Zinc supplementation to patients with celiac disease—is it required?

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Summary

Background and objectives: This study was conducted to evaluate plasma levels of zinc in children with celiac disease, to correlate plasma zinc levels among the celiacs with short stature and diarrhea and to compare plasma zinc levels in deficient patients on gluten-free diet (GFD) with or without 4 weeks of zinc supplementation.

Methods: A total of 134 patients underwent plasma zinc estimation at baseline and after a four week period. Zinc-deficient patients were randomly assigned to two groups. Group G ($n = 48$) received GFD without zinc supplementation, Group G + Z ($n = 48$) received GFD with zinc supplementation for 4 weeks.

Results: The rise in plasma Zinc levels was significant in each group regardless of zinc supplementation but similar when compared in the two groups after 4 weeks. Mean zinc levels at baseline and increase in zinc levels were statistically similar at 4 weeks in patients with diarrhea and short stature.

Conclusions: Zinc levels rise with GFD irrespective of zinc supplementation.

Key words: celiac disease, zinc, diarrhea, short stature.

Introduction

Celiac disease (CD) is characterized by small intestinal malabsorption of nutrients as a consequence of ingestion of wheat gluten or related proteins from rye and barley in a genetically predisposed individual which is characterized by villous atrophy of the small intestinal mucosa, prompt clinical and histological improvement following adherence to strict gluten-free diet (GFD) and clinical relapse when gluten is reintroduced [1–3].

CD predominantly affects the proximal small intestine. The small intestine has a central role in maintaining zinc homeostasis. In patients with CD, zinc deficiency may result from a cumulative loss of insoluble zinc complexes with fat and phosphate, exudation of zinc protein complexes into the intestinal lumen and massive loss of intestinal secretions or impaired zinc absorption because of damaged intestinal epithelial cell membrane. Some of the symptoms of CD (e.g. anorexia and reduced growth rate) may be related, in part, to zinc deficiency.

In recent years zinc has emerged as a very important micronutrient for maintaining the integrity of intestinal mucosa, immunity and growth in children. Comparative data about the effects of GFD with or without zinc supplementation on intestinal mucosal healing and normalization of plasma zinc levels is scanty. So this study was conducted to evaluate the effects of GFD with or without zinc supplementation on plasma zinc levels in diagnosed CD patients with low plasma zinc levels.

Patients and Methods

Study population and design

All prospective patients coming to the Celiac Disease Clinic in the division of Pediatric Gastroenterology, Post Graduate Institute Medical Education and Research, Chandigarh, India, were enrolled for this study, between 1st July 2006 and 31st December 2007. Inclusion criteria comprised age of: patients aged less than 14, and newly diagnosed cases of CD as per the revised European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) criteria [4]. Inability of the legal guardian to provide informed consent was taken as only exclusion criterion.

Ethical clearance was obtained from the institutional ethics committee. Verbal consent was taken from the children and a written informed consent was obtained from the legal guardian(s) of each subject. All the subjects were studied for baseline demographic and social profile. Their detailed history and physical examination were recorded on pretested proforma. In all patients, blood investigations such as hemoglobin, total leukocyte count, differential leukocyte count, platelet count, reticulocyte count and peripheral smear for the type of anemia, liver function test including serum bilirubin, aspartate aminotransferase (AST)/alanine transaminase (ALT), serum alkaline phosphatase, serum proteins, serum calcium, serum phosphorus, anti-tissue transglutaminase (tTG) and plasma zinc levels were carried out.

Anti-tTG levels were evaluated using DRG tTG-A ELISA REF EIA-10503 kit. This test is based on recombinant human tTG as antigen. This is an indirect non-competitive enzyme immunoassay for determination of tTG antibodies in human sera or plasma. Reference cut-off of anti-tTG IgA was $15\,U\,ml^{-1}$. In case of clinical symptoms and signs but serologically negative patients, IgA levels were evaluated.

Upper gastrointestinal (GI) endoscopic examination was performed using GIF 160 Olympus endoscope. Patients received injection ketamine $1\,mg/kg$ of body weight as premedication. Endoscopic markers were recorded for grooving/scalloping of the duodenal folds, mosaic pattern of the mucosa, nodularity and reduction and thinning in the numbers (no more than 3-folds in straight line on endoscopic vision) or absence of Kerkring folds at maximum insufflation.

