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SHORT REPORT

# When women work: Endocrine reactivity in women during everyday physical activity at high altitude

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**Abstract**

**Objectives:** This study explores the acute endocrine reactivity of testosterone and cortisol in women engaging in everyday physical activity in a high altitude environment.

**Methods:** Data were collected from 35 women living in the Himalayas, with women recruited from both high (>10 000 ft.) and low altitude villages (<10 000 ft.). Saliva samples were collected at 3 time points (pre-activity, 30 and 60 minutes) and women wore the wGT3X-BT Actigraph during an hour of everyday work to assess the relationship between high altitude, endocrine reactivity, and physical activity. Saliva samples were then analyzed for testosterone and cortisol.

**Results:** Women living at high altitude had lower cortisol and testosterone levels, after controlling for moderate-to-vigorous physical activity, age, and sum of skinfolds.

**Conclusions:** Testosterone and cortisol increase allocation of energy to costly somatic tissues and the utilization of stored energy. Lower production of these hormones may be beneficial for heightened energetic demands at high altitude.

KEYWORDS

cortisol, endocrine reactivity, energetic costs, high altitude, physical activity, testosterone

High altitude (HA) environments are uniquely challenging ecologies in which human populations have lived for generations across the world. Populations living at HA experience pervasive and ever-present stressors (e.g., hypoxia) that are not easily modified by cultural or behavioral responses (Beall, 2014; Frisancho, 1993). The study of these populations has long served as a foundational paradigm in biological anthropology, representing a robust model to not only study genetic adaptation, but also the role of phenotypic plasticity under extreme environmental stress (Frisancho, 1993; Little, Thomas, & Garruto, 2013).

Significant work has been done on the physiology (i.e., oxygen-delivery systems) and physical growth and development of HA populations (Alkorta-Aranburu et al., 2012; Beall, 2014; Bogin, 1999; Frisancho, 2013; Moore, Charles, & Julian, 2011; Quinn, Diki Bista, & Childs, 2015). In comparison, relatively little is known about the ways in which HA populations accommodate the metabolic demands of day-to-day life, though basal energetic costs are thought to be heightened due to the challenges of HA (Beall, Henry, Worthmann, & Goldstein, 1996). Given the documented importance of how HA exposure shapes biological processes that contribute to metabolic function (e.g., oxygen delivery), constrained by energetic availability (growth), and leads to dynamic changes in body composition in resident populations (Beall, 2014; Beall et al., 1996), there is a need to understand HA population metabolic physiology during everyday activity, including endocrine function due to its role in energy allocation.

Testosterone (T) and cortisol (CORT) are endocrine signals that are acutely responsive to physical exertion and psychosocial demands, and help shape short-term metabolic function through their effects on freeing up stored energy from fat tissue and shunting energy to key tissues

(Frisancho, 1993; Sapolsky, Romero, & Munck, 2000; Tsai & Sapolsky, 1996). Assessing short-term hormonal reactivity over the course of minutes is important for understanding how the body acutely functions during regular physical activity while balancing longer-term environmental demands. When women, particularly, engage in demanding physical activity (i.e., weight bearing and/or endurance demands), their CORT and T tend to rise concurrently (Hale, Kosasa, Krieger, & Pepper, 1983; Webb, Wallace, Hamill, Hodgson, & Mashaly, 1984). Here, we tested the hypothesis that variation in hypoxic exposure at HA leads to differential CORT and T reactivity patterns during demanding physical activity among women in the Nubri Valley of Nepal. Specifically, due to the increased metabolic demands of HA, we hypothesized those high village (HV) women would experience greater increases in CORT and T than low village (LV) women while engaged in everyday work.

## 1 | METHODS

Participants were from the Nubri Valley, Nepal, a rural, HA area of Nepal. Historical records document at least 700 years of habitation; current inhabitants descend from migrants from the Tibetan Plateau and southern valleys (Childs, 2004). The villages were stratified into high (>10 000 ft.; mean = 11,151 ± 574 ft.; HV) and low villages (LVs) (<10 000 ft.; mean = 8,034 ± 923 ft.; LV), following existing research classification of Nubri Valley villages (Craig, Childs, & Beall, 2016). Village residents mainly practice small scale agriculture (barley, maize, potatoes, wheat), trade (timber, medicinal plants), animal husbandry (yaks and yak-cow cross-breeds), and, more recently, are engaged in tourism and mountaineering.

