Benjamin Campbell^a, Paul Leslie^b, and Kenneth Campbell^c^aDepartment of Anthropology, University of Wisconsin-Milwaukee, Sabin Hall, N. Downer Avenue, Milwaukee, WI; ^bDepartment of Anthropology, University of North Carolina, Alumni Hall, Chapel Hill; ^cDepartment of Biology, University of Massachusetts at Boston, Morrissey Blvd

ABSTRACT: To determine age-related patterns of gonadotropins and their relationship to energetic status in a subsistence population we analyzed urinary FSH, LH, and estrone-3-glucuronide (E-3-G) along with anthropometric measures among Turkana males of northern Kenya. Subjects were 134 nomadic and 109 settled males ages 20 to 80+. FSH, LH and E-3-G were significantly higher among the settled, compared to nomadic, males. LH, but not FSH, showed a significant increase across 10 year age groups among all the men. E-3-G increased across age groups only among the settled males. Controlled for age, FSH was inversely related to measures of fat free and body mass among the settled men. These findings suggest an unusual age profile of gonadotropins and estrogen metabolites that may reflect the impact of fluctuating food availability. More research is needed to address the impact of energetic and social factors on the male reproductive axis among energetically stressed populations.

INTRODUCTION

Age-related increases in gonadotropin secretion (Baker et al., 1976; Gray et al., 1991) are thought to be markers of male reproductive aging, along with declines in bioavailable testosterone (Gray et al., 1991; Harman et al., 2001) increasing rates of benign prostate hyperplasia (Berry et al., 1984), erectile dysfunction (Gray and Campbell, 2005; Feldman et al., 1994) and changes in spermatogenesis (Neaves et al., 1984). Age-related increases in FSH have been related to decreases in inhibin B, a marker of Sertoli cell function and spermatogenesis (Tenover et al., 1988), while

increases in LH represent a compensatory response to declining testicular sensitivity (Veldhuis et al., 2005). The earlier rise of FSH reported in some studies (Hadjji et al., 1994; Temmekoon and Karunanayake, 1993) is consistent with the maintenance of spermatogenesis over the production of testosterone, because of the more direct relationship of spermatogenesis to reproduction as well the low energetic costs of spermatogenesis compared to the anabolic effects of testosterone (Bribiescas, 2001).

Current evidence suggests similar age related patterns of gonadotropins in men across populations (Odabas et al., 2002; Peng et al., 1987). However, the recent demonstration of population variation in age-related declines of testosterone among men (Bribiescas, 2005; Campbell et al., 2006a, b; Campbell et al. 2003; Ellison

et al., 2002), suggests that age-related changes in gonadotropins may also vary across populations. At the simplest level, age-related patterns of LH might be expected to differ in association with variation in the rate of testosterone decline. In addition, it is well known that insulin plays an important role in gonadotropin regulation (Baecetti et al., 2002) and acute changes in LH release has been related to blood glucose levels (Oltmanns, 2001). Thus differences in nutritional status across populations as reflected in blood glucose and insulin might serve as a basis for population level differences in LH stimulation of testicular function. Whether similar differences in FSH would be expected is unclear.

In a study of age-related patterns of gonadotropins among in the Ache, a population of hunter-gathers, Bribiescas (2005) reported a significant increase in blood FSH and LH with age among 17 Ache hunter-gathers from Paraguay, ages 20 to 63. In contrast, salivary T and serum estradiol did not change with age. Gonadotropin levels were not associated with variation in anthropometric measures of energetic status, despite low levels of leptin. Bribiescas (2005) concludes that increases in LH and FSH may represent a more universal part of the aging process in men than does a decline in T.

The generalizability of Bribiescas's results across subsistence populations is limited by the relatively small sample size and limited age range. Thus, we chose to use data collected among the Turkana, pastoral nomads of Northern Kenya to address age-related patterns of gonadotropins in men. The Turkana are of particular interest to the study of the impact of energetic factors on male reproductive function. Not only do the

Turkana experience chronic undernutrition (Galvin and Little, 1999), but the presence of two genetically similar subpopulations that differ in their ecological context provides an additional contrast. Earlier results documenting lower levels of adiposity (Campbell et al., 2005) and increased urinary cortisol (Lukas et al., 2005) among the nomadic males, compared to their settled counterparts, provide evidence that the nomads are in a catabolic state while the settled males are in positive energy balance.

If Bribiescas (2005) is correct and age-related increases in gonadotropins among men are universal (or nearly so) across populations, Turkana males should exhibit age related increases in FSH and LH despite relatively limited age-related decline in blood T and FTI as demonstrated by Campbell et al. (2006b). At the same time, given evidence for the effects of blood sugar on LH pulse production (Oltmanns et al., 2001), we predict that LH levels will be significantly and inversely related to measures of energetic status, while FSH levels will be relatively unaffected.

