

The effect of zinc supplementation on parasitic reinfection of Guatemalan schoolchildren^{1,2}

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ABSTRACT One hundred thirty children (65–95 mo old) from a low-socioeconomic neighborhood of Guatemala City participated in a randomized, double-blind, controlled trial of zinc supplementation. One group received 10 mg Zn/d ($n = 65$) and the other group received a placebo ($n = 65$); 90 ± 9.2 doses were given over 120–150 d. Stools were examined for prevalence and intensity of helminths and prevalence of protozoa at the beginning and end of the study. The initial prevalence was 42% for helminths and 18% for protozoa, with no differences between groups. Mebendazole was administered to all children, and protozoal infections were treated specifically at the beginning of the study. The reinfection rates were 17% (11 of 65) for helminths and 12.3% (8 of 65) for protozoa in the zinc group and 15% (10 of 65) and 10.7% (7 of 65), respectively, in the placebo group ($P > 0.05$). Analysis by specific parasites revealed no treatment effect. We conclude that neither plasma or hair zinc status nor oral zinc supplementation had an effect on parasite status in children. *Am J Clin Nutr* 1993;57:673–8.

KEY WORDS Nematodes, tapeworms, protozoans, Kato-Katz, plasma zinc, hair zinc

Introduction

Differential environmental exposure to infective forms of pathogenic protozoa or human intestinal helminths can account for different rates of parasitic infection. When exposure is relatively constant, however, the determinants of susceptibility to parasitic infection have yet to be enumerated. Individual and familial epidemiological aspects (1), age (2, 3), sex (3), and genetic (4), nutritional (5), and immunological variables (6–8) can all be invoked as factors in differential vulnerability to parasitic infection.

Zinc is a trace metal nutrient that influences many physiological functions, including immunity (9, 10). Immune function can be involved in protection from parasites in several ways (11). For some helminths one possible role for zinc would be to modify the destruction of the infective larval forms during their tissue migration phase before establishing adult forms in the gut lumen (12). Evidence from Jamaica suggests that zinc nutritional status may influence infections with *Trichuris trichiura* (13), nevertheless, this relation has not been studied in depth.

Alternatively, specific nutrients can exert their effects by direct contact with organisms in the gastrointestinal lumen rather than by way of the nutritional status of the host. Zinc is poorly absorbed, showing an absorption efficiency ranging from 10% to

40% in various human studies that used isotopic tracers (14). Dental research has shown that zinc salts have antiseptic properties against the bacteria of plaque (15, 16). By analogy, other unicellular organisms such as protozoa might be influenced in their proliferation, virulence, or cyst-trophozoite transition by intraluminal ionic exposure. Zinc concentration in the gut may also have a direct effect on parasite (nematode) reproduction, as it has been known to affect reproduction in different ways in many other organisms (17–19).

A randomized, controlled, double-blind clinical trial of zinc supplementation in Guatemalan schoolchildren was used to test the hypothesis that increased dietary intake of zinc, either through an effect on nutritional status or through a change in the passage of unabsorbed zinc through the gastrointestinal tract, would influence the acquisition of parasites. We also determined whether plasma and hair zinc concentrations were correlated with parasite prevalence and infection intensity in children.

Subject and methods

Subjects

The study was conducted among the primary school children of the Fe y Alegria School in the northeastern sector of Guatemala City. The present work was derived from a larger collaborative study of the University of Guelph, Canada, and the Center for Studies of Sensory Impairment, Aging, and Metabolism (CeSSIAM) to determine the effect of zinc supplementation on growth and functional and biochemical variables related to zinc metabolism in Guatemalan children. The present observations were appended to, and subject to, the overall design of two studies (20, 21). We took the opportunity to examine parasite issues.

