Short Report

Testosterone and Male Cognitive Performance in Tsimane Forager-Horticulturalists

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Objective: Testosterone plays a vital role in brain function and behavior. Among humans, age-related decline in testosterone is associated with declining cognitive functioning, and aging men with higher testosterone maintain better cognitive performance. However, most research focuses on industrialized populations with widespread access to formal schooling, high testosterone, and low parasite and pathogen load. We examine whether men's testosterone is associated with cognitive performance among Tsimane forager-horticulturalists of Bolivia despite relatively lower levels of testosterone and higher immune burden.

Methods: Ninety-four Tsimane men aged 36–86 (median = 49) participated in a cognitive battery (assessing short- and long-term recall, digit span, semantic memory, and visual scan) and provided urine and blood samples to measure testosterone and markers of immune activation. Linear mixed effects regressions were used to model associations between cognitive performance and testosterone, controlling for age, years of schooling, Spanish fluency, and village residence. For a subset ($n = 66$) we included immune activation markers to examine mediator effects.

Results: Testosterone is positively associated with short- and long-term verbal memory ($\beta = 0.267$, $P = 0.018$; $\beta = 0.326$, $P = 0.005$ respectively) and visual scanning ($\beta = 0.306$, $P = 0.008$) after controlling for potential confounders. Markers of immune activation were negatively associated with cognitive function, but did not change the associations between testosterone and cognitive performance.

Conclusion: Tsimane men show positive associations between testosterone and cognitive performance, particularly for recall and visual scanning, despite higher immune burden. Testosterone may help motivate both physical and cognitive capacities that were essential for extracting the difficult-to-acquire, high-quality resources upon which humans relied over evolutionary history. Am. J. Hum. Biol. 27:582–586, 2015.

INTRODUCTION

Increased intelligence allowed early hominins to access difficult-to-acquire foods and develop social coalitions, facilitating the intergenerational transfer of calories, knowledge and culture (Kaplan et al., 2000; Muehlenbein and Flinn, 2011). By examining proximate mechanisms underlying cognitive ability, it is possible to better understand the constraints that shaped the evolution of human intelligence. Androgens are one such proximate mechanism; neuro-hormones like testosterone impact human brain development, function, and behavior (Janowsky, 2006; Schulz et al., 2009). Research on human sex differences in cognitive performance often focuses on spatial cognition, with men generally showing enhanced performance on spatial tasks compared to women (Leonard and Winsauer, 2011). Androgens are often cited as a potential mediator of these sex differences, but there are also many other hormonal, physiological, and cultural sex differences; and even cognitive domains that show no sex differences are impacted by testosterone (Janowsky, 2006). Women tend to perform better on semantic memory tasks (e.g., category fluency) than men, with estradiol and not testosterone supporting many aspects of female cognitive functioning (Yaffe et al., 2000).

Age-related declines in testosterone are associated with declining cognitive functioning, and aging men with higher testosterone maintain higher cognitive performance (Ackermann et al., 2012; Barrett-Connor et al., 1999; Janowsky, 2006; Moffat et al., 2002). Endogenous male testosterone predicts cognitive performance, including short-term and long-term memory, in both cross-sectional (Barrett-Connor et al., 1999) and longitudinal analyses (Moffat et al., 2002). Hypogonadal men perform poorly on verbal memory tasks (Alexander et al., 1998), and androgen deprivation therapy reduces performance in verbal memory and inhibition tasks (Janowsky, 2006). Exogenous testosterone administration yields mixed effects on cognition (Cherrier et al., 2001; Gray et al., 2005), possibly due to varying levels of testosterone and other androgens. Functional magnetic resonance imaging (fMRI) studies suggest several biological pathways by which testosterone could influence cognitive function. Testosterone administration may result in heightened reactions to threat processing in the amygdala, and increased potential for aggressive behavior in men (Goetz et al., 2014). Increased activity in the amygdala (Ackermann et al., 2012; Goetz et al., 2014) and hippocampus (Janowsky, 2006) can increase the probability of memory formation. In addition to acute or activating influences of testosterone on brain activity, testosterone organizes brain tissues and sets

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androgen receptor densities during development (Schulz et al., 2009).

