Am. J. Trop. Med. Hyg., 73(1), 2005, pp. 178–187

THE REPRODUCTIVE ENDOCRINE RESPONSE TO *PLASMODIUM VIVAX* INFECTION IN HONDURANS

MICHAEL P. MUEHLENBEIN,* JACKELINE ALGER, FRANK COGSWELL, MARK JAMES, AND DONALD KROGSTAD

Department of Anthropology, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin; Departamento de Laboratorios Clinicos, Hospital Escuela, Tegucigalpa, Honduras; Department of Bacteriology and Parasitology, Tulane National Primate Research Center, Covington, Louisiana; Department of Tropical Medicine, Tulane University School of Public Health and Tropical Medicine, New Orleans, Louisiana

Abstract. Reproductive physiology and endocrinology change with the onset of illness and injury in a variety of species, including humans. To assess the human reproductive endocrine response to malaria, serial serum samples were collected from 8 male and 9 female residents of Honduras infected with *Plasmodium vivax* (plus 19 male and 23 female healthy age-matched controls) and were analyzed for associations between testosterone, parasitemia, and cytokine levels. Because testosterone has been negatively associated with measures of immune function under various circumstances, it was hypothesized that testosterone would be directly associated with *P. vivax* parasitemia and inversely associated with proinflammatory cytokine levels. The findings presented here suggest that 1) testosterone levels are positively associated with *P. vivax* parasitemia in adult males, and 2) males infected with *P. vivax* exhibit significantly lower testosterone levels and significantly higher cortisol levels than healthy individuals. Depressed androgen levels during physiologic perturbations may be an advantageous, adaptive host response to ameliorate immunosuppression by higher testosterone levels and to curb the use of energetic resources for metabolically expensive anabolic functions.

INTRODUCTION

Malaria ranks as one of the most common and deadly diseases ever known, with more than one third of the world's population at risk of being infected, a global incidence estimated to be more than 300 million cases per year,[1] and an annual death toll of more than 3 million people, mostly children.[2] It is therefore vitally important to understand all aspects of the malarial disease process, including the endocrine response that regulates stress, immune, reproductive, and somatic metabolic functions in mammals.[3] Optimal performance of host immune responses is influenced by steroid hormones, and assessing changes in key hormones, such as testosterone, and determining their interaction with immune factors during infection may have important implications for understanding the optimization of testosterone levels under varying environmental conditions.

Diversion of metabolic energy to bolster immunocompetence can reduce the energy available for reproduction.[3–5] Likewise, maintaining high androgen levels may induce fitness costs by causing immunosuppression. This, in turn, may increase susceptibility to parasitic infection, which would be balanced against the reproductive benefits of testosterone. Understanding of how various hormones, particularly testosterone, mediate immune-endocrine interactions depends on accurate testing of the hypothesis that testosterone is immunosuppressive. The immunoregulatory roles of testosterone have been assessed mainly through four different means: 1) comparing male and female differences in immunocompetence,[6–11] 2) examining associations between circulating endogenous testosterone levels and measurements of immune function, such as size of immune organs or leukocyte counts in healthy or parasitized animals,[12–16] 3) experimentally manipulating testosterone levels through castration or supplementation and observing subsequent effects on immunocompetence,[17–24] and 4) performing *in vitro* analyses of immune-endocrine interactions.[25–31]

Unfortunately, few field studies have yielded consistent associations between testosterone levels and measures of immunocompetence or parasitic infection.[3] To better describe the associations between immunocompetence and testosterone levels in humans, we examined correlates of gonadal function (testosterone levels) with physiologic measures of disease progression (signs and symptoms) and soluble indicators of immune function (cytokines) during *Plasmodium vivax* infection in Honduran residents. We hypothesized that significant, positive and negative correlations would exist between testosterone levels and *P. vivax* parasitemia and proinflammatory cytokine levels, respectively. We also hypothesized that, because of the differences in hormone levels between the sexes, males and females infected with *P. vivax* would exhibit significantly different immune and hormone profiles, with males having significantly higher parasitemia and significantly lower proinflammatory cytokine levels than females.

MATERIALS AND METHODS

Study location. The study was conducted at Tocoa Hospital, located in the town of Tocoa, Honduras, 40 km south of Trujillo on the Caribbean coast, equidistant from the country's northernmost borders with Guatemala and Nicaragua. The region is warm and humid with a temperature ranging from 28°C to 35°C, with a rainy season from May to November and an approximate elevation of 150 m above sea level. The community is situated in the banana- and palm-growing region of the country, and most inhabitants are subsistence farmers and plantation workers.[32] In 2001, the region was populated by approximately 68,000 persons (with 31,000 between the ages of 15 and 49) (Municipio de Tocoa, unpublished data).

Case detection and recruitment. The study used an epidemiologic passive detection design in which those seeking treatment of malaria-like symptoms at Tocoa Hospital were diagnosed via examination of thick and thin blood smears and were recruited into the study following a signed informed consent and health and demographic questionnaires. Inclu-

* Address correspondence to Michael P. Muehlenbein, Department of Anthropology, University of Wisconsin-Milwaukee, P.O. Box 413, Milwaukee, Wisconsin 53201. E-mail: mpm1@uwm.edu

sion criteria were positive diagnosis with *Plasmodium vivax* in native adults (male or female, ≥ 18 years of age). Exclusion criteria included history of known endocrine disorder, current or recent use of hormone therapy or immunosuppressive drugs, pregnant or postmenopausal, history of immunosuppressive disease, history of chronic disease (e.g., chronic obstructive pulmonary disease, congestive heart failure, etc.), recent surgery, and current prescription treatment of any disease other than *Plasmodium*. Permission to conduct this research was granted by the Health Area No. 4, Health Region No. 6, Departamento de Colon, Honduras. The project was approved by the Yale University Human Subjects Committee.

In Honduras, *P. vivax* (transmitted mainly by *Anopheles darlingi*) is responsible for approximately 97% of malarial cases (2003 status report on malaria programs in the Americas, 44th Directing Council, 55th Session of the Regional Committee, Pan American Health Organization, Washington, D.C.: available at http://www.paho.org/english/gov/cd/cd44-inf3-e.htm). The majority of malaria cases are concentrated in the Northern coastal region, including Tocoa. In 2003, approximately 15% of the Tocoa population (8,744 of 57,573) was examined for malaria (using Giemsa-stained thick or thin blood smears); 1543 were positive for *P. vivax* and 81 were positive for *P. falciparum* (approximately equal distribution between the sexes) (Secretaría de Salud Pública, Tocoa, Honduras, unpublished data). Therefore, 2.68% of the population was diagnosed as positive with *Plasmodium vivax*.

During the first phase of the current study (June through August 2001), 271 slides were examined in the Tocoa Hospital Laboratory, and only 39 were positive for *P. vivax*. Of these 39, only 10 (5 males and 5 females) were over the age of 18. Enrollment continued from January through June, 2003, and the final sample size was 8 adult males and 9 adult females infected with *P. vivax* in addition to 19 male and 23 female healthy age-matched control subjects. Average ages of males and females, cases and controls did not significantly differ from one another (Table 1).

