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ORIGINAL ARTICLE

Bioavailability of zinc from NutriSet zinc tablets compared with aqueous zinc sulfate

NW Solomons[1], M-E Romero-Abal[1], G Weiss[2], B Michalke[3] and K Schümann[4]

[1]*Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM), Guatemala City, Guatemala;* [2]*Department of Internal Medicine I, Clinical Immunology and Infectious Diseases, Medical University of Innsbruck, Innsbruck, Austria;* [3]*Helmholtz Center Munich, German Research Center for Environmental Health, Institute of Ecological Chemistry, Neuherberg, Germany and* [4]*Center Institute for Nutrition and Food Research, Technische Universität München, Freising, Germany*

Background/Objectives: The apparent widespread extent of zinc (Zn) deficiency in developing countries and the efficacy of oral Zn supplements as an adjunct to oral rehydration therapy make oral Zn supplementation an increasingly important modality in clinical medicine and public health. In this study we aimed to compare the relative bioavailability of oral doses of 30 mg of Zn in two dosing forms

Subjects/Methods: In total, 10 healthy male volunteers ingested oral Zn doses with 200 ml plain water at about 0830 hours in the fasting state on two occasions, once as 30 mg of Zn in an aqueous solution of reagent grade zinc sulfate ($ZnSO_4$) and another time as 1.5 NutriSet Zn tablets (Nutriset, Malaunay, France); on a third occasion, only plain water was consumed. Venous blood specimens were collected at baseline, 60, 120, 180 and 240 min after ingestion and the plasma Zn was measured for each sample.

Results: The relative bioavailability of oral Zn from a commonly used, tableted (NutriSet) form is only about half of that of a reference dose of aqueous $ZnSO_4$ as indicated by the area under the curve of serial plasma Zn excursion and maximal change in circulating Zn.

Conclusions: Reduced or absent functional outcomes in Zn intervention trials may derive, in part, from a lower than anticipated intestinal uptake of the Zn in the tableted form.

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Introduction

The dependence of much of the World's population on coarse, unrefined cereal grains, which contain high contents of phytic acid, a potent inhibitor of zinc (Zn) absorption, combined with recurrent infectious episodes, is thought to be the basis of widespread Zn deficiency in developing countries (International Zinc Nutrition Consultative Group (IZiNCG) *et al.*, 2004; Hess *et al.*, 2009). Oral zinc has been shown to reduce recurrence of respiratory and diarrheal diseases (Baqui *et al.*, 2002, 2003). It mitigates the symptoms of acute, dehydrating diarrheal diatheses (Bhandari *et al.*, 2008; Lazzerini and Ronfani, 2008), leading to recommendations of adjunctive supplementation for both developing (WHO, 2004) and developed (King *et al.*, 2003) country settings. Although oral Zn supplementation has been suggested to enhance linear growth in some estimates (Brown *et al.*, 1998, 2002), but not in others (Ramakrishnan *et al.*, 2009), policies and programs for daily Zn supplementation in populations with high stunting rates are under consideration.

Zinc from supplemental forms must be absorbed across the intestine and taken up into the systemic circulation. Zinc sulfate ($ZnSO_4$) has been the reference compound for Zn bioavailability, because of its widespread use and low cost (Rosado *et al.*, 2005; Brown *et al.*, 2007). However, some

Correspondence: Dr NW Solomons, Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM), 17 Avenida No. 16-89 (interior), Zona 11 (Anillo Periferico), Guatemala City 01011, CeSSIAM in Guatemala, PO Box 02-5339, Section 3163/Guatemala, Miami, FL 33102-5339, USA.
E-mail: cessiam@guate.net.gt
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doubts have arisen recently about whether all formulations of this compound are equally available for nutritional purposes when provided as supplements. This concern has arisen around the unexpectedly low increases in circulating Zn concentrations observed in major field intervention studies with Zn, with tablets provided from NutriSet (Rivera, 2007). We report here the results of an experimental study in healthy male volunteers, to compare the relative bioavailability of Zn from aqueous $ZnSO_4$ and dispersed NutriSet tablets.

