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Testosterone is positively and estradiol negatively associated with mucosal immunity in Amazonian adolescents

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Abstract

Objectives: A core assumption of life history theory and the immunocompetence handicap hypothesis (ICHH) is that testosterone (T) upregulates energetic investment in mating effort at the expense of immunity. This tenet, along with observed positive relationships between estrogens and immunity, may contribute to the higher observed morbidity and mortality of males. In the present study, we examine the association between sex steroid hormones and mucosal immunity as well as sex differences in immunity in a rural Amazonian population of immune-challenged Bolivian adolescents.

Methods: Salivary steroid hormones (T [males only] and estradiol [E_2, females only]), Tsimane-specific age-standardized BMI z-scores, and salivary mucosal immunity (sIgA, secretory IgA) were measured in 89 adolescent males and females.

Results: Males had significantly higher sIgA levels than females, which may be due to the observed immune-endocrine associations found in the present study. Controlling for age and phenotypic condition, higher T significantly predicted higher sIgA; whereas higher E_2 was associated with lower sIgA in females.

Conclusions: Results stood in contrast to common interpretations of the ICHH, that is, that T should be inversely associated with immunity. Findings from the present study support the notion that the endocrine system likely affects immunity in a regulatory fashion, upregulating certain aspects of immunity while downregulating others. An important remaining question is the adaptive reason(s) for sex differences in endocrine-mediated immuno-redistribution.

1 | INTRODUCTION

From a life history perspective, finite energy availability forces organisms to trade-off between investments in reproduction, survival (e.g., immunity), and growth across different phases of the life course (Kaplan, Hill, Lancaster, & Hurtado, 2000; Stearns, 1992). The trade-off between immune functioning and reproductive effort in particular represents a strong source of energetic conflict for both males and females (Abrams & Miller, 2011; Lassek & Gaulin, 2009; Lochmiller & Deerenberg, 2000; Muehlenbein & Bribiescas, 2005; Sheldon & Verhulst, 1996). For males, these compromises may contribute to higher mortality and morbidity when selection biases energy toward mating effort at the expense of longevity (Klein, 2004; Nunn, Lindenfors, Pursall, & Rolff, 2009). For females, energy balance and stress play key roles in mediating resource allocation to immune investment and reproduction (Abrams & Miller, 2011).

The endocrine system plays an important role in orchestrating the distribution of energy to these different demands (Bribiescas, 2001; Da Silva, 1999; Ellison, 2001; Folstad & Karter, 1992; McDade, 2003; Muehlenbein & Bribiescas, 2005;

Roberts, Buchanan, & Evans, 2004; Schuurs & Verheul, 1990). Testosterone (T), in particular, strongly upregulates energetic investment in mating and reproductive effort among males, partially by shunting energy away from immunity (Muehlenbein & Bribiescas, 2005). Researchers have relied heavily on Folstad and Karter's (1992) immunocompetence handicap hypothesis (ICHH) to frame mating-immunity trade-offs in males. According to the ICHH, masculinizing androgens shunt energy away from (ie, suppress) the immune system, therefore only males with superior genetic quality can afford high levels of these hormones. Thus, phenotypic manifestations of high T (i.e., increased expression of secondary sexual characteristics) may reveal underlying genetic quality to potential mates and competitors. Due to the influence of ICHH in spurring research, T has featured prominently in studies on sexual selection and the origins of sexual dimorphism (Folstad & Karter, 1992; Muehlenbein & Bribiescas, 2005).

Predictions derived from the ICHH and life history theory typically forecast an inverse association between mating effort and immunity (controlling for overall energy budget; Folstad & Karter, 1992; Roberts et al., 2004; cf. Braude, 1995). Given the diverse and complex nature of both mating effort and the immune system, it is not surprising that these predictions are not always supported. Experimental manipulation of androgens in animal models indicate elevated levels of T downregulate both humoral and cell-mediated immune function (Casto, Nolan Jr, & Ketterson, 2001; Duffy, Bentley, Drazen, & Ball, 2000). Males with higher T show reduced antibody response to vaccines (Furman et al., 2014) and administration of vaccine diminishes T levels 2 weeks later (Simmons & Roney, 2009). However, results are inconsistent (Saino, Incagli, Martinelli, & Moller, 2002). For example, in a study of in vitro stimulation of blood from pathogen stressed humans, samples with higher T showed lower responsiveness to a T-cell mitogen, but not to a B-cell mitogen (Trumble et al., 2016). In humans, T therapy among those with Klinefelter's syndrome (Koçar et al., 2000) is negatively associated with T; however, for those with systemic lupus erythematosus and rheumatoid arthritis (Holroyd & Edwards, 2009; Kanda, Tsuchida, & Tamaki, 1997; Schmidt et al., 2005) certain aspects of immunity are positively affected by administration of exogenous T. Finally, compared with in vitro and in vivo designs, research on naturalistic relationships between endogenous male T and immune markers also shows inconsistent findings (Granger, Booth, & Johnson, 2000; Roubenoff et al., 2002; van Anders, 2010), prompting many to characterize T as immune-modulatory rather than suppressive (Da Silva, 1999; McDade, 2003; Muehlenbein & Bribiescas, 2005). However, a clear theory of which aspects of immunity would be adaptively upregulated and downregulated in the context of male reproductive strategies has not yet been offered, but would be a desirable goal.