Measurements and sample collection. The sampling regimen was designed to collect specimens at four time points: day of diagnosis, 24 hours post-diagnosis, 48 hours post-diagnosis, and 8 days post-diagnosis. This sampling schedule was chosen to correspond with the three consecutive treatment dosages of chloroquine, the standard dosage used in this region. The final sample served as a baseline self-control. At each time point, physical measurements (e.g., body temperature, height, weight, and skinfolds) were made, a health questionnaire was used to determine the presence and severity of malaria symptoms, a capillary blood sample was obtained to prepare blood smears and determine hemoglobin and hematocrit, and approximately 10 mL of blood was collected from the brachial artery using a 10-mL SST Vacutainer Collection Tube with serum separator (Beckton-Dickinson, Franklin Lakes, NJ) and a 21-gauge needle. The blood was centrifuged, and the serum frozen. The blood samples were stored at the Tocoa Hospital until transfer for analysis to the United States.

Of the 8 male cases, between 1 and 4 samples were obtained for each with an average of 3.67 samples per case. Mean number of days between their first and second samples was 1.3; between first and third samples was 2.7; between first and fourth samples was 8.0. Of the 9 female cases, between 2 and 4 samples were obtained for each with an average of 3.70 samples per case. Mean number of days between their first and second samples was 1.0; between first and third samples was 2.3; between first and fourth samples was 7.8. Therefore, the timing between repeat samples for the male and female cases was approximately the same.

Laboratory analyses. Serum samples were shipped from Honduras to Yale University and Tulane University for analysis. No samples thawed in transit. At Yale, samples were stored at –80°C until assayed for total testosterone and cortisol levels using coated-tube radioimmunoassay kits (DSL-4000 and DSL-2100) from Diagnostic Systems Laboratories (DSL; Webster, TX). The sensitivities of the assays were 0.08 ng/mL for testosterone and 0.3 ng/dL for cortisol. The correlation coefficients for each of the curves were better than 0.99. High and low level DSL controls were included in each standard curve. Results for the controls in each assay were within established confidence limits (testosterone: low 0.5 ± 0.2 ng/mL, high 5.0 ± 2.0 ng/mL; cortisol: low: 4.0 ± 1.0 ng/mL, high 20.0 ± 5.0 ng/mL). Intra-assay coefficients of variation were assessed using the mean coefficients of variation of control duplicates. Intra-assay coefficients of variation were 6.0% for testosterone and 8.5% for cortisol. Inter-assay coefficients of variation were assessed using the mean coefficients of variation of control duplicates in two separate assays. Inter-assay coefficients of variation were 8.1% for testosterone and 10.0% for cortisol.

At Yale University, serum samples were analyzed for IL-10, TNF-α, and IFN-γ using the solid phase sandwich enzyme linked immunosorbent assay (ELISA) kits from Biosource International Inc. (Camarillo, CA). At Tulane University, serum samples were also analyzed for IL-1β, IL-4, and IL-12 using the solid phase sandwich enzyme linked immunosorbent assay (ELISA) kits from Biosource International Inc. All assays were run according to the manufacturer's instructions. The sensitivities of the assays were IFN-γ: < 4 pg/mL; TNF-α: 1.7 pg/mL; IL-1β: 1 pg/mL; IL-4: < 2 pg/mL; IL-10: <

TABLE 1
Case description

Variable	Male cases	Female cases	Male controls	Female controls
Sample size	8	9	19	23
Mean age (years)	28.5	33.3	30.1	31.4
Mean no. previously reported malarial episodes	3.3	3.6	2.0	1.9
% Reported fever on day of diagnosis	100%	100%		
% Reported chills on day of diagnosis	87.5%	100%		
% Reported headache on day of diagnosis	100%	89%		
% Reported muscle aches on day of diagnosis	87.5%	89%		
% Reported paroxysms on day of diagnosis	100%	100%		

1 pg/mL; IL-12: < 2 pg/mL. Intra-assay coefficients of variation were assessed using the mean coefficients of variation of control duplicates. Intra-assay coefficients of variation were IFN-γ: 5.3%; TNF-α: 4.5%; IL-1β: 4.5%; IL-4: 3.0%; IL-10: 4.1%; IL-12: 3.9%. Inter-assay coefficients of variation were assessed using the mean coefficients of variation of control duplicates on three plates. Inter-assay coefficients of variation were IFN-γ: 6.0%; TNF-α: 8.0%; IL-1β: 6.9%; IL-4: 4.4%; IL-10: 3.5%; IL-12: 3.9%.

Parasitemia estimates were performed at Hospital Escuela, Tegucigalpa, Honduras. Estimation of parasitemia was based on microscopic examination of thick and thin blood smears stained with Giemsa. Parasitemia on thick smears was estimated by counting parasites and white blood cells (WBC) per field at 100× magnification. One hundred microscope fields were observed (200 observation fields were counted if only one parasite was identified within the first 100 WBC). Parasitemia on thin smears was estimated by first counting the number of parasites in a total of 50 fields, and the amount of parasites was estimated as a percentage of 100 red blood cells (RBC). Because the average numbers of white and red blood cells were not known for each individual patient, constants of 5,000,000 RBC/μL and 8,000 WBC/μL were assumed. The numbers obtained from the thick and thin smears were transformed into parasites per microliter of blood via the following method: for thick smears, divide the number of parasites by the number of observed white blood cells and multiple by 8,000; for thin smears, divide the percentage of observed infected red blood cells by 100 and multiple by 5,000,000. Samples were deemed negative if no parasite was found after counting 200 fields in either the thick or thin smears.

For the samples collected in year 2001, hemoglobin levels were determined using a portable HemoCue Beta-Hemoglobin Photometer system (HemoCue AB, Angelholm, Sweden) at Tocoa Hospital. Hematocrit was determined by multiplying hemoglobi values by three. For the samples collected in year 2003, hemoglobin and hematocrit were determined via a standard hemogram at Laboratorio Portillo, Tocoa.

Statistical analyses. Data were entered into an Access database that was then imported into SAS for statistical analysis with SAS/STAT software.[33] Differences in parasitemia categories between the male and female cases were assessed using the Fisher's exact χ^2. Differences in variable levels between male and female cases and between cases and controls were assessed using Wilcoxon rank sum exact test, the nonparametric counterpart of the independent *t* test. Differences between sampling days within individual cases were assessed using the Wilcoxon signed ranks test, the nonparametric equivalent of the paired *t* test. In this case, a new variable was created reflecting the difference between the two paired observations. If the Wilcoxon signed ranks test statistic was negative, then the values on day four were higher than the values on day one, and *vice versa* if the test statistic was positive. For the repeated measures analyses (testosterone/parasitemia association over time), Friedman's two-way nonparametric ANOVA was used. In this case, parasitemias were ranked over time within each case, and testosterone levels were categorized in the following manner: < 3 ng/mL, 3–5.99 ng/mL, ≥ 6 ng/mL. Cortisol levels were controlled for in all statistical analyses for two important reasons: 1) cortisol exhibits mainly inhibitory effects on immune function,[34–37] and 2) cortisol is often associated with suppression of the hypothalamic-pituitary-gonadal axis.[38,39]

Power analyses were calculated for all tests that revealed non-significant results (*P* value greater than 0.05) to assess the likelihood of false negatives. Power analyses for the Wilcoxon signed rank tests were performed with the SAS/STAT Analyst Sample Size application for paired *t* tests.[31] Power analyses for the Wilcoxon rank sum tests and all correlations were performed with the program G-Power.[40] For those variables that were non-normally distributed, G-Power analyses for parametric data were used with a 95% correction.[41] Results of statistical tests that yielded *P* values greater than 0.10 are still reported as "non-significant," or rather, "not consistent with the hypothesis," even if power was less than 80%. However, power of less than 80% should suggest to the reader that the data (with the present sample size) are not strong enough to prove that the null hypothesis was not true.