All patients were subjected to endoscopic punch biopsy starting from the second part of duodenum and specimens were taken for histopathological examination. These samples were carefully oriented on filter paper and fixed in 10% formalin. Biopsies were embedded in paraffin wax, cut in sections and stained with hematoxylin and eosin. Histopathology was expressed according to the Marsh Classification of 1992 [5].

Measurement of Plasma Zinc Levels

Plasma zinc level was estimated by using atomic absorption spectrophotometer (PERKLIN ELMER 400). The blood samples (2 ml) were collected in zinc-free vials under aseptic conditions in fasting patients and were centrifuged to isolate plasma and stored at $-20°C$ until further analysis. Plasma was diluted in 1:5 ratio with distilled water. Acetylene gas was used for the burner. Monochromatic light, with wavelength of 213.9 nm and slit-width of 1 mm, was used for zinc. Standard solutions (Sigma Aldrich) containing 25, 50 and $100\,\mu g\,dl^{-1}$ of zinc were used for standardization and calibration. The diluted

samples were analyzed in triplicates in serial order. Mean value obtained for each sample was reported. Values in the range of $70–110\,\mu g\,dl^{-1}$ were taken as normal (standardized by our lab). After starting GFD, deficient patients were randomly assigned to two groups by computer allocator:

Group G: Received GFD without zinc supplementation

Group G + Z: Received GFD with zinc supplementation

Zinc supplementation was administered for 4 weeks based on previous studies which looked into the role of zinc in CD and chronic diarrhea [6]. A dose of 20 mg elemental zinc was given as zinc sulphate in powder or tablet form [6]. All children received the same dose and formulation. Compliance was reinforced and tablets were counted in case of any doubt. Plasma zinc levels were measured in both groups after 4 weeks and compared. Zinc levels were also compared at baseline and at 4 weeks (to identify zinc deficiency) in patients who presented with diarrhea and short stature alone. All patients were put on GFD and dietary counseling was done by a physician and a trained dietician.

Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences software (SPSS, version 13, Chicago, IL, USA). For continuous data from the entire study population, mean, standard deviation and range were calculated. For categorical data, number and percentages were calculated. The baseline parameters between the two randomized groups and baseline and post-treatment plasma zinc and percentage changes between the two groups were analyzed using the unpaired t-test. Pre- and post-treatment changes in plasma zinc levels within the groups were analyzed by the paired t-test. For comparing categorical data between randomized groups, the chi-square test was used.

A p-value of ≤ 0.05 was considered to be statistically significant.

Results

A total of 134 children diagnosed with CD who came for regular follow-up were included in the study. Follow-up evaluation was carried out at 4 weeks. Plasma zinc samples were taken at baseline and repeated at 4 weeks in those who were zinc deficient at the commencement of treatment. As mentioned, zinc-deficient patients were divided in two groups (Fig. 1).

Mean age for onset of symptoms was 3.4 years (range: 0.5–11.5) and mean age for presentation was 6.2 years (range: 1–14) with mean period of delay in diagnosis of 33.4 months (range: 0–132). Mean age at cereal introduction was 7.2 months (range: 2–29). In 77% of patients, symptoms

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┌─────────────────────────┐
│ Diagnosed celiac disease│
│        patients         │
│        (N=149)          │
└─────────────────────────┘
          │        ┌──────────────────────┐
          │───────▶│ Refused to participate│
          │        │  in study (N=15)      │
          ▼        └──────────────────────┘
┌─────────────────────────┐
│ Participated in study   │
│        (N=134)          │
└─────────────────────────┘
          │        ┌──────────────────────┐
          │───────▶│ Baseline serum zinc  │
          │        │       levels         │
          ▼        └──────────────────────┘
┌─────────────────────────┐
│ Patients deficient in   │
│      zinc (N=96)        │
└─────────────────────────┘
          │        ┌──────────────┐
          │───────▶│  Randomized  │
          ▼        └──────────────┘
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| GFD without zinc supplementation (N=48) (G Group) | GFD with zinc supplementation (N=48) (G+Z group) |

