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C. Duggan
Children's Hospital, Boston

W.B. MacLeod
Boston University

N.F. Krebs
University of Colorado Health Sciences Center

J.L. Westcott
University of Colorado Health Sciences Center

W.W. Fawzi
Harvard School of Public Health

See next page for additional authors

Authors
C. Duggan, W.B. MacLeod, N.F. Krebs, J.L. Westcott, W.W. Fawzi, Zul Premji, V. Mwanakasale, J.L. Simon, K. Yeboah-Antwi, and D.H. Hamer

Plasma Zinc Concentrations Are Depressed during the Acute Phase Response in Children with Falciparum Malaria[1]

Christopher Duggan,*† William B. MacLeod,** Nancy F. Krebs,‡ Jamie L. Westcott,‡ Wafaie W. Fawzi,† Zul G. Premji,†† Victor Mwanakasale,‡‡ Jonathon L. Simon,** Kojo Yeboah-Antwi,**¶ Davidson H. Hamer,**§2 and the Zinc Against Plasmodium Study Group[3]

*Division of Gastroenterology and Nutrition, Children's Hospital, Boston, MA; †Department of Nutrition, Harvard School of Public Health, Boston, MA; **Center for International Health and Development, Boston University School of Public Health, Boston, MA; ‡Section of Nutrition, Department of Pediatrics, University of Colorado Health Sciences Center, Denver, CO; ††Muhimbili University College of Health Sciences, Dar es Salaam, Tanzania; ‡‡Tropical Diseases Research Centre, Ndola, Zambia; ¶Malaria Consortium, Accra, Ghana; and §Gerald J. and Dorothy R. Friedman School of Nutrition Science and Policy, Tufts University, Medford, MA

ABSTRACT Plasma concentrations of some micronutrients are altered in the setting of acute infectious or inflammatory stress. Previous studies have provided conflicting evidence concerning the extent and direction of changes in plasma zinc concentrations during the acute phase response. We carried out an observational cohort study in 689 children enrolled in a randomized trial of zinc supplementation during acute falciparum malaria in order to evaluate the relation between plasma zinc concentration and the acute phase response. Plasma zinc was measured by atomic absorption spectrophotometry. On admission, 70% of all subjects had low plasma zinc (<9.2 fLmol/L). Multivariate analysis of predictors of admission plasma zinc showed that admission C-reactive protein (CRP), parasite density, and study site were the most important predictors. Predictors of changes in plasma zinc from admission to 72 h included baseline CRP, change in CRP, treatment group, study site, and baseline zinc concentration. In children with acute malaria infection, baseline plasma zinc concentrations were very low and were inversely correlated with CRP (r = −0.24, P < 0.0001) and the degree of parasitemia (r = −0.19, P < 0.0001). Even when CRP and time were taken into account, zinc supplementation increased plasma zinc concentration from admission to 72 h. When available, plasma zinc concentrations should be interpreted with concurrent measures of the acute phase response such as CRP. In children whose age, diet, and/or nutritional status place them at risk of zinc deficiency, those with low plasma zinc levels should be supplemented with oral zinc and followed for clinical and/or biochemical response. J. Nutr. 135: 802–807, 2005.

KEY WORDS: • *malaria* • *zinc* • *Plasmodium falciparum* • *child* • *acute phase response* • *C-reactive protein*

THE RELATION BETWEEN PLASMA MICRONUTRIENT CONCENTRATIONS AND THE EXTENT OF THE BODY'S ACUTE PHASE RESPONSE IS AN IMPORTANT FACTOR IN THE DESIGN AND CONDUCT OF STUDIES IN THE FIELD OF HUMAN NUTRITION. TWO MICRONUTRIENTS FOR WHICH THIS RELATION HAS BEEN OF PARTICULAR INTEREST ARE VITAMIN A AND ZINC. BLOOD CONCENTRATIONS OF VITAMIN A ARE DEPRESSED DURING THE ACUTE PHASE RESPONSE (1–5), AND IT IS DEBATABLE WHETHER THESE CHANGES ARE RELATED TO VITAMIN A REDISTRIBUTION THROUGHOUT THE BODY, INCREASED EXOGENOUS LOSS (6,7), AND/OR INCREASED METABOLIC NEEDS (8). BECAUSE CLINICAL SIGNS OF VITAMIN A DEFICIENCY CAN OCCUR AMONG WOMEN (9) AND CHILDREN (5) WITH DECREASED RETINOL CONCENTRATIONS AND ELEVATED ACUTE PHASE RESPONSE MARKERS, IT SEEMS INCORRECT TO ASCRIBE THESE LOW BLOOD LEVELS TO MERELY A PHYSIOLOGIC RESPONSE TO INFECTIOUS OR INFLAMMATORY STRESS.

SIMILAR, ALTHOUGH FEWER, DATA HAVE BEEN DESCRIBED FOR THE RELATION BETWEEN ZINC BLOOD CONCENTRATIONS AND THE ACUTE PHASE RESPONSE. MOST EXPERIMENTALLY INDUCED INFECTIONS IN ANIMALS AND HUMANS SHOW A DECREASE IN PLASMA ZINC CONCENTRATIONS (10), WITH MORE SEVERE INFECTIONS LEADING TO A MORE SIGNIFICANT DECREASE. EXTENSIVE EXPERIMENTAL WORK HAS DEMONSTRATED THAT HEPATIC METALLOTHIONEIN IS INVOLVED IN THE RESPONSE TO STRESS. METALLOTHIONEIN CAN BE INDUCED BY INFUSION OF DEXAMETHASONE OR OTHER GLUCOCORTICOIDS, ENDOTOXIN, OR CYTOKINES (11–13). INCREASES IN METALLOTHIONEIN AND METALLOTHIO-

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[2] To whom correspondence should be addressed. E-mail: dhamer@bu.edu.