RESULTS

Case descriptions. Table 1 reports mean ages and mean number of previous malarial episodes experienced by the male and female cases and controls. Values did not significantly differ between males and females or cases and controls.

Parasitemia. Parasitemia was measured as a continuous variable, but also graduated as follows for comparative purposes: low < 800 parasites/μL; moderate 801–2,399 parasites/μL; high > 2,400 parasites/μL. Results were not consistent with the hypothesis: there were no significant differences in parasitemia categories between the male and female cases ($P = 0.22$; Fisher's exact χ^2). Mean parasitemia for the male cases on day of diagnosis was 5,012 parasites/μL (range: 308–10,923 parasites/μL). Mean parasitemia for the female cases on day of diagnosis was 6,733 parasites/μL (range: 162–25,600 parasites/μL). Results were not consistent with the hypothesis: mean parasitemia for the female cases was not significantly different than that of the male cases ($T = 62.5$, $P = 0.78$, power = 7%; Wilcoxon rank sum exact).

Mean parasitemia for the male cases on second sample day was 706 parasites/μL (range: 0–3,500 parasites/μL). Mean parasitemia for the female cases on second sample day was 894 parasites/μL (range: 0–4,863 parasites/μl). Male and female cases did not significantly differ in mean parasitemia on their second sample day ($T = 50$, $P = 0.53$, power = 5%; Wilcoxon rank sum exact). On the third sample day, only one case (a male) had a parasitemia greater than zero, and his parasitemia was 350 parasites/μL. On the fourth sample day, no cases presented with a parasitemia greater than zero.

Hormones, hemoglobin, and hematocrit. Figures 1 and 2 illustrate mean testosterone, cortisol, and hemoglobin levels for the cases (days one through four) and controls. Table 2 also presents mean hormone, hemoglobin, and hematocrit values. Testosterone levels were significantly higher in male controls than in male cases on their day of diagnosis ($T = 59$, $P = 0.004$; Wilcoxon rank sum exact). In the male cases, testosterone levels were significantly higher on their fourth sample (average of 8 days post-diagnosis) than on their day of diagnosis ($T = -14$, $P = 0.016$; Wilcoxon signed rank). Cortisol levels were also significantly higher in the male and female cases on their day of diagnosis compared with their controls (males: $T = 144$, $P = 0.047$; females: $T = 142$, $P = 0.033$; Wilcoxon rank sum).

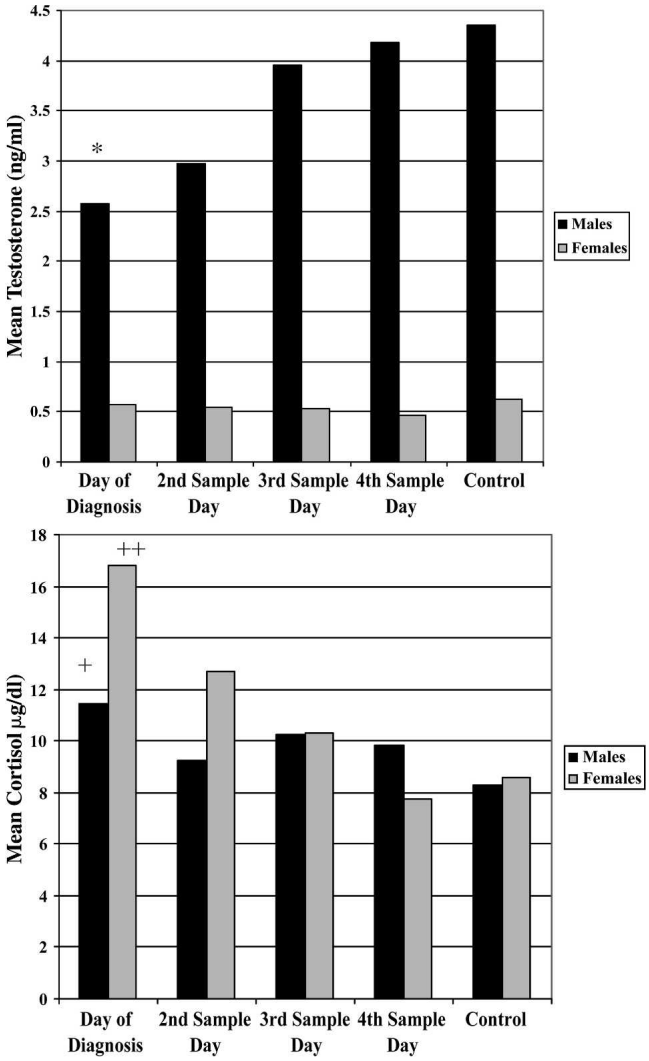

FIGURE 1. Mean testosterone and cortisol levels in male and female cases and controls. *, In male cases, testosterone levels on their day of diagnosis were significantly lower than on their fourth sample (average of 8 days post-diagnosis) (T = −14, $P = 0.016$; Wilcoxon signed rank) and their age-matched healthy controls (T = 59, $P = 0.004$; Wilcoxon rank sum exact). +, In male cases, cortisol levels were significantly higher in the male cases on their day of diagnosis compared with the male controls (T = 144, $P = 0.047$; Wilcoxon rank sum). ++, In the female cases, cortisol levels on the day of diagnosis were significantly higher than those of female controls (T = 142, $P = 0.033$; Wilcoxon rank sum).

In the male cases, hemoglobin and hematocrit levels were significantly different between their fourth sample (average of 8 days post-diagnosis) and their day of diagnosis (hemoglobin: T = 10.5, $P = 0.031$; hematocrit: T = 10.5, $P = 0.031$). Hemoglobin and hematocrit levels in male cases on their fourth sample day were significantly lower than levels in male controls (hemoglobin: T = 42.5, $P = 0.028$; hematocrit: T = 42.5, $P = 0.028$). Hemoglobin and hematocrit levels were not significantly different in the male cases on their day of diagnosis compared with the male controls (hemoglobin: T = 80, $P = 0.124$, power = 13%; hematocrit: T = 83, $P = 0.172$, power = 13%).

In the female cases, hemoglobin and hematocrit levels were not significantly different between their fourth sample (average of 7.8 days post-diagnosis) and their day of diagnosis

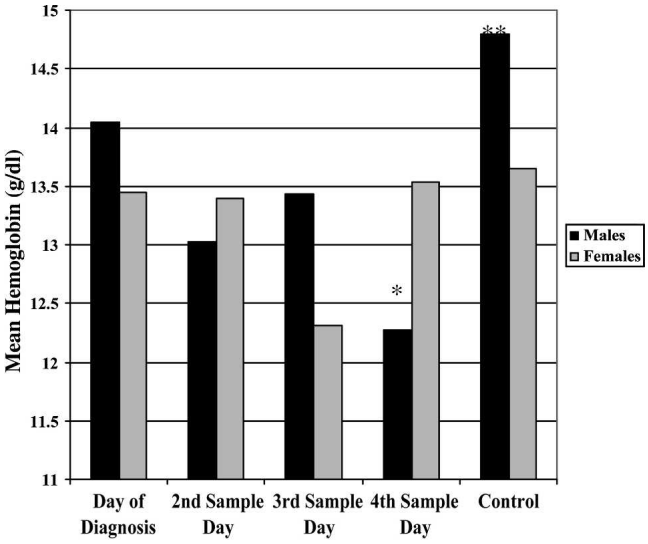

FIGURE 2. Mean hemoglobin levels in male and female cases and controls. *, In the male cases, hemoglobin on their fourth sample day (average of 8 days post-diagnosis) was significantly lower than on their day of diagnosis (T = 10.5, $P = 0.031$) and their age-matched healthy controls (T = 42.5, $P = 0.028$). **, Mean hemoglobin levels were significantly higher in male controls than female controls (t = 3.41, $P = 0.002$).