Children were eligible for inclusion in the study if they were between 65 and 95 mo of age at the beginning of the study and were not receiving vitamin and/or mineral supplementation at home in the last 2 mo. One hundred sixty-two children fit the

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inclusion criteria. Of these 162 children, 130 completed the plasma, hair, and fecal sample data collection at the beginning and end of the study. The data presented in this article are based specifically on this latter group. None of the participating children had any obvious chronic disease. The protocol was approved by the Human Ethics Committee of the University of Guelph and by the Human Studies Committee of CeSIAM. Children were pair matched by sex and age to constitute two groups; thereafter, a coin toss was used to assign the groups to one or the other coded treatment. On the basis of the prevalence of parasite infection of $\approx 50\%$ found in the general population of the studied group and by using a significance level of 5% with a power of 80% for a bilateral test, and considering 25% as an important difference in prevalence for both groups, it was necessary to have 58 subjects in each group to test this hypothesis (22). The final n was 65 subjects for each group. The group receiving zinc supplementation is designated +Zn; the group receiving the placebo is designated -Zn.

Zinc supplementation

Two sets of color-coded tablets were prepared by a pharmaceutical company in Canada (CE Jamieson and Co, Windsor, Ontario). One set was blue and the other was orange. One tablet contained only vitamins and minerals, excluding zinc; the other tablet contained 10 mg Zn as an amino acid chelate along with the remaining micronutrients. A uniform single dose of 10 mg Zn/d was used for obvious operational reasons; moreover, the justification for using 10 mg Zn/d as a single dose was based on the recommendations of the National Research Council of the United States (23). Because the US recommended dietary allowance for infants varies between 5.5 and 6.3 mg Zn/d and because the recommendations of the World Health Organization (WHO) for adult males is 11 mg Zn/d (24), the 10-mg dose was considered adequate for male and female children aged 5–8 y and of various weights. In addition, the use of zinc as an amino acid chelate improves intestinal absorption and the utilization of zinc in the diet compared with other zinc preparations.

The tablets were administered to the children between 0900 and 1000 on days that the children attended classes, excluding holidays and weekends. The identity of the pills was not known by the investigators until the study ended and the code was broken. The content of the pills was confirmed by direct analysis. The +Zn and placebo supplements provided 0.27 mg vitamin A (acetate), 3.3 mg vitamin E (*d*- α -tocopheryl succinate), 100 mg vitamin C (ascorbic acid), 100 μ g folic acid, 1.5 mg thiamin (mononitrate), 1.2 mg riboflavin, 10 mg niacinamide, 1.0 mg vitamin B-6 (pyridoxine hydrochloride), 6 μ g vitamin B-12 (cyanocobalamin), 10 μ g vitamin D (ergocalciferol), 10 mg pantothenic acid (calcium pantothenate), 7 mg Fe (ferrous fumarate), 50 μ g Cu (amino acid chelate), 110 μ g I (from kelp), 50 μ g Se (sodium selenate), 2 mg Cr (amino acid chelate), and 110 mg Mg (oxide). Strictly from an intestinal parasite point of view, zinc alone would have been sufficient to test the hypothesis, but because this study had a nutritional design, associated micronutrient deficiencies would have confounded our ability to detect a unique effect of zinc on growth.

Evaluation of zinc status

Plasma zinc analysis. Fasting blood samples (4–7 mL) were drawn from an antecubital vein through 22-gauge stainless steel needles into a 10-mL plastic disposable syringe, with subjects in

a sitting position. Blood was transferred immediately into 7-mL trace metal-free heparinized evacuated tubes (Vacutainer, Becton Dickinson, Rutherford, NJ) previously checked for contamination, and was centrifuged at $1112 \times g$ for 15 min. The plasma was separated, transferred into zinc-free plastic tubes, and frozen at -20°C until transported to Guelph, Canada. The plasma specimens were stored at -70°C until analysis. Zinc content in plasma was determined by flame atomic-absorption spectrophotometry with a Varian AAS (model Spectra-30; Varian Techton, Georgetown, Ontario), by using the method of Smith et al (25). Plasma was diluted 1:4 in double-distilled deionized water and standards were prepared in glycerine (5% vol:vol) to compensate for plasma viscosity. Concentration were expressed in $\mu\text{mol/L}$. A cutoff point of 10.71 $\mu\text{mol/L}$ was used. This cutoff point is frequently cited in the literature and is considered the lower limit for normal adults and children in these laboratories (26, 27). The lower tertile of the distribution in this case 13.2 $\mu\text{mol/L}$ was also chosen as a particular cutoff point to separate between low and normal plasma zinc values.

