Extracellular Calcium Dictates Onset, Severity, and Recovery of Diarrhea in a Child with Immune-Mediated Enteropathy

Johnathan Fraebel, Regino Gonzalez-Peralta†, Maryann Maximos, Genie L. Beasley, Christopher Douglas Jolley and Sam Xianjun Cheng*

Department of Pediatrics, Gastroenterology, Hepatology, and Nutrition, University of Florida, Gainesville, FL, United States

Diarrhea causes monovalent and divalent ion losses that can influence clinical outcome. Unlike the losses of monovalent ions, such as Na⁺, K⁺, Cl⁻, and HCO₃⁻, which are generally large in quantity (osmoles) and therefore determine the severity of diarrhea, the losses of divalent ions are relatively small in osmoles and are often overlooked during diarrheal treatment. Studies now suggest that despite divalent ions being small in osmoles, their effects are large due to the presence of divalent ion-sensing receptors and their amplifying effects in the gut. As a result, losses of these divalent ions without prompt replacement could also significantly affect the onset, severity, and/or recovery of diarrheal disease. Herein, we report a case of a malnourished child with an immune-mediated enteropathy who developed episodes of “breakthrough” diarrhea with concurrent hypocalcemia while on appropriate immunotherapy. Interestingly, during these periods of diarrhea, stool volume fluctuated with levels of blood Ca²⁺. When Ca²⁺ was low, diarrhea occurred; when Ca²⁺ levels normalized with replacement, diarrhea stopped. Based on this and other observations, a broader question arises as to whether the Ca²⁺ lost in diarrhea should be replaced promptly in these patients.

Keywords: calcium, calcium metabolism, calcium-sensing receptor, diarrhea, immune-mediated enteropathy, inflammatory bowel disease, intestinal barrier function, ion transport

BACKGROUND

Diarrhea causes both monovalent and divalent ion losses (1, 2). Without prompt replacement, both affect the outcome of diarrheal disease. While losses of monovalent ions Na⁺, K⁺, Cl⁻, and HCO₃⁻ are large and therefore determine the severity of diarrhea, losses of divalent ions are small but large in biologic effect due to the presence of divalent ion-sensing receptor-mediated signal amplification (3, 4). As a result, without prompt replacement, the loss of divalent ions can also likely affect the onset, severity, and recovery of diarrheal disease. For example, Zn²⁺, acting via Zn²⁺-sensing receptor (ZnSR), can reduce the severity, duration, and recurrence rate of diarrhea (5). This Zn²⁺ effect is particularly important in mal- and undernourished children in whom an underlying negative balance of Zn²⁺ metabolism often exists. However, despite the recent advances in Ca²⁺ and Ca²⁺-sensing receptor (CaSR) research, limited information is available on the role and function of this important divalent ion in diarrhea.
In this communication, we report a malnourished child with an immune-mediated enteropathy. Despite adequate immunotherapy, he developed episodes of “breakthrough” diarrhea with concurrent hypocalcemia. Interestingly, during these disease flare-up episodes, diarrhea symptoms inversely fluctuated with levels of blood Ca\^{2+}. When blood (serum) Ca\^{2+} was low, diarrhea occurred; when Ca\^{2+} levels normalized with replacement, diarrhea quickly stopped. In light of this and other observations, we propose that, similar to Zn\^{2+} deficiency, the loss of Ca\^{2+} without prompt replacement may compromise the diarrhea-protective capability of Ca\^{2+} and CaSR in the gut and lead to severe, protracted, and recurrent diarrhea.

**CASE PRESENTATION**

The patient is a 6-year-old African-American male with autoimmune enteropathy (diagnosed 12 months prior to admission, based on the following criteria: intractable diarrhea, small bowel villous atrophy, presence of circulating anti-enterocyte antibodies, and responsiveness to immunosuppressive treatment) who was hospitalized for worsening non-bloody, watery diarrhea, severe malnutrition, and hypocalcemia. Initially, he responded well to glucocorticosteroid monotherapy. However, for the 6 months prior to this admission, social issues gradually led to interruption of this therapy with recurrence of diarrhea (Figure 1A) and weight loss. Despite re-initiation of glucocorticoids, diarrhea persisted, so tacrolimus was added to his treatment regimen. He responded well with combination therapy, but 6 weeks prior to admission, he developed recurrent diarrhea and weight loss (despite appropriate administration of medications and consistent therapeutic tacrolimus levels). He had no other medical problems and had no relatives with gastrointestinal or immunological disease. Findings on the initial physical examination revealed a moderately malnourished (height Z = −0.56, weight Z = −2.25, BMI Z = −2.85) child with hypothermia (temperature 97.5 °F), hypotension (blood pressure 83/54 mmHg), tachycardia (pulse 103 beats per minute), dry mucus membranes, sunken orbits, temporal wasting, and a protuberant but otherwise benign abdomen. His laboratory values are shown in Figures 1B–E. He was initially resuscitated with intravenous fluids. However, his diarrhea (Figure 1A) and metabolic acidosis (Figure 1E) remained while hypokalemia (Figure 1D) worsened. All enteral intakes were withheld and total parenteral nutrition was initiated, while intravenous solumedrol and oral tacrolimus were continued. Despite good therapeutic blood levels of tacrolimus (Figure 1C), the diarrhea persisted (Figure 1A). Pan cultures of stool, urine, and blood revealed no abnormal growth. Stool ova and parasites analysis, viral, and *Clostridium difficile* toxin A/B studies were negative. On hospital day 4, he developed severe hypocalcemia with tetany (contractures of the hands and lower extremities), and worsening hypokalemia and metabolic acidosis. He was transferred to the intensive care unit for a closer monitoring and given three consecutive doses of q 3-h intravenous calcium chloride (0.5 mEq elemental Ca\^{2+}/kg/dose), in addition to intravenous fluids and other electrolyte replacements. Remarkably, as his serum-ionized Ca\^{2+} normalized, his diarrhea resolved (Figure 1A). In fact, after 3 days of calcium therapy, he became constipated.