(hemoglobin: T = 3, $P = 0.719$, power = 5%; hematocrit: T = 3, $P = 0.719$, power = 5%). Hemoglobin and hematocrit levels were not significantly different in the female cases on their day of diagnosis than in female controls (hemoglobin: T = 113.5, $P = 0.746$, power = 6%; hematocrit: T = 113.5, $P = 0.746$, power = 6%). Hemoglobin and hematocrit levels in female cases on their fourth sample day were not significantly different from levels in female controls (hemoglobin: T = 98, $P = 0.701$, power = 8%; hematocrit: T = 98, $P = 0.701$, power = 8%).

Comparing male and female cases, hemoglobin and hematocrit levels on day of diagnosis did not significantly differ (hemoglobin: T = 75.5, $P = 0.760$, power = 8%; hematocrit: T = 75.5, $P = 0.760$, power = 8%). Likewise, hemoglobin and hematocrit levels on the fourth sample day did not differ between the sexes (hemoglobin: T = 36.5, $P = 0.295$, power = 15%; hematocrit: T = 36.5, $P = 0.295$, power = 15%). As expected, mean hemoglobin and hematocrit levels were significantly higher in male controls than female controls (hemoglobin: T = 3.41, $P = 0.002$; hematocrit: T = 3.24, $P = 0.003$).

Cytokines. The type 1 cytokines (cell-mediated immune responses) examined here were IL-1β, IFN-γ, TNF-α, and IL-12, and the type 2 cytokines (antibody-mediated immunity) were IL-4 and IL-10. Figure 3 illustrates mean cytokine levels for the cases (Days 1 through 4) and controls. IL-4 is not included because values for this cytokine were not significantly greater than zero. Table 3 also presents mean cytokine values.

IL-10 ($P = 0.02$) and TNF-α ($P = 0.03$) levels were significantly lower on fourth sample day compared with those on the day of diagnosis for male cases. IL-12 ($P = 0.02$), IL-10 ($P = 0.01$), and TNF-α ($P = 0.01$) were significantly lower on fourth sample day compared with the day of diagnosis for female cases. Mean levels of IL-10 ($P < 0.0001$), IL-12 ($P < 0.01$), TNF-α ($P = 0.003$), and IFN-γ ($P = 0.08$) were sig-

TABLE 2
Mean hormone, hemoglobin and hematocrit levels in cases and controls

Variable	Male cases	Female cases	Male controls	Female controls
Day of diagnosis				
Testosterone (ng/mL)	2.57	0.57		
Cortisol (μg/dL)	11.43	16.78		
Hemoglobin (g/dL)	14.05	13.44		
Hematocrit (% RBCs)	42.28	40.37		
Second sample day				
Testosterone (ng/mL)	2.98	0.54		
Cortisol (μg/dL)	9.26	12.69		
Hemoglobin (g/dL)	13.03	13.40		
Hematocrit (% RBCs)	38.94	39.56		
Third sample day				
Testosterone (ng/mL)	3.95	0.53		
Cortisol (μg/dL)	10.28	10.31		
Hemoglobin (g/dL)	13.43	12.31		
Hematocrit (% RBCs)	40.30	36.94		
Fourth sample day (or control)				
Testosterone (ng/mL)	4.18	0.46	4.35	0.63
Cortisol (μg/dL)	9.83	7.72	8.27	8.58
Hemoglobin (g/dL)	12.27	13.53	14.77	13.14
Hematocrit (% RBCs)	36.80	40.58	44.21	39.41

nificantly lower in male controls than in male cases on day of diagnosis. Mean levels of IL-10 ($P < 0.0001$), TNF-α ($P = 0.02$) and IFN-γ ($P = 0.01$) were significantly lower in female controls than in female cases on day of diagnosis. Thus, *P. vivax* infection in Honduran residents appeared to be characterized by a proinflammatory cytokine response. There were no other significant differences in mean cytokine levels between males and females or between any of the other sample days. However, power for these analyses was less than 80%.

Repeated measures analysis. Over time (from sample days one [diagnosis] to four [baseline recovery]), testosterone categories were positively associated with parasitemia in the male cases ($F = 10.45$, $P = 0.0012$). A mixed model analysis (which allows for continuous effects [non-categorized testosterone levels] in the model statement) produced similar results ($F = 22.36$, $P = 0.0002$).

DISCUSSION

The data presented here are consistent with the suppositions that 1) *P. vivax* infection in Honduran residents is characterized by a proinflammatory cytokine response; 2) testosterone levels are positively associated with *P. vivax* parasitemia in adult males; and 3) males infected with *P. vivax* exhibit significantly lower testosterone levels and significantly higher cortisol levels than age-matched healthy controls.

Cytokine responses. A number of cytokines, both type 1 and type 2, are associated with *P. vivax* infection in humans.[42] For example, TNF-α was positively associated with disease severity in Sri Lankans infected with *P. vivax*.[43] Likewise, TNF-α was elevated during feverish paroxysms but fell shortly afterwards (3,000 to 15 pg/mL in 3 hours) in a separate nonimmune population.[44] In contrast, Seoh and others[45] found no association between degree of pyrexia (pyrexic = axillary temperature ≥ 37°C versus hyperpyrexic = axillary temperature ≥ 40°C) and serum TNF-α level in a cohort of 162 male Korean military personnel.

IFN-γ can inhibit parasitemia in *Plasmodium chabaudi, Plasmodium vinckei, Plasmodium cynomolgi,* and *Plasmodium vivax* infections[46–49] and can activate macrophages that produce endogenous pyrogens (i.e., TNF-α).[48] However, in at least one case, IFN-γ was not associated with TNF-α concentrations in Soloman islanders infected with *P. vivax*.[49] Most patients had little detectable IFN-γ in their sera, and neither IFN-γ nor IL-1 were associated with illness whereas TNF-α was.[49] However, in a different sample of *P. vivax* patients, IFN-γ was directly associated with fever magnitude.[50] In contrast, IL-12, another type 1 cytokine, was inversely associated with *P. falciparum* disease severity and parasitemia in a population of Papua New Guineans.[51]

The type 2 cytokine profile, dominated by IL-4 expression, is less frequently associated with *P. vivax* infection and is usually noticeable only after prolonged exposure to the parasite.[52] However, IL-10 may be elevated during *Plasmodium* infection to "prevent both hyper-parasitemia and host pathology."[52] That is, a strong proinflammatory cytokine response should be balanced by a strong anti-inflammatory cytokine response.[45]

In the current study, various cytokine levels were significantly higher in male and female cases on day of diagnosis as compared with 8 days post-diagnosis (following chloroquine treatment) or when compared with the age- and sex-matched controls. That is, prior to treatment (day of diagnosis), mean levels of IL-10, IL-12, TNF-α, and IFN-γ were significantly higher in male cases, as were IL-10, TNF-α and IFN-γ in female cases. In a study of Colombians that involved similar sample sizes, Praba-Egge and others[52] identified a similar type 1 proinflammatory cytokine response to *P. vivax* infection: in contrast to high IL-12 and TNF-α levels (and moderately increased IFN-γ levels), there was no IL-4 expression with moderate IL-10 levels.

