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RESEARCH ARTICLE

# Does exposure to parasites modify relationships between diurnal cortisol and leukocytes among Honduran women?

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## Abstract

**Background:** Altered hypothalamic–pituitary–adrenal (HPA) function and related changes in circulating glucocorticoids have been implicated in the pathogenesis of numerous diseases that involve dysregulated immune function. Glucocorticoid hormones have both direct and indirect modulatory effects on both pro- and anti-inflammatory aspects of the immune system, including granulocytic and lymphocytic leukocyte subsets. However, past findings are complicated by inconsistencies across studies in how glucocorticoids and immune markers interact and relate to disease risk. Some incongruencies are likely due to an overreliance on single-unit (e.g., HPA or one immune marker) measures, and a failure to consider ecological exposures that may shape the base levels or correspondence between these systems. Here, we test single-unit and diurnal measures of HPA axis and immune system interactions in a less-industrial ecological setting with relatively high parasite loads.

**Methods:** In a sample of 114 Honduran women (mean age = 36 years), morning and evening blood samples were analyzed to quantify granulocytes, lymphocytes, and immunoglobulin-E (IgE). Saliva was collected over 2 days (8 samples per woman) to measure peak cortisol, cumulative cortisol, and slope of decline. These repeated measures of saliva and venous blood were used to investigate associations between single-point and diurnal salivary cortisol and leukocytes, under variable levels of past parasite exposure (proxied by IgE).

**Results:** Individuals with less of a decline in cortisol (i.e., "flatter" decline) show less of an increase in lymphocytes (2.27% increase in cells/μL/hr; 95% CI: 0.91–7.29; $p = .01$) across the day compared to those with steeper cortisol decline (7.5% increase in lymphocytes; 95% CI: 5.79–9.34; $p < .001$). IgE levels did not modify this association. Interestingly, IgE did moderate relationships between measures of cortisol and granulocytes: diurnal cortisol was positively associated with granulocytes, only in

  

individuals with high previous exposure to parasites. There were no consistent relationships between single-unit measures of cortisol, lymphocytes or granulocytes, regardless of past parasite exposure.

**Discussion:** Results demonstrate that the relationship between HPA function and immune modulation cannot be fully understood without an understanding of local disease ecology. These results highlight the importance of research that seeks to identify etiologies of disease across environmental contexts.

**KEYWORDS**

diurnal immune function, ecological immunology, HPA–immune interactions, Latin American, salivary cortisol

## 1 | INTRODUCTION

Alterations to the hypothalamic–pituitary–adrenal (HPA) axis and its primary hormones, glucocorticoids, have been linked to a wide range of diseases from cardiometabolic and psychiatric pathologies to cancers (Brunner et al., 2002; Sephton, 2000; Weinrib et al., 2010). It is increasingly evident that the link between HPA function and poor health is often mediated by actions of glucocorticoids on the immune system (Chrousos, 1995; Dhabhar, Miller, Stein, McEwen, & Spencer, 1994; McEwen et al., 1997). Dysregulation of HPA and proinflammatory immune signaling (i.e., glucocorticoid resistance), for example, is a major precursor to many diseases (Jarcho, Slavich, Tylova-Stein, Wolkowitz, & Burke, 2013; Miller et al., 2008). However, as evidenced by cross-cultural studies, there are inconsistencies regarding how HPA function, the immune system, and health status are linked across different populations and disease ecologies (Gurven et al., 2009; Kaplan et al., 2017b; Souza-Talarico, Plusquellec, Lupien, Fiocco, & Suchecki, 2014). Some inconsistencies may be due to an over-reliance on single-unit, or single-system, measures of diurnal biomarkers—whose relationships are dynamic across the day.

Local disease ecology (e.g., exposure to parasites and other pathogens) also shapes the development of the immune system and adult immune responses (Blackwell et al., 2011, 2016a; Gluckman, Hanson, Spencer, & Bateson, 2005; McDade, 2012). A largely unexplored and potentially important question is the extent to which differences in ecological exposure may further influence how the body's HPA axis and immune system themselves interact. For example, we know little about how these systems interact under conditions of higher pathogen exposure.

The major aim of the present study is to identify how diurnal patterns and single-unit measures of HPA and immune function relate under variable levels of past and present parasitic exposure. To accomplish this aim, we evaluate associations between multiple measurements of salivary cortisol and peripheral leukocyte subsets (lymphocytes and granulocytes) among individuals living in a relatively parasite-rich setting, on the island of Utila.

### 1.1 | Bidirectional HPA and immune pathways

Though HPA activation was previously considered to be immunosuppressive (Lindberg & Frenkel, 1977; Veen et al., 2009), in the context of normal physiology, HPA activation is better conceptualized as "immunomodulatory," enhancing and suppressing different aspects of the immune system, dependent upon the type of response and immune cell type (Silverman, Pearce, Biron, & Andrew, 2005). Numerous in vitro studies find that in response to acute natural or psychosocial stress, or exogenous administration, glucocorticoids redistribute lymphocytes away from the periphery and suspend apoptosis of neutrophils (the most abundant type of granulocytic cell) (Davis, Maney, & Maerz, 2008; Fauci, 1975; Padgett & Glaser, 2003). Glucocorticoids further enhance immunoglobulin production, biasing in favor of the development of T cells that produce Th2 (anti-inflammatory) cytokines (Elenkov, 2004; Petrovsky, 2001).

Many components of the HPA axis and immune system also exhibit circadian rhythmicity, varying in activity across the day (Dimitrov et al., 2009; Haus & Smolensky, 1999; Lowrey & Takahashi, 2004; Man, Loudon, & Chawla, 2016; Trifonova, Zimmer, Turner, & Muller, 2013). A number of cells and signaling molecules of the immune system follow antiphasic diurnal rhythms to—and are largely controlled by— circulating glucocorticoids (Chrousos, 1999; Cermakian et al., 2013; for a comprehensive review: Labrecque & Cermakian, 2015). For example, diurnal fluctuation in glucocorticoids, in conjunction with non HPA-derived hormones (e.g., melatonin, 17β-oestradiol), modulate the expression of various chemokines, β-adrenergic receptors and chemokine receptors, which mediate circadian homing patterns in leukocytes (Butcher & Picker, 1996; Ince, Weber, & Scheiermann, 2019; Straub, Cutolo, Buttgereit, & Pongratz, 2010). In humans, peripheral lymphocytes are at their lowest early in the day, in parallel with the morning cortisol spike, and peak during evening hours when cortisol levels are falling (Ackermann et al., 2012; Haus & Smolensky, 1999). The HPA axis and immune system together are responsible for restoring or maintaining metabolic homeostasis through these regulatory interactions (Dumbell et al., 2016; Straub et al., 2010).

Diurnal oscillations in the proinflammatory immune system likely coevolved in coordination with the HPA axis and other circadian regulators, to optimize immune responses and minimize vulnerability to runaway inflammation (e.g., autoimmunity or sepsis) (Man et al., 2016). This occurs through a series of signaling and negative feedback processes which control the expression and activity of key regulators of adaptive immune function, including some cellular and tissue repair mechanisms (Chrousos, 1999; McEwen et al., 1997; Vukelic et al., 2011).

Importantly, communication across the HPA axis and immune system is bidirectional: the abundance and class of cytokines, immunoglobulins, interleukins, and other molecules present exert influences on neuroendocrine function and the subsequent immunological cascade (Chrousos, 1999; Dhabhar & McEwen, 1997; Fauci, 1975; Petrovsky, 2001; Vukelic et al., 2011). For example, upon immune activation, the immune system releases various cytokines via innate and adaptive immune channels, including proinflammatory cytokines like tumor necrosis factor-alpha (TNF-α), interleukins (IL) 1 and 6, and type I interferons (IFN α/β), and anti-inflammatory proteins like the T-cell cytokines IL-2 and type II interferons, which activate the HPA axis and result in the release of adrenal glucocorticoids (Silverman et al., 2005). In turn, glucocorticoids negatively feedback on immune cells to suppress further synthesis and release of cytokines. Thus, differences in overall exposure to pathogens may also coincide with distinct relationships between glucocorticoids and immune markers.

