Plasma Zinc Concentration Increases within 2 Weeks in Healthy Senegalese Men Given Liquid Supplemental Zinc, but Not Zinc-Fortified Wheat Bread

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Abstract
The responsiveness of plasma zinc concentration to zinc fortification is uncertain. Our objective in this study was to determine whether plasma zinc concentration changes in response to consuming zinc-fortified foods or liquid zinc supplements. We conducted a 4-wk double-blind, randomized trial among 132 healthy Senegalese men aged 18 yr. Participants received 1 of 4 interventions: (1) control, 200 g/d of wheat bread fortified with iron and folic acid, but not zinc, and a liquid multivitamin supplement without zinc between meals; (2) zinc supplement, the same bread and the same multivitamin supplement with 15 mg zinc as ZnSO_4 added; (3) moderate zinc fortification, the same bread fortified with 7.5 mg zinc as ZnO and the same multivitamin supplement without zinc; or (4) high zinc fortification, the same bread fortified with 15 mg zinc as ZnO and the same multivitamin supplement without zinc. Fasting blood samples were collected twice at baseline and at d 15 and 29 of the intervention. There was no significant interaction between group and study day (P = 0.11). However, at d15, the mean change in plasma zinc concentration in the zinc-supplemented group was greater than in the placebo and fortification groups (0.72 μmol/L vs. −0.09 to 0.03 μmol/L; P = 0.05). At d 29 there were no significant group-wise differences. Across all time points, the zinc-supplemented group was the only group where plasma zinc concentration increased from baseline (P = 0.006). These results suggest that plasma zinc concentration may not be a sufficiently sensitive indicator to evaluate short-term responses to zinc fortification. J. Nutr. 141: 1369–1374, 2011.

Introduction
Zinc is an essential micronutrient, which is required for normal growth, immune function, neuro-behavioral development, and pregnancy outcomes (1,2). Despite the biological importance of zinc, sensitive and specific biochemical indicators of individual zinc status are lacking. For population-based assessments, several international agencies and expert groups have proposed that the concentration of zinc in blood plasma (or serum) is the best available biomarker to assess a population’s risk of zinc deficiency (1–3). Likewise, 2 recent reviews concluded that plasma zinc concentration is a useful biomarker of a population’s response to zinc supplementation (4) and plasma zinc concentration responds to both zinc depletion and repletion in adult volunteers (3). Moreover, a recently completed study in U.S. men found that plasma zinc concentration responded within just a few days to both the initiation and discontinuation of zinc supplementation (5).

Less information is available with regard to the response of plasma zinc concentration following zinc fortification interventions, but several studies found no impact on plasma zinc concentration (6,7). Because of the limited number of available studies, it is not possible to determine whether the observed differences between responses to supplementation and fortification in previous studies were related to age, the presence of infection, the presence of other micronutrient deficiencies, the types of zinc-fortified products that were provided, the level of zinc fortification, or other factors. In addition, technical factors surrounding the collection and analysis of blood samples may have been responsible for some of the inconsistent responses that were observed. Thus, additional information is needed to de-
termine whether plasma zinc concentration is a useful biomarker to assess population responses to zinc fortification programs. The objective of the present study was to determine whether plasma zinc concentration of adult males changed in response to additional zinc consumption when provided as either zinc-fortified wheat bread products or liquid zinc supplements.

**Participants and Methods**

**Experimental design and study site.** The study was designed as a double-blind, randomized, clinical trial. The clinical phase of the project was carried out from August 2009 to December 2009 in a community clinic based in a low-income urban neighborhood in Dakar, Senegal. Eligible participants were randomly assigned to 1 of 4 dietary treatment groups using a computer-generated block randomization scheme with a block length of 4 (8). Study participants reported to a feeding center 6 d/wk for 4 consecutive wk. Fasting venous blood samples were drawn on 2 occasions within 1 wk prior to the start of the 4-wk intervention and 15 and 29 d after initiating the assigned dietary treatment. The rationale for collecting 2 blood samples prior to the intervention was to account for normal day-to-day variation in plasma zinc concentrations (9). The mean of the preintervention blood draws (controlling for potential confounding factors) served as the baseline plasma zinc concentration for subsequent analyses.