Comparisons between sexes. The current project sampled a semi-immune population in a malaria-endemic region. It was not possible to determine exactly how long prior to diagnosis each case had acquired their most recent malarial infection (which was assessed in this project) nor was it possible to recruit more than a few individuals which had not previously

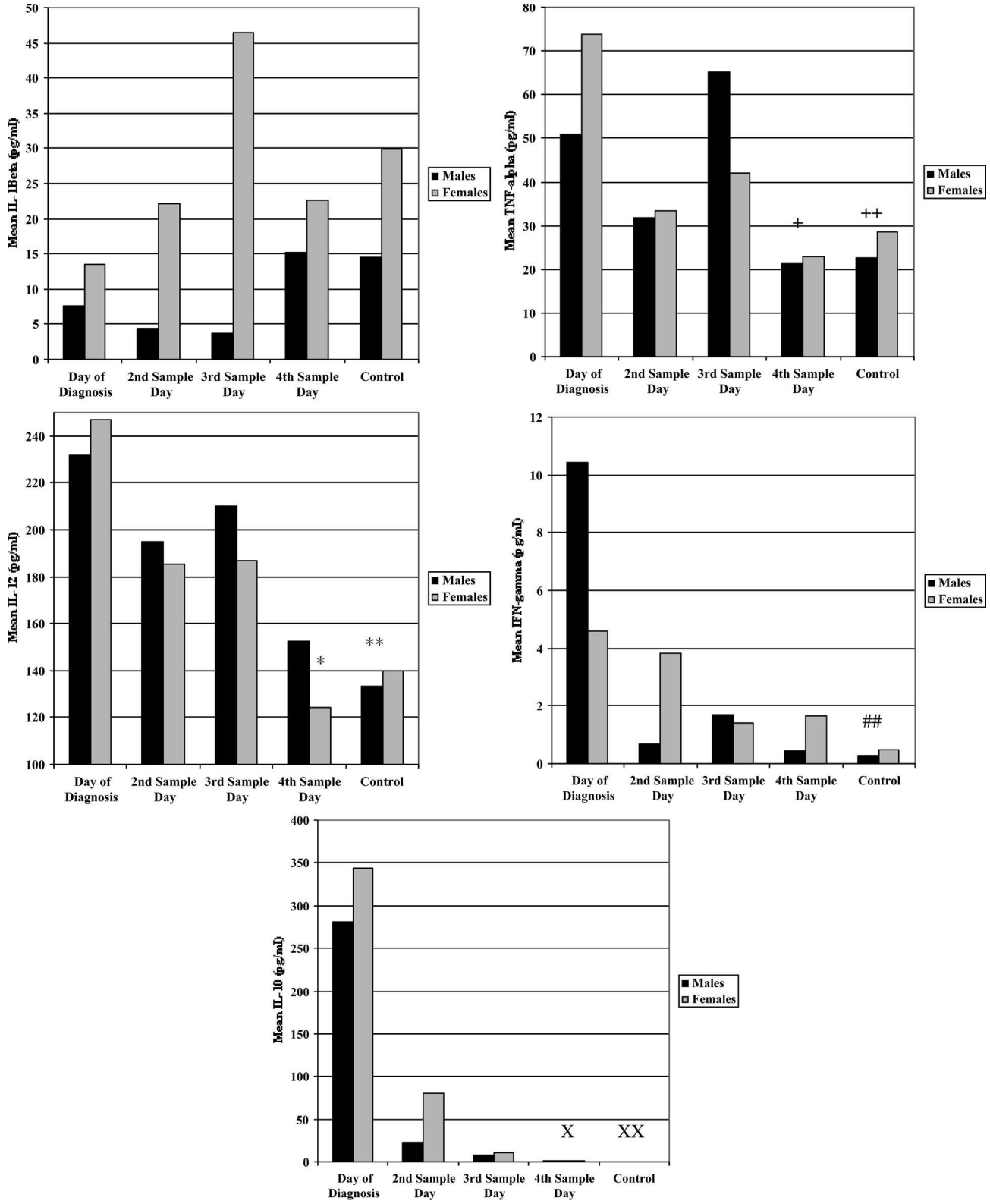

FIGURE 3. Mean cytokine levels in male and female cases and controls. *, IL-12 levels were significantly lower on fourth sample day compared with day of diagnosis for females ($P = 0.02$). **, Mean levels of IL-12 were significantly lower ($P < 0.01$) in male controls than in male cases on day of diagnosis. +, TNF-α levels were significantly lower on fourth sample day compared with day of diagnosis for males ($P = 0.03$) and females ($P = 0.01$). ++, Mean levels of TNF-α were significantly lower in male controls than in male cases on day of diagnosis ($P = 0.003$) and were significantly lower in female controls than in female cases on day of diagnosis ($P = 0.02$). ##, Mean levels of IFN-γ were significantly lower in male controls than in male cases on day of diagnosis ($P = 0.08$) and were significantly lower in female controls than in female cases on day of diagnosis ($P = 0.01$). ^{X}, IL-10 levels were significantly lower on fourth sample day compared with day of diagnosis for males ($P = 0.02$) and females ($P = 0.01$). ^{XX}, Mean levels of IL-10 were significantly lower in male controls than in male cases on day of diagnosis ($P < 0.0001$) and were significantly lower in female controls than in female cases on day of diagnosis ($P < 0.0001$).

TABLE 3
Mean cytokine levels for cases and controls

Cytokine (pg/mL)	Male cases	Female cases	Male controls	Female controls
Day of diagnosis				
IL-1β	7.60	13.46		
IL-12	231.71	246.96		
TNF-α	50.94	73.90		
IFN-γ	10.42	4.61		
IL-4	0	0		
IL-10	280.94	344.16		
Second sample day				
IL-1β	4.36	22.19		
IL-12	195.11	185.39		
TNF-α	31.78	33.51		
IFN-γ	0.70	3.84		
IL-4	0	1.35		
IL-10	22.14	80.52		
Third sample day				
IL-1β	3.75	46.41		
IL-12	210.13	186.93		
TNF-α	65.24	41.89		
IFN-γ	1.70	1.39		
IL-4	0	9.21		
IL-10	7.75	10.82		
Fourth sample day (or control)				
IL-1β	15.15	22.65	14.58	29.83
IL-12	152.49	124.22	133.15	139.94
TNF-α	21.28	22.78	22.63	28.63
IFN-γ	0.45	1.66	0.27	0.49
IL-4	0	1.00	0	0
IL-10	0.97	1.43	0.12	0.51

been infected with *P. vivax*. Furthermore, for obvious ethical reasons each case was treated for malaria using a standard chloroquine regiment, and therefore it was impossible to examine changes in immuno-endocrine profiles throughout further development of infection or natural convalescence. Thus, this project was unable to identify immuno-endocrine interactions in infection-naïve, non-chloroquine-treated individuals, nor was it able to assess testosterone-mediated susceptibility to acquiring infection. However, the male and female cases recruited in this study were comparable in that their average number of previous malarial cases as well as the timing between consecutive samples was similar, making comparisons between the two sexes feasible.

Results of the analyses were not consistent with the hypotheses that males and females differ in their *P. vivax* parasitemia and cytokine levels. It was expected that higher testosterone levels in the male cases would potentially contribute to higher parasitemia and lower cytokine levels compared with female cases. Although low power in many of the analyses precluded definitive conclusions with the current sample size, males and females appear to have differed very little in all measures except testosterone. However, comparisons between the sexes do not adequately test for testosterone-mediated differences in immunocompetence.

