Am. J. Trop. Med. Hyg., 62(6), 2000, pp. 663–669

THE INFLUENCE OF ZINC SUPPLEMENTATION ON MORBIDITY DUE TO *PLASMODIUM FALCIPARUM*: A RANDOMIZED TRIAL IN PRESCHOOL CHILDREN IN PAPUA NEW GUINEA

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Abstract. Zinc is crucial for normal immune function and can reduce morbidity from multiple infectious diseases. To determine the influence of zinc on malaria morbidity we conducted a randomized placebo-controlled trial of daily zinc supplementation in children residing in a malaria endemic region of Papua New Guinea. A total of 274 preschool children aged 6 to 60 months were given 10 mg elemental zinc (n = 136) or placebo (n = 138) for 6 days a week for 46 weeks. Slide-confirmed malaria episodes were detected by surveillance of cases self-reporting to a local health center. Cross-sectional surveys were conducted at the beginning, middle, and end of the study to assess infection rates, parasite density, spleen enlargement, and hemoglobin levels. Zinc supplementation resulted in a 38% (95% CI 3–60, $P = 0.037$) reduction in *Plasmodium falciparum* health center-based episodes, defined as parasitemia ≥ 9200 parasites/μl with axial temperature ≥ 37.5°C or reported fever. Episodes accompanied by any parasitemia were also reduced by 38% (95% CI 5–60, $P = 0.028$), and episodes with parasitemia ≥ 100,000/μl were reduced by 69% (95% CI 25–87, $P = 0.009$). There was no evidence of the effects of zinc on *Plasmodium vivax* morbidity or on health center attendance for causes other than *P. falciparum.* Zinc had no consistent effect on cross-sectional malariometric indices. Although *P. falciparum* prevalence tended to be lower at the end of the study in children given the placebo, such changes were absent at the mid-study survey. These results suggest that improved dietary zinc intake may reduce morbidity due to *P. falciparum.*

INTRODUCTION

Plasmodium falciparum (*Pf*) results in 400 million clinical malaria episodes and 2.5 million deaths annually.[1] Many of the 2 billion people living in endemic areas are at risk for micronutrient deficiencies that could impair protective immunity or exacerbate pathology. Because zinc (Zn) is essential for normal immune function[2] and has been shown to reduce the incidence of diarrhea and pneumonia,[3] we hypothesized that Zn supplementation might also reduce *Pf* morbidity.

Zinc is essential for a variety of lymphocyte functions implicated in resistance to *Pf* malaria, including production of IgG, IFN-γ, and TNF-α and microbicidal activity of macrophages.[2,4] Cross-sectional studies amongst school-age children in Papua New Guinea[5] and pregnant women in Malawi[6] have reported inverse associations between measures of Zn status and *Pf* parasitemia. In addition, a placebo-controlled trial of Zn supplementation in preschool children in The Gambia documented a 30% reduction in *Pf*-attributable health center attendance,[7] although this was not statistically significant. Lastly, mildly Zn-deficient mice experienced mortality from a normally non-lethal strain of *Plasmodium yoelii*,[8] and Zn supplements decreased markers of oxidative stress during infection with *Plasmodium berghei.*[9]

These studies were consistent with the hypothesized role for Zn in reducing *Pf* malaria morbidity in humans. We therefore carried out a double-blind placebo-controlled trial of Zn supplementation of preschool children in an endemic area of Papua New Guinea to evaluate the potential of Zn to reduce *Pf* morbidity.

MATERIALS AND METHODS

Study area. The study was conducted between November 1995 and September 1996 in the North Wosera District of East Sepik Province, in northwestern Papua New Guinea. North Wosera is an area of intense and perennial malaria transmission, with entomological inoculation rates of *Pf* approaching 100 bites/person/year and a prevalence of asymptomatic parasitemia in preschool children of approximately 55% for *P. falciparum*, 25% for *Plasmodium vivax*, and 10% for *Plasmodium malariae.*[10] Amongst preschool children, enlarged spleen rates exceed 50% and malaria is responsible for 44% of all fevers and 33% of fatalities, making it the most frequent cause of death.[11] Malaria control in the region consists of the use of untreated bed nets and presumptive treatment at local health centers. Treatment, as per government policy, is amodiaquine or chloroquine,[12] and combined quinine and Fansidar for severe or resistant cases, resulting in a 90% cure rate.[13] Adjunct treatment with iron and folic acid is provided for cases of anemia. There is virtually no self-treatment because anti-malarials are readily available free of charge at the health center and there are no local commercial suppliers.[10]

Study design. The study was a randomized double-blind placebo-controlled trial of daily (6 days/week) oral supplementation with 10 mg Zn for 46 weeks amongst 274 children from 6 to 60 months of age. The primary outcome measure was the number of slide-confirmed *Pf*-attributable febrile episodes amongst children seen at the local health center. Pretrial surveillance estimated the frequency of such clinic-based episodes at 0.6 per child-year, yielding a targeted sample size of 270 children (135 per group) followed for 1 year. This enabled detection of a 35% reduction in episodes with 80% power (alpha = 0.05), assuming an 80% follow-up rate. Seven villages consisting of ~2,300 inhabitants with 291 children between 6 and 60 months of age in October 1995 participated in this trial and formed a contiguous geographic area near a centrally located health center.