Among females, research suggests that estrogens also influence investment in reproductive effort in response to energy availability (Ellison, 2001), and therefore must interface with the immune system (Kahn & Ahmed, 2016; Kovats, 2015), which is also energetically costly (McDade, 2005). Rather than shifting energy away from immunity in favor of reproduction (as described for T), estrogens are usually characterized as immune-boosting (Abrams & Miller, 2011). These hormones positively affect many aspects of the immune system, primarily via differential expression of estrogen receptors on immune cells and estrogen-mediated immune-signaling pathways (Kovats, 2015). The elevation of females' immune response can include increases in type 1 interferon activity, T- and B-cell numbers, and antibody responses (Bouman, Heineman, & Faas, 2005). In terms of innate immune response, evidence shows females have a greater Th2 cytokine response following viral and parasitic infections as well as greater upregulation of the expression of antiviral and proinflammatory genes in T cells compared to men (Klein, Marriott, & Fish, 2015). As a result, females in general are characterized as having more robust immune systems than males, which, along with the inhibitory action of T, contributes to sex differences in morbidity and mortality (Klein, 2004; Nunn et al., 2009; Read, Troendle, & Klebanoff, 1997). As a consequent trade-off, females are more susceptible to overactive or dysregulated immune systems, resulting in increased rates of autoimmune disease (Kahn & Ahmed, 2016; Whitacre et al., 1999).

1.1 | The present research

The goal of the present study is to examine associations between reproductive effort [via T in males and estradiol, an estrogen steroid hormone (E2), in females] and immunity in terms of predictions derived from life history theory and the ICHH. We target an understudied branch of the immune system—mucosal immunity, as measured by secretory IgA (sIgA), a dimeric form of IgA containing an anti-proteolytic secretory component. Pathogens often achieve entry to the body via mucosal surfaces and sIgA is the most abundant immunoglobulin in the secretions covering these surfaces (including tears, saliva, colostrum, as well as the surfaces of the genitourinary, gastrointestinal, and respiratory tracts; Brandtzaeg, 2007). As the initial line of defense, sIgA binds to invading pathogens to prevent entry to the epithelium, helps maintain homeostasis using humoral and cellular responses, responds to pathogens through noninflammatory mechanisms, and prevents developing mucosal immunity systems from reacting disproportionately to new pathogens (Brandtzaeg, 2009; Corthésy, 2007; Corthésy, 2013; Corthësy, 2009; Mantis, Rol, & Corthésy, 2011). The amount of secretory IgA in saliva depends on several factors, including the presence of local plasma cells and secretory component, and not

simply on the amount of IgA in serum (Brandtzaeg, 2009). In addition, secretory IgA may be a poor activator of the complement immune system (Mestecky, 1993). Thus, it may be necessary to consider mucosal immunity separately from other aspects of immune function.

Due to its immune-response role in the upper respiratory pathway, sIgA may functionally participate in preventing upper respiratory tract infections (URTI). Previous research, especially on athlete populations, has found that lower concentration of sIgA can lead to an increased risk of URTI (Fahlman & Engels, 2005; Gleeson et al., 1999; Gleeson et al., 2002; Gleeson, Bishop, Oliveira, McCauley, & Tauler, 2011; Neville, Gleeson, & Folland, 2008). A year-long longitudinal study of American college football players found a drop in sIgA and its secretion rate under intense conditioning, which led to an increase in URTI incidence (Fahlman & Engels, 2005; see also Moreira et al., 2014). Men with lower sIgA levels also report greater cold/flu symptoms (Gettler, McDade, Agustin, Feranil, & Kuzawa, 2014). Lower sIgA levels may be the reason why children frequently contract URTIs, as healthy children have lower nasal secretion of sIgA compared to healthy adults (Bellussi, Cambi, & Passali, 2013). A study using prepubertal and late-pubertal girls found that, although the rate of URTI cases was the same across the two groups, sIgA levels were higher in the late-pubertal group (Corbett et al., 2010).

Previous research suggests that sIgA may be associated with steroid hormone levels, although evidence is somewhat conflicting. Our own previous research showed a positive association between dehydroepiandrosterone sulfate (DHEA-S), a precursor of both T and E_2, and sIgA (Hodges-Simeon, Prall, Blackwell, Gurven, & Gaulin, 2017). In several studies comparing sIgA before and after exercise, it was found that T increased along with sIgA levels (Fornieles et al., 2014; Peñailillo, Maya, Niño, Torres, & Zbinden-Foncea, 2015). One compelling longitudinal study showed that sIgA levels decreased among men concomitant with the decline in T associated with changes in fatherhood status (Gettler et al., 2014). However, a study on exogenous T administration observed associated negative changes in sIgA (Koçar et al., 2000). Thus far, two additional studies have examined the association between endogenous T and sIgA in men; one showed a positive correlation (Arnocky, Hodges-Simeon, Ouellette, & Albert, 2018), and the other found no association (van Anders, 2010). The latter study, however, did not describe a correction for secretion rate, which is a necessary control (Kugler, Hess, & Haake, 1992).

van Anders (2010) also described a positive relationship between E_2 and sIgA (although note our methodological criticism above). Investigators studying the effect of E_2 and progesterone on sIgA levels along the menstrual cycle found that women had higher levels of sIgA than men and that the pattern of sIgA in women followed E_2 levels across the menstrual cycle, indicating a possible relationship between E_2 and sIgA in the parotid gland (Gómez et al., 1993). In contrast, in experimental studies on female rats, administration of E_2 decreased levels of cervicovaginal IgA (Wira & Sullivan, 1985).