In addition to gonadotropins, recent research also suggests that estrogen has important effects on male reproductive function. These include an impact on sperm production (Rosselli and Skinner, 1992) and sperm maturation (Hess, 2003), as well as effects of estradiol on the hypothalamus (Bagatell et al., 1994; Vermeulen et al., 2002). In addition, increased subcutaneous abdominal fat has been associated with higher levels of free serum estradiol (Vermeulen et al., 2002) suggesting that fat gain with aging may contribute to increasing estrogen levels.

Thus to determine the age related patterns of estrogen and its relationship to adiposity among Turkana male, we

*Address correspondence to: Benjamin Campbell, Department of Anthropology, University of Wisconsin-Milwaukee, 290 Sabin Hall, 3413 N. Downer Avenue, Milwaukee, WI 53211 Tel: 617-876-0491. E-mail: campbe@uwm.edu

measured estrone-3-glucuronide (E-3-G), the major metabolic of estradiol in urine (Feinberg et al., 2006). Because of the ease of daily urine collection, urinary E-3-G has been used primarily to study change in estrogen over the menstrual cycle in women, including detecting ovulation (Baird et al., 1995), and assessing reproductive aging (Miro et al., 2005).

In contrast, we used a urinary assay for E-3-G because of the difficulties of obtaining serum under field conditions. Little if any data is available on E-3-G in men, and our study represents the first use of E-3-G among males in a subsistence population. We predict that settled males with their greater level of adiposity will exhibit higher levels of E-3-G than the nomadic males.

MATERIALS AND METHODS

FIELD SITE

The Ngisonyoka Turkana studied here live in the lower third of Turkana District, Kenya. The environment can best be described as arid and seasonally fluctuating (Little et al., 1999). Their subsistence is based largely on animals, including goats, sheep, cattle, camels and donkeys, and their diet consists primarily of animal products including milk, blood and meat. Total caloric intake is limited, with dietary estimates of between 1300–1600 kcal/day for adults. However, protein intake is quite high with per capita estimates of 69 grams of crude protein per day, three times the FAO/WHO requirements (Galvin and Little, 1999).

Important diseases among the Turkana include hydatid cysts and tuberculosis, the latter of which may be transmitted through untreated milk leading to wide-

spread exposure of the population. Malaria is considered holoendemic and reports of diarrheal disease and acute respiratory infection (ARI) are also common (Shell-Duncan et al., 1999).

The settled men in this study come from Moruim, which is primarily a agriculture scheme dating to the 60's now run by World Vision, a non-governmental organization. Most of the men were nomads who settled in Moruim as the result of drought or raiding that depleted their herds. Thus, the settled males represent a sub-population that shares its genealogy and part of its individual life history with the nomads, but differs in current diet and disease exposure. The specific living conditions in Moruim are not well-documented, though there is evidence for reduced meat and increased grain consumption, and greater exposure to malaria relative to nomadic Turkana (Campbell et al., 1999).

SUBJECTS

A total of 275 men, 154 nomads and 131 settled men participated in the study. Nomads were recruited at watering locations in the bush in July–Aug of 1992, while settled men were recruited from the village of Moruim in March–April of 1993. Urinary FSH determinations were available for 88 nomadic and 74 settled males, LH determinations 99 nomadic males and 97 settled males, urinary E-3-G determinations for 110 nomadic and 97 settled males.

July and August 1992 was a time of drought with nomads sampled preceding the distribution of food relief, and the settled population sampled in March–April of 1993, three months after the food relief became available (Deluca, 1997). Thus differences in nutritional status between the two sub-populations

here reflect short-term as well as long-term conditions.

Human subjects clearance was obtained from both the University of North Carolina–Chapel Hill and the University of Massachusetts at Boston Institutional Review Boards. Permission to conduct the study was obtained from the Kenyan government and the local chiefs. Oral consent was obtained from each subject. Subjects were given a modest gift for their participation.

AGE ESTIMATES

Age estimates of the participants, ranging from 20 to 90, were obtained based on an event calendar (Leslie et al., 1999 Appendix) and comparison with subjects of similar but established ages. Ages above 80 should be regarded with skepticism.

ANTHROPOMETRIC MEASURES

Nutritional status was assessed using standard anthropometric measures, including height, weight, mid upper arm, waist, and hip circumference (Lohman et al., 1988). Six skinfold measures were obtained: triceps, subscapular, mid-axillary, pectoral, suprailiac and midcalf. Derived measures include Body Mass Index (BMI) calculated as $(\text{wt (kg)} / \text{ht}^2 (\text{m}^2))$; total lean mass $(1 - \% \text{body fat}) \times \text{weight}$; and MPBA calculated as $[(\text{muac} - (\text{TI} \times \text{tcsf}/10))]^2 / 4$

where muac is the mid upper arm circumference and tcsf is the triceps skinfold (Gurney and Jelliffe, 1973). Body fat was determined using the Durim-Wommersley equations (Durim and Wommersley, 1974). Two BMI values, three waist circumference measurements and three % body fat measurements were more than three SD's from the mean and were removed from the data set.