Hair zinc analysis. Hair samples (20–50 mg) cut close to the occipital portion of the scalp with stainless steel scissors were collected. Samples were washed by using a modification of the nonionic detergent procedure of Harrison et al (28) and then were analyzed for zinc and copper by instrumental neutron-activation analysis as described previously (29). The cutoff points used as indicative of suboptimal hair zinc status were $< 1.07 \mu\text{mol/g}$ (26) and the lower tertile of the distribution, $< 1.34 \mu\text{mol/g}$.

Fecal examinations. Stools were collected in cardboard cartons at home and brought in the morning to the school. The fecal samples were examined microscopically on the same day in duplicate for the presence of protozoa by using a concentration technique (light microscope 40 \times). A modification of the Kato-Katz quantitative procedure for determining the intensity of helminth infection, as number of eggs per gram of stool, was performed (30, 31). Approximately 50 mg of a glycerine-treated stool sample was spread evenly over a defined 2-cm² area on a glass slide, with use of malachite green for contrast coloration. The modification of Forrester and Scott (31) was used, in which the number of eggs was recorded in a transect of 10 low-power (10 \times) fields of the sample. If no eggs were found in the first 10 fields (5 mg stool), the entire 50-mg sample was examined. Egg counts were expressed as eggs/g stool, based on the average of the duplicate samples, by using appropriate conversion factors. This technique is not sensitive for detecting < 1 egg/50 g stool.

Treatment with antiparasitic drugs. After the fecal sample was obtained, all the children received mebendazole (100 mg twice a day for 3 d), and children who had protozoans received specific therapy with metronidazole (40–50 mg \cdot kg⁻¹ \cdot d⁻¹ divided in three doses for 10 d for the presence of *Entamoeba histolytica* and 20–30 mg \cdot kg⁻¹ \cdot d⁻¹ divided in three doses over a 5-d period in the case of *Giardia lamblia* infection). Those positive for *Hymenolepis nana* received treatment with niclosamide at the end of the study.

Statistical analysis

Chi-square analyses were used to test differences between proportions (positive or negative for parasite). For the analysis of the intensities, Student's two-tailed *t* test for paired and nonpaired data, as appropriate, was used. Previously, the data were trans-

formed into logarithms because the distributions were not normal. Odds ratios were used to establish the relative risk of being infected or reinfected with parasites, given a higher or lower plasma zinc concentration. A linear regression was also run between plasma and hair zinc concentrations and for each of these indexes vs the intensities of *Trichuris* and *Ascaris* after logarithmic transformation to normalize the data. The data are presented as mean \pm SD.

Results

One hundred thirty children (ages 81.5 ± 7 mo) who fulfilled the inclusion criteria were included in the study. Sixty-five children from each group gave fecal samples at the beginning and end of the study; only these samples are considered in the data analysis.

Zinc status

The initial concentration of plasma zinc was 14.3 ± 2.3 $\mu\text{mol/L}$ for 130 subjects from whom blood samples were obtained. The +Zn and -Zn groups had initial zinc concentrations of 14.1 ± 2.3 and 14.5 ± 2.4 $\mu\text{mol/L}$, respectively (NS). Seven percent of the children had plasma zinc concentrations < 10.71 $\mu\text{mol/L}$. The plasma zinc concentrations in the +Zn and -Zn groups at the end of the study were, respectively, 16.1 ± 2.4 and 14.9 ± 2.3 $\mu\text{mol/L}$; the difference between the two groups was significant ($P < 0.05$). Only the +Zn group had plasma zinc concentrations at the end of the study that were significantly different than those at the beginning of the study ($P < 0.05$). In this second collection, 10% of the children had plasma zinc concentrations < 13.2 $\mu\text{mol/L}$ and only five children (3.7%) had concentrations < 10.71 $\mu\text{mol/L}$. Four of these five children were in the -Zn group.

