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Associations between intestinal mucosal function and changes in plasma zinc concentration following zinc supplementation[1]

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Abstract

Objectives—Subclinical environmental enteropathy is associated with malabsorption of fats, carbohydrates, and vitamins A, B12 and folate; however, little information is available on mineral absorption. We therefore investigated the relationship between intestinal mucosal function (measured by the lactulose:mannitol permeability test and plasma citrulline concentration), and zinc absorption, as estimated by the change in plasma zinc concentration (PZC) following short-term zinc or placebo supplementation.

Methods—We conducted a randomized, partially-masked, placebo-controlled trial among 282 apparently healthy children 6–23 mo of age in Burkina Faso. After completing baseline intestinal function tests, participants received either 5 mg zinc, as zinc sulfate, or placebo, daily for 21 d.

Results—At baseline, mean ± SD PZC was 62.9 ± 11.9 μg/dL; median (IQR) urinary lactulose:mannitol (L:M) recovery ratio and plasma citrulline concentration were 0.04 (0.03 – 0.07) and 11.4 (9.0 – 15.6) μmol/L, respectively. Change in PZC was significantly greater in the zinc supplemented *versus* placebo group (15.6 ± 13.3 μg/dL vs. 0.02 ± 10.9 μg/dL; P < 0.0001), and was negatively associated with initial urinary L:M recovery ratio (−1.1 μg/dL per 50%

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increase in urinary L:M recovery ratio; P = 0.014); this latter relationship did not differ between supplementation groups (P = 0.26). Baseline plasma citrulline concentration was not associated with change in PZC.

Conclusions—Although altered intestinal permeability may reduce dietary zinc absorption, it likely does not undermine the efficacy of zinc supplementation, given the large increases in PZC following short-term zinc supplementation observed in this study, even among those with increased urinary L:M recovery ratios.

Keywords

intestinal permeability; lactulose; mannitol; citrulline; zinc

INTRODUCTION

Subclinical environmental enteropathy (EE) is a chronic condition of unknown etiology, characterized by changes in small bowel morphology, including villous atrophy, hyperplasia of crypt cells and infiltration of the lamina propria by inflammatory cells (1, 2). The lactulose:mannitol (L:M) intestinal permeability test provides a non-invasive measure of the absorptive surface area and paracellular permeability of the small intestine, and can serve as a proxy for intestinal biopsy in the diagnosis of EE (3, 4). In addition, plasma citrulline, a non-essential amino acid synthesized by enterocytes, is a novel biomarker of enterocyte mass, intestinal epithelial cell loss and absorptive function (5–7).

Estimates of the prevalence of impaired intestinal permeability range from 62 – 96% among infants and young children in developing countries in sub-Saharan Africa and Asia (urinary L:M (mg:mg) concentration ratio > 0.10–0.12) (4, 8–10). Altered intestinal permeability is associated with acute and chronic malnutrition, growth faltering, and acute and persistent diarrhea (3, 9, 11–15). Perturbed intestinal function is associated with the malabsorption of macro- and micronutrients, including fat, carbohydrates, and vitamins A, B12 and folate (1, 16–21); however, limited knowledge exists as to the relationships between intestinal permeability and mineral status and absorption.

Preventive zinc supplementation has been shown to reduce morbidity from diarrhea and pneumonia, lower all-cause mortality, increase linear growth and weight gain and increase plasma zinc concentrations among infants and young children in populations at risk of zinc deficiency (22). However, there is considerable heterogeneity of these results, possibly related to differences in the prevalence of enteropathy and related effects on zinc absorption.

Zinc homeostasis is achieved by the regulation of dietary zinc absorption, in relation to zinc intake, and endogenous zinc secretion via active transporter-mediated pathways (23). It is not certain whether EE may perturb zinc balance. Although altered intestinal permeability is not associated with plasma zinc concentrations in cross-sectional studies (10, 11, 24), zinc supplementation improved intestinal permeability in some studies, either by reducing paracellular permeability (25, 26) or by increasing absorptive surface area (27). Recent studies using zinc stable-isotope techniques have indicated that impaired intestinal

permeability may affect zinc absorption (28) or homeostasis (10, 29), but the results are inconsistent.

Using data from a recently conducted short-term zinc supplementation study designed to evaluate the efficacy of different physical formulations of zinc supplements on changes in plasma zinc concentration in young children in Burkina Faso (30), we investigated the relationship between intestinal mucosal function (L:M permeability and plasma citrulline concentrations) and changes in plasma zinc concentration following zinc supplementation.

SUBJECTS AND METHODS

Subjects

The parent study was designed as a randomized, partially-masked, placebo-controlled intervention trial, conducted from September to December 2009 in rural Burkina Faso. The details of the study population and methods were presented previously (30). Briefly, infants and young children were invited to participate in the intervention trial if they met the following inclusion criteria: age 6–23 mo, currently breast-feeding, hemoglobin ≥ 60 g/L, and no fever or diarrhea (>3 liquid or semi-liquid stools in a 24 h period) reported in the past week. Children who were currently consuming vitamin or mineral supplements or zinc-fortified infant formulas, or who demonstrated bipedal edema or other serious medical conditions, were excluded. 451 eligible study participants were randomly assigned to 1 of 3 treatment groups: (1) dispersible Zn tablet, containing 5 mg Zn as zinc sulfate (Nutriset S.A.S, Malauney, France; ZnTab), (2) liquid Zn supplement, containing 5 mg Zn as zinc sulfate per 5 ml (ZnLiq), or (3) liquid placebo supplement. After the baseline assessment period, all children received a daily dose of supplement for 21 days, under the supervision of a study fieldworker.