Follow up for 4 weeks

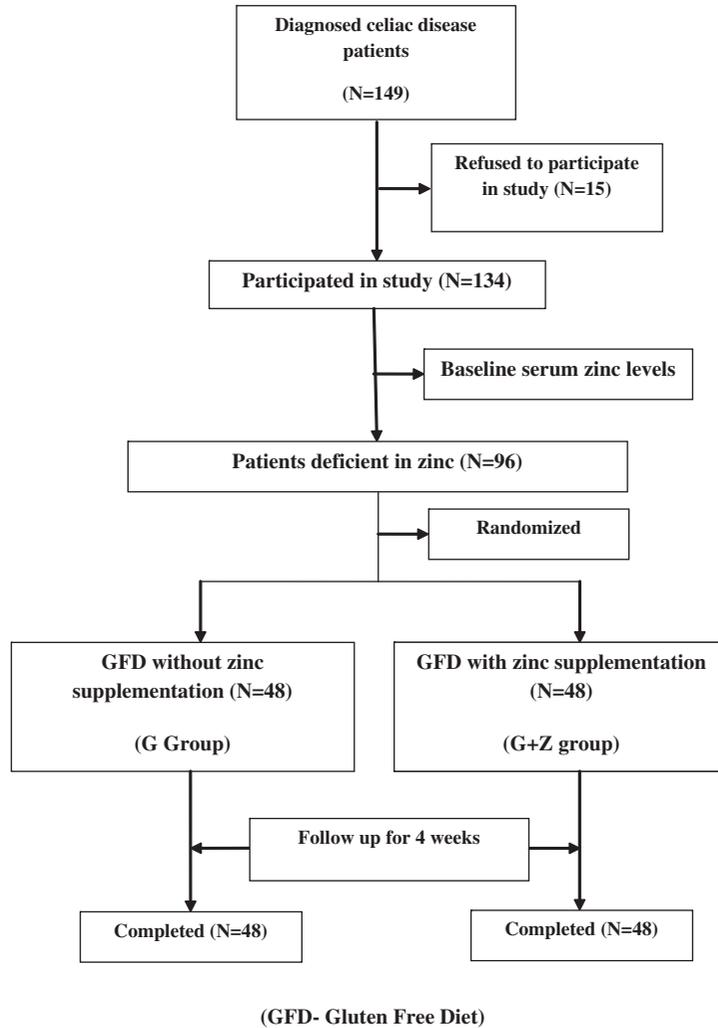| Completed (N=48) | Completed (N=48) |

(GFD- Gluten Free Diet)

FIG. 1. Design of study.

appeared early, i.e. <5 years of age (mean age: 3.36 years) but 55.2% presented to the hospital late, i.e. >5 years of age (mean age: 6.19 years) with mean delay in diagnosis of 33.4 months.

Presenting symptoms included diarrhea in 54.5%, failure to thrive in 52.2%, features suggestive of anemia in 40.3%, short stature in 20.9%, abdominal pain in 19.4%, vomiting in 15.7% and constipation in 2.2% of the study population. Abdominal distension was the presenting feature in 41% of cases. At presentation, the mean weight was 14.6 ± 5.5 kg. The mean length/height at the time of presentation was 102.6 ± 17.2 cm. Exactly 14.2% of the patients had weight >80% of the 50th percentile specified by the National Center for Health Statistics (NCHS) for that age and sex at the time of diagnosis;

24.6% patients had weight for age <60% of normal and 60.4% of patients had length/height below the 3rd percentile of the NCHS specification for that age and sex. All patients showed significant improvement in their clinical parameters after introduction of GFD.

Two groups were almost equally distributed with regard to the clinical spectrum and baseline investigations (Tables 1–3), except anemia as presenting symptom which was more common in the group randomly assigned to receive the supplemental zinc along with GFD (G + Z).

