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# Dehydroepiandrosterone Sulfate Levels Associated with Decreased Malaria Parasite Density and Increased Hemoglobin Concentration in Pubertal Girls from Western Kenya

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**In areas where *Plasmodium falciparum* malaria is endemic, parasite density, morbidity, and mortality decrease with increasing age, which supports the view that years of cumulative exposure are necessary for the expression of maximal protective immunity. Developmental changes in the host also have been implicated in the expression of maximal resistance. To further evaluate the contribution of host developmental factors in malaria resistance, we examined the relationship between *P. falciparum* parasitemia and pubertal development in a cross-sectional sample of 12–18-year-old schoolgirls from an area of intense transmission in western Kenya. Among pubertal girls, dehydroepiandrosterone sulfate (DHEAS) levels were significantly associated with decreased parasite density, even after adjustment for age. DHEAS levels also were related to increased hemoglobin levels, even after accounting for age and other determinants of hemoglobin level. These findings support the hypothesis that host pubertal development, independent of age and, by proxy, cumulative exposure, is necessary for maximal expression of resistance to malarial infection and morbidity, as assessed by hemoglobin level.**

*Plasmodium falciparum* malaria remains one of the most important causes of morbidity and mortality in sub-Saharan Africa, accounting for the deaths of up to 1 million children/year [1] and contributing to the economic instability of the region [2]. Understanding the epidemiology of malaria-attributed illness and death remains vital for the effective targeting of scarce public health resources.

The magnitude and age distribution of malaria-at-

tributable morbidity and mortality varies with the intensity of transmission. In areas with intense, stable malaria transmission, the risk of morbidity declines rapidly within the first years of life [3–5]. Where transmission is less intense, clinical immunity develops at a slower pace, and children remain at risk of severe disease and death for a longer time [6]. Under conditions of stable transmission, adolescents are at a markedly reduced risk of severe malaria morbidity and mortality, compared with preschool children [3, 4]. Despite this reduced risk, adolescents continue to suffer from frequent asymptomatic infections and periodic clinical illness and death [1, 7].

The age-related decline in parasite density, morbidity, and mortality observed during childhood has supported the view that years of cumulative exposure, potentially to multiple parasite strains, are necessary for the production of protective immune responses. Recently, studies of transmigrant populations in Irian Jaya (now West Papua) and longitudinal cohort studies in Kenya have challenged this hypothesis. Baird et al. [8,

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9] demonstrated that nonimmune, adult transmigrants moving from Java, where malaria is not endemic, to parts of Irian Jaya where malaria is endemic acquired immunity faster than did their transmigrant children. Kurtis et al. [10] demonstrated that, among Kenyan males, resistance to reinfection after antimalarial treatment increased during puberty and was predicted by levels of the pubertal steroid dehydroepiandrosterone sulfate (DHEAS), independently of age and, by proxy, cumulative exposure.

In the present study, we examined the relationships among DHEAS levels, malaria parasitemia, and anemia in a group of 12–18-year-old schoolgirls from an area of western Kenya where malaria is holoendemic. We hypothesized that, as in males, DHEAS levels in girls would be associated with reduced parasite densities, even after adjustment for age. We further hypothesized that DHEAS would predict decreased malarial morbidity, as measured by increased hemoglobin (Hgb) levels.

## SUBJECTS, MATERIALS, AND METHODS

**Study area and population.** Two cross-sectional surveys were conducted between October 1998 and March 1999 at public primary schools in Asembo, Rarieda Division, Bondo District, located on the shores of Lake Victoria in Nyanza Province, western Kenya. The study area is representative of many parts of sub-Saharan Africa, with intense perennial malaria transmission. Details of the study site have been published elsewhere [11, 12]. In brief, ~55,000 people live in an area covering 200 km<sup>2</sup>. Malaria transmission is year-round, and the number of infective bites per person per year is comparable with the highest rates documented worldwide (estimated to be 60–300 bites/person/year). Between 60% and 90% of children <5 years old are anemic at any time (Hgb level, <11 g/dL), and 10%–25% have moderate-to-severe anemia (Hgb level, <7 g/dL) [13]. Malaria is one of the most important determinants of anemia in young children in this area. Approximately 60% of 10–14-year-old children were found to be parasitemic at any time, most with asymptomatic low parasite density infections [3]. The present study coincided with a large community-based, randomized controlled study of the efficacy of insecticide-treated bed nets (ITNs) [11, 14], and half the girls we studied were sleeping under ITNs.