Subjects and methods

Enrollment of experimental volunteers

In total, 10 men, ranging in age from 18 to 55 years, volunteered for the study. Inclusion criteria included having an adequate hemoglobin concentration, general good health and no history of nutritional supplement use. The study was approved by the Human Subjects Committee of CeSSIAM and subjects gave written, informed consent after the nature, purpose, inconveniences, risks and benefits of participation were explained. Subjects were compensated for their participation.

Conduct of the zinc bioavailability tests

Subjects arrived after an overnight fast of at least 8 h. A baseline (zero-time) sample of whole blood was collected from an antecubital vein using a Vacutainer needle sheath into 6 ml trace-metal-free blue-stopper evacuated tubes containing oxalate anticoagulant (Becton-Dickinson, Oxnard, CA, USA). The collection tube was centrifuged for 10 min at 3000 r.p.m. to separate plasma from red cells, and the supernatant was carefully pipetted with plastic pipettes into Zn-free, screw-top collection vials (Polypropylene Wheaton Vials, Wheaton Science products, Millville, NJ, USA).

Immediately following the basal blood sampling, subjects consumed one of three oral regimens, as determined by random sequence allocation, of either a no-Zn placebo or 30 mg of elemental Zn in one of two formats. Given that the NutriSet tablets are scored for easy breaking into 10 mg of Zn dosages for young children, our options were a single tablet (20 mg) or one-and-a-half tablets (30 mg), taking advantage of the scoring. The 30 mg dosage of Zn was chosen based on considerations of the expected magnitude of response to be seen, and differentiated among treatment variants based on the experience with 25 mg of elemental Zn in prior research (Solomons and Jacob, 1981). Treatment A consisted of 200 ml of plain bottled water in a disposable plastic cup. Treatment B consisted of one-and-a-half NutriSet 20 mg Zn tablets, compounded from $ZnSO_4$ monohydrate. In accordance with the instructions on the packet insert, the tablet and fragment were dissolved in 10 ml of water to full dispersion. This was then transferred into a plastic cup, and filled to make a total volume of 200 ml for consumption. Treatment C consisted of 200 ml of water, to which 82.4 mg of monohydrated $ZnSO_4$ powder (36.4% elemental Zn by weight), containing 30 mg of elemental Zn (Fluka, Sigma Aldrich Chemie, GmBH, Steinheim, Germany), had been dissolved. After the initial 200 ml prescribed dose of liquid had been consumed, the subjects were allowed to drink plain water, *ad libitum*, throughout the 4-h period.

Venupunctures were repeated at 60, 120, 180 and 240 min after the ingestion of the assigned treatment, and samples were handled and processed for plasma in an identical manner as the zero-time sample. In addition to water *ad libitum*, a standard light breakfast snack of 120 g of pancakes with syrup and 120 g of papaya or melon was provided, immediately after the fourth blood sample (at 180 min interval) had been collected. Later, after the final 240-min blood extraction, concluding the test, subjects were offered a full, 800–1000 kcal brunch prior to leaving the clinical center.

The protocol called for a series of three separate days of test participation at 7-day intervals. It was noted that, on the day on which the subjects were give Zn in an aqueous solution, most experienced nausea and 4 of the 10 vomited at some point between 60 and 180 min after ingesting the Zn solution. Consent was obtained from 8 of the 10 subjects to repeat the aqueous Zn treatment in an additional test session during a fourth week of the protocol period. All plasma specimens were color-coded by experimental treatment and stored frozen at $-20\,^{\circ}C$ until being shipped to Munich, Germany, for chemical analysis.

Quantitative analysis of plasma zinc

In the Munich laboratory, the masked frozen plasma samples were thawed gently at room temperature, vortexed to homogeneity and diluted 1:10 with Milli-Q water for direct measurement by inductively coupled plasma atomic emission spectrometry (ICP-AES) on a Spectro Ciros Vision ICP-AES system (Spectro, Analytical Instrument, Kleve, Germany), equipped with a Spetec peristaltic pump, equipped with an 'anti-pulse head' (Erding, Germany) and an argon (Ar) system from Air Liquide (Gröbenzell, Germany). The radio frequency power was set to 1200 W with plasma gas flow set at 15 l Ar/min and nebulizer gas flow set at 0.6 l Ar/min.

