Short Report

Dehydroepiandrosterone and Multiple Measures of Functional Immunity in Young Adults

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Objectives: Human immune function is strongly influenced by variation in hormone concentrations. The adrenal androgens dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEA-S) are thought to be beneficial to immune function and disease resistance, but physiologically interact with testosterone and cortisol. We predict that DHEA and DHEA-S will interact with these other hormones in determining immunological outcomes. Understanding the interactive effects of these hormones will aid in understanding variability in immunocompetence and clarify discrepancies in human studies of androgen–immune interactions.

Methods: Thirty-eight participants collected morning saliva over three days, from which concentrations of DHEA, DHEA-S, testosterone, and cortisol were measured, as well as salivary bacteria killing ability to measure innate immune function. From blood collection, serum was collected to measure innate immune function via a hemolytic complement assay, and whole blood collected and processed to measure proliferative responses of lymphocytes in the presence of mitogens.

Results: DHEA was negatively correlated with T cell proliferation, and positively correlated with salivary bacteria killing in male participants. Additionally, using regression models, DHEA-S was negatively associated with hemolytic complement activity, but interaction variables did not yield statistically significant relationships for any other outcome measure.

Conclusions: While interactions with other hormones did not significantly relate with immune function measures in this sample, DHEA and DHEA-S did differentially impact multiple branches of the immune system. Generally characterized as immunosupportive in action, DHEA is shown to inhibit certain facets of innate and cell-mediated immunity, suggesting a more complex role in regulating immunocompetence. Am. J. Hum. Biol. 27:877–880, 2015.

Understanding the determinants of immunocompetence is crucial when evaluating trade-offs in human life history decisions and influences on human health. Typically, hormones like testosterone and cortisol have been explored for their roles in mediating these trade-offs, as they respond dynamically to external stimuli and modulate immune function in direct appreciable ways. However, "weak" androgens like dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEA-S) have also been associated with immune function and disease outcomes in some contexts. Integrating DHEA and DHEA-S into immunocompetence in humans will aid in understanding how shifts in androgen concentrations alter life history decisions.

Testosterone is generally considered immunosuppressive, found to inhibit various immune components *in vitro*, and negatively related to immune activity in some contexts (Muehlenbein and Bribiescas, 2005). In humans, the relationship is much less consistent, with cross-sectional studies inconclusive and contradictory. These discrepancies may be due to the actions of unmeasured hormones, such as the DHEA and DHEA-S. Stimulation of DHEA and DHEA-S synthesis occurs via the hypothalamic–pituitary–adrenal (HPA) axis. Acute social stress causes elevations in both DHEA and DHEA-S alongside cortisol (Lennartsson et al., 2012). *In vitro* examination of DHEA indicates it also has immunological properties, modulating a number of immune factors (Hazeldine et al., 2010). It is likely that these hormones have important impacts in human immune function, and inclusion of analysis alongside testosterone can have important implications for explaining variation in immune function.

Beyond androgens, cortisol also has well defined immunological actions, dependent on the degree of HPA stimulation (McEwen et al., 1997), but its effects may be mediated by other hormones. For example, cortisol has significant interactions with testosterone which influence immunological responses to hepatitis B vaccination in humans (Rantala et al., 2012). Additionally, DHEA exerts interactive properties, acting as an anti-glucocorticoid in rodent experimentation (Ben-Nathan et al., 1992) and is related to disease and health outcomes in humans (Arlt et al., 2006; Phillips et al., 2010a,b). Exploring the immunological interactions between DHEA and cortisol would yield a greater understanding of how HPA activation mediates immune function.

