Zinc homeostasis and gut function in children with celiac disease

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ABSTRACT

Background: Celiac disease (CD) is an immunologic enteropathy triggered by the intake of gluten. It is thought that the enteropathy impairs gut function and absorption.

Objective: We assessed the zinc-absorption capacity and small-bowel integrity in children with CD.

Design: Children in whom a diagnosis of CD was considered clinically and either confirmed (n = 16; Marsh score ≥3) or not (n = 22; Marsh score of 0) with a small-bowel biopsy (SBB) were recruited. The fractional absorption of zinc (FAZ) was determined by the administration of an oral 67Zn dose (2.5 mg) and an intravenous 70Zn dose (0.2 mg) 2 h before and during the SBB, respectively. Spot urine samples were collected, and zinc isotopic ratios were determined by ion-coupled plasma mass spectrometry. Gut health was assessed by the ingestion of 13C-sucrose (20 g) after an overnight fast, and breath samples were collected and analyzed by isotope ratio mass spectrometry.

Results: There was no difference in FAZ between children with a Marsh score ≥3 (mean ± SEM: 0.68 ± 0.05) and children with a Marsh score of 0 (0.74 ± 0.05). The exchangeable zinc pool (EZP) was significantly (P < 0.05) lower in children with a Marsh score ≥3 (2.6 ± 0.8 mg/kg) than in children with a Marsh score of 0 (3.8 ± 1.4 mg/kg). Gut function in children with a Marsh score ≥3 (4.5 ± 0.7% cumulative dose recovered at 90 min) was lower than the lower cutoff of a normal gut-function breath test (5.06% cumulative dose recovered at 90 min) but not significantly different from that in children with a Marsh score of 0 (4.9 ± 0.4%). There was a significant (P < 0.01) correlation between zinc absorption and gut function in children with CD.

Conclusions: Zinc absorption did not appear below usual amounts in subjects with CD. Children with CD have impaired gut function that may affect their zinc nutritional status as shown by a smaller EZP; however, the EZP decrease in children with CD was not that may affect their zinc nutritional status as shown by a smaller

INTRODUCTION

CD is a common disorder that affects up to 1 in every 100 individuals (1, 2). In susceptible individuals, CD is triggered by an immune response to the ingestion of gluten-containing cereals, wheat, barley, and rye. The diagnosis of CD requires a histologic examination of the intestinal mucosa obtained by a duodenal biopsy. Infants with CD may present with anorexia, pallor, abdominal distention, vomiting, diarrhea, peripheral edema, and iron-deficiency anemia, and a significant portion can present with a short stature as a result of severe damage to the small intestinal mucosa, which impairs nutrient absorption.

Zinc is a trace element involved in numerous enzymatic reactions, biochemical functions, and immune responses (3, 4) and is, therefore, required for growth and cellular function. The results from meta-analyses and pooled analyses of multiple high-quality worldwide intervention studies have shown that the impairment of zinc absorption alone contributes substantially to growth failure (5). Human studies have measured plasma zinc concentrations to assess zinc absorption; however, this method is generally considered a qualitative test at best (6). Jones and Peters (7) also showed that some patients with CD who failed to respond to diet, steroids, and nutritional supplements made a recovery when zinc was administered, which suggested that these CD subjects were likely to be zinc deficient (8). A study that applied zinc stable-isotope techniques in patients with CD was performed by Crofton et al (9), who showed that untreated CD patients had an increased turnover and loss of intestinal endogenous zinc.

Zinc stable-isotope techniques provide a safe and potentially powerful test for zinc metabolism, which is an essential prerequisite for the estimation of zinc requirements. The FAZ, which is measured by the dual-isotope tracer ratio technique, has been widely used as an important variable of zinc homeostasis (10–12). It was hypothesized that children with CD will have impaired zinc homeostasis. Therefore, the aim of this study was to assess the efficiency of zinc absorption and homeostasis in patients with...
proven CD and biopsy-negative control subjects by determining the FAZ.