**Study design.** A multistage, random-sample design, with primary schools as first-stage units and schoolgirls as second-stage units, was used [15]. Schools were ranked by geographic location, to allow for equal distribution of the schools over the study area, and were selected by random sampling with probability proportional to size [16]. A total of 840 12–18-year-old girls in 28 schools were randomly selected to participate. Complete data were collected on 669 individuals (80%): 116 girls refused (median, 6 girls/school; interquartile range [IQR], 2.5–8 girls/school), and 55 had moved away or were absent from school

on the day of survey (median, 2 girls/school; IQR, 0–2.5 girls/school). No attempt was made to follow up randomized girls who refused to participate or who were absent. During data cleaning, 21 girls who reported that they were ≥12 years old were excluded from the final data set, because analysis of age using date of birth revealed that they were actually <12 years old. Six hundred forty-eight girls were included in the final data set: 312 in the first survey and 336 in the second survey.

Written consent was obtained from each participating student, her parents, and the parent-teacher association of each school. The study was approved by the ethical committee of the Kenya Medical Research Institute and the Academic Medical Center of the University of Amsterdam.

The cross-sectional surveys were originally designed to study the prevalence of and risk factors for anemia, malaria, and malnutrition in adolescent school girls in an area of intense malaria transmission. During the course of the study, data from a cohort study conducted in the same study area, demonstrating that puberty and DHEAS levels predict resistance to malaria in males, were published [10]. Therefore, we chose to determine whether this same relationship operated in our cross-sectional sample of adolescent girls.

**Cross-sectional surveys.** The first of 2 cross-sectional surveys (14 schools) was conducted in October–November 1998 (after the rainy season), and the second (14 different schools) was conducted in February–March 1999 (after a prolonged dry period). Data were collected by a trained local team. A variety of demographic, clinical, laboratory, and anthropometric data were collected via a questionnaire, a finger-prick blood sample was obtained, and a basic clinical examination, including assessment of pubertal development through a modified Tanner staging based on breast development only [17], was done. Pubertal development was quantified on an ordinal scale from B1 (prepubescent) to B5 (postpubescent).

Heights and weights were measured using standard anthropometric procedures [18]. Height-for-age *z* scores and body mass index (BMI)-for-age *z* scores were calculated using the EpiNut program in EpiInfo 2000 with Centers for Disease Control and Prevention 2000 reference data [19].

**Laboratory methods.** A finger-prick blood sample (250–500 μL) was obtained for determination of hematologic markers, plasma analytes, and malaria parasitemia. Blood was collected into heparinized tubes and kept on ice in cooler boxes until transport to the laboratory the same day. After a complete blood count was obtained by use of a Coulter Counter (model ACT 10; Coulter), samples were centrifuged, and cells and plasma were separated and stored at –18°C.

Thick blood smears were stained with Giemsa and examined for malaria parasites. Parasites and leukocytes were counted in the same fields until 300 leukocytes or 500 parasites were counted. Parasite densities (parasites/mm<sup>3</sup>) were calculated using the ac-

**Table 1. Descriptive statistics of participants with and without available dehydroepiandrosterone sulfate (DHEAS) level data.**

