

SHORT REPORT



Replicability of leptin associations with testosterone, estradiol, follicle-stimulating hormone, and luteinizing hormone in healthy Ache men of Paraguay: A multiple daily assessment

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Abstract

Objective: Associations between leptin and male reproductive hormone levels have been reported in men. However, few of these investigations have focused on associations in healthy men without obesity or overweight or nonindustrial societies.

Methods: We test hypotheses that leptin is associated with testosterone, estradiol, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) within healthy Ache men, an indigenous nonindustrialized South American community using archived data.

Results: Archived data of serum FSH, LH, leptin, and salivary testosterone and estradiol levels collected from healthy Ache men ($n = 17$, mean age = 37.1 ± 14.2) on two separate days revealed leptin was correlated with FSH (D1 $p = .07$, D2 $p = .009$) and PM testosterone (TsalPM, D1 $p = .05$, D2 $p = .05$). However, controlling for age, associations with FSH were not significant. Mean comparisons and linear regression of values over 2 days resulted in leptin ($t = 0.08$, $p = .94$, $r^2 = .58$, $p = .0009$), LH ($t = 1.16$, $p = .26$, $r^2 = .11$, $p = .27$), FSH (U = 131.5, $p = .88$, $r^2 = .63$, $p = .0002$), AM testosterone (TsalAM, $t = 4.0$, $p = .001$, $r^2 = .02$, $p = 0.75$), and TsalPM ($t = 2.99$, $p = .01$, $r^2 = .56$, $p = .01$).

Conclusion: We conclude (a) FSH, TsalPM, and leptin levels within individual men are relatively invariant over a span of days; (b) despite small sample sizes, results suggest ecological and lifestyle variation can contribute to variation in leptin associations with male reproductive hormones.

1 | INTRODUCTION

Leptin is a hormone that is produced predominantly in adipose tissue. Through its role in monitoring of body fat reserves, leptin affects food intake and energy balance via communication with the hypothalamus and has associations with aging, reproduction, and immune system functions. Leptin is associated with kisspeptin levels,

downstream effects on hypothalamic function, gonadotropin (luteinizing hormone [LH] and follicle-stimulating hormone [FSH]) production, and has been reported to be associated with male reproductive function (Landry et al., 2013; Lima et al., 2020).

Little is known about associations between leptin and male reproductive hormones in healthy men across varied ecologies and lifestyles. Among Nigerian men,

testosterone was lower and leptin higher among men with metabolic syndrome, however, associations between leptin and reproductive hormones were not reported (Fabian et al., 2016). Similar associations have been found in studies with men from Tibet (pubertal transition) (Xi et al., 2011), Pakistan (with/without infertility problems) (Amjad et al., 2019), and Lebanon (elderly with/without metabolic syndrome) (Gannage-Yared et al., 2006). Male reproductive hormone levels differ between communities depending on ecological variation (Bribiescas, 2001a). Therefore, associations between leptin and male reproductive function may also vary in these populations. Moreover, interday variation has been shown to contribute to assessments of male reproductive hormones (Brambilla et al., 2007). We test the hypothesis that leptin is associated with male reproductive hormone levels within healthy Ache men, an indigenous South American community.

2 | METHODS

Archived data from assays conducted between 1999 and 2000 and reported in earlier publications was combined for this analysis (Bribiescas, 2001b, 2005). Details on sample collection and analysis have been reported previously (Bribiescas, 2001b, 2005). Hormone values are based on serum and saliva samples collected 10 days apart from the same participants. FSH, LH, and leptin values were derived from serum samples collected from the same participants on collection days one (D1) and two (D2). Some participants were unavailable on one collection day resulting in unequal sample sizes for each day.

Morning (TsalAM) and evening (TsalPM) testosterone were measured from salivary samples collected concurrently with serum samples on both days. Salivary morning (E2AM) and evening (E2PM) estradiol were measured D1 only due to sample volume limitations. To assess within participant variation across days, comparisons of mean hormone values were conducted using log transformed data using two tailed unpaired *t* tests due to unequal sample sizes for some hormones. Welch's correction was applied when variances were significantly different. Mann Whitney tests were deployed when log transformation was ineffective. The effect of log transformation on data normalization was assessed using Anderson-Darling test.