SUBJECTS AND METHODS

Recruitment of subjects and informed consent

Children aged 2–18 y who underwent routine endoscopy for the diagnosis or exclusion of CD were recruited. All ethnic groups and both sexes were eligible. All potential subjects who were identified and had met the inclusion and exclusion criteria were given the opportunity to participate. All children admitted to the CYWHS to undergo an endoscopic investigation to diagnose or exclude CD were included. The exclusion criteria were the CYWHS to undergo an endoscopic investigation to diagnose or exclude CD were included. The exclusion criteria were children undergoing an endoscopic investigation <2 y of age, children who had consumed zinc supplements in the 2 mo before the study, children who has diabetes, and parents or guardians who were unable to provide informed consent. Informed consent was obtained from parents or guardians before study. Parents and guardians were given the opportunity to review the consent form and ask questions about the study. Parents and guardians were asked to summarize, in their own words, what participation in the study involved and whether they were comfortable with the risks and benefits of participation.

Ethics

This study was approved by the CYWHS Human Research Ethics Committee in Adelaide, Australia.

Zinc stable-isotope preparation

As previously described (10), accurately weighed quantities of zinc oxide enriched with $^{67}$Zn and $^{70}$Zn (Trace Sciences International) were dissolved in 0.5 M H$_2$SO$_4$ to prepare a stock solution. For the preparation of an orally administered dose ($^{70}$Zn), the stock solution was diluted with triply deionized water and titrated to pH 5.0 with metal-free ammonium hydroxide. For the intravenously administered dose ($^{67}$Zn), the pH of the stock solution was adjusted to pH 6.0 with ammonium hydroxide, and the stock solution was diluted with sterile isotonic sodium chloride. Oral and intravenous solutions were filtered through a 0.2-$\mu$m filter. Zinc concentrations of these solutions were measured by atomic absorption spectrophotometry with a mass correction factor applied. The intravenous solution was tested for sterility and pyrogens. Accurately weighed quantities were stored at 4°C in polypropylene tubes (oral doses) or sealed sterile vials (intravenous doses) until use.

Measurement of zinc absorption

A test was conducted to determine if there was abnormal zinc absorption in children with and without villous atrophy on a SBB. Before the SBB procedure, the subject was given 5 ml zinc sulfate solution that contained 2.5 mg zinc solution in a test tube to be taken orally followed by an oral rinse 2–3 times with deionized water. The solution contained a standard oral dose of zinc labeled with stable isotope (total of 2.5 mg, which included 0.5 mg $^{67}$Zn and 2 mg natural abundance zinc) mixed in 5 ml sterile water. Approximately 2 h after the oral zinc dose (at the time of the SBB), the physicians quantitatively administered (by using a slow intravenous push) an intravenous dose of $^{70}$Zn (~0.25 mg) through an intravenous line that was already in place as part of the standard SBB procedure. The intravenous-dose administration was usually done before the SBB procedure.

SBB

The SBB was performed by the staff specialist in the Gastroenterology Procedure Theater at the CYWHS as a standard upper intestinal endoscopy (Olympus Pediatric Videoendoscope Gastrointestinal Fibrascope 160/180; Olympus) under general anesthesia [volatile cevolflurane with or without nitrous oxide with oxygen or propofol (2 mg/kg) and/or remifentanil] with biopsies taken from the duodenum with standard oral forceps (2.8-mm diameter and 2300-mm working length). All children were monitored in standard manner with pulse oximetry. Blood pressure and vital signs were checked at least every 5 min during the SBB procedure and for 30 min after the procedure.

