

Zinc Metabolism in Adolescents with Crohn's Disease

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ABSTRACT

Low serum zinc concentrations have been reported in Crohn's disease (CD) and overt zinc deficiency has been described, but little is known about the effect of CD on zinc metabolism in adolescents. The aim of this study was to measure zinc absorption, endogenous fecal zinc excretion, urinary zinc excretion, and zinc balance in children with stable CD and in matched controls. Subjects were 15 children, ages 8–18 y, with stable CD, and 15 healthy matched controls. Subjects were adapted to diets providing 12 mg/d elemental zinc for 2 wk, and then admitted for a 6-d metabolic study. Stable zinc isotopes were given intravenously and orally, and urine and feces collected for 6 d. Fractional zinc absorption, endogenous fecal zinc excretion, and zinc balance were calculated using established stable isotope methods. In subjects with CD, zinc absorption ($10.9\% \pm 6.1$ versus

23.4 ± 15.8 , $p = 0.008$) and plasma zinc concentration ($0.85 \text{ mg/dL} \pm 0.15$ versus 1.25 ± 0.35 , $p = 0.004$) were significantly reduced, compared with controls. Despite this, there were no significant differences in endogenous fecal zinc excretion ($2.0 \text{ mg} \pm 1.5$ versus 1.5 ± 1.5 , $p = 0.34$) or urinary zinc excretion ($0.9 \text{ mg} \pm 0.7$ versus 1.0 ± 0.7 , $p = 0.47$). Zinc balance was significantly lower in CD ($-1.5 \text{ mg} \pm 1.5$) than in controls ($+0.6 \text{ mg} \pm 3.1$, $p < 0.0001$). In conclusion, adolescents with CD have significantly reduced zinc absorption. Despite this, they were unable to reduce endogenous fecal zinc excretion to restore normal zinc balance and had a significantly worse zinc balance and lower plasma zinc concentration than controls. (*Pediatr Res* 56: 235–239, 2004)

Crohn's disease (CD) is a chronic, progressive inflammatory disorder that can affect the entire length of the gastrointestinal tract. Children with CD often present with growth stunting, weight loss, and nutritional deficiencies due to anorexia, poor intestinal absorption, and increased losses of nutrients in the gastrointestinal tract (1).

Zinc is a component of over 200 enzymes and is essential for normal immune function, DNA and RNA synthesis, and gene transcription. Zinc is absorbed along the length of the small intestine and is transported to the liver in the portal circulation. It is excreted into the gut (endogenous fecal zinc

excretion), and pancreatic and biliary secretions contain large amounts of zinc, most of which is subsequently reabsorbed. Zinc homeostasis is maintained by changes in fractional zinc absorption and in endogenous fecal zinc excretion (2). Endogenous fecal zinc excretion increases during periods of high zinc intake (3) and decreases during periods of zinc restriction (4).

Severe zinc deficiency leads to clinical features of acrodermatitis such as alopecia, anorexia, diarrhea, dermatitis, poor growth, and impaired immune function, and children are at especially high risk for zinc deficiency due to their high requirements for normal growth (2). Zinc deficiency was first reported in CD in the 1970s (5–7), and, in extreme cases, the clinical features of acrodermatitis have been described (8–11). The incidence of milder forms of zinc deficiency has been difficult to assess. Although several investigators have reported low plasma zinc concentrations in patients with CD (8, 12–18), very little body zinc is in the plasma and it is a poor measure of zinc status (2). Some studies have also demonstrated decreases in zinc-dependent enzymes [such as thymulin in the plasma (19) and metallothionein in the gut mucosa (20)], reduction in muscle zinc concentration (19), and poor taste acuity (6, 21) in patients with CD.

