

## Testosterone, cortisol, and secretory immunoglobulin-A within a single day and across two sequential days among *trans*- and *cis*-gender men



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### ABSTRACT

**Background:** Previous research on the association between testosterone (T) and immunity has produced conflicting results.

**Objectives:** We address two potential reasons for these empirical inconsistencies in the present research. First, the association between T and immunity may depend on which branch of the immune system is considered. Here, we examine secretory IgA (sIgA), a measure of mucosal immunity functionally related to respiratory infection risk. Second, the association between T and immunity may depend on a third regulatory variable. Therefore, we examine the interaction between T and cortisol (CORT) as well as their independent and combined effects on mucosal immunity. To do this, we explore intra-individual associations between sIgA, CORT, and T within a single day (i.e., morning vs. evening) and across 2 sequential mornings. We target two samples of men: (1) cisgender (i.e., born and identifying as men), and (2) transgender (i.e., born female but identifying as men) undergoing T therapy for gender realignment.

**Materials and methods:** One hundred and forty-eight adult men (transgender  $n = 29$ ) provided saliva samples at three time points: (1) upon waking, (2) before sleep on the same day, and (3) upon waking the following day. Samples were assayed in duplicate for sIgA, T and CORT.

**Results:** For cisgender men, sIgA, T, and CORT exhibited clear circadian rhythms and were significantly related within and between samples. For transgender men, evidence for circadian change was found for sIgA and CORT, but not T. Further, sIgA was associated with CORT, but not T.

**Discussion and conclusions:** This study provides the first evidence that salivary T and sIgA concentrations are associated within a single day and across sequential days for cisgender men. Differences between cis- and transgender men suggest that this may only be true for T levels driven by endogenous production; however, future studies should employ a larger sample size.

### 1. Introduction

Among human and animal models, sex-specific modulation of immune responses are associated with androgens, particularly

testosterone (T; e.g., [50–51,71,102–103,114]). T deprivation (e.g., through pharmacological or surgical castration) tends to extend the lifespan for many species, both non-human [65,80] and human ([52,82,119]; however, see [64,86]), likely reflecting tradeoffs between

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T production and longevity. Because sex differences in immunocompetence (and thus morbidity and mortality) often favor females across vertebrates, including humans [43,70,88,98,107], T is generally characterized as an immune-suppressing hormone (e.g., [103]).

In support of this view, men<sup>1</sup> produce fewer antibodies in response to vaccines than women (see review [126]), and men with higher T show diminished antibody response compared to men with lower T (e.g., [43]). In comparison to women, men also exhibit less resistance to most infections and less intense cellular and humoral immune responses [11,50]. Further, T administration studies across a variety of taxa support the idea that T has a detrimental, causal effect on immunity. For example, European starlings administered T produced a significantly reduced response to a novel antigenic challenge, including lower levels of antibodies and secretory immunoglobulin-G [32]. Male song sparrows administered T had reduced cell-mediated and humoral immune responses compared to those administered a placebo [35,35]. In humans, T therapy among those with Klinefelter's syndrome (who are typically low in T) and systemic lupus erythematosus resulted in diminished cellular and humoral immunity [67,73].

Although T is generally thought to be immunosuppressive, not all research findings demonstrate this association. Some studies of androgen administration in humans and non-human animal models suggest that certain aspects of immunity are positively affected by administration of exogenous T (e.g., [41,78,96]). *In vivo* designs have shown mixed results, with findings indicating positive (e.g., [51], negative (e.g., [102]), and null (e.g., [51,116]) relationships between T and various measures of immunity. Finally, 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 [114]—suggesting that only specific aspects of immunity may be affected by T.

Given the diverse and complex nature of the immune system, it is not surprising that a unilateral T-immunity relationship has not been found, leading some to characterize T as immune-modulatory rather than suppressive [27,79,85]. Additionally, it remains unclear which aspects of immunity are potentially up- or downregulated by T and the extent to which T concentrations due to exogenous administration compared to those from endogenous production may differentially impact immunity. Therefore, the first goal of this research is to test the association between T and one understudied branch of the immune system—mucosal immunity, measured via secretory IgA (sIgA). Second, several researchers have posited that the association between T and immunity is opaque because we have ignored co-regulatory dynamics—in particular, T and glucocorticoids [e.g., cortisol (CORT); [16,20]], which likely affect immunity in tandem [35,83,97]. Thus, an additional goal of this research is to examine the joint effect of T and CORT on mucosal immunity among a healthy (i.e., non-clinical) sample of young adult cisgender men (herein also referred to as “cismen”). Because a T-immunity relationship has important implications for those undergoing T therapy, we include a second sample of transgender males

<sup>1</sup> Because transgender men are underrepresented in research, we thought it was important to clarify the language used to describe our populations. Throughout, “cisgender men” (or cismen) refers to those born and identifying as men and “transgender men” refers to those born female but identifying as men or closer to the masculine side of the gender spectrum. However, in the introduction and discussion, we use the conventional terms “men or man” and “women or woman” to refer to participants in studies where gender identity was *not* assessed by the researchers (i.e., the vast majority of research). In other words, when these researchers recruited “men” for their research, we have no way of knowing if these men were cis- or transgender. Because transgender men number approximately 0.6% of the general population (Flores et al., 2016), we can assume a similar percentage in these participants. In contrast to the vast majority of research, we specify cis- or transgender in the results and discussion because we recruited as such.