Characteristic	DHEAS level data available		DHEAS level data not available		P
	No. of subjects evaluated	Value	No. of subjects evaluated	Value	
Age, median years (IQR)	264	14.0 (13.1–15.1)	384	14.3 (13.2–15.4)	.149 <sup>a</sup>
Maturity rating, median (IQR) <sup>b</sup>	263	B3 (B2–B4)	384	B3 (B2–B4)	.024 <sup>a,c</sup>
DHEAS level, geometric mean ng/mL (95% CI)	264	968 (834–1122)		NA	NA
ITN use	263	144 (53.9)	384	197 (51.0)	.819 <sup>d</sup>
Prevalence of malaria parasitemia	222	48 (21.6)	353	116 (31.0)	.091 <sup>d</sup>
Parasite density for positive blood films only, geometric mean parasites/mm <sup>3</sup> (95% CI)	48	178 (129–246)	116	159 (100–253)	.699 <sup>e</sup>
Anemia <sup>f</sup>	263	74 (27.6)	371	61 (17.0)	.066 <sup>d</sup>
Hemoglobin level, mean g/dL (95% CI)	263	12.6 (12.2–12.9)	371	13.2 (12.8–13.4)	.019 <sup>e</sup>
Any helminth infection	237	117 (48.8)	303	136 (45.1)	.611 <sup>d</sup>
Hookworm	237	29 (11.9)	303	46 (15.1)	.390 <sup>d</sup>
<i>Schistosoma mansoni</i>	237	23 (9.9)	303	39 (13.0)	.454 <sup>d</sup>
<i>Trichuris trichiura</i>	237	43 (18.1)	303	47 (16.1)	.560 <sup>d</sup>
<i>Ascaris lumbricoides</i>	237	66 (26.9)	303	62 (19.9)	.302 <sup>d</sup>

**NOTE.** Data are no. (%) of subjects, except where noted. CI, confidence interval; IQR, interquartile range; ITN, insecticide-treated bed net; NA, not applicable.

<sup>a</sup> Wilcoxon-Mann-Whitney *U* 2-sample test.

<sup>b</sup> Modified Tanner staging of breast development only [17].

<sup>c</sup> The group for whom DHEAS level data were available was more mature than the group for whom DHEAS level data were not available.

<sup>d</sup>  $\chi^2$  test.

<sup>e</sup> Student's *t* test.

<sup>f</sup> Anemia is defined as a hemoglobin level <12 g/dL.

tual white blood cell count determined simultaneously by Coulter Counter. The limit of detection was ~10 parasites/mm<sup>3</sup>.

At the time of each survey, all study participants were asked to bring fresh (<24 h old) stool and urine samples to be examined for the presence of geohelminths (i.e., hookworm, *Ascaris lumbricoides*, *Trichuris trichiura*, and *Strongyloides stercoralis*), *Schistosoma mansoni*, and *Schistosoma haematobium*. Stool and urine samples were obtained from 540 and 554 volunteers, respectively. Samples were stored at 4°C and processed the day after collection. Stool samples were microscopically examined by concentration methods, using a modification of the formol-ether and ethyl-acetate techniques, and by Kato-Katz methods [20–22]. Urine samples were examined using a filtration-based concentration method [23].

Quantitative plasma DHEAS, ferritin, and C-reactive protein (CRP) assays were performed using PANTEX EIA kits (ICN Pharmaceuticals), for DHEAS assays, and solid-phase ELISA kits (ICN Pharmaceuticals), for ferritin and CRP assays. Immunoassays were done for a subsample of 264 of 648 girls (36/312 from the first survey and 228/336 from the second survey), because clusters of plasma samples (packed per school) were lost in transport during a prolonged power failure in Kisumu in 1999. Descriptive statistics of subjects with and without a

DHEAS immunoassay result are presented in table 1. Girls with inflammation, defined as a plasma CRP level >8.2 mg/L [24], were excluded from statistical analyses that included ferritin as the end point or as a covariate, because ferritin levels increase as an acute phase response and no longer reflect the component of stored iron [25]. Iron deficiency was defined as a plasma ferritin level <12  $\mu$ g/L [26].

**Definitions.** Adolescence was defined as age 12–18 years. Malaria parasitemia was defined as the presence of *P. falciparum* asexual stage parasites in thick smears of peripheral blood. Nonfalciparum parasitemia was not detected in any of the volunteers in this study. Prepuberty was defined as having no breast development (i.e., a breast development score <B2). Puberty was defined as having a breast development score  $\geq$ B2.