HORMONAL MEASURES

Subjects collected their own first morning urine and delivered them promptly to project field staff. Urine specimens were dried on filter paper (Schleicher and Schnell; catalogue #903) in the field; a 1 in X 1 in square of paper saturates with 250 μL . Dry specimens were stored in glassine sleeves in notebooks places in plastic bags with desiccant. These were kept in a cool location in the field.

The samples were transported to the University of Massachusetts Boston where the filter paper was rehydrated and the filter paper removed using cellulase (Campbell, 1994; 1997). Once extracted from the filter paper, FSH, LH and E-3-G were determined using standard assays. Assay characteristics are shown in Table 1. Interassay variability for LH was $7.7 \pm 9.1\%$, FSH $9.5 \pm 8.0\%$ and E-3-G $17.7 \pm 9.1\%$ (see Table 1 for assay characteristics).

TABLE 1
ASSAY CHARACTERISTICS

ASSAY	TYPE	SOURCE	PRECISION ^a	SENSITIVITY ^b	ACCURACY ^c
LH	IRMA	ICN	7.7 \pm 9.1	1.08 \pm 0.48 IU/L	+0.7 \pm 11.1
FSH	IRMA	ICN	9.5 \pm 8.0	0.55 \pm 0.33 IU/L	+1.1 \pm 12.4
E-3-G	EIA	Munroe	17.7 \pm 9.1	5 ng/ml	4.7 \pm 17.74

^aInterassay CV.

^bLimit of detection.

^cBased on estimation of standards.

Urinary creatinine was obtained by measuring creatinine-piurate adducts (affe reaction) (Campbell et al., 2000). The creatinine results were used to correct urinary hormone values for subject hydration.

STATISTICAL ANALYSIS

For comparison with western reference values we report the original hormonal concentrations. Differences in the raw hormonal measures between settled and nomadic groups were compared using standard two-tailed, unpaired *t*-tests. For all other analyses, we use log transformed hormonal values to ensure a normal distribution.

To determine the effect of age on FSH, LH and E-3-G we used general linear models (GLM) to test for differences in log transformed values for each hormone across 10 year age groups, with residence as a control; 10 year age groups have the benefit of reducing the error associated with uncertain age estimates. Post hoc tests using the Bonferroni correction were used to test for differences between specific age groups.

The relationship of FSH, LH, and E-3-G to nutritional status and body composition

was determined using regression models with measures of body composition as a predictor, and age as a control. Predictors included waist circumference and suprailiac skinfolds as measures of abdominal fat; per cent body fat was used as a measure of overall adipose storage. Fat free mass, BMI and MPPBA were used as measures of non-adipose tissues and muscle. Finally, height was used as a predictor because of its role as a measure of overall childhood growth and development.

RESULTS

Table 2 shows average urinary gonadotropin and E-3-G values, as well as anthropometric measures for settled and nomadic groups. Average levels of urinary FSH, LH and E-3-G are all significantly higher among the settled vs. the nomadic men at the $p < 0.001$ level. In terms of anthropometric measures, the settled males show significantly more adiposity as measured by skinfold measures, but there is no difference in weight or lean mass among the two groups.

Overall values for urinary FSH among the settled males (18.4 ± 16.1 IU/g Cr) are

TABLE 2

COMPARISON OF URINARY GONADOTROPINS AND E-3-G IN NOMADIC VS. SETTLED MEN

VARIABLE	Group		Difference
	Nomadic	Settled	
Urinary FSH IU/gm cr	7.7 ± 9.6(88)	18.4 ± 16.1(74)	$p < 0.001$
Urinary LH (IU/gm cr)	7.3 ± 6.8(99)	13.7 ± 15.2(97)	$p < 0.001$
E-3-G (ng/mg cr)	28.4 ± 21.8(110)	61.8 ± 47.1(97)	$p < 0.001$
Height (cm)	174.7 ± 7.1(109)	172.0 ± 7.4(99)	$p < 0.001$
Weight (Kgs)	53.3 ± 6.8(109)	53.6 ± 7.1(100)	$p = 0.005$
Body Mass Index (kg/m ²)	17.4 ± 1.6(109)	17.9 ± 1.7(97)	$p = 0.593$
MPPBA (cm ²)	36.0 ± 13.0(109)	35.0 ± 8.5(99)	$p = 0.007$
Waist Circumference (mm)	71.6 ± 4.6(109)	75.4 ± 4.7(98)	$p < 0.001$
Fat Free Mass (kg)	50.2 ± 6.0(109)	48.8 ± 5.9(99)	$p = 0.149$
Body Fat (%)	5.8 ± 1.9(108)	9.0 ± 3.2(98)	$p < 0.001$
Triceps Skinfold (mm)	4.0 ± 0.7(109)	5.4 ± 1.7(100)	$p < 0.001$
Suprailiac Skinfold (mm)	4.6 ± 1.0(109)	6.2 ± 2.6(100)	$p < 0.001$

elevated compared to the western reference range of 2 to 15 IU/g Cr (Tieiz 1986) while those for the nomadic males (7.7 ± 9.6 IU/g Cr) are not. Urinary LH levels for the nomads (7.3 ± 6.8 IU/g Cr) are at the low end of the western reference ranges of 6 to 30 IU/gm Cr (Tieiz 1986) while those of the settled males (13.7 ± 15.2) are in more fully in the normal range. We were not able to find reliable reference range for E-3-G, so our results here are restricted to comparison of settled and nomadic males.