The study protocol was approved by the Institutional Review Boards of the Centre Muraz in Bobo-Dioulasso, Burkina Faso and the University of California, Davis, and registered as a clinical trial (www.ClinicalTrials.gov; NCT00944853). Informed consent was obtained from a parent of each child before enrollment in the study.

Biochemical and anthropometric assessments

As described previously (30), capillary blood samples were obtained from each study participant at baseline (on the day of enrollment) to measure hemoglobin (Hb) concentration. Venous blood samples were drawn at baseline and after the 21 d supplementation period to measure plasma concentrations of zinc, citrulline, C-reactive protein (CRP), alpha-1-acid glycoprotein (AGP) and histidine-rich protein II (HRP2), a *Plasmodium falciparum* specific antigen. The blood was collected in evacuated, trace element-free polyethylene tubes containing lithium heparin (Sarstedt AG & Co, Numbrecht, Germany); standard collection and processing techniques, as recommended by the International Zinc Nutrition Consultative Group (IZiNCG), were followed to prevent zinc contamination (31). Weight and length were measured following standard techniques (32). Weight-for-age (WAZ), length-for-age (LAZ) and weight-for length (WLZ) z-scores were calculated according to the WHO 2006 growth standards (33).

Lactulose:Mannitol intestinal permeability assessments

The L:M intestinal permeability tests were conducted on the day following study enrollment, prior to the start of zinc supplementation. All children were breastfed upon arrival at the study clinic. At ~1 h after breastfeeding (median = 65 minutes, range = 52 – 121 minutes), children were given 2 ml/kg body weight of a sugar solution containing 400 mg lactulose (Duphalac, Duphar Laboratories Ltd. Southampton UK) and 100 mg mannitol (Sigma-Aldrich Corporation, St. Louis, MO) per 2 ml water. The sugar solution was administered by the mother under the close supervision of the field team to ensure that the prescribed amount was consumed. 30 min after administration of the sugar test dose, children were allowed to consume water; after an additional 30 min, they were allowed to consume breast milk and food *ad libitum*, according to their usual dietary practices. All of the urine was collected for 5 h post-dosing in sterile adhesive pediatric urine bags (Briggs Corporation, Des Moines, IA). Each time the child urinated, the time and mass of the void were recorded and the specimen was placed into a collection bottle containing 0.5 ml of 0.1% thimerosal (1g/L, Sigma-Aldrich Corporation, St. Louis, MO) to prevent bacterial growth. 2 ml aliquots of the pooled urine samples were stored at −20°C until laboratory analysis. L:M tests were considered unsuccessful if a child did not consume all of the sugar solution, or if the urine leaked from the collection bag or became contaminated with feces; these samples were excluded from the analyses.

Laboratory assays of blood and urine samples

Urinary lactulose and mannitol concentrations (mg/dL) were measured at the Division of Biological Anthropology, University of Cambridge (Cambridge, UK) by using enzyme kinetic assays, as described previously (34, 35), and adapted for analysis with an iEMS Reader MF (Labsystems Ltd. Helsinki, Finland). All reagents were supplied by Sigma-Aldrich (Poole, UK), except for mannitol dehydrogenase, which was obtained from Biocatalysts Ltd (Cardiff, UK). Mean coefficients of variation (CV) for lactulose standards (20, 50, 80 mg/dL) and mannitol standards (25, 100, 150 mg/dL) were 10.1% and 6.6%, respectively. To adjust for renal clearance, urinary creatinine was measured colorimetrically using a commercially available kit (Creatinine Jaffé Gen.2 Urine; Roche Diagnostics, Indianapolis, IN) and a COBAS Integra 400 Plus autoanalyzyer (Roche Diagnostics, Indianapolis, IN) at the USDA Western Human Nutrition Research Center (Davis, CA).

Hemoglobin concentration at baseline was analyzed immediately following the capillary blood collection with a HemoCue 201^+ photometer (HemoCue AB, Angelholm, Sweden). Plasma zinc concentrations were analyzed by inductively coupled plasma optical emission spectrophotometry (ICP-OES, Vista; Varian Inc., Walnut Creek, California) at the Children's Hospital of Oakland Research Institute (Oakland, CA) (30). Analyses of plasma citrulline concentrations were performed using a Waters Aquity UPLC system with UV detection and pre-column derivatization of amino acids using the Waters AccQTag (6-amino-quinolyl-N-hydroxycuccinimidyl carbamate) fluor method at Boston Children's Hospital (Boston, MA). The inter-assay CV was < 6%. Plasma CRP and AGP concentrations were measured by a combined sandwich ELISA at the VitA-Iron Lab (Willstaett, Germany), to control for the presence of sub-clinical infection (36). The inter-assay CV for a control sample was 3.5% for CRP and 4.1% for AGP. Plasma HRP2

concentrations, indicative of current or recent *Plasmodium falciparum* malaria parasitemia, were measured colorimetrically using a commercially available kit (Malaria Ag CELISA, CeLLabs Pty. Ltd., Brookvale, Australia); plasma HRP2 concentrations > 0.75 ng/mL were considered to be positive for *P. falciparum* antigen, as per kit instructions.

Outcome variable

The major outcome variable was the change in plasma zinc concentration following three weeks of zinc supplementation to determine whether the response to treatment was modified by initial markers of intestinal function. A detailed description of the overall effect of the intervention on plasma zinc concentration has been published elsewhere (30).