[3] The ZAP study group is composed of Fernando Sempértegui, Bertha Estrella, Franklin R. Toapanta, Darwin S. Torres, and Dheyanira E. Calahorrano (Ecuador); Emmanuel Addo-Yobo, Paul Arthur (deceased), and Sam Newton (Ghana); Mloka Hubert and Cyprian S. Makwaya (Tanzania); Freddie Ssengooba, Joseph Konde-Lule, and Emmanuel Mukisa (Uganda); and Modest Mulenga, Thomas Sukwa, and John Tshiula (Zambia).

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NEIN MRNA ARE CORRELATED WITH INCREASED HEPATIC ZINC AND A CORRESPONDING REDUCTION OF CIRCULATING ZINC.

SOME ANIMAL MODELS OF INFECTION, HOWEVER, HAVE SHOWN AN INCREASE IN PLASMA ZINC WITH INFECTIOUS/INFLAMMATORY STRESSES (14,15), AND THE RESULTS OF CLINICAL STUDIES HAVE NOT BEEN CONSISTENT. PLASMA ZINC CONCENTRATION WAS NOT SIGNIFICANTLY AFFECTED BY COMMON INTERCURRENT INFECTIONS SUCH AS DIARRHEA AND RESPIRATORY TRACT INFECTION IN 3 COMMUNITY-BASED STUDIES IN PRESCHOOL- AND SCHOOL-AGE CHILDREN (16).

ACUTE MALARIA INFECTIONS, ESPECIALLY THOSE DUE TO *Plasmodium falciparum*, ARE NOTABLE FOR VERY HIGH FEVERS AND A SEVERE ACUTE PHASE PROTEIN RESPONSE (17,18). PREVIOUS STUDIES HAVE LINKED LOW CIRCULATING LEVELS OF VITAMINS A AND E WITH THE ACUTE PHASE RESPONSE OF MALARIA (19,20). HOWEVER, THE IMPACT OF THE ACUTE PHASE RESPONSE ON BLOOD ZINC CONCENTRATIONS IN THE SETTING OF ACUTE MALARIA HAS NOT BEEN REPORTED. USING DATA FROM A CLINICAL TRIAL OF ZINC SUPPLEMENTATION IN CHILDREN WITH ACUTE MALARIA (21), WE SOUGHT TO EVALUATE THE RELATION BETWEEN PLASMA ZINC CONCENTRATION AND THE ACUTE PHASE RESPONSE IN SUBJECTS ENROLLED IN THIS TRIAL. IN ADDITION, WE WERE ABLE TO EVALUATE THE RESPONSE OF BLOOD ZINC CONCENTRATION TO ORAL ZINC SUPPLEMENTATION.

METHODS

Study population. AS PREVIOUSLY DESCRIBED (21), THE STUDY WAS A MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED CLINICAL TRIAL OF SUPPLEMENTAL ZINC AMONG CHILDREN WITH UNCOMPLICATED FALCIPARUM MALARIA. SUBJECT ENROLLMENT TOOK PLACE BETWEEN DECEMBER 1998 AND MAY 2000 AT THE FOLLOWING SITES: HOSPITAL DELFINA TORRES (ESMERALDAS CITY, ECUADOR), KOMFO ANOKYE TEACHING HOSPITAL (KUMASI, GHANA), KISARAWE DISTRICT HOSPITAL (KISARAWE, TANZANIA), MPIGI HEALTH CENTER (MPIGI, UGANDA), AND ARTHUR DAVISON CHILDREN HOSPITAL (NDOLA, ZAMBIA). CHILDREN BETWEEN THE AGES OF 6 AND 60 MO WHO PRESENTED WITH FEVER (AXILLARY TEMPERATURE > 37.5°C) AND >2000/fLL ASEXUAL FORMS OF *P. falciparum* IN A THICK BLOOD SMEAR WERE RANDOMLY ASSIGNED EITHER ZINC (20 MG/D FOR CHILDREN <12 MO AND 40 MG/D FOR CHILDREN AGED 12–60 MO) OR PLACEBO FOR THE FIRST 3 D OF THE STUDY. CLINICAL AND PARASITOLOGIC OUTCOMES WERE NOTED AT 3, 7, 14, AND 28 D. EXCLUSION CRITERIA INCLUDED HEMOGLOBIN < 70 G/L; SEVERE MALARIA AS DEFINED BY THE PRESENCE OF ANY OF THE FOLLOWING: CEREBRAL MALARIA, SEVERE ANEMIA, RENAL FAILURE, PULMONARY EDEMA, HYPOGLYCEMIA, SHOCK, SPONTANEOUS BLEEDING, REPEATED CONVULSIONS (22); NONFALCIPARUM OR MIXED *Plasmodium* INFECTIONS; CONCURRENT SEVERE INFECTIONS (I.E., LOWER RESPIRATORY INFECTION, ACUTE OTITIS MEDIA, PYELONEPHRITIS, TYPHOID FEVER, BLOODY DIARRHEA, MENINGITIS, OR MEASLES); SEVERE DEHYDRATION; MALNUTRITION AS DEFINED BY THE WELLCOME CRITERIA (23) (I.E., MARASMUS, KWASHIORKOR, OR MARASMIC KWASHIORKOR); INABILITY TO TOLERATE ORAL MEDICATIONS OR FLUIDS; CHRONIC ILLNESS (INCLUDING TUBERCULOSIS, AIDS, SEVERE CONGENITAL ANOMALIES, SICKLE CELL DISEASE); AND PRIOR PARTICIPATION IN THIS TRIAL.