**Statistical analysis.** Analysis was done using SAS for Windows (version 8.02; SAS Institute); SUDAAN software (SAS callable version; Research Triangle Institute) was used to allow for correlation among observations taken from the same school (cluster-unit). To maintain the assumption of an equal probability sample, weighting was used to adjust for unequal cluster size due to variation in the number of absentees or refusals between clusters [16].

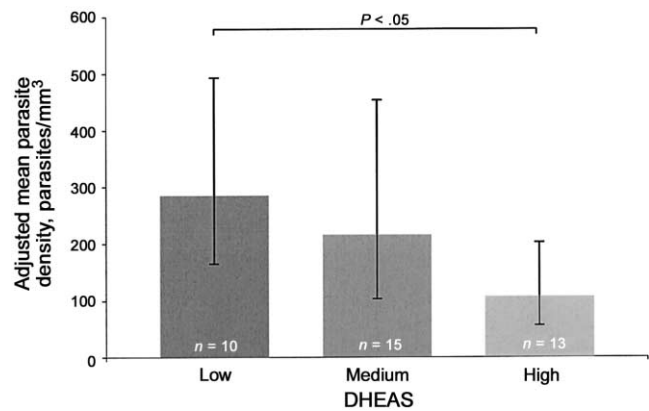
All analyses were adjusted for clustering in schools. Differences

between proportions were compared by the  $\chi^2$  test. The Mantel-Haenszel test for linear association (1 *df*) was used to test linear relations between 2 ordinal variables. Normally distributed continuous data were compared by the Student's *t* test and analysis of variance. Data that did not conform to a normal distribution were compared by the Wilcoxon-Mann-Whitney *U* 2-sample test and the Kruskal-Wallis 1-way analysis of variance. Correlations were assessed by Pearson's or Spearman's  $\rho$  for normally or nonnormally distributed data, respectively. Multivariate linear regression models were used to control for confounding and effect modification of the relationships among DHEAS levels, parasite density, and Hgb levels. A multivariate logistic regression model was used to control for other determinants of anemia. Nonnormally distributed variables of interest, DHEAS levels, malaria parasite density, and ferritin levels were transformed by calculating the natural logarithm (Ln) of the variable + 1, for use in regression analyses. The least-squares means procedure was used to obtain adjusted means for Hgb and Ln-transformed parasitemia for high, medium, and low tertiles of DHEAS level, as presented in figure 1 and 2. For figure 2, mean parasitemia data were back-transformed to their original scale (no. of parasites/mm<sup>3</sup>). DHEAS tertiles were based on the distribution in all available samples (*n* = 264) (low, <699 ng/mL; medium, 699–1426 ng/mL; and high,  $\geq$ 1427 ng/mL). Two-sided *P*  $\leq$  .05 was considered to be statistically significant.

Analysis showed significant effect modification of DHEAS levels on parasite density, by pubertal status (*P* = .002), and of DHEAS levels on Hgb levels, by pubertal status (*P* = .032). Therefore, separate multivariate regression models for pubertal and prepubertal girls were used in the analysis of the associations between DHEAS levels and the end points of parasitemia and Hgb level.

## RESULTS

**Descriptive statistics.** A total of 648 girls participated in the 2 cross-sectional surveys. Details of the prevalence and determinants of anemia and malnutrition for this same study population will be published elsewhere. DHEAS levels were successfully measured for a subsample of 264 girls. The median age in the subsample was 14.0 years (IQR, 13.1–15.1 years), and the median maturity rating was B3 (IQR, B2–B4). The geometric mean DHEAS level was 968 ng/mL (95% confidence interval [CI], 834–1122 ng/mL) and was significantly correlated with age (Spearman's  $\rho$ , 0.41; *P* < .001), maturity level (Spearman's  $\rho$ , 0.37; *P* < .001), height-for-age *z* score (Spearman's  $\rho$ , 0.30; *P* < .001), and BMI-for-age *z* score (Spearman's  $\rho$ , 0.15; *P* = .013). The geometric mean DHEAS level was 1038 ng/mL (95% CI, 892–1208 ng/mL) among pubertal girls and 655 ng/mL (95% CI, 464–926 ng/mL) among prepubertal girls. The