### 1.2 | HPA–immune interactions in ecological context

The low-pathogen urban environments in which many people now live are an evolutionary novelty. More than 99% of human history was spent living under conditions thought to be characterized by chronic exposure to a diverse array of parasites and microbes (Brachman, 2003). Parasitic and microbial infections are associated with a shift toward Th2-biased (anti-inflammatory) immune responses (Chandramathi, Suresh, Sivanandam, & Kuppusamy, 2014; Yazdanbakhsh, Van Den Biggelaar, & Maizels, 2001). A growing body of literature suggests that the higher prevalence of chronic inflammatory diseases in developed regions may be due to the loss of parasitic and microbial exposures, resulting in a Th1-biased (inflammatory) immune system (Georgiev, Kuzawa, & McDade, 2016; McDade, 2012; McDade et al., 2017; Raison, Lowry, & Rook, 2010; Rook, Lowry, & Raison, 2013; Yazdanbakhsh, Kremsner, & van Ree, 2004), and that early exposure to parasites and microbes promotes effective immunomodulation, decreasing the vulnerability of runaway inflammatory responses (Böbel et al., 2018; McDade, Hoke, Borja, Adair, & Kuzawa, 2012). Past research has suggested that high exposure to parasites and microbes can be evidenced by differences in base levels of immunoglobulin-E (IgE), an antibody synthesized by the immune system in response to parasitic infection. IgE levels increase at the onset of infection and typically fall after treatment (Hagel, Cabrera, Sanchez, Rodrigues, & Lattouf, 2006). However, in high pathogen environments where individuals experience chronic parasitic infections, individuals' total IgE levels remain elevated compared to those from industrialized populations, suggesting persistent changes in host immune function (Blackwell et al., 2011; Iancovici Kidon et al., 2005).

The vast majority of our understanding of how glucocorticoids and immune markers relate to each other is derived from experimental in vitro lab-based studies or in vivo studies in the United States and Europe, where there is overall low exposure to pathogens. There has been little empirical research in humans on the potential variability of relationships between diurnal glucocorticoids and immune markers under a range of ecological conditions. Given the bidirectional nature of HPA and immune signaling, differences in background immunomodulation by parasites and microbes may influence absolute levels of hormones and immune markers. However, stable coordination *between* these systems is critical for regulating numerous features related to metabolic homeostasis and somatic maintenance, regardless of infection history.

In this study, we evaluate repeated measures of salivary cortisol (a key human glucocorticoid hormone) and leukocyte subsets (i.e., lymphocytes and granulocytes) across the day to investigate associations between single-unit (i.e., single-shot and aggregate) measures and diurnal changes in salivary cortisol and leukocytes, under variable levels of past parasite exposure (proxied by IgE level). We propose that, given their critical role in maintaining metabolic homeostasis, the antiphasic relationship between the diurnal rhythms of cortisol and the proinflammatory immune system should not vary substantially due to differences in past exposure, whereas absolute levels of hormones or immune markers (e.g., single-shot or aggregate measures) are likely calibrated according to localized ecological and physiological demands, and may thus relate differently depending on level of past exposure. We test this hypothesis in a sample of mainland Honduran women living a relatively high pathogen context on the island of Utila.

## 2 | METHODS

### 2.1 | Participants and recruitment

Utila is the smallest of the Honduran bay islands and is home to native Utilians (of British and American ancestry) and immigrants from mainland Honduras. Pathogen prevalence on mainland Honduras is relatively high compared to higher-income countries with 40.6% of Honduran municipalities having >50% prevalence of soil-transmitted helminths (Sanchez et al., 2014). While possibly lower than numbers from mainland Honduras, parasitic infections on Utila are still relatively high: in this sample, 33% of Honduran immigrant women tested positive for at least one strain of helminthic or protozoal infection based on microscopy analysis from fecal samples (Garcia, 2018).

Data for this study were collected as a part of a larger project assessing the effects of social adversity on diurnal cortisol and immune profiles. Stratified sampling was used to recruit a sample of 123 Honduran mainland immigrant women. Only Honduran women

**TABLE 1** Descriptive information for participants in the sample, reported as mean (*SD*), except for Immunoglobulin-E, which is reported as geometric mean (geometric *SD*)

| Characteristic | Distribution |
|---|---|
| Age | 36 years (18–82) |
| Body mass index | 29.1 kg/m$^2$ (6.13) |
| Waist-to-hip ratio | 0.85 (0.07) |
| Medical diagnoses | |
| Diabetic | 7.4% |
| High blood pressure | 15.7% |
| Pregnant | 3.3% |
| Asthma or allergies | 22.3% |
| Medicine use | |
| Hormonal birth control | 27% |
| Diabetes medication | 4.4% |
| Blood pressure medication | 7.1% |
| Smoking | 4.1% smoke daily |
| | 1.7% smoke occasionally |
| Hours of sleep | 7.61 hr (1.63) |
| Quality of sleep | 25.2% sleep well |
| | 25.2% might wake up once |
| | 39.5% wakes up multiple times |
| | 10.1% does not sleep well |
| Bathroom type | |
| Private, with flush | 69.0% |
| Private, no flush | 20.4% |
| Shared, with flush | 8.0% |
| Shared, no flush | 12.5% |
| Latrine | 3.0% |
| Immunoglobulin-E | 298.08 IU/ml (2.88) |
| Current parasitic infection ($n$ = 99) | |
| Helminthic | 5.6% |
| Protozoal | 27.1% |
| Cortisol decline slope | −0.128 log pg/ml/hr |
| Cumulative cortisol (AUCg, logged) | 11.36 (0.33) |
| Peak cortisol (logged) | 9.46 (0.38) |
| Morning leukocyte subset counts | 5.10 (1.81) × 10$^3$ granulocytes |
| | 2.43 (0.71) × 10$^3$ lymphocyte/monocytes |
| Evening leukocyte subset counts | 5.42 (1.83) × 10$^3$ granulocytes |
| | 2.92 (0.88) × 10$^3$ lymphocyte/monocytes |
| Lymphocyte/granulocyte proportion | |
| Morning | 33.2% (7.25) |
| Evening | 35.5% (6.75) |

*Note:* Data on medicine use, smoking, sleep, bathroom type, and pregnancy status also included, though not included in final models.

are included in the study sample as a consequence of the broader research project, which focuses on evaluating stress and immune activity in metabolic profiles among this demographic. Individuals were recruited through the community clinic, word of mouth, and outreach. Individuals gave informed verbal consent before participating. All data collection protocols were approved by the Institutional Review Board at University of California Santa Barbara (UCSB, #1-18-0545). The local Honduran governance and the community health center on Utila also approved the project and all protocols. Individuals were compensated for any part of the research in which they participated, with those that participated in all aspects paid 200 Honduran Lempira ($9 USD or ~2.5 hr of typical wage labor). Of our initial sample of 123 women, data were incomplete for nine women, due to accidental omission by the interviewer (inadvertently missing questions or forgetting to take anthropometric measurements), reducing the final sample size to 114 women. The sample captures a wide range of socioeconomic and ecological variation within the immigrant community on the island (Table S1) (García, Gurven, & Blackwell, 2017).