Sample size calculations and data analysis

Sample size calculations were based on the primary outcome of change in plasma zinc concentration following different forms of zinc supplementation. To detect supplementation-related differences having an effect size of 0.40 SD, using three-way group-wise comparisons, a sample size of 150 participants per group was necessary ($\alpha = 0.05$, $\beta = 0.20$, 20% estimated attrition). The expected final sample size (N = 122/group) was estimated to be sufficient to allow detection of a correlation (r) of ~0.23 between baseline urinary L:M recovery ratio and the change in plasma zinc concentration, within each study group. Since plasma Zn responses did not differ for the two forms of Zn supplement (P = 0.78) (30), the two Zn supplemented groups were combined for the purposes of the subsequent analyses. Plasma citrulline concentrations were analyzed in a sub-set of 80 children (N = 32–48/group) with CRP concentrations < 10mg/L. This sample size was estimated to be sufficient to allow detection of a correlation (r) of ~0.4 – 0.45 between citrulline and plasma zinc concentrations, within each study group.

Urinary L:M recovery ratios were calculated as the ratio of the percentage recoveries of the oral doses of each test sugar. To facilitate comparisons with other published data, we also calculated the ratios of the concentrations of lactulose:mannitol in the urine, henceforth referred to as urinary L:M concentration ratios. Percent recoveries of lactulose and mannitol, and the ratios of the concentrations of mannitol:creatinine (M:C) and lactulose:creatinine (L:C) in the urine (mg:mg) were also calculated to establish the type of intestinal alteration. Children who did not urinate following the first 120 min of the test (N = 17) were excluded from the analyses because children in this subgroup had extremely low percent recoveries of lactulose. Given the lack of consensus regarding reference cut-off values to distinguish between normal and abnormal intestinal mucosal function, altered (increased) intestinal permeability was defined on the basis of urinary L:M recovery ratios of either ≥ 0.07 (37) or ≥ 0.03 (equivalent to urinary L:M concentration ratio ≥ 0.28 or ≥ 0.12, respectively) (38); a greater L:M ratio is indicative of impaired gut function. Plasma citrulline concentrations below 14 μmol/L were considered to be low for children 6–23 mo (39).

Descriptive statistics were calculated for all variables; variables not normally distributed (Shapiro-Wilk statistic, W < 0.97) were arithmetically transformed prior to subsequent analysis. Relationships between baseline characteristics and markers of intestinal mucosal function (percent recovery of lactulose or mannitol, urinary L:M recovery ratio, urinary L:C

or M:C concentration ratios, or plasma citrulline concentration) were compared within and between treatment groups. Continuous variables were compared by linear regression, analysis of variance (ANOVA), the non-parametric Kruskal-Wallis test and t-tests. Categorical variables were compared by chi-square test or Fisher's exact test when the expected number in any cell was ≤ 5. Analysis of covariance (ANCOVA) was used to assess the relationships between markers of intestinal mucosal function and baseline plasma zinc concentration, as well as the effects of subject characteristics and supplementation group on change in plasma citrulline concentration, controlling for covariates (age, sex, elevated acute phase proteins, and positive HRP2 status) as appropriate. The effects of intestinal mucosal function on changes in plasma zinc concentrations following the intervention were assessed by ANCOVA, with baseline intestinal mucosal function (urinary L:M recovery ratio, L:C or M:C concentration ratio, or plasma citrulline concentration) as the main effect and controlling for covariates (supplementation group, age, initial plasma zinc concentration, anthropometric measurements, methodological factors related to blood collection such as time of day and time since last breastfeed, elevated acute phase proteins and HRP2, and morbidity) as appropriate. Means were compared post-hoc using least-square means with the Tukey-Kramer adjustment. The covariates which remained significant in the ANCOVA models were used to calculate adjusted within-group partial correlation coefficients.

All statistical analyses were completed using SAS System for Windows release 9.3 (SAS Institute, Cary, North Carolina). A P value < 0.05 was considered statistically significant. Data are presented as medians (IQR) or means ± SD for all analyses except ANCOVA, which are presented as means ± SEM, unless otherwise noted.

RESULTS

Of 451 participants enrolled in the main zinc supplementation study, the L:M test was completed successfully in 282 (63%) Predominant reasons for unsuccessful tests were incomplete consumption of test sugars (N = 70, 16%) and leakage or fecal contamination of urine samples (N = 67, 15%) (Figure 1). There were no differences between treatment groups at baseline with respect to the children's sex, age, socio-economic indicators, and anthropometric and biochemical indicators of nutritional status, except that a higher percentage of children in the placebo group had elevated HRP2 concentrations than those in the Zn group (58.2% vs. 42.1%; P = 0.012) (Table 1).

The baseline values for urinary recovery of lactulose, mannitol, and their ratios, and for plasma citrulline concentrations, are shown in Table 2; these were not different between treatment groups. Depending on the cut-off applied (urinary L:M recovery ratio ≥ 0.07 or ≥ 0.03), the prevalence of altered (increased) intestinal permeability was 25.5% and 75.5%, respectively, and 70% of children were identified as having low plasma citrulline concentrations. Urinary L:M recovery ratios and plasma citrulline concentrations at baseline, when evaluated as either continuous or categorical variables, were not significantly associated with sex, anthropometric indicators or socio-economic status. Citrulline concentrations, but not L:M recovery ratios, were positively associated with age in months (P = 0.020). Urinary L:M recovery ratios were positively correlated with urinary L:C concentration ratios (r = 0.65; P < 0.001) and negatively correlated with urinary M:C

concentration ratios (r = −0.42; P < 0.001). Urinary L:M recovery ratios and urinary L:C and M:C concentration ratios were not correlated with plasma citrulline concentrations (L:M ratio, r = −0.16, P = 0.20; N = 68).