**Figure 1.** Adjusted mean parasite density, by plasma level of dehydroepiandrosterone sulfate (DHEAS), in parasitemic pubertal girls from western Kenya (*n* = 38). Bars, mean parasite density (least-squares means procedure) for low (<699 ng/mL), medium (699–1426 ng/mL), and high ( $\geq$ 1427 ng/mL) tertiles of DHEAS level, adjusted for effects of age, insecticide-treated bed net use, survey number, and body mass index-for-age *z* score. Error bars, 95% confidence interval for the mean.

prevalence of malaria was 21.6%, and the geometric mean parasitemia in those with a positive blood slide result was 178 parasites/mm<sup>3</sup> (95% CI, 129–246 parasites/mm<sup>3</sup>). The prevalence of anemia was 27.6%, and the mean Hgb level was 12.6 g/dL (95% CI, 12.2–12.9 g/dL). Table 1 compares descriptive statistics of the subjects with available DHEAS data with those without DHEAS data. The 2 groups were comparable with respect to most determinants of parasite density and Hgb, with the exception that the group with DHEAS data was slightly more mature (table 1). In addition, because of the loss of stored plasma samples, the first survey was underrepresented in the subsample with available DHEAS data. Of importance, the relationship between the predictors (age and maturity rating) and the outcome variables (parasite density and Hgb level) did not differ significantly for individuals with or without DHEAS results (data not shown).

**DHEAS and parasitic infections.** In the whole sample population, the prevalence of malaria decreased with increasing age and maturity level (*P* = .020, for linear association between malaria prevalence and age, and *P* = .005, for linear association between malaria and maturity [Mantel-Haenszel test]). In the subsample, we saw no association between malaria prevalence and DHEAS level (*P* = .679, Student's *t* test).

DHEAS level was negatively correlated with parasite density in parasitemic pubertal girls (Spearman's  $\rho$ , -0.40; *P* = .013). In multivariate linear regression analyses, this relationship remained statistically significant, even after controlling for age, survey number (i.e., seasonal variation), ITN use, and BMI-for-age *z* score (*P* = .012; table 2). The residual plot of this relationship showed a linear trend across all levels of DHEAS

**Table 2. Multivariate linear regression analysis of the association between dehydroepiandrosterone sulfate (DHEAS) level and natural logarithm-transformed (Ln) malaria parasite density (parasites/mm<sup>3</sup>) in parasitemic pubertal school girls (n = 38).**

Characteristic	Regression coefficient (95% CI)	P
Ln DHEAS level, ng/mL	-0.72 (-1.24 to -0.20)	.012
Age, years		
12	Reference	
13	-1.87 (-5.03 to 1.29)	.257
14	-1.62 (-4.80 to 1.57)	.328
15	-1.74 (-5.00 to 1.51)	.304
≥16	-1.78 (-4.84 to 1.28)	.265
ITN use	0.23 (-0.89 to 1.35)	.689
Survey number <sup>a</sup>	0.46 (-0.23 to 1.16)	.199
BMI-for-age z score	0.01 (-0.40 to 0.43)	.957

**NOTE.** BMI, body mass index; CI, confidence interval; ITN, insecticide-treated bed net.

<sup>a</sup> First survey vs. second survey.

(data not shown). No association between DHEAS level and parasite density was seen in the prepubertal girls (regression coefficient, -0.07; 95% CI, -0.38 to 0.24; *P* = .656). Adjusted mean parasite density in pubertal girls with a DHEAS level in the upper tertile was 62% lower than adjusted mean parasite density in girls with a DHEAS level in the lower tertile (*P* = .048; figure 1).

High DHEAS level was associated with a lower prevalence of helminth infection (all types combined) in bivariate analysis (crude odds ratio [OR] for high vs. low tertile, 0.50; 95% CI, 0.27–0.94; *P* = .032); however, after adjustment for age, this association was no longer significant (adjusted OR, 0.56; 95% CI, 0.28–1.11; *P* = .092). DHEAS had a similar association with *T. trichiura* infection alone (*P* = .095), and this association also was attenuated after controlling for age (*P* = .121). No associations were found between DHEAS level and *S. mansoni*, hookworm, or *A. lumbricoides* prevalence or intensity of infection.

