Plasma Zinc Concentration Responds Rapidly to the Initiation and Discontinuation of Short-Term Zinc Supplementation in Healthy Men1–4

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Abstract

To assist with the evaluation of zinc (Zn) intervention programs, information is needed on the magnitude and velocity of response of plasma Zn concentration following changes in Zn intake. Our objective in this study was to measure plasma Zn concentration of healthy adult men before and after initiation and discontinuation of 1 of 2 dosages of Zn supplements or placebo. We conducted a randomized, double-blind, placebo-controlled trial in 58 apparently healthy males aged 19–54 y. Participants received 1 of 3 liquid supplements daily for 21 d: 10 or 20 mg Zn/d, as Zn sulfate, or placebo. Fasting plasma Zn concentrations were measured on 14 occasions before, during, and after supplementation. Data were analyzed using mixed-model ANCOVA. The plasma Zn concentration was related to day of study (P < 0.0001) and study group (P < 0.0001). Controlling for baseline concentrations, plasma Zn concentrations were consistently elevated above baseline by d 5 among individuals in both of the Zn-supplemented groups compared with those receiving placebo supplements, regardless of their initial plasma Zn concentration. There were no significant group-wise differences between those who received either 10 or 20 mg/d Zn. Plasma Zn concentrations of supplemented individuals declined following withdrawal of supplementation and within 2 wk no longer differed from those of the placebo group. Change in the plasma Zn concentration is a useful indicator to monitor compliance with, and possibly effectiveness of, Zn supplementation programs. To ensure accurate interpretation of the results, samples should be collected while the intervention is still in progress. J. Nutr. 140: 2128–2133, 2010.

Introduction

Plasma or serum zinc (Zn) concentration is the most widely used indicator of individual and population Zn status. In healthy individuals, fasting plasma Zn concentrations are homeostatically maintained. Significant increases in individual and population mean plasma Zn concentrations have been consistently observed following Zn supplementation (1), and controlled studies of dietary Zn depletion and repletion have found a strong correlation between changes in total body Zn content and changes in plasma Zn concentration (2).

Many factors independent of Zn status, including the duration of fasting, circadian rhythms, age, sex, pregnancy, use of hormonal contraceptives, and the presence of infection and inflammation, are known to influence plasma Zn concentration, thereby affecting its interpretation for assessing Zn status (3,4). Despite these limitations, plasma/serum Zn concentration is currently the only biochemical indicator recommended by the WHO, UNICEF, International Atomic Energy Agency, and the International Zinc Nutrition Consultative Group for the assessment of population Zn status (1). Likewise, the European Micronutrient Recommendations Aligned Network of Excellence recently concluded that plasma Zn concentration is the only valid biomarker to measure Zn status in both Zn-supplemented and Zn-depleted individuals (5). However, information is lacking on the time course of changes in plasma Zn concentration following the introduction of interventions to enhance Zn intake. Similarly, the duration of such changes following discontinuation of Zn interventions is unknown. Finally, the relationship between the dosage of Zn consumed and changes in plasma Zn concentration has received relatively little attention. These issues are critical for the proper interpretation of plasma Zn concentrations for assessing population Zn status and determining the potential impact of Zn intervention programs.

Our objective in this study was to investigate the effects of short-term Zn supplementation, and discontinuation of supplementation, on the magnitude and velocity of change in plasma Zn concentration.
Zn concentrations in apparently healthy adult males. We assessed the effects of each of 2 dosages of Zn supplement (10 and 20 mg/d) compared with a placebo supplement.

Methods

Participants. Fifty-eight males between the ages of 19 and 54 y (median = 27 y) participated in this study. None of the participants had a history of acute or chronic illnesses at the start of the study or were taking medications that might affect Zn metabolism. All of them had normal baseline hematological and blood chemistry profiles (complete blood count with auto-differential and comprehensive metabolic panel). The study was conducted at the USDA Western Human Nutrition Research Center (WHNRC), on the campus of the University of California, Davis (UC Davis). The study was approved by the Institutional Review Board of UC Davis and written informed consent was obtained from all participants.

Experimental design. The study was conducted as a randomized, double-blind, placebo-controlled trial. Individuals were randomly assigned to 1 of 3 dosage regimens: 0 mg/d Zn (placebo), 10 mg/d Zn, or 20 mg/d Zn, as Zn sulfate. The study was divided into 2 phases: a 21-d supplementation period (study d 0–20) and a 21-d postsupplementation period (study d 21–42). The participants were scheduled to attend the study clinic for an initial screening and on 12 subsequent occasions during the course of the 43-d study period (d 0, 1, 2, 5, 9, 14, 21, 22, 23, 26, 35, and 42). Group assignments remained unknown to all participants and research staff members until biochemical tests and preliminary data analyses were completed.