In the subset of 282 children with complete L:M results, the overall mean unadjusted plasma Zn concentration at baseline was 62.9 ± 11.9 μg/dL. Baseline plasma zinc concentrations were not significantly associated with baseline urinary L:M recovery ratios or plasma citrulline concentrations (P = 0.17, P = 0.84; respectively). The mean change in plasma Zn concentrations was significantly greater among children who received Zn supplements compared with children who received the placebo supplement (15.6 ± 13.3 μg/dL vs. 0.02 ± 10.9 μg/dL; P < 0.0001). Several child characteristics and study methodological factors were significantly associated with the change in plasma Zn concentrations, independent of treatment group. Specifically, initial plasma Zn concentration, child age and HAZ, change in WAZ, and final AGP ≥ 1 g/L, HRP2 ≥ 0.75 ng/mL, and time of day, were negatively related to change in plasma Zn concentration; baseline AGP ≥ 1 g/L and elapsed time since last breast-feed (final) were positively related to change in plasma Zn concentration (Supplemental Table 1). Controlling for the aforementioned factors, baseline urinary L:M recovery ratio was negatively associated with the change in plasma Zn concentration (P = 0.014), and was independent of supplementation group (P for interaction = 0.26). For every 50% increase in the urinary L:M recovery ratio, the change in plasma Zn concentration was 1.1 μg/dL less (Figure 2). There was no significant relationship between percent recoveries of lactulose and mannitol, urinary L:C or M:C concentration ratios, or baseline plasma citrulline concentration, and change in plasma Zn concentration. Median plasma citrulline concentrations did not change from baseline following short-term zinc or placebo supplementation (0.4 (−2.2 – 2.6) μmol/L) and did not differ by supplementation group (P = 0.48); change in plasma citrulline concentration was not correlated with change in plasma zinc concentration (P = 0.11). Controlling for baseline plasma citrulline concentrations, change in plasma citrulline concentration was significantly associated with change in weight-for-age Z score over the course of the 3 week intervention period, independent of study group (r = 0.34, P = 0.0035) (Figure 3).

DISCUSSION

The results of the present study indicate that both altered intestinal permeability, as measured by elevated urinary L:M recovery ratios and/or low plasma citrulline concentrations, and low plasma zinc concentrations, are prevalent among Burkinabe children 6–23 months of age. Short-term zinc supplementation significantly increased plasma zinc concentrations among children who received supplements containing 5 mg zinc daily for 21 d in comparison to those who received placebo supplements, with an overall effect size of 1.25 SD (95% CI, 0.98–1.52) (30). Children with more severe alterations in intestinal permeability at baseline, as measured by an increased urinary L:M recovery ratio, had significantly smaller increases in mean plasma zinc concentrations than children with more normal intestinal permeability; this relationship did not differ between children who received either zinc or placebo supplements. However, given the large effect of zinc supplementation on change in plasma zinc concentration, zinc supplemented children with altered intestinal permeability still experienced a significant increase in plasma zinc

concentration from baseline compared to the placebo. The highly standardized and rigorous implementation of the L:M test, collection and processing of plasma zinc samples and large sample size lend strength to these findings.

The use of indirect methods to assess intestinal mucosal function (L:M intestinal permeability and plasma citrulline concentration) and zinc absorption (change in plasma zinc concentration), is a limitation of this study. However, results of dual-sugar permeability assays have been shown to correlate with abnormal histological findings of villous atrophy and increased mucosal and intraepithelial lymphocyte densities in Gambian children (3, 40), and plasma citrulline has recently been validated as a biomarker of enterocyte dysfunction in patients with various disease states (5). Altered intestinal mucosal function in this population, as diagnosed with the L:M intestinal permeability test or plasma citrulline concentration, is likely related to subclinical EE given that other underlying diseases potentially responsible for these changes would be expected to be fairly uncommon in a population-based study. Although plasma zinc concentration is generally homeostatically maintained, it responds rapidly to zinc supplementation (41), and controlled studies of dietary zinc depletion and repletion have found a strong correlation between plasma zinc concentration and changes in whole-body zinc (42). Therefore, following acute changes in zinc intake, such as occurs during supplementation interventions, the change in plasma zinc concentration may be used as an indicator of zinc absorption, when controlling for confounding factors (e.g. infection, time of day, fasting status).

We found a high prevalence of altered intestinal function in this population; estimates based on urinary L:M recovery ratios were 25.5% and 75.5% depending on the cutoff applied (≥ 0.07 and ≥ 0.03, respectively); these results are comparable to the prevalence reported among asymptomatic infants and young children in other developing countries (8–11, 13), although prevalence > 90% have been observed in some studies (3, 8, 12). Altered intestinal permeability in the present study (increased urinary L:M recovery ratio) was likely attributable to both a decreased absorption of mannitol (6.5% in present study compared with up to 20% in healthy individuals) and an increased absorption of lactulose (0.30% in present study compared with < 0.25% in healthy individuals) (3). Mean plasma citrulline concentrations were generally low, compared to published norms among healthy Canadian infants and children undergoing elective surgery (39). These data suggest a high prevalence of EE in our population. In contrast to the results of most previous studies on intestinal permeability status in infants and young children in developing countries, this study found no consistent associations between urinary L:M recovery ratios and age (3, 4, 9, 11, 40) or anthropometric status (3, 9, 11, 12). Although subclinical enteropathy is associated with mucosal inflammation, intestinal permeability was not associated with systemic inflammation, as measured by elevated acute phase proteins (AGP and CRP) in the present study, consistent with results of some (9, 43), but not all, previous studies (28, 43). This lack of association may be due to the numerous different causes of infection in this region, including a high prevalence of malaria (47.5% of children had HRP2 concentrations > 0.75 ng/ml). However, even among children with no evidence of recent or current malaria parasitemia, elevated AGP and CRP were not associated with intestinal permeability.