## 2.2 | Past exposure to parasites

We use plasma IgE level as a proxy for past exposure to parasites. Individuals were stratified as having low (IgE <500 IU/ml) or high (IgE ≥500 IU/ml) parasite exposure. This cutoff point was chosen to differentiate "normal" levels of IgE in non-infected, non-atopic, adults, from IgE levels that suggest a phenotypic shift driven by chronic exposure to parasites throughout life (Blackwell et al., 2011). Though IgE is variable and is altered by a number of environmental and physiological factors, the "normal" range for healthy adults typically falls below 100 IU/ml across most studies, with an upper limit between 150–458 IU/ml (Bazaral, Orgel, & Hamburger, 1971; Wittig, Belloit, De Fillippi, & Royal, 1980). The geometric mean for IgE in this sample is 297.08 IU/ml; 23.5% of individuals have high IgE. The typical Honduran woman in this sample has six-fold higher IgE than age-matched American woman (Blackwell et al., 2011).

## 2.3 | Data collection

### 2.3.1 | Procedures for blood collection and interview

Morning venous blood samples were collected into EDTA-coated vacutainers by a trained phlebotomist from the community clinic.

Following collection of the blood sample and anthropometrics (height and weight), individuals were interviewed about their health history for known major ailments and diagnoses, current or recent illness, sleep quality and quantity, reproductive history (including date of last menstruation), and medication use, including hormonal birth control. Approximately 8 hr later, a second blood sample was taken from each participant, following the same procedures as the initial blood draw. Self-reported time of waking and exact collection times were noted for each sample.

### 2.3.2 | Procedure for saliva collection

Four saliva samples were collected from participants for two consecutive days at (a) waking, (b) 30 min, (c) 45 min, and (d) 8 hr after waking (a total of 8 samples per individual) to establish diurnal cortisol profiles. Saliva was collected by passive drool (García et al., 2017) and stored in a −20°C freezer within 12 hr of collection until the end of the field season (up to 4 months). Salivary cortisol shows strong diurnal variability, which makes precise collection times critical for calculating slopes and area under the curve (AUC). As such, trained researchers collected samples from all but three participants, who collected their own samples at the specified times. Participants were asked what time they usually woke up, and researchers arrived at their house a few minutes prior to their typical waking time to collect the first sample. The median sampling times were: (a) waking: 1.2 min post-waking, (b) 31.2 min post-waking, (c) 46.2 min post-waking, and (d) 8 hr, 3 min post-waking (refer to Figure S1 for range distributions). Waking time and exact collection times were recorded for all samples and included in cortisol models.

### 2.3.3 | Procedure for fecal collection and parasite analysis

During their visit to the community clinic for a blood draw, participants were provided with sterile plastic polypropylene specimen collection cups to independently collect a fecal sample. Samples were returned to the laboratory in the community clinic the same or the following day. Fecal samples were analyzed for the presence of helminth eggs and larvae and protozoa by direct identification on the same day that they were brought to the laboratory by a trained laboratory parasitologist (MM). Mounts were prepared with 0.9% saline solution and iodine solution, respectively, and examined at 100× and 400× for presence of helminth eggs and larvae, and protozoa. Infections by helminthic parasites consistent mainly of *T. Trichiura* (whipworm), and to a lesser extent of *Taenia spp*. (tapeworm) and *A. lumbricoides* (roundworm); protozoal infections include *G. lamblia* (giardia) and common amoebae (e.g., *E. nana*, *E. coli*, and *E. histolytica*, and *I. buetschlii*). Parasite infection was recorded as presence or absence of each species (Garcia, 2018).

## 2.4 | Biological assays

### 2.4.1 | Blood samples

Within 2 hr of venipuncture, total leukocyte counts, granulocyte counts, and lymphocyte/monocyte counts were determined by a complete blood count assay with two-part differential using a QBC Autoread Plus dry hematology system (Drucker Diagnostics, PA). These analyses were conducted by the lead author (AG) and the laboratory director and co-author (MM) at the community clinic. Henceforth, we refer to lymphocyte/monocyte values solely as lymphocytes because they comprise the vast majority of this category, accounting for up to 50% of all leukocytes, whereas monocytes represent only 2–10% in industrial populations, and <1% in high pathogen contexts (Blackwell et al., ).

Plasma samples were stored at −20°C for up to 4 months, then transported on dry ice and kept at −80°C in the UCSB Human Biodemography Lab until analysis. Total IgE was analyzed from morning plasma samples using commercially available Human IgE ELISA quantification assay by Bethyl Laboratories (catalog #E80-108). IgE was converted from ng/ml to IU/ml (2.4 ng/ml = 1 IU/ml) to follow typical reporting standards. The within and between assay CVs for IgE ($n$ = 4 plates) were 2.75 and 2.80% for the high (263.37 ng/ml), and 1.54 and 1.32% for the low (61.76 ng/ml) in-house controls, respectively.

### 2.4.2 | Saliva samples

Saliva samples were stored and transferred to UCSB with plasma samples. Samples were centrifuged at 1500$g$ for 20 min, and the aqueous layer assayed in duplicate using a validated in-house enzyme immunoassay utilizing C. Munro's R4866 anti-cortisol antibody (Munro & Stabenfeldt, 1985; Trumble, Brindle, Kupsik, & O'Connor, 2010). The limits of detection for this assay are 78.125 to 10,000 pg/ml (Munro & Stabenfeldt, 1985). The within and between assay CVs for cortisol ($n$ = 27 plates) were 5.63 and 2.04% for the high (4,622 pg/ml), and 5.94 and 3.39% for the low (726 pg/ml) in-house controls, respectively.

## 2.5 | Diurnal cortisol and leukocyte modeling

Cortisol values were natural log transformed before analysis due to skewed distributions and because a log-linear model fit the pattern of cortisol decline across the day better than a linear model. To create an easily interpretable way of modeling the shape of the cortisol profile with terms for the cortisol awakening response (slope of the increase from waking to peak morning cortisol, that is, CAR), and the slope of decline during the latter part of the day, we modeled cortisol relative to the within-sampling-day peak cortisol value. The peak was defined as the sample for a particular individual on a particular day with the highest cortisol value collected <1.5 hr after waking. This cutoff was chosen in order to capture the peak during the early part of the day

(which usually occurs within the first hour of waking), and was based on collection times for the morning samples: >95% of the first three of the day were collected within this time frame, and because a few individuals peaked in the evening, possibly due to evening shift work. Random effects include intercepts for individual and sampling day (nested within individual). Cortisol models were fit in R 3.5.1 (R Core Team, 2018) using the *lme* function in the *nlme* package (Pinheiro, Bates, DebRoy, Sarkar,, & R Core Team, 2019). Using the values from these models (CAR and "slope"), along with the time of the cortisol peak, AUC was calculated from waking to 12 hr post-waking using the *integrate* function in R. Due to the slight left skew, AUC was log-transformed before analyses; however, the results were consistent in direction and significance when using non-logged AUC. Also note that though CAR is described here as a part of the overall cortisol modeling, this article focuses exclusively on the slope of change from peak to afternoon (diurnal slope) as a measure diurnal cortisol, and peak cortisol and AUC for measures of "single point" and "cumulative" cortisol measures, respectively. For each measure of diurnal change in leukocyte subset counts (lymphocyte and granulocyte count and proportion), slopes were calculated using a basic formula for a two-point slope, converted into percent change (i.e., $[(m_2 - m_1)/m_1/(t_2 - t_1)]$). Due to the causal direction in analyses (cortisol predicting leukocytes), to meet the requirements of using generalized linear models, diurnal slope, peak cortisol, and AUC values were averaged across the two sampling days to provide one measure per individual. However, results did not differ in effect size of statistical significance using estimating models that include random effects versus generalized linear models. Figure S2 shows the individual level diurnal cortisol fits and distribution of estimated components for the full sample. See Supplementary Materials Data (S1) for full description of cortisol and leukocyte modeling.

