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 Clínicos, 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.4–6 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-
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; 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>
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<th>Table 1</th>
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<tr>
<td><strong>Case description</strong></td>
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<td><strong>Variable</strong></td>
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<tr>
<td>Sample size</td>
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<tr>
<td>Mean age (years)</td>
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<tr>
<td>Mean no. previously reported malarial episodes</td>
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<tr>
<td>% Reported fever on day of diagnosis</td>
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<tr>
<td>% Reported chills on day of diagnosis</td>
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<tr>
<td>% Reported headache on day of diagnosis</td>
</tr>
<tr>
<td>% Reported muscle aches on day of diagnosis</td>
</tr>
<tr>
<td>% Reported paroxysms on day of diagnosis</td>
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</table>
Parasitemia was measured as a continuous variable. Table 1 reports mean ages and mean hormone levels using the 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, and 2) cortisol is often associated with suppression of the hypothalamic-pituitary-gonadal axis.

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. Power analyses for the Wilcoxon rank sum tests and all correlations were performed with the program G-Power. For those variables that were non-normally distributed, G-Power analyses for parametric data were used with a 95% correction. 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 test). 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).
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: \( T = 3, P = 0.719 \), 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-
A number of cytokines, both type 1 and type 2, are associated with *P. vivax* infection in humans. For example, TNF-α was positively associated with disease severity in Sri Lankans infected with *P. vivax*. 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. In contrast, Seoh and others found no association between degree of pyrexia (pyrexic = axillary temperature ≥ 37°C or 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 vivax*, *Plasmodium cynomolgi*, and *Plasmodium vinckei*, and can activate macrophages that produce endogenous pyrogens (i.e., TNF-α). However, in at least one case, IFN-γ was not associated with TNF-α concentrations in Soloman islanders infected with *P. vivax*. 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. However, in a different sample of *P. vivax* patients, IFN-γ was directly associated with fever magnitude. 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.

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. However, IL-10 may be elevated during feverish paroxysms but fall shortly afterwards (3,000 to 15 pg/mL in 3 hours) in a separate nonimmune population. 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

### Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male cases</th>
<th>Female cases</th>
<th>Male controls</th>
<th>Female controls</th>
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<tbody>
<tr>
<td>Day of diagnosis</td>
<td></td>
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<td></td>
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<tr>
<td>Testosterone (ng/mL)</td>
<td>2.57</td>
<td>0.57</td>
<td>4.35</td>
<td>0.63</td>
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<tr>
<td>Cortisol (µg/dL)</td>
<td>11.43</td>
<td>16.78</td>
<td>12.31</td>
<td>8.58</td>
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<tr>
<td>Hemoglobin (g/dL)</td>
<td>14.05</td>
<td>13.44</td>
<td>12.17</td>
<td>13.14</td>
</tr>
<tr>
<td>Hematocrit (% RBCs)</td>
<td>42.28</td>
<td>40.37</td>
<td>36.94</td>
<td>39.41</td>
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<td>Second sample day</td>
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<tr>
<td>Testosterone (ng/mL)</td>
<td>2.98</td>
<td>0.54</td>
<td>4.35</td>
<td>0.63</td>
</tr>
<tr>
<td>Cortisol (µg/dL)</td>
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<td>12.69</td>
<td>12.31</td>
<td>8.58</td>
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<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.03</td>
<td>13.40</td>
<td>12.17</td>
<td>13.14</td>
</tr>
<tr>
<td>Hematocrit (% RBCs)</td>
<td>38.94</td>
<td>39.56</td>
<td>36.94</td>
<td>39.41</td>
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<tr>
<td>Third sample day</td>
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<tr>
<td>Testosterone (ng/mL)</td>
<td>3.95</td>
<td>0.53</td>
<td>4.35</td>
<td>0.63</td>
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<tr>
<td>Cortisol (µg/dL)</td>
<td>10.28</td>
<td>10.31</td>
<td>12.31</td>
<td>8.58</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.43</td>
<td>12.31</td>
<td>12.17</td>
<td>13.14</td>
</tr>
<tr>
<td>Hematocrit (% RBCs)</td>
<td>40.30</td>
<td>36.94</td>
<td>36.94</td>
<td>39.41</td>
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<tr>
<td>Fourth sample day (or control)</td>
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<tr>
<td>Testosterone (ng/mL)</td>
<td>4.18</td>
<td>0.46</td>
<td>4.35</td>
<td>0.63</td>
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<tr>
<td>Cortisol (µg/dL)</td>
<td>9.83</td>
<td>7.72</td>
<td>8.27</td>
<td>8.58</td>
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<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.27</td>
<td>13.53</td>
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<tr>
<td>Hematocrit (% RBCs)</td>
<td>36.80</td>
<td>40.58</td>
<td>44.21</td>
<td>39.41</td>
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</tbody>
</table>

**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. For example, TNF-α was positively associated with disease severity in Sri Lankans infected with *P. vivax*. 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. In contrast, Seoh and others found no association between degree of pyrexia (pyrexic = axillary temperature ≥ 37°C or 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 vivax*, *Plasmodium cynomolgi*, and *Plasmodium vinckei*. However, IL-10 may be elevated during feverish paroxysms but fall shortly afterwards (3,000 to 15 pg/mL in 3 hours) in a separate nonimmune population. 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

**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 ($P < 0.0001$).
been infected with \textit{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 \textit{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.\textsuperscript{6,10,53,54} For example, Poulin\textsuperscript{7} found that males of various vertebrate taxa were more often infected with helminths than females, Moore and Wilson\textsuperscript{11} found that males were more likely to be parasitized than females in 8 of 10 orders examined, and Brabin\textsuperscript{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.\textsuperscript{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.\textsuperscript{59} For example, sex differences in contact with either the vector, intermediate host, or infected individuals may explain sex-biases in infection.\textsuperscript{60} Alternatively, androgens may promote behaviors, such as aggression and competition, that increase the likelihood of exposure to parasites or other contact-related infections.\textsuperscript{60–62}

\textbf{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 \textit{P. chabaudi}.\textsuperscript{21} Female mice placed on a testosterone-supplemented diet exhibit reduced capacity of peritoneal macrophages to generate reactive oxygen intermediates during \textit{P. chabaudi} infection.\textsuperscript{29} However, levels of \textit{P. mexicanum} infection in the western fence lizard (\textit{Sceloporus occidentalis}) did not differ between castrated or control males.\textsuperscript{63} Associations between testosterone and \textit{Plasmodium} infection have been assessed even less in humans. In one case, testosterone was a significant positive predictor of resistance to \textit{P. falciparum} parasitemia in male Kenyans.\textsuperscript{64} In contrast, Campbell and others\textsuperscript{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.\textsuperscript{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. Testicular atrophy and azoospermia have been reported from men who died of AIDS and azoospermia has been associated with SIV infection in young male rhesus macaques. Serum testosterone decreases during the onset of various conditions, including sepsis, burns, myocardial infarction, and surgery in humans. 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.

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. 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). 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 adrenocorticotropic levels, especially within the first eighteen hours after being admitted for treatment compared with uninjured controls. 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, as well as to redirect energetic resources specifically away from metabolically expensive anabolic functions during a time in which all available energy should theoretically be used for immunocompetence.

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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@cablcolor.hn. Frank Cogswell, Department of Entomology 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

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