Plasma zinc levels
Plasma zinc estimation was done in all patients enrolled in the study. Mean zinc level in study

TABLE 1
*Comparison of clinical characteristics in the CD patients in randomized groups (*n = 48 each)

Characteristics	G	G + Z	*p*-value
Mean age (range) (years)	6.26 ± 2.78 (2–13)	6.71 ± 3.45 (2–14)	NS
Sex, *n* (%)			
Males	27 (56.3)	30 (62.5)	NS
Females	21 (43.8)	18 (37.5)	NS
Mean age of onset of symptoms (years)	4.17	3.39	NS
Mean age of presentation (years)	6.24	6.56	NS
Mean delay in presentation (months)	40.6	31.3	NS
Diarrhea, *n* (%)	25 (52.1)	32 (66.7)	NS
Short stature, *n* (%)	32 (66.7)	27 (56.3)	NS
Failure to thrive, *n* (%)	27 (56.3)	22 (45.8)	NS
Anemia, *n* (%)	13 (27.1)	27 (56.3)	0.004
Pain abdomen, *n* (%)	10 (20.8)	8 (16.7)	NS
Vomiting, *n* (%)	5 (10.4)	11 (22.9)	NS
Constipation, *n* (%)	3 (6.3)	2 (4.2)	NS
Diabetes mellitus, *n* (%)	1 (2.1)	0	NS
Liver disease, *n* (%)	0	1 (2.1)	NS
Abdominal distension, *n* (%)	18 (37.5)	22 (45.8)	NS

NS, not significant.

TABLE 2
*Clinical characteristics (physical examination) in the randomized groups (*n = 48 each)

Characteristics	G	G + Z	*p*-value
Pallor, *n* (%)	48 (100.0)	48 (100.0)	NS
Clubbing, *n* (%)	8 (16.7)	10 (20.8)	NS
Pedal edema, *n* (%)	2 (4.2)	2 (4.2)	NS
Icterus, *n* (%)	0	1 (2.1)	NS
Lymphadenopathy, *n* (%)	0	0	
Skin changes, *n* (%)	0	0	
Hepatomegaly, *n* (%)	5 (10.41)	2 (4.16)	NS
Short stature in total, *n* (%)	32 (66.7)	27 (56.3)	NS
Short stature with diarrhea, *n* (%)	16 (33.3)	18 (37.5)	NS
Short stature alone, *n* (%)	16 (33.3)	9 (18.8)	NS

NS, not significant.

TABLE 3
*Comparison of the biochemical parameters in the randomized groups (*n = 48 each)

Parameters	G	G + Z	*p*-value
Hemoglobin (g dl^{-1})	8.6 ± 1.7	8.7 ± 2.1	NS
TLC	9013.7 ± 2918.9	8274.3 ± 2985.4	NS
Platelets (×1000)	414.0 ± 212.0	380.6 ± 237	NS
Total proteins (g dl^{-1})	6.9 ± 1.1	6.7 ± 0.8	NS
Albumin (g dl^{-1})	3.6 ± 0.7	3.5 ± 0.7	NS
Globulin (g dl^{-1})	3.3 ± 0.6	3.2 ± 0.6	NS
Bilirubin (mg dl^{-1})	0.7 ± 0.1	0.7 ± 0.1	NS
SGOT (IU)	58.2 ± 24.4	77.2 ± 70	NS
SGPT (IU)	45.4 ± 14.1	60.0 ± 52.1	NS
SAP (IU)	211.5 ± 80.5	225.2 ± 101.4	NS
Calcium (mg dl^{-1})	9.1 ± 0.5	8.8 ± 0.6	NS
Phosphorus (mg dl^{-1})	4.1 ± 1	4.1 ± 1.1	NS

Data expressed as mean ± SD. TLC, total leukocyte count; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; SAP, serum alkaline phosphatase; NS, not significant.

group was $67.2 \pm 37.4 \,\mu g\, dl^{-1}$. At the baseline, 96 of the 134 (71.6%) patients were found to be zinc deficient and 48 patients were randomly assigned to each group. Only 10 zinc-deficient patients had albumin levels $<2.5 \,g\, dl^{-1}$ ($p = 0.346$).

Mean baseline zinc levels were $52.3 \,\mu g\, dl^{-1}$ in the G and $51.2 \,\mu g\, dl^{-1}$ in the G + Z groups. These values were statistically similar. Mean zinc levels after 4 weeks were $71.9 \,\mu g\, dl^{-1}$ and $74.9 \,\mu g\, dl^{-1}$, respectively, and again the difference was statistically non-significant (Table 4). Mean rise in plasma zinc level was $19.6 \,\mu g\, dl^{-1}$ in the G group and $23.8 \,\mu g\, dl^{-1}$ in the G + Z group after 4 weeks. This change was significant in groups when considered separately, but when compared to each other the increases in zinc levels were statistically similar.