Within the Turkana, log urinary LH and log urinary FSH were significantly related across all individuals as expected ($n = 157$; $r = 0.57$, $p < 0.001$). Log E-3-G was significantly related to both log urinary LH ($n = 195$; $r = 0.39$, $p < 0.001$) and log urinary FSH ($n = 162$; $r = 0.32$, $p < 0.001$).

Figure 1 shows age related patterns of urinary FSH, urinary LH and urinary E-3-G. Urinary LH increases across age groups in both sub-populations. GLM models indicate an overall effect of both group and age group ($n = 196$; overall model adj. $r^2 = 0.18$; group $F = 20.9$, $p < .001$; age group $F = 4.1$, $p = 0.003$). There was no difference in the age-related pattern between the two groups as indicated by a non-significant interaction term (group*age group $F = 1.6$, $p = 0.17$). Post hoc tests show that urinary LH among men in 60+ year age group was significantly higher than that of men in their 20's (mean difference = 0.31, $p = 0.02$) and 30s (mean difference = 0.32, $p = 0.001$).

Urinary FSH levels were clearly higher among the settled males but exhibited no consistent age-related change in either sub-population, as confirmed by GLM models ($n = 162$; overall model adj. $r^2 = 0.21$; group $F = 33.7$, $p < 0.001$; age

group $F = 1.2$, $p = 0.32$). However, a difference in age-related patterns between the two groups is indicated by a significant group* age group interaction term ($F = 2.4$, $p = 0.054$).

Separate analysis of the two groups showed no significant differences between age groups among nomads ($n = 89$; adj. $r^2 = -0.02$; $F = 0.67$, $p = 0.62$), while a significant difference between age groups among the settled males ($n = 74$; adj. $r^2 = 0.09$; $F = 2.71$, $p = 0.037$) is attributable to the difference between the 50 and 60+ year age groups (mean difference = 0.49, $p = 0.038$).

E-3-G shows different age-related patterns among nomadic and settled males, with average levels increasing across all age groups among the settled males, but declining after the 30 year age group for the nomadic males. GLM shows a significant difference in overall levels between nomadic and settled males, with no overall difference between age groups ($n = 199$; overall model adj. $r^2 = 0.25$; group $F = 53.5$, $p = 0.001$; age group $F = 1.7$, $p = 0.14$).

However, the existence of a significant group* age group interaction ($F = 3.3$, $p = 0.012$) indicates that the age-related patterns of E-3-G differ between the settled vs. nomadic groups. Separate analysis of the two groups indicate that the settled men show a significant overall difference among age groups ($n = 90$; adj. $r^2 = 0.11$; $F = 4.0$; $p = 0.005$). Post hoc tests indicate that values for men in the 50 and 60+ year age groups are significantly greater than those in their 20s (mean difference = 0.31, $p = 0.042$ and 0.39, $p = 0.003$, respectively). The nomadic men, on the other hand, do not exhibit a significant overall effect of age group ($n = 109$; adj. $r^2 = 0.002$; age group $F = 0.94$; $p = 0.44$) and post hoc tests showed no significant differences between specific age groups.