In the present study, baseline plasma zinc concentration and altered intestinal permeability were not significantly related; this is consistent with the findings of previous studies of intestinal L:M permeability conducted among asymptomatic children in developing countries (10, 11, 24). However, children with more severe alterations in intestinal mucosal function at baseline, as assessed by urinary L:M recovery ratios, had significantly smaller increases in plasma zinc concentration post-supplementation. For example, in the zinc supplemented group, children with urinary L:M recovery ratios in the 10^{th} and 90^{th} percentiles (urinary L:M recovery ratios = 0.02 and 0.10, respectively) would be expected to have a change of 17.0 μg/dL and 14.5 μg/dL in plasma zinc concentration, respectively. In the placebo group, expected change in plasma zinc concentration would be 2.6 μg/dL and −2.4 μg/dL, respectively. The fact that the association between baseline intestinal permeability and change in plasma zinc concentration occurred in both the zinc supplemented and the placebo groups suggests that this phenomenon was not modified by supplemental zinc intake, but may be reflective of general processes involving zinc absorption and homeostasis or indicate the involvement of additional intervening variables. Impaired intestinal mucosal function may increase the risk of zinc deficiency in infants and young children in developing countries due to reduced absorption of dietary zinc, increased excretion of endogenous zinc, or reduced re-absorptive capacity in the distal bowel for endogenously secreted zinc. Alternatively, zinc deficiency could exacerbate alterations in intestinal permeability, as zinc is essential for normal enterocyte proliferation and differentiation, maintaining tight junction barrier function, regulating ion transport and controlling the effects of oxidative stress (44). It is possible that the zinc supplementation itself could have modified the relationship between urinary L:M recovery ratios and zinc uptake, as previous studies have shown zinc supplementation to improve intestinal permeability (25–27). However, there was no evidence for this in the present short-term study, as indicated by the lack of significant group-wise differences in the correlation coefficients for the relationship between urinary L:M recovery ratios and change in plasma zinc concentration. In addition, change in plasma citrulline concentration following supplementation, perhaps indicative of an increase in enterocyte mass and absorptive function, did not differ between groups. Therefore, it appears that the current results reflect the impact of baseline intestinal permeability status on change in plasma zinc concentration.

The results of the present study are interesting in light of recent stable isotope tracer studies which have demonstrated possible associations between intestinal mucosal function and zinc absorption and homeostasis, although the studies have yielded conflicting results. In a recent study of 25 Malawian children 2–5 y of age, urinary L:M concentration ratios were found to be positively correlated with endogenous fecal zinc excretion, indicating a potential association between altered intestinal permeability and perturbed zinc homeostasis (10). In pediatric patients with celiac disease, those with impaired gut function, as assessed by a ^{13}C-sucrose breath test, were more likely to have impaired zinc absorption (reduced fractional absorption of zinc) and, the authors concluded, potentially lower zinc status (29). However, in a study conducted among vitamin A depleted women of child-bearing age in Bangladesh, those with altered intestinal permeability, due mainly to increased lactulose absorption, had higher fractional and total absorption of zinc than women with normal intestinal permeability, suggesting increased zinc absorption with impaired intestinal barrier function

(28). Endogenous zinc losses were not measured in that study, so the total effect on zinc balance could not be determined. Additional studies using stable isotope tracer studies and kinetic modeling to determine internal zinc pool mass and flux among different metabolic compartments are necessary to clarify relationships between intestinal permeability and zinc status.

Urinary L:M recovery ratios and plasma citrulline concentrations were not significantly related in this study, suggesting that mucosal mass (as measured by citrulline) may not necessarily correlate with intestinal permeability (as measured by L:M). This is consistent with a small study of chemotherapy induced mucosal barrier injury in children, as well as a study of HIV-associated villous atrophy, both of which found no correlation between plasma citrulline concentrations and lactulose:rhamnose ratios (45, 46). In addition, urinary M:C concentration ratios and plasma citrulline concentrations were not related, even though both are generally thought to be reflective of the absorptive surface area of the small intestine, and enterocyte mass. Thus, it is possible that L:M tests and plasma citrulline concentrations are measuring two different phenomena, or that mucosal alternations identified by changes in the recovery of urinary lactulose and mannitol are more subtle than those that would be required to cause changes in plasma citrulline concentrations. Further studies of the relationship between plasma citrulline, as an amino acid marker of mucosal mass, and other measures of gastrointestinal function and nutritional status are warranted.

Change in plasma zinc concentrations, within- and between- supplementation groups, were not related to plasma citrulline concentrations at baseline, or to the change in plasma citrulline concentrations post-supplementation. This indicates that the change in plasma zinc concentrations may not be causally related to enterocyte mass, or may only be related when enterocyte losses are more severe than those detected in this population. Independent of zinc supplementation group, weight gain (increase in WAZ) over the 21 d intervention period was associated with an increase in plasma citrulline concentration in the present study. The direction of causality in this relationship cannot be established; it is possible that an increase in enterocyte mass, and thus absorptive function, may lead to an increased rate of weight gain. Alternatively, an increased rate of weight gain may lead to an increased rate of generation of mucosal mass.