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Zinc deficiency in CD must presumably result from low gastrointestinal absorption or increased zinc losses (either in the urine or feces) because the self-selected diets of adults with CD typically contain a similar amount of zinc to that consumed by control subjects (22). A number of studies have examined zinc absorption in CD and have shown either normal (23, 24) or low zinc absorption (17, 18, 25). However, we are unaware of any study that has measured endogenous fecal zinc excretion in CD. Without this data, the absorption measurements are very difficult to interpret because endogenous fecal zinc excretion is an important site of zinc homeostasis. Subjects with CD might adapt to low zinc absorption by reducing endogenous fecal zinc excretion. Alternatively, they may have increased endogenous fecal zinc excretion, increasing their susceptibility to zinc deficiency.

The objectives of the current study were to test the hypotheses that fractional zinc absorption is significantly reduced, and endogenous fecal zinc excretion is significantly increased, in adolescents with CD when compared with healthy age-matched controls.

SUBJECTS AND METHODS

Study population. Fifteen patients with stable CD, 8–18 y of age, were recruited from the Inflammatory Bowel Disease Clinic of Texas Children's Hospital, Houston, TX, U.S.A. They were matched one-to-one with healthy age-, ethnicity-, and gender-matched adolescents.

Subjects with CD were considered eligible if they were aged 8–18 y, had a clinical diagnosis of CD, a history of good compliance with medical treatments and clinic visits, and had a CD Activity Index (CAI) between 150 and 250 (representing stable and relatively nonsevere disease). Subjects were ineligible if they had been hospitalized with an acute exacerbation of CD, required an escalation of medical therapy within 2 mo of the study, had undergone bowel resection, or had received systemic corticosteroids within 2 mo of enrollment. All subjects had previously documented small intestinal disease, either by endoscopic study or contrast radiology.

Controls were considered eligible if they were healthy, not on any regular medications (including vitamin, mineral, and zinc supplements), and were within 1 y of age of their match, the same ethnic group, and the same gender.

Ethical approval was obtained from the Baylor College of Medicine and Affiliated Hospitals Institutional Review Board. Parental consent was obtained in all cases, as was assent (as age appropriate) from the study subject.

Isotope preparation. Zinc-70 (74% enrichment by mass) and zinc-67 (90% enrichment by mass) produced in Russia were purchased from Trace Sciences Inc. (Toronto, ON, Canada) as the oxide. Aqueous solutions were prepared by the Investigational Drug Service of Texas Children's Hospital as described elsewhere (26). Solutions were tested for sterility before use, and intravenous isotopes were also tested for pyrogenicity.

Study protocol. After informed written consent was obtained, the subjects had a structured interview with a registered dietitian, who estimated their usual zinc intake. If required, a

dietary plan was devised to modify the zinc intake to approximately 12 mg/d. Typically, this involved increasing zinc intake by substituting a zinc-fortified breakfast cereal for an unfortified one, increasing chicken or beef intake, or both. At the time the study was carried out, the U.S. recommended dietary allowance (RDA) for zinc was 10 mg/d for children 7–10 y, 15 mg/d for males aged 11–18 y, and 12 mg/d for females 11–18 y (27).

Subjects remained on this diet for 2 wk before being admitted to the General Clinical Research Center of Texas Children's Hospital in Houston, TX, or the Metabolic Research Unit of the Children's Nutrition Research Center, Houston, TX, for a 6-d study period. In the week preceding admission, subjects kept a weighed record of all the food they consumed on two weekdays and one weekend day.

Subjects were admitted at approximately 0700 h and asked to empty their bladders. They then had 0.5 mg of an aqueous zinc-70 enriched tracer infused intravenously and 5–10 mL of blood drawn. Blood samples for plasma zinc analysis were collected in zinc-free tubes anticoagulated with ammonium heparin (Monovette 9 mL AH, Sarstedt, Newton, NC, U.S.A.). Subjects consumed a standard breakfast including 120 mL of orange juice to which 2.0 mg of an aqueous zinc-67 enriched tracer had been added 18–24 h earlier. A complete 6-d urine and fecal collection was then begun. Urine was collected and analyzed in 8-h pools. Each fecal collection was analyzed separately.