Sex differences in sIgA seem to mirror this general pattern, although, again, results are somewhat inconsistent. Although females are generally characterized as having stronger immune systems than males (Kahn & Ahmed, 2016), several previous studies have shown that women have lower sIgA concentrations compared to men, in athletic populations as well as the general public (Allgrove, Geneen, Latif, & Gleeson, 2009; Eliasson, Birkhed, Österberg, & Carlén, 2006; Evans et al., 2000; Phillips, Carroll, Drayson, & Der, 2015). Further, women have an increased risk of respiratory tract infections (Falagas, Mourtzoukou, & Vardakas, 2007), which might be related to women having lower sIgA levels than men. However, a few studies have shown the opposite to be true or that there is no difference in concentration (Gomez et al, 1993; Birkett, Johnson, & Gelety, 2017; Rutherfurd-Markwick, Starck, Dulson, & Ali, 2017).

In addition to targeting mucosal immunity, we implement three additional improvements to previous studies of relationships between endogenous sex steroids and immunity. First, we target adolescence, an important phase when individuals transition to adult levels of reproductive effort, with associated upregulation in the production of sex steroid hormones. Most prior research focuses on adults; however, different life history trade-offs occur across the life course (Ellison, 2001). Adolescents face large energetic demands from high-velocity growth and a developing immune system (McDade, 2003). By targeting a period of dramatic endocrine change for the present research, we may highlight concomitant changes in immunity. Second, we sample a population with anthropological and evolutionary relevance. The Tsimane offer an opportunity to assess hormone-immune relationships among individuals with large energy and pathogen burdens, where trade-offs are likely to be more acute, and hence, easier to identify. Few studies have examined trade-offs between immunity and steroid hormones in energetically stressed populations. Several studies have reported lower adult T in such groups (Bribiescas, 1996; Campbell, Gillett-Netting, & Meloy, 2004; Christiansen, 1991; Ellison, Lipson, & Meredith, 1989; Trumble et al., 2012), as well as higher immune biomarkers (e.g., IgE, CRP, VSG; Blackwell, Snodgrass, Madimenos, & Sugiyama, 2010; Blackwell et al., 2016; Vasunilashorn et al., 2010). Our research may help to clarify the developmental origins of such population differences. Finally, we attempt to control for potential "phenotypic correlation" (i.e., a positive correlation between life history traits) that results from interindividual variation in energy budgets. For instance, individuals with a high energy budget (either due to access to greater calories or lower energy expenditure) can invest more in *both* reproduction and survival

than an individual with a low energy budget, obscuring the expected trade-offs between them. Because of the inconsistent previous findings concerning the relationship between sIgA and sex steroid hormones, our a priori predictions derive from the classic interpretations of the ICHH. All the following control for age and adjust for differences in energy budget (a proxy of phenotypic correlation):

1. Males will show compromised immunity (i.e., lower sIgA levels than females).
2. Among males, T will be inversely associated with mucosal immunity.
3. Among females, E_2 will be positively associated with mucosal immunity.

2 | METHOD

2.1 | Population

The Tsimane are Amazonian forager-horticulturalists residing in central Bolivia (Gurven, Kaplan, & Supa, 2007). They live in a high-pathogen tropical environment and manifest high rates of infection, gastrointestinal and respiratory disease, and anemia (Gurven, Kaplan, Winking, Finch, & Crimmins, 2008; Vasunilashorn et al., 2010). Physical growth is also stunted at all ages relative to WHO standards (Blackwell et al., 2017). Between 1950 and 2002, half of all deaths were due to infection, with the largest subset categorized as respiratory infections (Gurven et al., 2007).

2.2 | Participants

Participants were initially screened using the pubertal development scale (PDS; Petersen, Crockett, Richards, & Boxer, 1988, see section 2.6 for more detail) and only those ranked "2" or above were included in this study. The aim was to target those individuals who had already begun to exhibit pubertal changes and were, therefore, within the window of development where rapid endocrine change occurs (e.g., males' T increases by an order of magnitude between the initiation of puberty and late adolescence; Butler et al., 1989). These criteria were met by 45 males (mean age = 15.3 ± 3.1) and 44 non-breastfeeding females of which 22 had menstruated (mean age = 13.1 ± 1.9). Data were collected in accordance with procedures approved by the IRB at the University of California, Santa Barbara, including informed consent by parents, participants and the Tsimane government.

2.3 | Saliva collection

Participants did not eat in the hour before testing and rinsed their mouths with clean water before filling a 1 mL polystyrene cryotube with bubble-free saliva via passive drool. Samples were stored in liquid nitrogen (LN_2) 20–40 min after collection, and kept frozen in LN_2 until transported on dry ice to the University of California, Santa Barbara, where they remained frozen at −80°C until shipment on dry ice to Salimetrics LLC, State College, Pennsylvania, for analysis.

2.4 | Endocrine and immune assays and data treatment

Samples were assayed in duplicate using a highly sensitive competitive enzyme immunoassay (EIA) protocol (Salimetrics LLC, State College, Pennsylvania).

To control for potential blood contamination, transferrin was assayed using EIA (M = 0.91, SD = 0.89, Range: 0.08-5.0). Transferrin levels showed no significant correlation with T ($r = 0.21$, $P = .15$), E_2 ($r = -.17$, $P = .27$), or sIgA ($r = 0.13$, $P = .23$); nevertheless, those with levels >3 SDs (N = 3) were excluded.