In conclusion, subclinical environmental enteropathy is common among infants and young children in this rural setting in Sub-Saharan Africa, as has been observed in other low income countries, and may contribute to the risk of zinc deficiency. The magnitude of this contribution may be small, but could be important for individuals with marginal zinc status or dietary adequacy. Nevertheless, zinc supplementation efficaciously increases plasma zinc concentration despite the evidence of impaired intestinal mucosal function. The extent to which subclinical enteropathy would affect the efficacy of zinc fortification interventions, in which change in plasma zinc concentrations are likely to be less, warrants further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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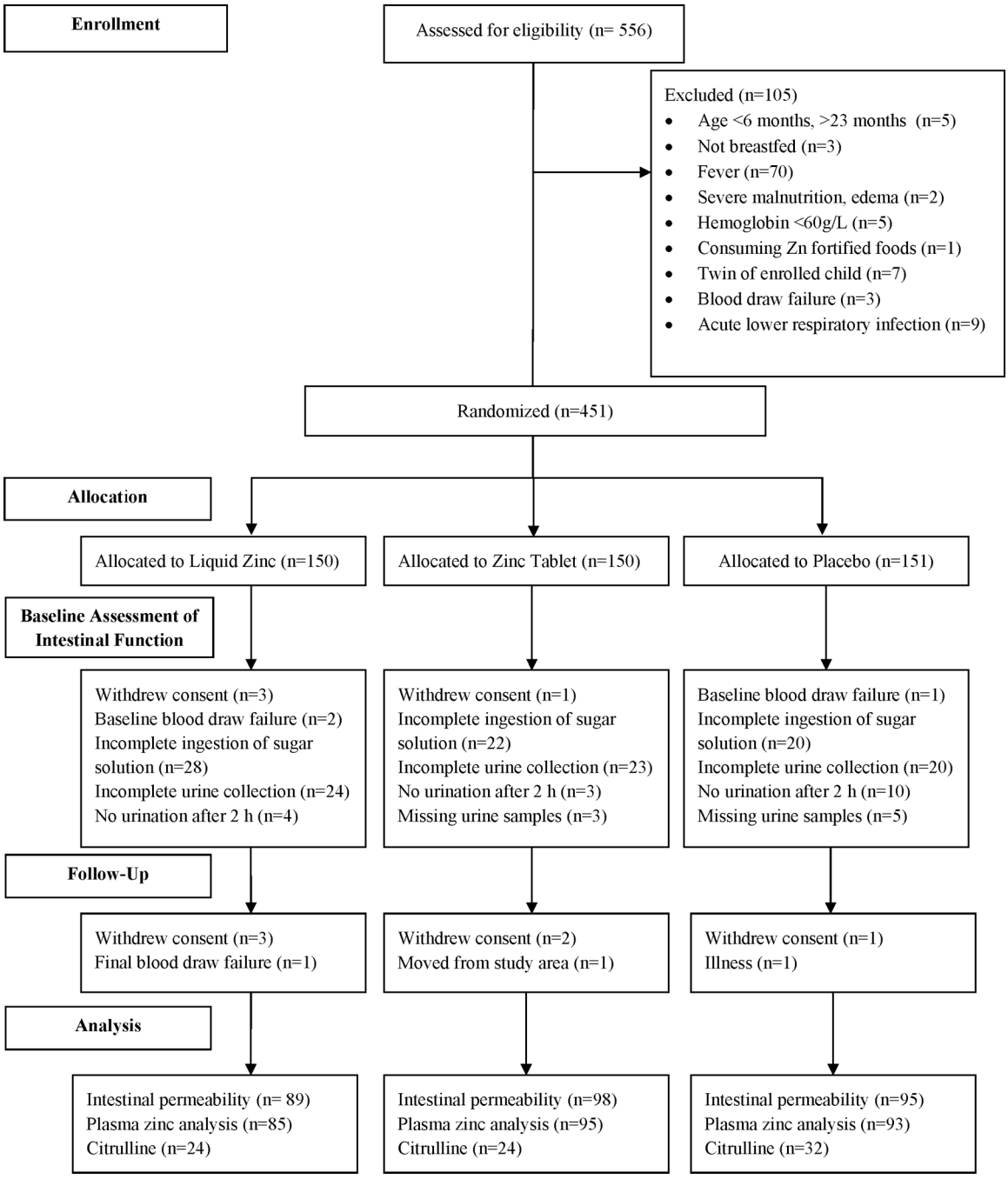

Figure 1.
Consort flow diagram detailing the number of children who were enrolled and completed the study, by treatment group.