**Participants.** Apparently healthy males ≥ 18 y were invited to participate in the study. Participants were recruited by word of mouth from the catchment neighborhoods surrounding the clinic. The rationale for choosing adult males was to assess the impact of zinc fortification among individuals who could consume relatively large amounts of zinc-fortified bread and to avoid possible female hormone-related fluctuations in plasma zinc concentrations throughout the month (1,10,11). Individuals who satisfied the following inclusion criteria were identified during the preliminary screening sessions: hemoglobin concentrations > 10.0 g/L; no history of chronic illnesses, no acute illnesses or medication use for 2 wk preceding the intervention, no use of vitamin or mineral supplements, and no consumption of commercially available zinc-fortified foods. All men attending the screening sessions received a single 400-mg dose of the antihistamine drug albuterol. All participants provided their written informed consent to participate in the study. The Institutional Review Boards at the University of California, Davis and the University Cheikh Anta Diop, Dakar, Senegal and the human participants review committee of the Senegalese Ministry of Health approved the study protocol.

**Interventions.** The 4 treatment groups and the specific interventions were as follows: 1) negative control group (control) received 200 g/d of wheat bread fortified with iron and folic acid, but not zinc, and a liquid multivitamin supplement without zinc between meals; 2) zinc supplementation group (SZn15) received the same bread product and the same liquid multivitamin supplement between meals with 15 mg zinc as zinc sulfate added to the liquid supplement; 3) moderate zinc fortification group (FZn7.5) received the same bread product fortified with 7.5 mg zinc as zinc oxide per 200-g serving and the same liquid multivitamin supplement without zinc between meals; or 4) high zinc fortification group (FZn15) received the same bread product cofortified with 15 mg zinc as zinc oxide per 200-g serving and the same liquid multivitamin supplement without zinc between meals. The bread products were prepared following a Senegalese recipe for baguettes using 55% extraction wheat flour (595 g/kg dough), water (381 g/kg dough), salt (12 g/kg dough), yeast (9 g/kg dough), and a baking enzyme additive (3 g/kg dough). In accordance with current national recommendations in Senegal (12), the wheat flour used in all breads was fortified with 1370 mg iron as ferrous fumarate and 1.5 mg folic acid per kg of flour, corresponding to 1.8 mg iron and 178.5 μg folic acid/200-g serving of bread. The intrinsic zinc and phytate contents per 200-g serving of this bread product were ~1.1 mg and ~60 mg, respectively, which corresponded to an estimated phytate:zinc molar ratio of 5.5. For the FZn7.5 and FZn15 breads, wheat flour was fortified with the same amounts of ferrous fumarate and folic acid and with either 63 or 126 mg zinc as zinc oxide/kg of flour, corresponding to either 7.5 or 15 mg zinc/200-g serving of bread. The estimated phytate:zinc molar ratios for the FZn7.5 and FZn15 breads were 0.7 and 0.3, respectively. A 5% overage was added to account for mineral losses incurred during the fortification process. The acceptability of the bread products was confirmed in a sensory evaluation study among adult Senegalese panelists (13). Samples were analyzed periodically to ensure that fortification levels were appropriate.

The liquid multivitamin supplements were masked for taste and were prepared from a strawberry-flavored syrup concentrate that was diluted with purified water. The liquid vitamin supplements (with or without zinc) provided the following amounts of vitamins/10 mL dose: vitamin B-6, 0.4 mg; vitamin B-12, 0.8 mg; biotin, 9.9 μg; vitamin C, 29.7 mg; niacin, 5.3 mg; riboflavin, 0.4 mg; pantothenic acid, 1.7 mg; and thiamin, 0.4 mg. The supplement with added zinc provided 15 mg zinc as zinc sulfate monohydrate/10 mL dose. The mineral contents of the supplements were verified by an independent laboratory.

Participants consumed under supervision the respective bread products during a morning meal. Breads were served with fruit jam or butter, depending on individual preference, and a non-zinc-containing juice prepared from purified water and fruit juice concentrate. Research staff gave the liquid vitamin (or vitamin + zinc) supplement to participants after a 90-min fast following the meal. Participants were allowed to leave the feeding center after consuming the supplements; however, they were requested to fast for an additional 30 min to permit maximum absorption of the supplement. During the course of the study, participants were instructed to continue eating as they normally would during home meals but to avoid any zinc-fortified food products and any vitamin-mineral supplements. Adherence to study protocols was assessed by research staff through interviews with participants during each clinic visit. Non-compliant participants, as well as participants who were absent for >2 consecutive days, were dropped from the study.