It is clear that cortisol, testosterone, and DHEA independently have potent relationships with immunological components, and that these relationships modulate immunological outcomes relevant to understanding life

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TABLE 1. Comparison of mean (and standard deviation) endocrine and immune results by sex

	Male	Female
Hemolytic complement (CH50)	89.150 (11.087)	89.895 (23.953)
Salivary BKA (% killing)	74.0% (17.7%)	69.6% (19.5%)
LPA response to PHA	5.338 (1.903)	5.685 (1.733)
LPA response to Con A	3.898 (1.406)	3.751 (1.072)
DHEA (pg/ml)	219.373 (227.476)	127.8 (88.641)
DHEA-S (pg/ml)	8890.838 (6,232.011)	5883.542 (4,001.703)
Cortisol (ug/dl)	0.403 (0.191)	0.435 (0.179)
Testosterone (pg/ml)	168.362 (74.064)	66.9 (15.705)

No statistically significant sex differences were found in DHEA, DHEA-S, cortisol, or any immune measures ($P > 0.09$)

history allocations. As a precursor hormone on the HPA axis, DHEA and DHEA-S synthesis is inherently tied to testosterone and cortisol. These relationships have not yet been adequately explored in humans. In order to understand how DHEA and DHEA-S mediate immunological outcomes between these two axes, we examined the contributions of baseline concentrations of these hormones with multiple measures of immune function in young adults from a college-aged population. We predict that DHEA will be positively related with immune outcomes, while cortisol and testosterone will be negatively related with immune outcomes.

METHODS

Participants were recruited and sampled at the Evolutionary Physiology and Ecology Laboratory at Indiana University, Bloomington, following a rigorous exclusion and inclusion criteria. Participants were instructed to collect waking saliva samples via passive drool (in duplicate) on three non-consecutive days, before storing samples in an at-home freezer. Upon return of materials to the lab, anthropometrics were collected, and each participant underwent a standard blood draw from the forearm. Collected sera were stored at −80°C until analysis, while whole blood was processed immediately as described below.

To minimize the effects of anticipatory stress related to blood collection procedures, saliva samples were used to assess hormone concentrations. The three morning saliva samples were pooled and analyzed for DHEA, DHEA-S, testosterone, and cortisol concentrations using enzyme immunoassay kits according to manufacturer's instructions (Salimetrics #1-1202, #1-1252, 1-2402, and #1-3002, respectively). The intra-assay coefficients of variation, calculated by taking the mean coefficient of variation of the sample duplicates, were 6.1% for DHEA, 10.8% for DHEA-S, 6.1% for testosterone, and 3.7% for cortisol. The controls for assays were all within established limits. When necessary, samples that were out of range for an assay kit were diluted and re-run. Ultimately, four DHEA-S and three cortisol results were excluded due to high coefficients of variation or were out of range.

Pooled saliva was also used to assess functional salivary innate immunity via an *ex vivo* bacteria killing assay (BKA), with a 1:2 dilution using methods outlined elsewhere (Millet et al., 2007; Muehlenbein et al., 2011). Serum samples were used to assess innate immune function via a hemolytic complement assay, which measures the classical pathway of complement activity to lyse a known quantity of sheep red blood cells (SRBC) in the presence of antibodies. Samples were diluted 1:90 and 1:180, and assays were run using previously published methods, with results expressed in CH50 units (Sinclair and Lochmiller, 2000).

For the lymphocyte proliferation assay (LPA), lymphocytes were separated from whole blood using Ficoll-Paque following manufacturer's instructions, and cells resuspended in CellVation cryopreservation medium (MP Biomedicals #092030046) at an estimated concentration of 1 million cells/ml, and stored on liquid nitrogen until analysis. Thawed cells were resuspended in AIM V cell culture media (Gibco #12055-083) and a volume equal to 100,000 cells was diluted (in duplicate) in cell culture media on a 96-well flat bottom plate. Phytohemagglutinin (PHA) and concanavalin A (ConA) were added to their respective wells to reach a mitogen concentration of 10 μg/ml. Each sample was also run without mitogen (zero stimulation), and each plate contained background absorbance wells with mitogens and media only. Some samples did not recover enough cells to run all zero stimulation and mitogen wells in duplicate, and for these only single wells were used. Following incubation, Promega CellTiter (#G3582) was used to measure stimulation of cells, according to manufacturer's instructions.