(herein also called “transmen”) that were assigned female at birth and who are currently taking T for the purpose of gender identity alignment [3,25,56,89,120,125]. This underrepresented sample population provides a novel comparison group to further delineate the relationship between sIgA levels and T concentrations among those both born and identifying as men (cismen) as well as those born female but identifying as men (transmen).

## 2. Mucosal Immunity: Secretory Immunoglobulin-A (sIgA)

Pathogens often achieve entry to the body via mucosal surfaces (i.e., saliva, colostrum, and surfaces of the genitourinary, gastrointestinal, and respiratory tracts), where sIgA is the most abundant immunoglobulin [13,76]. Salivary sIgA is produced by plasma B cells residing within salivary glands, and it is secreted into the saliva, providing an initial defense against oral pathogens (particularly those that cause respiratory infection; [38,46–48,87]). The primary role of sIgA is immune exclusion, which involves binding to invading pathogens to prevent entry to the epithelium; however, it also plays a role in maintaining homeostasis between host and commensal bacteria [13,22–24,77]. Several studies show lower concentrations of sIgA may lead to an increased risk of upper respiratory tract infections and cold/flu symptoms [38,45–48,87]. In a 19-year-long longitudinal study of men and women, sIgA was negatively correlated with mortality, particularly from death due to cancer and respiratory diseases [93], suggesting that it may serve as a useful proxy measure of mortality risk, which is one index of overall health.

The purpose of the current investigation is to explore the independent and combined effects of T and CORT on sIgA. Previous research on T and sIgA suggests that the association may be positive (rather than negative, as has been previously characterized for other branches of immunity). For example, Hodges-Simeon et al. [58] found a positive association between dehydroepiandrosterone sulfate (DHEA-S), a precursor of T, and sIgA. In several studies comparing immunity prior to and following exercise, T increased alongside sIgA after exercise [40,90]. Additionally, a longitudinal analysis showed that sIgA levels decreased among men concomitant with fatherhood-related declines in T [45]. Changes in T with puberty parallel changes in sIgA, such that older adolescents have both higher T and higher sIgA compared with younger adolescents [59]. Further, these males also had significantly higher sIgA levels than females, controlling for age and energetic condition. Two additional studies have examined the association between endogenous T and sIgA in men; one showed a positive correlation [6], and the other found no association [116]. The latter study, however, did not describe a correction for secretion rate, which is a necessary control [74]. Conversely, one study on exogenous T administration observed negative changes in serum IgA [73]. Therefore, it remains unclear whether T and sIgA are positively or negatively related.

The association between CORT and sIgA is similarly inconclusive. Hucklebridge et al. [63] demonstrated that the decline in sIgA is significantly correlated with the post-waking rise in CORT. Zeier et al. [124] found an increase in both sIgA and CORT in response to increased physical and psychosocial stress among air traffic controllers; however, the increase in sIgA was not related to the increase in CORT. Similarly, Dimitriou et al. [28] found that although sIgA and CORT decreased from morning to evening, the changes in concentrations of sIgA and CORT were not related. Moreover, sIgA concentrations have been found to increase in response to an acute stressor [18,37,124], but decrease in response to prolonged stress [44]—both of which are likely linked to CORT levels [105]. Finally, the relationship between T and CORT is also inconsistent. Some studies examining their circadian rhythms have found significant positive relationships between the concentrations of these hormones [54,113], whereas other studies have not [49,112].

### 3. Aims of the current study

We add to the current literature on T, CORT, and sIgA relationships by exploring intra-individual associations between T, CORT, and sIgA within a single day (i.e., morning to evening) and across two sequential mornings (i.e., morning 1 to morning 2). This approach is facilitated by known diurnal patterns in T, characterized by a morning peak(s) followed by an evening decline (e.g., [12,29,49,69,104]). CORT follows a similar diurnal pattern to T: high in the morning, peaking approximately thirty minutes after waking, with an evening nadir [28,49,75]. Participants provided two morning samples and an evening saliva sample, affording us the ability to test whether changes in sIgA across the day/days are associated with changes in T and CORT. Based on previous research [6,40,45,58,73,90,110], we hypothesize that within-individual diurnal changes in T will be positively associated with within-individual changes in sIgA and CORT.

For all analyses, we target two samples of men: a sample of young cisgender men and a sample of young to middle-aged transgender men. Transgender individuals experience a distressing incongruence between their assigned sex at birth and their experienced gender identity [3]; (Zucker et al., 2016); [120]. As a result, many transgender men seek T therapy to bring their physical appearance into closer alignment with their gender identity. Quality of life and well-being improvements have been reported due to greater gender congruence, particularly from T therapy, thereby underscoring the importance of this intervention [25,56,89,120]. As such, we add to the literature and understanding of biological phenomena occurring during T therapy.

Because no published studies have examined the relationships between T, CORT, and sIgA in transgender males, making predictions is difficult. Previous studies have shown that men have higher sIgA than women [4,34–35,59,93]; therefore, T therapy in transmen may increase sIgA to the levels seen in cismen. On the other hand, higher levels of CORT resulting from transition-related stress [31] may suppress sIgA levels in this vulnerable population (however, see [132] for evidence of lower cortisol after T therapy). Further, to our knowledge, no other studies have reported diurnal changes in salivary T in transgender males undergoing T therapy.

