Int J Primatol (2014) 35:787–804
DOI 10.1007/s10764-014-9749-5

Testosterone, Immune Function, and Life History Transitions in Filipino Males (*Homo sapiens*)

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Received: 18 June 2013 / Accepted: 21 October 2013 / Published online: 15 February 2014

Abstract Testosterone contributes to male life history trade-offs through effects on behavior and energy usage. Testosterone's role as a trade-off mediator is often discussed as manifesting partly through a negative impact on investment in survival, via immune suppression. Studies across species also show that testosterone in males commonly fluctuates with social changes, providing natural experiments to evaluate any potential immune impacts of intraindividual changes in testosterone. Using longitudinal data from Metropolitan Cebu City, the Philippines, we recently showed that men transitioning to fatherhood experienced substantial declines in testosterone over a 4.5-yr period. Drawing on a subsample of the same men here (N=330), we evaluate whether these socially mediated changes in testosterone are paralleled by changes in immune function as reflected in salivary secretory immunoglobulin A (SIgA), a localized marker of mucosal immunity. Men reporting more cold/flu symptoms had lower testosterone and a trend toward lower SIgA in cross-section. Intraindividual changes in testosterone between baseline and follow-up 4.5 yr later were strong, positive predictors of changes in SIgA. Men becoming new fathers did not differ in ΔSIgA compared to other men. The positive relationship between ΔSIgA and ΔT in this sample runs counter to the expectation of a mating–maintenance trade-off, and may reflect direct effects of androgens on SIgA production. Our results add to the dialogue

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on the complex relationships between the reproductive and immune axes, providing additional evidence that in humans testosterone is not uniformly immunosuppressive.

Keywords Immunity · Life history trade-offs · Polymeric immunoglobulin receptors · Reproductive physiology · Secretory immunoglobulin A

Introduction

In the vast majority of mammalian species (*ca.* 95–97 %), including most primates, male reproductive strategy equates solely to mating effort (Clutton-Brock 1991; Kleiman and Malcolm 1981). Testosterone is a common mediator of those mating investments, contributing to enhanced musculature, libido, conspecific aggressivity, and courtship (Archer 2006; Bribiescas 2001; Hart 1974; Hull and Rodriguez-Manzo 2009). To the extent that these testosterone-driven energetic and behavioral investments might reduce male longevity and survivability, such as through behavioral exposures that increase injury/morbidity, testosterone is also a primary facilitatory of the costs of male reproduction (Bribiescas 2006b; Bribiescas and Ellison 2008; Hau 2007; Muehlenbein and Bribiescas 2005).

In this vein, there is much interest in the possibility that testosterone is immunosuppressive, facilitating trade-offs between mating and survival. This idea has intuitive appeal, given that both mating and maintenance functions are competing for the same finite resource pool, and there is empirical evidence that testosterone directly downregulates the production or function of many immune defenses, although it is important to note that testosterone does not universally suppress all components of immunity. In both animal and human models, studies employing *in vitro* designs or administration of exogenous testosterone (*in vivo*) provide compelling data supporting testosterone's role in decreasing aspects of immunity (Muehlenbein and Bribiescas 2005; Zuk and Stoehr 2010). For example, testosterone appears to negatively impact certain components of the humoral immune response, as it has been shown to reduce immunoglobulin (antibody) production by human B-lymphocytes and to inhibit B-cell differentiation in response to antigen (both *in vitro*) (Kanda *et al.* 1996; Sthoeger *et al.* 1988). Animal models also indicate that testosterone can impair B-lymphocyte development in bone marrow, potentially reducing immune capacities (Olsen and Kovacs 2001; Sakiani *et al.* 2012). In multiple avian species, administration of exogenous testosterone has been shown to decrease *in vivo* antibody production, through various physiological pathways (Casto *et al.* 2001; Duffy *et al.* 2000; Evans *et al.* 2000), though findings do vary (Hasselquist *et al.* 1999; Saino *et al.* 2002). Several of these studies also indicate that experimentally elevated testosterone reduces male birds' abilities to mount effective cell-mediated immune responses to mitogen challenge (Casto *et al.* 2001; Duffy *et al.* 2000). Despite strong support from such experimental designs, data indicating that endogenous, i.e., not experimentally altered, testosterone suppresses immunity under natural conditions are less robust, with studies often reporting null results or, more rarely, positive correlations. Notably, there are few field studies of primates in this area (Muehlenbein and Bribiescas 2005).

The inconsistent naturalistic evidence for endogenous testosterone suppressing immunity might be due to substantial methodological challenges in documenting such

physiological trade-offs (McDade 2003; Muehlenbein and Bribiescas 2005;). As outlined by Prall and Muehlenbein (2013), repeated sampling is ideal for establishing reliable profiles of endocrine and immune status, and the simultaneous measurement of multiple, diverse immune markers provides a more rigorous profile of an individual's immunity. To date, few field studies of primates have operationalized these methodological considerations, perhaps because of logistical constraints, but they would substantially increase power to detect potential intraindividual testosterone-immune trade-offs. Importantly, factors that can differ widely between individuals, such as energetic status, diet, psychosocial stress, and age, affect both immunity and reproductive function (Bribiescas 2006a; Chandra 1997; McDade 2003; Miller 1996; Muehlenbein and Bribiescas 2010; Sapolsky *et al*. 2000). To the extent that these unmeasured characteristics influence both immunity and testosterone levels, large variation in these factors across subjects can obscure trade-offs (Hill and Hurtado 1996). For instance, if individuals vary substantially in the size of energy and resource budgets, those who are energy-replete will have more to invest in all physiological demands, and high testosterone will co-occur in the same individual with high immunity, leading to positive relationships across subjects (phenotypic correlation) (McDade 2003; Muehlenbein and Bribiescas 2005). This issue is common in observational research. It is at present not clear whether some null findings in studies of T-immunity trade-offs reflect this type of confounding by unmeasured third factors, rather than the actual direction of physiologic regulation.