Quantitative analysis of zinc tablets

Five whole tablets from a 10-pill blister pack of the original lot of NutriSet tablets were chosen at random for analysis and transferred into trace-element-free quartz vessels into which 10 ml 0.5% suprapure nitric acid (HNO_3) in Milli-Q water was added. After complete dissolution of the tablet, the solution was further diluted with Milli-Q water for Zn analysis. The direct ICP-AES determination was conducted as described for blood plasma, with results reported as mg Zn per original tablet.

Quality control for ICP-AES

Serial and day-to-day precision and quantitative accuracy were established by replicate measurements ($n = 10$) of a

control serum with a target value of Zn concentration of 2275 μg/l (Recipe, Munich, Germany). Precisions were <1% and accuracy was 99.5%. Within runs of plasma and tablet samples, at regular intervals after every 10 measurement samples, three blank determinations and a control determination of a certified Zn standard were performed. Calculation of Zn concentration or content was carried out on a computerized management system, relating the sample measurement to the calibration curves and the blank and control internal standards.

Miscellaneous laboratory analyses
On the first and final study days, blood was analyzed for hemoglobin concentration on an automated hematological profile analyzer (CELL-DYN Ruby, Abbott Diagnostics, Santa Clara, CA, USA) and for C-reactive protein, both at the Nuestra Señora del Pilar Hospital in Guatemala City. Plasma ferritin was determined on the thawed samples by turbimetric tests (Roche, Mannheim, Germany) in Innsbruck.

Data handling and statistical analysis
The starting point for interpretive analysis was the absolute concentrations of plasma Zn in samples, expressed in μg per 100 ml. Descriptive statistics of mean, s.d., and minimum and maximum limits were computed for the plasma Zn at baseline and across the 4-hourly collection time points. Repeated-measures analysis of variance (multivariate analysis of variance) was performed using general linear models on SPSS version 15.0 (Statistical Program for the Social Sciences, Chicago, IL, USA) on the indicators of plasma Zn response for each of the 10 subjects who had the primary randomized series of three tests. Multivariate analysis of variance was similarly performed across the four tests (including the additional aqueous Zn 'make-up' test) in those eight subjects undergoing the full series. The *post-hoc* test was the 'Least Significant Difference' test. A probability level of 5% was accepted as the criterion for statistical significance.

Results

Characteristics of the subjects
The characteristics of age, body mass index, initial and final hemoglobin and C-reactive protein are provided in Table 1. The subjects were men with age ranging from 18 to 55 years, with an average weight of 78 ± 17 kg, and a range of body mass index from 21 to 33 kg/m^2. All subjects had normal iron stores, as reflected by baseline ferritin concentrations and adequate hemoglobin levels throughout the study. Two subjects had mild inflammation with C-reactive protein levels above 5 mg/l. The mean initial fasting Zn concentration of the subjects on entry to the study was 99 ± 33 μg per 100 ml, with no value falling below 70 μg per 100ml.

Relative oral zinc bioavailability responses
The mean changes in plasma Zn, using time zero as the reference point for each individual, are shown as time-dependent curves in Figure 1. Zinc concentrations fell below initial fasting levels, on aggregate, with the plain water treatment, but rose modestly with the NutriSet Zn tablet and dramatically after the administration of aqueous Zn solution on both occasions. The mean (and s.d.) of the maximal change over the 4-hourly blood collection period following each of the three treatment arms are shown in Table 2. We performed repeated-measures analysis of variance (multivariate analysis of variance) across the results of the original treatment arms for the 10 subjects completing the original series; we found statistically significant difference between each of the three treatments. Similar results were found when all four testing situations, including the repeat aqueous Zn, were compared for the eight individuals with the four tests, with the exception of no difference between the two tests performed with aqueous Zn. The mean and s.d. for our proxy for area under curve, that is, the cumulative sum of serial, hourly changes in circulating Zn concentration during the individual tests, are also shown in Table 2. We again performed repeated-measures multivariate analysis of

