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Original Research Article

Urinary Cortisol and Muscle Mass in Turkana Men

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ABSTRACT To determine the role of cortisol in modulating the effects of energetics on muscle mass in a subsistence society, measures of cortisol and body composition were obtained from a sample of Turkana men. Subjects were 63 settled and 69 nomadic men, ages 24 and older. Urinary cortisol was determined along with measures of muscle mass. Cortisol/creatinine ratio was higher among nomadic men (57.8 ± 56.8 vs. 34.5 ± 44.6; $P < 0.001$). Controlled for age, urinary cortisol was inversely related to arm muscle plus bone area (MPBA) among the nomadic ($\beta = -0.28$; $P = 0.04$), but not the settled ($\beta = -0.04$; $P = 0.78$) sample. Urinary cortisol was not related to any other measures of body composition. These results suggest that even in chronically undernourished populations, cortisol may be elevated primarily under conditions of acute nutritional stress. However, the catabolic effects of cortisol on muscle in our results may be confounded by other energetic factors, including energy availability. Am. J. Hum. Biol. 17:489–495, 2005.

Cortisol, a steroid secreted by the adrenal gland, is a major mediator of physiological function in humans (Weiner, 1992). Cortisol has the net effect of liberating glucose for rapid mobilization during physical activity (Weiner, 1992). Under glucocorticoid stimulation, the liver converts glycogen to glucose, whereas lipids in fat cells and proteins in muscle cells are broken down into glycerol, fatty acids, and amino acids as substrates for gluconeogenesis (Young et al., 1992; Wernerman et al., 1993; Hickson et al., 1996).

Cortisol's role in muscle catabolism noted previously makes it a major lever of physiological function and energetic allocation. Not only is skeletal muscle the single largest tissue type in proportion to total body mass in humans and most other vertebrates (Calder, 1996), it is plastic both in terms of size (Welle, 1999) and histological composition (Hamilton and Booth, 2000) and is highly energy-consumptive during periods of high activity (Korzeniewski, 1998). Most importantly for present purposes, it is the excess mass of skeletal muscle of men in relation to women that is the costliest component of differential somatic allocation between the sexes (Bribiescas, 2001). A large portion of sexual dimorphism in muscle mass is due to the greater muscularity of the upper appendicular system in men, including arm muscle mass (Bribiescas, 2001).

Yet, despite the importance of cortisol to muscle catabolism, the relationship of cortisol levels to energetic status is complex and related to the intensity and duration of the conditions of energy expenditure and intake (Friedl et al., 2000; Morgan et al., 2000; Fernandez-Garcia et al., 2002). Both increased (Friedl et al., 2000; Soliman et al., 2000) and decreased (Buffenstein et al., 2000) resting levels of cortisol have been reported in studies of long-term low-energy diets in human subjects from Western populations.

We expect the role of cortisol in energetic allocation to be more clearly detectable in subsistence populations, for whom limited energetic availability would produce more direct tradeoffs between physiological systems.Yet, little data on the relationship of cortisol to measures of energetic status in these subsistence populations exist (see Flinn and England [1997] for the role of cortisol in mediating the impact of psychosocial

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stress on immune function in a Caribbean village).

Hence, we decided to explore the relationship of cortisol and muscle mass among the Turkana, pastoral nomads of northern Kenya who are characterized by chronic undernutrition, relatively high protein intake (Galvin and Little, 1999), and moderate levels of physical activity (Curran and Galvin, 1999). A community of settled Turkana, who belong to the same population as the nomads but have access to more food and a somewhat more sedentary lifestyle (Campbell et al., 1999), were also included in the investigation. Our investigation is guided by the following hypotheses.

HYPOTHESES

1. Cortisol levels will be higher (1) among the Turkana population compared with Western populations and (2) in the nomadic Turkana compared with the settled Turkana, because cortisol secretion is increased during periods of physiological stress.
2. Cortisol levels will be negatively associated with measures of energetic status, as well as measures of muscle, because of the catabolic actions of cortisol. Of these measures, arm muscle mass, because of its greater degree of sexual dimorphism, should be most closely related to cortisol.

