increased significantly in both DEF and ADQ upon acute altitude exposure, although the rise in DEF was more modest than that of the ADQ group (+3.14 ± 1.20 vs. +6.23 ± 2.13 \( \mu g/24 \, h \) by day 1, respectively, \( P < 0.05 \)). After the initial peak, urinary epinephrine levels steadily declined toward baseline SL values, returning to normal within 7 days after ascent to 4,300 meters. Urinary norepinephrine levels were slower to rise in both groups, reaching peak values between days 4 and 7 of HA exposure (Fig. 6B) and remaining high throughout the remainder of the stay at Pikes Peak.

Other analytes. Changes in total cholesterol and triglyceride concentration are listed in Table 5. Initial blood lipid levels were significantly higher in HYPO compared with DEF and ADQ (\( P < 0.05 \)), but there was no difference in baseline values between the HA groups. Total cholesterol in DEF increased significantly upon acute exposure to altitude, whereas ADQ experienced a more gradual rise. HYPO experienced a steady decline in total cholesterol, attaining lower levels than baseline by the end of the study. There was no discernible pattern in high- or low-density lipoprotein among the three groups over the course of the intervention (data not shown). Triglyceride levels in both altitude groups increased slightly, but insignificantly, upon acute exposure at Pikes Peak. Triglycerides in the ADQ group continued rising, reaching levels >100% greater than baseline by day 21. Triglycerides did not show any significant changes in the DEF group throughout the investigation. HYPO exhibited a significant reduction of triglycerides by day 5 of the diet intervention.

DISCUSSION

Group comparisons in this study isolated the influence of hypoxia from that of CR on the neuroendocrine system. As predicted, some of the endocrine responses at altitude in the energy-deficient group (DEF) tended to be muted by CR relative to adequately fed subjects (ADQ), although a few interesting exceptions emerged. Those altitude-induced hormonal responses that were altered by CR, such as epinephrine and insulin, could cause an inhibition of the normal altitude acclimatization process. The unanticipated deviation in DEF of many hormones, especially the adipocytokines, from their SL response to CR suggests that the priority of function among hormones may be altered during times of hypoxic stress. This theory warrants further discussion and investigation.

Hormone Responses with Distinct Interactive Effects: Support for Dietary Modulation

CR has been shown to reduce blood glucose concentration and circulating insulin levels at SL (14), and both effects were evident in the HYPO group. In contrast, glucose and insulin both transiently increased at HA in ADQ and DEF, although the rise was less robust in DEF subjects. Our findings suggest that CR at altitude dampens the rise in blood glucose availability normally experienced at increased elevations (23, 35). Lower blood glucose concentrations contribute to a shift in substrate selection from carbohydrate to alternate fuel sources such as fat or ketones to support energy needs. Indeed, previ-
The muted glucoregulatory response in DEF subjects was even though may not yield the peak change value. Positive stimulus on the hypothalamic-gonadotroph-testicular axis throughout the exposure. However, in accord with our hypothesis, the chronic altitude influence on testosterone levels seemed to be mitigated by CR in DEF. Measurements from previous studies taken after periods of intense trekking or diminished caloric intake (4, 34) often show a decrease in calorie-restricted subjects may bypass the transient rise in IR that hypoxia has been shown to elicit (23). Negative energy balance per se influences insulin sensitivity as evidenced by previous studies in obese subjects (11, 33) and confirmed by our HYPO group results. The transient impairment of insulin action at altitude has been attributed to acute increases in stress hormones (23) with an additive effect from hypoxia itself (35). The acute rise in insulin concentration upon altitude exposure may facilitate oxygen availability over time via increased hematocrit/Hb (12) and erythropoiesis (31). Insulin has been shown to stimulate hypoxia-inducible factor–α1, a factor responsive to both hypoxia and iron deficiency that promotes transcription of proteins such as EPO and vascular endothelial growth factor (31). Insulin may also be a growth factor for erythroid precursors as demonstrated in cell culture models (32). The greater increase in both insulin levels and EPO concentration experienced by ADQ compared with DEF subjects supports the hypothesis that the acclimatization process may be impaired in calorie-restricted individuals.