### 2.5.1 | Potential confounding variables for cortisol, leukocytes, and IgE models

In models that tested predictions regarding associations between diurnal cortisol and leukocytes, we included diabetic status as a covariate, due to its common co-occurrence with glucocorticoid resistance (GCR), that is, a breakdown in signaling between these systems (Carvalho et al., 2015; Silverman et al., 2005; Silverman & Sternberg, 2012). A covariate for self-reported current illness (coded as a binary variable where 1 = currently feeling "very sick" and 0 = not feeling "very sick") was included to adjust for potential effects of immune activation on GCR (Miller et al., 1997). To account for potential variation in leukocyte slopes due to the lag between when an individual woke up and when the first sample was taken, we included "time since waking" measured in hours (or fractions of) as a covariate, (Cermakian et al., 2013; Hriscu, 2005). Finally, to rule out the possibilities that IgE was driven primarily by either current parasitic infection, asthma or allergies (Wittig et al., 1980), we ran additional models controlling for current infection with helminthic parasites, analyzed via fecal microscopy (33% of sample, data available for $n$ = 99) and self-reported allergies or asthma (22.3% of full sample) for models assessing the interactive effects of IgE and cortisol.

### 2.6 | Statistical analyses

Multiple linear regression models were used to analyze variation in the distribution of hematological parameters (lymphocyte and granulocytes counts and percentage of lymphocytes) as a function of the main cortisol parameters (peak cortisol, AUC, and slope), adjusting for covariates. Due to skewed distributions, morning and evening granulocyte counts and evening lymphocyte counts were square-root transformed, and morning and mean lymphocyte counts were exponentiated to the negative one-half ($n^{-1/2}$). Cortisol and leukocyte measures were standardized for analyses, to enable comparison across models. To assess any moderation effects of IgE on the relationship between cortisol and leukocytes, a dichotomous interaction term between IgE and measures of cortisol was added to models. For biological interpretation, for statistically significant findings, we reran models using non-transformed DVs and standardized cortisol parameters using robust regression models, using the R package "MASS" (Venables & Ripley, 2002), and report both statistically and biologically relevant results in each section. Finally, please see the Supplement for information on data sharing and access.

## 3 | RESULTS

Table 1 presents descriptive information about the women in the study. The mean age of participants was 36 years old (range: 18–82 years) and mean body mass index was 29.1 kg/m$^2$ (6.1 SD). Eight women in the study responded that they were diagnosed diabetic, and four responded that they were pregnant. Neither self-reported diabetic nor pregnant women differed substantially from the larger sample across most variables of interest, though it is possible that this is due to the small number of individuals in each group (Table S1). However, omitting these individuals did not significantly alter the main effects or direction across any model, so they were included in all subsequent analyses. A covariate for diabetic status was included to adjust for any relevant differences.

### 3.1 | Cortisol, leukocyte subsets, and IgE

Overall, cortisol shows the expected diurnal pattern in this sample: there is a sharp increase in cortisol shortly upon wakening and a decline over the afternoon (Figure S2).Cortisol levels peak at ~32 min after waking (mean), and the mean slope of decline is –0.128 log (pg/ml)/hr. Adjusting for time differences between waking and when the first sample was taken, both lymphocyte ($\beta$ = 60 cells/μL/hr, $p$ < .001) and granulocyte ($\beta$ = 70 cells/μL/hr, $p$ = .01) counts significantly increase across the day (Figure 1); however, lymphocyte counts increase almost twice as much as granulocytes (5.1% increase in lymphocytes compared to 2.8% increase in granulocytes between

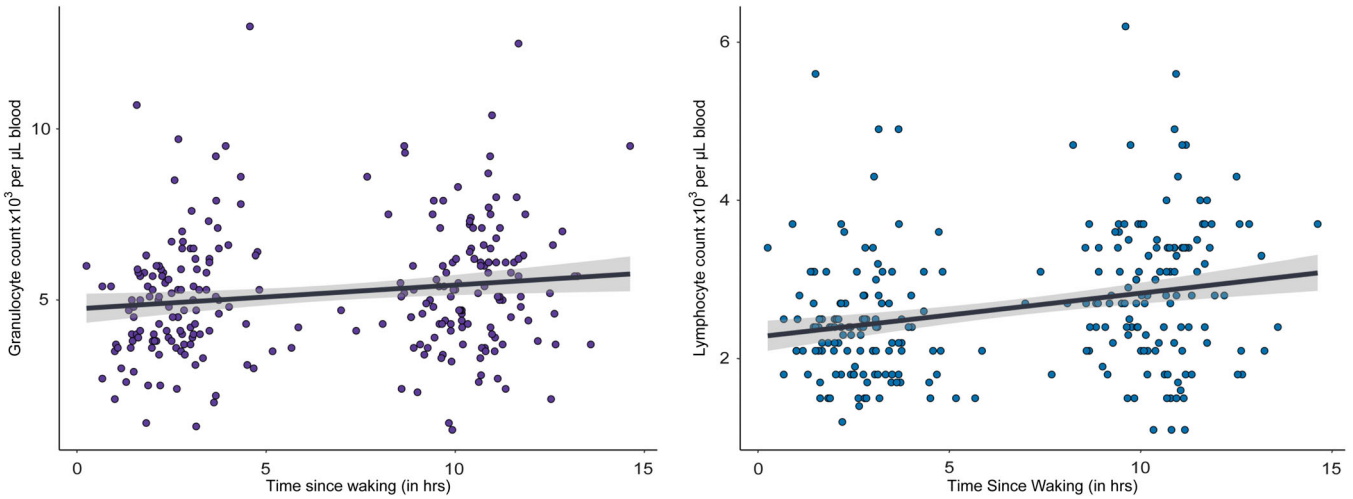


**FIGURE 1** Diurnal change in lymphocyte and granulocyte levels from morning to afternoon. Models adjust for variation in time (in hours) between waking and first sample

**TABLE 2** Linear models of associations between diurnal cortisol and leukocyte subsets, across different levels of past exposure to parasites

| | Model 1 | | Model 2 | | Model 3 | | Model 4 | | Model 5 | | Model 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Base Model 1: % change lymphocyte count | | Base Model 1 + IgE | | Base Model 2: % change granulocyte count | | Base Model 2 + IgE | | Base Model 3: Diurnal L:G ratio | | Base Model 3 + IgE | |
| IV | *β* | *p*-Value | *β* | *p*-Value | *β* | *p*-Value | *β* | *p*-Value | *β* | *p*-Value | *β* | *p*-Value |
| Cortisol slope | **−.239** | **.01** | **−.244** | **.02** | .064 | .46 | .011 | .91 | **−.183** | **.04** | **−.174** | **.09** |
| High IgE* | — | — | −.206 | .37 | — | — | **−.392** | **.06** | — | — | .327 | .15 |
| Cortisol slope: High IgE | — | — | .021 | .93 | — | — | **.506** | **.03** | — | — | −.299 | .23 |
| **Covariates** | | | | | | | | | | | | |
| Age (in years) | −.001 | .92 | −.001 | .85 | −.005 | .41 | −.006 | .34 | .006 | .37 | .006 | .40 |
| Diabetes (yes = 1) | **−.810** | **.03** | **−.860** | **.02** | −.154 | .65 | −.174 | .61 | −.418 | .25 | −.373 | .31 |
| Time since waking | .103 | .20 | .077 | .35 | .035 | .64 | .042 | .58 | .073 | .36 | .065 | .43 |
| Current illness | **1.000** | **.01** | **.968** | **.01** | .477 | .18 | .300 | .40 | .221 | .55 | .338 | .37 |
| Intercept | −.218 | .49 | −.076 | .82 | .108 | .72 | .196 | .52 | −.376 | .24 | −.419 | .20 |

*Note:* Base models (Models 1, 3, and 5) report associations between diurnal cortisol slope and diurnal change in lymphocyte and granulocyte counts, and lymphocyte to granulocyte ratio across the day. Dependent variables and cortisol slope are standardized. Models 2, 4, and 6 include interaction terms with IgE (high IgE ≥500 IU/ml) to assess the effects of different levels of past exposure to parasites on associations between diurnal cortisol and leukocyte subsets.

morning to afternoon samples). There are no significant differences in diurnal or single-unit measures of cortisol and leukocyte subsets between individuals who have high versus low levels of IgE (Table S2).