Among adult males, T is higher in the morning than the evening; therefore, saliva collection time was analyzed as a potential confound. Research took place around participants' school and family obligations, so that the average time of day for saliva collection was 1:22 PM (SD = 169 min, range = 8:39 AM to 6:09 PM); however, collection times clustered in the morning (M = 10:27, N = 67) and afternoon (M = 15:35, N = 88). Time of day was not significantly associated (linearly or nonlinearly) with T; nevertheless, sample collection time was included in all statistical models and eventually removed because it explained no additional variance.

For free T (males only) the average intra-assay and inter-assay coefficients of variation were 4.6% and 9.8%, with a lower limit of sensitivity of 1.0 pg/mL and a standard-curve range from 6.1 to 600 pg/mL. For E_2 (females only) the average intra-assay and inter-assay coefficients of variation were 7.1% and 7.5%. The lower limit of sensitivity was 0.1 pg/mL. The standard-curve range was 1.0 to 32.0 pg/mL.

Saliva flow rate (i.e., volume of saliva per unit of time), which affects sIgA (Kugler et al., 1992), was also recorded during testing sessions. sIgA is sensitive to flow rate and is appropriately analyzed as a secretion rate (Kugler et al., 1992). In all analyses, "sIgA" refers to sIgA secretion rate (μg/min). For sIgA, the average intra-assay and inter-assay coefficients of variation were 5.6% and 8.8%. The lower limit of sensitivity was 2.5 μg/mL. The standard-curve range was 2.5 to 600 μg/mL. As a further check, flow rate was included in statistical models and then removed because it explained no additional variance.

2.5 | Energetic status

Standard anthropometric procedures were used to measure height and weight (Lohman, Roche, & Matorell, 1988). Body

mass index (BMI) was calculated for males (M = 20.3 ± 2.4) and females (M = 20.0 ± 2.4). Age-and-sex-standardized z-scores were then calculated for BMI using Tsimane-specific BMI-for-age curves (TBAZ; Blackwell et al., 2017), which may be more accurate than WHO standards for within population comparisons (Martin, Blackwell, Kaplan, & Gurven, 2019). We use this measure as a proxy for energetic budget to adjust for potential phenotypic correlation.

2.6 | Developmental stage

Adolescent development was assessed using the PDS (Petersen et al., 1988), a self-report questionnaire aimed at assaying variation in adolescent phenotypic change. The PDS has been shown to be a reliable approximation of the Tanner stages (Brooks-Gunn & Warren, 1985; Petersen et al., 1988). As per section 2.2, the PDS was used to exclude potential participants showing no pubertal development.

2.7 | Statistical analyses

Alpha level was set to 0.05. Natural log transformations were applied to normalize data distributions for T, E_2, sIgA, and age. Other variables were normally distributed (Shapiro-Wilk, $P > .05$). For all models, variance inflation factors (VIFs) were small (1.0-3.7), suggesting minimal collinearity. Interactions were explored by computing cross-products of centered variables. Only one statistically significant interaction was found (see section 3.2).

3 | RESULTS

3.1 | Associations with age

Several variables were correlated with age in the present sample. Among males, sIgA ($r = .34$, $P < .05$) and T ($r = .77$, $P < .001$) were associated with age. Among females, E_2 was significantly correlated with age ($r = .39$, $P < .01$), while sIgA approached significance ($r = -.25$, $P < .10$). Therefore, we controlled for age in all analyses to highlight maturational relationships rather than to track simple age-related change. See Table 1 and Figure 1.

3.2 | Do males show compromised mucosal immunity levels relative to females during adolescence?

No. To examine sex differences, we used sex to predict sIgA in a multiple-regression model controlling for age and Tsimane-specific BMI-for-age scores (TBAZ). Males had significantly *higher* sIgA levels (ß = .31, $P < .01$; M = 28.5 g/mL/min; raw data) than females (M = 19.6 g/mL/min; raw data). A significant sex-by-age interaction (ß = .52, $P < .01$) revealed that sIgA was positively associated with age for males (ß = .35, $P < .05$) but negatively associated with age for females (ß = −.33, $P < .05$). In this model, age was a significant predictor of sIgA (ß = −.39, $P < .05$) while TBAZ approached significance (ß = −.18, $P = .07$). See Model 1 of Table 2, and Figure 2.

3.3 | Among males, is T inversely associated with mucosal immunity?

No. Without controls for age and TBAZ, the pairwise correlation between T and sIgA was *positive* (r = .43, $P < .01$). This association was not explained by greater T and sIgA with age; in a model predicting sIgA, T remained positively associated with sIgA in males (ß = .50, $P < .05$), controlling for both age (ß = −.04, $P = .88$) and TBAZ (ß = −.18, $P = .26$). Interactions between T and both age and TBAZ were not significant. See Figure 3 and Model 2 of Table 2.

3.4 | Among females, is E_2 positively associated with mucosal immunity?

No. Without controls for age and TBAZ, E_2 was *negatively* correlated with sIgA (r = −.53, $P < .001$) in females. In a model predicting sIgA, E_2 remained a significant *negative* predictor of sIgA (ß = −.43, $P < .01$), controlling for age (ß = −.13, $P = .40$) and TBAZ (ß = −0.20, $P = 0.16$).