IN ACCORDANCE WITH NATIONAL TREATMENT GUIDELINES AT THE TIME OF THE TRIAL, CHLOROQUINE (10 MG/KG ON D 0, 10 MG/KG ON D 1, AND 5 MG/KG ON D 2) WAS GIVEN AS FIRST-LINE TREATMENT FOR MALARIA. TREATMENT FAILURE WAS DEFINED AS THE PRESENCE OF AXILLARY TEMPERATURE > 37.5°C AND PARASITEMIA > 25% OF THE BASELINE LEVEL AT 72 H. PARASITOLOGIC FAILURE WAS DEFINED AS PARASITEMIA > 25% OF THE BASELINE LEVEL WITH RESOLUTION OF FEVER (I.E., TEMPERATURE < 37.5°C AT 72 H). IF EITHER A TREATMENT OR A PARASITOLOGIC FAILURE OCCURRED, SUBJECTS WERE CHANGED TO A STANDARD DOSE OF EITHER AMODIAQUINE OR SULFADOXINE PYRIMETHAMINE AS SECOND-LINE ANTIMALARIAL THERAPY. ALL SUBJECTS RECEIVED STANDARD MEDICAL CARE FOR ANY CONCURRENT ILLNESSES THAT WERE PRESENT AT BASELINE OR DEVELOPED DURING THE STUDY. THIS INCLUDED APPROPRIATE ANTIMICROBIAL THERAPY FOR ACUTE RESPIRATORY INFECTIONS, DYSENTERY, AND OTHER TREATABLE INFECTIONS.

ETHICAL APPROVAL OF THE STUDY WAS OBTAINED FROM THE INSTITUTIONAL REVIEW BOARDS AT EACH SITE AND THE HARVARD SCHOOL OF PUBLIC HEALTH. WRITTEN INFORMED CONSENT WAS OBTAINED FROM THE PARENT OR GUARDIAN OF EACH SUBJECT.

Laboratory methods. BLOOD FOR PLASMA ZINC MEASUREMENT WAS TAKEN ON D 0 BEFORE THE ADMINISTRATION OF THE STUDY DRUG AND THEN AT 72 H BEFORE THE LAST DOSE OF ZINC OR PLACEBO WAS GIVEN. SAMPLES WERE OBTAINED JUST BEFORE MEALS. VENOUS BLOOD WAS DRAWN WITH ZINC-FREE SYRINGES AND PLACED INTO HEPARINIZED ZINC-FREE TUBES. BLOOD WAS IMMEDIATELY CENTRIFUGED AND PLASMA WAS TRANSFERRED INTO ZINC-FREE TUBES WITH A PLASTIC ZINC-FREE PIPET AND FROZEN AT −20°C. PLASMA ZINC WAS ASSAYED BY ATOMIC ABSORPTION SPECTROPHOTOMETRY AT THE PEDIATRIC NUTRITION LABORATORY AT THE UNIVERSITY OF COLORADO HEALTH SCIENCES CENTER (24). PLASMA C-REACTIVE PROTEIN (CRP)[4] WAS MEASURED VIA IMMUNOTURBIDIMETRIC ASSAY (ROCHE DIAGNOSTICS). DUE TO LOGISTICAL CONSTRAINTS, WE ANALYZED PLASMA ZINC AND CRP CONCENTRATIONS FROM 3 OF THE 5 SITES (GHANA, TANZANIA, AND ZAMBIA).

Data analysis. WE USED SAS SOFTWARE, VERSION 8.2 (SAS INSTITUTE), FOR STATISTICAL ANALYSIS. UNIVARIATE CORRELATES OF BASELINE PLASMA ZINC WERE COMPARED USING PEARSON CORRELATION COEFFICIENTS. BASELINE CHARACTERISTICS WERE COMPARED USING LOGISTIC REGRESSION FOR CATEGORICAL VARIABLES USING PROC LOGISTIC AND ANOVA FOR CONTINUOUS VARIABLES USING PROC ANOVA ALLOWING FOR ADJUSTMENT OF MULTIPLE COMPARISONS, USING THE SCHEFFE TEST (25). MANTEL-HAENSZEL RELATIVE RISKS FOR THE DIFFERENCES IN ZINC DEFICIENCY WERE CALCULATED USING PROC FREQ.

PREDICTORS OF BASELINE PLASMA ZINC WERE MODELED USING A GENERALIZED LINEAR MODEL USING PROC REG. VARIABLES ELIGIBLE FOR INCLUSION INTO THE MODEL WERE ADMISSION CRP, TREATMENT GROUP, SITE, AGE IN MONTHS, ANTHROPOMETRIC MEASUREMENTS (WEIGHT FOR AGE *Z*-SCORE (WAZ), HEIGHT FOR AGE *Z*-SCORE (HAZ), WEIGHT FOR HEIGHT *Z*-SCORE (WHZ), MEAN UPPER ARM CIRCUMFERENCE), TREATMENT FAILURE, PRESENCE OF OTHER ILLNESS, PARASITEMIA, AND ADMISSION TEMPERATURE. EACH VARIABLE (OR GROUP IN THE CASE OF INDICATOR VARIABLES) WAS ENTERED INTO THE MODEL SEQUENTIALLY AND THEN REMOVED, AND THE ONE WITH THE LARGEST *F* VALUE WAS RETAINED. THIS CONTINUED UNTIL ALL THE VARIABLES WITH A *P* VALUE < 0.05 WERE RETAINED IN THE MODEL. AT THIS POINT, 2-WAY INTERACTION TERMS WERE ENTERED INTO THE MODEL USING THE SAME CRITERIA. NONE OF THE 2-WAY INTERACTIONS HAD A *P* VALUE < 0.05 TO BE RETAINED IN THE MODEL.