## 3.2 | Diurnal cortisol slope is associated with diurnal increase in lymphocytes

Models 1, 3, and 5 in Table 2 presents linear models of percent change in lymphocyte count (cells/μL), granulocyte count (cells/μL), and proportion (lymphocyte to granulocyte ratio) from morning to evening as a function of diurnal cortisol slope after adjusting for potential confounders. There is a statistically significant inverse association between diurnal cortisol and lymphocytes: the steeper the decline in cortisol, the greater the increase in lymphocyte count across the day ($\beta$ = −.24, $p$ < .01; Figure S3a) and proportion ($\beta$ = −.18, $p$ = .04). An inverse relationship between cortisol and lymphocyte slopes supports the notion that diurnal cortisol functions to regulate peripheral lymphocyte levels across the day. To better visualize these relationships, we ran models that compare individuals with a "flatter"

diurnal cortisol slope (less than 5% decline in pg/ml/hr) to those with a "steeper" diurnal cortisol slope (more than 5% decline in pg/ml/hr), based on *SDs* from the mean within-population distribution of cortisol slopes. A "flatter" cortisol slope suggests less of a change in peripheral cortisol between morning and evening, whereas "steeper" indicates a greater change between morning and evening. Those with a flatter cortisol decline have significantly less of an increase in lymphocytes (2.27% increase in cells/μL/hr; 95% CI: 0.91–7.29; $p = .01$) compared to those with steeper cortisol decline (7.5% increase in cells/μL/hr; 95% CI: 5.79–9.34; $p < .001$). Despite the lower diurnal lymphocyte increase in the "flatter" cortisol group, comparing R-squared between models shows that the model for those with flatter cortisol decline is a substantially better-fit than the model for individuals with steeper cortisol decline (flatter decline: $R^2 = 0.21$, $p = .04$ and steeper decline: $R^2 = 0.11$, $p = .24$, respectively). Comparing coefficients between models, it appears that being diabetic has a substantial impact on diurnal lymphocytes for those with flatter cortisol decline, but not among the steeper cortisol group, which likely explains the better-fit of the former model. We find no relationship between diurnal cortisol and diurnal granulocytes ($\beta = .06$, $p = .46$; Figure S3b).

### 3.3 | Assessing the effects of parasite exposure on diurnal cortisol–leukocyte associations

Including an interaction term with IgE (a proxy for past exposure to parasites), the association between diurnal cortisol and lymphocyte measures is slightly diminished, though effect sizes of associations remain nearly unchanged for both lymphocyte count ($\beta = -.24$, $p = .02$) and proportion ($\beta = -.17$, $p = .09$). There are no main or interactive effects of IgE group on these relationships (Table 2: *Models 2 and 6*; Figure 2a). Interestingly, though IgE does not independently influence associations between diurnal cortisol and percent change in granulocyte count, it does have an effect on the association between granulocytes and cortisol slope ($\beta = .51$, $p = .03$; Figure 2b): decline in cortisol is only associated with a diurnal change in granulocytes across the day in individuals with high IgE, and this relationship is focused around the negative slopes of both variables (Figure S4). In other words, a steeper decline in cortisol across the day is associated with a steeper decline in granulocytes, only among individuals with high past exposure to parasites. For these individuals, an *SD* decrease in cortisol slope is associated with an 8% decrease in granulocytes. Adjusting for current helminthic infection strengthens the independent associations between cortisol slope and diurnal change in lymphocyte count ($\beta = -.37$, $p < .01$) and proportion ($\beta = -.27$, $p = .04$), and the interactive association with granulocytes ($\beta = .53$, $p = .04$; Table S3). Similarly including self-reported asthma or allergies strengthens existing associations with lymphocyte count ($\beta = -.28$, $p < .01$) and proportion ($\beta = -.19$, $p = .08$), and also strengthens the interactive effect of IgE on the relationship between diurnal cortisol and granulocytes ($\beta = .54$, $p = .02$; Table S3). On their own, neither helminthic infection nor self-report allergies or asthma had an interactive effect on diurnal cortisol in relation to diurnal leukocytes. Furthermore, there was no difference in mean levels of IgE between either group (helminthic infection versus no infection: $t = 0.599$, $p = .56$, and allergies/asthma versus none: $t = -1.320$, $p = .25$).

### 3.4 | No associations between single or aggregate measures of cortisol and leukocyte subsets

Most studies of glucocorticoid-immune interactions use one-shot or aggregate measures. To examine such cross-sectional data, we also ran models limiting the data to single-unit cortisol measures (cumulative [AUC] and peak cortisol) on morning, afternoon, and mean levels of granulocytes and lymphocytes, and lymphocyte to granulocyte ratio, adjusting for potential confounders. In this sample, we find no associations between cumulative (AUC) or peak cortisol and granulocytes or lymphocytes across any of these models (Tables 3 and 4). Furthermore, none of the traditional covariates track consistently with levels across the day.

To evaluate whether past exposure to parasites moderates any relationships between cortisol and leukocytes we then included an interaction with IgE. In these models, associations remain statistically non-significant (Tables S4a and S4b). However, after adjusting for current helminthic infection, there are statistically significant positive associations between AUC cortisol and all measures of granulocyte counts (morning: $\beta = .19$, $p = .08$; evening: $\beta = .24$, $p = .03$; mean levels: $\beta = .26$, $p = .02$), and negatively associated with morning lymphocyte counts ($\beta = -.25$, $p = .04$) (Table S5a). Biologically, these associations translate into 340 cell/μL (morning), 400 cell/μL (evening), and 380 cell/μL (mean) increases in granulocytes, and a 150 cell/μL decrease in morning lymphocytes per *SD* increase in AUC cortisol. However, for both evening and mean levels of granulocytes, there is a negative interaction between IgE and AUC in these models: the positive association between AUC cortisol and granulocytes exists only for individuals with low IgE (Figure 3a). Again, helminthic infection does not, on its own, explain cortisol-immune relationships either independently or interactively (data not shown). Similarly, the moderating effect of IgE on the association between peak cortisol and granulocytes exists only after adjusting for current helminthic infection (Table S5b; Figure 3b). Correspondence between peak cortisol and granulocytes appears to be reversed dependent upon level of IgE: among individuals with low IgE there is a positive relationship between peak cortisol and granulocytes, whereas among individuals with high IgE, there is a negative relationship. Adjusting for allergies or asthma does not significantly alter the relationships between peak cortisol and granulocytes or lymphocytes.