Table 1 Characteristics of the experimental subjects

Subject	*Age (years)*	*Weight (kg)*	*BMI (kg/m^2)*	*Initial Hb (g/l)*	*Final Hb (g/l)*	*Initial (ferritin) (μg/l)*	*CRP (mg/l)*	*Initial fasting (zinc) (μg per 100 ml)*
A	23	77	28	160	162	98	<5	127
B	18	58	22	162	147	104	6	99
C	36	67	27	162	155	110	<5	96
D	26	91	27	149	156	143	<5	97
E	34	100	33	154	154	55	5	134
F	55	98	31	125	169	211	22	116
G	25	94	30	179	161	77	<5	111
H	23	68	23	163	158	73	<5	92
I	21	61	21	175	170	55	<5	94
J	44	94	30	161	168	136	<5	119
Mean	31	78	27	159	160	106	—	99
s.d.	12	17	4	15	7	48	—	33

Abbreviations: BMI, body mass index; CRP, C-reactive protein; Hb, hemoglobin.

variance as for the latter index, and an identical pattern of statistical differences among treatment arms was observed.

Zinc content of the zinc tablets

The specified Zn content per tablet on the package label was 20 mg. The average content measured by ICP-AES determinations for five tablets was 20.8 ± 1.0 mg per tablet.

Discussion

The radiation hazards of the initial radioisotopic tracer absorption studies with ^{65}Zn (Prasad *et al*., 1963) led to the 'zinc tolerance curve' approach, which was initiated in the 1970s (Oelshlegel and Brewer, 1977). Used extensively throughout the ensuing years in Guatemala (Solomons *et al*., 1979a, b, c, 1983a, b; Solomons and Jacob, 1981) and elsewhere (Capel *et al*., 1982; Boosalis *et al*., 1983; Sturniolo *et al*., 1983; Abu-Hamdan *et al*., 1986, 1988; Solis-Herruzo *et al*., 1989), it reigned until the advent and perfection of more sophisticated and physiological techniques of stable isotopic tracers (King *et al*., 1978; Turnlund *et al*., 1982; Hambidge *et al*., 1998). Although there is no strict correlation with quantitative Zn absorption (Valberg *et al*., 1985; Tran *et al*., 2004), the Zn tolerance approach still serves as a useful indicator of the relative bioavailability among treatment situations in the same subjects when, as here, the issue is uptake from oral supplements.

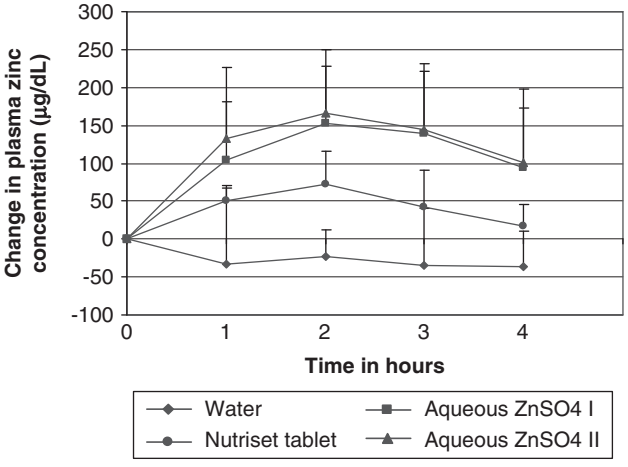

Figure 1 The mean (s.d.) of the serial changes in circulating zinc concentration, expressed in µg per 100 ml over 4-hourly blood samplings after oral ingestion of pure water (diamond symbol), 30 mg of elemental zinc from one-and-a-half NutriSet 20 mg Zn tablets (circle symbol) and 30 mg of elemental Zn from 82.4 mg of zinc sulfate monohydrate on the first occasion (square symbol), and a second occasion (triangle symbol). Only 8 of 10 original subjects completed the second administration of the aqueous zinc sulfate absorption test. A light snack was provided after the 180 min blood sampling.

