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 \textit{Plasmodium falciparum} health-center-based episodes, defined as parasitemia \( \geq 9200 
\) parasites/\( \mu l \) with axial temperature \( \geq 37.5^\circ C \) or reported fever. Episodes accompanied by any parasitemia were also
reduced by 38% (95% CI 5–60, \( P = 0.028 \)), and episodes with parasitemia \( \geq 100,000/\mu l \) were reduced by 69% (95% CI
25–87, \( P = 0.009 \)). There was no evidence of the effects of zinc on \textit{Plasmodium vivax} morbidity or on health
center attendance for causes other than \textit{P. falciparum}. Zinc had no consistent effect on cross-sectional malarialometric
indices. Although \textit{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 \textit{P. falciparum}.

INTRODUCTION

\textit{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 immu-
ny 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-\( \gamma \), and TNF-\( \alpha \) and microbicidal activity of macrophages.\(^4,5\) Cross-sectional studies amongst school-age children
in Papua New Guinea\(^6\) 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 \textit{Plasmodium yoelii},\(^8\) and Zn supplements decreased markers of oxidative stress during infection with \textit{Plasmodium berghei}.\(^9\)

These studies were consistent with the hypothesized role for Zn in reducing \( Pf \) malaria morbidity in humans. We therefore
conducted 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 asympto-
tomatic parasitemia in preschool children of approximately 55% for \textit{P. falciparum}, 25% for \textit{Plasmodium vivax}, and 10% for \textit{Plasmodium malariacea}.\(^10\) 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 govern-
ment 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 supple-
mentation 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. Pre-
trial surveillance estimated the frequency of such clinic-based episodes at 0.6 per child-year, yielding a targeted sam-
ple 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 attendance 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, 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), and 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 had 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 visits 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, 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), and thick and thin blood slides were taken, and 5 ml venous blood was drawn for nutrient and immunologic analyses.

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, 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. An episode was thus defined as axillary temperature $\geq 37.5^\circ C$ or 72-hour fever history and parasitemia $\geq 9,200/\mu l$, which was the lowest density satisfying the predefined criterion of 80% specificity, and had a sensitivity of 90%. Additional definitions of fever $\geq 37.5^\circ C$ and any parasitemia (having 67% specificity and 100% sensitivity), or parasitemia $\geq 100,000/\mu l$ (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 $\geq 7.0 \text{ g/dl}$).

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, 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. 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. 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 ≤ 7.0 g/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 malarriometric indices or anthropometric indices were seen between groups. In the PL and Zn groups, respectively, P. falciparum prevalence increased to 43% 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 malarriometric indices were observed. There was a tendency toward higher P. falciparum 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.

Malaria morbidity. Supplementation with Zn reduced by 38% (95% CI 3–60, P = 0.037) the incidence of clinic-based P. falciparum episodes, defined as fever with P. falciparum parasitemia ≥ 9,200/μl (shown in Table 2 as RR = 0.62, 95% CI 0.40–0.97). Episodes accompanied by any P. falciparum 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 P. falciparum episodes ≥ 9,200/μl, only 1 in the Zn group was also positive for P. vivax so that protection against pure P. falciparum 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 P. falciparum 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 P. falciparum episodes was 36, 9, and

![Flow diagram of enrollment and follow-up of patients by supplementation group.](image-url)
Table 1
Baseline and end-of-study cross-sectional survey data