One approach that reduces the likelihood of such confounding is to compare changes in the same individual through time using longitudinal data, which eliminates the influence of any unmeasured stable or permanent differences between individuals (Duncan *et al*. 1998). With respect to the hypothesized trade-off between testosterone and immunity, studies that track individuals whose testosterone is shifting owing to recent changes in social position provide a natural experiment of sorts, allowing assessment of whether the intrasubject change in testosterone predicts a corresponding intrasubject change in immunity. In theory, this could help identify trade-offs that might be otherwise obscured in cross-sectional comparisons (Duncan *et al*. 1998; Williams 2008). For example, in species with rigid hierarchies, higher ranking males frequently have elevated testosterone relative to subordinates, particularly during periods of social instability when males must compete for social standing (Bercovitch 1993; Higham *et al*. 2013; Holekamp and Smale 1998; Muehlenbein *et al*. 2004; Muller and Wrangham 2004; Setchell *et al*. 2008). Although longitudinal data from species with biparental care are notably sparse, there are also a few well-characterized examples of large changes in mammalian males' testosterone as they transition from mating to parenting (Bales *et al*. 2006; Gettler *et al*. 2011a; Nunes *et al*. 2001; Reburn and Wynne-Edwards 1999; Schradin and Yuen 2011; Ziegler *et al*. 2009), though not in all mammals with bi-parental care (Wynne-Edwards and Timonin 2007), including certain nonhuman primates (Ziegler and Snowdon 2000). Humans are one of the best studied mammalian species in the domain of paternal physiology, as fathers often have lower testosterone than childless men across diverse cultural settings, especially if they invest in offspring care (Alvergne *et al*. 2009; Gettler *et al*. 2011a, 2012a; Gray *et al*. 2006; Muller *et al*. 2009; Perini *et al*. 2012).

Using longitudinal data from the Philippines, we recently showed that men experienced substantial declines in testosterone during the transition to first-time fatherhood (Gettler *et al*. 2011a), particularly compared to men remaining single and childless over

the same time period. Here we build on this work to evaluate whether this socially mediated change in testosterone within the same individuals, whose baseline state serves as control, has parallel effects on a marker of immune function [salivary secretory immunoglobulin A (SIgA)].

SIgA acts as a first-line of defense against pathogens at mucosal surfaces, including the glandular secretions of the oral cavity, from which salivary SIgA can be assessed. Rather than serving as an overarching indicator of immune health, SIgA is a relatively localized marker of immunity that is specific to the tissue from which it is drawn (in our case, the oral cavity) (Brandtzaeg 2007). Hence, there is an expectation that salivary SIgA should confer protection against pathogenic agents that might enter the body via the mouth, and, indeed, a number of studies have shown that individuals with higher salivary SIgA (Gleeson *et al*. 1999; Jemmott and McClelland 1989; Klentrou *et al*. 2002; Neville *et al*. 2008; Volkmann and Weekes, 2006) or greater SIgA secretion rates (Fahlman and Engels 2005; Nakamura *et al*. 2006) are less prone toward upper respiratory tract infections. The interface between testosterone and SIgA is likely complex. Testosterone might reduce B-cell development (Olsen and Kovacs 2001), potentially affecting circulating serum IgA (the ultimate source of SIgA) (Kocar *et al*. 2000). However, animal models also indicate that androgens might upregulate the production of SIgA in some tissues, particularly the ocular glands and the reproductive tract (Kaetzel 2005). Data on the relationships between salivary SIgA and testosterone are limited. In the only prior study to date, to our knowledge, no correlation was observed between the two analytes among a pool of healthy U.S. subjects that included men and women (van Anders 2010).

Here we test for longitudinal relationships between testosterone and SIgA, including across a major life history transition (first-time fatherhood), among men enrolled in the Cebu Longitudinal Health and Nutrition Survey, a birth cohort study that began in 1983–1984 in the Metropolitan Cebu area of the Philippines. We draw from sociodemographic, behavioral, and salivary hormonal (testosterone) and immune (SIgA) data collected at two time points, 2005 (age: 21.5±0.3 yr) and 2009, in a subsample of men from this study. We first explore whether SIgA and testosterone are lower among men reporting greater symptoms of acute illness. In light of evidence from animal models suggesting that androgens can enhance the production of SIgA (Kaetzel 2005), we then test whether intraindividual changes in testosterone positively predict changes in salivary SIgA over the 4.5-yr follow-up period (N=281). These models control for relevant covariates that might have also shifted between surveys, including changes in adiposity, psychosocial stress, and sleep time. Because testosterone in the present sample has been shown to relate positively with cortisol (Gettler *et al*. 2011b), which is often immunosuppressive, we also include cortisol as a covariate in cross-sectional models from baseline. Finally, we draw on the subsample of men who were non-fathers at baseline (N=240) to evaluate whether a key life history transition, i.e., becoming a first-time father vs. remaining a non-father, predicts differential patterns of SIgA change. We hypothesize that new fathers will have greater declines in SIgA, given our past results showing large declines in testosterone among newly married new fathers, relative to single non-fathers (Gettler *et al*. 2011a), and that lifestyle adjustments, e.g., psychosocial stress, sleep, due to parenting demands might negatively impact immune function.