Supplementation. The Zn and placebo supplements were prepared using an Emerson Cherry Compounding Syrup (Humco) by a research pharmacist at the UC Davis Medical Center Investigational Drug Service and were coded by individual participant identification number. Daily doses were individually packaged by study personnel prior to being distributed to study participants. The Zn content of each supplement was verified by inductively coupled plasma atomic emission spectrometry (ICP-AES).

During the supplementation phase (d 0–20), the participants were instructed to consume preprepared doses of liquid supplements once daily upon waking, followed by a fast of ≥30 min before their first meal to permit maximum absorption of the supplement. The participants kept a log certifying daily ingestion of the supplement, the time of meal to permit maximum absorption of the supplement. The participants kept daily morbidity and medication intake logs, which were collected weekly and cross-checked by study personnel. During each clinic visit, the following signs and symptoms of illness were recorded by the study staff: fever, diarrhea (stool number and consistency), vomiting/nausea, cough, nasal secretion, flu-like symptoms, earache, and difficulty breathing/respiratory distress. Any participant-reported medical consultations were noted along with the respective diagnosis and treatment.

Sample collection and processing. Blood samples were drawn by certified phlebotomists on 2 occasions prior to the start of supplementation: once at the time of initial screening (between 6 and 15 d prior to the start of the intervention) and again on d 0, just before the first supplement was administered. Subsequent blood samples were scheduled on d 1, 2, 5, 9, 14, and 21 (supplementation period) and on d 22, 23, 26, 30, 35, and 42 (d 1, 2, 5, 9, 14, and 21 postsupplementation). If a participant was unable to attend a scheduled blood drawing, this was rescheduled and included in the analysis if the sample was obtained within 1–2 d of the scheduled visit, depending on which sample was affected. All blood samples were collected in the morning between 0700 and 1020 h following an overnight fast of at least 8 h. For each blood sampling event, 7.5–15 mL of blood was drawn from the antecubital vein using stainless steel needles according to procedures recommended by the International Zinc Nutrition Consultative Group (3,6). The blood was collected into 1 or 2 trace element-free, evacuated polyethylene tubes containing Zn-free lithium heparin anticoagulant (Sarstedt AG & Co; no. 01.1604.400) and immediately placed horizontally in a cold box over ice. Plasma was separated from heparinized blood within 1 h of blood collection by centrifuging at 1140 × g (Allegra 6R centrifuge with Beckman Coulter) for 10 min at 4°C. Plasma was aliquoted and stored at −20°C prior to laboratory analysis. Standard protocols and trace element-free materials were used to avoid Zn contamination of samples (3,6).

Sample analyses. The plasma Zn concentration was measured by ICP-AES (Vista AX CCD Simultaneous ICP-AES with an SPS5 autosampler, Varian). In brief, plasma samples were diluted 13.5-fold in 1 N nitric acid (Trace Metal Grade, Fisher Scientific) and allowed to sit overnight at 4°C. Samples were then centrifuged at 4°C for 15 min at 2200 × g and the supernatant was separated for analysis. Each batch of samples run on the ICP-AES was analyzed with the following research tools, which were prepared in a manner identical to that of the clinical samples: Seronorm Trace Elements Serum L-1 (Lots NO0371y and JL4409; Cat-SR-218405, Accurate Chemical and Scientific) and an internal pooled plasma control. A bovine liver standard (no. SRM 1577b; National Institute of Standards and Technology) was also run with each batch of samples. Samples were analyzed in duplicate and all samples from a particular participant were analyzed within the same analytic run. Plasma C-reactive protein (CRP) concentrations were analyzed by using radial immunodiffusion (GTO44.3, The Binding Site Limited).

Sample size estimation and statistical analyses. The sample size was estimated based on the results of an unpublished trial conducted previously in our laboratory, in which we found a 0.9-μmol/L within-subject SD in the plasma Zn concentration during the course of a supplementation intervention. Using this information, we estimated that a sample size of 16 participants per group would be sufficient to detect a 2.3-μmol/L difference among study groups in the change in plasma Zn concentration based upon a 5% level of significance and 80% power.