TABLE 1 Partial correlations (controlling for age) for males (lower left triangle) and females (upper right triangle) with descriptive statistics below

	sIgA	T	E_2	TBAZ
sIgA	–	–	−0.48***	−0.34*
T	0.26†	–	–	–
E_2	–	–	–	0.32*
TBAZ	−0.04	0.44**	–	–
Male $\bar{x}$:	28.5 µg/min	69.1 pg/mL	–	0.21
Female $\bar{x}$:	19.6 µg/min	–	1.7 pg/mL	0.22
Male SD:	24.4 µg/min	39.6 pg/mL	–	0.95
Female SD:	15.7 µg/min	–	1.2 pg/mL	0.90
Male min:	4 µg/min	10.4 pg/mL	–	−1.40
Female min:	4 µg/min	–	0.2 pg/mL	−1.62
Male max:	141 µg/min	174.0 pg/mL	–	1.97
Female max:	67 µg/min	–	5.8 pg/mL	2.79

Note: Means and SD for raw data.

Abbreviations: sIgA, secretory IgA secretion rate; T, Testosterone; E_2, Estradiol; TBAZ, Tsimane-specific BMI-for-age z-scores.

*$P < .05$; **$P < .01$; ***$P < .001$, †$P < .10$.

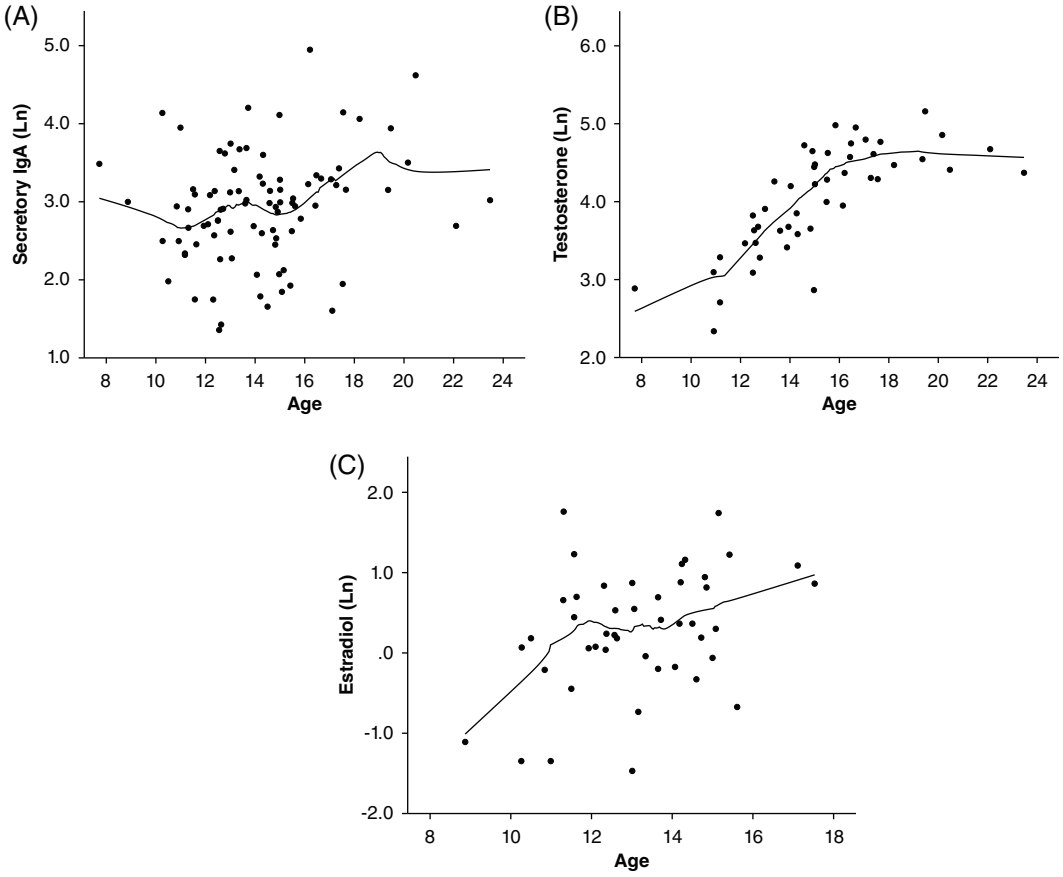

FIGURE 1 Secretory IgA (A), testosterone (B), and estradiol (C) by age
Caption: *Note.* Solid lines indicate LOWESS curves (using Epanechnikov kernel) fit to 50% of the data

Interactions between E_2 and both age and TBAZ were not significant. See Figure 4 and Model 3 of Table 2.

4 | DISCUSSION

The goal of the present research was to examine ICHH-informed associations between steroid hormones (T and E_2) and mucosal immunity (sIgA) in a nonindustrialized population during a period of known rapid endocrinological change (i.e., adolescence). Results revealed that adolescent males had significantly higher levels of sIgA, which, may be explained by two other findings: a significant positive correlation between T and sIgA in males and a significant inverse association between E_2 and sIgA in females.

4.1 | Sex differences in mucosal immunity

As an indirect test of immune-endocrine associations (Nunn et al., 2009), we examined sex differences in sIgA, finding significantly higher levels among adolescent males than females. Previous research suggests that sIgA is higher in male infants (Miller & McConnell, 2012) and adults (Gleeson et al., 2011), but not children (Sonesson, Hamberg, Lundin Wallengren, Matsson, & Ericson, 2011). A second T-associated rise in sIgA during male puberty, such as observed here, would account for the higher sIgA among male adults not found in children (Sonnesson et al., 2011). These findings suggest that the standard prediction derived from the ICHH (i.e., that males have depressed immunity) does not extend to sIgA. Indeed, recent evidence suggests that T is better thought of as immunomodulatory rather than immunosuppressive (Da Silva, 1999; McDade, 2003; Muehlenbein & Bribiescas, 2005; Trumble et al., 2016). In terms of proximate hormonal effects, males may have higher sIgA than females either because of androgen enhancement, estrogenic suppression, or both.