Data were collected from women between the ages of 18 and 45 years ($n = 35$), excluding women who were pregnant or lactating. Given the remoteness of the villages in which they lived, women had no access to hormonal contraceptives. The age range was chosen to capture post-pubescent, pre-menopausal women engaging in everyday work. Members of the research team collected anthropometric data and general demographics. Two research assistants collected all anthropometrics on individuals in the sample (inter-individual reliability = 4.3%). Each anthropometric measure was taken 3 times. The averages of these 3 measurements are reported here. Participants were fitted with a waist-worn portable accelerometer (wGT3X-BT Actigraph). The wGTRX-BT Actigraph measures physical activity level and estimates total energy expenditure using activity counts, which represents the frequency and amplitude of acceleration events over time (Rothney, Schaefer, Neumann, Choi, & Chen, 2008). Women wore the actigraph for 1-3 hours during which physical activity data were recorded while the women engaged in everyday work. Daily work was not restricted to any specific activity with the intent of capturing typical daily activity; women were asked through an activity recall what work they engaged in while wearing the actigraph. Common activities included harvesting barley, collecting wood, washing dishes, carrying water, and cooking. Paired with the actigraphy data, women were asked to provide 3 saliva samples, (1) pre-activity, (2) 30 minutes into activity, and (3) 60 minutes into activity. Women provided saliva samples in polypropylene tubes via passive-drool.

### 1.1 | Salivary CORT and T analysis

Salivary CORT (μg/dL) and salivary T (pg/mL) assays for all samples were run at the Hormones, Health, and Human Behavior Lab at the University of Notre Dame using enzyme immunoassay protocols developed for use with saliva samples (Salimetrics, State College, PA; CORT: Kit No. 1-3002; T: Kit No. 1-2402). Interassay coefficients of variation (CV) were 8.33% and 6.34% for high and low kit-based controls for CORT; the intra-assay CV for CORT was 6.02%. The CVs for the high and low kit controls were 6.48% and 3.66% for T; the intra-assay CV for T was 4.60%. One individual was eliminated from the CORT analyses due to a % CORT value 6+ SD above the mean.

### 1.2 | Statistical analysis

Data were analyzed using STATA 14.1 (Stata Corporation) and R software packages. Endocrine values were log-transformed for normality in all statistical analyses. Hormonal reactivity was calculated as percentage change. Comparisons between sampling time points were analyzed using repeated-measures ANOVA and those between groups (HV and LV) used 1-way ANOVA. Age, sum of skinfolds, time of day, and percentage of time spent in moderate-to-vigorous physical activity (%MVPA) were included as covariates in all models focusing on T and CORT.

## 2 | RESULTS

We report descriptive statistics for the sample in Table 1, stratified according to HV vs LV. There was a statistically significant difference in percentage of time spent in MVPA between HV and LV ($P < .01$); however, HV and LV did not differ in terms of energy expenditure (kCal spent) ($P > .2$). Women in HV and LV did not significantly differ from one another in short-term variation in percent change in CORT and T (all $P > .3$). Women's %MVPA and the time of day their sampling occurred did not significantly predict their percent change in CORT and T (all $P > .2$). We also note that the HV and LV groups did not significantly differ in the time of day their sampling occurred (Table 1; $P > .8$).

When compared with LV, HV women had lower CORT at pre-activity ($F$ [1,29] = 10.45, $P < .01$), 30 minutes into activity ($F$ [1,29] = 4.40, $P < .05$), and 60 minutes into

**TABLE 1** Characteristics of the sample stratified by HV vs LV

| | Full sample (m ± SD) | HV ($n = 16$) | LV ($n = 19$) | df | $F$ |
|---|---|---|---|---|---|
| **Age and Anthropometrics**[a] | | | | | |
| Age (years) | 31.29 ± 9.59 | 29.19 ± 10.26 | 33.05 ± 8.88 | 1 | 1.43 |
| Sum of skin folds (mm) | 51.18 ± 11.33 | 55.49 ± 9.81 | 47.77 ± 11.52 | 1 | 3.42* |
| BMI | 22.82 ± 1.73 | 22.93 ± 1.63 | 22.73 ± 1.85 | 1 | 0.09 |
| **Physical activity**[a] | | | | | |
| %MVPA | 25.9% ± 21.2% | 16.5% ± 9.7% | 34.2% ± 25.1% | 1 | 7.77*** |
| kCal/hour | 38.99 ± 38.65 | 29.14 ± 17.26 | 47.74 ± 49.65 | 1 | 1.36 |
| **Sampling time** | 11:45 ± 2.89 hours | 11:52 ± 3.50 hours | 11:39 ± 2.35 hours | 1 | 0.05 |
| **Hormones**[b] | | | | | |
| *Cortisol (μg/dL)*[c] | | | | | |
| Pre-activity | 0.25 ± 0.28 | 0.10 ± 0.05 | 0.37 ± 0.33 | 1 | 10.45*** |
| 30 minutes | 0.20 ± 0.22 | 0.11 ± 0.06 | 0.29 ± 0.28 | 1 | 4.40*** |
| 60 minutes | 0.18 ± 0.20 | 0.11 ± 0.09 | 0.26 ± 0.25 | 1 | 7.57** |
| % change (pre to 30 minutes) | −4.1% ± 64.2% | 9.7% ± 66.8% | −15.1% ±62.2% | 1 | 0.29 |
| % change (pre to 60 minutes) | −9.7% ± 62.9% | −0.4% ± 51.8% | −17.2% ± 71.5% | 1 | 0.02 |
| *Testosterone (pg/mL)* | | | | | |
| Pre-activity | 31.67 ± 15.25 | 26.17 ± 8.33 | 36.85 ± 18.48 | 1 | 5.08** |
| 30 minutes | 29.26 ± 13.25 | 25.59 ± 7.17 | 32.51 ± 16.48 | 1 | 2.01 |
| 60 minutes | 31.55 ± 17.81 | 25.82 ± 8.97 | 35.60 ± 21.40 | 1 | 3.55* |
| % change (pre to 30 minutes) | −3.7% ± 28.5% | −2.5% ± 31.2% | −4.9% ± 26.7% | 1 | 0.58 |
| % change (pre to 60 minutes) | 3.0% ± 32.3% | 5.8% ± 40.4% | 1.0% ± 25.8% | 1 | 0.01 |