Testosterone thus appears to play a strong role in men's cognitive performance. Men in industrialized populations achieve higher levels of testosterone during young adulthood than men in subsistence populations, resulting in steeper declines at older ages among the former (Ellison et al., 2002; Trumble et al., 2012). Consistently high testosterone is difficult to maintain when energetic surplus is low. When facing infectious disease or caloric restriction, male testosterone decreases rapidly (Muehlenbein and Bribiescas, 2005; Wingfield et al., 1990). Lower levels of testosterone coupled with high pathogen burden and reduced energetic status may independently or jointly contribute to reduced cognitive capacity in subsistence populations (Boivin and Giordani, 1993; Eppig et al., 2010; Jonassaint et al., 2014). Subsistence populations also lack widespread access to formal schooling, which can protect against some degenerative cognitive diseases of aging in modern societies (Wilson et al., 2009).

This study examines co-variation in testosterone and cognitive performance among the Tsimane, who practice a relatively traditional subsistence lifestyle in the Bolivian Amazon. While much research focuses on testosterone and aggressive behavior, the cognitive impacts of testosterone could be beneficial for male subsistence productivity and social relationships, and thus reproductive success. We hypothesized that men's testosterone is positively associated with their cognitive performance, particularly spatial ability and memory. Enhanced spatial ability is beneficial for route finding and hunting, and enhanced short- and long-term recall benefits a number of production and social domains (e.g., remembering previous encounters, defectors in social contracts). Previous research finds that immune activation and parasite loads decrease both testosterone levels (Muehlenbein and Bribiescas, 2005), as well as cognitive function (Boivin and Giordani, 1993; Eppig et al., 2010; Finch, 2010).

METHODS

Population

Ninety-four Tsimane men aged 36–86 (median = 49) completed a cognitive battery and provided urine specimens for hormonal analysis. Approximately, half of participants ($n = 44$) had no formal schooling, and though years of schooling ranged from 0 to 12 (median = 1), only nine individuals completed more than four years.

Cognitive battery

Participants completed a series of cognitive tasks adapted from commonly used cognitive batteries. The first task examined short- and long-term verbal memory via word recall; participants were asked to listen to and repeat a list of eight Tsimane words immediately and once again after 10 min had lapsed. A second set of tasks assessed working memory using three digit span tasks; participants were asked to repeat a series of digits in both Tsimane and Spanish that increased in length until failure on two consecutive trials. In the third digit span task (tactile), the interviewer touched a sequence of numbered boxes on a sheet of paper; participants were asked to touch the boxes in the same order. The next task examined semantic memory (category fluency) with three locally salient categories: animals, plants, and fish. Participants generated a list of items from memory that matched each category (e.g., all animals) during a 2-min period. The final task was a visual scan where participants were shown a random array of symbols, and asked to highlight all instances of a target symbol. All tests were conducted by a trained Tsimane translator at participants' homes and in the Tsimane language, with the exception of the Spanish numbers in the digit forward test. All participants provided informed consent, and the procedures were approved by the institutional review boards at the University of California Santa Barbara and University of New Mexico (UNM).

Urinary hormone analysis

First morning void urine specimens were collected during routine medical examinations as a part of a larger study on aging by the Tsimane Health and Life History Project, on the morning that participants engaged in the cognitive battery. Specimens were stored in liquid nitrogen before transfer on dry ice to UNM where they were stored at −80°C until assay. Testosterone was analyzed with an in-house enzyme immunoassay using a polyclonal antibody (R156/7) and signal provided by C. Munro at the University of California Davis Clinical Endocrinology Laboratory; this antibody cross-reacts 100% with testosterone, 57.4% with 5α-dihydrotestosterone, 0.27% with androstenedione, and less than 0.05% with other androgens, see Muir et al. (2001) for assay validation information. The within and between plate coefficients of variation were 4.7% and 12.2% for the high and 4.7% and 14.9% for the low controls.