Diets during the 6-d admission were designed to provide 12 mg/d zinc. Complete records of all food and drink consumed during the study period were collected. These records, together with the food records for the 3 d before admission, were used to calculate the subjects' actual zinc intake using the Minnesota Nutrition Data System (University of Minnesota, version 2.91, Food Database 12A, Nutrition Database 27).

Sample preparation and analysis. Urine and fecal samples were digested in nitric acid, purified using anion exchange columns, and loaded on to rhenium filaments for mass spectrometry analysis as described elsewhere (28). Zinc isotope ratios were measured by thermal ionization magnetic sector mass spectrometry (Finnigan MAT 261, Thermo Finnigan, Bremen, Germany). Ratios were expressed with reference to the nonadministered isotope zinc-66 and mathematically corrected to the zinc-64/zinc-66 ratio to compensate for temperature-specific effects of fractionation (28). This method is capable of measuring isotope ratio with a relative standard deviation of <0.2% (29). Isotope ratios were converted to tracer:tracee ratios (TTR) (30). Plasma, fecal, and urinary zinc concentration were measured by atomic absorption spectroscopy. A plasma zinc concentration of <0.8 mg/L was taken to be suggestive of suboptimal zinc status.

Measurement of zinc absorption. Zinc absorption was measured using a modification of the dual stable isotope method described by Friel *et al.* (31) from the ratio of the fractional excretion of the oral and intravenous isotopes in the 8-h urinary aliquot collected 48 h after tracer administration, using the equation,

$$\text{Zinc absorption} = \frac{\text{TTR for oral tracer/dose of oral tracer}}{\text{TTR for i.v. tracer/dose of i.v. tracer}}$$

RESULTS

Measurement of endogenous fecal zinc excretion. Endogenous fecal zinc excretion was measured using methods previously described for calcium (32), where endogenous fecal zinc excretion is given by

Endogenous fecal zinc excretion =

$$\frac{\text{urinary zinc excretion in time } t \times \text{fecal excretion of i.v. tracer in time } t}{\text{urinary excretion of i.v. tracer in time } t}$$

Endogenous fecal zinc excretion was measured from the complete 6-d urine and fecal collection. Two subjects with CD did not collect any feces during the 6-d in-patient study, so endogenous fecal excretion was not calculated for them.

Zinc balance. Approximate zinc balance, ignoring losses in sweat, hair, and desquamated skin cells, was estimated from the equation

Zinc balance = (Zinc intake \times fractional zinc absorption)

– endogenous fecal zinc excretion – urinary zinc excretion

Statistical analysis. Statistical analysis was carried out using StatView v5.1 for Macintosh (SAS Institute, Cary, NC, U.S.A.). Comparisons between the two groups were made using the unpaired *t* test (for continuous variables) and the Fisher exact test (for categorical variables). Zinc balance was compared with zero balance using the one-sample *t* test (hypothesized mean = 0). The relationship between plasma zinc concentration and zinc absorption, endogenous fecal zinc excretion, urinary zinc excretion, and zinc balance was assessed using simple regression analysis. Data are given as mean \pm SD, unless otherwise stated, and statistical significance was assumed at $p < 0.05$. Ninety-five percent confidence intervals (95% CI) for the differences between the two groups were calculated.

Subject characteristics. Fifteen subjects with CD and 15 age-, gender-, and ethnicity-matched controls were recruited. Most CD subjects were receiving treatment with 5-aminosalicylic acid, allopurinol, and acetaminophen or a nonsteroidal anti-inflammatory agent. No subjects were taking oral steroids. Subjects with CD tended to have lower Hb concentrations and had significantly higher red cell distribution widths (Table 1). The zinc intakes for the two groups were similar (12.0 ± 2.5 mg/d versus 13.5 ± 2.2 ; $p = 0.09$; 95% CI, -0.29 to 3.19 mg/d).