NutriSet supplies the standard 20 mg $ZnSO_4$ tablets for intervention programs of the United Nations agencies as an adjuvant for routine oral rehydration. They formulated the active treatment (5 mg Zn) and placebo tablets for intervention studies in Tanzania (Sazawal *et al*., 2007), Nepal (Tielsch *et al*., 2007) and Guatemala (Mazariegos *et al*., 2010). Rivera (2007) commented on the first two by noting that, at the end of the intervention, the difference in average circulating Zn concentration between Zn supplement participants and controls in the subsamples with blood extraction (Sazawal *et al*., 2007; Tielsch *et al*., 2007) was only minimal (<10%). Such small increments were below the range of effect sizes generally seen in similar age groups receiving daily Zn doses of 5–10 mg/day for comparable durations. He speculated that the lack of growth effects in these studies was related to poor absorption of the interventions' Zn source (Rivera, 2007). In a trial in Guatemala, with 5 mg of daily Zn and placebo from the same supplier, administered through the second semester of life, reported a similarly low, ~10% greater increase in the treatment infants (Mazariegos *et al*., 2010).

The NutriSet tablets were confirmed to contain the elemental Zn content declared by the manufacturers. Nevertheless, we observed a significantly lower plasma Zn response to the NutriSet tablets, dispersed in water, as compared with dissolved reagent-grade $ZnSO_4$ powder, and conclude that there might be differential bioavailability in the two presentations. Our conclusions on the absorption efficiency of Zn from NutriSet tablets contrast with those of the only other relevant experience, that of Kraushaar *et al*. (2008). Any direct comparison between studies, of course, is impossible. The latter employed a single 10 mg dispersible

Table 2 Comparison of plasma zinc responses (maximal change, cumulative sum of 3-hourly changes) for the different treatment arms of the study, each conducted in healthy, male subjects

Treatment	*Water* (n = *10*)	*NutriSet tablet* (n = *10*)	*Aqueous $ZnSO_4$ I* (n = *10*)	*Aqueous $ZnSO_4$ II* (n = *8*)
Dose of oral Zn (mg)	0	30	30	30
Maximal changes in [Zn] (µg per 100 ml)	23 ± 62*,a (6)	76 ± 39^{b} (84)	178 ± 78^{c} (150)	191 ± 85^{c} (213)
∑3-hourly changes in [Zn] (µg per 100 ml)[a]	−41 ± 126^{a} (−27)	182 ± 137^{b} (227)	490 ± 245^{c} (416)	544 ± 298^{c} (659)

Abbreviations: Zn, zinc; $ZnSO_4$; zinc sulfate.
Superscript letters represent MANOVA comparison. Values not sharing the same superscript letter within a row are statistically different using the least significant difference *post-hoc* test ($P<0.05$).
*Mean ± s.d. (median) (all such values).

NutriSet tablet with extrinsic 0.5 mg of Zn^{70} in a single subject and determined true quantitative uptake with a reference intravenous Zn^{67} dose and compared the result with a pooled historical database of reference Zn absorption (Kraushaar *et al*., 2008). In contrast, we used the scored 20 mg NutriSet Zn tablets, that is, the standard formulation for the current World Health Organization recommendations for dehydrating diarrhea therapy (WHO, 2004), adjusted to a 30 mg dosage, in a relative bioavailability paradigm using the plasma Zn response; we studied 10 subjects, who served as their own comparisons through three arms of a placebo-controlled design.

The exact mechanisms of action for Zn in ameliorating the diarrheal state are not currently understood (King *et al*., 2003). On the basis of an 'antiseptic' effect of Zn salts in the treatment of gingivitis (Schaeken *et al*., 1994, 1996), Roberto Schneider in Guatemala in the 1970s advanced the notion for an intraluminal site of action for Zn in acute diarrhea (RE Schneider, unpublished observations, 1979). In practical terms, for the use as an adjuvant to reduce the duration and severity of acute diarrheal episodes, it is not immediately evident that reduced Zn bioavailability impair effectiveness, as the effect might be intraluminal. For the issues of growth and immune restoration in the aforementioned field trials (Sazawal *et al*., 2007; Tielsch *et al*., 2007; Mazariegos *et al*., 2010), however, efficient systemic uptake of the Zn would seem crucial for effect.