METHODS AND MATERIALS

Study population

As pastoral nomads, the Turkana depend largely on their animals, including goats, sheep, cattle, camels, and donkeys, for subsistence in an arid and fluctuating environment. Their diet is based primarily on animal products, including milk, blood, and meat, with up to 90% of calories in some wet seasons consumed in the form of milk (Little, 1989). During the dry season, consumption of grains, particularly maize, as well as blood and gathered food, substitutes for lower consumption of milk. Caloric intake is limited throughout the year (Little, 1989); however, protein intake is quite high (Galvin and Little, 1999).

The settled Turkana sampled here are from Morulem, which consists largely of an agriculture scheme now run by World Vision, a nongovernmental organization. Unfortunately, we have much less evidence documenting the living conditions in Morulem, although there is evidence for reduced meat and increased grain consumption and greater exposure to malaria (Campbell et al., 1999). Previous work has shown that among adults, settled men are shorter, exhibit higher levels of fat, and report more health complaints (Barkey et al., 2001).

Most of the Morulem population has settled since the 1960s as the result of loss of animals to drought or raiding. Thus, Morelum represents a subpopulation who share their genealogy and part of their individual life history with the nomads but differ in terms of their current diet and disease exposure. In addition, the data presented here were collected during a time of drought, with the data from the nomads preceding the distribution of food relief, whereas the data from the settled population was collected 3 months after the food relief became available (DeLuca, 1997). Thus, the differences between the two subpopulations here reflect short-term more than long-term conditions.

Human subjects approval was obtained from Human Use Committees at the University of North Carolina in Chapel Hill, the University of Massachusetts at Boston, and the Kenyan Medical Research Institute.

Data collection

Anthropometrics, sample collections, and interviews were performed in July–August 1992 for 155 nomads and in April 1993 for 129 settled Turkana. In both settings, data collection was done close to an encampment, and the subjects came from the immediate area to minimize any disruption of the subjects daily routine and the possible impact of stress on cortisol levels.

Six skinfolds (calf, midaxillary, periumbilical, subscapular, suprailiac, triceps), height, weight, and mid-calf and mid-upper arm circumferences were measured. Anthropometrics were taken in a standard procedure (Lohman et al., 1988). Skinfolds were taken with standard calipers to the nearest millimeter, circumferences to the nearest tenth of a centimeter, weight with a beam scale to the nearest tenth of a kilogram, and height with a standard anthropometer to the nearest centimeter.

Subjects were given urine collection cups and were instructed to collect their first void after arising the next day. They then promptly delivered the samples to project field staff, who stored them in a cool location.

Subjects younger than 23 years old (when Turkana men cease linear growth, signifying skeletal adulthood) were removed from the population sample to eliminate the impact of adolescent change, and only individuals with measurable cortisol were included, leaving a sample size of 69 nomads and 63 settled men.

Sample storage and extraction

Urine specimens were dried on Schleicher and Schuell No. 903 filter paper in the field soon after collection; square inch of paper saturates with 250 µL. Dry specimens were placed in glassine sleeves in notebooks inside plastic bags with desiccant. These were kept in cool locations in the field and refrigerated after transport to the United States. In the laboratory, strips of paper containing the samples were shredded and then digested with cellulase. The extracts were centrifuged and brought into a dilution of 10× their original volume, at which point they were used directly for assay (Campbell, 1994; Evindar, 1999).

Cortisol assays

Cortisol was measured with RIA kits (07-221105 cortisol coated tube assay kit, ICN Pharmaceuticals Inc., Costa Mesa, CA; minimum detectable dose, 0.15 µg/dL). To assay cortisol, urine extracted from filter paper was sonicated for 1 h with 1 mL ethyl acetate, then 500 µL of the ethyl acetate layer was pipetted off and centrifuged to separate the aqueous and organic layers. The solvent was evaporated, and the solvent extract was rehydrated with 25 µL saline buffer. The cortisol in the treated samples represented unconjugated cortisol in urine derived from total cortisol in the circulation. Hence, urinary cortisol is an accurate and useful indicator of physiological glucocorticoid activity. Sample recovery during steroid extraction from the initial aqueous phase (ethyl acetate extraction) ranged from 70–120%.

The cortisol assays had a coefficient of variation (CV) of 8.15%. The lower limit of detection of assays varied from 0.20–1.81 µg/dL. Approximately one fifth of the samples were removed from the final statistical regression analyses because their signal was below the lower limit of detection. The interassay CV of the 10-µg/dL part of the standard curve was 14.81%. In addition, the mean CV of intraassay replicates for experimental samples (percent bound) was 7.62%.