Multiple and individual linear regression was used to assess the contributions of LH, FSH, TsalAM, TsalPM, E2AM, E2PM, and age on leptin variation. Height, weight, body fat percentage, and body mass index (BMI) have previously been demonstrated to not be associated with any of the predictor variables (FSH, LH, estradiol,

testosterone) in Ache men so were not included in models (Bribiescas, 2005). A repeated measures mixed linear model with collection day set as a random effect was performed to assess the interactions of hormone variables and age on leptin variability. Alpha was set at .05. Statistical analysis was conducted using JMP 15 for Macintosh (SAS Software).

3 | RESULTS

Log transformation was effective at normalizing data for all hormone data (Anderson-Darling $p > .05$) except D1 FSH (Anderson-Darling, $p = .041$). Nonparametric comparisons of means (Mann Whitney U) were therefore conducted for FSH. Height, weight, BMI, body fat percentage, and age have been previously reported (Bribiescas, 2001b, 2005).

Comparisons of means and between day correlations are presented in Table 1.

No significant differences were noted between the D1 and D2 means of FSH, LH, or leptin. However, significant differences between testosterone were evident. Analysis using paired samples from individuals yielded similar results. Low Hedge's *g* for leptin and FSH suggest low sample sizes impacted the analysis. Correlations between collection days were significant for FSH, TsalPM, and leptin suggesting consistent measurement replicability for these hormones.

Individual linear regressions were conducted between leptin and hormone predictor variables. Significant associations were evident between leptin and TsalPM and FSH (Table 2). Since age correlated with leptin and FSH, linear regression of residuals from age*leptin and age*FSH resulted in no significant association, suggesting that age is a common variable driving this association (residuals D1 leptin*residuals D1 FSH, $r^2 = <.001$, $p = .96$; D2 $r^2 = .16$, $p = .13$). Multiple linear regression of all hormones and age with leptin revealed high variance inflation factor (VIF) for age (19.8) with all other D1 VIFs <7.0 . Removal of age from model resulted in remaining D1 VIFs reduced to <3.0 and AICc lowered from 131.3 to 41.9. The most parsimonious model for D1 leptin included only TsalPM ($r^2 = .31$, $p = .05$, AICc = -22.3). The D2 model resulted in high VIFs for LH (19.9) and FSH (11.2). Exclusion of LH resulted in VIFs <3.0 . The most parsimonious model for D2 included age only ($r^2 = .31$, $p = .03$, AICc = -25.5).

Mixed linear model of repeated measures with collection day set as a random effect and FSH, TsalPM, and age as fixed effects resulted in the total interaction of all three variables (FSH*TsalPM*age) to be the only significant result ($\beta = 12.2$, $SE = 4.38$, 95% confidence

TABLE 1 Summary of data used in present analysis

| | Collection day one | | Collection day two | | t | p | Hedge's g | Correlation between collection days r^2 , p | |
|--------------------------|--------------------|--------------------|--------------------|--------------------|------------------------|------|-----------|---|----------|
| | N | Mean \pm SD | N | Mean \pm SD | | | | | |
| Age (years) | 17 | 37.1 \pm 14.2 | | | | | | | |
| Weight (kg) | 15 | 56.1 \pm 5.5 | | | | | | | |
| Height (cm) | 15 | 153.4 \pm 6.3 | | | | | | | |
| BMI (kg/m ²) | 15 | 23.8 \pm 1.4 | | | | | | | |
| Body fat (%) | 15 | 18.2 \pm 1.5 | | | | | | | |
| Leptin (ng/ml) | 17 | 1148.8 \pm 400.7 | 16 | 1120.6 \pm 273.1 | 0.08 | .94 | 0.08 | .58, .0009 ^a | .08, .28 |
| FSH (ng/ml) | 17 | 15.3 \pm 6.1 | 16 | 16.4 \pm 8.7 | U ^b = 131.5 | .88 | 0.15 | .63, .0002 | |
| LH (ng/ml) | 14 | 9.5 \pm 5.5 | 15 | 7.7 \pm 3.6 | 1.16 | .26 | 0.39 | .11, .27 | |
| AM testosterone (pmol/l) | 14 | 156.9 \pm 112.6 | 11 | 324.7 \pm 145.4 | 4.00 | .001 | 1.31 | .02, .75 | |
| PM testosterone | 14 | 189.1 \pm 78.9 | 12 | 299.8 \pm 104.5 | 2.99 | .01 | 1.21 | .56, .01 | |
| AM estradiol (pmol/l) | 15 | 29.8 \pm 19.4 | – | – | – | – | – | – | – |
| PM estradiol | 15 | 26.0 \pm 24.5 | – | – | – | – | – | – | – |

Abbreviations: AM, morning collection; BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PM, afternoon collection.