Informed consent and enrollment procedures. Approval was granted by the Institutional Review Board of the Johns Hopkins School of Medicine and the Papua New Guinea Medical Research Advisory Committee. Village meetings were held in the local language to explain the purpose, methodology, and risks of the study. Consent forms were given and explained to caretakers of children who were 6 to 60 months of age on pre-assigned enrollment days, and caretakers actively brought their children for enrollment on the designated day. Eligibility requirements were age as described, planning to reside in the Wosera for at least one year, no signs of severe zinc deficiency or malnutrition, and no apparent chronic or debilitating condition.

At enrollment, a standardized seven-day morbidity history was obtained with aid of the caretaker for fever, cough, difficulty breathing, diarrhea, and stomach ache. History of night blindness and use of a mosquito net on the previous night were also recorded. The number of health center attendances in the last year was abstracted from each child's clinic book, which was required for all health center visits. Axillary temperature by electronic thermometer, spleen size by Hackett grade,[14] middle upper arm circumference, triceps skin fold, height or length, and weight were recorded. Hemoglobin levels were measured by HemoCue machine (HemoCue Inc., Mission Viejo, CA), thick and thin blood slides were taken, and 5 ml venous blood was drawn for nutrient and immunologic analyses.

Randomization and supplementation procedures. Following enrollment, children were stratified by age (greater or less than the median of 28 months), and by 7 geographical regions, termed biotopes which were defined by village of residence, distance from the health center, and proximity to local swamps. Children within these strata were individually allocated to computer-generated randomly permuted 4-person blocks of two codes, Zn or placebo (PL). Comparability of groups at baseline was examined by testing for differences between the predetermined indices of age, spleen rate, hemoglobin level, health center visits, and mosquito net use.

Supplements were tablets containing 10 mg elemental Zn as Zn gluconate. Placebos were indistinguishable from the supplements in color, size, or taste (Jameson, Inc., Windsor, Canada). The tablets were encoded and the assignment held off-site by personnel not involved in the study. Trained village-based field workers directly administered the supplements 6 days a week, Sunday being the exception. To facilitate consumption, tablets were dissolved in 3 ml of an orange-flavored drink that was prepared daily.

Morbidity surveillance. Patients reporting to the local health center were referred to a study nurse if the child's clinic book was marked with a fluorescent green decal indicating participation in the study. The standardized seven-day morbidity history was recorded along with axillary temperature, respiratory rate, spleen size, and hemoglobin levels by HemoCue; and thick and thin blood smears were prepared. Children were diagnosed and treated in accordance with government and health center policy, as described above.

Cross-sectional surveys. Complete cross-sectional surveys of the study population were performed at mid-study (5 months), and end-of-study (10 months). These were identical in methodology to the enrollment survey.

Laboratory and data handling procedures. Venous blood was kept in a dark box at ambient temperature no more than 6 hours prior to centrifugation and cryopreservation of plasma at −70°C. Samples were transported to Baltimore, Maryland, on liquid nitrogen and Zn levels were determined using inductively coupled plasma analysis.

Thick and thin Giemsa-stained blood films were read for species-specific density per 200 white blood cells (WBC). Slides were declared negative if no parasites were seen in 100 thick film fields. Slides with densities below 5 per 200 WBC, along with a randomly selected 10% of all slides, were routinely reexamined to verify at least 75% concordance of positivity, species, and density ± 10%. Parasites per μl blood were calculated for cross sectional surveys using the actual WBC count for each child at that survey,[15] whereas densities for slides taken at the health clinic were calculated from the mean WBC count determined from pooled cross-sectional data for each child. White blood cell counts were not affected by Zn supplementation.

Data forms were edited for completeness and validity prior to computer entry. A FoxPro v2.6x (Microsoft Corp., Seattle, WA) data entry program was designed for double entry with real time notification of numeric and logical inconsistencies. The study code was broken after closing the databases following double entry of all data.

Outcome definitions. The primary outcome was the number of *Pf*-attributable febrile episodes reporting to the local health center. The analytical algorithms and the required 80% specificity of the primary parasite density-dependent case definition were established prior to breaking the study code. To determine the density cut-off we modeled, using non-linear logistic regression,[16,17] the fever risk as a function of parasite density amongst clinic-based febrile episodes from the longitudinal surveillance and afebrile control children from the three cross-sectional surveys. The density-dependent probability that an observed fever with a given parasitemia was or was not attributable to malaria was assigned to each episode. The sum of these probabilities amongst all episodes represented the total number of true malaria and non-malaria febrile episodes. The sum above any given density cut-off allowed calculation of specificity and sensitivity for that cut-off.[16] An episode was thus defined as axial temperature ≥ 37.5°C or 72-hour fever history and parasitemia ≥ 9,200/μl, which was the lowest density satisfying the predefined criterion of 80% specificity, and had a sensitivity of 90%. Additional definitions of fever ≥ 37.5°C and any parasitemia (having 67% specificity and 100% sensitivity), or parasitemia ≥ 100,000/μl (having 99% specificity and 34% sensitivity), were also used since they represented traditional case definitions of mild and heavy infections, respectively. Secondary trial outcomes were number of children with at least one *Pf* episode, and time to first *Pf* episode, as well as cross sectional indices of parasite prevalence, density, spleen enlargement, hemoglobin levels, and anemia (hemoglobin levels ≤ 7.0 g/dl).