Statistical analyses were completed by using SAS for WINDOWS software (version 9.1; SAS Institute). Descriptive statistics were calculated for each continuous variable, which were then tested for normality. Intra-individual differences in plasma Zn concentration at baseline were assessed using a paired t-test and Pearson correlation and variance components analyses. Group-wise differences at baseline were compared using ANOVA. Mixed model ANCOVA was used to test for significant differences in changes over time in plasma Zn concentration among groups after controlling for relevant baseline variables (initial plasma Zn concentration, BMI, hematological indicators, and age), time-dependent variables (CRP and morbidity), and methodological factors (time of day of blood draw and elapsed times between blood draw and last food intake, time of day of last supplement intake, and elapsed time between each blood sample collection and separation of plasma from whole blood). When overall results were significant, group means were compared using least-square means with the Tukey-Kramer adjustment. Differences of P < 0.05 were considered significant. Data are presented as means ± SE for mixed-model ANCOVA and means ± SD for all other analyses unless otherwise noted. Treatment groups remained masked until all statistical analyses were completed.
Results

Accuracy and precision of laboratory tests. Reference standards were analyzed with each batch of study samples during all 31 analytical runs to assess the accuracy and precision of the laboratory tests; Zn concentrations of all reference materials were found to be within the acceptable ranges according to the manufacturers’ specifications. The inter-run CV of Seronorm Trace Elements Serum L-1, lots NO0371y and JL4409, were 3.0 and 3.4%, respectively. The inter-run CV of the Zn concentration in the pooled plasma samples was 4.8%. The concentration of Zn in bovine liver standard across all analytical runs was 17.7 ± 0.5 μmol/L.

Enrollment, attrition, and baseline characteristics. Of the 72 individuals assessed for eligibility, 58 participants were enrolled and 53 (91%) completed the study (Supplemental Fig. 1). At baseline, there were no significant differences in mean age, BMI, or initial plasma Zn concentration between those who completed the study and those who did not. Data from participants who did not complete the study were included in the analyses until the day of attrition (range = 0–21 d). There were no significant differences in mean baseline age, BMI, hematological indicators, or plasma Zn concentration by study group (Table 1). Baseline plasma Zn concentrations were normally distributed. The participants’ 2 preintervention measurements of fasting plasma Zn concentrations, obtained 6–15 d apart (median = 8 d), were strongly correlated (r = 0.74; P < 0.001) and did not differ from one another (P = 0.57), indicating that the participants’ fasting plasma Zn concentrations were fairly stable (Fig. 1). In particular, the percent difference within participants between screening and d 0 was 7.3% ± 6.6% (n = 53). Variance components analysis of baseline fasting plasma Zn concentrations (n = 57) indicated a between-person SD of 1.46 μmol/L and a within-person SD of 0.75 μmol/L. The baseline plasma Zn concentration was not significantly related to the participants’ BMI or age. The person-specific means of the 2 baseline measures were used for all further analyses to increase the precision of the baseline plasma Zn concentration and enhance the ability to detect change.

Factors associated with plasma Zn concentration. We next examined factors other than supplementation that might have been associated with the participants’ plasma Zn concentrations. The blood samples were drawn at 0816 h ± 41 min, which was 12.2 ± 1.4 h since the last food intake and 24.2 ± 1.3 h since the last dose of supplement consumed. The plasma samples were separated from blood within 28.5 ± 15.0 min after the blood was drawn. Three samples were excluded from data analysis, because they were not within the preestablished criteria for these methodological factors and were >5 SD from the mean. Of these methodological factors, the time between last food intake and the blood drawing was the only one that was significantly (positively) related to the plasma Zn concentration (P = 0.006 ANCOVA; r = 0.16; P < 0.001), so this was included as a covariate in subsequent analyses. Specifically, the plasma Zn concentration increased by ~0.11 μmol/L with each additional hour of fasting between 7 and 19 h (Fig. 2).

There was no effect of reported morbidity or medication intake on plasma Zn concentration, although these conditions were reported on very few study days (range = 3–47 d of a possible total of 685 person-days of observation). On the other hand, samples with elevated CRP (>10 mg/L; 0.92% of all samples, n = 8) had lower plasma Zn concentrations (10.43 ± 0.41 vs. 12.86 ± 0.11 μmol/L, respectively; P < 0.0001); these samples were excluded from subsequent analyses.