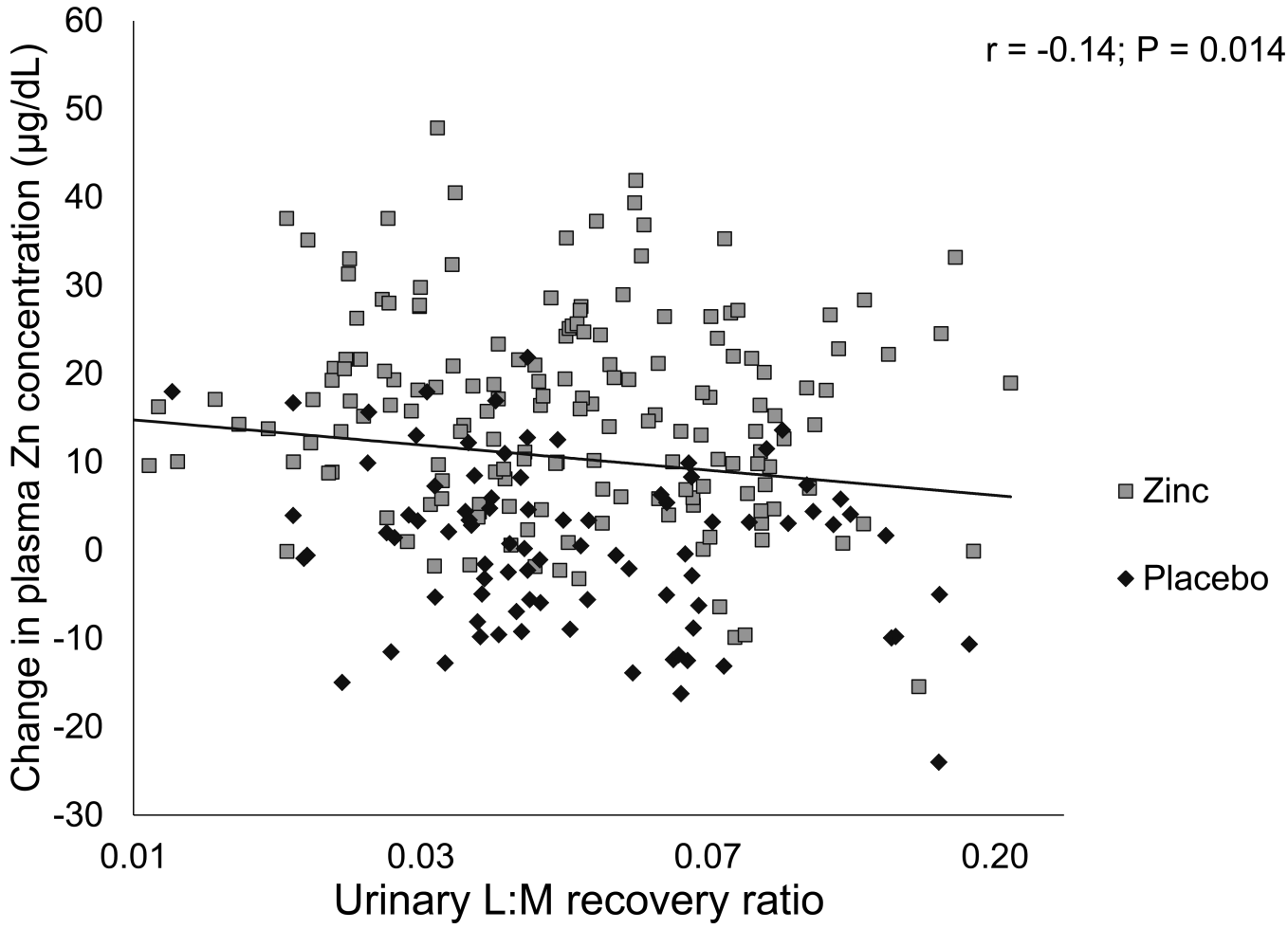

Figure 2.

Relationship between change in plasma zinc concentration from baseline to post-supplementation and urinary L:M recovery ratio (natural log scale), by study group. The partial correlation coefficient is adjusted for differences in mean change in plasma zinc concentrations among groups, initial plasma zinc concentration, child age, height-for-age Z score, change in weight-for-age Z score (baseline to final), baseline and final AGP ≥ 1 g/L, final HRP2 > 0.75 ng/mL, time of day (final) and elapsed time since last breastfeed (final). Urinary L:M recovery ratio and change in plasma zinc concentration were correlated (r = −0.14, P = 0.014); the relationship did not differ between study groups (ANCOVA, P for interaction = 0.26). Number of participants by study group: Zn, N = 168, Placebo, N= 86.