Plasma zinc concentration. Fasting plasma zinc concentration was significantly lower in subjects with CD than for controls (0.85 ± 0.15 mg/L versus 1.24 ± 0.35 ; $p = 0.0003$; 95% CI, 0.19 to 0.59 mg/L). Plasma zinc concentration was <0.8 mg/L in three subjects with CD compared with none of the controls ($p = 0.48$, Fisher's exact test).

Zinc absorption, endogenous fecal zinc excretion, and urinary zinc excretion. Zinc absorption in the subjects with CD ($10.9 \pm 6.1\%$) was significantly lower than in the controls subjects ($23.0 \pm 16\%$; $p = 0.0003$; 95% CI for difference 3.47 to 21.5%). There was no difference in urinary zinc excretion (0.86 ± 0.65 mg/d versus 1.04 ± 0.69 ; $p = 0.47$; 95% CI for difference -0.32 to 0.68 mg/d) or in endogenous fecal zinc excretion (2.0 ± 1.5 mg/d versus 1.1 ± 1.5 ; $p = 0.34$; 95% CI for difference -1.76 to 0.64 mg/d). Zinc balance was significantly negative in the CD subjects (-1.5 ± 1.5 mg/d, one-sample *t* test hypothesized mean = 0, $p = 0.0019$) and significantly lower than in the control subjects (0.6 ± 3.1 mg/d; $p = 0.030$).

Effect of plasma zinc concentration. There was no significant correlation between plasma zinc concentration and fractional zinc absorption, endogenous fecal zinc excretion, or urinary zinc excretion for either the CD group or for the control group ($r^2 < 0.20$, $p > 0.20$ for all comparisons).

Table 1. Baseline blood chemistry values of the Crohn's disease and controls subjects

| Parameter | Controls | Crohn's disease | <i>p</i> Value |
|---|-----------------|-----------------|----------------|
| Age (y) | 14.8 ± 1.5 | 15.4 ± 1.7 | 0.35 |
| Height (cm) | 163 ± 15 | 159 ± 11 | 0.48 |
| Weight (kg) | 61.2 ± 10.4 | 54.6 ± 12.7 | 0.21 |
| Hemoglobin (g/dL) | 13.0 ± 1.6 | 11.9 ± 1.3 | 0.06 |
| Hematocrit (%) | 37.7 ± 4.9 | 35.4 ± 3.3 | 0.16 |
| Mean cell volume (fl) | 82.5 ± 5.7 | 83.8 ± 6.0 | 0.57 |
| Mean cell hemoglobin (pg) | 28.5 ± 1.3 | 28.3 ± 2.4 | 0.81 |
| Mean cell hemoglobin concentration (g/dL) | 34.6 ± 1.2 | 33.8 ± 1.1 | 0.09 |
| Red cell distribution width (%) | 13.2 ± 0.5 | 14.4 ± 1.6 | 0.015 |
| Serum sodium (mmol/L) | 141 ± 2 | 140 ± 2 | 0.12 |
| Serum potassium (mmol/L) | 4.3 ± 0.1 | 4.1 ± 0.4 | 0.18 |
| Serum chloride (mmol/L) | 105 ± 2 | 103 ± 4 | 0.10 |
| Serum bicarbonate (mmol/L) | 25.3 ± 1.9 | 23.8 ± 3.2 | 0.18 |
| Blood urea nitrogen (mg/dL) | 13.0 ± 3.6 | 10.7 ± 4.0 | 0.12 |
| Creatinine (g/dL) | 0.7 ± 0.1 | 0.6 ± 0.2 | 0.13 |
| Alkaline phosphatase (IU) | 190 ± 97 | 144 ± 77 | 0.19 |
| Serum albumin (g/dL) | 4.2 ± 0.3 | 4.1 ± 0.3 | 0.32 |

Continuous variables are given as mean \pm SD and compared using two-sample *t*-tests.