Creatinine

Urinary creatinine was obtained by measurement of creatinine-picrate adducts (Jaffe reaction) (Jaffe, 1886). Results were used to correct for urine concentration.

Statistical analysis

SPSS (Statistical Package for Social Sciences) software was used for statistical analysis of assay results. *T* tests were performed to determine whether there were significant differences between the nomadic and settled populations. Multiple regression models were used to determine the within-population relationships between cortisol and measures of body composition as specified in the hypotheses. For parsimony, linear relationships between the variables were tested. The level of significance was set at 0.05. Because many anthropometric traits change during aging, age was included in most regression analyses.

RESULTS

Descriptive statistics

Descriptive statistics for anthropometric and hormonal variables among Turkana men are depicted in Table 1. The mean body mass index (BMI) for the two groups are 18.1 (±1.9) for the settled and 17.4 (±1.8) kg/m^2 for the nomads, consistent with previous measures of the Turkana (Little et al., 1999) and similar to that of obtained from other East African pastoralists (Campbell et al., 2003). It is important to note that the BMI for both the nomadic and settled groups are below the clinical standard of chronic energy deficiency of 18.5 kg/m^2 (Ferro-Luzzi et al., 1992). Percent body fat for the two groups is 7.1% (±5.6%) for the settled and 4.5% (±7.6%) for the nomads, another indication of how lean these men are.

The mean cortisol/creatinine ratios of 34.5 (±44.6) µg/g for the settled and 57.8 (±56.8) µg/g for the nomads fall in the middle of the clinical reference range of 20–90 µg cortisol/g creatinine (Greenspan and Gardner, 2000). They are also similar to the mean morning cortisol/creatinine ratio of normotensive urban Kenyans, 51 µg/g (Beaman-Mbaya and Ogola, 2000).

TABLE 1. Summary of Turkana anthropometrics and analytes

A. Settled

	N	Min.	Max.	Mean	SD	Skew.	Kurtosis
Age, years	63	24.0	80.0	47.64	14.57	0.20	−0.76
Body mass index, kg/m^2	63	12.94	23.16	18.12	1.88	0.07	0.37
Arm muscle plus bone area, cm^2	63	19.12	54.63	39.35	6.98	−0.16	0.14
Waist Circumference cm^2	63	57.70	86.80	75.90	5.40	−0.35	1.16
% Body fat	63	4.51	14.09	7.60	2.08	1.05	0.82
Cortisol, μg/dL	63	0.58	31.94	4.76	5.92	3.18	11.6
Cortisol/creatinine, μg/g	63	3.30	225.6	34.15	44.6	2.63	7.55

B. Nomads

	N	Min.	Max.	Mean	SD	Skew.	Kurtosis
Age, years	69	23.0	90.0	47.99	15.27	0.55	−0.24
Body mass index, kg/m^2	69	13.41	21.44	17.43	1.80	−0.12	−0.52
Arm muscle plus bone area, cm^2	68	21.07	60.37	35.62	7.66	−0.75	1.64
Waist Circumference cm^2	69	62.40	84.90	71.52	4.36	0.26	0.254
% Body fat	69	3.75	7.11	5.616	0.63	−0.21	0.17
Cortisol, μg/dL	69	0.61	27.68	6.95	6.94	1.51	1.82
Cortisol/creatinine, μg/g	69	4.48	259.9	57.77	56.76	1.76	2.91

However, the cortisol/creatinine ratio in both the settled and nomadic Turkana was highly skewed (2.63 and 1.76, respectively). Hence, the median micrograms cortisol/grams creatinine ratios for the settled and the nomads, 18.21 and 40.11, respectively, were correspondingly lower than the means. Log transformation (natural logs) reduces skew and kurtosis in both settled and nomadic cortisol/creatinine ratios. Settled log transformed cortisol/creatinine skew and kurtosis was 0.400 and −0.528, respectively. Nomadic log transformed cortisol/creatinine skew and kurtosis was −0.046 and −0.773, respectively.

Subpopulation comparisons

The mean ages of the settled and nomadic groups were 47.6 years (±14.6 SD) and 47.99 years (±15.3), respectively. The mean ages of the two groups were not significantly different (two-tailed t test; $P = 0.89$); hence comparison on subpopulation differences was done with simple t tests.