Elevated blood lipid concentration experienced by ADQ subjects may also be a result of the rise in insulin levels. Young et al. (56) documented a similar increase in plasma triglyceride concentration at altitude and attributed the dyslipidemia to the influence of elevated insulin levels on hepatic triglyceride production. However, the time course in this study suggests that hyperinsulinemia may not be the sole cause of the increased blood lipids in ADQ, since the rise in HOMA-IR was acute and transient while triglycerides were not significantly elevated until day 5. Studies in animal models have demonstrated that hypoxia can induce dyslipidemia (25), and this effect in humans may be exacerbated by a shift in diet macronutrient content. A diet high in simple carbohydrates as consumed by our ADQ subjects has been shown to raise triglycerides even with no change in the percentage of dietary fat (36).

The chronic testosterone response to HA may also be modulated by caloric consumption. Acutely, testosterone levels increased in all HA subjects regardless of energy intake. The ADQ subjects who remained weight stable and adequately fed at altitude continued to experience a gradual rise in serum testosterone concentration, suggesting that hypoxia may exert a positive stimulus on the hypothalamic-gonadotroph-testicular axis throughout the exposure. However, in accord with our hypothesis, the chronic altitude influence on testosterone levels seemed to be mitigated by CR in DEF. Measurements from previous studies taken after periods of intense trekking or diminished caloric intake (4, 34) often show a decrease in

Table 5. Blood lipid data measured at baseline and over the 21-day intervention

<table>
<thead>
<tr>
<th></th>
<th>Baseline (SL)</th>
<th>Day 3 (HA or SL)</th>
<th>Day 5 (HA or SL)</th>
<th>Day 10 (HA or SL)</th>
<th>Day 21 (HA or SL)</th>
<th>Change from Baseline,* %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total cholesterol, mg/dl</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADQ</td>
<td>135.4±8.4†</td>
<td>136.4±10.3†</td>
<td>131.9±9.4†</td>
<td>148.0±5.3*</td>
<td>159.3±7.6†</td>
<td>+13.7</td>
</tr>
<tr>
<td>DEF</td>
<td>135.5±9.0†‡</td>
<td>148.8±11.4*†‡</td>
<td>149.6±10.6*†‡</td>
<td>144.7±9.6*†‡</td>
<td>147.4±10.4*‡</td>
<td>+8.8</td>
</tr>
<tr>
<td>HYPO</td>
<td>171.2±7.6</td>
<td>163.4±6.2</td>
<td>160.8±7.8*</td>
<td>155.6±6.2*</td>
<td>143.0±6.7*</td>
<td>−16.5</td>
</tr>
<tr>
<td><strong>Triglycerides, mg/dl</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADQ</td>
<td>86.1±11.0</td>
<td>109.6±16.7</td>
<td>114.4±13.2*†</td>
<td>151.1±19.0*†</td>
<td>174.0±25.4*†</td>
<td>+102.1</td>
</tr>
<tr>
<td>DEF</td>
<td>81.6±4.2†</td>
<td>98.3±14.6</td>
<td>84.2±5.7‡‡</td>
<td>77.5±5.1‡‡</td>
<td>89.7±4.2†‡</td>
<td>+9.9</td>
</tr>
<tr>
<td>HYPO</td>
<td>109.3±10.2</td>
<td>87.7±8.0</td>
<td>78.8±7.8*</td>
<td>79.1±8.4*</td>
<td>78.7±10.9*</td>
<td>−28.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, †significant from HYPO, ‡significant from ADQ, and *significant from SL baseline. *Represents % change between baseline and day 21 even though day 21 may not yield the peak change value.
pituitary-testicular hormone release that may be the result of the confounding influences of a negative energy state rather than altitude. The steady decline of testosterone levels over time in both HYPO and DEF may represent an adaptive response of the reproductive system to a low-energy, catabolic state (13).

**Catecholamines: a Varied Response**

In our study, altitude exposure led to a rapid rise in urinary epinephrine concentration between days 1 and 3 in ADQ subjects. Epinephrine also increased acutely in DEF, but peak levels in DEF were significantly less than those experienced by the ADQ group. In contrast, norepinephrine levels rose gradually over the first 4–7 days of exposure, indicating that sympathetic nervous activity increased similarly in both HA groups over time (7, 30, 41). These results are consistent with the theory that there is a dissociation between the adrenal medullary and sympathetic response to HA exposure (3, 28). Hypoxia per se can act directly on the adrenal system to secrete epinephrine based on the severity of hypoxemia, even before sympathetic activity is elevated (2). The muted epinephrine rise in DEF subjects supports a negative interactive effect between hypoxia and CR on the adrenal medulla. Conversely, the similarity in norepinephrine levels between DEF and ADQ subjects throughout the intervention implies that the sympathetic nervous system may be activated during hypoxia independent of energy intake status. We did not obtain HYPO group catecholamine data, but previous investigations conducted at SL have reported both significant (16) and insignificant (44) decreases in epinephrine concentrations after a period of clinically controlled hypocaloric dieting. A dampened epinephrine response upon acute altitude exposure may diminish the body’s compensatory response to reduced arterial oxygen, placing calorie-restricted sojourners at a greater risk for altitude sickness and decrements in physical performance. Furthermore, the negative influence of CR on epinephrine availability could also reduce muscle glycogenolysis directly and hepatic glucose production indirectly, thus decreasing carbohydrate availability and use upon acute altitude exposure.