We acknowledge limitations of our study, related to sample size and our choice of Zn absorption method. Although small, a sample of 10 individuals is a typical number of subjects for this type of oral zinc-response study; in both repeated-measures and intergroup designs, published studies often have had 15 or fewer subjects per group (Solomons *et al*., 1979a; Solomons and Jacob, 1981; Boosalis *et al*., 1983; Abu-Hamdan *et al*., 1986, 1988; Castillo-Duran and Solomons, 1991a, b). As discussed in the Subjects and methods section, the 30 mg dosage of Zn was chosen, to exceed slightly the 25-mg dose used in previous Guatemalan studies (Solomons and Jacob, 1981) out of caution to detect a robust response. The alternative would have been using a single 20-mg tablet. In fact, a 'ceiling effect' seems to occur within this dosing range (Tran *et al*., 2004).

Wallock *et al*. (1993) showed that meal consumption produces a progressive decline in circulating Zn concentration. Although the peak rise in Zn concentration occurred by the end of the third hour of study, that is, before the snack, the downward deflection of Zn concentration of all three treatments to the final hour may have been influenced. Any artifactual effect of breaking the subjects' fast, however, would be equally distributed across treatments.

Our subjects experienced unanticipated nausea and vomiting with the 30 mg of Zn in aqueous solution. Vomiting did not occur with either the 20 or the 30 mg dose in any of the subjects in a dose–response study in Colorado (Tran *et al*., 2004; CD Tran, personal communication, 2009), nor was it seen frequently, if at all, with 25 mg reference doses in the earlier studies (Solomons and Jacob, 1981; NW Solomons, unpublished observations, 1979–1984). As indicated by the strict overlapping of the two response curves for repeat Zn challenges in Figure 1, a greater and lesser vomiting experience has negligible effect on the resultant serum Zn response, and any regurgitated Zn from the sulfate compound would only have mitigated the true magnitude of its superiority over the tablet form. However, vomiting after ingestion of oral Zn is not totally unknown. It was noted as a side effect by the World Health Organization office promoting its use with oral rehydration (Fontaine, 2001) and confirmed to be more prevalent than with conventional oral rehydration therapy in meta-analysis (Lazzerini and Ronfani, 2008). With respect to the propensity of Zn salts to induce nausea and vomiting, at least at the 30 mg supplementation dose used here, the presentation in tablet form was obviously less emetic and better tolerated than the reagent grade Zn sulfate. This is a potential point in its favor when used adjunctively in acute diarrhea wherein absorptive efficiency may be secondary.

From the relative magnitudes of responses in this basic, but practical, indicator of post-supplementation uptake of Zn, we see that the NutriSet tablets provide more uptake than a placebo situation, although with only about half of the response of that of an aqueous solution of $ZnSO_4$. We have no ready explanation for either the difference in relative bioavailability or that of relative emetic responses between the two formulations. A facile explanation would relate to the excipient ingredients in the tablet. Although the pill is predispersed in water before ingestion, it retains some sort of 'coating' effects of the excipient. Such 'shielding' of the Zn might improve its gastric tolerance but impairs its uptake lower down in the small intestine. The trade-off for better tolerance is reduced systemic uptake of the Zn. Thus, the less-than-expected cumulative effects to raise Zn concentrations above background levels in intervention studies involving NutriSet (Sazawal *et al*., 2007; Tielsch *et al*., 2007; Mazariegos *et al*., 2010) may be explained in part by a reduced bioavailability of the tableted form. The full implications for achieving maximal efficacy in field studies or community programs with supplemental oral Zn need further elucidation.

Conflict of interest

The authors declare no conflict of interest.

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