IN ORDER TO MODEL THE PREDICTORS FOR CHANGE IN PLASMA ZINC FROM ADMISSION TO 72 H, WE USED A REGRESSION MODEL WITH THE CHANGE IN PLASMA ZINC FROM ADMISSION TO 72 H AS THE BASELINE USING PROC REG. WE INCLUDED ADMISSION PLASMA ZINC AS ONE OF THE PREDICTORS TO ACCOUNT FOR THE DIFFERENCES IN PLASMA ZINC AT BASELINE. THE DETAILS OF THIS MODEL CONSTRUCTION WERE SIMILAR TO THOSE DESCRIBED ABOVE. VARIABLES ELIGIBLE FOR INCLUSION INTO THE MODEL WERE ADMISSION CRP, THE CHANGE IN CRP FROM ADMISSION TO 72 H, TREATMENT GROUP, SITE, AGE IN MONTHS, TREATMENT FAILURE, PRESENCE OF OTHER ILLNESS, ANTHROPOMETRIC MEASUREMENTS (WAZ, HAZ, WHZ, MEAN UPPER ARM CIRCUMFERENCE), ADMISSION PARASITEMIA, ADMISSION TEMPERATURE, AND CHANGE IN PARASITEMIA FROM ADMISSION TO 72 H.

ALL VARIABLES INCLUDED IN THE FINAL MODELS WERE DETERMINED TO BE INDEPENDENT BY ASSESSING COLINEARITY WITH THE EIGEN VALUE (25).

RESULTS

BASELINE CHARACTERISTICS OF THE STUDY GROUPS SHOWED COMPARABLE AGE AND SEX DISTRIBUTIONS AMONG THE 3 SITES (Table 1). MORE CHILDREN IN TANZANIA WERE BREASTFED (OR 1.45, 95% CI 1.05 TO 2.0), USED BEDNETS (OR 12.6, 95% CI 8.3 TO 19.2), AND WERE GIVEN ANTI-MALARIAL MEDICATION IN THE 7 D BEFORE ADMISSION (OR 2.22, 95% CI 1.58 TO 3.13) THAN CHILDREN IN GHANA AND ZAMBIA. NUTRITIONAL STATUS, INCLUDING WEIGHT, HEIGHT, AND ARM ANTHROPOMETRICS, WAS COMPARABLE AMONG ALL SITES, AS WERE ADMISSION TEMPERATURE AND THE PEAK TEMPERATURE IN THE FIRST 24 H. AT ADMISSION, TANZANIAN SUBJECTS HAD LOWER CRP (P ≈ 0.002), HIGHER PLASMA ZINC (P < 0.0001) AND LOWER HEMOGLOBIN (P < 0.0001) CONCENTRATIONS THAN SUBJECTS IN GHANA AND ZAMBIA. CHILDREN IN GHANA HAD LOWER LEVELS OF PARASITEMIA THAN THOSE IN TANZANIA AND ZAMBIA (P < 0.0001).

[4] Abbreviations used: CRP, C-reactive protein; HAZ, height for age *Z*-score; WAZ, weight for age *Z*-score; WHZ, weight for height *Z*-score.

TABLE 1

Baseline characteristics of subjects[1]

Variable	Tanzania (*n* = 221)	Zambia (*n* = 260)	Ghana (*n* = 208)	All (*n* = 689)
Gender (% male)	115 (52)	140 (54)	108 (52)	363 (53)
Breastfeeding	106 (48)	109 (42)	73 (35)	288 (42)
Bednet use by child	118 (53)	16 (6)	23 (11)	157 (23)
Prior antimalarial use in previous 7 d	89 (40)	63 (24)	46 (22)	198 (29)
Age, *mo*	24.0 (12 to 36)	24.0 (13 to 39)	24.0 (14 to 40)	24.0 (13 to 38)
WAZ	−1.2 (−2 to −0.3)	−1.4 (−2 to −0.6)	−1.1 (−2 to −0.5)	−1.2 (−2 to −0.5)
HAZ	−1.2 (−2 to −0.3)	−1.5 (−2 to −0.6)	−1.1 (−2 to −0.2)	−1.2 (−2 to −0.4)
WHZ	−0.4 (−1 to .37)	−0.5 (−1 to .07)	−0.4 (−1 to .20)	−0.4 (−1 to .21)
Mid upper arm circumference, *cm*	15.0 (14 to 16)	15.0 (14 to 16)	15.0 (14 to 16)	15.0 (14 to 16)
Axillary temperature, *°C*	38.3 (38 to 39)	39.1 (39 to 40)	38.6 (38 to 39)	38.7 (38 to 39)
Parasitemia, (asexual forms of P. falciparum), *n/fLL*	44,000 (7,040 to 153,000)	46,680 (17,980 to 110,380)	12,200 (3,540 to 49,120)	34,400 (8,200 to 88,760)
Parasitemia 0–9999	68 (31)	40 (15)	93 (45)	201 (29)
Parasitemia 10,000–99,999	70 (32)	149 (57)	110 (53)	329 (48)
Parasitemia > 100,000	83 (38)	71 (27)	5 (2)	159 (23)
Hemoglobin, *g/L*	83 (76 to 92)	91 (81 to 100)	92 (81 to 100)	88 (79 to 100)
Plasma zinc, fL*mol/L*	9.55 (7.96 to 11.48)	6.76 (5.51 to 8.11)	6.84 (5.20 to 9.64)	7.59 (5.97 to 9.64)
Low plasma zinc[2] (<9.2 fLmol/L), *n/total n*	81/183 (44)	206/233 (88)	139/194 (72)	426/610 (70)
Plasma CRP, *mg/L*	55 (30 to 100)	78 (43 to 130)	70 (32 to 110)	68 (35 to 120)
Maximum 24-h axillary temperature, *°C*	38.7 (38 to 40)	39.3 (39 to 40)	39.0 (38 to 40)	39.1 (38 to 40)