The initial hair zinc content was 1.6 ± 0.49 $\mu\text{mol/g}$ (median 1.6) for the total group, 1.58 ± 0.45 $\mu\text{mol/g}$ for the +Zn group, and 1.61 ± 0.54 $\mu\text{mol/g}$ for the -Zn group. Thus, the difference in hair zinc contents was not statistically significant nor were there significant differences at the end of the study between the +Zn and the -Zn groups. The global hair zinc content of 1.38 ± 0.43 $\mu\text{mol/g}$ was significantly lower at the end of the study (paired t test < 0.001). No correlation was found between plasma and hair zinc concentrations ($r = -0.03$).

Zinc status vs parasite status

No difference in the prevalence of fecal helminths (*Ascaris* and *Trichuris* together) was detected at the beginning of the study between the children with plasma zinc concentrations < 13.2 $\mu\text{mol/L}$ ($n = 36$) and those with plasma zinc concentrations ≥ 13.2 $\mu\text{mol/L}$ ($n = 94$) ($P = 0.20$). There were no differences in the prevalence of parasites, by chi-square test, when < 10.71 $\mu\text{mol/L}$ was used as the cutoff point for zinc plasma concentrations. Differences in *Ascaris* and *Trichuris* intensities were not found between the children with normal and low plasma and hair zinc status. Pearson product-moment correlations were performed between plasma or hair zinc concentrations and the logarithmic transformations of the intensities of *Ascaris* and *Trichuris*; they were not significant ($r = -0.02$ to $+0.21$, $P > 0.05$).

Parasite status and zinc treatment

At the beginning of the study the 130 children sampled had an overall infection rate of 53% for one or more species of all

helminths and protozoa, with infection rates of 52% and 54% for the +Zn and -Zn groups, respectively. Of these, 38% (13 of 34) of the +Zn group and 29% (10 of 35) of the -Zn group were infected with multiple organisms. The individual species detected in the initial survey are listed in Table 1. The prevalence for helminth infection for the +Zn group was 43% (28 of 65) and was 41% (27 of 65) for the -Zn group. The prevalence of predominant helminths for the total group was 21% (21 of 130) for *Ascaris lumbricoides* and 28% (36 of 130) for *Trichuris trichiura*. Initially, 11 subjects in the +Zn group were found to be infected with protozoa compared with 10 children in the -Zn group. The predominant protozoal species was *Giardia lamblia*. No significant differences in initial parasite status were seen between the two treatment groups.

After mebendazole therapy, and after a 120–150-d interval, 15% (10 of 65) of the +Zn group and 14% (9 of 65) of the -Zn group manifested a helminthic infection. This represents a 65% reduction from the prestudy rate for the former group, and a 66% reduction for the latter. No statistical difference between treatments was seen with regard to the final parasite status. Table 1 shows the specific organisms present in the second sampling. No differences in infection intensities were found in the +Zn or -Zn groups either at the beginning or at the end of the study.

Predisposition to parasitic reinfection

We examined the data for any predisposition for infection that may have been detected at the end of the observation period. Because no treatment effect was shown, the entire data set was pooled for the subsequent analyses. The chance that a child was infected or reinfected on the second sampling was twice as high if the child had had a parasite in feces at the baseline time point. For roundworms, the predisposition was five times higher for the children who had nematodes at the first examination. We did not find an increased risk of having a protozoal infection at the end of the supplementation if protozoa had been found at the beginning of the study.

A twofold increase in the risk of being infected with *H. nana* was documented among children who had been previously infected with other helminths as compared with those without helminths at the first examination. Nevertheless, the number of cases was small.

TABLE 1

Absolute number of parasites identified before and after treatment in unsupplemented (-Zn) and zinc-supplemented (+Zn) groups*

Parasite	Initial		Final	
	-Zn	+Zn	-Zn	+Zn
<i>Ascaris lumbricoides</i>	15	13	4	4
<i>Trichuris trichiura</i>	16	20	4	5
<i>Uncinaria</i>	2	0	0	1
<i>Oxiuros</i>	1	1	0	0
<i>Giardia lamblia</i>	10	8	7	6
<i>Entamoeba histolytica</i>	2	4	0	2
<i>Hymenolepis nana</i>	1	2	2	3

* $n = 65$ for each group. Parasites were identified in stool samples by microscopic examination.