## 4. Method

### 4.1. Participants

Cisgender male participants included 119 male students from Boston University (BU) between the ages of 18 and 30 ( $M_{\text{age}} = 21.19$ ,  $SD = 2.88$ ). We targeted younger men for the current study because younger men are more likely to have more dramatic declines in T from morning to evening than older men, who exhibit lower serum and salivary morning T concentrations and a shallower diurnal decline [12,29]. The ethnic composition of the sample was as follows: Caucasian (40.5%), Asian (19.0%), South Asian (14.9%), Latin American (10.7%), Black (7.4%), Arab West Asian (4.1%), Southeast Asian (1.7%). Cismen were recruited via advertisements placed throughout the BU campus, and through online job ads for BU students. No participant indicated that they were taking prescribed steroid medications. Remuneration was \$35 USD.

Data on transgender men were collected as part of a larger study on the somatic, physiological, and psychological effects of T therapy on transmen. Participants were 29 individuals from the greater Boston area between the ages of 20 and 40 ( $M_{\text{age,years}} = 25.87$ ,  $SD = 5.22$ ). The ethnic composition of the sample was as follows: Caucasian (86.7%), Asian (3.3%), Latin American (3.3%) and Multiple Ethnicities (3.3%). All participants were assigned female at birth and were currently undergoing hormone replacement therapy (HRT) with T for at least 9 months ( $M_{\text{HRT,months}} = 41.50$ ,  $SD = 25.45$ ) through different administration routes (intramuscular = 13, every 7–14 days; subcutaneous = 11, every 5–14 days; subdermal pellets = 4, every 3–4

months; transdermal = 1, daily). Participants were recruited using digital flyers and notifications on private or closed LGBTQ+ or transgender Facebook groups, physical flyers posted at the 2017 Boston Pride festival, as well as through referral sampling. Remuneration was \$40 USD.

### 4.2. Procedure

Participants provided three saliva samples: one upon waking the morning following the first study appointment (hereafter referred to as “Day 1 AM”), a second that evening before going to sleep (“Day 1 PM”), and a third upon waking on the day of the second study appointment (“Day 2 AM”). Samples were analyzed using commercially available enzyme immunoassay kits (DRG International, NJ, USA) for T, CORT, and sIgA. Full details of the procedure, including measures taken to avoid blood contamination, can be found in the Supplement.

### 4.3. Data analysis

Averages for sIgA, T, and CORT were Log10-transformed to adjust for non-normality. We controlled for flow rate in our analysis by multiplying sIgA sample concentrations by their respective flow rates prior to the Log10 transformation of these variables, as is standard practice in research involving sIgA concentrations [34,74]. We computed the difference in concentrations of sIgA, T, and CORT between adjacent sample times (designated by  $\Delta$ ) to test if sIgA concentrations conformed to a diurnal pattern that was related to daily fluctuations in T and CORT. Additionally, we computed difference variables between the AM samples. Given that we were interested in the joint effects that CORT and T had on the secretory immune system, we computed the product of T and CORT (hereafter  $T \times \text{CORT}$ ) for each sample provision time and then computed difference variables of these products in the same way as detailed above.

Analyses were conducted in R studio (3.5.1; [95]). One-sample  $t$ -tests were conducted using the *stats* package [95] to determine if the changes in sIgA concentration from Day 1 AM to Day 1 PM, and the  $\Delta$  sIgA from Day 1 PM to Day 2 AM were significantly different from zero, providing evidence for change in concentration over time. Further, we conducted one-samples  $t$ -test comparing  $\Delta$  sIgA from Day 1 AM to Day 2 AM to determine if waking sIgA concentrations were similar across sequential days. We conducted linear regressions using the *stats* package [95] to determine if the change in sIgA concentration from sample provision Day 1 AM to Day 1 PM was a significant predictor in the  $\Delta$  sIgA concentration from Day 1 PM to Day 2 AM on the following day. These analyses were repeated for T and CORT.

To get a more in-depth understanding of the relationships between sIgA, T, and  $T \times \text{CORT}$ , we conducted observed variable path models using the *lavaan* package in R [101]. We did this for the cismen only, as our sample of transmen was too small to conduct path models. We analyzed the extent to which sample concentrations of sIgA, T, CORT, and  $T \times \text{CORT}$  provided by participants on Day 1 AM predicted their concentrations on Day 2 AM, and the extent to which Day 1 PM sample concentrations mediated this effect. To analyze the relationships between the concentrations of T, CORT,  $T \times \text{CORT}$  and sIgA across time, we specified covariances between sIgA and T, sIgA and CORT, as well as sIgA and  $T \times \text{CORT}$ , at each sample provision time. To test if the  $\Delta$  sIgA,  $\Delta$  T,  $\Delta$  CORT and  $\Delta$   $T \times \text{CORT}$  were related, we conducted path models using the difference variables of sIgA, T and CORT concentrations, as well as the product of T and CORT concentrations from Day 1's AM and PM samples, and Day 1 PM to Day 2 AM samples. By conducting path models using the difference variables, we were testing if the change in concentration over time among these hormones and sIgA were related.