With weaning of calcium supplementation, there was a recurrence of diarrhea, hypocalcemia, and metabolic acidosis. Five days after discontinuing calcium, his daily stool output increased to more than 2 L—close to that before calcium supplementation. The diarrhea resolved within 2 days of administration of oral calcium carbonate suspension (1.3 mEq elemental Ca\^{2+}/kg/day) (Figure 1A). Of note, he received a combination of
glucocorticoid-tacrolimus (at therapeutic levels) therapy during the entire hospitalization. At discharge, the patient was prescribed vitamin D in addition to calcium supplementation to restore normal Ca\(^{2+}\) balance. He was also placed on an unrestricted diet. At his recent 3-month follow-up clinic visit, he had no diarrhea, and the serum calcium levels remained normal.

**DISCUSSION**

Management of pediatric diarrhea remains challenging, particularly in children with malnutrition or undernutrition, in whom diarrheal episodes are often severe, protracted, and recurrent. Based on the previous Ca\(^{2+}\) metabolic balance studies in diarrhea (1, 2) and the recent work on the influence of Ca\(^{2+}\) and CaSR in reversing both secretory (6–10) and inflammatory diarrheas (11–13), we propose that the inadequate replacement of Ca\(^{2+}\) ions and their corresponding divalent ion-sensing receptors (i.e., Ca\(^{2+}\)SR) may be responsible for the severity and persistence of diarrhea symptoms in these malnourished patients.

According to earlier metabolic balance studies (1, 2), diarrhea results in losses of not only Na\(^{+}\), K\(^{+}\), Cl\(^{-}\), and HCO\(_3\)^{−}\), which are normally replaced with oral rehydration solution (ORS), but also Ca\(^{2+}\), Mg\(^{2+}\), and Zn\(^{2+}\), which are not routinely included in these solutions, possibly because of their relatively small quantities compared to monovalent ion losses (see summary in Table 1).

It is important to note that the absolute concentrations of divalent ion losses in diarrhea are smaller than those of monovalent ones, and they contribute less to the severity of dehydration. However, divalent mineral losses may greatly affect the course of diarrhea, leading to prolonged duration and frequent recurrence of the disease. Unlike monovalent ions, divalent ions such as Ca\(^{2+}\) are “functional” nutrients. In addition to their nutritional values, they also function as hormones or first messengers, binding to their corresponding divalent ion-sensing receptors (i.e., Ca\(^{2+}\) and Mg\(^{2+}\) binding to CaSR (3, 4, 13) and Zn\(^{2+}\) binding to ZnSR (5)) in the enterocytes of the gastrointestinal tract and inhibiting pathophysiologic processes that result in diarrhea. Indeed, as shown in this present case, diarrhea volume inversely correlated with serum levels of Ca\(^{2+}\) (Figure 1). Similar findings were also observed in children with infectious diarrheal diseases (14), as well as in healthy adult volunteers who were infected with enterotoxigenic *Escherichia coli* resulting in secretory diarrhea (15).

Considering the chronic nature of the diarrhea prior to admission and the absence of gastrointestinal infection, our patient’s diarrhea was most likely related to the underlying autoimmune enteropathy. Indeed, the diarrhea responded well to appropriate immunotherapy while calcium levels were normal. However, it recurred with hypocalcemia and again resolved with normalization of this element’s serum levels. The direct correlation between calcium concentration and stool volume (Figure 2) strongly implicates hypocalcemia as the cause of “breakthrough” diarrhea in this patient. The fact that the intensification of immunosuppression alone (with low calcium levels) early during his hospitalization failed to curtail diarrhea also lends support to this conclusion.

There are multiple reasons that our patient would have a negative balance of Ca\(^{2+}\) metabolism. First, he has malnutrition. It is common for children with malnutrition to have a negative calcium balance. Second, this child had a history of enteropathy that could impair Ca\(^{2+}\) absorption and induce Ca\(^{2+}\) loss. Third, he also has milk-dairy intolerance and was consuming a dairy-free diet. The long restriction of Ca\(^{2+}\)-rich dairy intake could lead his Ca\(^{2+}\) metabolic balance to a further negative direction, precipitating hypocalcemia and diarrhea.