In addition, a significant sex-by-age association was found; sIgA was positively associated with age for males but negatively associated with age for females. Several studies have reported that sIgA develops during infancy and childhood, peaking in early childhood, and it is only after puberty that nasal anatomical and immunological function fully matures (Bellussi et al., 2013, Ben-Aryeh, Fisher, Szargel, & Laufer, 1990; Kugler et al., 1991). Unfortunately, adolescents have not been adequately sampled in other studies. The few studies that have measured sIgA in adolescents studied only athletes and yielded inconsistent results. One study examining

TABLE 2 Multiple regression models predicting sIgA

	Predicting sIgA			
	Unstandardized Beta	**SE**	**Standardized Beta**	**t statistic**
Model 1 (N = 89):				
Sex	0.49	0.15	0.31	2.91**
Age	−1.43	0.69	−0.39	−2.07*
TBAZ	−0.15	0.08	−0.18	−1.84†
Sex × Age	2.47	0.84	0.52	2.96**
Model 2 (males only; N = 45):				
Testosterone	0.47	0.22	0.50	2.09*
Age	−0.11	0.72	−0.04	−0.16
TBAZ	−0.13	0.11	−0.18	−1.14
Model 3 (females only; N = 44):				
Estradiol	−0.41	0.14	−0.43	−2.90**
Age	−0.60	0.74	−0.13	−0.85
TBAZ	−0.17	0.12	−0.20	−1.43

Abbreviations: sIgA, secretory IgA; TBAZ, Tsimane-specific BMI-for-age z-scores.
*$P < .05$; **$P < .01$; †$P < .10$.

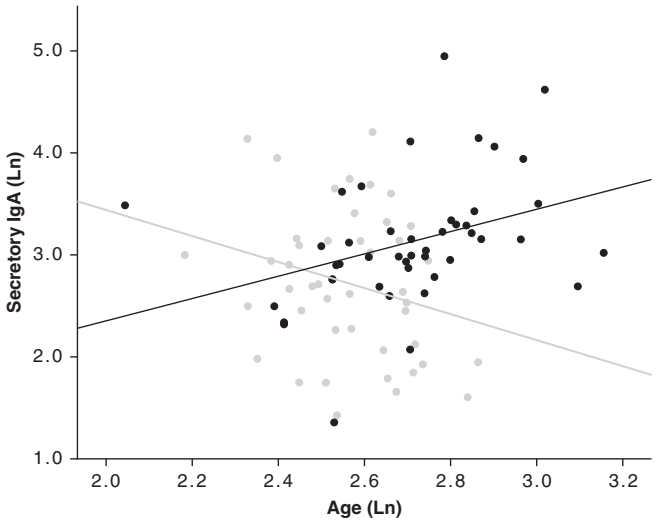

FIGURE 2 Secretory IgA by age for males (black) and females (gray)

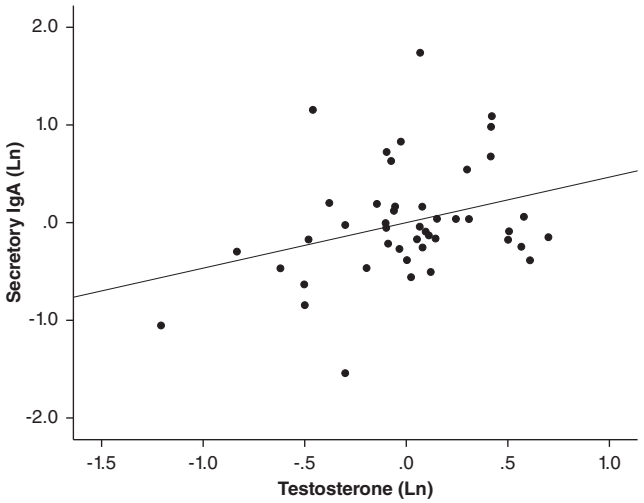

FIGURE 3 Secretory IgA by testosterone (controlling for age and TBAZ). TBAZ, Tsimane-specific BMI-for-age z-scores

pre-pubertal and late-pubertal girls found that sIgA levels were higher after pubertal maturation (Corbett et al., 2010), which contrasts with the findings presented here.

4.2 | Association between T and mucosal immunity

To the best of our knowledge, this is the first study investigating the relationship between T and sIgA among male adolescents in a nonindustrialized population. Regardless of whether we control for age and/or phenotypic correlation (measured here as age-standardized BMI z-scores), T and sIgA are positively associated in our male sample, which accords with previous findings from longitudinal (Gettler et al., 2014), pseudo-experimental (Fornieles et al., 2014; Koçar et al., 2000; Peñailillo et al., 2015), correlational (Arnocky et al., 2018; Hodges-Simeon et al., 2017), and nonhuman animal (Sullivan & Allansmith, 1987) data, possibly due to mechanisms suggested by Kaetzel (2005).