DISCUSSION

We found significant changes in zinc metabolism in children with stable CD compared with healthy age-, gender-, and ethnicity-matched controls. All subjects were adapted to a diet similar to the U.S. RDA for zinc at the time the study was carried out (27) and similar to the usual intakes of children in these age ranges (33). Subjects with CD had significantly lower fractional zinc absorption, lower plasma zinc, and worse zinc balance than the controls.

Although much remains to be learned about the absorption and cellular uptake of zinc in humans, a recent advance is the identification of a range of zinc/iron-regulated transporters (ZIP) superfamily of proteins (34) that transport a variety of cations in fungi, plants, and humans. They include the SLC39 family of transporters (SLC38A1-A4) (35). The gene SLC39A4 codes for the protein hZIP4 that is highly expressed in stomach, small intestine, and colon. Mutations in this gene have recently been shown to be the cause of the autosomal recessive disease of zinc uptake, acrodermatitis enteropathica (36, 37). Although some of the mutations described in this gene would be expected to have very significant effects on protein confirmation and function (38), acrodermatitis enteropathica responds clinically to high-dose zinc supplementation (39). This suggests that other, lower-affinity, or less-specific, transport proteins are involved in gastrointestinal absorption of zinc.

Zinc homeostasis is affected both by fractional zinc absorption and by zinc losses into the gut (endogenous fecal zinc absorption) (40). Studies in swine have demonstrated that an important metabolic adaptation to zinc deficiency is a decrease in zinc losses in pancreatic and biliary secretions (41). This is supported by previous studies of Chinese women that have shown that the principal adaptation to marginal zinc intake is decreased endogenous fecal zinc excretion (4). Therefore, one would expect that the poorer zinc status in the CD children should have led to reduced endogenous fecal zinc excretion. However, this was not the case. It appears that the children with CD were unable to down-regulate their endogenous fecal zinc excretion. Taken together, these findings suggest that children with CD are at risk of zinc deficiency due to reduced zinc absorption and an inability to compensate with a reduction in endogenous fecal zinc excretion.

Fasting plasma zinc was lower in the CD subjects than the controls, consistent with other studies in CD with (19) or without gut resection (6, 8, 14, 16, 17, 42). It has been suggested that the lower plasma (or serum) zinc in CD might result from a lower serum albumin concentration (12, 13). However, in our study, the serum albumin concentration was not different between the groups. It appears, therefore, that lower fasting zinc concentration in children with CD in our study truly reflects poor zinc nutritional status.

Several studies have suggested that urinary zinc excretion is lower in CD patients (17, 42–44), presumably as an adaptation to suboptimal zinc status. In our study, urinary zinc excretion was similar in the CD subjects and the controls. However, we studied subjects with stable CD, so we might have seen a difference in subjects with more severe CD or with more severe zinc deficiency (45).

The Institute of Medicine estimates that children aged 9–13 y need to absorb 0.2 mg/d for normal growth, whereas those aged 14–18 y require 0.2 mg/d (males) or 0.1 mg/d (females) (45). If we assume that zinc absorption, endogenous fecal zinc losses, or urinary zinc excretion would not change with increasing zinc intake, children with CD in our study would need total zinc intakes of 27–28 mg/d to achieve this. However, such intakes are close to, or above, the tolerable upper limit at these ages (9–13 y = 23 mg/d, 14–18 y = 34 mg/d) (45). The tolerable upper limit is based on possible adverse effects of high zinc intakes on copper status (45). Zinc intakes this high may raise concern for their adverse effect on copper status or retention in children with CD. These intakes cannot, therefore, be recommended without prospective, randomized studies to evaluate the effect of zinc supplementation at this level on zinc balance, copper status, and other markers such as disease activity and immune function.

In summary, this study demonstrates that children with mild stable CD are at risk of zinc deficiency due to low zinc absorption and inappropriately high endogenous fecal zinc losses. Further studies are needed to determine the optimum level of zinc supplementation for children with CD.

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