The mechanisms whereby hypocalcemia precipitates diarrhea in an inflammatory gut have been described in animal models and not in humans. Several studies have reported that Ca\(^{2+}\) is required for the maintenance of epithelial tight junction integrity, a critical determinant of intestinal barrier function and inflammatory diarrhea (16–25). Decreases in extracellular Ca\(^{2+}\) concentration caused cell–cell junction destruction and opening of the paracellular pathway (17, 18, 20–22, 25). As a result, animals on high Ca\(^{2+}\) diets were shown to be more resistant to the development of enterocolitis caused by barrier function disrupters, such as invading pathogens (26–29), chemical colitogens (30), and immune-mediated processes (31). By contrast, animals on reduced Ca\(^{2+}\) diets were more prone to induced intestinal inflammation, resulting in colitis and diarrhea (32). Emerging data are now linking this phenomenon to the activity of the CaSR, as activation of this special G-protein-coupled cell surface receptor increased the epithelial tight junction barrier (12, 25) while its inactivation [as in CaSR-deficient mice (11)] disrupted intestinal barrier, leading to increased gut inflammation. In these CaSR-deficient mice, diarrhea responses to induced gut inflammation were more severe and persisted longer than their wild-type littermates. Based on these experimental observations, we propose that Ca\(^{2+}\)-therapeutic effects on inflammatory diarrhea are mediated via the CaSR, augmenting the epithelial barrier function that is so often disrupted in these conditions.

Of note, the localization of CaSR in the gut has been described on both the apical and the basolateral sides of the plasma membrane of the enterocyte (8), and receptors in both membrane domains of the polarized enterocyte are functionally active and can be activated by Ca\(^{2+}\), calcimimetics such as R568, and other polycations such as spermine with similar potency and EC\(_{50}\).
values (7–9). It is therefore possible that the efficacy of oral Ca2+ supplementation results from the activation of the CaSR at the intestinal brush border, whereas the efficacy of intravenous Ca2+ supplementation results from the activation of the receptor at the blood side of the enterocyte. These observations demonstrate that the anti-diarrheal activity of Ca2+ can be readily achieved by either route. Thus, depending upon the severity of symptoms and the presence or absence of emergencies, the replacement of Ca2+ can be given either intravenously or orally, as in the case of this patient. However, it is worth noting that, while the repletion via intravenous route raises serum Ca2+ quickly, this quickly raised serum calcium is lost quickly due to its prompt activation of CaSR in parathyroid glands and kidneys (which increases Ca2+ excretion). By contrast, while oral replacement raises serum Ca2+ relatively slowly, it produces local therapeutic anti-diarrheal (and other) actions and helps restore Ca2+ balance without causing much unwanted systemic adverse effects. Given this, the oral route is considered both safer and more physiological. Whenever clinically allowed, prompt switching from intravenous to oral supplementation of Ca2+ is recommended.

### CONCLUDING REMARKS

Given the wealth of accumulating data on the role and importance of Ca2+ and CaSR in both secretory and inflammatory diarrheal conditions, we suggest that Ca2+ stool losses should be routinely replaced as is currently done for Na+, K+, Cl−, HCO3−, and Zn2+. As summarized in Table 2, an ideal ORS composition would contain both monovalent ions and divalent minerals. The monovalent ions aim at replacing electrolyte losses, and correcting both hypovolemia and metabolic acidosis, thereby reducing diarrhea-associated mortality. By contrast, divalent ions would replace mineral losses, restoring the anti-diarrheal activities of CaSR and ZnSR, and thereby reducing the onset, duration, and recurrence of diarrhea. ORS use has been declining over the past decade, with fewer than 33% of children with diarrhea under the age of 5 using this therapy (33). Because it has little effects in decreasing stool volume, caregivers are reluctant to use ORS and instead prefer using anti-microbial agents, which increases the risk of developing drug resistance (3). If the findings presented in this and other studies are confirmed, adding divalent minerals to, or concurrently supplementing these ions along with ORS, would likely increase compliance with this oral therapy. Large randomized-controlled trials are warranted to further test the efficacy and safety of this therapeutic strategy.

### CONSENT FOR PUBLICATION

The written consent for publication was obtained from the child’s parent.

### ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the University of Florida Health Center’s IRB Guidelines and Privacy Rules about case reports, and it has been reviewed.
by UF IRB with written informed consent from the guardian of the studied subject.

**AUTHOR CONTRIBUTIONS**

SXC conceptualized and designed the work, JF and SXC collected and analyzed the data, RG, MM, GLB, CDJ, and SXC interpreted the data, JF and SXC drafted and RG, MM, GLB, and CDJ revised this manuscript, and all authors approved the final version and agreed to be accountable for the content of this work.

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**Conflict of Interest Statement:** The authors declare that the submitted work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.