Materials and Methods

Study Population

We collected the present data in 2005 and 2009 as part of the Cebu Longitudinal Health and Nutrition Survey (CLHNS), a population-based cohort study of mothers and their infants born in 1983–1984 in the Metro Cebu region of the Philippines (Adair *et al*. 2011). Metro Cebu encompasses the urban center of Cebu City and other large adjacent cities as well as rural, mountainous areas. CLHNS researchers conducted the original 1983–1984 survey in 17 urban and 16 rural barangays (neighborhoods). As of 2009, 70 % of the participants in the present study resided in urban barangays. Project members collected sociodemographic, health, and behavioral data during in-home interviews administered in the local dialect (Adair *et al*. 2011). Here, we define men as "partnered" if they identified themselves as being legally married or cohabitating (Gettler *et al*. 2012b; Kuzawa *et al*. 2009) and as fathers if they reported having one or more biological children (Gettler *et al*. 2011a). As we have previously reported (Gettler *et al*. 2012a), having adopted or stepchildren is rare for Cebuano men in their 20s, and 99 % of men characterizing themselves as fathers had at least one biological child in the present analysis.

We conducted this research under conditions of informed consent with human subject clearance from the Institutional Review Boards of the University of North Carolina at Chapel Hill and Northwestern University.

Sample Selection The men analyzed here are those with salivary SIgA data and represent a subsample of the overall CLHNS participants. To construct the subject pool for SIgA analysis, we used Stata's "sample" command (Stata Corporation) to draw a random sample of 355 participants who had full data from both 2005 and 2009. Of these men, 333 had sufficient saliva from both time points for SIgA analysis. Compared to men (N=574) who were not included in the SIgA pool but had otherwise full data (2009), the random sample did not differ in terms of likelihood of being employed, partnered (married/cohabitating), or fathers. The generated sample also did not differ in education level, body composition (body mass index, triceps skinfold), AM testosterone, psychosocial stress, or incidence of chronic or acute illness (not shown). We eliminated men who had abnormal sleeping patterns, consistent with shift work, which may increase the likelihood of disrupted circadian rhythms for testosterone (Touitou *et al*. 1990), particularly if linked with disrupted sleep (Leproult and Van Cauter 2011), from the analysis (2005, N=20; 2009, N=23). We have previously shown that men with sleep–wake times consistent with shift work have lower testosterone compared to men keeping regular sleep–wake patterns (Gettler *et al*. 2013). We excluded five subjects because of SIgA values 6+ SD above the sample mean. The total sample size, summed across all analyses we present here, is 330 subjects, while 281 individuals have full data for our longitudinal models that examine change in SIgA and testosterone between baseline and follow-up. This discrepancy exists because some subjects in our sample pool have sufficient data to be included in either the 2005 or the 2009 cross-sectional analyses, but are lacking data or are subject to exclusion from the longitudinal analyses. Wherever possible, we have included all available data points, rather than limiting the entire analysis to those 281 subjects with the full repertoire of longitudinal data.

Salivary Collection and Biomarker Measurement

We used the same saliva collection procedures in 2005 and 2009. We provided each participant with two polypropylene tubes for saliva collection and instructed them to collect the first sample immediately before bed and the second sample immediately upon waking the following morning (AM) and to report the times of saliva collection. The present analysis focuses only on biomarker data assayed from AM samples. Mean AM sampling times were 6:29 AM±1:16 (SD) in 2005 and 6:51 AM±1:30 (SD) in 2009. An interviewer collected the saliva tubes on the second day by and stored at −35 °C until shipment on dry ice to Northwestern University, where they were stored at −80 °C.

Salivary Testosterone SIgA, and Cortisol Assessment We used enzyme immunoassay protocols developed for use with saliva samples (Salimetrics, State College, PA; testosterone Kit No. 1-2402; SIgA Kit No. 1-1602) to determine testosterone and SIgA concentrations at the Laboratory for Human Biology Research at Northwestern University. We ran testosterone analyses for 2005 and 2009 samples at different time points. Interassay coefficients of variation for testosterone were 13.7 % and 11.5 % for high (200 pg/ml) and low (20 pg/ml) kit-based controls, respectively, for 2005 analyses and 7.8 % and 17.9 % for high and low controls, respectively, for 2009 analyses. We ran SIgA analyses for 2005 and 2009 contemporaneously and only for AM samples. Interassay coefficients of variation for SIgA were 6.5 % and 3.7 % for high (236 μg/ml) and low (26 μg/ml) kit-based controls, respectively.

A separate laboratory (Trier, Germany) assayed the waking saliva samples for cortisol (2005) using a time-resolved immunoassay with fluorometric detection (DELFIA). The interassay coefficients of variation for high and low controls ranged from 7.1 % to 9.0 %. Follow-up (2009) cortisol was not measured.

Covariates

We quantified self-reported psychosocial stress in the month preceding sample collection via a modified version of the 10-item Perceived Stress Scale (PSS) (Cohen *et al.* 1983), assessed sleep duration via participants' self-reports of total sleep time, and measured triceps skinfold thicknesses (mm) using standard anthropometric techniques (Lohman *et al.* 1988). We calculated changes in PSS, sleep time, and triceps skinfolds by subtracting 2005 values from 2009 values.