Analysis and statistical methods. All analyses were by intention to treat. The relative risk (RR) of a *Pf* episode in those given Zn was the ratio of incidence densities between Zn and PL groups, where incidence densities were defined as the total number of malaria episodes divided by the total days under study. Relative risks, 95% confidence intervals,

and *P* values were derived by negative binomial regression, which inflates confidence intervals to allow for inter-person variability and within-child clustering of episodes,[18] and were adjusted for age and biotope. To avoid inclusion of recrudescent malaria episodes, children were removed from the numerator and denominator for 28 days after experiencing an episode.[19] Other methods to adjust for within-child clustering of episodes or simple Poisson regression, which assumes independence of events, did not materially alter the outcome of the analysis, nor did the use of exclusion intervals for recrudescent cases ranging from 7 to 56 days.

Standard logistical regression was used to compare other dichotomized outcomes, and t-tests were used to compare continuous normal or log-normalized data as in the case of parasite density and triceps skin fold. Weight, height and age data were converted to Z-scores based on the National Center for Health Statistics standard.[20] Changes in prevalence, spleen rates, and hemoglobin values were adjusted for baseline status. Kaplan-Meier analysis using a log-rank test was used to determine the effect of Zn on the time to first or only malaria episode. All analyses were performed with SAS (SAS Institute, Cary, NC).

RESULTS

Enrollment, follow-up, and compliance. As seen in Figure 1, 274 children were seen at enrolment and randomized to Zn or PL. Mid-study and end-of-study cross sectional follow-up rates were 81% (222 of 274) and 77% (212 of 274), respectively, and similar for Zn and PL. Compliance data indicated that 85% (27,454 of 32,154) and 84% (25,415 of 30,078) of scheduled supplements were administered to PL and Zn groups, respectively. Additional analysis of compliance-related data revealed no differential reporting of side effects. During the trial 15 enrollees refused to continue, 43 migrated out of the study area, and 4 died. Verbal autopsy and clinical data indicated the one death in the PL group was due to malaria, and in the Zn group one was due to dysentery, one to malaria, and one died overnight with no preceding symptoms or illness.

Baseline comparability and cross sectional follow-up surveys. Table 1 indicates that PL and Zn groups were similar at baseline with respect to age, clinic-seeking behavior, indicated by the frequency of clinic attendance in the year prior to the study, and untreated bed-net use. Likewise, no differences were observed between groups for parasite prevalence or density, anthropometric indices, mean hemoglobin levels or number of anemic (Hb ≤ 7.0g/dl) children. Plasma zinc concentrations were mildly lower by 10% (95% CI 2–18, $P = 0.02$) in children randomized to the Zn group.

At the mid-study survey (data not shown), taken 5 months later during the high malaria transmission season, no significant differences in malariometric or anthropometric indices were seen between groups. In the PL and Zn groups, respectively, *Pf* prevalence increased to 45% and 41%, parasite density to 1660/μl and 1659/μl, spleen rates to 74% and 81%, and hemoglobin levels decreased to 8.2 ± 1.4 and 7.9 ± 1.5 g/dl.

At the end-of-study survey (Table 1), no differences between the PL and Zn groups were observed for anthropometric indices, mean Hb levels, or anemia. No differences in malariometric indices were observed. There was a tendency toward higher *Pf* prevalence in the Zn group (42%) compared to the PL group (29%) ($P = 0.050$). Zn had no effect on prevalence or density of *P. vivax* or *P. malariae*. Plasma Zn levels increased by 4.0 μg/dl for children given Zn with no change for those given PL.

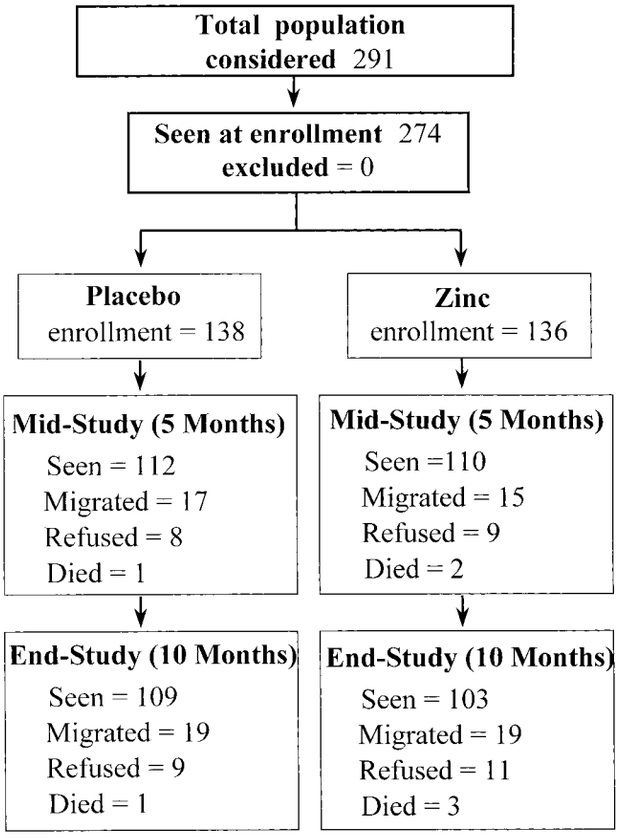

FIGURE 1. Flow diagram of enrollment and follow-up of patients by supplementation group.