Normality for all variables was assessed via Shapiro–Wilk tests. Sex differences were compared with an independent samples *t*-test, and homogeneity of variances was examined using Levene's test for equality of variances. One significant outlier (greater than two standard deviations from the mean) was removed to attain normality for CH50. Associations between hormones and immune variables were assessed via Pearson's correlations, unless variables could not be transformed into normality, in which case Spearman's correlations were used. Combined effects of hormones on immune variables were assessed via multiple regressions, using the Durbin–Watson statistic to test for independence of observations, and tolerance values to test for multicollinearity. Where interactions were included in the model, variables were centered to reduce multicollinearity by subtracting each variable from its mean.

RESULTS

Hormones

A total of 20 males and 18 females completed the study. Average values and sex differences for all endocrine and immune variables are in detailed in Table 1. Several of the hormones were correlated (Table 2), but there was no relationship between hormone concentrations and mean salivary collection time or age ($P > 0.10$).

Innate immunity

Date of blood collection was negatively correlated with CH50 (rho = −0.516, $P = 0.001$), indicating no loss of complement action with extended freezing. Additionally, there was no correlation between blood draw time and CH50, indicating no effect of diurnal variation ($r = -0.198$, $P = 0.241$). CH50 was not associated with any hormones or anthropometrics ($P > 0.10$).

Salivary BKA was not correlated with any variable examined, however, when separated by sex, BKA in males was positively correlated with DHEA and DHEA-S ($r = 0.538$, $P = 0.015$; $r = 0.514$, $P = 0.042$). Multiple regression to predict CH50 from transformed hormone values yielded a statistically significant model [F(25,

TABLE 2. Correlation coefficient values between endocrine variables

	DHEA	DHEA-S	Testosterone	Cortisol
DHEA	–			
DHEA-S	0.480[a]	–		
Testosterone	0.508[a]	0.384[a]	–	
Cortisol	0.508[a]	0.191	0.122	–

[a]$P < 0.05$

TABLE 3. Summary of multiple regression analyses for predicting CH50

Variable	*B*	SE_B	β
Androgens and cortisol used to predict CH50			
Constant	2.054	0.105	
Testosterone	0.073	0.040	0.329[a]
DHEA	0.047	0.043	0.236
DHEA-S	−0.092	0.026	−0.690[b]
Cortisol	0.044	0.052	0.157
Androgens and androgen interactions used to predict CH50			
Constant	2.016	0.055	
DHEA	0.082	0.038	0.452[b]
DHEA-S	−0.074	0.032	−0.482[b]
Testosterone	−0.088	0.095	0.345
DHEA*DHEA-S	0.058	0.116	0.106
DHEA*T	−0.140	0.180	−0.167
Sex	−0.052	0.037	−0.495

[a]$P < 0.10$;
[b]$P < 0.05$; *B* = unstandardized coefficient; SE_B = standard error of coefficient; β = standardized coefficient

4) = 3.515, $P = 0.021$, adjusted $R^2 = 0.258$]. Only DHEA-S contributed significantly to the model, while testosterone was marginally predictive (Table 3). Multiple regression to predict transformed BKA found no significant relationship for these variables ($P = 0.195$).

In order to understand the interactive effects of hormones on immunological outcomes, additional multiple regressions with interaction variables were run for hormones and sex. When examining the interactions among DHEA, DHEA-S, and testosterone on effects on CH50, multiple regression yielded a significant model [$F(26,6) = 2.811$, $P = 0.030$, adjusted $R^2 = 0.253$], with both DHEA and DHEA-S as significant predictors, although in opposite directions (Table 3). Other regressions for BKA and CH50 as dependent variables were not statistically significant ($P > 0.07$).