Biomarkers of parasite and pathogen load

For a subset of $n = 66$ men, fasting morning blood draws were also collected the day of the cognitive battery. White blood cell count (WBC) and hemoglobin concentration were calculated with a QBC Autoread Plus Dry Hematology System (Drucker Diagnostics, Port Matilda, PA), and eosinophils (%) were measured as a part of a manual five-part differential blood count.

Statistical analysis

Urine specimens were corrected for specific gravity, and testosterone concentrations normalized by log transformation. Because villages have varying access to the market town of San Borja that could confound the associations examined here, village ID was included as a random effect, and mixed effects regression models were used to estimate associations between testosterone and cognitive performance. Two sets of regression models (separate models for each cognitive test) were run, Model A examining associations between log testosterone and each task controlling for age, years of schooling, and Spanish fluency ($n = 94$). Model B represents exploratory analyses examining the same associations on the subset of men for whom WBC, hemoglobin, and eosinophil data were also available ($n = 66$). Eosinophils are a marker of parasitic infection, while WBC and hemoglobin respond to both parasite and pathogen load. It should be noted that Model B is an exploratory first pass; power analyses indicate that a sample size of $n = 103$ would be necessary to achieve sufficient power. All cognitive measures were transformed into Z-scores to make comparisons across measures possible. In the present sample, testosterone was not associated with age, nor highest grade of schooling attained (all P's > 0.09). We also find no evidence of an

TABLE 1. Regression models examining associations between testosterone (logged) and measures of cognitive performance among Tsimane men (n = 94), controlling for age, years of schooling, Spanish fluency, and village ID

	Short-term recall	Long-term recall	Digit forward (Tsimane)	Digit forward (Spanish)	Digit forward (Tactile)	Category fluency	Visual scan correct
Log testosterone	0.267*	0.326**	0.097	0.224	0.136	0.049	0.306**
Age	−0.023**	−0.042***	−0.017†	−0.023**	−0.022**	−0.018*	−0.037***
Years schooling	0.075*	0.043	0.028	−0.041	0.053	−0.018	0.098**
Spanish fluency	−0.254***	−0.046	0.110	−0.014	0.228**	−0.096	0.021

*≤0.05, **≤0.01, ***≤0.001, †≤0.1.

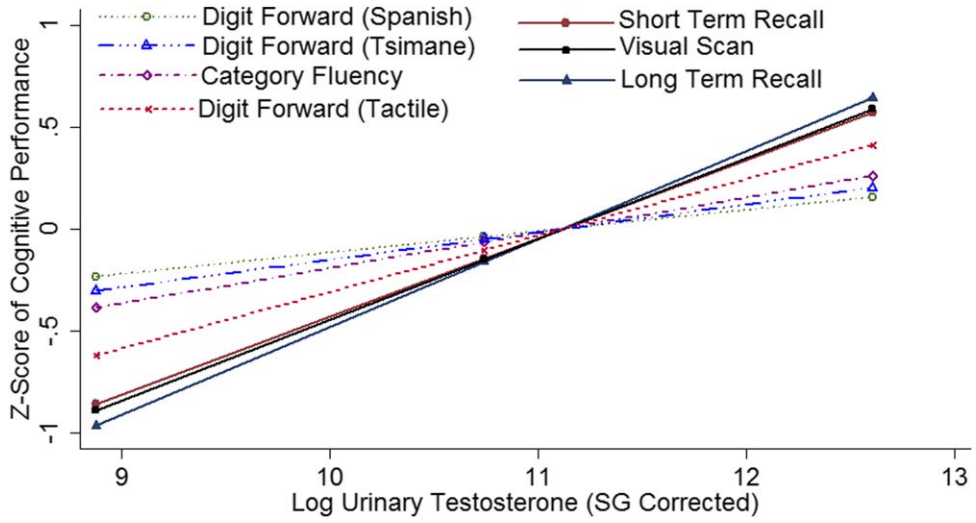

Fig. 1. Fitted associations between testosterone (logged) and measures of cognitive performance among Tsimane men ($n = 94$) controlling for age, years of schooling, Spanish fluency, and village ID. Solid lines indicate statistically significant associations between cognitive performance and log testosterone.

interaction between testosterone and age in any model (all P's > 0.23).