Prevalence and intensity of parasitic infection is often higher in male mammals and birds than in females.[6,10,53,54] For example, Poulin[9] found that males of various vertebrate taxa were more often infected with helminths than females, Moore and Wilson[11] found that males were more likely to be parasitized than females in 8 of 10 orders examined, and Brabin[55] found that men showed higher incidence of filarial infection than did women in 43 of 53 studies examined. Likewise, malaria is more common in the males of various lizard species.[56–58] However, male biases in parasitism may not be a general rule, and several factors, such as exposure rates, social behavior, habitat, diet, *and* hormone levels may account for these differences.[59] For example, sex differences in contact with either the vector, intermediate host, or infected individuals may explain sex-biases in infection.[60] Alternatively, androgens may promote behaviors, such as aggression and competition, that increase the likelihood of exposure to parasites or other contact-related infections.[60–62]

Associations between testosterone and immunocompetence. The effects of testosterone on malarial infection have received little prior attention. In rodents, testosterone reduces resistance to the malarial parasite *P. chabaudi*.[21] Female mice placed on a testosterone-supplemented diet exhibit reduced capacity of peritoneal macrophages to generate reactive oxygen intermediates during *P. chabaudi* infection.[29] However, levels of *P. mexicanuum* infection in the western fence lizard (*Sceloporus occidentalis*) did not differ between castrated or control males.[63]

Associations between testosterone and *Plasmodium* infection have been assessed even less in humans. In one case, testosterone was a significant positive predictor of resistance to *P. falciparum* parasitemia in male Kenyans.[64] In contrast, Campbell and others[65] found that testosterone was a marginally significant predictor of spleen complaints (interpreted by the authors as malarial infection; not a clinical diagnosis) among a group of settled Turkana men in Northwest Kenya. However, no such correlation was found among a nomadic population, despite poorer nutritional status among this group.[65]

In the present study, male testosterone levels (whether assessed as a continuous variable or categorically) were significantly and positively associated with parasitemia in the re-

peated measures analyses. That is, throughout the four consecutive samples taken during convalescence, higher testosterone levels were associated with higher parasitemia. Furthermore, testosterone levels were significantly depressed on the day of diagnosis for male cases compared with their fourth sample day (an average of 8 days post-diagnosis) or compared with their age-matched healthy controls. In various vertebrate species, male reproductive hormone function can be compromised following the onset of illness and somatic injury.[66,67] Testicular atrophy and azoospermia have been reported from men who died of AIDS,[68] and azoospermia has been associated with SIV infection in young male rhesus macaques.[69] Serum testosterone decreases during the onset of various conditions, including sepsis, burns, myocardial infarction, and surgery in humans.[67] Experimental *Trypanosoma brucei brucei* infection in rats has been associated with significant declines in serum luteinizing hormone (LH) and testosterone, as well as a loss in testicular responsiveness to exogenous gonadotropin and a decline in testicular LH receptors.[70]

In general, decreased testosterone synthesis and release may be caused by a variety of factors. Activated macrophages can secrete nitric oxide, which at high concentrations can inhibit Leydig cell steroidogenesis.[71] Corticotropin releasing hormone (CRH), glucocorticoids, and cytokines could possibly suppress gonadotropin releasing hormone (GnRH) secretion and decrease stimulation of Leydig cells by luteinizing hormone (LH).[38,68,72] The primary mechanism may involve a stress-induced increase in glucocorticoids in response to illness or injury, resulting in suppressed androgen synthesis. For example, in an investigation of sixty-two wounded soldiers in the former Yugoslavia, men with high injury severity scores exhibited significantly lower testosterone levels and higher adrenocorticotropin levels, especially within the first eighteen hours after being admitted for treatment compared with uninjured controls.[66] In the current study, cortisol levels were significantly higher in cases on day of diagnosis compared with their age-matched healthy controls, and thus glucocorticoid inhibition of testicular steroidogenesis may account for at least a portion of the depressed testosterone levels. Whether lowered testosterone levels are the result of physiologic alterations at the level of the testis or hypothalamus remains to be seen. Most importantly, depressed androgen levels during physiologic perturbations may be an advantageous, adaptive host response to prevent immunosuppression by higher testosterone levels,[20] as well as to redirect energetic resources,[3–5] specifically away from metabolically expensive anabolic functions during a time in which all available energy should theoretically be used for immunocompetence.

Received January 4, 2005. Accepted for publication February 25, 2005.

Acknowledgments: Permission to conduct this research was granted by the Health Area No. 4, Health Region No. 6, Dept. de Colon, Honduras. This project was approved by the Yale University Human Subjects Committee. The following individuals provided valuable logistical support: Nadia Abdala, Rosa Acosta, Richard Bribiescas, Susan Durham, Norma Funez, Daisy Guardiola, Robert Heimer, Reyna Hernandez, Rafael Linares, Leann Myers, Jaime Rosales, Geoffrey Routh, Stephen Stearns, Walker Umaña, David Watts, and the staff of Tocoa Hospital. This work would not have been possible without the gracious participation of the Honduran people.

Financial support: Funding was provided by the Department of Bacteriology and Parasitology at Tulane National Primate Research Center, the Department of Tropical Medicine and Center for Infectious Diseases at Tulane University, and the Williams Fund and Graduate School Summer Study Fund from Yale University.

Authors' addresses: Michael P. Muehlenbein, Department of Anthropology, University of Wisconsin-Milwaukee, P.O. Box 413, Milwaukee, WI 53201, Telephone (414) 229-6250, Fax: (414) 229-5848, E-mail: mpm1@uwm.edu. Jackeline Alger, Departamento de Laboratorios Clinicos, Hospital Escuela, P.O. Box 3051, Tegucigalpa, Honduras, E-mail: malaria@cablecolor.hn. Frank Cogswell, Department of Bacteriology and Parasitology, Tulane National Primate Research Center, 18703 Three Rivers Road, Covington, LA 70433, Telephone: (985) 871-6224, E-mail: cogswell@tpc.tulane.edu. Mark James, Department of Tropical Medicine, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA 70112, Telephone: (504) 584-2503, E-mail: mjames@tulane.edu. Donald Krogstad, Department of Tropical Medicine, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA 70112, Telephone: (504) 584-3552, E-mail: krogstad@tulane.edu.