Ultimately, it remains to be discovered what adaptive function elevated sIgA might serve for adolescent and adult males, and why T is associated with an increase in sIgA. Among Ariaal infants, higher sIgA was associated with lower

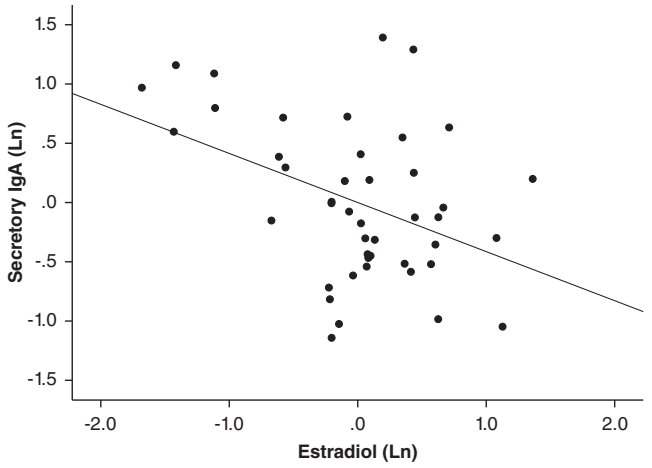

FIGURE 4 Secretory IgA by estradiol (controlling for age and TBAZ). TBAZ, Tsimane-specific BMI-for-age z-scores

height-for-age (Miller & McConnell, 2012), suggesting that mucosal immunity may be upregulated among slow or stunted growers, or that this aspect of immunity has costs in a different developmental arena. Miller and McConnell (2012) suggested the possibility that elevation of sIgA may compensate for immune suppression in cell-mediated immunity resulting from malnutrition. Similarly, elevation of sIgA in adolescent males may compensate for androgenic suppression of other branches of immunity (Granger et al., 2000; Roubenoff et al., 2002). Braude (1995) suggests that intrasexual combat may pose specific immune defense risks (e.g., increased probability of transmission), and that male immune systems may be designed to address these heightened risks. Others suggest that risky behaviors, larger mate-search ranges, and other sexually selected behaviors expose males to different immune challenges than females (Rolff, 2002). Consistent with this, Trumble et al. (2016) found that T in Tsimane males was correlated with reductions in T-cell mediated immune responses, but uncorrelated with B-cell and macrophage related responses. Thus, upregulation of mucosal immunity by T can be thought of as a reallocation of immune effort to match risks associated with male adulthood.

After increasing throughout adolescence, Tsimane males' sIgA reached approximately 147 μg/mL, which is the predicted value of an 18-year-old using linear regression (note that this does not correct for secretion rate to facilitate comparison with van Anders). In one sample of healthy North American adult males, sIgA ranged from ~40 to 84 μg/mL (van Anders, 2010). Thus sIgA may be higher among Tsimane than in populations in industrialized countries. Tsimane have elevated serum antibodies (Blackwell et al., 2016), but two aspects of Tsimane disease ecology may account for elevations in sIgA in particular. First, respiratory infections are common. Two-thirds of early childhood deaths are due to infectious disease, of which respiratory infections account for half (Gurven et al., 2007). Lower sIgA has been associated with increased respiratory infections (Drummond & Hewson-Bower, 1997; McClelland, Alexander, & Marks, 1982). Second, the Tsimane experience a high rate of dental caries, which are epidemiologically associated with higher sIgA (Thaweboon, Thaweboon, Nakornchai, & Jitmaitree, 2008). Thus, Tsimane adolescents may upregulate sIgA as an adaptive response to higher levels of respiratory and cariogenic pathogens. The facultative regulation of immune functioning as an adaptive response to environmental heterogeneity is an important area of current and future research in human ecological immunology (Blackwell et al., 2010; McDade, 2003; McDade, 2005).

4.3 | Association between E_2 and mucosal immunity

With or without controls for age and phenotypic correlation, we find that E_2 is inversely associated with sIgA and this association may contribute to the observed sex differences in mucosal immunity. This accords with experimental research on female rats (Wira & Sullivan, 1985). In contrast to the present results, van Anders (2010) found a positive association between E_2 and sIgA among healthy US adult women. Although van Anders (2010) did not control for secretory flow rate, a potentially important confound in analysis of sIgA (Bishop & Gleeson, 2009; Kugler et al., 1992), at least one other study agrees with van Ander's finding. Ali, Diebel, and Liberati (2012) found that the administration of E_2 significantly increased IgA transport and decreased bacterial passage. In some studies, it is suggested that the transport of IgA by polyimmunoglobulin receptors is upregulated by Toll-like receptor 4, which is itself upregulated by E_2 (Ali et al., 2012; Diebel, Diebel, & Liberati, 2011). Further research is needed to understand the relationship between E_2 and mucosal immunity.

4.4 | Limitations

One potential limitation of the present study is that menstrual cycle day was not collected. E_2 varies widely across the menstrual cycle, increasing substantially before ovulation (Gandara, Leresche, & Mancl, 2007). Interpretation of the present results, however, does not require menstrual cycle day; our findings show that higher E_2 (whether due to age and/or menstrual cycle) is associated with lower sIgA. These findings suggest that sIgA may vary across the menstrual cycle with changes in E_2. Gómez et al. (1993) noted that during the follicular phase, sIgA was higher than the luteal phase, but not significantly so (however, the sample size was 10). Future researchers should target intra-cycle variation in sIgA among adult participants; however, this type of study may be difficult in adolescent samples (especially among energy-limited populations) given the high degree of menstrual irregularity and anovulation in this

age group (Apter, Viinikka, & Vihko, 1978). Moreover, future studies may wish to measure progesterone as well, as interactions between E_2 and progesterone may play a role (Gómez et al., 1993; Wira & Sullivan, 1985).