SBT

The SBT test has been validated by the co-investigators at the Gastroenterology Unit of the CYWHS as a site-specific marker of small intestinal mucosal integrity (13–15). On a separate day, after an overnight fast, a baseline breath sample was collected in duplicate. Breath samples were collected by having patients blow through a straw into 2 evacuated tubes and quickly capping the lid. A 100-mL solution that contained 20 mg $^{13}$C-sucrose (SBT kits with $^{13}$C-enriched sucrose; Nidor Pty Ltd) was administered orally over a 5-min period. Breath samples were collected in duplicate at 15-min intervals for 90 min. Sips of water were allowed after 30 min of breath testing, and food was only allowed after the test was completed. Breath samples were analyzed by isotope ratio mass spectrometry at the CYWHS. Results were expressed as the percentage cumulative dose recovered at 90 min.

Intestinal biopsy histology

Before a routine endoscopy for diagnosis of CD, all subjects were consuming a regular diet and were only advised to adhere to a gluten-free diet by their clinician if the diagnosis was positive. SBB specimens, 2–4 in number, were interpreted by a pediatric pathologist (as part of the diagnosis) who was not aware of specific clinical information. Biopsy specimens were scored according to the system described by Marsh (16). The scoring system was as follows: normal = 0; increased numbers of intraepithelial lymphocytes = 1; intraepithelial lymphocytes plus enlarged crypts = 2; and villous atrophy = 3 (subclassified as partial = 3a, subtotal = 3b, and total = 3c). A Marsh score of 0 was considered normal, whereas a Marsh score $\geq 3$ was classified as having CD.

Urine collections

To determine zinc stable-isotope enrichment, subjects were asked to collect spot urine samples (100 mL) in standard zinc-free urine containers twice daily (am and pm) for 4 d starting 3 d after the administration of the zinc stable isotope. Urine was transferred into zinc-free nalgene bottles for storage at $-20^\circ$C until analysis.
Determination of zinc stable-isotope ratios in urine

Urine samples were digested by using an MDA-2000 microwave sample preparation system (CEM Corp). A 5-mL urine sample was placed into an Advanced Composite Vessel (CEM Corp) and combined with 1 mL concentrated HNO₃; the pressure was gradually increased to a maximum of 120 psi. The total digestion time was 90 min. Digested samples were transferred to a 50-mL beaker, evaporated to dryness on a hot plate, and reconstituted in 2 mL ammonia acetate buffer (pH 5.6). Zinc in the sample was purified by its chelation with trifluoroacetylacetone and extraction of the chelate with hexane (17). Organics were removed by heating the samples to complete dryness. The purified zinc was reconstituted in 4 mL 2% (vol:vol) nitric acid.

Eight mL of 50 ppb Zn solution in 2% (vol:vol) nitric acid were prepared from each sample. The solution was introduced into an inductive coupled plasma mass spectrometer (VG Plasma Quad 3, VG Elemental; Thermo Electron Corp) for measurement of zinc stable-isotope ratios ($^{67}$Zn/$^{66}$Zn and $^{70}$Zn/$^{66}$Zn).

Calculation of FAZ and EZP

For the measurement of the FAZ, the ratio of the urinary isotopic enrichment of the intravenously administered $^{70}$Zn to the orally administered $^{67}$Zn was used in the following equation (18):

$$\text{FAZ} = \frac{\text{enrichment (oral ÷ intravenous)}}{\text{dose (intravenous ÷ oral)}}$$

(1)

The EZP was defined as the estimate of the total size of the combined pools of zinc that exchanged with zinc in plasma in ≤3 d. The EZP was calculated by dividing the mass of intravenous isotope dose by the enrichment value at the y intercept of the linear regression of a semilogarithmic plot of urine-enrichment data from days 3–7 after isotope administration. This method of estimation of the EZP size relied on treatment of the individual zinc pools in the EZP as a single homogeneously mixed pool and assumed that losses also occurred at a monoexponential rate (19).

Statistics

All results are presented as means ± SEMs unless otherwise stated. All statistical analyses were performed by using GraphPad Prism (version 3.01; GraphPad Software Inc). Data of Marsh scores 0 compared with ≥3 were assessed by using a 2-sample t test. Multiple means were analyzed by using an ANOVA with a Bonferroni post hoc test. Proportions (Table 1) between data for Marsh scores 0 compared with ≥3 were compared by using the chi-square test. Differences were considered to be significant at $P < 0.05$.