## 5. Data screening

Prior to the data analyses, all cases and study variables were

**Table 1**  
Descriptive statistics.

	Cisgender males		Transgender males	
	M	SD	M	SD
<b>Testosterone (T; pg/mL)</b>				
Day 1 AM	152.10	60.46	239.41	156.22
Day 1 PM	85.42	54.76	246.02	227.04
Day 2 AM	152.96	57.15	329.73	468.64
<b>Cortisol (CORT; pg/mL)</b>				
Day 1 AM	4.11	3.38	4.05	2.39
Day 1 PM	0.74	1.41	1.09	1.88
Day 2 AM	4.30	3.16	3.48	2.28
<b>Secretory IgA (sIgA; µg/mL)</b>				
Day 1 AM	1.14	1.81	0.67	1.29
Day 1 PM	0.60	1.21	0.42	0.54
Day 2 AM	1.22	1.66	1.04	1.64

Note. Means (M) and standard deviations (SD) for raw values. Units for sIgA are in µg/mL controlling for participants' flow rate mL/minute.

examined for missing values and violations of the assumptions of multivariate analyses (i.e., multicollinearity, normality, linearity, and homogeneity of variance). Full detail on data screening and analysis can be found in the Supplement. Means and standard deviations were computed and can be found in Table 1.

**Goodness of fit indices.** For all path models, we evaluated the goodness of fit using the global  $\chi^2$  test of fit, the standardized root mean square (SRMR), the Tucker-Lewis Index (TLI; [115]), and the Comparative Fit Index (CFI; [133]). Acceptable model fit was defined as follows: a non-significant  $\chi^2$ , SRMR < .08, CFI > .95, and TLI > .95. We elected to interpret multiple indices because they provide different information for evaluating model fit.

**Table 2**  
Summary of results.

Diurnality in sIgA, T, and CORT <sup>a</sup>						
	Day 1 AM to Day 1 PM		Day 1 PM to Day 1 AM		Day 1 AM to Day 2 AM	
	Cisgender	Transgender	Cisgender	Transgender	Cisgender	Transgender
Δ sIgA	3.48***	1.56 <sup>†</sup>	-7.28***	2.48**	1.96 <sup>†</sup>	1.46
Δ T	10.32***	1.35 <sup>†</sup>	-11.80***	0.81	0.69	-0.48
Δ CORT	15.10***	9.32***	16.21***	5.09***	1.45	-1.21
Within-sample associations with sIgA <sup>b</sup>						
	Day 1 AM		Day 1 PM		Day 2 AM	
	Cisgender	Transgender	Cisgender	Transgender	Cisgender	Transgender
T	0.21*	0.08	0.21*	-0.09	0.41***	0.05
CORT	-0.02	0.14	0.22*	0.45**	0.25**	0.11
T × CORT	-0.02	0.13	0.21*	0.43**	0.26**	0.16
Between-sample associations with sIgA <sup>c</sup>						
	Day 1 AM to Day 1 PM		Day 1 PM to Day 2 AM		Day 1 AM to Day 2 AM	
	Cisgender	Transgender	Cisgender	Transgender	Cisgender	Transgender
Δ T	0.26**	0.22	0.37***	0.22	0.37***	0.30
Δ CORT	0.18 <sup>†</sup>	0.29	0.29**	-0.48**	0.28**	0.55**
Δ T × CORT	0.18 <sup>†</sup>	0.34 <sup>†</sup>	0.31**	-0.48**	0.31**	0.56**

Note. <sup>†</sup>p < .10; \*p < .05; \*\*p < .01; \*\*\*p < .001. <sup>a</sup>t-statistic; <sup>b,c</sup>Beta values for cisgender men, Pearson correlations for transgender men.

## 6. Results

### 6.1. Do sIgA, T, and CORT follow a consistent diurnal pattern in young adult males?

**Cisgender men.** We conducted one-sample t-tests to determine if the decline in sIgA from AM to PM on Day 1, and the rise in sIgA from Day 1 PM to Day 2 AM, were significantly different from 0. Both tests were significant, which suggests a diurnal pattern (see Table S1 in Supplement). The Δ sIgA concentration from Day 1 AM to Day 2 AM approached statistical significance, suggesting that cisgender men's sIgA may vary some across sequential mornings, but likely remains relatively consistent. Further, a linear regression revealed that the decline in sIgA concentrations from AM to PM was a significant predictor of the rise in sIgA concentration from PM to AM for the following day (see Table S2). The model accounted for 23.8% explained variance ( $R^2_{adjusted}$ ). See also Table S4 for associations across time points for each analyte. We also tested if cisgender men in our sample demonstrated a typical diurnal T and CORT pattern [12,28–29,33,49]. In keeping with previous findings, the cisgender men's T and CORT concentrations declined from AM to PM and increased from PM to the following AM (Table S1). Cisgender men's concentrations of T and CORT did not differ across sequential mornings.

**Transgender men.** A similar approach was used for the transgender men group. The decline in sIgA from AM to PM approached significance while the rise in sIgA from PM to AM was significantly different from 0, indicating that transgender men also show some evidence of diurnal variation in sIgA (Table S1). The Δ sIgA concentration from Day 1 AM to Day 2 AM was not significantly different from 0, indicating that transgender men's sIgA concentrations remained relatively stable across mornings. A linear regression revealed that the decline in sIgA concentrations from Day 1 AM to PM was a significant predictor of the rise in sIgA concentrations from Day 1 PM to Day 2 AM. The model (Table S2) accounted for 12.7% explained variance ( $R^2_{adjusted}$ ).

Like our above analysis on the cisgender men, we tested if transgender men in our sample demonstrated a typical diurnal T and CORT pattern. Transgender men did not exhibit a significant decline in T from Day 1 AM to PM; however, they did experience a significant decline in CORT from Day 1 AM to PM. Similarly, transgender men did not exhibit a significant increase in T

from Day 1 PM to Day 2 AM; however, they did exhibit a significant increase in CORT across the same time period, indicating diurnality among transmen in CORT but not T (Table S2). The  $\Delta T$  and CORT concentrations from Day 1 AM to Day 2 AM was not significantly different from 0, suggesting that these hormone concentrations remained relatively stable across time. See Table 2 for a summary of results.