At both baseline and follow-up, subjects reported whether they were suffering from chronic illness. In 2005, men specifically reported chronic illnesses and disabilities. In 2009, men indicated their chronic illness history by reporting ailments suffered since the prior CLHNS survey (2007). The relevant chronic maladies listed by the subjects included asthma/respiratory diseases, e.g. sinusitis; insomnia; toothache; ulcer; tonsillitis; anemia; cardiovascular ailment; kidney ailment; and diabetes. Few men reported chronic disease at either baseline (5 %) or follow-up (3 %). During the 2009 saliva collection, men also provided a count (up to 6) of cold/flu type symptoms, including suffering from cold, cough, loose bowel movement, fever, muscle pain, and fatigue. We also added men reporting headache or sinusitis to the count. The vast majority (94 %) of men reported zero, one, or two symptoms. The highest reported count was four

symptoms. We grouped men reporting three or four symptoms (6 % of subjects) with those reporting two.

Statistical Analysis

We conducted all analyses using version 12.1 of Stata (Stata Corporation). We treated AM testosterone (pg/ml), SIgA (μg/ml), AM cortisol (nmol/l), total sleep time, PSS, triceps skinfolds, and educational attainment (highest grade) as continuous variables and cold/flu symptom count (2009) as a three-level variable (0, 1, or 2+ symptoms). For analytical purposes, particularly the construction of change in SIgA (ΔSIgA) for longitudinal modeling, we ascribed men with undetectable SIgA values of 2.5 μg/ml, which is the lower limit of assay sensitivity, according to the assay manufacturer (Salimetrics LLC). There were 54 (2005) and seven (2009) subjects with undetectable SIgA at the two measurement points. We log-transformed values of SIgA used in cross-sectional analyses because of skewed distributions. We adjusted our statistical models for saliva sampling time. We evaluated statistical significance at $P<0.05$, with relationships between $P<0.05$ and $P<0.10$ interpreted as a statistical trend. In all the analyses that follow, we converted our continuous independent variables to *z*-scores before running models to allow comparability of coefficients.

We applied tobit regression to models predicting SIgA in cross-section. Given the censoring and non-normality of the SIgA distribution, application of ordinary least squares regression procedures would likely result in biased and unstable parameter estimates, whereas tobit regression takes into account the censored nature of the distribution to provide more reliable parameter estimates (Greene 2000). We separately predicted SIgA (2009; tobit regression) and AM testosterone (2009; linear regression) in cross-section from 2009 cold/flu symptom count ($N=305$). We then predicted 2009 SIgA from AM testosterone, cold/flu symptom count, chronic morbidity, and other relevant covariates, e.g., PSS, sleep time, skinfolds, and education level. We similarly analyzed cross-sectional relationships between 2005 SIgA and AM testosterone ($N=307$), including an additional model with 2005 AM cortisol as a covariate ($N=302$).

In observational research, such as the present study, correlated non-measured factors can influence both predictor and outcome variables and thus lead to confounded associations. Here we used an econometric change, multiple linear regression approach ($N=281$) to predict ΔSIgA (2009 SIgA minus 2005 SIgA) from ΔT (2009 T minus 2005 T). This approach minimizes the likelihood of confounding because any permanent or stable factors that differ between men but that have not changed in the follow-up period are included within the error term of the linear regression models and thus are eliminated as potential influences on any ΔSIgA and ΔT experienced during the period of follow-up. We also controlled for changes in PSS, triceps skinfolds, and sleep time as potential confounders between ΔSIgA and ΔT.

Finally, drawing on men who were non-fathers at baseline, we used multiple linear regression to test whether life history transitions predicted ΔSIgA. To do so, we first stratified men according to whether they transitioned to first-time fatherhood by follow-up ($N=98$) or remained single non-fathers ($N=142$) and used this dichotomous variable to predict ΔSIgA, while also controlling for PSS, triceps skinfolds, and sleep time.

Table I SIgA and testosterone longitudinal sample characteristics among male *Homo sapiens* in Metro Cebu, Philippines (N=281)

Sample characteristics	Mean	SD
Demographic characteristics		
Age (years)	25.9	0.3
Education (highest grade)	10.5	3.3
Currently employed (%)	68.3	–
Urban barangay (%)	70.1	–
Married/cohabitating (%)	50.9	–
Father (%)	47.7	–
Biomarker values		
SIgA 2005 (μg/ml)	303.6	381.4
SIgA 2009 (μg/ml)	302.5	299.3
AM testosterone 2005 (pg/ml)	194.1	77.3
AM testosterone 2009 (pg/ml)	157.9	59.2
Anthropometric characteristics		
Triceps skinfold thickness (mm)	15.2	7.5
Body mass index (kg/m^2)	22.7	3.5
Morbidity reports		
Chronic morbidity (% yes)	3.3	–
Cold/flu symptoms (% 2+ symptoms)	26.6	–

Values are from follow-up (2009) unless otherwise noted. SIgA=secretory immunoglobulin A

Results

Table I shows descriptive statistics of the study participants. Self-reported incidence of chronic morbidity at follow-up was low (3 %). Subjects experienced acute illness at comparably higher rates, with a substantial percentage of males (27 %) reporting two or more acute cold/flu-like symptoms at the follow-up interview, while 29 % reported zero symptoms.