RESULTS

Between May 2007 and April 2010, 51 subjects were recruited, and 24, 4, 3, and 20 subjects had a Marsh score of 0, 1, 2, and ≥3, respectively. However, 2, 1, 1, and 4 subjects with a Marsh score of 0, 1, 2 and ≥3, respectively, did not complete either the zinc-absorption test or the breath test and were excluded from the analyses (Table 1). Of 16 subjects with a Marsh score ≥3, 4, 7, and 5 subjects had a Marsh score of 3a, 3b, and 3c respectively. Subjects with a Marsh score of 0 were considered control subjects (negative by biopsy), whereas subjects with a Marsh score ≥3 were considered CD positive by biopsy. There was no significant difference in the mean age, weight-for-age z scores, height-for-age z scores, BMI z scores, symptoms, and family history of CD between the 4 groups (Table 1). However, there was a significant ($P < 0.05$) difference in the celiac serology, in particular transglutaminase and endomyosial antibodies between subjects with Marsh scores of 0 and ≥3 (Table 1).

FAZ

There was no significant difference in the FAZ between children with a Marsh scores ≥3 and 0 (Figure 1A). There were no significant differences in the FAZ between children with a Marsh score of 0 and the subcategorization of Marsh scores 3a, 3b, and 3c (Figure 1B).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Demographic profile of subjects with Marsh scores of 0, 1, 2, and ≥3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Marsh score of 0 (n = 22)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>9.0 ± 4.2</td>
</tr>
<tr>
<td>Sex (F:M)</td>
<td>16:6</td>
</tr>
<tr>
<td>Height-for-age z score</td>
<td>0.47 ± 1.2$^1$</td>
</tr>
<tr>
<td>Weight-for-age z score</td>
<td>0.03 ± 1.1</td>
</tr>
<tr>
<td>BMI z score</td>
<td>−0.37 ± 1.2</td>
</tr>
<tr>
<td>Symptoms [n (%)]</td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>18 (82)</td>
</tr>
<tr>
<td>Gastroesophageal reflux</td>
<td>5 (23)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>7 (32)</td>
</tr>
<tr>
<td>Indigestion</td>
<td>4 (18)</td>
</tr>
<tr>
<td>Constipation</td>
<td>5 (23)</td>
</tr>
<tr>
<td>Celiac serology [n (%)]</td>
<td></td>
</tr>
<tr>
<td>Transglutaminase IgA antibody</td>
<td>9 (41)</td>
</tr>
<tr>
<td>Endomyosial IgG antibody</td>
<td>1 (0.05)</td>
</tr>
<tr>
<td>Gliadin IgA antibody</td>
<td>4 (18)</td>
</tr>
<tr>
<td>Family history [n (%)]</td>
<td>7 (32)</td>
</tr>
</tbody>
</table>

$^1$ Mean ± SD (all such values).

$^2$ Significant difference between subjects with Marsh scores of 0 and ≥3, $P < 0.05$ (chi-square test).
EZP

There was a significant difference in the EZP between children with Marsh scores ≥3 and 0 (Figure 2), with CD children having an ~32% smaller pool size of EZP.

SBT

There was no significant difference in gut integrity as determined by the SBT between children with Marsh scores ≥3 and 0 (Figure 3A). Furthermore, there were no significant differences in SBT results between children with a Marsh score of 0 and the subcategorization of Marsh scores 3a, 3b, and 3c (Figure 3B). The average SBT of normal healthy children (aged 11.2 ± 0.8 y) was 8.5 ± 0% cumulative dose recovered at 90 min (20). The dashed line in Figure 3 (A and B) indicates the lower cutoff of a normal SBT, which was 5.06% cumulative dose recovered at 90 min on the basis of a mean – 2 SD of the average SBT.