## 7. Are sIgA, T, and CORT related within individual saliva samples?

*Cisgender men.* To examine sIgA, T, and CORT relationships, we conducted a mediation model and specified covariances between the three biomarkers within the three sample times. This model fit the data well (see Table S3 in Supporting Information for all fit indices, see Table S4 for the standardized and unstandardized coefficients,  $z$ -statistic, standard errors and  $p$ -values). In Model 2 (Table S3, S4 and Fig. S2), we then substituted T and CORT concentrations with their cross-product ( $T \times CORT$ ). Significant positive covariances between T and sIgA, and  $T \times CORT$  and sIgA across sample provision times would extend findings from previous research (e.g., [6,57,59]), as they would provide evidence that the relationships between these biomarkers are stable across time.

In Model 1, the covariances between T and sIgA for Day 1 AM, Day 1 PM, and Day 2 AM were all significant (Table S6). Likewise, the covariance between CORT and T were significant across samples for: Day 1 AM, Day 1 PM, and Day 2 AM. The covariance between CORT and sIgA at Day 1 AM was not significant; however, covariances within the remaining samples were significant (see Fig. S1).

In Model 2, we then specified covariances between sIgA and  $T \times CORT$  within the same sample. This model fit the data well (see Table S3 for all fit indices). The covariance between  $T \times CORT$  and sIgA at Day 1 AM was not significant; however, there were significant covariances for Day 1 PM and Day 2 AM. See Fig. S2 for a visual representation of Model 2. Refer to Table S6 for the standardized and unstandardized coefficients,  $z$ -statistic, standard errors and  $p$ -values for each covariance. In order to understand the nature of this interaction, we plotted sIgA by T for high and low cortisol men using a median split (see Fig. 1), which indicated that sIgA and T were more closely, positively associated for low cortisol men.

*Transgender men.* Because of the small sample size, we used Pearson correlations for the transgender group rather than path models as described for cisgender men. To control for experiment-wise Type I error, we implemented Holm's sequential Bonferroni correction ( $\alpha = .05$ ). Because we conducted four Pearson correlations within each sample

time, we divided our alpha level from one to four and produced four alpha levels ( $0.05/1 = 0.05$ ,  $0.05/2 = 0.025$ ,  $0.05/3 = 0.0167$ ,  $0.05/4 = 0.0125$ ; [61]). Regarding the Day 1 AM sample, sIgA was not correlated with T, CORT, or  $T \times CORT$  (all  $r_s \leq .23$ , all  $p_s \geq .36$ ), and T was not correlated with CORT ( $r = .25$ ,  $p = .19$ ). For Day 1 PM, sIgA was positively correlated with CORT ( $r = .46$ ,  $p = .01$ ) and  $T \times CORT$  ( $r = .43$ ,  $p = .02$ ), but not T ( $r = .13$ ,  $p = .49$ ). Again, T was not correlated with CORT ( $r = .17$ ,  $p = .37$ ). For the Day 2 AM sample, sIgA was not significantly correlated with T, CORT, or  $T \times CORT$  (all  $r_s \leq .21$ , all  $p_s \geq .50$ ). The relationship between T and CORT was also not significant ( $r = .17$ ,  $p = .37$ ). Again, these findings should be approached with caution as the small sample size leaves the analyses underpowered to detect bivariate associations. See Table 2 for a summary of the results.

## 8. Is within-individual variation in sIgA related to within-individual change in T, CORT and $T \times CORT$ ?

*Cisgender men.* Although the above results provide evidence that sIgA, T, CORT, and  $T \times CORT$  are related to each other within multiple time points, they do not necessarily indicate whether these biomarkers fluctuate to the same degree over a 24-hour period. Therefore, we conducted another set of path models using the change in concentration ( $\Delta$ ) from Day 1 AM to PM and Day 1 PM to Day 2 AM, to determine if  $\Delta$  sIgA,  $\Delta T$ , and  $\Delta CORT$  were related to each other, and if  $\Delta$  sIgA was related to  $\Delta T \times CORT$ .

Similar to the previous analyses, we began by specifying a model in which the decline in sIgA, T, and CORT concentrations from Day 1 AM to PM predicted the rise in sIgA, T, and CORT concentrations from Day 1 PM to Day 2 AM (Fig. S3). To test for these relationships, we specified covariances between  $\Delta$  sIgA,  $\Delta T$ , and  $\Delta CORT$  within the same time period (Model 3; see Table S7). This model fit the data well (see Table S3). The decline in sIgA, T, and CORT from Day 1 AM to Day 1 PM significantly predicted the rise in sIgA, T, and CORT from Day 1 PM to Day 2 AM.

Inspecting the covariances amongst the difference variables (Table S8) revealed that the relationship between the decline in sIgA and CORT from Day 1 AM to Day 1 PM approached conventional significance levels. The rise in CORT and sIgA from Day 1 PM to Day 2 AM was significant. The declines in T and CORT from Day 1 AM to Day 1 PM were significantly positively related, and so was the rise in T and CORT from Day 1 PM to Day 2 AM. The declines in sIgA and T were significantly positively related and so was the subsequent rise in sIgA and T (see Fig. S3).