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Zinc</th>
<th>Placebo</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of children</td>
<td>138</td>
<td>136</td>
<td>109</td>
<td>103</td>
</tr>
<tr>
<td>Age (months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6–12</td>
<td>15</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12–24</td>
<td>36</td>
<td>36</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>24–36</td>
<td>40</td>
<td>31</td>
<td>32</td>
<td>27</td>
</tr>
<tr>
<td>36–48</td>
<td>16</td>
<td>20</td>
<td>29</td>
<td>24</td>
</tr>
<tr>
<td>48+</td>
<td>31</td>
<td>31</td>
<td>34</td>
<td>36</td>
</tr>
<tr>
<td>(sex (% male)</td>
<td>51</td>
<td>43</td>
<td>49</td>
<td>47</td>
</tr>
<tr>
<td>Health center visits in last year (%)</td>
<td>57, 38, 5</td>
<td>56, 42, 2</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Reported mosquito net use on the previous night (no.)</td>
<td>52% (72/138)</td>
<td>46% (62/136)</td>
<td>61% (66/109)</td>
<td>56% (58/103)</td>
</tr>
<tr>
<td>Anthropometric indices: no. (±SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height-for-age Z score</td>
<td>−1.95 (1.2)</td>
<td>−1.85 (1.02)</td>
<td>−1.96 (1.07)</td>
<td>−1.88 (1.19)</td>
</tr>
<tr>
<td>Height-for-age Z score</td>
<td>−0.74 (0.92)</td>
<td>−0.85 (0.83)</td>
<td>−0.76 (0.82)</td>
<td>−0.69 (0.82)</td>
</tr>
<tr>
<td>MUAC (cm)</td>
<td>14.1 (1.1)</td>
<td>13.9 (1.3)</td>
<td>14.6 (1.0)</td>
<td>14.6 (1.0)</td>
</tr>
<tr>
<td>triceps skinfold (mm)</td>
<td>6.8 (1.4)</td>
<td>7.1 (1.3)</td>
<td>6.8 (1.3)</td>
<td>6.8 (1.4)</td>
</tr>
<tr>
<td>Biochemical indices: no. (±SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>10.5 (1.7)</td>
<td>10.4 (1.8)</td>
<td>8.7 (1.7)</td>
<td>8.6 (1.3)</td>
</tr>
<tr>
<td>anemia (Hb ≤ 7.0 g/dl)</td>
<td>3.9% (5/129)</td>
<td>3.2% (4/125)</td>
<td>14% (15/108)</td>
<td>13% (13/100)</td>
</tr>
<tr>
<td>Plasma zinc (µg/dl)</td>
<td>74 (19)</td>
<td>67 (12)§</td>
<td>74 (18)</td>
<td>71 (13)</td>
</tr>
<tr>
<td>Plasma zinc ≤ 60 µg/dl</td>
<td>24% (14/58)</td>
<td>28% (15/54)</td>
<td>25% (14/56)</td>
<td>26% (11/54)</td>
</tr>
<tr>
<td>Malariometric indices (no.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pf positive</td>
<td>40% (54/136)</td>
<td>39% (50/129)</td>
<td>29% (31/108)</td>
<td>42% (42/101)§</td>
</tr>
<tr>
<td>Pv positive</td>
<td>27% (37/136)</td>
<td>32% (41/129)</td>
<td>31% (34/109)</td>
<td>33% (33/101)</td>
</tr>
<tr>
<td>Pm positive</td>
<td>3.7% (5/136)</td>
<td>1.5% (2/129)</td>
<td>0% (0/108)</td>
<td>2.0% (2/101)</td>
</tr>
<tr>
<td>Enlarged spleen</td>
<td>40% (48/121)</td>
<td>49% (58/119)</td>
<td>39% (62/105)</td>
<td>68% (63/93)</td>
</tr>
</tbody>
</table>

† for positive slides; na = not applicable.
§ P < 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

<table>
<thead>
<tr>
<th>Morbidity indicators</th>
<th>Placebo</th>
<th>Zinc</th>
<th>RR or 2× [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Health center visits*</td>
<td>156</td>
<td>114</td>
<td>0.79 [0.54–1.14]</td>
</tr>
<tr>
<td>Plasmodium falciparum episodes (fever and Pf density ≥)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/µl</td>
<td>82</td>
<td>48</td>
<td>0.62 [0.40–0.95]§</td>
</tr>
<tr>
<td>9,200/µl</td>
<td>57</td>
<td>34</td>
<td>0.62 [0.40–0.97]§</td>
</tr>
<tr>
<td>100,000/µl</td>
<td>24</td>
<td>7</td>
<td>0.31 [0.13–0.74]§</td>
</tr>
<tr>
<td>non-Plasmodium falciparum visits (Plasmodium falciparum = 0/µl)*</td>
<td>63</td>
<td>57</td>
<td>1.01 [0.50–2.02]</td>
</tr>
<tr>
<td>GMD Plasmodium falciparum density/µl [95% CI]†</td>
<td>16,131</td>
<td>13,320</td>
<td>Δ = 17% [–60–71]</td>
</tr>
<tr>
<td>Mean hemoglobin (g/dl ± SD)</td>
<td>8.6 (1.8)</td>
<td>8.8 (1.8)</td>
<td>Δ = 2.2% [–3.2–7.5]</td>
</tr>
<tr>
<td>Anemia (Hb ≤ 7.0 g/dl)</td>
<td>23% (30/132)</td>
<td>19% (19/101)</td>
<td>0.83 [0.50–1.38]</td>
</tr>
<tr>
<td>Spleen enlargement</td>
<td>28% (39/139)</td>
<td>30% (31/102)</td>
<td>1.08 [0.73–1.61]</td>
</tr>
</tbody>
</table>