Malaria morbidity. Supplementation with Zn reduced by 38% (95% CI 3–60, $P = 0.037$) the incidence of clinic-based *Pf* febrile episodes, defined as fever with *Pf* parasitemia ≥ 9,200/μl (shown in Table 2 as RR = 0.62, 95% CI 0.40–0.97). Episodes accompanied by any *Pf* parasitemia were also reduced by 38% (95% CI 5–60, $P = 0.028$). Malaria episodes accompanied with heavy parasitemia, i.e. ≥ 100,000/μl blood, were reduced by 69% (95% CI 25–87, $P = 0.009$). Amongst the 91 *Pf* episodes ≥ 9,200/μl, only 1 in the Zn group was also positive for *P. vivax* so that protection against pure *Pf* episodes was 39% (95% CI 5–61, $P = 0.027$).

To further characterize the protective effects of Zn, we compared the number of first or only *Pf* episodes ≥ 9,200/μl in PL and Zn groups, which were 46 and 29, respectively, indicating 36% protection (RR = 0.64, 95% CI 0.43–0.95, $P = 0.024$) amongst those given Zn. The distribution of children experiencing 1, 2, or 3 *Pf* episodes was 36, 9, and

TABLE 1
Baseline and end-of-study cross-sectional survey data

	Baseline		End-of-study	
	Placebo	Zinc	Placebo	Zinc
Number of children	138	136	109	103
Age (months)				
6–12 (number of children)	15	18	0	0
12–24	36	36	14	16
24–36	40	31	32	27
36–48	16	20	29	24
48+	31	31	34	36
Sex (% male)	51	43	49	47
Health center visits in last year (% having 0–1, 2–6, or >6 visits)	57, 38, 5	56, 42, 2	na	na
Reported mosquito net use on the previous night (no.)	52% (72/138)	46% (62/136)	61% (66/109)	56% (58/103)
Anthropometric indices: no. (±SD)				
Height-for-age Z score	−1.95 (1.2)	−1.85 (1.02)	−1.96 (1.07)	−1.88 (1.19)
Weight-for-height Z score	−0.74 (0.92)	−0.85 (0.83)	−0.76 (0.82)	−0.69 (0.82)
MUAC (cm)	14.1 (1.1)	13.9 (1.3)	14.6 (1.0)	14.6 (1.0)
triceps skinfold (mm)	6.8 (1.4)	7.1 (1.3)	6.8 (1.3)	6.8 (1.4)
Biochemical indices: no. (±SD)				
Hemoglobin (g/dl)	10.5 (1.7)	10.4 (1.8)	8.7 (1.7)	8.6 (1.3)
anemia (Hb ≤ 7.0 g/dl)	3.9% (5/129)	3.2% (4/125)	14% (15/108)	13% (13/100)
Plasma zinc (μg/dl)	74 (19)	67 (12)§	74 (18)	71 (13)
plasma zinc ≤ 60 μg/dl	24% (14/58)	28% (15/54)	25% (14/56)	20% (11/54)
Malariometric indices (no.)				
Pf positive	40% (54/136)	39% (50/129)	29% (31/108)	42% (42/101)§
GMD/μl [95% CI]†	982 [513–1881]	1300 [721–2345]	1854 [1012–3397]	1700 [962–3000]
Pv positive	27% (37/136)	32% (41/129)	31% (34/109)	33% (33/101)
GMD/μl [95% CI]†	437 [270–706]	622 [387–1000]	706 [355–1404]	667 [353–1258]
Pm positive	3.7% (5/136)	1.5% (2/129)	0% (0/108)	2.0% (2/101)
GMD/μl [95% CI]†	471 [138–1603]	230 [11–4591]	na	701 [167–2942]
Enlarged spleen	40% (48/121)	49% (58/119)	59% (62/105)	68% (63/93)

† for positive slides. na = not applicable.
§ $P \leq 0.05$ compared to placebo, see text for exact P values.
MUAC = middle upper-arm circumference; GMD = Geometric mean density; *Pf* = *Plasmodium falciparum*; *Pv* = *Plasmodium vivax*; *Pm* = *Plasmodium malariae*.

TABLE 2
The influence of zinc supplementation of *Plasmodium falciparum* episodes and general morbidity