Men reporting the greatest (2+) cold/flu symptoms (2009) showed a trend toward lower SIgA09 (log-transformed) compared to men reporting no symptoms [tobit regression: β (95 % CI)=–0.38 (–0.77, 0.00), P=0.051, Fig. 1a]. Men reporting one symptom did not significantly differ from those reporting 0 symptoms [β=–0.13 (–0.47, 0.22), P=0.474]. Men reporting one symptom [linear regression: β (95 % CI)=–16.38 (–32.29, –0.48), R^2=0.017, P=0.044] or 2+ symptoms [β=–18.33 (–36.21, –0.44), P=0.045] also had lower AM T09 than men reporting no symptoms (Fig. 1b).

We then predicted SIgA09 from AM T09 (z-scores), and found that men with elevated AM T09 had significantly higher SIgA09 [tobit regression: β=0.44 (0.31, 0.58), P<0.0001]. We controlled for potential confounders, which we hypothesized might contribute to a phenotypic correlation between SIgA and testosterone. Men with higher energetic status (triceps skinfolds, z-scores) had elevated SIgA09 [β=0.17 (0.03, 0.31), P=0.015]. Chronic [β=0.18 (–0.57, 0.93), P=0.640] and acute [β=–0.23 (–0.59, 0.14), P=0.222] morbidity, psychosocial stress [β=–0.05 (–0.18, 0.01), P=

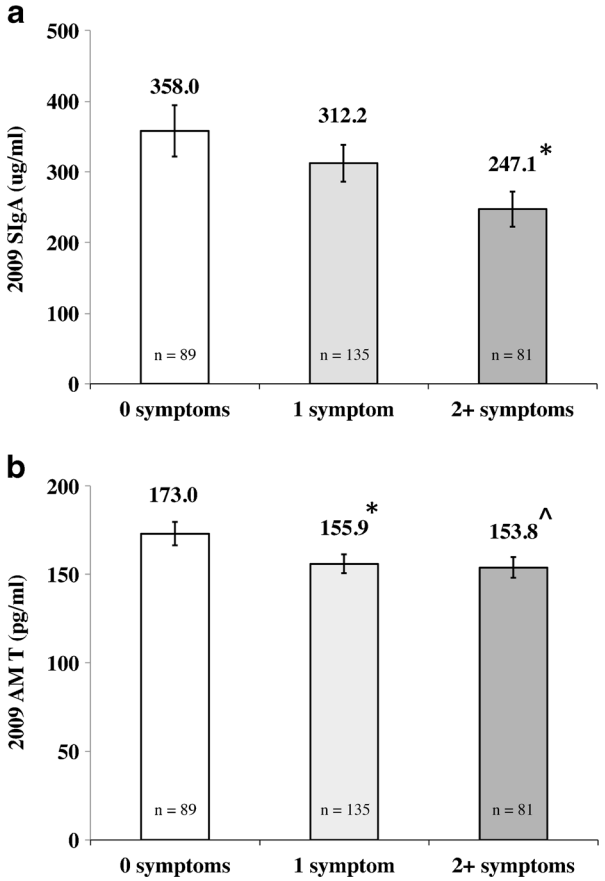

Fig. 1 **a** Follow-up (2009) salivary SIgA stratified according to acute cold/flu symptom count among male *Homo sapiens* in Metro Cebu, Philippines. Raw values are shown. Statistical comparisons reflect results from tobit regression with log-transformed SIgA (see Results for full model details). *P=0.051. *Error bars* indicate SEM. **b** Follow-up (2009) AM testosterone stratified according to acute cold/flu symptom count among male *Homo sapiens* in Metro Cebu, Philippines. Sample sizes in parentheses at column bases. *P=0.044, ^P= 0.045. *Error bars* indicate SEM.

0.477], total sleep [β=–0.12 (–0.27, 0.04), P=0.139], and education level [β=–0.03 (–0.08, 0.09), P=0.153] were nonsignificant predictors of SIgA09. The effect size for testosterone predicting SIgA was virtually unchanged with the addition of these controls [β=0.44 (0.31, 0.58), P<0.0001]. Removing men who were potentially acutely ill (reporting 2+ cold/flu symptoms) from the model also did not affect the results [β=0.45 (0.30, 0.60), P<0.0001; N=224].

Cross-sectional results for 2005 SIgA and 2005 AM testosterone were similar [β= 0.84 (0.58, 1.10), P<0.0001]. We added 2005 AM CORT to the model to test whether the SIgA05-AM T05 relationship would be strengthened. CORT did not predict SIgA05 [β=-0.07 (–0.34, 0.21), P=0.640] and did not substantially change the relationship between AM T05 and SIgA05 [β=0.86 (0.60, 1.13), P<0.0001].

In a preliminary change model, ΔAM testosterone positively predicted ΔSIgA (Table II). Men whose AM testosterone declined more between baseline and follow-up experienced concomitant greater decreases in SIgA (Fig. 2). To assess the potential

Table II Multiple linear regression models predicting ΔSIgA from ΔAM testosterone among male *Homo sapiens* in Metro Cebu, Philippines (N=281)

	Model 1		*P* value	Model 2		*P* value
ΔAM testosterone	178.19	(130.56, 225.82)	0.0001	179.29	(131.40, 227.18)	0.0001
ΔTriceps skinfolds				18.84	(–28.78, 66.46)	0.437
ΔPsychosocial stress				–5.56	(–53.08, 41.97)	0.818
ΔSleep time				–13.30	(–64.52, 37.92)	0.610
Model R^2		0.165			0.168	

Values are β (95 % CI) of ΔSIgA. Models adjusted for sampling times. All predictor variables are *z*-scores. SIgA=secretory immunoglobulin A

for confounding, we added change in triceps skinfolds, psychosocial stress, and total sleep time to the model. These variables were not significant predictors of ΔSIgA (Table II). The addition of these confounding variables did not change the effect size and significance for ΔAM testosterone predicting ΔSIgA (Table II). Removing men who were potentially acutely ill at follow-up (reporting 2+ cold/flu symptoms in 2009) from the model increased the effect size [β=199.43 (144.74, 254.12), R^2=0.205, P<0.0001; N=208].