Discussion

The concept of interaction of nutrition and infection was refined in the classic 1968 monograph by Scrimshaw et al (32). The specific message was a potential for the nutritional state of the host to influence susceptibility to infection. This was usually synergistic, in which malnutrition increases the incidence, duration, or severity of the infection; however, often there was an antagonistic interaction, i.e., one in which malnutrition decreased infection, presumably because of a lesser availability of nutrients for the parasite. Parasites and obligate intracellular organisms were the most likely to show the antagonistic interaction with a host's nutrition (32).

Parasitic infection is widespread in developing countries where there is a lack of hygienic education and the sanitation infrastructure found in industrialized nations. Experiences at CESSIAM have shown rates of nematode infection from 14% to 43% in Guatemalan urban schoolchildren (33). Zinc deficiency was first discovered in juvenile boy populations in the Middle East (34, 35), where they consumed a diet high in fiber and phytate. The staple food of Guatemala is corn tortilla, which has a high fiber content and interferes with the uptake of zinc (36). Moreover, Guatemalan children grow well below their theoretical growth potential, and it was worth exploring whether some of the growth limitation was related to zinc insufficiency. The overall research effort was directed at this question and the parasite observations were derivative. Zinc in the circulation represents < 1% of the total body pool (37) and it is influenced by factors unrelated to nutritional status, i.e., infection or other stresses could depress plasma zinc concentration (38).

The fact that the plasma zinc concentration rises with supplementation is not a *prima facie* indication of a preexisting deficiency, as circulating concentrations are poorly regulated. Hair zinc has been proposed as an index of the chronic status of trace mineral nutrition. Nevertheless, it should be taken with caution because it is influenced by the mean rate of hair growth and environmental contamination by external sources (39). The poor correlation between hair zinc and plasma zinc concentrations has also been stressed (40). An epidemiological interpretation of our biochemical data suggests that $\geq 7\%$ of the children were deficient, with plasma zinc concentrations < $10.71 \mu\text{mol/L}$ in the original survey.

On the basis of the results obtained in the functional assessment of the zinc variables in the longitudinal study (21), it was noted that the most consistent and notable changes observed post-supplementation were in the Z scores for triceps skinfold thickness and midarm circumference: children who received the zinc supplement gained more fat and lost less muscle. A significant increase in linear growth was not detected in the +Zn group possibly because the study was not long enough to observe this change; the change in fat deposition showed that at least some degree of zinc deficiency was observed in this group.

We supported a two-tailed hypothesis as to the direction of influence of human zinc status on parasitic infections. Low zinc status could either favor or not favor the reestablishment of an infection, and it could act differently on different classes of parasites. The evidence suggesting a relationship between zinc status and parasitic infections is meager. Bundy and Golden (13), in Jamaica, reported a slight relationship between plasma zinc concentrations and *Trichuris* infection intensities in children. Researchers in Quebec have demonstrated that challenge infec-

tions, but not primary infections, with the murine nematode *Heligmosomoides polygyrus*, are affected by a moderate dietary zinc deficiency (41). Worm burdens and egg production are increased in zinc-restricted mice on second exposure to helminths. Our assignment of children to treatments resulted in an absolute initial comparability of groups in terms of percentage of infection and number and relative distributions of parasites. Exposure to parasitic infection was widespread, as demonstrated by the 53% prevalence of all infections at the baseline sampling and the rapid renewal of reinfections after treatment. Although the dose of zinc, its form of administration, and the duration of treatment may not have been optimal to unmask whatever zinc-parasite reinfection interaction might exist in nature, the study design and high compliance of the patients allowed us to make certain conclusions about the influence of zinc on the posttreatment evolution of reinfection in this population. The zinc effect per se was clearly null. If a true differential response to parasitic exposure exists between zinc-deficient and zinc-adequate subjects, the lack of a sufficient number of zinc-depleted subjects could account for a less-than-complete testing of the nutritional aspect of the hypothesis.