Another limitation is that only one salivary sample was collected. Salivary T may vary by hour, day, and week (Dabbs, 1990) in response to circadian (Gupta, Lindemulder, & Sathyan, 2000), social (e.g., Archer, 1991; Trumble et al., 2012), energetic (Trumble, Brindle, Kupsik, & O'Connor, 2010), and other environmental and intra-individual factors (e.g., Trumble, Smith, O'Connor, Kaplan, & Gurven, 2014). Again, the exact cause of variation in T may not be critical to interpretation of the present results; our findings show that T may be positively associated with mucosal immunity, and that any circadian, social, or environmental factor that affects T may also affect sIgA. Given other evidence of this relationship (Arnocky et al., 2018; Fornieles et al., 2014; Gettler et al., 2014; Peñailillo et al., 2015), and probable mechanisms linking T and sIgA (Parr, Ren, Russell, Prins, & Parr, 1992), this is a hypothesis worth pursuing in future research. Future research should target the distinction between "state" and "trait" T and sIgA using intra-individual, longitudinal research design.

A third potential limitation is that saliva collection times were not standardized across participants; these times were set to accommodate family and school obligations in order to minimize the study's disruption to the community. Nevertheless, time of day was not significantly associated with steroid levels, which may be due to several factors. First, saliva was collected four or more hours after waking (and 100% after 2 hours) for around 85% of participants. This is when T would be leveling off for those with an established diurnal pattern (Butler et al., 1989). Second, salivary T levels of boys from industrialized societies do not exhibit diurnal rhythms until at least Tanner genital stage 3 (Butler et al., 1989), after which further diurnality develops by adulthood. In a previous analysis of these data using a broader age range (Hodges-Simeon, Gurven, & Gaulin, 2015), there was no significant difference in T between morning and afternoon sessions, even after boys were grouped into developmental stages (pre-, peri-, and post-pubertal). It may be that diurnality in T occurs later in non-industrialized, developmentally delayed populations like the Tsimane. Among the Ache, peak AM:PM T ratio did not occur until the fourth decade (Bribiescas & Hill, 2010). Third, among adolescents, age-related change in T is so substantial that it likely captures most of the inter-individual variation. To our knowledge, no previous studies have examined the development of diurnal T rhythms among energetically stressed populations; this is an important area for future research.

Finally, the present sample size is relatively small with 45 males and 44 females and includes four males between the ages of 19 and 23. There are several reasons to include these ages in males in particular and classify them as adolescents for the purposes of the present research. First, while adolescence has a relatively clear beginning coinciding with the initiation of puberty, the end of adolescence is ambiguous. While Western societies arbitrarily set this at a certain age (e.g., 18 in the United States), life history theory marks the end of adolescence with the end of growth and birth of first offspring. The end of growth may occur in the early 20s in societies where energy demands are higher (Bogin, 1999), and first birth may depend on a myriad of factors, including gaining the skills necessary to provision offspring (Kaplan et al., 2000). Further, while linear growth may end in the late teens (but not always; Bogin, 1999), growth in other tissues (i.e., muscle mass) may continue after age 18 (Schutz et al., 2002). Third, and most important for this research, adolescent-typical endocrine activation, that is, rapidly increasing production of sex steroids, also often continues into the early 20s. Studies show that males' salivary T exhibits a sigmoidal growth pattern with low levels prepuberty, then sharply increasing levels following pubertal initiation. T then peaks in the 19 to 24 range, and declines throughout adulthood (Butler et al., 1989; Elmlinger et al., 2004; Kelsey et al., 2014; Hodges-Simeon et al., 2015). Given the present study's results, and with a larger range of ages from childhood through adulthood, we would likely observe a sigmoidal pattern in sIgA as well. The goal of the present research was to target a period where hormones are known to change substantially to look for concomitant change in mucosal immunity. We accomplished this goal by including participants whose T velocity was likely increasing (based on the presence of pubertal signs), as well as those that had likely reached peak T (i.e., ages 18 to 23).

4.5 | Conclusions

Based on the most common interpretation of the ICHH, males should show depressed immunity compared with females. Results of the present research, along with several other studies (Arnocky et al., 2018; Gettler et al., 2014), suggest that the arm of the immune system represented by sIgA is not compromised by male steroid hormones, and, in fact, may be enhanced by T. Further, E_2 may not universally enhance all aspects of the immune system. Work in ecological immunity (McDade, 2003; McDade, 2005; McDade, Georgiev, & Kuzawa, 2016; Muehlenbein & Bribiescas, 2005) and theoretical modeling (Stoehr & Kokko, 2006) suggest that the relationship between sex steroids and immunity—or the degree of sex differences in immunity—may depend on a myriad of assumptions and facultative contingencies. For instance, reductions in energy supply may not affect all aspects of immunity in the same manner, and allocation of immune investment may depend on the prevailing array of pathogen-specific risks (McDade, 2003; McDade et al., 2016). Here we show that the relationship between sex steroid hormones and immune function depends on the branch

of immunity under investigation. Practically speaking, these possibilities require attention to the ways that nutritional and disease ecology affect the development of different components of the immune system across the life course (Blackwell et al., 2010; McDade, 2003; McDade, 2005; McDade et al., 2016). Understanding the nature of the relationship between steroid hormones and immunity will take careful study of particular cases in order to understand how and why various ecological, pathogenic, nutritional, and other inputs affect different aspects of immunity. In particular, future empirical and theoretical work should focus on differential immunity-relevant selection pressures on males and females to understand the adaptive relationships between steroid hormones and mucosal immunity.

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AUTHOR CONTRIBUTIONS

CRHS designed the study, directed implementation and data collection, analyzed the data and drafted the manuscript. MG and SJCG provided necessary planning and logistical support for data collection. CRHS, MG, SJCG, and ADB edited the manuscript for intellectual content and provided critical comments on the manuscript.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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