[1] Values are *n* (%) or medians (interquartile range).
[2] For this variable the total *n* differs because of incomplete sampling collection and/or hemolysis.

PLASMA ZINC AND CRP CONCENTRATIONS CHANGED SIGNIFICANTLY BETWEEN BASELINE AND 72 H (Figs. 1 AND 2) THE PROPORTION OF CHILDREN WITH LOW PLASMA ZINC (<9.2 fLMOL/L) WAS 66% IN THE ZINC GROUP AND 73% IN THE PLACEBO GROUP (RR 0.91, 95% CI 0.82 TO 1.01) (70% FOR THE 2 GROUPS COMBINED) ON ADMISSION. THE PROPORTION OF CHILDREN WITH LOW PLASMA ZINC AT 72 H DECREASED TO 30 AND 41% IN THE ZINC AND PLACEBO GROUPS, RESPECTIVELY (RR 0.75, 95% CI 0.60 TO 0.93).

BASELINE PLASMA ZINC CONCENTRATION WAS SIGNIFICANTLY ASSOCIATED WITH SEVERAL FACTORS, INCLUDING AGE (PEARSON r = −0.12, P = 0.002), WHZ (r = 0.08, P < 0.05), PARASITE DENSITY (r

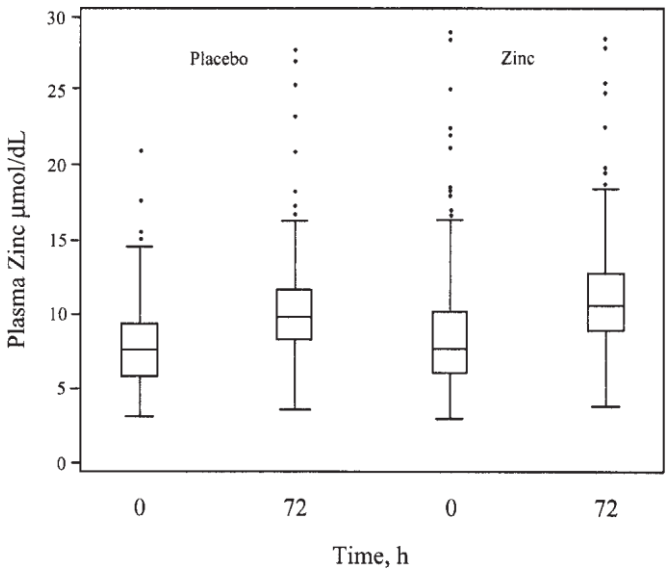

FIGURE 1 Box plots of plasma zinc at time 0 and 72 h by placebo and zinc groups. Values are illustrated by box plots with the box representing the 25th and 75th percentiles (ends of boxes). The upper and lower whiskers are drawn from the box to the most extreme point within 1.5 interquartile range. The median is represented by the horizontal line in the box. Outliers are represented by dots. The mean concentration of plasma zinc at 72 h differed from that at time 0 h for both placebo (P < 0.0001) and zinc groups (P < 0.0001).

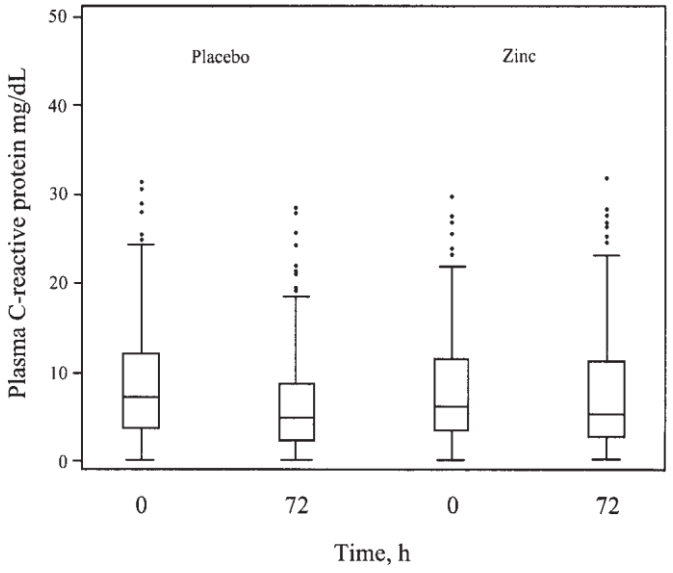

FIGURE 2 Box plots of plasma CRP at time 0 and 72 h by placebo and zinc groups. See Figure 1 for box plot legend. The mean concentration of plasma CRP at 72 h differed from that at time 0 h for the placebo group (P = 0.0002), although the zinc group showed no difference between baseline and 72 h (P = 0.81).

$\rho = -0.19$, $P < 0.0001$), BASELINE CRP ($r = -0.24$, $P < 0.0001$), AND PEAK BODY TEMPERATURE IN THE FIRST 24 H ($r = -0.16$, $P < 0.0001$).