To test for relationships between the  $\Delta$  sIgA, and  $\Delta T \times CORT$ , we specified covariances between difference variables from Day 1 AM to Day 1 PM and from Day 1 PM to Day 2 AM (Model 4; Fig. S4). This model fit the data well (see Table S3). The decline in  $T \times CORT$  from Day 1 AM to Day 1 PM was a significant predictor of the rise in  $T \times CORT$  from Day 1 PM to Day 2 AM (see Table S7). The decline in  $T \times CORT$  from Day 1 AM to Day 1 PM was marginally related to the decline in sIgA. The rise in  $T \times CORT$  from Day 1 PM to Day 2 AM was positively related to the rise in sIgA (see Table S8).

We conducted separate path models to test if  $\Delta$  sIgA,  $\Delta T$ ,  $\Delta CORT$ , and  $\Delta T \times CORT$  from Day 1 AM to Day 1 PM significantly predicted change in these biomarkers across days (i.e., from Day 1 AM to Day 2 AM). We first specified a model in which  $\Delta$  sIgA,  $\Delta T$ ,  $\Delta CORT$ , from Day 1 AM to Day 1 PM predicted the change in these concentrations of these biomarkers across days (Model 5; Fig. S5). This model fit the data well (see Table S3). Men's decline in sIgA, T, and CORT from Day 1 AM to Day 1 PM significantly negatively predicted the change in these biomarkers across days (see Table S9). All covariances between the change in sIgA, T, and CORT across days were significant (see Table S10). The change in sIgA from Day 1 AM to Day 2 AM was significantly related to the change in T, and to change in CORT. Change in T from Day 1 AM to Day 2 AM was significantly related to change in CORT. The covariances

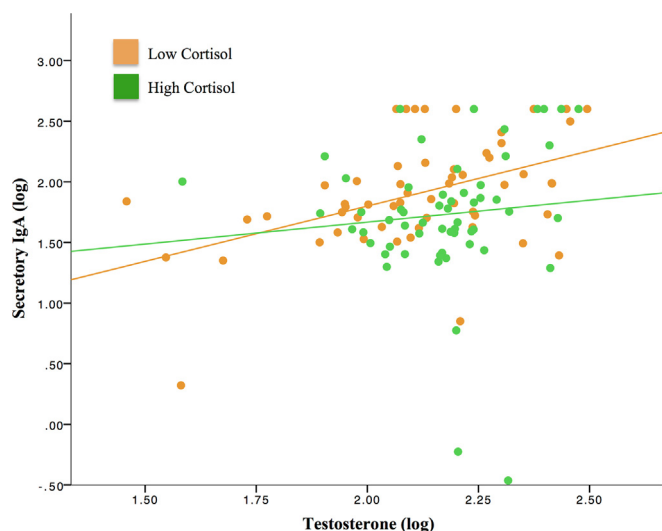


Fig. 1. Testosterone by Cortisol ( $T \times CORT$ ) interaction for cisgender men (evening sample).

amongst sIgA, T, CORT for the change in concentrations from Day 1 AM to Day 1 PM are similar to those of Model 3.

To test for relationships between the  $\Delta$  sIgA and  $\Delta$  T  $\times$  CORT, we specified covariances between difference variables from Day 1 AM to Day 1 PM and from Day 1 AM to Day 2 AM (Model 6; Fig. S6). This model fit the data well (see Table S3). Men's decline in T  $\times$  CORT, from Day 1 AM to Day 1 PM significantly negatively predicted the change in T  $\times$  CORT across days, (Table S9). The change in sIgA from Day 1 AM to Day 2 AM was significantly related to the change in T  $\times$  CORT (Table S10).

**Transmen  $\Delta$  concentrations.** To assess if  $\Delta$  sIgA,  $\Delta$  T,  $\Delta$  CORT and  $\Delta$  T  $\times$  CORT were related to each we compared these difference variables to each other within the same time point. Again, we implemented Holm's sequential Bonferroni correction ( $\alpha = .05$ ). The  $\Delta$  sIgA concentration from Day 1 AM to Day 1 PM was not significantly correlated with the  $\Delta$  T ( $r = .22, p = .27$ ),  $\Delta$  CORT ( $r = .29, p = .13$ ) and  $\Delta$  T  $\times$  CORT ( $r = .34, p = .08$ ) over the same time period. The  $\Delta$  T from Day 1 AM to Day 1 PM was not significantly related to the change in CORT over the same time period ( $r = .14, p = .49$ ). The  $\Delta$  sIgA concentration from Day 1 PM to Day 2 AM was significantly related to  $\Delta$  CORT concentration ( $r = -.48, p = .01$ ) and  $\Delta$  T  $\times$  CORT, ( $r = -.48, p = .01$ ), but not  $\Delta$  T ( $r = .22, p = .26$ ). The  $\Delta$  T from Day 1 PM to Day 2 AM was not significantly related to the  $\Delta$  CORT over the same time period ( $r = -.34, p = .08$ ). The  $\Delta$  sIgA from Day 1 AM to Day 2 AM was significantly related to  $\Delta$  CORT concentration ( $r = .55, p = .002$ ) and  $\Delta$  T  $\times$  CORT ( $r = .56, p = .002$ ), but not  $\Delta$  T ( $r = .30, p = .12$ ). The relationship between  $\Delta$  T and  $\Delta$  CORT from Day 1 AM to Day 2 AM approached conventional levels of statistical significance ( $r = .32, p = .09$ ). See Table 2 for a summary of results.