[a] Statistical comparisons reflect 1-way ANOVA. Model for age and sampling time includes no covariates. Models for sum of skinfolds and BMI control for age. Physical Activity models control for age and sum of skinfolds.

[b] Raw values reported for means; statistical comparisons reflect 1-way ANOVA for hormonal values controlling for age, sum of skinfolds, and time of sampling; pre-activity, 30, and 60 minutes were log-transformed.

[c] One individual excluded due to CORT changes 6+ SD above the mean.

$^*P < .1$. $^{**}P < .01$. $^{***}P < .05$.

activity ($F$ [1,27] = 7.57, $P < .05$) controlling for time of day, age, sum of skinfolds, and %MVPA (see Table 1; Figure 1). Women living in HV had significantly lower T pre-activity ($F$ [1,30] = 5.08, $P < .05$) and marginally lower T 60 minutes into activity ($F$[1,26] = 3.55, $P = .07$) controlling for time of day, age, sum of skinfolds, and %MVPA.

## 3 | DISCUSSION

LV women spent a greater percentage of time in MVPA than HV women, most likely due to the harvesting season that coincided with our sampling at those villages. However, we observed potential differences in CORT and T production between HV and LV, even after we controlled for variation in physical activity intensity. Prior studies of females' hormonal reactivity during intense activity have shown that that women's CORT and T often acutely increase during short-term endurance bouts (Casto, Elliott, & Edwards, 2014; Consitt, Copeland, & Tremblay, 2002; Cumming & Rebar, 1985). Here, we did not find that women had comparable significant increases in CORT or T in either of the groups. Running counter to our hypothesis, we also did not find that altitude predicted different reactivity patterns (ie, percentage change) in CORT or T in HV compared to LV women, including when we controlled for relevant covariates, specifically age, sum of skinfolds, and physical activity.

In contrast to those results, we found that HV women had lower pre-activity CORT and T and also had lower CORT 30 and 60 minutes into the activity compared to LV women, including in models controlling for covariates. The basal production of CORT and T diverges in different populations partly due to variation in early environmental psychosocial stress, energetic, and pathogenic exposures that help program physiology (Ellison et al., 2002; Vitzthum, 2009; Worthman, 1999). Consistent with interpretations of past studies of endocrine function at HA (Vitzthum, Ellison, Sukalich, Caceres, & Spielvogel, 2000; Vitzthum & Wiley, 2003), one potential explanation for our findings is that HA ecology may influence the set-points and thus output of key endocrine axes.

One limitation of this study is that we cannot account for work done in the hours prior to sampling, which could influence the levels of CORT and T and their reactivity in response to the activities in our study. We attempted to address this issue by controlling for time of day, which is likewise an important confounder for CORT and T, as they exhibit diurnal curves, with their production being greatest around waking or shortly thereafter (Edwards, Clow, Evans, & Hucklebridge, 2001; Rickenlund, Thoren, Carlstrom, von Schoultz, & Linden Hirschberg, 2004). An