Effects of supplementation. The reported compliance with supplement intake was 99.7% of possible doses. Controlling for

| TABLE 1 | Baseline characteristics and plasma Zn concentrations in apparently healthy adult males, 19–54 y of age, by intervention group1 |
|----------------|----------------|----------------|
|               | Placebo         | 10 mg/d Zn     | 20 mg/d Zn     |
| n              | 20              | 19             | 18             |
| Age, y         | 26.9 ± 7.0      | 30.8 ± 9.2     | 26.9 ± 4.1     |
| BMI, kg/m2     | 23.1 ± 2.7      | 24.9 ± 4.1     | 24.7 ± 4.3     |
| Hemoglobin, g/L| 15.4 ± 0.9      | 15.2 ± 0.8     | 15.3 ± 0.7     |
| Plasma Zn, μmol/L | 12.5 ± 1.8 | 12.4 ± 1.4 | 12.3 ± 1.5 |

1 Values are mean ± SD.
2 Mean of 2 baseline samples per individual calculated prior to calculating group-wise mean. Plasma sample when individual had CRP > 10 mg/L (n = 1) was excluded from analysis.

FIGURE 1 Association between participants’ baseline fasting plasma Zn concentrations obtained 6–15 d (median = 8 d) apart (n = 53).

FIGURE 2 Association between time between last food intake and the blood drawing and plasma Zn concentration (n= 741 observations on 53 participants).
The magnitude of the change in plasma Zn concentration from baseline to any particular day of the supplementation period depended on the mean baseline value for each subject (Fig. 4, d 14). Notably, participants with higher mean initial plasma Zn concentrations had smaller changes over time, and vice versa, even in the placebo group, which is likely due to regression to the mean. Nevertheless, at any level of mean initial plasma Zn concentration, the participants in the Zn-supplemented groups had a greater change in plasma Zn concentration than those in the placebo group ($P < 0.0001$). Among individuals who received Zn supplements, the magnitude of change in plasma Zn concentration did not differ for participants with lower or higher initial mean plasma Zn concentration. Inter-individual variability in change in plasma Zn concentration from either baseline or the final day of supplementation (d 21) did not differ between study group on any study day or postsupplementation study day, respectively ($0.12 < P < 0.97$).

**Discussion**

The results of this study indicate that the plasma Zn concentration increases within 2–5 d of starting each of the doses of Zn supplements that were studied (10 and 20 mg/d Zn), remains elevated during the period of supplementation, and declines to baseline concentrations within 14 d of discontinuing supplementation. The randomized, double-blind clinical trial design, highly standardized and rigorous collection and processing of plasma Zn samples, and multiple and frequent blood draws lend strength to these findings.

We found that unsupplemented individuals have fairly stable fasting plasma Zn concentrations, possibly due to consistent dietary intake and/or genetic factors controlling Zn homeostasis. Numerous individual and methodological factors (including age, sex, CRP concentration, fasting status, time of day of blood draw, and sample processing) have been shown to significantly affect plasma Zn concentration in previous studies (3). Therefore, we standardized the plasma collection procedures throughout the course of the present study to limit the possible impact of methodological factors on plasma Zn concentrations. Nevertheless, we also evaluated the effects of these aforementioned factors on plasma Zn concentrations.
variables and adjusted the Zn concentrations statistically as necessary to account for these factors.

Consistent with previous studies, the change in plasma Zn concentration (in both the groups that received 10 or 20 mg/d Zn as well as the placebo group) was related to the initial status, so baseline plasma Zn concentrations should be controlled when interpreting the effects of interventions (1). It is not certain whether this observation is due to physiological control of the response to Zn supplementation or to regression to the mean, but the fact that this phenomenon also occurred in the placebo group suggests that the latter explanation is more likely. Irrespective of initial plasma Zn concentration, we found a significant and rapid rise in the mean plasma Zn concentration in both of the Zn-supplemented groups within the first 5 d of supplementation, as has been observed during previous studies of short-term Zn supplementation in apparently healthy adults (1,7–9). In the present study, this elevation in mean plasma Zn concentrations seemed to be more rapid in the group that received the higher dosage of Zn and persisted throughout the entire supplementation period in both the Zn-supplemented groups compared with the placebo group, a phenomenon observed in most prior short- and long-term Zn supplementation studies (1,7–10). However, these results differ from 2 previous short-term supplementation studies by Sullivan et al. (7,8), who noted an early peak in plasma Zn concentration on d 6 of the supplementation period (>180% of baseline) followed by a decline on d 15 (to ~112–120% of baseline or placebo group). This pattern may represent a metabolic adaptation to the relatively high dose of supplements that were tested in these studies, which provided 50 mg/d supplemental Zn, almost 5 times the RDA for adult men (11). In comparison, mean plasma Zn concentrations in the current study were never elevated more than 20% above baseline, which may explain the lack of an early peak followed by a subsequent decline in plasma Zn concentrations.