Reprint requests: Michael P. Muehlenbein, Laboratory for Evolutionary Physiology and Parasitology, Department of Anthropology, University of Wisconsin-Milwaukee, 3413 N. Downer Ave., Milwaukee, WI 53211, Phone: (414) 229-6250, Fax: (414) 229-5848, E-mail: mpm1@uwm.edu

REFERENCES

1. Trigg PI, Kondrachine AV, 1998. The current global malaria situation. Sherman PW, ed. *Malaria: Parasite Biology, Pathogenesis, and Protection*. Washington, DC: ASM Press, 11–24.
2. World Health Organization, 1997. World malaria situation in 1994: population at risk. *Wkly Epidemiol Rec 72:* 269–276.
3. Muehlenbein MP, 2004. *Testosterone-Mediated Immune Function: An Energetic Allocation Mechanism Evaluated in Human and Non-Human Primate Males.* Ph.D. Thesis, Department of Anthropology, Yale University.
4. Wedekind C, Folstad I, 1994. Adaptive or nonadaptive immunosuppression by sex-hormones. *Am Nat 143:* 936–938.
5. Sheldon BC, Verhulst S, 1996. Ecological immunology: costly parasite defenses and trade-offs in evolutionary ecology. *Trends Ecol Evol 11:* 317–321.
6. Folstad I, Nilssen AC, Halvorsen O, Andersen J, 1989. Why do male reindeer (*Rangifer tarandus tarandus*) have higher abundance of second and third instar larvae of *Hypoderma tarandi* than females? *Oikos 55:* 87–92.
7. Tiuria R, Horii Y, Makimura S, Ishikawa N, Tsuchiya K, Nawa Y, 1995. Effect of testosterone on the mucosal defence against intestinal helminths in Indian soft-furred rats, *Millardia meltada* with reference to goblet and mast cell responses. *Parasite Immunol 17:* 479–484.
8. Olsen NJ, Kovacs WJ, 1996. Gonadal steroids and immunity. *Endocr Rev 17:* 369–384.
9. Poulin R, 1996. Sexual inequalities in helminth infections: a cost of being a male? *Am Nat 147:* 287–295.
10. Zuk M, McKean KA, 1996. Sex differences in parasite infections: patterns and processes. *Int J Parasitol 26:* 1009–1023.
11. Moore SL, Wilson K, 2002. Parasites as a viability cost of sexual selection in natural populations of mammals. *Science 297:* 2015–2018.
12. Weatherhead PJ, Metz KJ, Bennett GF, Irwin RE, 1993. Parasite faunas, testosterone and secondary sexual traits in male red-winged blackbirds. *Behav Ecol Sociobiol 33:* 13–23.
13. Saino N, Moller AP, 1994. Secondary sexual characters, parasites and testosterone in the barn swallow, *Hirundo rustica. Anim Behav 48:* 1325–1333.
14. Saino N, Moller AP, Bolzern AM, 1995. Testosterone effects on the immune system and parasite infestations in the barn swallow (*Hirundo rustica*): an experimental test of the immunocompetence hypothesis. *Behav Ecol 6:* 397–404.
15. Hasselquist D, Marsh JA, Sherman PW, Wingfield JC, 1999. Is avian humoral immunocompetence suppressed by testosterone? *Behav Ecol Sociobiol 45:* 167–175.
16. Duffy DL, Ball GF, 2002. Song predicts immunocompetence in

male European starlings (*Sturnus vulgaris*). *Proc R Soc Lond B Biol Sci 269:* 847–852.
17. Weinstein Y, Bercovich Z, 1981. Testosterone effects on bone marrow, thymus and suppressor T cells in the (NZB x NZW) F1 mice: its relevance to autoimmunity. *J Immunol 126:* 998–1002.
18. Schuurs AHWM, Verheul HAM, 1990. Effects of gender and sex steroids on the immune response. *J Steroid Biochem Mol Biol 35:* 157–172.
19. Olsen NJ, Watson MB, Henderson GS, Kovacs WJ, 1991. Androgen deprivation induces phenotypic and functional changes in the thymus of adult mice. *Endocrinology 129:* 2471–2476.
20. Folstad I, Karter AJ, 1992. Parasites, bright males and the immunocompetence handicap. *Am Nat 139:* 603–622.
21. Benten WP, Wunderlich F, Herrmann R, Kuhn-Velten WN, 1993. Testosterone-induced compared with oestradiol-induced immunosuppression against *Plasmodium chabaudi* malaria. *J Endocrinol 139:* 487–494.
22. Hadid R, Spinedi E, Daneva T, Grau G, Gaillard RC, 1995. Repeated endotoxin treatment decreases immune and hypothalamo-pituitary-adrenal axis responses—effects of orchiectomy and testosterone therapy. *Neuroendocrinology 62:* 348–355.
23. Zuk M, Johnsten TS, Maclarty T, 1995. Endocrine-immune interactions, ornaments and mate choice in red jungle fowl. *Proc R Soc Lond B Biol Sci 260:* 205–210.
24. Duffy DL, Bentley GE, Drazen DL, 2000. Effects of testosterone on cell-mediated and humoral immunity in non-breeding adult European starlings. *Behav Ecol 11:* 654–662.
25. Grossman CJ, Roselle GA, Mendenhall CL, 1991. Sex steroid regulation of autoimunity. *J Steroid Biochem Mol Biol 40:* 649–659.
26. Chao TC, Van Alten PJ, Walter RJ, 1994. Steroid sex hormones and macrophage function: modulation of reactive oxygen intermediates and nitrite release. *Am J Reprod Immunol 32:* 43–52.
27. Chao TC, Van Alten PJ, Greager JA, Walter RJ, 1995. Steroid sex hormones regulate the release of tumor necrosis factor by macrophages. *Cell Immunol 160:* 43–49.
28. Grossman CJ, 1995. The role of sex steroids in immune system regulation. Grossman CJ, ed. *Bilateral Communication between the Endocrine and Immune Systems*. New York: Springer-Verlag, 1–11.
29. Benten WP, Ulrich P, Kuhn-Velten WN, Vohr HW, Wunderlich F, 1997. Testosterone-induced susceptibility to *Plasmodium chabaudi* malaria: persistence after withdrawal of testosterone. *J Endocrinol 153:* 275–281.
30. Straub RH, Cutolo M, 2001. Involvement of the hypothalamic-pituitary-adrenal/gonadal axis and the peripheral nervous system in rheumatoid arthritis: viewpoint based on a systemic pathogenetic role. *Arthritis Rheum 44:* 493–507.
31. Wunderlich F, Benten WP, Lieberherr M, Guo Z, Stamm O, Wrehlke C, Sekeris CE, Mossmann H, 2002. Testosterone signaling in T cells and macrophages. *Steroids 67:* 535–538.
32. Palmer CJ, Makler M, Klaskala WI, Lindo JF, Baum MK, Ager AL, 1998. Increased prevalence of *Plasmodium falciparum* malaria in Honduras, Central America. *Rev Panam Salud Publica 4:* 40–42.
33. SAS Institute Inc, 2001. SAS System for Windows. Cary, NC: SAS Institute Inc.
34. Elenkov IJ, Papanicolaou DA, Wilder RL, Chrousos GP, 1996. Modulatory effects of glucocorticoids and catecholamines on human interleukin-12 and interleukin-10 production: clinical implications. *Proc Assoc Am Physicians 108:* 374–381.
35. Norbiato G, Bevilacqua M, Vago T, Taddei A, Clerici M, 1997. Glucocorticoids and the immune function in the human immunodeficiency virus infection: a study in hypercortisolemic and cortisol-resistant patients. *J Clin Endocrinol Metab 82:* 3260–3263.
36. Elenkov IJ, Chrousos GP, 1999. Stress, cytokine patterns and susceptibility to disease. *Baillieres Best Pract Res Clin Endocrinol Metab 13:* 583–595.
37. Turnbull AV, Rivier CL, 1999. Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. *Physiol Rev 79:* 1–71.
38. Doerr P, Pirke KM, 1976. Cortisol-induced suppression of plasma testosterone in normal adult males. *J Clin Endocrinol Metab 43:* 622–629.
39. Bambino TH, Hsueh AJ, 1981. Direct inhibitory effect of glucocorticoids upon testicular luteinizing hormone receptor and steroidogenesis in vivo and in vitro. *Endocrinology 108:* 2142–2148.
40. Faul F, Erdfelder E, 1992. *GPower: a priori, post-hoc, and compromise power analyses for MS-DOS.* Bonn, FRG: Bonn University, Department of Psychology.
41. Lehmann EL, 1975. *Nonparametrics. Statistical Methods Based on Ranks.* San Francisco: Holden-Day.
42. Park JW, Park SH, Yeom JS, Huh AJ, Cho YK, Ahn JY, Min GS, Song GY, Kim YA, Ahn SY, Woo SY, Lee BE, Ha EH, Han HS, Yoo K, Seoh JY, 2003. Serum cytokine profiles in patients with *Plasmodium vivax* malaria: a comparison between those who presented with and without thrombocytopenia. *Ann Trop Med Parasitol 97:* 339–344.
43. Karunaweera ND, Carter R, Grau GE, Mendis KN, 1998. Demonstration of anti-disease immunity to *Plasmodium vivax* malaria in Sri Lanka using a quantitative method to assess clinical disease. *Am J Trop Med Hyg 58:* 204–210.
44. Mendis KN, Carter R, 1992. The role of cytokines in *Plasmodium vivax* malaria. *Mem Inst Oswaldo Cruz 87:* 51–55.
45. Seoh JY, Khan M, Park SH, Park HK, Shin MH, Ha EH, Lee BE, Yoo K, Han HS, Oh S, Wi JH, Hong CK, Oh CH, Kim YA, Park JW, 2003. Serum cytokine profiles in patients with *Plasmodium vivax* malaria: a comparison between those who presented with and without hyperpyrexia. *Am J Trop Med Hyg 68:* 102–106.
46. Mendis KN, Naotunne TD, Karunaweera ND, Del Giudice G, Grau GE, Carter R, 1990. Anti-parasite effects of cytokines in malaria. *Immunol Lett 25:* 217–220.
47. Stevenson MM, Tam MF, Belosevic M, Meide Pvd, Podoba JE, 1990. Role of endogenous gamma interferon in host response to infection with blood-stage *Plasmodium chabaudi* AS. *Infect Immun 58:* 3225–3232.
48. Artavanis-Tsakonas K, Tongren JE, Riley EM, 2003. The war between the malaria parasite and the immune system: immunity, immunoregulation and immunopathology. *Clin Exp Immunol 133:* 145–152.
49. Butcher GA, Garland T, Ajdukiewicz AB, Clark IA, 1990. Serum tumor necrosis factor associated with malaria in patients in the Solomon Islands. *Trans R Soc Trop Med Hyg 84:* 658–661.
50. Brown AE, Teja-Isavadharm P, Webster HK, 1991. Macrophage activation in vivax malaria: fever is associated with increased levels of neopterin and interferon-gamma. *Parasite Immunol 13:* 673–679.
51. Boutlis CS, Lagog M, Chaisavaneeyakorn S, Misukonis MA, Bockarie MJ, Mgone CS, Wang Z, Morahan G, Weinberg JB, Udhayakumar V, Anstey NM, 2003. Plasma interleukin-12 in malaria-tolerant papua new guineans: inverse correlation with *Plasmodium falciparum* parasitemia and peripheral blood mononuclear cell nitric oxide synthase activity. *Infect Immun 71:* 6354–6357.
52. Praba-Egge AD, Montenegro S, Arevalo-Herrera M, Hopper T, Herrera S, James MA, 2003. Human cytokine responses to meso-endemic malaria on the Pacific Coast of Colombia. *Ann Trop Med Parasitol 97:* 327–337.
53. Fedigan LM, Zohar S, 1997. Sex differences in mortality of Japanese macaques: Twenty-one years of data from the Arashiyama West population. *Am J Phys Anthropol 102:* 161–175.
54. Travi BL, Osorio Y, Melby PC, Chandrasekar B, Arteaga L, Saravia NG, 2002. Gender is a major determinant of the clinical evolution and immune response in hamsters infected with *Leishmania* spp. *Infect Immun 70:* 2288–2296.
55. Brabin L, 1990. Sex differentials in susceptibility to lymphatic filariasis and implications for maternal child immunity. *Epidemiol Infect 105:* 335–353.
56. Ayala SC, Spain JL, 1976. A population of *Plasmodium colombiense* sp. N. in the iguanid lizard, *Anolis auratus. J Parasitol 62:* 177–189.
57. Schall JJ, Vogt SP, 1993. Distribution of malaria in Anolis lizards of the Lupuillo Forest, Puerto Rica: implications for host community ecology. *Biotropica 25:* 229–235.