**Hormones with Overriding Altitude Effects**

There appears to be no strong interactive effect of CR and altitude on thyroid, cortisol, and adipocytokine concentrations, since there were little or no differences between the ADQ and DEF groups in these hormone levels. Both the TSH and fT4 results confirm previous HA studies that document a TSH-independent rise in thyroxine upon acute hypoxic exposure (21, 37, 43). Thyroid hormones are known to increase levels of 2,3-diphosphoglycerate in erythrocytes, facilitating the unloading of oxygen to tissues through a rightward shift in the oxyhemoglobin dissociation curve (45). However, the concomitant rise in thyroid concentration and basal energy expenditure that Surks et al. (46) have shown at HA may be detrimental when combined with periods of prolonged CR in lean subjects. During CR at SL, investigators have shown fT4 to remain unchanged (38) or to decrease (10) depending on the severity of energy deficit, findings consistent with data from our HYPO group. However, our results suggest that the hypoxic stimulus at altitude is capable of overriding the fall in fT4 induced by CR.

Altitude also exerts a strong influence over the hypothalamic-pituitary-adrenal axis. In this study, fasting serum cortisol was elevated in both HA groups at Pikes Peak, although CR tended to augment the acute rise. The pattern suggests that an increase in cortisol is accentuated when altitude exposure is associated with another stress, such as CR. Results from previous altitude studies show either a significant rise in cortisol (1, 18) or no change from SL values (4, 42). This discrepancy in the literature and the difference in acute cortisol response between our HA groups may reflect a varying response based on substrate availability. Cortisol is catabolic to fat and protein and is known to increase levels of serum free fatty acids (FFA), glycerol, and amino acids in circulation (8, 9). Although these substrates were not directly measured in this study, energy deficiency at altitude has been shown to increase dependence on lipid metabolism (55). Elevated cortisol concentration may have helped compensate for the early muted glucose response in DEF subjects by providing FFA as an alternate fuel and/or stimulating gluconeogenesis via elevated precursor availability (glycerol and amino acids). By day 5 at Pikes Peak, the between-group differences in both fasting glucose and cortisol concentrations disappeared. Although a causal relationship cannot be determined with these data, the parallel pattern is suggestive of an interaction between cortisol and glucose availability. The sustained increase in cortisol in both HA groups contrasts with the observed response in subjects at SL where prolonged CR either does not alter (20) or suppresses plasma cortisol concentrations (15), possibly preserving lean mass. Although these prior studies were conducted in obese rather than lean subjects, our HYPO group demonstrated similar results.

The adipocytokine response to CR also appears to be overridden by hypoxic exposure. We had anticipated that CR and loss of adipose tissue at altitude would translate into alterations in adiponectin and leptin concentrations. Adiponectin, abundant in the circulation of healthy, normal-weight volunteers, has an established role in improving insulin sensitivity and lipid profiles (52, 53). In our study, subjects on a restricted diet at SL experienced a gradual decline (−26%) in adiponectin levels over the course of the intervention period. This observed fall of adiponectin in HYPO conflicts with many previous studies investigating CR in obese subjects (33, 54). However, the one previous study in nonobese subjects by Wolfe et al. (52) measuring the adiponectin response to prolonged CR showed a similar decrease of adiponectin with reduced energy intake. In contrast to SL results, a fall in adiponectin with CR was not evident at altitude. Studies in animal models have demonstrated a direct and significant correlation between adiponectin levels and microvascular flow (47), suggesting a potential role for adiponectin in improving oxygen delivery during times of hypoxic stress. Therefore, it is possible that the energy homeostasis role of adiponectin becomes secondary to its regulation of the microvasculature at HA. However, further studies will be necessary to understand adiponectin’s function and mechanism of action during adaptation to a HA environment.