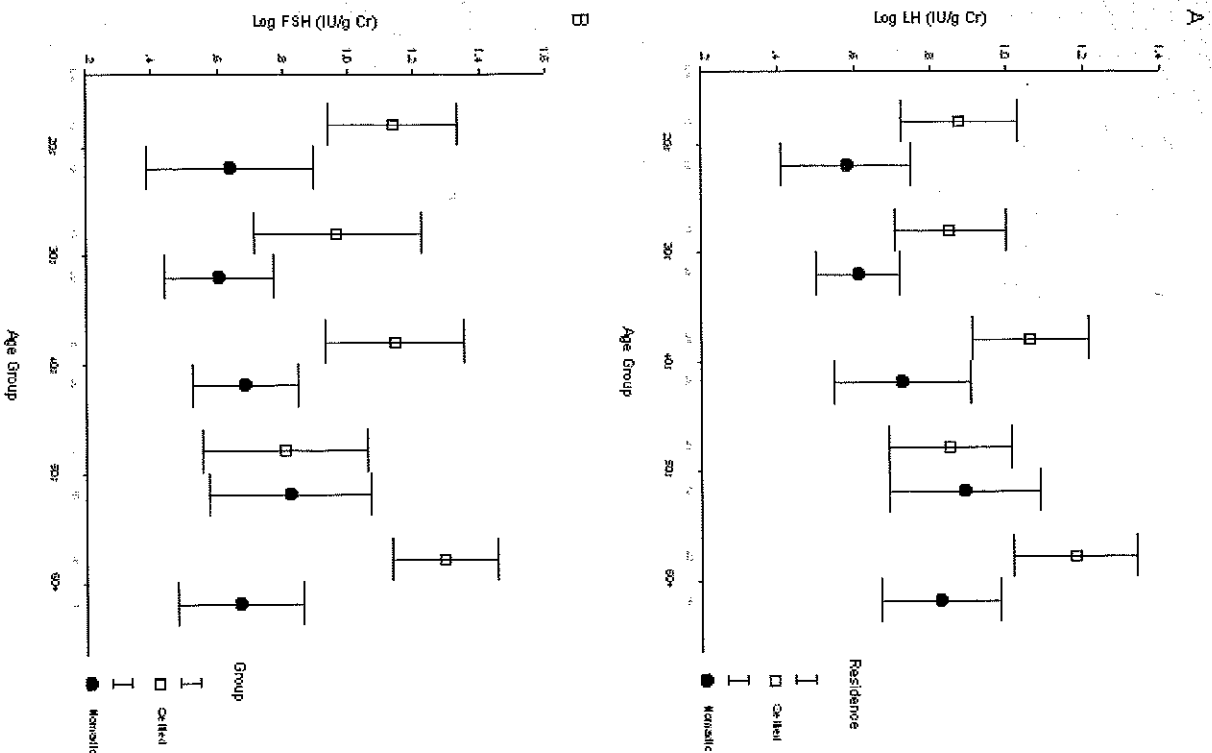


FIG. 1.—Age-related patterns of urinary gonadotropins and E-3-G. A) Log urinary LH is significantly higher among settled males and shows an increased across age groups. Post hoc tests show that males in the 50 and 60+ year age groups have significantly higher levels of LH than do men in their 20s. B) Log urinary FSH is significantly higher among the settled males, but shows no consistent increase across age groups. C) Log urinary E3G is higher among the settled males, and shows an age-related increase among the settled males, but not among the nomadic males.

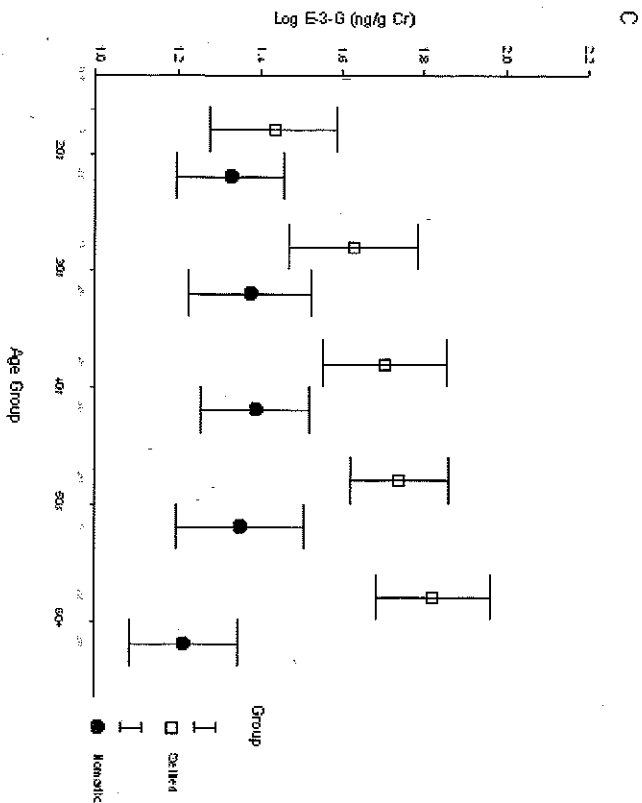


FIG. 1.—(Continued).

TABLE 3
RELATIONSHIP BETWEEN URINARY LH, FSH AND E3G AND BODY COMPOSITION (CONTROLLED FOR AGE)

VARIABLE	URINARY LH ^a		URINARY FSH		URINARY E3G	
	Settled (n = 96) b ²	Nomadic (n = 98) b	Settled (n = 73) b	Nomadic (n = 87) b	Settled (n = 95) b	Nomadic (n = 108) b
Height (cm)	-0.085	0.017	-0.144	0.104	-0.067	-0.157
Weight (kgs)	-0.123	-0.048	-0.236*	0.002	0.045	-0.170
BMI (kg/m ²)	-0.022	-0.093	-0.161	-0.102	0.189 ⁺	-0.107
MPBA (cm ²)	0.014	0.065	-0.295*	-0.069	0.024	-0.035
Waist (mm)	-0.066	-0.021	-0.121	-0.040	0.075	0.188*
FPM (kgs)	0.129	-0.052	-0.240*	0.013	0.041	0.179 ⁺
Body Fat (%)	-0.062	0.075	-0.232*	-0.089	0.002	0.015
Triceps SF (mm)	-0.092	0.033	-0.124	-0.044	-0.055	0.047
Suprailiac SF (mm)	0.040	0.027	0.004	-0.096	0.169	0.002

^ap < 0.1; ^bp < 0.05.
⁺Dependent hormonal variables are log transformed.
^{b2}p coefficients represent results of multiple regression models, controlling for age.
 Age is a significant predictor in all models predicting LH, while age is not a significant predictor in any of the models predicting FSH.