^aWith the exclusion of an extreme outlier (3.2 SD).

^bFSH comparison of means conducted using nonparametric test (Mann Whitney U test).

TABLE 2 Linear regression analysis of associations between leptin and reproductive hormones on each collection day

| | Collection day | Lower, upper 95% confidence interval | SE | r^2 | p |
|-----------------|----------------|--------------------------------------|------|--------|------------------|
| AM testosterone | 1 | –0.22, 0.31 | 0.12 | .01 | .72 |
| | 2 | –0.60, 0.37 | 0.21 | .04 | .60 |
| PM testosterone | 1 | –0.78, 0.01 | 0.18 | .28 | .05 ^a |
| | 2 | –0.13, 0.59 | 0.16 | .19 | .18 |
| FSH | 1 | –0.002, 0.59 | 0.14 | .23 | .05 ^a |
| | 2 | –0.06, 0.43 | 0.11 | .15 | .14 |
| LH | 1 | –0.39, 0.38 | 0.18 | < .001 | .98 |
| | 2 | –0.15, 0.33 | 0.11 | .05 | .42 |
| AM estradiol | 1 | –0.12, 0.41 | 0.12 | .10 | .26 |
| PM estradiol | 1 | –0.17, 0.19 | 0.08 | .001 | .90 |

Note: Pooling data from days one and two, even with potential pseudo-replication, did not result in any significant correlations (LH, $r^2 = .02$, $p = .49$).

Abbreviations: AM, morning collection; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PM, afternoon collection.

^aExclusion of leptin outlier resulted in FSH $r^2 = .21$, $p = .07$ and TsalPm $r^2 = .31$, $p = .047$. All other regressions did not change significantly.

interval = 3.03, 21.35, VIF = 1.5, $p = .01$). Collection day as a random effect accounted for 11.0% of the total variation.

4 | DISCUSSION

Our hypothesis that associations between leptin and reproductive hormones in Ache men are similar to other studies met with mixed support. Although FSH and

TsalPM were significantly associated with leptin, age was the primary driver of the correlation with FSH and TsalPM. These results suggest (a) limited interaction between leptin and male reproductive hormones in this population and (b) associations between leptin and reproductive hormones vary across populations, possibly as the result of developmental and lifestyle factors that influence leptin and reproductive hormone physiology. For example, Sharrock et al. (2008) demonstrated extremely low leptin levels in association with adiposity

in Tsimane children and adolescents. Similarly, Kuzawa et al. (2007) reported low leptin levels in Filipino adolescents suggesting that pre-adult conditions may have an effect on adult leptin. While the leanness and low variation in adiposity among Ache men may be influential in this study, comparisons with comparatively lean athletes suggests otherwise (Bribiescas & Hickey, 2006). Testosterone can also affect leptin levels. Increases in testosterone levels due to puberty and/or supplementation both suppress leptin production, mostly likely through decreases in adipose tissue (Xi et al., 2011).

The consistency of leptin between collection days is similar to previous results among Ache women (Bribiescas et al., 2008). LH and testosterone often exhibit a robust range of daily pulsatility compared to FSH (Spratt et al., 1988). However, FSH and TsalPM measures revealed notable consistency suggesting that pulsatility may differ among Ache men, but additional research is necessary. Salivary steroid measurements are subject to greater variability, likely due to pulsatility and lower levels that are more sensitive to variation compared to serum values.

The small, opportunistic sample size is a limitation of this study. Larger sample sizes from a variety of non-industrialized populations would be optimal although similar sample sizes have yielded noteworthy comparative results (Bribiescas & Hickey, 2006). In conclusion, we suggest that men living in industrialized societies exhibit increased responsiveness between leptin and the hypothalamic–pituitary–testicular axis as a result of recent changes toward high-calorie, low-nutrient foods and increased sedentism. Further investigation into the association between leptin and other metabolic hormones with male reproductive hormones across a variation of populations and settings would be informative.

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AUTHOR CONTRIBUTIONS

Victoria Harries: Conceptualization; formal analysis; writing-original draft; writing-review & editing. **Richard Bribiescas:** Conceptualization; data curation; formal analysis; writing-original draft; writing-review & editing.

CONFLICT OF INTEREST

The authors received no funding for this research and declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data

are not publicly available due to privacy or ethical restrictions.

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