## 9. Discussion

The central goal of the present research was to examine the unique and combined effect of salivary T and CORT levels on one measure of mucosal immune function, sIgA, among populations of cis- and transmen. Our results show that sIgA is consistently and positively related to T and CORT within and between samples for cismen but not for transmen. These results are supported by similar findings on cisgender males from longitudinal [45], quasi-experimental [40,73,90], correlational [6,58], and non-human animal [110] studies, which have also shown positive associations between T and sIgA. This body of work suggests that T may play a role in influencing sIgA variation, which may have implications for explaining individual differences in mortality [93] and disease risk [38,45,47–48,87] in cismen.

Transmen's T levels were not significantly related to sIgA, although most of the associations were in the predicted positive direction. CORT levels were significantly associated with sIgA in half of the tests we employed. These novel results provide evidence that the associations between T, CORT, and sIgA may not be generalizable to other populations; however, a larger sample of transmen is needed to make more definitive conclusions. Future research may consider the source of T (i.e., endogenous vs. exogenous administration), the fact that medications for transgender T therapy are primarily testosterone esters rather than T itself (i.e., 83% of our transgender participants), and the specific testosterone ester administered as factors that may explain the differing relationships between sIgA, CORT, and T levels among cis- and transmen.

Previous research has typically assessed relationships between T and sIgA using a single sample [6,57–59]. We sought to extend these findings by examining intraindividual relationships between sIgA and T within three time points, as well as the relationships of individual change in concentrations across sequential time points. To this end, we conducted path models in which we specified covariances between all markers within each sample provision time. In all models, T and sIgA were positively related at all sample times for cismales. Because we demonstrated these relationships at two time points within a single day

and across two sequential days, it is unlikely that previous findings of a positive sIgA-T relationship are an artefact of sample time, but an indication of a relationship between the concentrations of T and sIgA. Therefore, the above findings provide some of the most robust evidence to date that T is associated with sIgA within young cisgender men.

This approach was facilitated by known diurnal variation in T characterized by a morning peak(s) followed by an evening decline (e.g., [12,29,49,69,104]). In the present study, we demonstrated that sIgA also exhibits a diurnal rhythm like T and CORT, characterized by a morning peak and then decline. Several studies have found variation in sIgA at different collection times [7,28]; however, this is the first study to our knowledge to directly assess the diurnality of salivary sIgA in young men. We found evidence of diurnal change in sIgA and CORT, but not T, among transgender men. We are not aware of any other studies on diurnality of T among transgender males; however, we posit that trans T levels are more likely influenced by administration schedule, route, and frequency.

Our second main goal was to assess the impact of CORT on sIgA and to assess whether CORT may interact with T. Consistent with previous findings [19], among cismales, we found positive relationships between T and CORT concentrations across all three sample times, and positive relationships between sIgA and CORT for the evening and second morning sample. Additionally, sIgA concentrations were positively related to T  $\times$  CORT for the evening and second morning sample. The relationship between the decline in sIgA and CORT from the morning to the evening was not significant; however, the remaining relationships between change in CORT and change in sIgA and T were all positively related. Unlike previous investigations examining relationships in the circadian rhythms of T and CORT [49,112], we found positive associations between T and CORT concentrations for both morning samples and the evening samples.

We also investigated the interaction of T and CORT (T  $\times$  CORT) and its effect on variation in sIgA. Previous research indicates that CORT may interfere, suppress, or antagonize T's action on target tissues in the body and the brain (see *dual-hormone hypothesis* for review, [19]), including immunity [97,100], and that T may act on HPA functioning [134]. Overall, our findings show general support for a relationship between sIgA and T  $\times$  CORT in both cis- and transgender men such that the positive relationship between sIgA and T is stronger for those lower in cortisol. The decline in sIgA from morning to evening on Day 1 was related to the change in T  $\times$  CORT in cis- but not transmen; however, the remainder of the results showed significant, positive relationships. Both samples show a positive relationship between sIgA and T  $\times$  CORT across subsequent days and from evening to the following morning.

Our unique sample of transgender men provided us with an opportunity to explore the relationship between T concentrations and secretory immunity among those exposed to regular T administration. The findings presented here suggest that sIgA is not strongly associated T in transmen, but may be associated with T  $\times$  CORT. Given the small sample size, however, these results should be treated with caution. Our results show that among both cis- and transmen, sIgA (and likely CORT) exhibit diurnality, and that these two biomarkers vary together with and between days. However, only in cismen does T show diurnal change, and only cismen's T and sIgA are related, suggesting that perhaps the association between them is an artifact of their similar diurnal pattern. On the other hand, the null relationship between T and sIgA may be due to the unique circumstances that affect transmen's T levels. Different routes of T administration, duration of T therapy, and dosages among transmen could lead to variation in how testosterone is metabolized, impacting intracellular communication and biological activity. A full description of the mechanism that connects salivary T with sIgA is a necessary step in understanding the relationship between them. Research utilizing a mouse model suggest that endogenous T affects the amount of sIgA in the urethral glands [135] and tears [136] by increasing the availability of secretory component, which is necessary for the transfer of IgA from epithelial plasma cells into the lumen and the

integrity of IgA in the presence of salivary proteolytic enzymes [137].

## 10. Implications

In sum, this research adds to our understanding of individual differences in respiratory infection risk and potentially mortality, which have both been linked to variation in sIgA [30,38,45–48,87,93,127,128]). Because T varies across individuals, time, and context [5,26,138], associations with mucosal immunity (and therefore the risk of respiratory infection) have practical implications, such as potential short- and long-term effects of T therapy on immunity [41,62,67,73,78]. Therefore, this research should be of interest not only to researchers in biology and medicine, but also those in exercise, public health, and other disciplines focused on the relationship between androgens and health.