† Geometric mean parasite density for positive slides only.
§ P < 0.05, see text for exact P values.

* The respective surveillance periods were 37,513 child-days for the placebo group and 35,991 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.
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 $\geq 7,800/\mu l$ and $\geq 10,000/\mu l$, 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/\mu l 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 effect.

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 $\geq 3,800/\mu l$ (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 recrudescence Pf episodes (which were not considered as new Pf cases in Table 2). Clinic attenders given Zn also had a 17% (95% CI 0–71, $P = 0.43$) 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 $\geq 100,000/\mu l$, 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 malarialmetric 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 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. 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.

In a concurrent study in neighboring villages of the Wos-
era, we have demonstrated that vitamin A supplementation reduced \textit{Pf} episodes by 30%.\textsuperscript{21} Although both Zn and vitamin A reduced the incidence of \textit{Pf} episodes, there are several important qualitative differences in their effects. First, the protective efficacy of Zn was greatest for \textit{Pf} episodes \( \geq 100,000/\mu l \), whereas that of vitamin A began to wane at densities \( \geq 30,000/\mu l \). Second, whereas Zn had no effect on spleen enlargement and parasite density, vitamin A tended to reduce these malarialmetric 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 malarialmetric indices in those given vitamin A.

As mentioned, despite the 38\% reduction in \textit{Pf} clinical-based episodes in children given Zn, consistent effects were not observed against other malarialmetric indices. Indeed, \textit{Pf} prevalence at the end of the study 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,\textsuperscript{21} the 29\% \textit{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 \textit{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 \textit{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 \textit{Pf} illness. In fact, Zn administration during infection has been shown to potentiate the febrile response,\textsuperscript{22} which can suppress \textit{in vitro} growth of \textit{Pf}\textsuperscript{23} and \textit{Pf} parasitemia \textit{in vivo}.\textsuperscript{24,25} Alternatively, given that Zn is a known antioxidant and a potent inhibitor of apoptosis,\textsuperscript{26} children given Zn may better endure the immunopathologies associated with a \textit{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 \textit{Plasmodium berghei} is consistent with this idea.\textsuperscript{26} Sequestration of mature parasitized erythrocytes within the microvasculature is a pathogenec process associated with \textit{Pf} and not \textit{P. vivax}. If Zn selectively reduced \textit{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 \textit{Pf} attacks in the Zn group but in only 3.5\% (2 of 57) of those given PL (RR = 4.18 [95\% CI 0.28–186.76, \( P = 0.054 \)). Lastly, the immunopotentiating activities of Zn\textsuperscript{2} could enhance acquired immunity following exposure to \textit{Pf}. The magnitude and specificity of the Zn-mediated effects strongly implicates some form of specific immunity. Preliminary assessments of \textit{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\textsuperscript{27} and respiratory morbidity.\textsuperscript{28} Although the 38\% reduction in clinic-based \textit{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.\textsuperscript{29}

At present the protective efficacies of Zn and vitamin A against clinical \textit{Pf} episodes is similar to protection seen with insecticide-treated bed nets (48\%),\textsuperscript{30} and appear to exceed the most optimistic 23% efficacy estimates of the first phase III-tested experimental malaria vaccine SP666.\textsuperscript{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 chemophrophylaxis, 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 \textit{Pf} malaria. This study suggests that improved Zn intake, via supplementation or otherwise, may reduce \textit{Plasmodium falciparum} morbidity.

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