RESULTS

Age and cognitive performance

Age was significantly and negatively associated with every cognitive task (all $P < 0.045$), except digit forward task in Tsimane ($P = 0.056$), where age was negatively associated but did not achieve statistical significance (see Table 1).

Parasite and pathogen load and cognitive performance

Men in this sample had a mean hemoglobin of 14.20 (SD = 1.09) g/dl, a mean WBC count of 9,187 (SD = 2,255) per ul, and a mean eosinophil percentage of 20% (SD = 8.29). Binary variables denoting low hemoglobin (<13 g/dl, 11% of participants), high WBC count (>10,000 per ul, 29% of participants) were created using standard clinical cutoffs. In industrial populations eosinophils counts above 5% are considered elevated; in this sample only 1 participant fell within the normal industrial range, so a median split was employed to create a binary high eosinophil variable.

Testosterone and short- and long-term memory

Model A: Log testosterone was positively associated with performance on short- ($\beta = 0.267$, $P = 0.018$), and long-term recall ($\beta = 0.326$, $P = 0.005$) after controlling for age, schooling, and Spanish fluency (Table 1, Fig. 1). Model B: the addition of biomarkers of parasite and pathogen load somewhat attenuated the association between testosterone and short-term ($\beta = 0.182$, $P = 0.078$) and long-term memory ($\beta = 0.210$, $P = 0.090$), though with a larger sample it is likely that this would have reached statistical significance (Table 2).

Testosterone and working memory (digit forward)

Model A: Testosterone was positively though not significantly associated with performance on digit forward tasks (whether conducted in Tsimane or Spanish, or the tactile version). Model B: In this subset of men, testosterone was positively and significantly associated with the Spanish digit forward task (Table 2); the signs and significance did not differ from Model A for the Tsimane and tactile versions.

Testosterone and semantic memory (category fluency)

Testosterone was positively though not significantly associated with performance on a semantic memory task for either Model A or Model B.

Testosterone and spatial cognition (visual scan)

Log testosterone was positively associated with number of items correct on the visual scan for both Model A ($\beta = 0.306$,

TABLE 2. Regression models examining associations between testosterone (logged) and measures of cognitive performance among a subset of Tsimane men (n = 66) for whom markers of immune activation were also collected (controls include age, years of schooling, Spanish fluency, low hemoglobin (<13 g/dl), high white blood cell (WBC) count (>10,000 per ul), high eosinophil count (above median) and village ID

	Short-term recall	Long-term recall	Digit forward (Tsimane)	Digit forward (Spanish)	Digit forward (Tactile)	Category fluency	Visual scan correct
Log testosterone	0.182†	0.210†	0.075	0.271*	0.102	0.048	0.270**
Age	−0.023**	−0.036***	−0.023+	−0.030**	−0.027**	−0.022†	−0.043***
Years schooling	0.067**	0.037	0.033	−0.039	0.045	−0.036	0.086**
Spanish fluency	−0.352***	−0.196†	0.131	−0.021	0.188	−0.104	−0.042
Low hemoglobin	−0.151	−0.236	0.533	0.994**	−0.320	0.416	−0.572†
High WBC count	−0.424*	−0.315	−0.047	−0.145	0.173	−0.165	−0.018
High eosinophils	−0.244	−0.165	0.001	−0.153	−0.384†	−0.109	−0.375†

*≤0.05, **≤0.01, ***≤0.001, †≤0.1.

$P = 0.008$), as well as Model B ($\beta = 0.270$, $P = 0.029$). Testosterone was not associated with items incorrectly marked in the visual scan (all $P > 0.6$) for either model.