**Data collection procedures.** The main outcome of the study was the change in plasma zinc concentration following the start of the intervention. Data were also collected on the plasma concentrations of the acute phase proteins α1-acid-glycoprotein (AGP) and C-reactive protein (CRP), self-reported morbidity, as well as cigarette smoking, because these have been demonstrated to affect the plasma zinc concentration (1). Weight, height, and hemoglobin concentrations were measured during the preliminary screening sessions. Weight was measured to ±0.5 g precision on a frequently calibrated scale (Ohaus Model DP150; Ohaus) and height was measured to ±0.1-cm precision (Seca Model 225; Seca). Hemoglobin concentration in capillary blood was measured with a HemoCue photometer (Hemocyte model 201; Hemocue). During each clinic visit, a detailed history of morbidity during the intervening period was collected, which included any signs or symptoms of illness, such as fever, diarrhea (stool number and consistency), cough, nasal secretions, and respiratory distress. Participant-reported consultations with an external medical doctor were recorded, along with symptoms, diagnosis, and treatment. If a participant reported illness symptoms, temperature was measured and the individual was referred to a primary care physician based at the clinic.

Venous blood specimens were collected from participants after an overnight fast following the International Zinc Nutrition Consultative Group (IZiNCG)-recommended procedures (1). For participants with symptoms of illness on the day of a scheduled intervention blood drawing, the blood draw was postponed until after recovery from the illness. If the illness occurred after the intervention started, feeding was continued and the blood test was postponed until 2 d after recovery from the illness, resulting in a prolongation of the total duration of the study for these participants. The times of the blood draws and prior food intakes were recorded. A tournaire was placed for a standardized

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8 Abbreviations used: AGP, α1-acid-glycoprotein; FZn7.5, moderate zinc fortification group; FZn15, high zinc fortification group; CHORI, Children’s Hospital of Oakland Research Institute; CRP, C-reactive protein; ICP-AES, inductively-coupled plasma atomic emission spectrometry; IZiNCG, International Zinc Nutrition Consultative Group; SZn15, zinc supplementation group.
amount of time (<1 min) prior to the blood draw while participants remained in a seated position. At each blood draw, 7 mL of blood was drawn from the antecubital vein using stainless steel needles (Ref: 367281; BD) and collected into a single trace element-free polyethylene tube containing zinc-free heparin (Ref: 01.1604.400; Sarstedt). The tubes were placed into an insulated cooler over ice immediately following the blood draw and plasma was separated from heparinized blood within 1 h of blood collection by centrifuging at 985 $\times$ g (EBA 20 centrifuge model 2002; Andreas Hettich) for 12 min. Plasma samples were aliquoted into plastic-capped polyethylene tubes (Ref: 2840; Perfection Scientific) on site, transported to the local university laboratory over ice, and stored at $-80^\circ$C. Plasma samples were transported with dry ice to the Children’s Hospital Oakland Research Institute (CHORI) for analysis.

**Biochemical analyses.** Plasma zinc concentrations were determined by inductively-coupled plasma atomic emission spectrometry (ICP-AES) (14) following overnight digestion in 70% nitric acid (OmniTrace; VWR International). Samples were diluted in trace element-free water to 5.5% nitric acid concentration, vortexed for 20 s, and then clarified by centrifugation at 3220 $\times$ g for 10 min at room temperature (5810R centrifuge with swing bucket rotor A-4-81; Eppendorf). Prepared samples were then introduced into the ICP-AES (Vista Pro with SPS5 autosampler; Varian) for determination of zinc along with the reference materials Seronorm Trace Elements Serum L-1 and L-2 (Accurate Chemical and Scientific) and an internal pooled plasma control. Elemental values were calibrated using National Institute of Standards and Technology traceable standards. All samples were run in duplicate and all samples from the same participant were analyzed in the same ICP-AES run. All reagents and materials used during the analyses were either certified trace metal-free or were frequently tested for contamination. AGP and CRP were analyzed in blood plasma using radial immunodiffusion (Kent Labs for AGP and The Binding Site for CRP).