Our overt question was whether zinc supplementation would alter parasitosis. There are at least three possible scenarios: 1) zinc in the lumen influences infection, 2) zinc status (as a continuous variable) influences parasitosis, and 3) prevalence of zinc deficiency (as a dichotomous variable with a defined cutoff point) influences parasitism. If the latter mechanism is in fact operative in nature, the underlying prevalence of zinc deficiency in the study population may have caused difficulty in detecting an effect with the present sample size. However, it is equally, or more likely that any effect of zinc on parasites is a local intestinal effect or one that operates both above and below any conventional cutoff point for zinc deficiency.

We observed a rapid reinfection rate during the 120-150 d. We call this reinfection, although our design did not allow us to confirm the absence of fecal pathogens immediately after the course of anthelmintic and antiprotozoal therapies. The drug courses were those specified for the respective agents. Our dosage of mebendazole has been reported to be between 84% and 100% effective against *A. lumbricoides* (42, 43) and up to 90% effective against *T. trichiura* (44). Metronidazole is very active against luminal amebiasis, but recurrences after treatment with this drug alone are common. Resistance of *E. histolytica* to metronidazole is unknown (45). This drug is also the treatment of choice for *G. lamblia* (46). We treated *H. nana* with niclosamide at the end of the course. We accept in our design that this tapeworm is the one organism that should have been unaffected by the main treatment.

For the sake of the interaction analyses we are assuming that the majority of infections had disappeared and infections detected in the second sampling were primarily new, with the exception of *H. nana*. The minimum interval from ingestion of an infective egg to the production of eggs by new adult female worms is 60 d for *A. lumbricoides* (47), 70-90 d for *T. trichiura* (48), and 42 d for *H. nana* (48). These are consistent with the possibility of detecting the new infection during the 120-150-d interval between treatment course and reevaluation. In this context we found a slight tendency for the subjects with any type of parasitic infection (protozoal or helminthic) to have some type of infection on the second round. However, if we restrict our analysis of recurrence to the two nematodes with a similar life cycle,

which soil-incubated eggs must be ingested, i.e. *A. lumbricoides* and *T. trichiura*, we find a much stronger relationship between initial presence of parasites and reinfection with either of the helminths. One is five times more likely to have one or the other nematode after treatment if one had a roundworm infection originally. The findings with regard to *H. nana* are interesting and consistent with the recognized biology and life cycle of this organism. These tapeworms can become established by autoinfection, either internally (by the development of eggs to adult forms released in the small intestine) or externally from a faecal-oral route. Females have a maximum life span of 10 wk; therefore, new females would have been producing the eggs observed after 5 mo whether effective treatment for this organism had been administered or not. Thus, the fact that 75% of those children originally excreting *H. nana* ova still had an infection with the same organism is not surprising, because children were not treated for *H. nana* until the end of the study. Of interest is the twofold greater new infection rates with *H. nana* among children previously infected with other helminths as compared with those without helminths at the first examination. This again implies a predisposition among traditional hosts of helminths but also may suggest that *H. nana* is truly a solitary species. It may require that the intestine be free of other helminths, as it would be after mebendazole treatment, for infective *H. nana* eggs to become established in the host.

Children in urban environments are susceptible to parasitism by infective cysts and ova. Altering dietary intake of zinc, with its presumed effect on nutritional reserves of the host and intraluminal ion concentration, could be postulated to influence the process of infections with protozoa and helminths on a number of theoretical grounds. Our data, however, are free of any evidence of a treatment effect. On the other hand, certain insights into the influence of a previous parasite status (be it infected or parasite free) on predisposition to new infections were revealed. A specific subsegment of the population seems to be more susceptible to repeat infections with helminths in general and nematodes in particular. The tapeworm *H. nana* appears to have the inclination to occupy niches in intestinal tracts that are free of other helminths.

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