TABLE 2. Hormonal predictors of MPBA

A Cortisol	Nomadic	Settled
Adjusted R^2	0.203	−0.02
N	63	47
Predictor	β	β
Age	0.17	0.16
Cortisol	0.43*	0.06

*$P < 0.05$.

Comparison of anthropometric measures between the two populations revealed that the nomadic men were leaner and had less muscle mass than their settled counterparts. Mean BMI of the settled men was 18.1 (±1.9) compared with 17.4 (±1.8) kg/m^2 for nomadic men, a significant difference at $P = 0.02$. The settled and nomadic mean arm muscle plus bone areas (MPBAs) were 39.4 (±7.0) cm^2 and 35.5 (±7.4) cm^2, respectively, again significantly different ($P < 0.001$). Mean waist circumference (in centimeters), which is indicative of visceral fat levels, was 75.9 cm (±5.4) in the settled men; the mean of the nomads was 71.52 (±4.36) cm ($P < 0.01$). Mean percent body fat of the settled men was 7.6% (±2.1) and in the nomads it was 5.6% (±0.6), a significant difference at $P < 0.01$.

Cortisol/creatinine ratios were significantly higher among the nomadic men than their settled counterparts. For the cortisol/creatinine ratio, this is 57.7 (±56.76) μg/g vs. 34.15 (±44.59) μg/g ($N = 131$, $t = -3.744$, $P < 0.001$).

Age-related changes

BMI declines with age in both settled ($N = 63$, $\beta = -0.238$, $P = 0.060$) and nomadic Turkana ($N = 69$, $\beta = -0.230$, $P = 0.057$). MPBA declines significantly with age in both the settled ($N = 63$, $\beta = -0.260$, $P = 0.039$), and the nomadic

Turkana ($N = 68$, $\beta = -0.258$, $P = 0.034$). Despite the decline in BMI, waist circumference increases with age in both the settled men ($N = 62$, $\beta = 0.288$, $P = 0.022$) and in the nomads ($N = 68$, $\beta = 0.219$, $P = 0.071$). Percent body fat does not change with age in the settled ($N = 62$, $\beta = 0.008$, $P = 0.948$) but declines in the nomads ($N = 68$, $\beta = -0.406$, $P = 0.001$).

Hormones and anthropometrics

The prediction that cortisol would be negatively correlated with muscle mass in the Turkana was confirmed in nomads but not the settled, as shown in Fig. 1. When controlled for age, Ln (natural log) cort/creat predicted upper MPBA in the nomadic men ($N = 63$; adj. $r^2 = 0.038$; age $\beta = -0.258$, $P = 0.04$; Ln cort/creat $\beta = -0.28$, $P = 0.018$) but not in the settled men ($N = 68$; $r^2 = 0.118$; age $\beta = -0.231$, $P = 0.05$; Ln cort/creat $\beta = -0.035$, $P = 0.78$).

However, Ln cort/creat was not related to any other measure of body composition among either the nomadic or the settled men.

DISCUSSION

Cortisol levels

Contrary to our original prediction that cortisol levels would be elevated under the energetically stressful conditions experienced

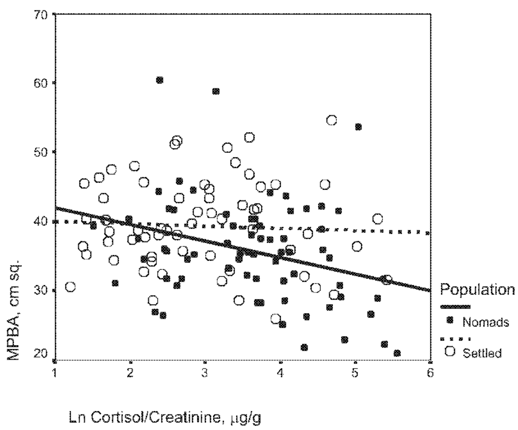

Fig. 1. Upper arm muscle plus bone area (MPBA) and cortisol/creatinine among Turkana men. When controlled for age, Ln cortisol/creatinine predicts MPBA in the nomadic ($N = 63$; adj. $r^2 = 0.038$; age $\beta = -0.26$; $P = 0.04$; ln cort/creat $\beta = -0.28$; $P = 0.018$) but not the settled men ($N = 68$; $r^2 = 0.118$; age $\beta = -0.23$; $P = 0.05$; ln cort/creat $\beta = -0.04$; $P = 0.78$).

by the Turkana, urinary cortisol levels do not seem elevated among Turkana men. In fact, the average cortisol/creatinine ratio in the nomadic Turkana was comparable to that of the mean values of 51µg/g for normotensive urban Kenyans (Beaman-Mbaya and Ogola 2000), whereas the average value for the settled Turkana is at the low end of the clinical reference range of 20–90 µg cortisol/g creatinine for U. S. samples (Greenspan and Gardner, 2000).