With the aim to analyze the plasma zinc status in cases of CD presenting with diarrhea and short stature separately, the number of cases presenting with diarrhea without short stature and vice versa were calculated and were 29 and 35, respectively. Mean plasma zinc levels in patients who presented with diarrhea alone (without short stature) was $62.9 \,\mu g\, dl^{-1}$ and in those with short stature alone (without diarrhea) was $63.7 \,\mu g\, dl^{-1}$. The difference between these two values was not significant (Table 5 and Fig. 2). Similar analysis was also done in patients of diarrhea and short stature alone in G and G + Z groups. Zinc levels in both the randomized groups in each category were statistically similar at baseline and at 4 weeks (Table 5).

Discussion

There is deficiency of both macronutrients and micronutrients in CD due to malabsorption resulting from mucosal atrophy. Zinc is the one of most important minerals and has been found to be low in CD. We studied plasma zinc levels in a large cohort of patients of CD from northwestern India. The measurement of plasma zinc levels has been the most widely used method to identify zinc deficiency as it is generally assumed that a low plasma zinc concentration is indicative, in the majority of cases, of zinc deficiency [7]. Values in the range of 70–110 $\mu g\, dl^{-1}$ was taken as normal [8–10].

In the present study, 71.6% cases were found to be deficient in zinc. Low plasma zinc values were observed in 54.2% of patients with CD presenting with short stature in a study by Altuntal et al. [11]. Reduced plasma zinc concentrations have also been found in patients with CD in other studies [12, 13] and it has been suggested that this may reflect a reduced nutritional zinc status secondary to intestinal malabsorption of zinc. Solomons et al. found that conditioned zinc deficiency may develop in patients with celiac sprue, zinc therapy greatly enhances the healing of both the intestinal and skin lesions and, theoretically, the rate and degree of recovery of mucosal integrity after successful treatment of sprue by gluten withdrawal could be influenced by a conditioned zinc deficiency which had developed prior to therapy [12]. Studies, in humans, in which the intestinal absorption of zinc was assessed by measuring

TABLE 4

Comparison of the plasma zinc levels at baseline and at 4 weeks after starting treatment in the randomized groups (n = 48 each)

	G	G + Z	Total	p-value
Number of cases	48	48	96	
Baseline Zn levels ($\mu g\, dl^{-1}$)	52.3 ± 12.3 (2.4–68.0)	51.2 ± 12.5 (1.2–66.0)	51.8 ± 12.3 (1.2–68.0)	NS
Zn levels- 4 weeks ($\mu g\, dl^{-1}$)	71.9 ± 19.3 (29.2–150.0)	74.9 ± 29.2 (48.8–217.0)	73.4 ± 24.7 (29.2–217.0)	NS
Difference ($\mu g\, dl^{-1}$)	19.6 ± 18.6	23.8 ± 29.8		NS

Data expressed as mean $\pm$ SD (Range). NS, not significant.

TABLE 5

Correlation of zinc levels in CD patients with diarrhea and short stature in randomized groups (n = 48 each)

Zinc levels	Diarrhea			Short stature		
	G	G + Z	p-value	G	G + Z	p-value
Baseline ($\mu g\, dl^{-1}$)	52.5 ± 8.1 (41–65)	52.4 ± 9.1 (31–62)	NS	57.4 ± 8.1 (33–68)	51.1 ± 11.7 (24–65)	NS
4 Weeks ($\mu g\, dl^{-1}$)	80.7 ± 23.2 (54–121)	71.7 ± 16 (49–100)	NS	73.3 ± 22 (60–150)	67.2 ± 6.4 (57–76)	NS
Difference ($\mu g\, dl^{-1}$)	28.3 ± 22.7 (4–69)	19.2 ± 14 (9–47)	NS	16.1 ± 22.5 (5–93)	16.1 ± 13.3 (0–48)	NS

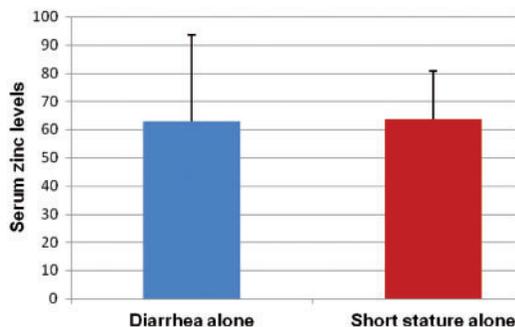Data expressed as mean $\pm$ SD (Range). NS, not significant.