FAZ and SBT

Overall, there was no significant correlation between the FAZ and SBT. However, there was a significant (P < 0.01) correlation between the FAZ and SBT in children with Marsh scores ≥1 (Figure 4). There was no significant correlation between the gut-function breath test and Marsh score. In addition, there was no significant correlation between the FAZ and Marsh score or between the FAZ and the most severe villous atrophy (3c).

DISCUSSION

The current study focused on the measurement of the FAZ, which examined the zinc-absorption capacity or efficiency in children with CD by using zinc stable-isotope techniques to provide useful perspective about the zinc nutritional status and zinc homeostasis. The primary determinants of zinc absorption are the amount of ingested zinc and the presence of the zinc-absorption...
inhibitor phytate. In the current study, the amount of ingested zinc was standardized, and phytate was not present. A key variable of zinc homeostasis such as the EZP, which was measured in the current study, is the sum of the combined pools that exchange with zinc in the plasma, which can be accurately calculated from urine-enrichment data after the intravenous administration of a zinc tracer provided that there is a simultaneous measurement of the FAZ (18). The size of the EZP is dependent on the zinc nutritional status (21), and this premise was supported by consistent observations of positive correlations between dietary and/or absorbed zinc (mg/d) and EZP size (19, 22, 23). In the current study, we showed that children with CD may have poor zinc nutritional status compared with that of control subjects as shown by a smaller size pool of the EZP. The findings suggested that children with CD may have impaired zinc homeostasis. It is possible that the lower EZP without the apparent impairment in zinc absorption suggests that the children may have a limited intake of dietary zinc. It is unclear whether gut inflammation has any effect on the EZP, although theoretically, the hepatic sequestration of zinc may not necessarily alter the EZP size. However, the EZP may be reduced by less exchange with zinc in the plasma. A fundamental limitation of the current study was a lack of normal healthy control subjects; however, our data suggested that the zinc status per se did not affect zinc absorption, and therefore, a comparison with a control group was less critical.

Abnormal zinc absorption in children with villous atrophy on the SBB was not observed compared with the zinc absorption in children with a Marsh score of 0. This result was an unexpected finding because children with severe villous atrophy would be inclined to have impaired zinc absorption. Our findings were not consistent with those of other authors researchers as Jameson (24), who reported a correlation between zinc deficiency and the severity of villous atrophy in adults. In addition, studies (25, 26) have concluded that serum zinc concentrations were low in newly diagnosed and severely malnourished children with CD. The authors speculated that some of the symptoms of CD (eg, anorexia and reduced growth rate) may be related, in part, to zinc deficiency. Furthermore, in patients with the genetic zinc-deficiency disorder acrodermatitis enteropathica, a duodenal biopsy clearly showed a small intestinal mucosal abnormality (27) that was similar to that of CD. Solomons et al (28) also reported that a decrease in plasma zinc was observed in untreated patients, and some patients who were otherwise in clinical remission also had impaired zinc nutrition. Another explanation for the similar FAZ between the 2 groups was that children with CD may have sufficient zinc transporters that remain on the luminal side of the small intestinal mucosa to absorb the small oral zinc dose (2.5 mg); however, the absorption may be compromised if a higher oral dose (>10 mg) is administered.

The FAZ was high in both groups, and this result would be expected because zinc was given as a single dose prebreakfast and as an inorganic salt. We (10) previously reported an FAZ of 0.8% in adults in the fasting state from a single inorganic oral dose of 2 mg Zn. Previous studies in children have reported an FAZ only with meals in the range of 0.15–0.35 (11, 29) or with dietary phytate in the range between 0.22 and 0.28 (30–32). To the best of our knowledge, the current study was the first time that the FAZ was reported in children from a single inorganic zinc dose after an overnight fast.

The lack of an apparent relation between the FAZ and EZP was consistent with a previous study (21) that suggested that fractional absorption was more reflective of the amount of zinc in the lumen together with the presence of any effectors of absorption (eg, phytic acid) than of the zinc status of the host per se. The authors (21) also concluded that the quantity of zinc absorbed each day did not seem to be regulated in response to changes in zinc status as reflected by the EZP size, but the EZP size varied directly with the quantity of zinc ingested and absorbed. However, the relation between the upper and lower amounts of absorption and EZP size was unclear.