## 4 | DISCUSSION

The main aims of this article are to evaluate HPA and immune interactions in a "natural ecological context" and to investigate how variation in parasite exposure influences correspondence between single- and diurnal-measures of HPA and immune function in

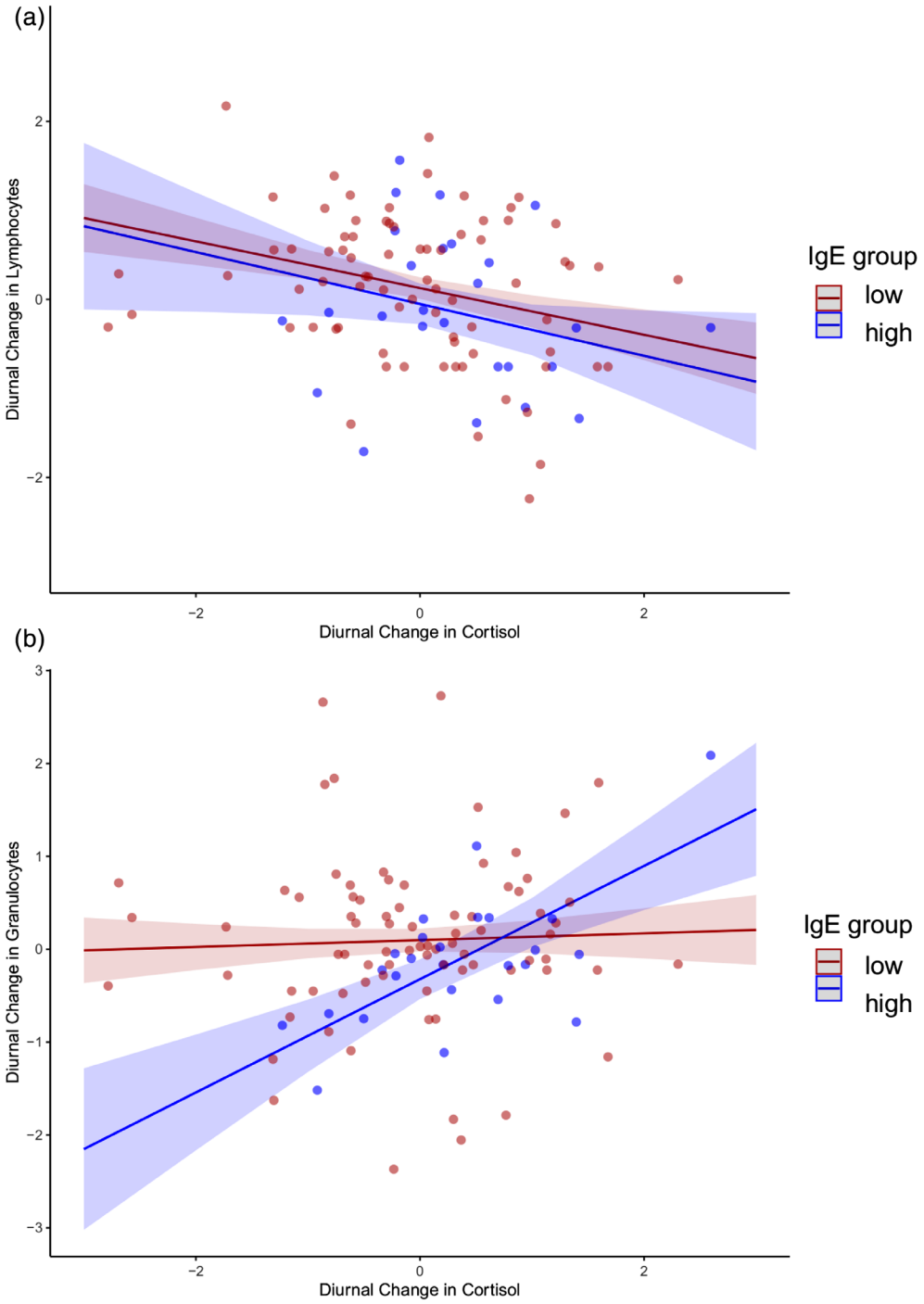


**FIGURE 2** Moderation effects of immunoglobulin-E (IgE) on association between diurnal cortisol and (a) lymphocyte and (b) granulocyte counts. Models adjust for age, diabetic status, current illness, current helminth infection, and time since waking

humans (Dhabhar et al., 1994; Jefferies, 1991; Souza-Talarico et al., 2014). To accomplish this, we tested whether cortisol and immune associations varied based on sampling period (single-shot versus diurnal) and differences in IgE and current helminthic infection among a sample of Honduran women.

We found partial support for our prediction that diurnal regulatory interactions involving glucocorticoids and the immune system should be relatively canalized and function similarly across environments. Regarding lymphocytes, results show an inverse association between the diurnal profiles of cortisol and lymphocytes that are congruent with similar findings from research conducted under more stringent, low-pathogen conditions, whereby the decline in cortisol stimulates an increase in lymphocytes. This inverse relationship describes cortisol's regulation of aspects of the proinflammatory immune system, known as "leukocyte trafficking" (Cermakian et al., 2013; Dimitrov et al., 2009; Trifonova et al., 2013). As cortisol declines across the day, it eases production of cytokines and chemokines that drive lymphocytes out of the periphery, which results in higher levels of those types of immune cells in the evening, when cortisol is at its nadir. For example, circadian rhythmicity of

**TABLE 3** Associations between cumulative cortisol exposure (AUC) and levels of granulocytes and lymphocytes

| Models 1–9 | Morning lymphocytes | Afternoon lymphocytes | Mean lymphocytes | Morning granulocytes | Afternoon granulocytes | Mean granulocytes | MorningL:G ratio | AfternoonL:G ratio | MeanL:G ratio |
|---|---|---|---|---|---|---|---|---|---|
| AUC cortisol | −0.134 | 0.071 | −0.103 | 0.050 | 0.099 | 0.097 | 0.074 | 0.001 | 0.044 |
| | (0.099) | (0.099) | (0.099) | (0.097) | (0.096) | (0.096) | (0.094) | (0.099) | (0.096) |
| Age (in years) | 0.001 | −0.002 | −0.0001 | −0.002 | −0.005 | −0.003 | −0.0003 | 0.007 | 0.004 |
| | (0.008) | (0.008) | (0.008) | (0.007) | (0.007) | (0.007) | (0.007) | (0.008) | (0.007) |
| Diabetic (yes = 1) | −0.076 | −0.453 | 0.289 | −0.703 | −0.746 | −0.773* | 0.891* | 0.376 | 0.740 |
| | (0.399) | (0.401) | (0.402) | (0.394) | (0.388) | (0.388) | (0.379) | (0.399) | (0.386) |
| Time since waking | 0.026 | 0.023 | 0.004 | 0.144 | 0.150 | 0.168 | −0.174* | −0.138 | −0.181* |
| | (0.088) | (0.088) | (0.088) | (0.087) | (0.085) | (0.085) | (0.083) | (0.088) | (0.085) |
| Current illness | −0.283 | 0.633 | −0.399 | 0.477 | 0.844* | 0.711 | −0.373 | −0.310 | −0.396 |
| | (0.415) | (0.417) | (0.419) | (0.410) | (0.404) | (0.404) | (0.394) | (0.416) | (0.402) |
| Constant | −0.112 | 0.025 | −0.014 | −0.298 | −0.199 | −0.312 | 0.448 | 0.126 | 0.370 |
| | (0.354) | (0.356) | (0.357) | (0.350) | (0.345) | (0.345) | (0.337) | (0.355) | (0.343) |

*Note:* All cortisol and leukocyte estimates are reported as standardized coefficients (*SE*). Other covariates are non-standardized.
*$p < .05$; **$p < .01$; ***$p < .001$.