Table 3 shows the results of multiple regression models analyzing the relationship of both gonadotropins and E-3-G to various anthropometric markers of energetic status, controlling for age. Log urinary LH is not significantly related to any of the anthropometric measures in either sub-population, while age is a significant

predictor in all the models predicting LH (β s not shown). In comparison, log urinary FSH is inversely related to several measures of energetic status including weight ($\beta = -0.236$, $p < .05$), arm muscle plus bone area ($\beta = -0.295$, $p < .05$), fat free mass ($\beta = -0.240$, $p < .05$) and overall per cent body fat ($\beta = -0.232$, $p < .05$) among the settled males, but shows no relationship to anthropometric measures among the nomadic males. Age is not a significant predictor in any of the models predicting FSH (β s not shown).

Among the nomads, log E-3-G does not show a significant association with any anthropometric measure that we used. Among the settled males, log E-3-G shows a significant (negative) relationship with waist circumference only ($\beta = -0.188$, $p < .05$).

DISCUSSION

The pattern of urinary gonadotropins reported here suggest alterations in the central reproductive axis among Turkana males in response to both long and short term energetic availability. Both nomadic and settled sub-groups exhibit suppressed urinary LH levels relative to western controls, suggesting the possibility of down-regulation of the LH-Leydig cell-testosterone loop. The settled males also exhibit elevated urinary FSH and higher levels of E-3-G relative to the nomads, suggesting additional up-regulation of Sertoli cell function and/or potentially elevated aromatase activity.

Furthermore, among the settled males FSH levels are associated with measures of body mass, but not adiposity, suggesting that Sertoli cell stimulation is associated with energetic status, but not current energy stores. Together with evidence for negative energy balance among the nomads and positive energy balance among the

settled males (Campbell et al., 2005; Lukas et al., 2005), the current findings suggest that Turkana men may experience significant changes in the HPG axis as a result of weight changes associated with environmental fluctuation.

AGE-RELATED CHANGES IN GONADOTROPINS

The age-related pattern of gonadotropins for Turkana males presented here suggests differences in those of FSH, but not LH compared to western samples. The findings of significantly increased urinary LH among Turkana men after 50 are consistent with results from western samples that demonstrate an increase in serum LH after the age of 40 (Baker et al., 1976; Gray et al., 1991). On the other hand, the lack of an age related increase in urinary FSH reported here contrasts with increases in serum FSH after the age of 40 reported for western populations (Baker et al., 1976; Gray et al., 1991) or the linear increase in serum FSH starting at age 24 reported by Pasqualotto et al. (2005).

POPULATION VARIATION IN GONADOTROPINS

Population differences in age-related patterns of gonadotropins has been previously reported. Haji et al. (1994) report that FSH levels in a sample of Japanese men begin to increase after 40, with LH levels increasing after 60. In the only similar non-western study, Tennekoon and Karunanayake (1993) report an increase in FSH before that of LH among Thai men, with FSH significantly higher from the 6th decade, and LH from the 7th decade. Bribescas (2005) reports a significant linear increase in serum FSH and LH among Ache hunter-gatherers of Paraguay, starting at age 20, but the data can not be interpreted to determine differences between specific age groups.

The results from the Turkana provide an interesting comparison with existing findings. As with the Ache, regression analysis indicates a significant linear increase in LH with age starting from age 20. However, significant differences among 10 year age groups are only apparent after the age of 50, earlier than for either the Japanese and Thai men. The age-related pattern of FSH differs more dramatically from that of the Ache, Japanese and Thais in showing no age-related increase. Thus, it appears that relative to other populations Turkana males may show an earlier rise in LH, coupled with a later or non-existent rise in FSH.

There are a number of different factors which might contribute to the apparent difference in age-related patterns of gonadotropins in the Turkana vs. other populations, including difference in biological fluids, including assay protocols, and statistical methods. At the same time, it is possible that the difference may reflect meaningful variation in the male reproductive axis across populations. Turkana men must pay dowries for their wives; as a result they exhibit a late age at the birth of their first child. In addition with polygyny, some Turkana men continue to contract additional marriages into their 60s (Leslie et al., 1999). This leads to a relatively late schedule of reproduction compared to the monogamous Japanese and Thai men. Continued fertility at older ages among Turkana men would be more directly related to the lack of increased FSH levels as markers of Sertoli cell function and spermatogenesis than it would be to increased LH stimulation of Leydig cell production of T for somatic maintenance (see discussion below).