**DHEAS, Hgb, and ferritin levels.** DHEAS level was positively correlated with Hgb level in pubertal girls (Spearman's  $\rho$ , 0.15; *P* = .022). Inclusion of known and potential determinants of Hgb level (menstruation, ITN use, helminth infections, survey number, BMI-for-age z score, and ferritin level) in a multivariate linear regression model did not attenuate the positive association between Hgb and DHEAS levels (table 3), nor did DHEAS attenuate the association of any of the parasitic infections with Hgb. The residual plot of this relationship showed a linear trend across all levels of DHEAS (data not shown). Girls in the upper tertile of DHEAS had Hgb levels 0.5 g/dL higher than those of girls in the lower tertile of DHEAS (figure 2). No association between DHEAS and Hgb was seen

in the least mature girls (i.e., Tanner score of B1) (linear regression coefficient, -0.04; 95% CI, -0.45 to 0.37; *P* = .847). DHEAS level was associated with a lower prevalence of anemia in all girls, although this result was not significant (adjusted prevalence OR for low-to-medium DHEAS level, 0.55 [95% CI, 0.24–1.27; *P* = .152]; adjusted prevalence OR for low-to-high DHEAS level, 0.78 [95% CI, 0.27–2.28; *P* = .645]).

DHEAS level was positively associated with ferritin level in all girls (Spearman's  $\rho$ , 0.13; *P* = .056). This association was still present after adjustment for potential determinants of ferritin level (menstruation, ITN use, helminth infections, survey number, and BMI-for-age z score) (adjusted mean difference for low-to-medium DHEAS level, 5.6  $\mu$ g/L [*P* = .091]; adjusted mean difference for low-to-high DHEAS level, 13.9  $\mu$ g/L [*P* = .004]). No association between DHEAS level and prevalence of iron deficiency was seen (data not shown).

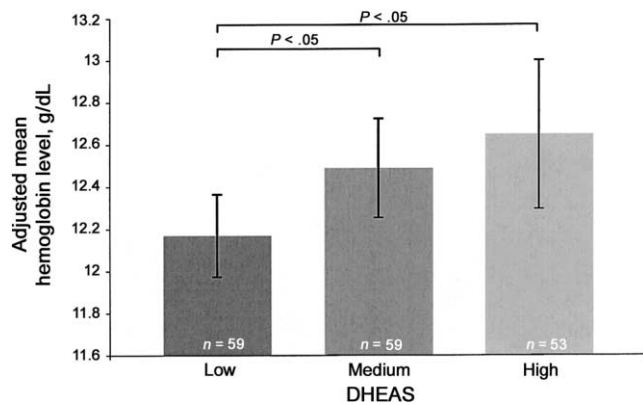
**DHEAS level, medication use, and clinical history.** Reported illness or fever in the month prior to the survey was common (66% and 54%) but not associated with DHEAS level (data not shown). Similarly reported conventional medication use (64%), including antimalarials (33%), in the month prior

**Table 3. Multivariate linear regression analysis of the association between dehydroepiandrosterone sulfate (DHEAS) level (ng/mL) and hemoglobin level (g/dL) in pubertal school girls (n = 171).**

Characteristic	Regression coefficient (95% CI)	P
Ln DHEAS level	0.39 (0.15 to 0.64)	.004
Age, years		
12	Reference	
13	-0.05 (-0.50 to 0.41)	.846
14	-0.18 (-0.64 to 0.27)	.432
15	-0.39 (-0.88 to 0.10)	.127
≥16	-0.56 (-0.99 to -0.13)	.017
Menstruation		
No	Reference	
Yes	-0.11 (-0.45 to 0.23)	.521
Heavy	-0.31 (-1.04 to 0.43)	.421
ITN use	0.63 (0.20 to 1.06)	.008
Helminth infections		
Hookworm	0.06 (-0.50 to 0.62)	.822
<i>Schistosoma mansoni</i>	-0.02 (-0.74 to 0.71)	.967
<i>Trichuris trichiura</i>	-0.13 (-0.54 to 0.28)	.542
<i>Ascaris lumbricoides</i>	-0.15 (-0.56 to 0.27)	.497
Survey number <sup>a</sup>	-1.00 (-2.16 to 0.15)	.103
Ln ferritin level	0.37 (0.18 to 0.57)	.001
BMI-for-age z score	-0.04 (-0.25 to 0.18)	.748