In this study, plasma Zn concentrations did not differ between the 10 and 20 mg/d Zn-supplemented groups during the study period, although both groups differed significantly from the placebo group. This study had the ability to detect differences between groups of 1.86 μmol/L, with 80% power. According to a recent review by Hess et al. (11), serum Zn concentrations appear to reach a plateau when Zn intakes approach ~25–30 mg/d. Although dietary Zn intake was not assessed in the present study, median dietary Zn intake in adult males in the United States is ~14 mg/d (12). Therefore, it is probable that the men included in this study had total Zn intakes of ~24–34 mg/d Zn when receiving the oral aqueous Zn supplements and it may not have been possible to detect any further increase in plasma Zn concentrations between the 2 supplemented groups. This speculation is consistent with the results of Zn absorption studies by Tran et al. (13) who found that Zn follows a saturable, dose-response model. They reported an abrupt decline in fractional Zn absorption when single, oral, aqueous doses > 10 mg were given.

This lack of a dose-dependent response in the present study contrasts with the conclusions of a recent meta-analysis, which reported that plasma Zn concentration responds in a dose-dependent manner over a wide range of supplemental Zn intakes (15–150 mg/d Zn) (5). However, this systematic review compared response to Zn supplementation across 40 different studies and a broad range of supplement dosages, and the data were highly heterogeneous, whereas the present study makes within-study comparisons of a fairly homogeneous group of participants. Additional research examining dose responses within a particular study population will be needed to resolve these inconsistencies.

Plasma Zn concentrations of the supplemented individuals declined following the withdrawal of Zn supplementation in the present study and there were no differences between supplemented and nonsupplemented groups within 2 wk. We found only 1 previous study in which significant differences in plasma Zn concentration existed between supplemented and nonsupplemented groups on the final day of supplementation and participants’ plasma Zn concentrations were measured following the termination of supplementation (7). Similar to the results of the present study, elevated plasma Zn concentrations were not sustained following discontinuation of the supplement. However, the authors of the former study observed an even more rapid decline in plasma Zn concentrations than in the present one and there were no differences between supplemented and nonsupplemented groups by 4 d following termination of supplementation. The reasons for these differences in the time of response to supplement withdrawal are unknown but may be related to differences in the amounts or duration of Zn supplementation.

There are several possible explanations for the fact that the increases in mean plasma Zn concentrations were not sustained following removal of the supplement. For example, the plasma Zn may have been redistributed to more slowly exchanging metabolic pools during this time period, resulting in increased total body Zn content but no sustained change in plasma Zn concentration. Alternatively, it is possible that urinary and fecal Zn excretion were increased during the supplementation period and this elevated excretion continued for several days post-supplementation, until a new equilibrium was achieved in response to the modified level of Zn intake (2). Additional studies of Zn absorption and kinetics are necessary to address these possibilities and determine the response of exchangeable Zn pools and total body Zn content to supplementation and their relations with functional responses to Zn supplementation.

Plasma Zn concentrations decline fairly rapidly following the termination of supplementation, although these concentrations may fluctuate during the first 1–2 wk post discontinuation of supplementation. Variations of just a few days in the timing of post-supplementation blood draws could affect the interpretation of plasma Zn concentrations, so it is advisable to collect relevant samples prior to the termination of supplementation. The relation between this rapid decline in plasma Zn concentrations after the termination of supplementation and total body Zn content is not known, nor is the relation between such changes in plasma Zn concentration and current or longer term functional responses to Zn supplementation (2,14). Additional research will be needed to elucidate these relationships.

In conclusion, the results of the present study indicate that individuals consuming their usual home diet have fairly stable fasting plasma Zn concentrations over 1- to 2-wk periods of observation. The findings confirm that plasma Zn concentrations reflect short-term changes in Zn intake and the results support most previous observations that increases in plasma Zn concentration in supplemented groups persist for the duration of the supplementation period. The study further indicates that plasma Zn concentrations return to baseline values within a fairly short 1- to 2-wk interval following discontinuation of supplementation. Despite this possible limitation of plasma Zn concentration as an indicator of longer term Zn status, the results of this study indicate that under carefully controlled conditions, plasma Zn concentration is a useful and responsive biomarker of change in Zn intake during supplementation. Monitoring plasma Zn...
concentration in appropriately selected study populations (and using proper precautions in sample collection and processing) is an appropriate strategy for evaluating compliance with, and possibly the effectiveness of, Zn supplementation interventions. Additional studies should be conducted to confirm these results in Zn-depleted populations.

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Literature Cited