MULTIVARIATE ANALYSIS OF PREDICTORS OF ADMISSION PLASMA ZINC (Table 2) SHOWED THAT ADMISSION CRP, PARASITE DENSITY, AND SITE WERE THE MOST IMPORTANT PREDICTORS. VARIABLES NOT SELECTED FOR INCLUSION IN THE MODEL INCLUDED AGE, BREASTFEEDING STATUS, TEMPERATURE, AND ANTHROPOMETRIC MEASURES. THE MODEL SHOWS THAT FOR EVERY INCREMENT IN CRP LEVELS BY 1.0 MG/L, PLASMA ZINC WAS 1.0 fLMOL/L LOWER. IN ADDITION, FOR EVERY 10,000 U INCREASE IN PARASITE DENSITY, PLASMA ZINC WAS 0.08 fLMOL/L LOWER. BOTH CRP AND PARASITE DENSITY WERE, THEREFORE, INDEPENDENTLY ASSOCIATED WITH PLASMA ZINC CONCENTRATION AT ADMISSION.

USING LINEAR REGRESSION, WE EXAMINED PREDICTORS OF CHANGE IN PLASMA ZINC FROM ADMISSION TO 72 H (Table 3) CONTROLLING FOR STUDY SITE. SUBJECTS WHO RECEIVED ZINC SUPPLEMENTATION HAD ON AVERAGE A GREATER INCREASE IN PLASMA ZINC BY 0.98 MMOL/L COMPARED TO THOSE WHO RECEIVED PLACEBO. THIS EFFECT OF ZINC SUPPLEMENTATION WAS INDEPENDENT OF BOTH TIME AND CRP CONCENTRATION. THE CHANGE IN CRP FROM ADMISSION TO 72 H VARIABLE SHOWS THAT CRP LEVELS WERE NEGATIVELY ASSOCIATED WITH PLASMA ZINC CONCENTRATIONS. THIS NEGATIVE RELATION BETWEEN THE 2 VARIABLES SHOWS THAT AS CRP DECLINED, PLASMA ZINC LEVELS INCREASED. PARASITEMIA, CHANGE IN PARASITEMIA, TREATMENT FAILURE, THE PRESENCE OF OTHER ILLNESS, ANTHROPOMETRIC MEASUREMENTS, ADMISSION TEMPERATURE, AND AGE WERE NOT SIGNIFICANT PREDICTORS OF CHANGE IN PLASMA ZINC BETWEEN BASELINE AND 72 H.

DISCUSSION

IN OUR COHORT OF 689 CHILDREN WITH ACUTE MALARIA, WE FOUND A VERY HIGH INCIDENCE OF LOW PLASMA ZINC CONCENTRATIONS, WITH 70% OF SUBJECTS HAVING PLASMA ZINC < 9.18 fLMOL/L (60 fLG/DL) ON ADMISSION, A CUTOFF COMMONLY USED TO DENOTE ZINC DEFICIENCY. WE ALSO FOUND SIGNIFICANT CORRELATIONS BETWEEN EVIDENCE OF ILLNESS SEVERITY (CRP, PARASITE DENSITY, AND BODY TEMPERATURE) AND BASELINE PLASMA ZINC CONCENTRATIONS. THESE CORRELATIONS WERE RELATIVELY LOW BUT IN THE EXPECTED DIRECTION (I.E., HIGHER CRP, PARASITE DENSITY, AND TEMPERATURE WERE ASSOCIATED WITH LOWER PLASMA ZINC). MULTIVARIATE MODELING CONFIRMED THAT CRP WAS A SIGNIFICANT PREDICTOR OF BASELINE PLASMA ZINC CONCENTRATION, IN ADDITION TO THE INDEPENDENT AND SIGNIFICANT EFFECTS OF PARASITE DENSITY AND STUDY SITE. CHANGES IN PLASMA ZINC OVER 72 H WERE RELATED TO STUDY SITE, WHETHER ZINC WAS ADMINISTERED, CHANGES IN CRP OVER TIME, AND BASELINE ZINC AND CRP CONCENTRATIONS. OUR DATA THEREFORE SUGGEST THAT THE FINDING OF LOW PLASMA ZINC ON ADMISSION WAS LARGELY BUT NOT EXCLUSIVELY DUE TO THE ACUTE PHASE RESPONSE OF MALARIA INFECTION. IN ADDITION, THE CHANGE IN PLASMA ZINC OVER 72 H WAS ASSOCIATED WITH THE CHANGE IN INFLAMMATION (I.E., CRP) OVER TIME AS WELL AS ZINC SUPPLEMENTATION.

PREVIOUS STUDIES THAT EXAMINED THE RELATION BETWEEN ZINC STATUS AND INFECTIOUS ILLNESSES GENERALLY CONCLUDED THAT DESPITE A HIGH INCIDENCE OF ACUTE INFECTIONS AMONG CHILDREN IN DEVELOPING COUNTRIES, PLASMA ZINC CONCENTRATIONS WERE STILL REASONABLE INDICATORS OF ZINC STATUS. INFECTIONS IN THESE STUDIES WERE VARIOUSLY DEFINED AS CLINICALLY APPARENT INFECTIONS SUCH AS DIARRHEA, DERMATITIS, AND RESPIRATORY AND OTHER INFECTIONS, AS WELL AS CLINICALLY SILENT INFECTIONS BASED ON ELEVATIONS IN SERUM CRP AND/OR WHITE BLOOD CELL COUNT. IN 3 CROSS-SECTIONAL COMMUNITY-BASED STUDIES AMONG AMBULATORY CHILDREN IN POOR COUNTRIES, MEAN DIFFERENCES IN PLASMA ZINC CONCENTRATION BETWEEN INFECTED AND NONINFECTED CHILDREN WERE 0 fLMOL/L (0 fLG/DL) IN ZIMBABWE, 0.6 TO 0.8 fLMOL/L (3.9 TO 5.2 fLG/DL) IN PERU, AND 0.6 fLMOL/L (3.9 fLG/DL) IN GUATEMALA (16).