**Hematologic and biochemical reference values.** Anemia was defined as a hemoglobin concentration < 110 g/L (15). Men with plasma AGP concentrations > 1.2 g/L or plasma CRP concentrations > 10 mg/L were defined as having subclinical infection. Low plasma zinc concentrations were defined as <10.7 $\mu$g/dL (<70 $\mu$g/dL), which is the reference value suggested by IZiNGC for fasting adult males at mid-morning (1).

**Sample size and statistical analyses.** We estimated that a total of 30 participants enrolled in each of the 4 groups would be sufficient to permit detection of inter-group differences with an effect size of $-0.9$ based upon a 5% significance level and 80% power. This effect size was selected based on results from a zinc supplementation study our group recently completed using similar doses of zinc provided as supplements to healthy American adult males (5).

Descriptive statistics were used to examine all variables. Baseline characteristics were compared by group using ANOVA for continuous variables and Pearson’s chi-square test for proportions. To assess intra-individual differences in plasma zinc concentration at baseline, paired $t$ tests were used, and Pearson correlation coefficients were computed. Linear mixed model repeated-measures ANCOVA (MIXED procedure) was used to compare changes in plasma zinc among groups from the mean of both baseline blood draws to the end of the study, with adjustment for baseline plasma zinc concentration and other relevant variables. The following classes of variables were evaluated: baseline characteristics (BMI and age), smoking behavior, methodological factors (time of blood draw, elapsed time between last food intake and blood draw, and elapsed time between separation of plasma from whole blood), and presence of elevated acute phase proteins (CRP and AGP). All interactions with group, study day, and group $\times$ study day were tested for significance and nonsignificant terms were removed following a stepwise procedure. Biochemical data from all participants who commenced the intervention were included in the data analyses. The Bonferroni post hoc multiple comparisons test was used if significant differences between group means were present. The level of significance for all tests was set at $P < 0.05$. Statistical analyses were performed using SPSS software (version 18; SPSS Institute). Group assignments remained masked until all biochemical and statistical analyses were completed. Values in the text are mean $\pm$ SD for unadjusted means and SE for adjusted means unless otherwise noted.

**Results**

**Accuracy and precision of laboratory tests.** Reference materials were analyzed with each ICP-AES run and were within acceptable ranges according to the manufacturer’s specifications. The CV for inter-assay precision of zinc concentration for Seronorm Trace Elements Serum L-1 and L-2 were 3.2 and 7.9%, respectively, over 11 analytical runs, and the CV for inter-assay precision for zinc concentration in the pooled plasma samples was 7.9%. The CV of intra-assay precision for zinc concentration was 7.9% ($n = 10$ in 1 run).

**Study profile.** Of the 160 adults originally screened, 144 participants were enrolled and 129 (90% of those enrolled) completed the study (Supplemental Fig. 1). We defined dropouts as those 15 men who were assigned to study groups (control: 6; SZn15: 2; FZn7.5: 3; FZn15: 4) but failed to complete all study protocols. Three of the 15 dropouts completed the d-15 blood draw (FZn7.5: 2; FZn15: 1). Data from these participants were included in subsequent analyses. There were no significant group-wise differences at baseline for age, BMI, or initial plasma zinc concentration among those who did or did not complete the study.

**Baseline characteristics.** All men assigned to study groups were included in the analysis of baseline characteristics (Table 1). There were no significant group-wise differences for age, anthropometric indices, or level of education; however, there was a smaller proportion of smokers in the SZn15 group compared with the other intervention groups ($P = 0.007$).

Plasma samples for preintervention zinc concentrations were collected on 2 occasions 1-2 d apart. Both measures were normally distributed, significantly correlated ($r = 0.61$; $P < 0.001$ (Fig. 1)), and not significantly different from one another (paired $t$ test, $P = 0.99$). The within-subject percent difference in baseline plasma zinc concentration was (mean $\pm$ SD) 9.5 $\pm$ 8.0% ($n = 144$). The within- and between-subject SD for the baseline plasma zinc measurements were 0.9 and 1.5 $\mu$mol/L, respectively. There were no significant group-wise differences among the mean baseline plasma zinc concentrations (Table 1), which ranged from $-9.7$ to 10.3 $\mu$mol/L among treatment groups (overall baseline mean 9.9 $\mu$mol/L). Overall, $\sim$72% of participants had plasma zinc concentrations that were below the cutoff of 10.7 $\mu$mol/L (70 $\mu$g/dL) suggested by IZiNGC (1).