**NOTE.** BMI, body mass index; CI, confidence interval; ITN, insecticide-treated bed net; Ln, natural logarithm-transformed.

<sup>a</sup> First survey vs. second survey.



**Figure 2.** Adjusted mean hemoglobin level, by plasma level of dehydroepiandrosterone sulfate (DHEAS), in pubertal girls from western Kenya ( $n = 171$ ). Bars, mean hemoglobin level (least-squares means procedure) for low (<699 ng/mL), medium (699–1426 ng/mL), and high ( $\geq 1427$  ng/mL) tertiles of DHEAS level, adjusted for effects of age, menstruation, insecticide-treated bed net use, helminth infections, body mass index—for age z score, ferritin level and survey number. Error bars, 95% confidence interval for the mean.

to survey was common but not associated with DHEAS level (data not shown).

## DISCUSSION

In areas where malaria is endemic, children and young adolescents suffer a disproportionately high burden of malaria-attributable morbidity and mortality, compared with adults. This observation has supported the view that resistance to malarial infection and disease results from years of cumulative exposure to multiple parasite strains. The majority of population-based studies documenting this observation have examined life-long inhabitants of areas where malaria is endemic. In these populations, host age is highly colinear with lifetime cumulative exposure, thus obscuring the relative contributions of these distinct variables on resistance.

Recently, Baird et al. [8, 9] have demonstrated that non-immune, adult transmigrants moving from Java, where malaria is not endemic, to parts of Irian Jaya where malaria is endemic acquire immunity faster than do their transmigrant children. Because their entire study population migrated to the area where malaria is endemic en masse, host age was decoupled from cumulative exposure. After 1–2 years of exposure, adult transmigrants showed resistance, as measured by cross-sectional prevalence of infection and parasite density, equivalent to that of life-long adult inhabitants of the area where malaria is endemic. The transmigrant children remained at equal risk as children raised in the area where malaria is endemic. These data, replicated in several distinct transmigrant populations, are not consistent with the notion that resistance in adults

results from cumulative exposure to multiple parasite strains. Instead, these data suggest that there is an intrinsic difference in the development of resistance between adults and children and that expression of this resistance in adults requires relatively little exposure to the parasite [27, 28].

Kurtis et al. [10] examined resistance to reinfection after antimalarial treatment in a longitudinal cohort study of 12–35-year-old males living in an area of western Kenya where malaria is holoendemic. Resistance to reinfection increased during puberty and was predicted by levels of the pubertal steroid DHEAS. The age at onset of puberty and the duration of puberty are heterogeneous. This heterogeneity permitted estimation of the influence of pubertal development on resistance to reinfection after adjustment for age and, by proxy, cumulative exposure. After adjustment for age, individuals with low DHEAS levels had significantly higher parasite densities than did individuals with higher DHEAS levels. DHEAS has potent immunomodulatory effects and may be in the causal pathway that permits the induction of protective immune responses. Therefore, increasing levels of DHEAS during adolescence could, in part, account for the greater resistance to malaria enjoyed by adults, compared with children.

In the present cross-sectional study of 12–18-year-old school-girls from western Kenya, we found that DHEAS level was an independent predictor of malaria parasite density in the parasitemic girls who had entered puberty (i.e., Tanner score  $\geq B2$ ). As in the earlier study of Kenyan males [10], this relationship remained significant even after adjustment for age. We did not detect this association in parasitemic prepubertal girls, likely because of a threshold effect due to significantly lower levels of DHEAS in these girls. Furthermore, we did not detect a relationship between cross-sectional prevalence of malaria and DHEAS level, possibly because of misclassification of girls unexposed to infective mosquito bites before the survey as being resistant to infection. Potential misclassification of “nonresistant” girls (i.e., not exposed and thus blood smear negative) together with truly resistant girls (i.e., exposed and negative blood smear) as resistant to infection would bias results toward the null.