THESE FINDINGS WERE NOTED TO BE IN CONTRAST WITH ANIMAL AND ADULT DATA THAT SHOWED A SIGNIFICANT REDUCTION IN PLASMA ZINC WITH ACUTE INFLAMMATORY STRESSES (10,26). OUR RESULTS, WHICH ARE MORE CONSISTENT WITH THIS PREVIOUS LITERATURE, ARE LIKELY DUE IN PART TO THE SEVERE NATURE OF THE ACUTE PHASE RESPONSE SEEN IN MALARIA, AS OPPOSED TO THAT OBSERVED AMONG CHILDREN WITH MORE MILD INFECTIOUS ILLNESSES. BY DEFINITION, SUBJECTS WERE ONLY INCLUDED IN OUR STUDY IF THEY PRESENTED WITH FEVER AND EVIDENCE OF MALARIA PARASITEMIA. THE CRP CONCENTRATION (MEAN + SD) AT ADMISSION WAS 71 + 2 MG/L IN OUR COHORT, WHICH IS SUBSTANTIALLY HIGHER THAN THAT OF OTHER STUDIES: 9.8 + 17 MG/L IN PERU (27), AND 14 OF 303 WITH CRP > 50 MG/L IN ZIMBABWE (28). OTHER STUDIES IN WHICH CRP WAS MEASURED TO EVALUATE THE ROLE OF THE ACUTE PHASE RESPONSE IN MICRONUTRIENT STATUS HAVE REPORTED CONCENTRATIONS RANGING FROM 3 TO 12 MG/L IN 90 CHILDREN IN PAPUA NEW GUINEA (29), 0.4 TO 1.6 MG/L AMONG PRESCHOOL INDONESIAN CHILDREN (5), AND 3 MG/L IN PREGNANT NEPALI WOMEN (9).

OUR FINDINGS EXTEND RECENT DATA ON PLASMA ZINC CONCENTRATIONS IN CHILDREN WITH INTERCURRENT ILLNESSES BY SPECIFICALLY ADDRESSING THE ROLE OF ACUTE MALARIA IN AFFECTING THIS INDICATOR OF ZINC NUTRITIONAL STATUS. AMONG CHILDREN IN NEPAL PRESENTING WITH ACUTE DIARRHEA, PLASMA ZINC WAS LOWER IN CHILDREN WITH DYSENTERY, FEVER, AND ELEVATED CRP CONCENTRATIONS (30). IN ADDITION, HYDRATION STATUS, SERUM ALBUMIN, AND THE PRESENCE OF HEMOLYSIS WERE ALSO CORRELATED WITH PLASMA ZINC CONCENTRATION.

TABLE 2

Multivariable linear regression of plasma zinc concentration (fLmol/L) at admission in plasma zinc and placebo groups

Variable	Coefficient (95% CI)
Intercept	11.29 (10.69, 11.89)
Admission CRP, *mg/L*	−1.0 (−1.4, −0.5)
Admission parasite density[1]	−0.08 (−0.11, −0.04)
Site	
Ghana	−2.82 (−3.44, −2.19)
Zambia	−2.52 (−3.19, −1.85)
Tanzania	—[2]

[1] In increments of 10,000 asexual forms of *P. falciparum* per microliter.

[2] Tanzania is reference site.

TABLE 3

Multivariable model for predicting change in plasma zinc concentrations in plasma zinc and placebo groups from admission to 72 h

Variable	Coefficient (95% CI)
Intercept	−12.98 (−14.39, −11.57)
Treatment group	
Zinc	0.98 (0.46, 1.50)
Placebo	—
Admission plasma zinc, fL*mol/L*	0.87 (0.79, 0.96)
Admission CRP, *mg/L*	1.2 (0.7, 1.7)
Change in CRP from admission to 72 h, *mg/L*	−1.6 (−2.0, −1.2)
Study site	
Ghana	1.62 (0.95, 2.29)
Zambia	2.09 (1.39, 2.78)
Tanzania	—[1]

[1] Tanzania is reference site.

WIERINGA ET AL. (31) REPORTED THAT AMONG INDONESIAN INFANTS (MEAN AGE 10.1 MO), 15% HAD ELEVATED CONCENTRATIONS OF CRP (DEFINED AS >10 MG/L). PLASMA ZINC CONCENTRATION WAS 15.5 + 4.8 fLMOL/L IN THOSE WITHOUT EVIDENCE OF AN ACUTE PHASE RESPONSE VERSUS 13.8 + 4.7 fLMOL/L (90.3 + 30.8 fLG/DL) IN THOSE WITH ELEVATED CRP ($P < 0.01$). THE INCIDENCE OF LOW PLASMA ZINC (DEFINED AS <10.7 fLMOL/L) WAS 33.3% IN THOSE WITH ELEVATED CRP AND 11% IN THOSE WITH NORMAL CRP AND a-1-ACID GLYCOPROTEIN. IN CONTRAST, OUR STUDY SHOWED A HIGH PREVALENCE OF LOW PLASMA ZINC (DEFINED AS <9.2 fLMOL/L, A MORE STRICT CRITERION) OF 70% AT BASELINE AND 30–41% AT 72 H.