The lack of elevated cortisol levels among Turkana men despite evidence for substantial chronic undernutrition suggests one of two overall responses of the adrenal axis to energetic stress: either the Turkana are (1) in state of energetic depletion, or (2) they are developmentally adapted to their energetic conditions. In the first case, fasting blunts cortisol secretion during the stress response (Kirschbaum et al., 1997). Hence, it is possible that under conditions of chronic undernutrition, the Turkana man's adrenal axis has been chronically suppressed.

On the other hand, the Turkana may be developmentally adapted to higher levels of nutritional, energetic, and pathogenic stress and so display normal cortisol levels under conditions that would result in elevated cortisol in Western populations. The development of the adrenal gland is thought to be responsive to nutrition (Phillips et al., 2000), and cortisol levels have been shown to vary among adults according to birth weight (Phillips et al., 2000; Kajante et al., 2002), although results are contradictory (Fall et al., 2002), making it unclear whether undernutrition among a population like the Turkana would alter the hypothalamopituitary axis.

Subpopulation comparison

Higher cortisol levels among the nomads compared with the settled group may be related to differences in diet or perhaps activity levels reflective of differences in the nomads and settled lifestyle (Barkey et al., 2001, Campbell et al., 2001). However, the differences in cortisol may not reflect subpopulation differences as much as temporal differences related to nutrition. At the time of sampling, the nomadic men were experiencing acute undernutrition as a result of drought (Barkey et al., 2001), which is most likely to be responsible for their higher levels of cortisol relative to the settled men. On the basis of a 48-h food recall, caloric intake among the nomadic men was estimated at roughly 800 kcal, whereas for the settled population, the estimated value was 1800 kcal (Muhleinbein, 1998).

Cortisol and MPBA

In addition to exhibiting higher cortisol/creatinine ratios than their settled counterparts, the nomads also displayed a correlation between cortisol and MPBA, as predicted. Our finding of a negative relationship between cortisol and MPBA in the nomads but not the settled men is consistent with the elevation in cortisol and reduction in muscle mass seen in conditions of acute undernutrition (Becker, 1983).

Although it is possible that their relatively higher cortisol may be the cause of reduced muscle mass in the nomads, a causal relationship between these factors cannot be determined from the data presently available. The negative relationship between cortisol and MPBA found here suggests that upper arm muscle may be an important site of cortisol-mediated dynamic energetic adjustment that might be apparent in other populations as well. The lack of a similar relationship between cortisol and MPBA among the settled men, who have a very low BMI but significantly higher levels of adiposity, suggests that substantial energetic stress is required for the a relationship between cortisol and upper arm muscle mass to be detectable.

One possible alternative explanation for the MPBA–cortisol relationship is that thinner men are more active and, hence, have higher cortisol levels. If that were the case, a negative relationship between BMI and cortisol would also be expected, but none was found. Other alternatives include pathogen loads or dietary differences between individuals within populations. Although both pathogens, intensity and diet, may have influenced cortisol level and MPBA, we do not have enough information to confirm or reject these hypotheses. It is also possible that differences in psychosocial stress might play a role in the subpopulation differences. However, it is unlikely that sample collection itself was more stressful for the nomads, thus leading to elevated cortisol levels.

CONCLUSION

Our results suggest that despite evidence for long-term undernutrition among the Turkana, urinary cortisol levels are not demonstrably elevated compared with other populations. On the other hand, higher cortisol levels among the drought-afflicted nomads relative to their settled counterparts suggests that cortisol levels do rise in response to acute undernutrition, although the effects of activity and diet cannot be ruled out. However, the fact that cortisol is negatively related to MPBA in the nomads, but not in the settled men, suggests the catabolic effects of cortisol under conditions of acute energetic stress. Further research is needed to determine whether cortisol is related to MBPA in other subsistence populations.

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