In these longitudinal analyses a total of 54 men had undetectable SIgA at baseline or follow-up. To ensure that these individuals were not biasing the relationship between ΔSIgA and ΔAM testosterone, we reran the analysis restricted only to men with detectable SIgA at both time points (N=227). The effect size of ΔAM testosterone predicting ΔSIgA was only modestly smaller and remained highly significant [linear regression: β=171.18 (117.56, 224.81), R^2=0.154, P<0.0001].

Lastly, analyzing the subsample of men who were non-fathers at baseline (N=240), we tested whether the transition to parenthood predicted ΔSIgA. Consistent with results in the broader sample, ΔAM testosterone significantly positively predicted ΔSIgA [linear regression: β=195.10 (144.04, 246.18), R^2=0.194, P<0.0001] across this subsample (N=240). Men transitioning from being non-fathers at baseline to first-time fathers at follow-up (N=98) experienced a significantly larger decline in testosterone over the study period compared to men who remained non-fathers [N=142; linear regression: β (95 % CI)=–25.25 (–48.75, –1.75), R^2=0.034, P=0.035]. However, counter to our predictions, we did not find that new fathers had more substantial declines in SIgA than men remaining non-fathers [β=30.68 (–86.43, 147.78), R^2=0.002, P=0.606], despite the former having a comparatively larger decrease in AM testosterone. We included key covariates that potentially change during the parenthood transition and might also affect immunity, i.e., shifts in adiposity [β=10.44 (–47.25, 68.13), P=0.722], psychosocial stress [β=–3.47 (–61.56, 54.63), P=0.907], and sleep time [β=–8.74 (–69.73, 52.26), P=0.778]. With these covariates included, new fathers and non-fathers still showed comparable changes in SIgA [β=30.24 (–88.67, 149.14), R^2=0.003, P=0.617].

Discussion

Here we found that intraindividual changes in men's salivary SIgA and change in salivary testosterone were positively related over a 4.5-yr follow-up window, meaning

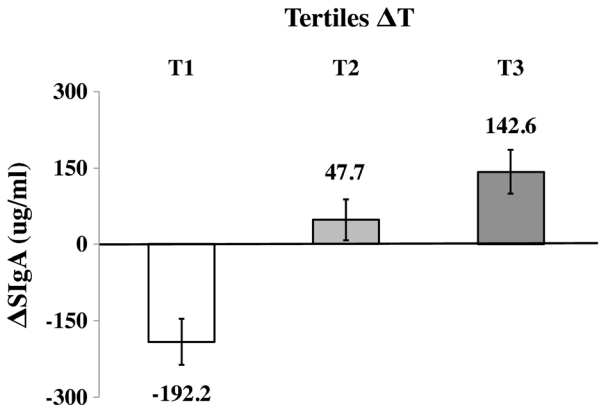

Fig. 2 Change in salivary SIgA (ΔSIgA) between baseline (2005) and follow-up (2009) stratified according to tertiles of change in AM testosterone (ΔT) over the same period, among male *Homo sapiens* in Metro Cebu, Philippines (N=281). We present tertiles of ΔT for visual purposes. We treated ΔT as a continuous variable in statistical analyses (see Table II for full model details). Lowest tertile of ΔT, range: –326.21 to –64.1 pg/ml. Middle tertile of ΔT: –64.1 to –2.7 pg/ml. Highest tertile of ΔT: –1.2 to 267.9 pg/ml. *Error bars* indicate SEM.

that men who experienced increases in testosterone over the study period also showed increases in SIgA, on average. This relationship did not change with the addition of key covariates that we hypothesized might contribute to positive SIgA-T correlations, such as changes in psychosocial stress, sleep time, and energetic status. A significant contribution of our study is the application of intraindividual, longitudinal change models, which eliminate the influence of unmeasured, time invariant characteristics that differ across subjects and that could relate to both SIgA and testosterone. In total our results add to the growing recognition that testosterone-immune system interactions are not ubiquitously antagonistic (Muehlenbein and Bribiescas 2005) and highlight the complexity of physiological systems and mechanisms that are germane to life history trade-offs, such as reproduction vs. maintenance.

In addition to longitudinal, positive relationships between SIgA and testosterone we found that men reporting multiple cold/flu symptoms tended to have lower SIgA in cross-section compared to men with no symptoms. Prior studies have shown that low salivary SIgA precedes upper respiratory tract infection and that total salivary SIgA is not necessarily elevated during active infection (Gleeson *et al.* 1999; Jemmott and McClelland 1989; Klentrou *et al.* 2002; Neville *et al.* 2008; Volkmann and Weekes 2006). This suggests that men with low SIgA might be more prone toward such acute infections in Cebu (*cf.* later). Men with greater cold/flu symptomology also had lower testosterone, which is potentially consistent with previous research suggesting that active infection suppresses testosterone (Muehlenbein 2006; Muehlenbein *et al.* 2005, 2010; Simmons and Roney 2009). However, we must be cautious in interpreting these findings for a variety of reasons. First, the results have relatively small effect sizes and wide point estimates in addition to being correlational in nature, raising questions for future research regarding their practical importance. Second, self-reported symptomology may provide only a modestly reliable indicator of biological illness (Cohen *et al.* 1995) and does not assess infection stage (Muehlenbein *et al.* 2005), with both factors being key to modeling testosterone and SIgA reliably in infected vs. noninfected individuals. Lastly, although there is compelling evidence that testosterone declines during acute infection and that low salivary SIgA is a risk factor for upper