DHEAS is a potent immunoactivator that modulates both T and B cell function [29–34] and augments antibody titers [35–37]. We speculate that DHEAS is associated with the expression of adultlike resistance, because it up-regulates the production of protective antimalarial immune responses. Longitudinal studies measuring the acquisition of protective immune responses during adolescence are necessary to evaluate this hypothesis. Alternately, DHEAS may simply be a marker for another, unmeasured, factor which is associated with host development and mediates resistance to parasitemia.

In pubertal girls, DHEAS level was also positively associated with Hgb level, even after adjustment for age and several known

determinants of Hgb level, including menstruation, ITN use, helminth infections, ferritin level, and malnutrition (table 3). Of importance, we detected this protective effect of DHEAS on Hgb level in pubertal girls, many of whom had undergone menarche. This finding is striking, because, in this age group and economic and nutritional context, Hgb levels frequently decrease with age [38]. To the best of our knowledge, only one earlier study has demonstrated an association, independent of age, between Hgb and DHEAS levels [39].

There are several mechanisms that could explain the protective effect of DHEAS on Hgb. Because DHEAS is associated with decreased parasite density, higher DHEAS levels would be associated with fewer circulating parasites, resulting in decreased parasite-induced erythrocyte lysis.

Malaria-associated anemia is due, in part, to the anemia of inflammation [40], which is characterized by dyserythropoiesis, shunting of iron to nonbioavailable forms, decreased erythropoietin production and responsiveness, and decreased erythrocyte survival [41, 42]. This form of anemia is mediated, in part, by the proinflammatory cytokines tumor necrosis factor- $\alpha$ , interleukin (IL)-6, and IL-1. DHEAS is a potent down-modulator of all 3 of these proinflammatory cytokines [30–34, 43–46] and may attenuate the deleterious consequences of parasite-associated proinflammatory cytokines on Hgb level.

Some design-related limitations of this study should be considered. Because no attempt was made to follow up randomized girls who were absent from school on the day of the survey (e.g., because of illness), the sample potentially missed girls with clinical malaria or severe anemia. It is unlikely, however, that this would have greatly influenced our results, because absenteeism was relatively uncommon (6.5%) and because reasons for absenteeism other than health (e.g., domestic duties, care for ill siblings, or failure to pay periodic tuition) are frequent. The unfortunate loss of plasma samples, as described in Subjects, Materials, and Methods, is also unlikely to have biased our results, because no significant differences between the predictors (age and maturity rating) and the outcome variables (parasite density and Hgb level) were seen for individuals with or without DHEAS data.

In summary, we detected a relationship between DHEAS level and decreased parasite density in parasitemic pubertal girls, even after adjustment for age. Our data are not consistent with the view that resistance to parasitemia results from years of cumulative exposure to multiple parasite strains. In contrast, our data are consistent with earlier studies of trans migrant populations and studies of DHEAS and resistance to *P. falciparum* reinfection in males. Together, these studies support a role for host development in the acquisition of adultlike resistance to falciparum malaria. These findings are of particular relevance to the development and implementation of a malaria vaccine, because they imply that vaccination of prepubertal children may not result in

adultlike immunity. This situation may require the use of immunoadjuvants, possibly including DHEAS [36, 37], specifically designed to boost adultlike immunity.

We also detected a relationship between DHEAS level and increased Hgb level in pubertal girls, even after adjustment for age and known determinants of Hgb. We hypothesize that DHEAS leads to improved Hgb levels by decreasing parasite-mediated erythrocyte lysis and attenuating malaria-associated anemia of inflammation. Understanding the precise relationship between puberty, DHEAS levels, and Hgb levels in the context of *P. falciparum* malaria infection will require focused longitudinal studies and should lead to a better understanding of malaria-associated morbidity.

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