**Factors associated with plasma zinc concentration.** The baseline plasma zinc concentrations were not related to age ($P = 0.18$), BMI ($P = 0.75$), or smoking status ($P = 0.21$). Blood samples were drawn at 0830 $\pm$ 43 min, which was 11:34 h $\pm$ 58 min since the last meal. Whole blood was centrifuged within 18 $\pm$ 17 min from the time of blood draw and plasma was separated from heparinized blood within 18 $\pm$ 5 min from the time of centrifuge. A sample from 1 blood draw was removed from consideration, because the participant was not in a fasted state at the time of the blood draw. Of these methodological variables, only the elapsed time since the last meal was correlated with the plasma zinc concentration ($r = 0.096$; $P = 0.025$) and was used as a covariate in subsequent analyses. Specifically, the plasma zinc concentration increased by $\sim$0.15 $\mu$mol/L with each additional hour of fasting between 8 and 19 h.

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There were no effects of self-reported morbidity or medication usage on plasma zinc concentrations (data not shown). However, baseline samples with elevated CRP concentrations (\(n=17\)) had plasma zinc concentrations that were 0.6 \(\mu\)mol/L lower compared with samples with normal CRP concentrations (9.3 ± 1.3 vs. 10.0 ± 1.3 \(\mu\)mol/L; \(P=0.06\)). The baseline plasma zinc concentrations did not differ for samples with normal or elevated AGP concentrations (\(n=45\)); however, we decided a priori that AGP would be included as a covariate in subsequent analyses. When controlling for both CRP and AGP concentrations, elevated CRP remained significant in the final ANCOVA model (data not shown).

**Effects of the intervention.** Compliance for the 129 participants who completed the study was 100%. Controlling for baseline plasma zinc concentration and relevant covariates, as described in “Methods,” there was no significant interaction between study group and study day when expressed as a categorical variable.

| Treatment group | Control | Szn15 | Fzn7.5 | Fzn15 | \(P\) | All participants |
|-----------------|---------|-------|--------|-------|------|------------------|
| \(n\)            | 39      | 34    | 36     | 35    |      | 144              |
| Age, y           | 25.2 ± 6.6 | 24.7 ± 8.3 | 25.0 ± 6.7 | 24.3 ± 5.5 | 0.94 | 24.8 ± 6.8      |
| Weight, kg       | 67.5 ± 11.0 | 65.3 ± 8.4 | 66.8 ± 8.4 | 66.2 ± 9.1  | 0.79 | 66.5 ± 9.3      |
| Height, m        | 1.78 ± 0.06 | 1.80 ± 0.08 | 1.78 ± 0.07 | 1.78 ± 0.07 | 0.71 | 1.78 ± 0.07     |
| BMI, kg/m²       | 21.2 ± 3 | 20.2 ± 2.2 | 21.2 ± 2.4 | 20.9 ± 2.9 | 0.36 | 20.9 ± 2.7      |
| Literate, %      | 84.6    | 85.3   | 86.5   | 85.7   | 0.99 | 85.5             |
| Education, %     |         |        |        |        | 0.81 |                  |
| None             | 2.6     | 0.0    | 2.7    | 5.7    | 2.8  |                  |
| Religious        | 5.1     | 5.9    | 0.0    | 0.0    | 2.8  |                  |
| Primary          | 28.2    | 26.5   | 29.7   | 22.9   | 26.9 |                  |
| Secondary        | 33.3    | 44.1   | 43.2   | 45.7   | 41.4 |                  |
| University       | 30.8    | 23.5   | 24.3   | 25.7   | 26.2 |                  |
| Smoking, %       |         |        |        |        | 0.007|                  |
| None             | 71.9    | 97.1   | 78.4   | 68.6   | 78.6 |                  |
| ≤5 cigarettes/d  | 15.4    | 0.0    | 0.0    | 14.3   | 7.6  |                  |
| >5 cigarettes/d  | 12.8    | 2.9    | 21.6   | 17.1   | 13.8 |                  |
| Hemoglobin, g/L  | 143 ± 17 | 137 ± 16 | 137 ± 17 | 140 ± 19 | 0.45 | 139 ± 17        |
| <110 g/L, %      | 0.0     | 5.9    | 5.4    | 5.7    | 0.51 | 4.1              |
| Plasma zinc,²,³ \(\mu\)mol/L | 10.2 ± 1.1 | 9.9 ± 1.15 | 9.6 ± 1.3 | 10.2 ± 1.6 | 0.18 | 10.0 ± 1.3 |
| <10.7 \(\mu\)mol/L, % | 64.1    | 76.5   | 80.6   | 85.7   | 0.32 | 71.5             |
| Elevated CRP, >10 mg/L, % | 6.4     | 5.9    | 6.9    | 4.3    | 0.92 | 5.9              |
| Elevated AGP, >1.2 mg/L, % | 15.4    | 20.6   | 16.7   | 10.0   | 0.39 | 15.6             |