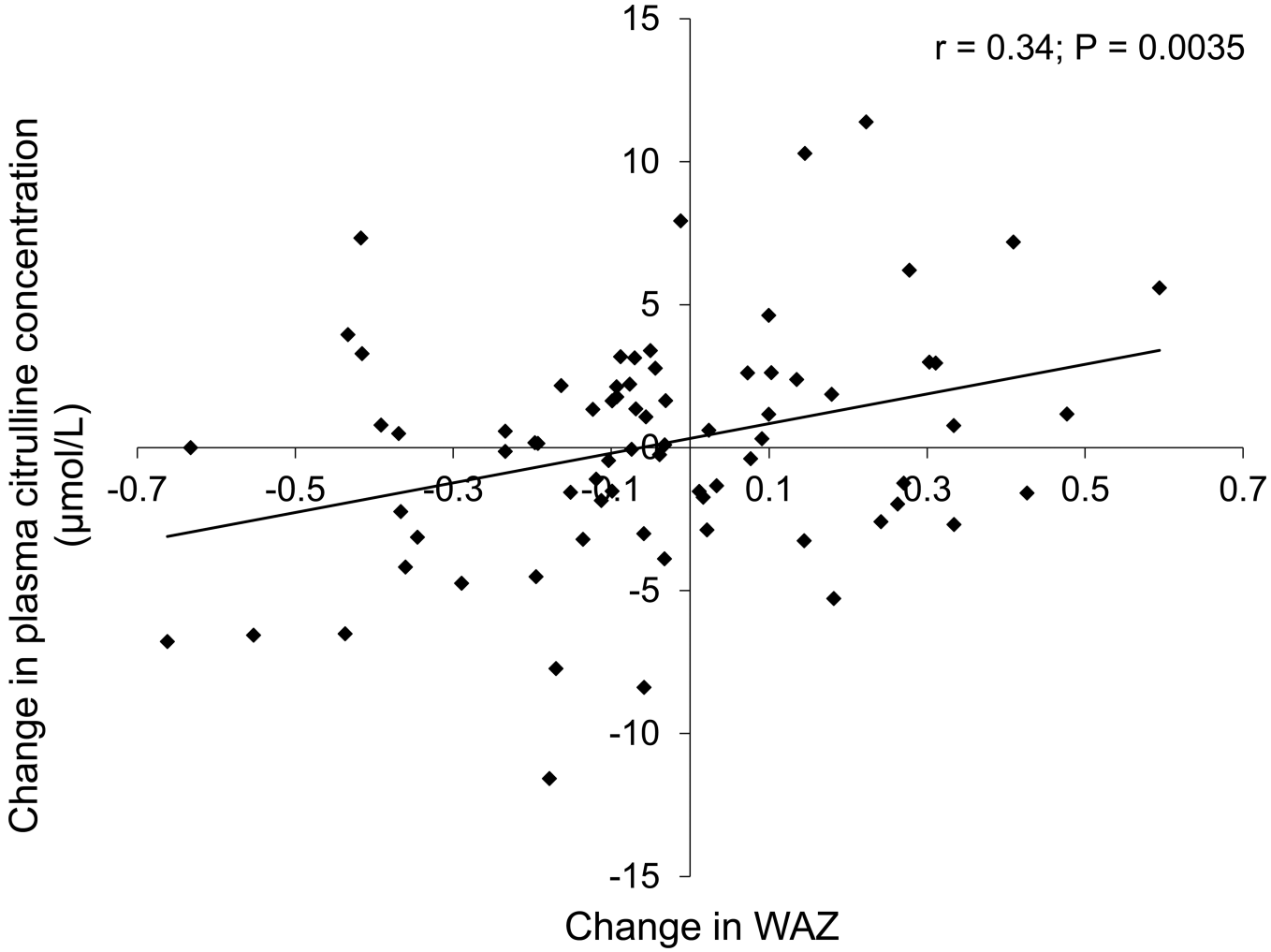

Figure 3.

Relationship between change in plasma citrulline concentration and change in weight-for-age Z score from baseline to post- supplementation. The partial correlation coefficient is adjusted for initial plasma citrulline concentration (r = 0.34, P=0.0035). N=75.

Table 1

Initial characteristics of study participants and their households

	All children (N=282)*
Male, n (%)	159 (56.4)
Age, mo†	14.6 ± 5.2
Socio-economic indicators	
Paternal education, n (%) completing primary school	79 (28.1)
Maternal education, n (%) completing primary school	32 (11.4)
Source of water for household, n (%)	
Surface	29 (10.3)
Well	152 (54.1)
Hand pump	100 (35.6)
Electricity, n (%)	49 (17.4)
Latrine facilities, n (%)	185 (65.8)
Anthropometric Measurements	
Length, cm	73.4 ± 5.5
Weight, kg	8.4 ± 1.5
LAZ	−1.5 ± 1.2
WAZ	−1.4 ± 1.1
WLZ	−0.8 ± 1.0
Biochemical Assessments	
Hb concentration, g/L	89.1 ± 14.9
Hb < 110 g/L, n (%)	253 (91.3)
AGP ≥1 g/L, n (%)	161 (57.5)
CRP ≥10 mg/L, n (%)	60 (21.4)
HRP2 > 0.75 ng/mL, n (%)	130 (47.5)
Initial plasma Zn concentration, μg/dL	62.9 ± 11.9
Initial plasma Zn < 65 μg/dL, n (%)	179 (63.7)

LAZ = length-for-age Z score; WAZ = weight-for-age *Z* score; WLZ = weight-for-length *Z* score; Hb = hemoglobin; AGP = α-1-acid-glycoprotein; CRP = C-reactive protein; HRP2 = histidine-rich protein II

*There were no statistically significant differences by treatment group among the children participating in the study for any of the variables examined, with the exception of HRP2 > 0.75 ng/mL (Zn: N=77/183, 42.1%; Placebo: N=53/91, 58.2%; P = 0.012). χ^2 or analysis of variance followed by Tukey multiple pairwise comparisons.

†Data are mean ± SD, unless otherwise indicated.

Table 2

Indicators of intestinal function among participants in the study at baseline

	All subjects N = 282*
Urinary lactulose recovery (% of oral dose)†	0.30 (0.19, 0.44)
Urinary mannitol recovery (% of oral dose)	6.5 (4.5, 9.1)
Urinary L:M recovery ratio‡	0.042 (0.030, 0.072)
Elevated urinary L:M recovery ratio (≥ 0.07), n (%)	72 (25.5)
Urinary L:M concentration ratio‡	0.17 (0.12, 0.29)
Elevated urinary L:M concentration ratio (≥ 0.12), n (%)	213 (75.5)
Urinary L:C concentration ratio§	0.68 (0.46, 1.05)
Urinary M:C concentration ratio§	3.6 (2.7, 5.2)
Plasma citrulline concentration (μmol/L)//	11.4 (9.0, 15.6)
Low plasma citrulline concentration, n (%)//¶	56 (70.0)

L:M = lactulose:mannitol; L:C = lactulose:creatinine; M:C = mannitol:creatinine

*There were no statistically significant differences by treatment group among the children participating in the study for any of the variables examined. (χ^2 or analysis of variance followed by Tukey multiple pairwise comparisons).

†Data are median (IQR) unless otherwise noted.

‡Urinary L:M recovery ratio (% of oral dose) is the ratio of the percentage recoveries of the oral doses of each test sugar.Urinary L:M concentration ratio (mg: mg) is the ratio of test sugars (mg) recovered in the urine

§Urinary L:C and M:C concentration ratios are calculated as the ratio in mg of test sugar:creatinine in the urine; N = 279

//N=80

¶Low citrulline concentration is defined as a citrulline concentration < 14 μmol/L.