Morbidity indicators	Placebo	Zinc	RR or Δ% [95% CI]
Health center visits*	156	114	0.79 [0.54–1.14]
Plasmodium falciparum episodes (fever and Pf density ≥)*			
1/μl	82	48	0.62 [0.40–0.95]§
9,200/μl	57	34	0.62 [0.40–0.97]§
100,000/μl	24	7	0.31 [0.13–0.74]§
non-*Plasmodium falciparum* visits (*Plasmodium falciparum* = 0/μL)*	63	57	1.01 [0.50–2.02]
GMD *Plasmodium falciparum* density/μl† [95% CI]	16,131 [10,249–25,388]	13,320 [7,705–23,027]	Δ − 17% [−60–71]
Mean hemoglobin g/dl ± SD)	8.6 (1.8)	8.8 (1.8)	Δ + 2.2% [−3.2–7.5]
anemia (Hb ≤ 7.0 g/dl)	23% (30/132)	19% (19/101)	0.83 [0.50–1.38]
Spleen enlargement	28% (39/139)	30% (31/102)	1.08 [0.73–1.61]
Symptom rates from 7-day recall (days reported)			
fever	33% (359/1071)	33% (258/770)	1.00 [0.85–1.17]
cough	16% (168/1071)	16% (122/770)	1.17 [0.75–1.80]
diarrhea	1.8% (19/1071)	2.1% (16/770)	0.99 [0.32–3.14]
stomach pain	1.5% (16/1071)	1.3% (10/770)	0.77 [0.19–3.07]

* The respective surveillance periods were 37,513 child-days for the placebo group and 35,091 child-days for the zinc group. Relative risk (RR) and 95% confidence intervals were derived using negative binomial regression and are adjusted for age and biotope.
† Geometric mean parasite density for positive slides only.
§ $P < 0.05$, see text for exact P values.

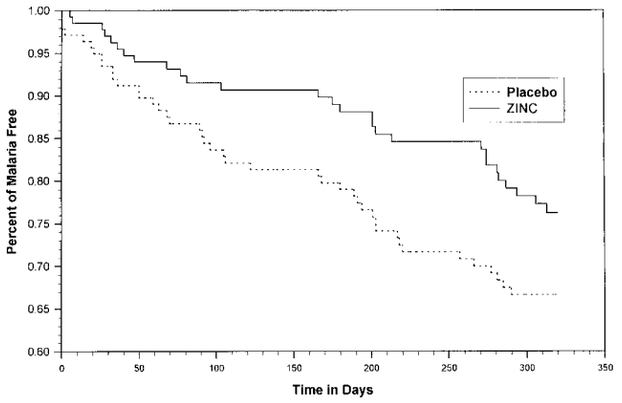

FIGURE 2. Kaplan-Meier survival analysis for first or only *Plasmodium falciparum* malaria episode. A *Pf* malaria episode was defined as a 72-hour fever history or an axial temperature ≥ 37.5° and parasitemia ≥ 9,200/μl. Analysis was performed using data from health center based case surveillance. Zinc supplementation tended to prolong time to first or only *Pf* malaria episode ($P = 0.053$).

1 in the PL group and 24, 5, and 0 in the Zn group, indicating that consistent reductions were observed in individuals with varying numbers of episodes. We also conducted an analysis of time to first or only *Pf* episode (Figure 2), which tended to be prolonged by Zn supplementation ($P = 0.053$).

It is possible that Zn may have altered the *Pf* pyrogenic density threshold for patients coming to the clinic, and that this could result in the observed effects. Therefore, we analyzed the PL and Zn cohorts separately and derived group-specific density cut-offs of 80% specificity, which were ≥ 7,800/μl and ≥ 10,000/μl, respectively, but were not statistically different. When we defined episodes by these iso-specific cut-offs, and included afebrile cases with densities between 7,800 and 10,000/μl for the Zn group, a reduction of 36% (95% CI 0–58, $P = 0.050$) was still observed in children given Zn. This indicates that a suppressed *Pf*-associated febrile response could not account for the observed effects.

We also examined the relationship between plasma Zn levels and *Pf* morbidity. Multiple logistic regression did not reveal any association between plasma Zn levels at the beginning or end of the study and *Pf*-attributable clinic attendance for either Zn or PL groups. This indicates that the 10% difference in plasma Zn levels between baseline PL and Zn groups was not associated with differential susceptibility to *Pf* episodes, and that post-supplementation plasma Zn levels were not predictive of the effects of Zn on *Pf* episodes.

The influence of Zn on *P. vivax* morbidity was also examined. No effect was seen on *P. vivax* episodes, defined as fever and any parasitemia, which were 38 in the PL group and 38 in the Zn group (RR = 1.03, 95% CI 0.60–1.77, $P = 0.58$). Using a density cut-off of ≥ 3,800/μl (80% specificity), there were 13 episodes in the PL group and 18 in the Zn group (RR = 1.33, 95% CI 0.48–3.68, $P = 0.58$), again suggesting no protection of Zn against *P. vivax.* The paucity of *P. malariae* episodes precluded meaningful analysis.

General clinic attendance. Overall, there was a non-significant 21% (95% CI −14–46, $P = 0.21$) decrease in health center visits amongst children given Zn (114 visits in Table 2) compared to PL (156 visits). Interestingly, there was no effect of Zn on clinic attendance amongst children who were not infected with *Pf* (RR = 1.01, 95% CI 0.50–2.02, $P = 0.98$). Of the 42 (156 PL minus 114 Zn) fewer clinic visits amongst children given Zn, 39 were associated in some way with *Pf*: 34 were febrile *Pf* infections, 3 were afebrile *Pf* infections, and 2 were recrudescent *Pf* episodes (which were not considered as new *Pf* cases in Table 2). Clinic attenders given Zn also had a 17% (95% CI −60–71, $P = 0.43$) lower *Pf* parasite density, 17% (95% CI −38–50, $P = 0.47$) reduced risk of anemia, and 2.2% (95% CI −3.2–7.5, $P = 0.43$) increase in Hb level, although none of these changes approached statistical significance. Likewise, no significant differences in symptom frequency from seven-day morbidity recalls were observed.