respiratory tract infection, it is less clear that salivary SIgA should remain low in actively infected subjects (Jemmott and McClelland 1989), and some studies have found the contrary (Isaacs *et al*. 1984). Thus, the fact that men with the highest symptom count had both low SIgA and low testosterone might be more indicative of a phenotypic correlation (rather than active infection), with such individuals being less physically robust. Given the limitations of our study design and these cross-sectional data, we cannot disentangle these possibilities in the present study.

Here we showed that men experienced strongly linked concomitant increases in salivary SIgA and testosterone over a 4.5-yr period, which persisted after we controlled for relevant confounders. This is the first demonstration of a positive relationship between testosterone and salivary SIgA in humans, to our knowledge, as the only prior study in this area found no relationship between SIgA and testosterone in a sample of U.S. men and women (van Anders 2010). The reasons for these discrepancies are unclear. As we discuss in the preceding text, our study does have the advantage of longitudinal data, allowing us to examine within-individual changes in SIgA and testosterone through time, whereas the aforementioned study was cross-sectional (van Anders 2010). Lack of concordance between the studies could also be explained by appreciating the inter- and intragroup differences across the two sample populations, which vary greatly in terms of their local ecological experiences. Differences in developmental energetic and pathogenic exposures, in particular, are thought to contribute to substantive variability between populations from affluent-industrialized and developing contexts in both immune and reproductive function (Bribiescas 2001; Kuzawa *et al.* 2010; McDade 2003; Muehlenbein and Bribiescas 2010). It seems plausible that such experiences might likewise impact the direct interaction between immunological and endocrine factors. In addition, we think it is likely that our sample, which is drawn from a birth cohort that was representative of the Metro Cebu population, comprises substantially greater intrasample variation in terms of socioeconomic status and living conditions, compared to the U.S.-based subject pool described in van Anders (2010). Paired with broad intersample ecological differences, this intrasample heterogeneity might increase the likelihood of detecting relationships between testosterone and SIgA. These issues, particularly considerations of developmental contexts, merit attention as research studies expand in this area.

Testosterone can affect the immune system through multiple pathways, including facilitating behavioral and somatic investments that reduce energy availability for immunity and directly affecting immune factor production/functionality by binding to androgen receptors expressed by immune tissues and circulating molecules/cells (Muehlenbein and Bribiescas 2005; Zuk and Stoehr 2010). Consistent with the latter, our results align with animal models showing that androgens can increase the output of SIgA and/or its transport protein [polymeric immunoglobulin receptor (PIGR)] at multiple mucosal surfaces in the body, including the reproductive tract and the lacrimal glands in the eye (Kaetzel 2005; Parr *et al*. 1992; Sullivan and Hann 1989; Sullivan *et al*. 1988). The contribution of androgens to enhanced SIgA occurs via a cellular transport mechanism. Epithelial cells produce PIGRs to transport IgA from serum to the mucosal surface (Brandtzaeg 2009). These epithelial cells express androgen receptors (ARs) and the genes encoding PIGRs have an androgen response element. Consequently, when testosterone binds to an AR, the latter can translocate to the nucleus and increase the production of PIGR. Because one PIGR is necessary for the emergence of

each SIgA molecule at the mucosal surface, this provides a plausible, well-described pathway through which SIgA and testosterone could be positively linked (Kaetzel 2005). However, the expression of this androgen–PIGR–SIgA pathway seems to vary by tissue, prominently influencing SIgA levels at some mucosal surfaces, e.g., the eye, while not being active at others (Lin *et al*. 1996; Sullivan *et al*. 1988). Human oral gland cells likely express androgen receptors (Laine *et al*. 1993; Ojanotko-Harri *et al*. 1992), and limited animal data suggest that the androgen–PIGR pathway might also operate in the oral cavity (Sullivan *et al*. 1988). More studies are needed, particularly in humans, before this can be definitively concluded. In light of the sparse data in this domain, we cannot rule out the possibility that a third, unmeasured variable changed between baseline and follow-up, interrelated with changes to both SIgA and testosterone, and thus contributed to the observed correlation between the two biomarkers. The two explanations (direct physiological regulation, phenotypic correlation) are not necessarily mutually exclusive.

In past research from this sample, we showed that men transitioning from being single and childless to being partnered fathers had substantial longitudinal decreases in testosterone, particularly compared to subjects remaining single non-fathers over that same time period (Gettler *et al*. 2011a). As expected, we found a similar pattern in the present analysis, as men who became first-time fathers had a greater decline in testosterone compared to men remaining childless. Given these results and the likelihood that new fatherhood might come with physiological costs in Cebu, where fathers are regularly involved with childcare, cosleep with their children, and commonly place value on their paternal roles as providers (Gettler *et al*. 2011a, 2012a; Medina 2001), we hypothesized that new fatherhood might cause a reduction in SIgA. However, this prediction was not borne out, as new fathers and childless men did not differ for changes in SIgA. The inconsistency in these patterns, i.e., new fathers showing larger declines in testosterone but similar changes in SIgA compared to non-fathers, is preliminarily suggestive that some correlate of parenthood might contribute to fathers maintaining their SIgA production in spite of their reduced testosterone. Because there has been little research, to date, exploring the importance of fatherhood status as a predictor of immune function, we cannot speculate so to what pathways might be relevant to this hypothesis. However, broadly consistent with the possibility that parenthood experiences might affect immunity, recent evidence from the United States suggests that parents are less susceptible to manifestations of the common cold when experimentally administered cold virus (Sneed *et al*. 2012). Here we analyzed an important but highly specific and localized marker of immunity; to understand "paternal immunology" better, future studies must include more rigorous, wide encompassing panels of immune and endocrine function.