58. Schall JJ, 1996. Malaria parasites in lizards: diversity and ecology. *Adv Parasitol 37:* 255–333.
59. Schalk G, Forbes MR, 1997. Male biases in parasitism of mammals. *Oikos 78:* 67–74.
60. Klein SL, 2000. The effects of hormones on sex differences in infection: from genes to behavior. *Neurosci Biobehav Rev 24:* 627–638.
61. Zuk M, 1996. Disease, endocrine-immune interactions, and sexual selection. *Ecology 77:* 1037–1042.
62. Wedekind C, Jakobsen PJ, 1998. Male-biased susceptibility to helminth infection: an experimental test with a copepod. *Oikos 81:* 458–462.
63. Eisen RJ, DeNardo DF, 2000. Life history of a malaria parasite (*Plasmodium mexicanum*) in its host, the western fence lizard (*Sceloporus occidentalis*): host testosterone as a source of seasonal and among-host variation? *J Parasitol 86:* 1041–1045.
64. Kurtis JD, Mtalib R, Onyango FK, Duffy PE, 2001. Human resistance to *Plasmodium falciparum* increases during puberty and is predicted by dehydroepiandrosterone sulfate levels. *Infect Immun 69:* 123–128.
65. Campbell BC, Lukas WD, Campbell KL, 2001. Reproductive Ecology of Male Immune Function and Gonadal Function. Ellison PT, ed. *Reproductive Ecology and Human Evolution.* New York: Aldine de Gruyter, 159–178.
66. Cernak I, Savic J, Lazarov A, 1997. Relations among plasma prolactin, testosterone, and injury severity in war casualties. *World J Surg 21:* 240–245.
67. Spratt DI, 2001. Altered gonadal steroidogenesis in critical illness: is treatment with anabolic steroids indicated? *Best Pract Res Clin Endocrinol Metab 15:* 479–494.
68. Dym M, Orenstein J, 1990. Structure of the male reproductive tract in AIDS patients. Alexander NJ, Gabelnick HL, Spieler JM, eds. *Heterosexual Transmission of AIDS*. New York: Alan R. Liss, 181–196.
69. Nadler RD, Manocha AD, McClure HM, 1993. Spermatogenesis and hormone levels in rhesus macaques inoculated with simian immunodeficiency virus. *J Med Primatol 22:* 325–329.
70. Soudan B, Tetaert D, Racadot A, Degand P, Boersma A, 1992. Decrease of testosterone level during an experimental African trypanosomiasis: involvement of a testicular LH receptor desensitization. *Acta Endocrinol (Copenh) 127:* 86–92.
71. Valenti S, Cuttica CM, Giusti M, Giordano G, 1999. Nitric oxide modulates Leydig cell function in vitro: is this a way of communication between the immune and endocrine system in the testis? *Ann N Y Acad Sci 876:* 298–300.
72. Hales DB, 1992. Interleukin-1 inhibits Leydig cell steroidogenesis primarily by decreasing 17 alpha-hydroxylase/C17-20 lyase cytochrome P450 expression. *Endocrinology 131:* 2165–2172.