DISCUSSION

This study indicates that Zn supplementation reduced *Pf*-attributable health center attendance by 38% in preschool children living in a hyperendemic area of Papua New Guinea. A 69% reduction was observed for *Pf* episodes of ≥ 100,000/μl, suggesting that Zn may preferentially protect against episodes with heavy parasitemia. In addition, there was a tendency for Zn to prolong the time to first or only *Pf* episode. No protection against *P. vivax* morbidity was observed. The apparent *Pf*-specificity of the effects of Zn is further underscored by the absence of an effect on all-cause clinic attendance for those who were *Pf*-negative at presentation. It is of interest that the effects of Zn on morbidity were not reflected in cross-sectional malariometric indicators, suggesting that Zn primarily affects immunological or pathological processes associated with clinical episodes. Interestingly, daily dietary intakes of Zn in the Wosera were less than ½ the RDA[5] of 10 mg and up to 28% of plasma Zn levels were below 60 μg/dl. Although supplementation resulted in moderate increases in plasma Zn concentration, plasma Zn levels alone were not predictive of susceptibility to malaria episodes for either the PL or Zn groups. It is also of interest that plasma Zn levels moderately improved in our study, but that malaria morbidity was strongly reduced, indicating that improved daily dietary Zn intake can modify resistance to *Pf*-episodes through pathways, perhaps at the cellular level, not fully reflected in plasma Zn levels.

The 38% protective efficacy of Zn is similar to the 30% ($P = 0.09$) reduction in *Pf*-attributable health center attendance observed in The Gambia following twice weekly Zn supplementation.[7] Indeed, combined analysis of original data from the Gambian trial (kindly provided by Dr. C.J. Bates) and our trial data indicates that Zn supplements resulted in an overall 36% (95% CI 9–55, $P = 0.012$) reduction in clinic-based malaria attacks accompanied by any *Pf* parasitemia. Moreover, as in our study, the Gambian study documented comparably weaker effects on non-*Pf*-attributable clinic attendance. The notion that malaria could be particularly sensitive to sub-optimal zinc intake is consistent with murine studies in which even moderate Zn deficiency resulted in mortality from the normally non-lethal rodent malaria *P. yoelii* 17X-NL.[8]

In a concurrent study in neighboring villages of the Wos-

era, we have demonstrated that vitamin A supplementation reduced *Pf* episodes by 30%.[21] Although both Zn and vitamin A reduced the incidence of *Pf* episodes, there are several important qualitative differences in their effects. First, the protective efficacy of Zn was greatest for *Pf* episodes ≥ 100,000/μl, however that of vitamin A began to wane at densities ≥ 30,000/μl. Second, whereas Zn had no effect on spleen enlargement and parasite density, vitamin A tended to reduce these malariometric indices. Third, protection due to Zn showed no clear age-dependent pattern, whereas vitamin A was most effective in 12–36 month-old children. These differences indicate that Zn and vitamin A could act through distinct sets of biologic effectors involved in selective resistance to either more severe episodes in persons given Zn or milder clinical episodes and asymptomatic malariometric indices in those given vitamin A.

As mentioned, despite the 38% reduction in *Pf* clinic-based episodes in children given Zn, consistent effects were not observed against other malariometric indices. Indeed, *Pf* prevalence at the end-of-study survey was lower in the PL group compared to the Zn group. It is, however, noteworthy that no differences in parasite density were observed, nor were such differences apparent at the mid-study survey. Moreover, in the context of the overall cross-sectional data and concomitant cross-sectional data from the adjacent vitamin A study area,[21] the 29% *Pf* point prevalence for the PL group at the end of the Zn trial reflects a drop in prevalence in the PL group rather than an exacerbation in the Zn group. Although it remains possible that Zn-supplemented children have higher *Pf* prevalence rates, we cannot rule out other possibilities. For example, greater rates of clinic attendance in the PL group could lead to higher consumption of anti-malarials, possibly resulting in the observed differences in cross-sectional prevalence.

The mechanisms whereby Zn affects *Pf* episodes remain unknown. While differences in pyrogenic threshold were not significant, and could not account for the observed effects, data from cases self-presenting to the health center may not reveal differences in the density-dependent early onset of *Pf* illness. In fact, Zn administration during infection has been shown to potentiate the febrile response,[22] which can suppress *in vitro* growth of *Pf*[23] and *Pf* parasitemia *in vivo*.[24,25] Alternatively, given that Zn is a known antioxidant and a potent inhibitor of apoptosis,[2] children given Zn may better endure the immunopathologies associated with a *Pf* episode and appear less ill to their parents, resulting in fewer clinic visits. The decreased oxidative stresses associated with Zn supplementation during murine infection with *P. berghei* is consistent with this idea.[26] Sequestration of mature parasitized erythrocytes within the microvasculature is a pathogenic process associated with *Pf* and not *P. vivax*. If Zn selectively reduced *Pf* morbidity by interfering with sequestration, we speculated that the presence of mature ring forms of parasitized erythrocytes would be more frequent in children given Zn. Although the data are limited, in this study mature ring forms were observed in 14.7% (5 of 34) of *Pf*-attacks in the Zn group but in only 3.5% (2 of 57) of those given PL (RR = 4.18 [95% CI 0.98–17.86, $P = 0.054$]). Lastly, the immunopotentiating activities of Zn^2 could enhance acquired immunity following exposure to *Pf*. The magnitude and specificity of the Zn-mediated effects strongly implicates some form of specific immunity. Preliminary assessments of *Pf*-specific T cell cytokine production suggest significant differences between children given Zn or PL. Additional study of the mechanistic basis for the effects of Zn may prove useful in elucidating mechanisms of immunity and pathophysiology of malaria.