Leptin is another adipose-derived hormone that plays a key role in the neuroendocrine regulation of energy homeostasis and appetite. Low levels of circulating leptin signal food deprivation and trigger the hypothalamic receptors to increase energy intake (48, 50). Our results showing a significant...
reduction in leptin concentration in calorie-restricted subjects at SL are consistent with the responses of other individuals after a severe energy-restricted diet (33, 52). In contrast to HYPO, neither altitude group experienced a marked change in leptin over the 21-day period. Although previous studies have shown a significant decrease in leptin concentration upon exposure to altitude (50, 57), to our knowledge, these prior investigations have not controlled dietary intake. In this report, the strength of altitude over the CR effects is inconclusive because the lack of change in leptin in the DEF group may have been confounded by significantly lower baseline values. However, the trend to increase leptin in ADQ and to fall insignificantly in DEF supports emerging data on a potential role for leptin in times of hypoxic stress. It has been shown that hypoxia can markedly augment leptin gene expression (17). Furthermore, leptin has been established to have pro-angiogenic activities and may be able to produce arterial relaxation via nitric oxide (47, 48). Tschop et al. (49) showed a similar increase in leptin concentration in individuals exposed to 4,559 meters and suggested that the elevated leptin levels contribute to the development of altitude-induced anorexia and weight loss. It is difficult to discern how the increased leptin concentration would have affected the appetite of subjects in this investigation since both HA groups were forced to consume a predetermined caloric load. However, our results may suggest that leptin concentrations are optimized to provide a balance between energy homeostasis and microcirculatory improvement upon altitude exposure.

Limitations of This Study

Despite our best efforts to match baseline characteristics, each subject’s availability to spend 3 wk in Colorado did factor into the initial group assignments. Unfortunately, this resulted in a SL HYPO group that consisted of less-fit, heavier individuals with elevated baseline energy balance-related hormone levels. The uneven group numbers resulting from the withdrawal of three ADQ subjects before altitude exposure also exacerbated discrepancies in group characteristics. To clarify comparisons among the three groups, we presented the data of certain hormones as a change from baseline levels. Although this method highlighted differences in the overall group responses to hypoxia and CR, it was difficult to determine if the observed trends were confounded by the higher (HYPO) or lower (DEF) starting values.

In addition, this study was part of a broader performance-based investigation with set exercise days that may have impacted the endocrine data. For example, prolonged cycling trials on day 1 and a V\textsubscript{O2 peak} test on day 2 may have impacted the hormone response on day 3. Similarly, prolonged exercise on day 18 may have caused a lingering influence on metabolic measurements taken at day 19. This effect may explain some of the acute fluctuations in hormone levels we observed between days 19 and 21. However, all three groups followed identical protocols, so scheduled exercise testing should not have impacted the group comparisons.

Finally, despite similar dietary restrictions and compensation for increased energy expenditure at altitude, DEF and HYPO subjects did not lose equivalent amounts of weight. Although this may indicate a synergistic effect of HA and CR on weight loss, either through increased fluid shift (6) or unexplained “energy requirement excess” (27), it could also be an issue of control. The DEF group resided on the summit of Pikes Peak throughout the intervention and had no access to additional food sources other than what was provided to them. In contrast, the HYPO subjects were permitted to leave the CSU for portions of most days, and their compliance to the dietary regimen may have been compromised during their absence. However, DEF lost significantly more weight than HYPO yet showed a muted effect from CR on the endocrine system. Therefore, the discrepancy in weight loss between HYPO and DEF does not impact our conclusions of a hypoxic overriding stimulus on the normal SL calorie restricted response of the endocrine system.

In conclusion, the altitude-induced rise of many hormones measured in the adequately fed subjects of this investigation provides evidence that acute exposure to hypoxia tends to stimulate the neuroendocrine system. A strong endocrine response can improve oxygen delivery via cardiorespiratory and hemopoietic adaptations. However, for some hormones, prolonged CR causes the body to modify or suppress the altitude-induced adaptive response in favor of enhanced energy preservation. Thus maintenance of an adequate energy intake may be necessary to facilitate the acclimatization process. Deviations in the CR endocrine response at altitude from the CR response observed at SL supports emerging data that the relative importance of certain hormones may shift in times of hypoxic stress. Further investigation is needed to elucidate whether the function of hormones such as adiponectin and leptin at altitude extends beyond their currently accepted roles in energy homeostasis.

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GRANTS

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REFERENCES