FIG. 2. Correlation of baseline zinc levels in the patients with diarrhea and short stature in the study group.

the increments of plasma zinc concentrations during zinc-tolerance tests indicate that the absorption of zinc is impaired at both pharmacological and physiological doses of zinc in untreated patients with CD [14].

In our study, the mean baseline zinc levels were statistically similar in groups randomized to receive GFD with or without zinc supplementation. Mean rise in plasma zinc level after 4 weeks was significant in groups when considered separately, but again when compared to each other; the rise in plasma zinc was statistically similar. So GFD alone led to significant rise in plasma zinc levels and this rise was comparable with that which occurred in patients receiving GFD along with zinc. In patients with CD, gluten withdrawal results both in improved absorption of dietary nutrients and in regeneration of the villi of the jejunal mucosa. Henker et al. observed that abnormally low values were found only in children with acute CD (50% at <2 SD), but not in children receiving a GFD [15]. Crofton et al. measured the sequential changes in plasma zinc concentration after an oral dose of zinc as an assessment of intestinal function after gluten withdrawal. They have shown that, in untreated patients with gluten-sensitive enteropathies, smaller increments in plasma zinc concentrations occur in response to a standard oral dose of zinc than in healthy volunteers and in most instances when the untreated and known CD patients commenced, under close supervision, a GFD, their apparent absorption of zinc improved significantly. Similar results were obtained by Naveh et al. [16] Lack of additional benefit with zinc supplementation on plasma zinc levels in patients on GFD may be related to mucosal recovery after commencing GFD, pharmacokinetic properties, distribution and storage of zinc in the body and duration of supplementation. As in previous studies [12], hypoalbuminemia did not correlate with plasma zinc

levels in our study as well, as only 10 zinc-deficient cases had albumin values $<2.5\,g\,dl^{-1}$.

Mean plasma zinc levels in patients who presented with diarrhea alone (without short stature) were statistically similar to those with short stature alone (without diarrhea). Previously, authors have looked into the status of zinc in patients of CD presenting with diarrhea or short stature. Guerrieri et al. evaluated plasma zinc concentrations in a group of children with chronic diarrhea, some of them affected with active CD and the others with protracted post-enteritis diarrhea. In the group of children with untreated CD, the mean value of plasma zinc concentration $(69\,\mu g\,dl^{-1})$ was significantly lower than that of the healthy children. Mean value for the chronic post-enteritis diarrhea group was not significantly different from that of the control group [7]. Similarly, analyzing cases of short stature with CD as an etiology, Altuntal et al. found low plasma zinc values in 54.2% of patients with CD [11]. Hambidge et al. observed an association between poor growth and unsatisfactory zinc status assessed by low hair-zinc levels [17]. The same association was reported by Halsted et al. in Iranian boys [18].

In our study, the change in plasma zinc levels after 4 weeks in patients of diarrhea and short stature was statistically similar when compared to each other, irrespective of whether they were randomly assigned to receive GFD with or without zinc, meaning thereby that two of the major presentations of CD have similar profiles of plasma zinc prior to and following commencement of treatment.

There are certain limitations in the present study. No blinding was done and this could have affected the results. The study demonstrates low plasma zinc that were corrected but plasma levels need not always necessarily indicate zinc deficiency. The higher prevalence of anemia in the zinc-supplemented group suggests that there might have been a difference in true deficiency between the groups.

In conclusion, plasma zinc levels are decreased in more than two-thirds of children with CD suggesting zinc deficiency. Supplementing zinc for 4 weeks along with GFD gives no additional benefit with respect to rise in plasma zinc levels although there is a significant rise in plasma zinc levels after starting GFD for 4 weeks in these patients. It is noteworthy here that the presentation of CD as diarrhea or short stature has no effect on the plasma zinc levels at baseline or after starting GFD with or without zinc supplementation.

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