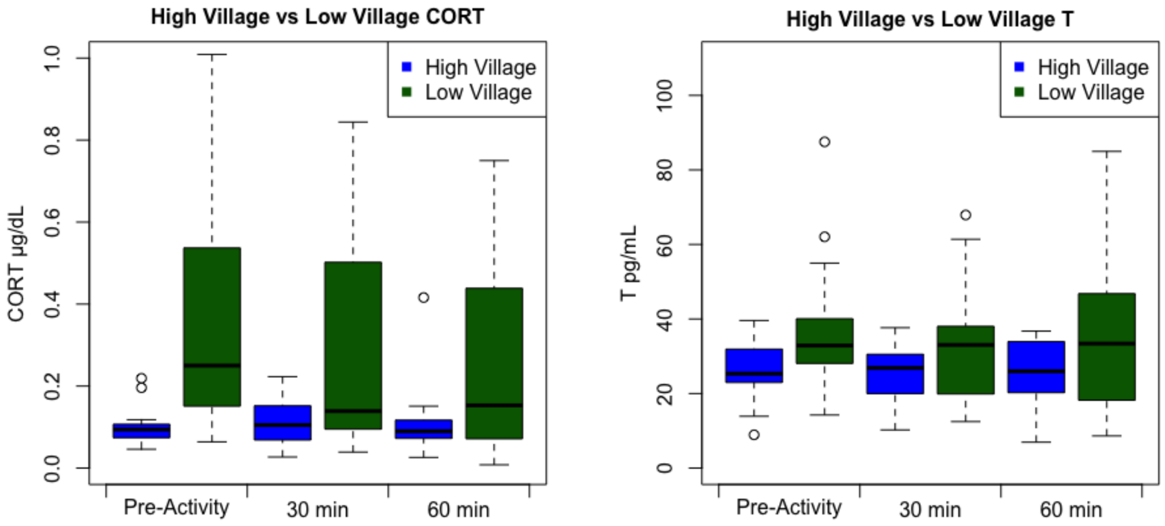

**FIGURE 1** Box and whisker plots of women's CORT and T in high villages (HV) and low villages (LV). In repeated measures ANOVA models, run separately for LV and HV, women's pre-activity hormone values did not significantly differ from their values 30 min and 60 min into physical activity (all $p > 0.2$). See Table 1 for comparisons of T and CORT between women residing in HV and LV. Bars represent the interquartile range, with error bars indicating the range of the hormone

additional limitation of these data is that, due to seasonality, LV women were primarily engaged in harvesting barley and wheat, skewing physical activity data for this group towards more vigorous activities, compared with HV women. However, when controlling for physical activity, living in a HV still predicted lower CORT and T (though not for all time points), regardless of the increased energetic costs of being active during hypoxic exposure.

Finally, our study is limited in that we do not have data on women's menstrual cycle phases. Basal CORT is generally thought to vary little across menstrual cycles, particularly for cycles with typical-length luteal phases (Nepomnaschy et al., 2011). Meanwhile, on average, women's T is highest around ovulation, although variation in T across the cycle has been described as sufficiently minimal that scholars have "concluded that [controlling for it] is unnecessary unless menstrual variation in T is itself of interest" (van Anders, Goldey, & Bell, 2014, p. 237). It is plausible that cycle fluctuations for T could affect our results if, by chance, there were a disproportionate number of LV women in or around the ovulatory phase of their cycles. If such a clustering occurred in the LV group it would not explain the observed patterns for CORT, which were consistent across, and statistically significant for, all 3 time points. Given the low likelihood of such clustering and the relatively small magnitude of T differences between cycle phases, we think it highly unlikely this drove our results for T. Further, research suggests menstrual cycle phase would have little impact on acute endocrine reactivity to intensive physical activity (Boisseau, Enea, Hospitalier, & Poitiers, 2013; Enea et al., 2009). Finally, relevant to our %MVPA results, past research suggests that menstrual cycle phase likely has minimal effects on daily work capacity among HA populations (Brutsaert et al., 2002).

In conclusion, these results are potentially consistent with emerging models suggesting that human energetic expenditure/metabolic costs are constrained regardless of levels of physical activity (Pontzer et al., 2016). CORT and T are relatively costly metabolic hormones, serving functions such as energy mobilization (CORT) and anabolic muscle building and catabolic effects on fat tissue (T) (Sapolsky et al., 2000; Tsai & Sapolsky, 1996). Thus, for resident populations who are thoroughly adapted and acclimated to the daily physical demands of hypoxic, cold ecologies, hormonal pathways like the HPA and HPG axes, and their physiological set-points, may be down-regulated to facilitate lower overall energetic/metabolic expenditure. In total, we suggest that long-term of residence in harsh environments and challenging ecological stressors may be accompanied by allostatic shifts in human physiological systems, including metabolic function, and these ideas merit exploration in future, larger studies.

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## AUTHOR CONTRIBUTIONS

*Collected the data*: MSS and EAQ

*Conducted the statistical analysis, wrote the article, and conducted the laboratory procedures*: MSS

*Primary investigators and conceived the research design of the overarching project*: GC and EAQ

*Contributed heavily to the framing, analysis, and editing of the article and oversaw the laboratory procedures*: LTG

*Edited the article for intellectual content and provided critical comments*: GC and EAQ.

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