Further, transgender individuals are a growing demographic: approximately 0.6% of the general population [39] and 1.2% of young adults [21] identify as transgender. For transmen, exogenous T therapy is the default treatment for masculinization, which has a positive impact on wellbeing [25,56,89,120]. Few studies, however, have empirically assessed how T therapy physiologically affects other systems, metabolic processes, or immunologic processes in the body (for reviews, see [60,118]). These and other findings suggest that T administration may have implications for immunity and health along with the expected observable changes in masculinity.

This research also has potential methodological implications. Our results provide initial evidence that sIgA, T and CORT exhibit similar, and potentially related, circadian rhythms; accordingly, research using these biomarkers and/or investigating relationships among them should sample concurrently (e.g., assay multiple markers from single saliva samples), while controlling for sample provision time. Furthermore, our results demonstrate the beneficial use of multiple sampling times for elucidating these relationships; and to date, represent the only such research to demonstrate the expected relationships between T and sIgA across multiple time-points.

## 11. Limitations

First, research on immune-endocrine associations suggests a complex immunomodulatory role for T (e.g., [114]); therefore, future studies would benefit from inclusion of biomarkers of both cellular and humoral immunity to achieve a broader picture of the relationship between T and immunity. Nevertheless, even as a single marker of immune function, sIgA has been shown to have important consequences for human health and mortality [30,38,45–48,87,93,93,127,128].

Second, only one saliva sample was collected in the morning, prohibiting assessment of the cortisol awakening response (CAR) which ideally requires participants to provide four samples, collected in 15-minute intervals over the first 60 minutes after waking (for a review see [129]). Precise assessment of CAR requires objective verification by researchers documenting waking times and morning time intervals of saliva collection [130] because evidence shows that participants often fail to accurately report their waking time, delay the time of providing the initial sample, or do not adhere to the required time intervals, all of which can result in false-high or -low estimates. One of the central aims of the present research, however, was to assess how cortisol and T covary with respect to IgA within individuals. That is, if cortisol is associated with T and/or sIgA, any variation in cortisol across the day—whether part of the CAR or not—should be associated with concomitant changes in T and/or sIgA. This conjecture is strengthened by research suggesting a testosterone awakening response [131], and is a promising avenue for future research.

Third, researchers have traditionally employed immunochemistry-based methods for analysis due to their high sensitivity and ease of use. However, future work would benefit from incorporating more sophisticated measures of salivary hormones that avoid cross-reactivity [91].

Liquid chromatography–mass spectrometry (LC-MS/MS) is considered the “gold standard” for salivary hormone analysis due to its superior selectivity compared to immunoassays [121]. This method benefits from analyzing multiple steroids simultaneously, is highly sensitive, and is considered the most technically sophisticated and accurate method available [72,108].

Fourth, participants for the present research were drawn exclusively from a healthy, “WEIRD” (Western, Educated, Industrialized, Rich and Democratic) population [55]. Future studies would benefit from sampling populations that inhabit an environment with more significant immunological burdens. For instance, research has shown that T is significantly lower in hunter-gatherer males than in North American males (e.g. [15]), and that these populations carry substantially larger pathogen burdens (e.g. Martin et al., 2013). Initial evidence suggests, however, that data from these types of populations may yield similar findings—i.e., a positive association between T and sIgA [59].

Finally, future studies on relationships between T and sIgA would benefit by including diverse samples, including cisgender women and/or cisgender men undergoing hormone therapy (e.g., hypogonadal men). We acknowledge that the circadian pattern of sIgA and T, although related in multiple study designs, may be independent of each other or jointly affected by a third variable. If the relationship between the circadian patterns of sIgA and T is not as pronounced in women as in men, then this may provide evidence for an interaction between men’s higher T and their concentrations of sIgA. This can only be determined by including a sample of age-matched women in future investigations. Similarly, future research should examine sIgA levels of hypogonadal men before and during T therapy to understand how sIgA changes with T dosage. These questions could also be addressed in an experimental design utilizing single-dose, short-term T administration (e.g., [17,122]).

## 12. Conclusions

Our findings revealed that among cisgender men, sIgA, T, and CORT exhibited clear circadian rhythms and were significantly related within and between samples. For transgender men, evidence for circadian change was found for sIgA and CORT, but not T. Further, sIgA was associated with CORT, but not T. This provides the first evidence that salivary T and sIgA concentrations are associated within a single day and across sequential days for cisgender men. Differences between cis- and transgender men suggest that this may only be true for T levels driven by endogenous production; however, future studies should employ a larger sample size.

### CRedit authorship contribution statement

**Carolyn R. Hodges-Simeon:** Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Writing - original draft, Resources, Visualization, Supervision, Funding acquisition. **Graham P.O. Grail:** Conceptualization, Methodology, Data curation, Investigation, Supervision, Resources, Funding acquisition, Writing - review & editing. **Graham Albert:** Conceptualization, Methodology, Formal analysis, Data curation, Visualization, Investigation, Writing - original draft. **Nicholas Landry:** Investigation. **Triana L. Ortiz:** Investigation, Writing - original draft. **Nicholas Landry:** Investigation. **Triana L. Ortiz:** Investigation. **Justin M. Carré:** Resources, Writing - review & editing. **Timothy S. McHale:** Writing - review & editing. **Steven A. Arnocky:** Resources, Writing - review & editing.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.steroids.2020.108640>.

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