DISCUSSION

Similar to studies in industrialized populations, older Tsimane men exhibited poorer cognitive performance than younger men (e.g., Barrett-Connor et al., 1999; Finch, 2010). Previous studies indicate that cognitive senescence is influenced by age-related changes in inflammation, infection, and nutrition (Finch, 2010), factors that are known to change across the life course both in industrial populations as well as among the Tsimane (Gurven et al., 2008). Despite having testosterone approximately one-third lower than age-matched US males (Trumble et al., 2012), along with low levels of formal education, Tsimane men show positive associations between testosterone and short- and long-term memory that are similar in size to what is reported in the US (Barrett-Connor et al., 1999; Moffat et al., 2002). This suggests that androgens and aging are both associated with cognitive function, regardless of subsistence regime, environmental conditions, or formal education. Previous research has shown that high parasite and pathogen burdens both decrease cognitive performance (Boivin and Giordani, 1993; Eppig et al., 2010), and down-regulate testosterone production (Muehlenbein and Bribiescas, 2005); thus phenotypic correlation between testosterone and cognitive function is a potential alternative explanation. However, we find that testosterone remains positively associated with cognitive function after controlling for immune burden, suggesting potential independent impacts of testosterone on cognitive function. Many hormone–behavior interactions, especially those related to testosterone and competition appear invariant across populations (Trumble et al., 2012), and even species (Wingfield et al., 1990). While this study cannot directly examine causality, the role of testosterone in cognitive function could be a foundation for many key androgen–behavior interactions (Goetz et al., 2014; Schulz et al., 2009).

This preliminary study has several limitations. First, this is a relatively small cross-sectional study that examines associations between testosterone and several aspects of cognitive function. Without experimentally manipulating testosterone, or the use of brain scanning equipment (both of which were not feasible in field conditions), it is not possible to infer strong causality. The markers of parasite and pathogen burden were admittedly crude; longitudinal data on a larger sample of Tsimane men and women are currently being collected in order to examine age-related changes in cognitive performance with more detailed health information including serum hormones, inflammatory cytokines, fecal parasite counts and species, and medical diagnoses. While fMRI studies suggest several viable pathways by which testosterone could impact some aspects of cognitive function and not others, such as our findings that testosterone impacts working memory and spatial cognition tasks but not a semantic memory task, this preliminary study must remain agnostic to the mechanism of action.

While testosterone enhances neural potentiation related to threat and influences aggressive and competitive behaviors (Goetz et al., 2014), the cognitive impacts of testosterone could be beneficial for male productivity and reproductive success beyond direct male–male competition; enhanced spatial ability would be beneficial to subsistence tasks like hunting, as would short- and long-term recall (e.g., recalling locations of prey sightings or tracks). Previous research among the Tsimane indicated acute changes in testosterone during physically intensive production strategies, like hunting and small scale horticulture (Trumble et al., 2013, 2014). The complementary effects of testosterone on increased physical abilities and cognitive function would be reproductively beneficial for early modern humans as they transitioned toward difficult to acquire, high-quality food extraction techniques like pursuit hunting which require both skill and strength (Kaplan et al., 2000; Muehlenbein and Flinn, 2011). Men in better condition who are able to maintain higher testosterone levels would have had greater economic productivity which would help maintain their condition, and increase reproductive success. Indeed, looking beyond the genus *Homo*, testosterone plays important roles in cognitive performance for our closest living relatives *Pan troglodytes* (Wobber and Herrmann, in press). Chimpanzees show positive associations between testosterone and cognitive ability, while bonobos show no association (Wobber and Herrmann, in press), suggesting that proximate neuro-hormones like testosterone played a vital role in the differentiation of cognitive architectures, social structures, and behaviors of early hominids. Future observational and experimental studies exploring how differences in the social and ecological niches occupied by humans, chimpanzees, and bonobos impact brain architecture and differential

response to testosterone stimulation may help elucidate underlying mechanisms by which testosterone enhances some cognitive domains and not others.

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