**TABLE 4** Associations between single-shot peak cortisol and levels of granulocytes and lymphocytes

| Models 1–9 | Morning lymphocytes | Afternoon lymphocytes | Mean lymphocytes | Morning granulocyte | Afternoon granulocytes | Mean granulocytes | MorningL:G ratio | AfternoonL:G ratio | MeanL:G ratio |
|---|---|---|---|---|---|---|---|---|---|
| Peak cortisol | −0.101 | 0.149 | −0.153 | 0.049 | 0.060 | 0.058 | 0.016 | 0.086 | 0.058 |
| | (0.115) | (0.114) | (0.114) | (0.113) | (0.111) | (0.111) | (0.109) | (0.114) | (0.110) |
| Age (in years) | 0.002 | −0.002 | −0.001 | −0.002 | −0.006 | −0.004 | −0.001 | 0.007 | 0.004 |
| | (0.008) | (0.008) | (0.008) | (0.007) | (0.007) | (0.007) | (0.007) | (0.008) | (0.007) |
| Diabetic (yes = 1) | −0.079 | −0.439 | 0.277 | −0.701 | −0.746 | −0.773 | 0.888* | 0.387 | 0.745 |
| | (0.401) | (0.399) | (0.401) | (0.395) | (0.390) | (0.390) | (0.380) | (0.399) | (0.387) |
| Time since waking | 0.025 | 0.039 | −0.009 | 0.146 | 0.148 | 0.166 | −0.180* | −0.124 | −0.176* |
| | (0.089) | (0.089) | (0.089) | (0.088) | (0.087) | (0.087) | (0.085) | (0.089) | (0.086) |
| Current illness | −0.272 | 0.713 | −0.457 | 0.482 | 0.821* | 0.688 | −0.415 | −0.233 | −0.377 |
| | (0.423) | (0.421) | (0.423) | (0.416) | (0.411) | (0.411) | (0.401) | (0.420) | (0.407) |
| Constant | −0.119 | −0.050 | 0.042 | −0.303 | −0.181 | −0.294 | 0.485 | 0.055 | 0.352 |
| | (0.362) | (0.360) | (0.362) | (0.356) | (0.352) | (0.352) | (0.343) | (0.360) | (0.349) |

*Note:* All cortisol and leukocyte estimates are reported as standardized coefficients (*SE*). Other covariates are non-standardized.
*$p < .05$; **$p < .01$; ***$p < .001$.

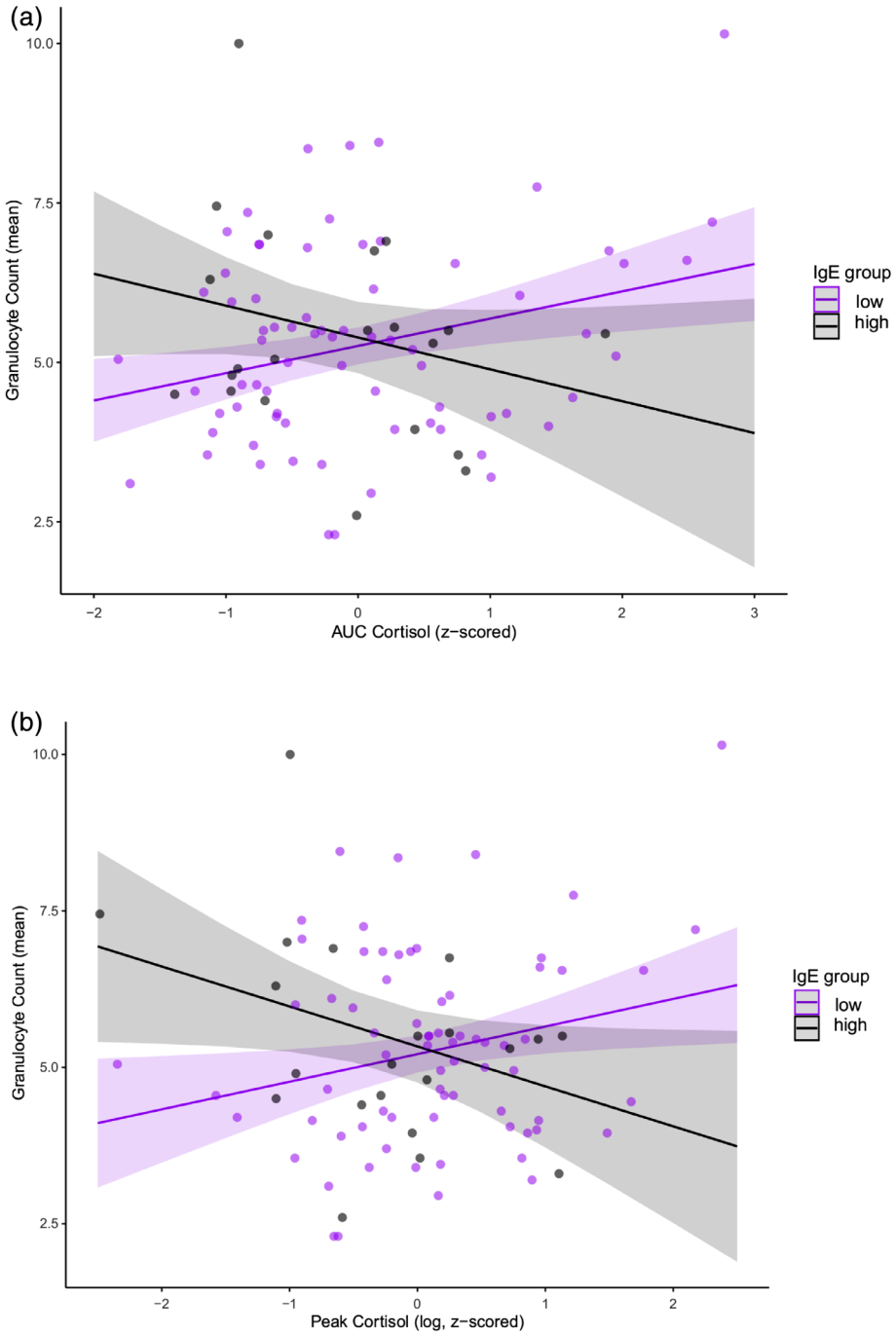


**FIGURE 3** Moderation effects of immunoglobulin-E (IgE) on association between the single-point measures of (a) peak and (b) area under the curve (AUC) cortisol on (b) mean granulocyte counts. Cortisol and granulocytes are transformed and *z*-scored. There is an opposite trend, dependent upon level of IgE: individuals with low IgE show positive associations between cortisol and granulocytes, while those with high IgE show negative associations between cortisol and granulocytes. Models adjust for age, diabetic status, current illness, current helminth infection, and time since waking

cortisol regulates the expression of CXCR4 on T cells, leading to their redistribution to bone marrow, the site of production of the CXCR4 ligand (Labrecque & Cermakian, 2015). A steeper decline in cortisol and a commensurate incline in lymphocytes could plausibly be linked to an increased capacity for immunological protection and repair at night. We also found that being diagnosed diabetic also contributed substantially to the overall fit of these models, in particular among those with flatter cortisol decline. Past work has found that glucose intolerance is linked to both dysregulation in both inflammatory processes (Cermakian et al., 2013) as well as HPA function (Champaneri et al., 2013). However, the biological impact of glucose intolerance on HPA and immune regulatory interactions has not been established. This finding suggests that a sharper focus at the interface of glucose tolerance, HPA and immune function may be important for disentangling how these factors interact in relation to metabolic disease risk. Furthermore, in accordance with our prediction, these associations are not significantly impacted by variation in past (or present) exposure to parasites. This finding also aligns with expectations of biological systems' signaling. The majority of lymphocytic cells (B and T cells) are regulated by the diurnal rhythmicity of glucocorticoids (Shimba et al., 2018). IgE levels, which are maintained through costimulatory actions involving B and T cells (often in coordination

with glucocorticoids), would not be expected to affect the sensitization or signaling of lymphocytes by glucocorticoids (Elenkov, 2004; Zieg, Harbeck, & Leung, 1994).

By contrast, and counter to our initial prediction, we found no broad correspondence between diurnal cortisol and change in granulocytes across the day. Interestingly, variation in past parasite exposure does appear to moderate a relationship: individuals with high IgE, suggestive of a greater history of exposure, show correspondence between diurnal cortisol slope and granulocytes, whereas individuals with less exposure have a lack of correspondence. Furthermore, unlike studies that have shown cross-sectionally that high glucocorticoid levels increase circulating number of granulocytes and decrease lymphocytes (Boomershine, Wang, & Zwilling, 2001; Dale, Fauci, Guerry IV, & Wolff, 1975; Ella, Csépányi-Kömi, & Káldi, 2016), single-unit measures of cortisol and leukocyte subsets are generally unrelated in this sample. In fact, relationships between these variables only emerge after accounting for variation in past and present exposure to parasites.