E-3-G

The substantially higher levels of urinary E-3-G reported among settled vs.

nomadic Turkana men here are notable. However, because E-3-G has not been widely used among men, and as a urinary metabolite it may reflect differences in either the production or metabolism of estrogens the difference between the two groups must be interpreted with caution.

The age related increase in E-3-G among the settled males is consistent with finding on bioavailable estrogen in western samples (Vermulen et al., 2002; Khosla et al., 2001), though most studies fail to find an increase in total serum estradiol (Belanger et al., 1994; Gray et al., 1991; Muller et al., 2003). Among men in western populations, age-related increases in free estrogen have been attributed to increased adiposity (Vermulen et al., 2002; Muller et al., 2003), as exhibited by the settled Turkana males. Furthermore, earlier findings demonstrated an age-related increase in blood SHBG among the settled males (Campbell et al., 2006b), consistent with findings of increasing adiposity, SHBG and aromatization of testosterone with age among western men (Vermulen et al., 2002).

On the other, the apparent decline in E-3-G with age among the nomadic males requires further explanation. Neither blood T (Campbell et al., 2006) nor urinary FSH or LH levels (as shown here) show a consistent decline with age. However, per cent body fat among the nomadic males declines from the 30's onward (Campbell et al., 2005), suggesting that the age-related declines in adiposity among the nomads may be related to the apparent decline in E-3-G.

In men, the production of estrogen is related to aromatization of testosterone within the testes (Vermulen et al., 2002) and/or adipose tissue (Belanger et al., 2002). Based on results from western

samples, 20 per cent of circulating estradiol among aging males is thought to come from the testes (Vermuelen et al., 2002), the rest comes from aromatization in peripheral tissues, which is now known to include muscle (Larionov et al., 2003) as well as fat. In particular, Vermuelen et al. (2002) report that free estradiol was highly positively correlated with subcutaneous, but not visceral, abdominal fat. In a group of 30-60 year old men, suggesting that subcutaneous abdominal fat may be a particularly active site of aromatization and estrogen production.

The low level of adiposity among nomadic Turkana men (average body fat = $5.8 \pm 1.9\%$) suggests relatively little adipose tissue for the peripheral conversion of testosterone to estrogen. Furthermore, FSH is known to stimulate aromatase production by Sertoli cells within the testes (Rosselli and Skinner, 1992). Thus decreased E-3-G levels in the nomadic males may reflect decreased FSH stimulation of the testes, as well as decreased aromatization of testosterone by peripheral tissues. In addition to FSH, insulin has also been shown to stimulate the production of aromatase in cultured human testicular cells (Berenstein et al., 1992). Thus to the extent that reduced adiposity reflects poorer overall energy status, including lower basal insulin levels, reduced adiposity among the nomadic males may play a role in relatively lower E-3-G levels.

EFFECTS OF ENERGETICS ON THE MALE REPRODUCTIVE AXIS

The central male reproductive axis is considered to be relatively insensitive to energetic status (Ellison, 2001; though see Campbell and Leslie, 1995). It has been argued that the energetic cost of spermatogenesis are sufficiently low and

the benefits of a potential insemination sufficiently high that there is little adaptive basis for linking variation in sperm production to energetic availability, except in the case of extreme energy deprivation when survival itself becomes an issue (Bribiescas, 2001). Empirical evidence from western populations supports this position. Marathon runners, for instance, show only isolated changes in reproductive function (Ayers et al., 1985; Bagatell and Bremner, 1990), while Indian men undergoing acute starvation do exhibit suppressed gonadotropin levels (Smith et al., 1975).

However, marathon runners who increase their mileage do show declines in testosterone and sperm quality (Roberts et al., 1993) suggesting that *changes* in energetic status may have an appreciable impact on the male reproductive axis in nutritionally stressed males. Ellison and Panter-Brick (1996) report that among Tamang males of Nepal, salivary testosterone is associated with indicators of energy status such as weight, biceps skinfold and mid arm circumferences, but only in winter, a low-nutrition period.

Thus the fact that settled Turkana males exhibit elevated FSH levels compared to their more poorly nourished nomadic males is surprising, as is the fact that urinary FSH is more highly associated with measures of body mass than urinary LH. These results suggest that chronic under nutrition may have a more direct effect on the central male reproductive axis, including sperm production, than previously considered.

Chang et al. (1994) report that serum LH and FSH were positively associated with waist hip ratio (WHR) in a sample of men with and without diabetes, suggesting that abdominal fat may have a direct effect on gonadotropin production,

even under conditions less energetically stressed than those experienced by the Turkana. However, in the Chang et al. (1994) study neither serum LH and FSH were associated with skinfold thickness, the measure of subcutaneous abdominal fat we use here.