It is well documented that zinc deficiency impairs growth in children, and it has been speculated that some of the symptoms of CD (eg, anorexia and reduced growth rate) may be related, in part, to zinc deficiency. Zinc is an essential micronutrient involved in growth. Several hundred zinc-containing nucleoproteins are involved in the gene expression of multiple proteins, which are important for growth (3). This effect suggests that compromised and/or impaired zinc homeostasis is a major contributor to growth failure. The impaired zinc homeostasis may be a consequence of poor gut integrity. Thus, a secondary aim of the current study was to use a newly developed breath test that was noninvasive, easy to use, rapid, and reliable to assess villous atrophy in children with CD. The use of sucrose, which was selectively enriched with $^{13}$C, as a breath-test substrate provided a direct and noninvasive assessment of small intestinal sucrase activity (13–15). When $^{13}$C-sucrose is digested by its brush border enzyme (sucrase), the increase in $^{13}$CO$_2$ can be measured in the breath and compared with that at baseline as a reflection of sucrase activity. With the use of the SBT, it was shown that children with CD had a low SBT that was similar to that of the lower cutoff of a normal breath test (20) compared with control subjects with a much higher SBT. This result was further reflected by the finding that increased severity of villous atrophy was associated with a decreased SBT which was markedly below that of the lower cutoff of a normal breath test, although there was no significant correlation with the Marsh score. The invasive SBB procedure for the diagnosis of CD has inherent risks to the patient, in addition to significant health care costs.
associated with these procedures. Suitable noninvasive screening tests would allow for the identification of the problem and possible severity of villous atrophy. The use of the SBT to measure the small intestinal sucrase activity as a marker of villous atrophy is important to establish a noninvasive test to assess and monitor the severity of gut damage in CD.

One result of the current study was that there was a significant positive correlation between the breath test and zinc absorption, but this correlation was only significant in children with CD and not in control subjects. This result suggested that children with CD who have a low SBT result (impaired gut function) are likely to have an impaired zinc-absorption capacity and, subsequently, a lower zinc status. The FAZ of \( \sim 0.80 \) was determined by extrapolation of the lower cutoff of the SBT (5.06% cumulative dose recovered after 90 min) from the y axis to meet the line of best fit and the extrapolation of that point toward the x axis. Thus, an FAZ <0.80 may suggest impaired zinc absorption and subsequent zinc deficiency. If this was the case, \( \sim 14 \) cases fell below this zinc-absorption cutoff, and an estimation of 67% of children with a Marsh score \( \geq 1 \) may have been zinc-deficient. This was consistent with the literature in which the elimination of gluten usually induced a clinical improvement within weeks, although histologic recovery may have taken months or even years, especially in adults, in whom a complete mucosal recovery may never have occurred (33, 34). Although we did not note any malnutrition in our cohort of subjects, it has been suggested that an impaired gut function and zinc-absorption capacity may result in significant malnutrition as shown by Corazza et al (35), who showed that 67% of patients with overt CD and 31% of patients with silent or subclinical CD had malnutrition at the time of diagnosis. Furthermore, Hallert et al (36) showed that CD patients who were on a gluten-free diet for 10 y continued to have nutritional deficiencies. Individuals with CD are more susceptible to lactose malabsorption, folic acid, vitamin B-12, iron, vitamin D, and zinc deficiencies (37, 38).

In conclusion, children with CD had impaired zinc homeostasis; however, zinc absorption was not compromised. Nevertheless, more research that uses normal healthy control subjects is warranted to confirm this observation. On the basis of the positive correlation between SBT and zinc absorption in children with CD, a high proportion of these children are likely to be zinc-deficient if they have impaired gut function. In addition, the SBT may provide a useful noninvasive tool to measure and monitor the gut integrity and function in children with CD.

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REFERENCES