To better understand these findings, we need to unpack more fine-grained aspects of glucocorticoid-granulocyte signaling under different immunotypic backgrounds. Granulocytes are comprised of neutrophils, basophils, eosinophils, and mast cells. The trafficking of granulocytes is complex, as some granulocytic cells are regulated by the action of glucocorticoids on the expression of circadian regulator genes (e.g., mast cells and basophils) (Pick, He, Chen, & Scheiermann, 2019), while others are regulated primarily through altering their lifespan or their adhesion to vascular walls, without directly influencing their expression (e.g., neutrophils) (Ince et al., 2019). Furthermore, IgE promotes mast cell and basophil survival and proliferation, and mast cells control actual trafficking of neutrophils (Burton & Oettgen, 2012).

A possible explanation for the IgE-dependent positive association between diurnal cortisol and granulocytes is that individuals with high IgE have a higher ratio of mast cells to neutrophils comprising total granulocytes. Since mast cells are more strongly regulated by circadian rhythmicity of glucocorticoids themselves (compared to neutrophils), this could explain why cortisol has a diurnal regulatory effect only under conditions of high IgE.

Furthermore, if **low** IgE is indicative of having proportionally higher neutrophilic granulocytes, this may also explain why cortisol is positively associated with granulocytes in these individuals cross-sectionally. Since glucocorticoids are linked to increased levels of neutrophils in the periphery through non-circadian pathways (e.g., suspension of apoptosis and increased demarginalization), it is logical that this relationship would be stronger among individuals whose granulocytes are comprised of a higher proportion of neutrophils (Ince et al., 2019; Schleimer, Freeland, Peters, Brown, & Derse, 1989).

The distinct patterns of cortisol-leukocyte associations found in this study may have implications for understanding the rise in chronic inflammatory diseases that accompanies industrialization (Natri, Garcia, Buetow, Trumble, & Wilson, 2019; Raison et al., 2010). It is currently common practice in medicine to use biomarkers of inflammation (e.g., C-reactive protein, leukocytes) at one single point during the day as risk factors predicting future inflammatory disease. However, cross-cultural studies challenge the appropriateness of these criteria, in finding that some populations with high systemic inflammation show low risk for chronic inflammatory diseases like heart disease and diabetes (Gurven et al., 2009; Kaplan et al., 2017a). Furthermore, emergent evidence suggests a number of metabolic diseases and their risk factors show circadian disruption that plays a key role in their pathogenesis (Champaneri et al., 2013; Maury, Hong, & Bass, 2014). Increased disease risk may be a consequence of a breakdown in coordination between the HPA axis and immune regulatory networks, more than just elevated levels of inflammation. Specifically, we found that individuals who have had less exposure to parasites have low diurnal coordination between cortisol and granulocytic cells, but positive cross-sectional association between absolute levels of cortisol and granulocytes. One interpretation of this relationship is that, in low pathogen environments, the simultaneous actions of cortisol promotes heightened innate inflammation (granulocytes) in the periphery, while having a diminished capacity to diurnally regulate the innate system, may lead to relative suppression of adaptive immune function and compromised immuno-repair activity, and result in higher inflammatory disease risk (Karin & Clevers, 2016; Li, Tan, Martino, & Lui, 2018; Martin, 1990).

Finally, although our results suggest that HPA and lymphocyte associations appear relatively stable regardless of infection history, ecological exposure could still influence the way in which these systems respond to other stressors (e.g., psychosocial). Rook et al. (2013) have suggested that the link between psychosocial stress and disease risk is heightened in industrialized environments due to reduced exposure to pathogens and lack of immunomodulation (Rook et al., 2013). The argument is that humans have an "evolved dependency" on pathogens that play immunomodulatory roles on HPA and proinflammatory immune responses, and the lack of priming may compromise immunoregulation and exacerbate links between psychosocial stress and disease risk. Coupled with our diurnal findings, this suggests that aspects of both the innate and adaptive arms of the immune system may be uniquely vulnerable to dysregulation in the absence of parasites. Incorporating the moderating role of the environment on how other factors (e.g., psychosocial stressors) influence HPA–immune interactions is an important consideration for future work.

### 4.1 | Limitations

Logistical constraints of subject recruitment and the use of multi-day sampling to calculate diurnal cortisol prohibited the collection of matched blood and saliva samples on the same day. Blood and saliva samples were instead taken within 1–3 days of each other. However, despite imperfect alignment, results still supported the predicted inverse associations between diurnal cortisol and lymphocyte profiles. These results are conservative given that error due to the timing of sample collection would more likely lead to Type II, rather than

Type I, errors. It is possible that the lack of a positive relationship between cortisol and granulocytes could have arisen due to error from the timing of sample collection, given the high variability and turnover of these cells, and difference in sampling day. Another potential issue are differences in oscillation frequency of granulocytes compared to cortisol, which also suggests a more complex network of regulatory processes that may have not be adequately captured (Ackermann et al., 2012; Labrecque & Cermakian, 2015). That the relationships between cortisol and granulocytes are altered, both diurnally and cross-sectionally, among individuals with different levels IgE, yet mean granulocyte counts and cortisol levels do not differ between low and high IgE groups, suggests that aspects of these relationships may be features of ecological variation in immune function, rather than simply noisy data.

Another limitation is the exclusive focus on women from mainland Honduras. Recent cross-cultural research has challenged some of the "typical" findings on sexual dimorphism in diurnal cortisol and immune function (Natri et al., 2019; Urlacher, Liebert, & Konečná, 2018). Future studies that include both men and women are important to clarify potential sex differences in HPA and immune activity, and build on this literature. There is no research that investigates the potential variability in diurnal cortisol and immune function interactions between sexes due to ecological context. Finally, individuals emigrate to Utila from the mainland for a number of reasons, including to seek a safer environment, or for work. Hondurans are a relatively marginalized group on the island. It is possible this sample overrepresents women who may have altered HPA function due to these factors associated with migration. Ongoing research among this population is aimed at teasing out the unique effects of psychosocial and ecological stressors on HPA–immune interactions.

## 5 | CONCLUSION

The common use of single or aggregate measures of neuroendocrine hormones or immune markers tends to overlook and underappreciate the dynamic relationships *between* the HPA axis and immune system, which are critical for maintaining homeostatic processes. Furthermore, while a number of experimental studies have shown alterations to one of these systems consequently affects the other (Besedovsky, Del Rey, Sorkin, Lotz, & Schwulera, 1985; Dhabhar et al., 1994; Petrovsky, 2001), no other study has looked at relationships between diurnal HPA and immune markers in a higher pathogen "natural" ecological context. In this way, the present study extends and contributes to an emerging literature in ecological immunology. Our results demonstrate that the relationship between HPA function and immune modulation cannot be fully understood without an understanding of contributing environmental factors, and highlight the importance of research that seeks to identify the etiologies of disease across diverse environmental contexts.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## AUTHOR CONTRIBUTIONS

**Angela Garcia:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; visualization; writing-original draft; writing-review and editing. **Ben Trumble:** Conceptualization; methodology; resources; supervision; writing-review and editing. **Thomas Kraft:** Formal analysis; methodology; supervision; writing-review and editing. **Sergio Murillo:** Data curation; investigation; methodology. **Mariela Marquez:** Data curation; investigation; methodology; project administration. **Michael Gurven:** Conceptualization; funding acquisition; methodology; supervision; writing-review and editing. **Aaron Blackwell:** Conceptualization; formal analysis; funding acquisition; resources; supervision; writing-review and editing.

## DATA AVAILABILITY STATEMENT

Because data from this project were collected as a part of the corresponding author's dissertation and are under a three-year embargo (to allow for publishing dissertation chapters), data are not provided. However, in support of open science, data can be made available upon request by emailing corresponding author: angela.garcia.2@asu.edu.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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