Zinc supplementation has been demonstrated in several settings to substantially reduce diarrheal[3,27] and respiratory morbidity.[3,28] Although the 38% reduction in clinic-based *Pf*-episodes reported here is encouraging, and the 69% reduction in episodes with heavy infections portends an effect on severe malaria, additional studies are needed to document the geographic regions and conditions of malaria transmission in which Zn might be effective. Importantly, if Zn is indeed selectively effective against more severe clinical episodes, such as those self-reporting to a health clinic, then daily community-based case surveillance, which tends to detect the earlier phase of a malaria episode, may not reveal a protective effect. Thus, future studies should include multiple case-detection strategies. Likewise, the Zn dose and delivery schedule may influence the outcome. The effects of other nutrients also require examination as indicated by the recently reported predisposition to severe and uncomplicated malaria due to thiamine deficiency.[29]

At present the protective efficacies of Zn and vitamin A against clinical *Pf*-episodes is similar to protection seen with insecticide-treated bed nets (48%),[30] and appear to exceed the most optimistic 23% efficacy estimates of the first phase III-tested experimental malaria vaccine SPf66.[31] Novel and integrated approaches may be required for control of malaria. Importantly, the potential for synergy between nutrients and other interventions, particularly bed nets, newer experimental vaccines, and chemoprophylaxis, may be worth exploring. In general, low cost, high safety, and potential efficacy of targeted nutritional supplementation or fortification suggest that a rational approach to development of such interventions might provide useful adjuncts to conventional preventive or therapeutic measures for *Pf* malaria. This study suggests that improved Zn intake, via supplementation or otherwise, may reduce *Plasmodium falciparum* morbidity.

Acknowledgments: We thank the citizens of North Wosera for their dedication and co-operation in this study. The indispensable logistical support of Manasseh Baea, slide reading by Nicky Gibson, anthropometry by Daina Lai, and hematology skills of William Deppsone are gratefully acknowledged, along with the careful clinic work by Dorothy Nawak and field supervision of Schola Pila. We also recognize the substantial contributions of Jack Taraika, Gertrude Kanawe, Linda Orare, Anita V. Shankar, Zachary Dimber, Clara Pomba, Jane Simbrandu, Jennifer Dipmawe, and Andrew Raiko, and the participation of Meza Ginny, Lucy Lavi, and Sarah Paget. We thank the Ministry of Health of Papua New Guinea and East Sepik Province for their support in carrying out this trial, Jameson Inc. for providing the zinc supplements and placebos, and Dr. Neal Alexander for the randomization routines and advice on the analysis. We also thank Dr. C.J. Bates for kindly providing the original study data from The Gambian trial. We appreciate the insights of Dr. Alan Scott and the encouragement of Dr. Robert Black in carrying out this work.

Financial support: The work was supported by Co-operative Agreements Nos. DAN 0045-A-00-5094-00 and HRN-A-00-97-00015-00 between the Johns Hopkins School of Hygiene and Public Health and the Office of Health and Nutrition, United States Agency for International Development (USAID), and an Australian Agency for

International Development (AusAID) grant to the Papua New Guinea Institute of Medical Research.