This study has multiple limitations that merit discussion. First, we collected single measurements of SIgA and testosterone at waking, with our baseline and follow-up surveys separated by 4.5 yr. Sampling across several days and using the average in analyses, rather than analyzing estimates from single samples, would have enhanced the reliability of our biomarker data as representative of men's baseline levels (Dabbs 1990). However, our single measurements of saliva do not introduce bias. Rather, this type of measurement error may increase the likelihood of type II error due to reduced statistical power when evaluating relationships between the biomarkers as well as with other variables. Second, salivary SIgA is influenced by saliva flow rate (Brandtzaeg

2007). Because our saliva sampling procedures were not originally designed for SIgA, we do not have this measure. However, salivary testosterone does not vary by flow rate (Vining *et al.* 1983); thus the influence of flow rate on SIgA should not contribute to the observed positive associations between SIgA and testosterone. Similar to the preceding reasoning, this limitation likely adds to measurement error, potentially reducing statistical power, but does not introduce bias, to our knowledge. These collective methodological drawbacks likely contributed to imprecision of our point estimates in some analyses, e.g., the relationship between SIgA and acute symptoms of infection, and wide confidence intervals thereof. Some studies that assess both absolute levels of SIgA as well as SIgA secretion rate have found that decreased secretion rate is the stronger predictor of acute infection risk (Fahlman and Engels 2005; Nakamura *et al.* 2006). It is plausible that the relationship we observed here between acute cold/flu symptoms would be strengthened with repeated measures of SIgA and its secretion rate. These are important methodological considerations for future studies in this domain (Brandtzaeg 2007).

In addition, we have very little data on oral health, which relates to salivary SIgA from a functional perspective, but could also contribute to positive correlations between SIgA and testosterone via blood leakage into saliva (in those with poorer oral hygiene), as both analytes exist at much higher levels in circulation than in saliva. However, these concerns would be more likely to manifest in cross-sectional analyses, in comparisons of analytes across subjects. Our use of a longitudinal design reduces the likelihood of blood leakage impacting our results. Any stable, time invariant intrasubject differences in oral health would not impact intrasubject change in SIgA or testosterone through time, but rather correlated change in the analytes would have to be due to a concomitant change in oral health (and thus blood leakage) from baseline to follow-up. In addition, we visually inspected our salivary samples for blood or other biological matter presence before analysis, which has been shown to be a modestly effective method at eliminating blood-contaminated samples (Kivlighan *et al.* 2004).

Finally, it should also be noted that although we control for change in total sleep time, our measures in this domain are somewhat limited. Although some studies link self-reported sleep to poor long-term health outcomes, it has comparatively less validity as a marker of sleep quality, relative to objective measures such as actigraphy (Lauderdale *et al.* 2008). Given evidence that men with disrupted sleep show lower testosterone production (Cote *et al.* 2012; Leproult and Van Cauter 2011) and impaired immune function (Bryant *et al.* 2004), future studies should aim to evaluate rigorously the impacts of sleep on relationships between immune and reproductive function.

In summary, we showed that men reporting greater numbers of cold/flu symptoms had reduced testosterone and a trend toward lower salivary SIgA in cross-section, compared to men with no symptoms. Although these findings are preliminary, they are consistent with the idea that low salivary SIgA might be a risk factor for acute infection and/or that active infection might reduce testosterone production in this sample. Alternatively, the two findings might also indicate phenotypic correlation. We also found a robust longitudinal, intraindividual relationship between men's change in salivary SIgA and change in salivary testosterone, such that men who experienced increases in testosterone over our 4.5-yr follow-up period also showed increases in SIgA, on average. This pattern is consistent with animal models suggesting that androgens can directly upregulate SIgA production. Our results run counter to the

expectation of a mating–maintenance trade-off and add to the growing dialogue on the complex relationships between the hypothalamic–pituitary–gonadal axis and the immune system. To allow for proper modeling of acute life history trade-offs between reproduction and maintenance as well as the potential selective pressures shaping them through evolutionary time, we must continue to expand our understanding of the physiological mechanisms underlying interactions between these systems.

Acknowledgments This work was supported by Wenner Gren Foundation (Gr. 7356; Gr. 8186) and the National Science Foundation (BCS-0542182; BCS-0962212). We thank Drs. Melissa Emery Thompson and Alexander Georgiev for organizing the conference symposium on which this special issue is based, inviting us to participate, and serving as the guest editors for our article. We also extend our gratitude to multiple anonymous reviewers whose extensive comments improved this contribution. Jim McKenna and Agustin Fuentes provided helpful conversation and remarks on the manuscript. Lauren Schmitt contributed to background research. Jeffrey Huang and Aaron Miller assisted with laboratory work, including assay procedures. We thank Linda Adair, and the many researchers at the Office of Population Studies, University of San Carlos, Metro Cebu, Philippines, for their role in study design and data collection, and the Filipino participants, who generously provided their time for this study.

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