Taken as a whole, our findings on Turkana men suggest that among the settled males weight gain after acute under nutrition may have resulted in a resumption of reproductive function with increased FSH stimulating Sertoli cell production of estrogen to promote the early stage of spermatogenesis (Rosselli and Skinner, 1992) and leading to increased circulating estrogen. Increased estrogen in turn may bias energy utilization toward the deposition of adipose tissue and away from muscle, in order to rebuild depleted energy stores.

In contrast, testosterone seems relatively unaffected by such changes in energetic status. Our previous results (Campbell et al., 2006b) show slightly lower, not higher levels of blood T among the settled males relative to the nomads, despite significantly greater adiposity. These findings are similar to those obtained from the Lese of the Iuri forest indicating a decline in salivary T associated with weight gain after the hunger season (Bentley et al., 1993).

Thus our results extend previous findings of the effects of energetics on male reproductive function among subsistence populations to include age-related patterns of gonadotropins and estrogen. Previous results from both the Turkana (Campbell et al. 2005), and the Ariaal (Campbell et al., 2003; Campbell et al., 2006a) suggest that compared to younger men, older males show greater fluctuation in adipose stores associated with changes in food availability. The results obtained

here suggest that such changes in body composition may be reflected in age-patterns of estrogens which in turn may play a role in the regulation of gonadotropin production (Bagatell et al., 1994).

IMPLICATIONS FOR FECUNDITY AND FERTILITY AMONG THE TURKANA

The lack of an age related increase in urinary FSH among Turkana males suggests that male fecundity among the Turkana may not decline substantially with age. Detailed studies of testes obtained at autopsy indicate age-related declines in several aspects of spermatogenesis (Neaves, 1984). However, Bohring and Krause (2003) found little change in the relationship of inhibin B to FSH across men aged 16 to 89 and conclude that there is little change in Sertoli cell function with age. Furthermore, Pasqualotto et al. (2005) report changes in sperm concentration and sperm morphology along with increasing FSH levels with age in a sample of 889 Brazilian men 24-67 years of age. By inference, a lack of increased FSH among Turkana men may reflect lack of a decline in sperm production. More direct assessment of sperm parameters are necessary to confirm this possibility.

Reproduction among the Turkana has several notable features, a high fertility rate, pronounced birth seasonality, and an extended male reproductive span (Leslie et al., 1999). Leslie and Fry (1989) have demonstrated a highly pronounced seasonality of births, associated with patterns of rainfall and changes in food availability. In addition, long term droughts have been linked to declines in age specific fertility rates (Leslie et al., 1999). These changes in fertility are thought to be mediated by the response of female ovarian function to changes in energetic

status (Leslie et al., 1999). Our results, however, suggest the possibility that variation in male reproductive function as a result of weight loss could play a role in seasonal variation in fertility as well.

Reproductive histories from Turkana men indicate a late onset set of reproduction; median age of first birth is 30 years (Leslie et al., 1999). Men continue to marry additional wives at relatively advanced ages; the average age of men at marriage to their 3rd wife is 55 years (Leslie et al., 1999). While there is a chance that children of higher order marriages are fathered by other men, ethnographic evidence suggests this is likely to be the case only for the very oldest men. Regardless, the lack of any marked age-related increase in FSH is consistent with continued fecundity among older men.

Campbell (1994) presents FSH and LH profiles among from Gajinj men of New Guinea, suggestive of early reproductive senescence. As with the Turkana, Gajinj males (as well as females) exhibit late reproductive maturation, but in contrast demonstrate early cessation of fertility. Thus, taken together the Turkana and Gajinj results suggest the possibility that population variation in age-related patterns of male fecundity with age may reflect the social structure of reproduction.

SUMMARY

Our results demonstrate unusual patterns of urinary gonadotropins and E-3-G among Turkana men associated with both chronic and acute undernutrition. Low levels of urinary LH among the nomads coupled with normal blood T (Campbell et al., 2006) together with increased FSH

and E-3-G among the settled males suggests the possible existence of hypergonadotropic hypogonadism associated with intermittent caloric restriction and deposition of adipose tissue with increased food availability.

Comparison with results from other populations (Haji et al., 1994; Tennekoon and Karunanayake, 1993) suggest that the lack of an age-related increase in FSH observed among the Turkana may be unusual, while the age-related pattern of LH observed among Turkana males may vary only in its relative timing. Comparison with existing results from subsistence populations (Bribiescas; 2005, Campbell, 1994) suggest that age-related patterns of gonadotropins may vary substantially across subsistence populations, as a reflection of energy availability and/or selection related to the social structuring of reproduction. However, given differences in bodily fluids assayed, and sample size in these two studies, results from additional subsistence populations are sorely needed before any conclusions about the extent of population variation in gonadotropins, its causes and their relationship to male fecundity, let alone its implications for variation in fertility, can be reliably drawn.

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