1 All values are mean ± SD or percent as indicated; to compare differences: ANOVA for continuous variables, Pearson’s chi-square test for proportions. \(P<0.05\) significantly different.

2 Mean of 2 preintervention values calculated prior to calculating group-wise mean.

3 To convert plasma zinc to \(\mu\)g/dL, divide by 0.153.

**FIGURE 1** Relation between plasma zinc concentrations in fasting, healthy Senegalese men at baseline in samples collected 1–2 d apart. Means did not differ, \(n=144\) (\(P=0.99\)).
(P = 0.11) (Table 2). However, across all time points, the zinc-supplemented group was the only group that increased in plasma zinc concentration from baseline (P = 0.006). Moreover, when comparing changes across groups by blood draw, at d 15, the adjusted mean change in plasma zinc concentration was −0.7 μmol/L greater in the SZn15 group than in the other groups (P = 0.05). However, at d 29, plasma zinc concentrations decreased by −0.3 μmol/L in the SZn15 group and increased by −0.3 μmol/L in the fortification group and 0.5 μmol/L in the control group; these group-wise differences were not significant at d 29 (P = 0.53).

Discussion

The results from this study show that the plasma zinc concentration responded to short-term zinc supplementation but not to short-term zinc fortification. We found a significant increase at d 15, but not d 29, in the plasma zinc concentration in the group that received additional zinc from a liquid zinc supplement, but not in the groups that received additional zinc from zinc-fortified bread products.

The strengths of the present study include its double-blind, randomized design and direct monitoring of the bread and supplement consumption. The study assessed the effects of consuming zinc-fortified bread products at moderate and high levels of zinc fortification and included both positive and negative control groups. In addition, the groups were not significantly different at baseline, except for smoking behavior, which was not associated with plasma zinc concentration, and the baseline characteristics of participants who completed (90%) or exited early from the study did not differ. Thus, the significant changes within the group that received additional zinc from a liquid zinc supplement are likely due to the intervention itself. One weakness of the study was that the sample size was powered to detect an effect size of −0.9, based on an earlier study of zinc supplementation of adult male volunteers (5), but the observed effect sizes following zinc supplementation only ranged from 0.4 to 0.6 in the current study (depending upon whether d 15 or 29 values were used for the calculation). Therefore, it is likely that the study was underpowered to detect the smaller differences that occurred in response to the intervention. However, because there was no evidence of a response to the intervention within the groups that received additional zinc from zinc-fortified breads, it seems unlikely that the overall conclusions would change with a larger sample size.

The magnitude and pattern of response in the group that received additional zinc from the liquid zinc supplement were somewhat surprising. The peak in plasma zinc concentration in the zinc-supplemented group at d 15 and subsequent decline at d 29 were consistent with the pattern of response in 2 earlier zinc supplementation studies by Sullivan et al. (16,17). In these studies, plasma zinc concentration peaked at d 6 and subsequently declined at d 15 in the group that received 50 mg/d supplemental zinc (as zinc gluconate). However, in a more recently completed zinc supplementation study that used similar doses of supplemental zinc to the present study (10 mg/d or 20 mg/d as zinc sulfate), the plasma zinc concentration responded in as few as 5 d in the zinc-supplemented groups and remained elevated throughout the 21-d supplementation period (5). The findings from the present study raise the possibility that the decline in the plasma zinc concentration in the supplementation group may be due to a homeostatic downregulation of zinc transporters over time (18–20). However, it is also possible that methodological factors, such as consuming the supplements 90 min after the test meal, may have affected zinc absorption or postabsorptive metabolism (5). Further research is needed to address these inconsistencies among different trials.