Cell-mediated immunity

Both Con A and PHA stimulation indices were positively correlated ($r = 0.729$, $P < 0.0005$), but neither variable was related with CH50 ($P > 0.10$), and PHA was significantly negatively correlated with BKA (rho = −0.383, $P = 0.017$). DHEA was negatively correlated with PHA ($r = -0.339$, $P = 0.037$; Supporting Information Fig. 1), and marginally with Con A ($r = -0.297$, $P = 0.07$), but LPA results were not significantly related with any other hormone ($P > 0.10$). However, when analyzed by sex, male DHEA was significantly correlated with both Con A and PHA ($r = -0.561$, $P = 0.010$; $r = -0.451$, $P = 0.046$ respectively). In females, there were no significant correlations between LPA results and any hormone ($P > 0.10$).

All four hormones as predictors did not generate a statistically significant regression model for Con A or PHA ($P > 0.3$). As with the innate immune measures, PHA and Con A were used as determinant variables in multivariate regressions to understand the effects of hormones in the HPA and androgen synthesis axis. Regression models using hormones and interactions related to the HPA axis and androgen synthesis failed to reach significance for either Con A or PHA ($P > 0.2$).

DISCUSSION

Associations between innate immune measures and endocrine variables appear to be sex-linked and dependent on the particular immune measure used. While there were no correlations between baseline hormones and innate immune responses, results from multiple regression models indicate that DHEA-S negatively influences, and testosterone positively influences, complement activity. However, when interactive variables are included in models using hormones related to androgen synthesis, DHEA positively predicts CH50. One interpretation is that DHEA is acting in a beneficial manner to this measure of immune function, and elevated DHEA-S reflects decreased conversion to the active form of the hormone. This is contrary to the findings of *in vitro* experimentation (Hidvégi et al., 1984), but results may be concentration dependent or influenced by additional factors not measured here.

Results from lymphocyte proliferation in relation to hormone levels were also sex specific, with DHEA moderately negatively associated with proliferative responses in the sample as a whole as well as in males, but not females. In some studies using rodents, DHEA acts to inhibit cellular immunity (Ben-Nathan et al., 1999; Padgett and Loria, 1994). In human lymphocytes subjected to various concentrations of DHEA *in vitro*, DHEA inhibited T cell proliferative responses, but increased B cell responses (Sakakura et al., 2006). The present study is the first to demonstrate this relationship using natural circulating concentrations of DHEA in assessing proliferative responses, and results suggest that DHEA downregulates responses of T cells.

Innate immune variables were not correlated in this sample, suggesting that different branches of innate immunity were measured. Surprisingly, lymphocyte responses to mitogens were moderately negatively related to salivary BKA, suggesting a possible trade-off between these immune components. Given that DHEA was positively correlated with BKA (in males), but negatively correlated with lymphocyte responses to T cell mitogens, it is possible that DHEA mediates these trade-offs, such that elevated DHEA results in higher secretory innate immunity but reduced cell-mediated immunity.

These results suggest a much more complex role for DHEA in immunocompetence than previously appreciated. While human studies indicate that DHEA acts as an anti-inflammatory and is protective against multiple types of parasitic infection, results from the current study suggest that DHEA has pleiotropic actions on immune function, and is not universally immunosupportive as sometimes suggested. Instead, DHEA preferentially upregulates particular facets, while downregulating others, acting in concert with other hormones to influence immune outcomes. These results make clear the notion that a cohesive understanding of hormone-mediated interactions requires analysis of more than a single hormone. DHEA in particular is interactive in that it is associated with both the HPA axis and sex steroid synthesis.

The role of DHEA in influencing immunocompetence may explain discrepancies in research investigating the immunosuppressive effects of testosterone, and can be used to understand changes in immunocompetence and disease susceptibility during aging. Future research should focus on understanding determinants of variability in DHEA concentrations within and between populations, and the immunological implications of this variation.

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