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REFERENCES

1. Krogstad DJ, 1996. Malaria as a reemerging disease. *Epidemiol Rev 18:* 77–89.
2. Shankar AH, Prasad AS, 1998. Zinc and immune function: the biological basis of altered resistance to infection. *Am J Clin Nutr 68:* 447S–463S.
3. Black RE, 1998. Therapeutic and preventive effects of zinc on serious childhood infectious diseases in developing countries. *Am J Clin Nutr 68:* 476S–479S.
4. Good MF, Kaslow DC, Miller LH, 1998. Pathways and strategies for developing a malaria blood-stage vaccine. *Annu Rev Immunol:* 57–87.
5. Gibson RS, Heywood A, Yaman C, Sohlstrom A, Thompson L, Heywood P, 1991. Growth in children from the Wosera subdistrict Papua New Guinea, in relation to energy and protein intakes and zinc status. *Am J Clin Nutr 53:* 782–789.
6. Gibson RS, Huddle JM, 1998. Suboptimal zinc status in pregnant Malawian women: its association with low intakes of poorly available zinc, frequent reproductive cycling, and malaria. *Am J Clin Nutr 67:* 702–709.
7. Bates CJ, Evans PH, Dardenne M, Prentice A, Lunn PG, Northrop-Clewes CA, Hoare S, Cole TJ, Horan SJ, Longman SC, 1993. A trial of zinc-supplemenation in young rural Gambian children. *Br J Clin Nutr 69:* 243–255.
8. Shankar AH, Kumar N, Scott AL, 1995. Zinc-deficiency exacerbates experimental malaria infection in mice. *FASEB Journal 9:* A4269
9. Arif AJ, Mathur PD, Chandra S, Singh C, Sen AB, 1987. Effect of zinc diet on Xanthine oxidase activity of liver of mice infected with *Plasmodium berghei. Indian J Malariol 24:* 59–63.
10. Genton B, al-Yaman F, Beck HP, Hii J, Mellor S, Narara A, Gibson N, Smith T, Alpers MP, 1995. The epidemiology of malaria in the Wosera area, East Sepik Province, Papua New Guinea, in preparation for vaccine trails. I. Malariometric indices and immunity. *Ann Trop Med Parasitol 89:* 359–376.
11. Genton B, al-Yaman F, Beck HP, Hii J, Mellor S, Rare L, Ginny M, Smith T, Alpers MP, 1995. The epidemiology of malaria in the Wosera area, East Sepik Province, Papua New Guinea, in preparation for vaccine trials. II Mortality morbidity. *Ann Trop Med Parasitol 89:* 377–390.
12. Anonymous, 1993. *Standard Treatment for Common Illnesses of Children in Papua New Guinea.* Fifth edition.
13. al-Yaman F, Genton B, Mokela D, Alpers MP, 1998. Resistance of *Plasmodium falciparum* in vivo to 3 days' treatment with quinine and single-dose Fansidar. *Papua New Guinea Med J 37:* 54–56.
14. Bruce-Chwatt LJ, 1985. *Essential Malariology*. Wiley Medical Books: 73–85.
15. Allen S, Genton B, Alexander N, Gibson N, Raiko A, 1995. Malaria vaccine in children under 12 months of age. *Lancet 346:* 1555–1556.
16. Smith T, Armstrong Schellenberg J, Hayes R, 1994. Attributable fraction estimates and case definitions for malaria in endemic areas. *Statistics in Medicine 13:* 2345–2358.
17. Armstrong-Schellenberg JRM, Smith T, Alonso PL, Hayes RJ, 1994. What is clinical malaria? Finding case definitions for field research in highly endemic areas. *Parasitol Today 10:* 439–442.
18. Williams TN, Maitland K, Bennett S, Ganczakowski M, Peto TE, Newbold CI, Bowden DK, Weatherall DJ, Clegg XX, 1996. High incidence of malaria in a-thalassaemic children. *Nature 383:* 522–525.
19. World Health Organization, 1997. Guidelines for the evaluation of *Plasmodium falciparum* vaccines in populations exposed to natural infection. *TDR/MAL/VAC/97:* 1–53.
20. World Health Organization working group, 1986. Use and intεpretation of anthropometric indicators of nutritional status. *Bull WHO 64:* 929–941.
21. Shankar AH, Genton B, Semba RD, Baisor M, Paino J, Tamja S, Adiguma T, Wu L, Rare L, Tielsch JM, Alpers MP, West KP Jr, 1999. Effect of vitamin A supplementation on morbidity due to *Plasmodium falciparum* in young children in Papua New Guinea: a randomized trial. *Lancet 354:* 203–209.
22. Braunschweig CL, Sowers M, Kovacevich DS, Hill GM, August DA, 1997. Parenteral zinc supplementation in adult humans during the acute phase response increases the febrile response. *J Nutr 127:* 70–74.
23. Kwiatowski D, 1989. Febrile temperatures can synchronize the growth of *Plasmodium falciparum* in vitro. *J Exp Med 169:* 357–361.
24. Rogier C, Commenges D, Trape JF, 1996. Evidence for an age-dependent pyrogenic threshold of *Plasmodium falciparum* parasitemia in highly endemic populations. *Am J Trop Med Hyg 54:* 613–619.
25. Gravenor MB, Kwiatowski D, 1998. An analysis of the temperature effects of fever on the intra-host population dynamics of *Plasmodium falciparum. Parasitol 117:* 97–105.
26. Arif AJ, Mathur PD, Chandra S, Singh C, Sen AB, 1997. Effect of zinc diet on xanthine oxidase activity of liver of mice infected with *Plasmodium berghei. Indian J Malariol 24:* 59–61.
27. Sazawal S, Black R, Jalla S, Bhan MK, Bhandari N, Sinha A, 1995. Zinc supplementation in young children with acute diarrhea in India. *New Engl J Med 333:* 839–844.
28. Sazawal S, Black R, Jalla S, Mazumdar S, Sinha A, Bhan MK, 1998. Zinc supplementation reduces the incidence of acute lower respiratory infections in infants and preschool children: a double-blind controlled trial. *Pediatrics 102:* 1–5.
29. Krishna S, Taylor AM, Supanaranond W, Pukrittayakamee S, ter Kuile F, Tawfiq KM, Holloway PA, White NJ, 1999. Thiamine deficiency and malaria in adults from Southeast Asia. *Lancet 353:* 546–549.
30. Lengeler C, 1998. Insecticide treated bednets and curtains for malaria control (Cochrane Review). *The Cochrane Library, Issue 4,* Oxford: Update Software.
31. Graves P, Gelband H, Garner P, 1998. The SPf66 malaria vaccine: what is the evidence for efficacy? *Parasitol Today 14:* 218–220.