The participants who received zinc-fortified breads in the present study had increases in plasma zinc concentrations of −0.3 μmol/L from baseline to d 29, although this did not differ from baseline values (FZn7.5: P = 0.89; FZn15: P = 0.37) and was within the range of expected day-to-day variation, as determined from the baseline samples. Moreover, the men in the control group had a similar increase in plasma zinc concentration from baseline to d 29. This lack of response in the groups that received additional zinc from zinc-fortified breads is consistent with 2 recently completed studies that used the plasma zinc concentration to assess the impact of zinc fortification interventions in young children. In 1 of these studies in which zinc-fortified cereal porridges were provided to young Peruvian children for 6 mo, those who received 3 mg zinc/d as a supplement had a significant increase in plasma zinc concentration, whereas those who received the same amount of zinc in a fortified porridge had no significant change in plasma zinc levels (21). In another recently completed 2-wk study among young Senegalese children, those who received 6 mg zinc/d as a liquid zinc supplement had a significant increase in plasma zinc concentration, whereas those who received the same amount of zinc from zinc-fortified cereal porridge did not respond (22).

In this study, the chemical form of additional zinc differed for the zinc supplement (zinc sulfate) and the zinc-fortified food

**Table 2** Changes in the plasma zinc concentration in healthy Senegalese men in the control, liquid supplemental zinc, and 2 zinc-fortified wheat bread groups

| Treatment group     | n  | Control | SZn15 | FZn7.5 | FZn15 | P^4 |
|---------------------|----|---------|-------|--------|-------|-----|
| Change in plasma zinc, μmol/L |    |         |       |        |       |     |
| Baseline to d 15    |    | 0.03 ± 0.23^a | 0.72 ± 0.24^b | −0.09 ± 0.23^b | −0.03 ± 0.23^b | 0.05 |
| Baseline to d 29    |    | 0.47 ± 0.23 | 0.44 ± 0.23 | 0.30 ± 0.23 | 0.32 ± 0.24 | 0.53 |
| P^5                |    | 0.10     | 0.006 | 0.89   | 0.37   |     |

1 Values are mean ± SE. Means in a row with superscripts without a common letter differ, P < 0.05
2 Data were analyzed by a mixed model ANCOVA with control for baseline plasma zinc concentration, CRP (>10 mg/L), AGP (>1.2 mg/L), and time since last meal.
3 To convert plasma zinc to μg/dL, divide by 0.153.
4 Between group.
5 Within group.
(zinc oxide). It is argued that the response of plasma zinc to fortification may depend on the chemical form of zinc or the use of potential enhancers of zinc absorption, such as exogenous phytase. However, based on the available evidence, zinc is equally well absorbed when foods are fortified with either zinc oxide or zinc sulfate (23) and exogenous phytase would be expected to enhance zinc absorption only when the phytate content of the diet is high (24). The phytate:zinc molar ratios of the 2 zinc-fortified breads were just 0.3 and 0.7, which would be expected to exert very little influence on total zinc absorption.

Results from the present study add to the existing knowledge on the usefulness of plasma zinc concentration for evaluating the impact of zinc fortification programs. Based on the information available, plasma zinc concentration may not be a suitable biomarker to assess the impact of short-term exposure to zinc fortification. However, results from a recently completed 3-y evaluation of a mass fortification program in China raise the possibility that plasma zinc concentration may respond to longer term fortification interventions. In the China evaluation, there was a small but significant increase in mean serum zinc concentrations among women of childbearing age after 24 and 36 mo of exposure to zinc fortification, but not after 12 mo (25). These findings raise several research questions. First, the duration of exposure to fortification interventions needs to undergo further investigation. Moreover, studies are needed to assess the metabolism of zinc absorbed from zinc-fortified foods and to determine whether functional benefits may be derived from zinc fortification even when there is no change in the serum zinc concentration.

In summary, findings from the present study indicate that the plasma zinc concentration may not be a sufficiently sensitive indicator to evaluate short-term responses to zinc fortification programs, even though the available evidence indicates that zinc fortification can increase dietary zinc intake and total daily zinc absorption (2). Because of these potential benefits of zinc fortification programs and the low cost of adding zinc to micronutrient premixes used in already planned or existing interventions, it is still advisable to include zinc in fortification programs implemented in populations with an elevated risk of zinc deficiency.

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