IN CHILDREN LIVING IN MALARIA ENDEMIC REGIONS, ELEVATED CRP CONCENTRATIONS ARE COMMON. IN RURAL CHILDREN FROM GHANA, MEAN CRP CONCENTRATIONS WERE 7 TO 8 MG/L (32). HURT ET AL. FOUND A MEDIAN CONCENTRATION OF 6 MG/L AMONG >600 RURAL TANZANIAN CHILDREN, WITH A MEDIAN VALUE OF 23.6 MG/L IN THOSE WITH TEMPERATURE > 37.4°C ON PRESENTATION (33). THEY ALSO FOUND A CORRELATION ($r = 0.24$, $P < 0.0001$) BETWEEN CRP AND MALARIA PARASITE DENSITY ON PERIPHERAL BLOOD SMEAR. ONLY A FEW STUDIES HAVE EXAMINED THE RELATION BETWEEN ZINC STATUS AND MALARIA. PREGNANT MALAWIAN WOMEN WITH A HIGH PREVALENCE OF MALARIA INFECTION WERE FOUND TO HAVE LOW PLASMA AND HAIR ZINC LEVELS, BUT THERE WAS NO RELATION BETWEEN PLASMA ZINC AND CRP LEVELS (34). MALARIA PREVALENCE WAS ASSOCIATED WITH HAIR BUT NOT PLASMA ZINC IN THIS COHORT (35).

PLASMA ZINC CONCENTRATIONS ARE AN IMPERFECT MEASURE OF ZINC NUTRITIONAL STATUS. PLASMA ZINC REPRESENTS ONLY A FRACTION OF TOTAL BODY ZINC, AND ALTERNATIVE MEASURES OF ZINC STATUS SUCH AS PLATELET, LYMPHOCYTE, OR TISSUE ZINC ARE NOT WELL-SUITED FOR LARGE FIELD TRIALS IN DEVELOPING COUNTRIES (36). MEASUREMENT OF METALLOTHIONEIN LEVELS IS A POTENTIALLY MORE SENSITIVE ALTERNATIVE TO PLASMA ZINC FOR THE ASSESSMENT OF ZINC STATUS. METALLOTHIONEIN PRODUCTION IS INDUCED BY AVAILABLE ZINC (37,38). MARGINAL ZINC INTAKE IN A SMALL HUMAN STUDY WAS ASSOCIATED WITH A 64% REDUCTION IN METALLOTHIONEIN MRNA CONCENTRATIONS, WHEREAS THERE WAS NO CHANGE IN PLASMA ZINC LEVELS (39). BOTH HUMAN AND ANIMAL STUDIES HAVE DEMONSTRATED THAT PRODUCTION OF METALLOTHIONEIN MRNA AND METALLOTHIONEIN SIGNIFICANTLY INCREASED AFTER DIETARY ZINC SUPPLEMENTATION (37,39,40). A ZINC SUPPLEMENTATION STUDY SHOWED THAT TOTAL RNA EXTRACTED FROM DRIED BLOOD SPOTS EXHIBITED A CHANGE IN MT MRNA COMPARABLE TO THAT OF PURIFIED MONOCYTES AND PERIPHERAL BLOOD MONONUCLEAR CELLS (41). DRIED BLOOD SPOT COLLECTION OFFERS THE ADVANTAGES OF CONVENIENCE AND FEASIBILITY IN FIELD SAMPLING SITUATIONS. CONSEQUENTLY, METALLOTHIONEIN MERITS FURTHER INVESTIGATION AS A POSSIBLE ALTERNATIVE MEASURE OF ZINC STATUS IN FIELD STUDIES.

WE HAVE SHOWN THAT AMONG CHILDREN WITH ACUTE MALARIA INFECTION, PLASMA ZINC CONCENTRATIONS ARE VERY LOW AND ARE INVERSELY CORRELATED WITH CRP, AS WELL AS OTHER MEASURES OF DISEASE SEVERITY SUCH AS BODY TEMPERATURE AND PARASITE DENSITY IN PERIPHERAL BLOOD. ALTHOUGH PART OF THE DEPRESSION IN PLASMA ZINC CONCENTRATION IS LIKELY RELATED TO THE REDISTRIBUTION OF ZINC IN THE ACUTE PHASE RESPONSE, ZINC SUPPLEMENTATION WAS EFFECTIVE AT IMPROVING THIS MEASURE OF ZINC STATUS, EVEN WHEN CRP AND TIME WERE TAKEN INTO ACCOUNT. THUS CHILDREN WITH ACUTE MALARIA AND LOW PLASMA ZINC CONCENTRATIONS MAY STILL BE AT RISK OF ZINC DEFICIENCY, AND ASCRIBING THIS DEPRESSION SOLELY TO THE ACUTE PHASE RESPONSE SEEMS UNWARRANTED. WE SUGGEST THAT LOW PLASMA ZINC LEVELS BE INTERPRETED WITH CONCURRENT MEASURES OF THE ACUTE PHASE RESPONSE SUCH AS CRP, WHEN AVAILABLE, ESPECIALLY AMONG CHILDREN WITH MODERATE TO SEVERE INFECTIOUS ILLNESSES. IN CHILDREN WHOSE AGE, DIET, AND/OR NUTRITIONAL STATUS PLACE THEM AT RISK OF ZINC DEFICIENCY, THOSE WITH LOW PLASMA ZINC LEVELS SHOULD BE SUPPLEMENTED WITH ORAL ZINC AND FOLLOWED FOR THE RESOLUTION OF THIS HYPOZINCEMIA.

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