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Bone deficits in parenteral nutrition-dependent infants and children with intestinal failure are attenuated when accounting for slower growth

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Abstract

Objective—To determine if bone mineral content (BMC) and density (BMD) of infants and children with parenteral nutrition (PN)-dependent intestinal failure (IF) is lower than healthy controls, and investigate potential causes of lower BMC and BMD.

Methods—We performed a cross-sectional study comparing infants and children with PN-dependent IF with duos of age, sex, and race matched controls. Lumbar spine BMC and BMD were measured by dual energy x-ray absorptiometry, and serum cytokines, aluminum, IGF-1, insulin-like growth factor-binding protein (IGF-BP)-3, parathyroid hormone, 25(OH) vitamin D, and 1,25(OH)₂ vitamin D were measured. Generalized estimating equation models accounting for matching were used for comparisons.

Results—BMC was 15% and BMD was 12% lower in IF participants than controls (p = 0.004). Group differences were attenuated to 3% and 7% and were not statistically significant (p=0.40 and p=0.07) when adjusted for length and weight; length- and weight-for-age were lower in IF than control participants (12.5% vs. 63%; 29.5% vs. 54%, p = 0.03). IF participants had higher serum aluminum (23 vs. 7 mcg/L, p<0.0001), IGF-1 (97 vs. 64 ng/mL, p=0.04), and 25(OH) vitamin D concentrations (40 vs. 30 ng/mL, p=0.0005), and lower IGF-BP3 (1418 vs. 1812 ng/mL, p<0.0001) and parathyroid hormone concentrations (51 vs. 98 pg/mL, p=0.0002) than controls. There was no difference in serum cytokine concentrations (p = 0.09).

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Conclusions—Growth retardation is a significant problem for PN-dependent IF patients. Additional investigation is needed to elucidate the cause and its impact on bone mass and density, especially the role of IGF-1 resistance and aluminum toxicity.

Keywords

pediatric; growth; bone mineral density; parenteral nutrition; DXA

Introduction

Children with intestinal failure (IF) have malabsorption due to loss of absorptive surface or intestinal dysfunction necessitating parenteral nutrition (PN) to sustain growth. The incidence of IF in developed countries is ~2–6.8 per 1,000,000 children (1).

Metabolic bone disease associated with PN leads to reduced bone mass in adults (2–4) and the risk of bone disease is dependent upon PN duration (5). PN-dependent patients are at risk for bone disease owing to continued exposure to aluminum in PN, low serum 25(OH) vitamin D (25(OH)D) (6, 7) and insulin-like growth factor (IGF)-1 and/or IGF-binding protein-3 (IGF-BP3) concentrations, and chronic inflammation (7, 8). To our knowledge, there is only one published study that systematically examined bone mineral density (BMD) of infants and children with PN-dependent IF. They found that BMD was lower in patients with PN-dependent IF compared to controls (9). However, they did not account for the delayed growth of PN-dependent IF patients, which may have confounded their study findings.

The purposes of this study were to assess BMD in infants and children with IF and to assess potential mechanisms that might contribute to low BMD. We hypothesized that infants and children with PN-dependent IF would have lower BMD when adjusting for growth and higher serum cytokines (tumor necrosis factor- α (TNF- α), interleukin-1 beta (IL-1 β), interleukin-6 (IL-6)) and aluminum concentrations, and lower serum concentrations of IGF-1, IGF-BP3, 25(OH)D, and 1, 25(OH) $_2$ vitamin D (1,25(OH) $_2$ D) than age, sex, and race matched controls.

Materials and Methods

Study Participants

Study participants with *PN-dependent IF*, defined by the inability to sustain growth without parenteral nutrition, were recruited from Cincinnati Children's Hospital Medical Center (CCHMC) (Cincinnati, OH), and Nationwide Children's Hospital (Columbus, OH). Inclusion criteria for IF participants were age between 6 months and 18 years who had PN-dependent IF. Exclusion criteria for IF participants were: clinically significant renal disease (<50% function for age as measured by creatinine (10); cerebral palsy or other disorders of the musculoskeletal system; use of medications affecting bone metabolism within the last 6 months for >1 week; antibiotic use for systemic infection within 1 week of enrollment; immunodeficiency; chronic inflammatory conditions; and orthopedic procedures that limited mobility.

Two control participants were recruited for each IF participant. Control participants were recruited from patients receiving ENT and urologic surgeries, and gastrointestinal endoscopic procedures who were having intravenous catheter placement facilitating phlebotomy. Control participants were pair matched by age, sex, and race to an IF participant. Age was matched as follows: 6–18 mo $\pm$ 1 mo, 19–36 mo $\pm$ 2 mo, 37–72 mo $\pm$ 3 mo, 73–120 mo $\pm$ 4 mo, 121–144 mo $\pm$ 5 mo, 145 $\pm$ 216 mo $\pm$ 6 mo. Exclusion criteria for control participants were clinically significant chronic medical conditions that may affect bone, use of medications affecting bone metabolism within the last 6 months for >1 week, and PN usage for >2 weeks at any point in time. There was no restriction regarding gestational age; however, non-premature controls (>37 weeks gestation) below the 5th percentile for length- (or height) or weight-for age were excluded.

Study Design

Data regarding age, sex, gestational age at birth, birth weight, medications, and fracture history were obtained by questionnaire. Information on diet at the time of study (percentage of calories as enteral vs. parenteral), amount and location of bowel resected, medical conditions, episodes of bacteremia, was obtained from the medical record for IF participants.

Height (ages > 2 y) or length (age ≤ 2 y) and weight were measured using a wall mounted stadiometer or length board, and digital scale, on the day of the study visit. Length-for age and weight-for-age percentiles and z-scores were determined using the CDC 2000 growth reference. Study participants provided a blood sample for measurement of serum concentrations of 25(OH)D, parathyroid hormone (PTH), IGF-1, IGF-BP3, cytokines (IL-1 β , IL-6, TNF- α), 1,25(OH) $_2$ D, and aluminum. Blood samples from IF participants were obtained when they came to clinic; the duration of time from last parenteral and enteral feeding was variable. Blood samples were obtained from control participants in a fasted state (> 8 h for solid foods and > 6 h for clear liquids for ages > 1 y; > 6 h for formula and breast milk for ages < 1 y) before surgery.

BMC and BMD of the lumbar spine were measured by dual energy x-ray absorptiometry (DXA) using a Hologic Discovery A. DXA scans were analyzed with the infant spine software version 12.7 (ages ≤ 36 months) or auto low density software version 12.7.3.1 (ages > 36 months). The reproducibility of lumbar spine BMC and BMD measurements were 3% and 2% for children ≤ 36 months, and 2.5% and 1% for children 6–9 years.

Informed consent was obtained from the parents of all study participants. This study was approved by the Institutional Review Board and the Scientific Advisory Committee of the Clinical and Translational Research Center at CCHMC.

Laboratory Methods

Serum concentration of 25(OH)D was measured by a direct competitive chemiluminescence immunoassay (Diasorin Liaison, Stillwater Minnesota). The intra-assay coefficients of variation (CV) were 6.3% and 8.6% for mean concentrations of 21 and 65 ng/mL, respectively. The respective inter-assay CVs were 9.4 ± 1.6 % and 7.7 ± 4.2 %.

Serum 1,25(OH)₂D was measured by a modified radioimmunoassay (Diasorin, Stillwater, Minnesota). The analytical measurement range was 6 – 230 pg/mL. Inter and intra-assay CVs were <8.2% at 17 pg/mL and <15.1% at 90 pg/mL, respectively.

Intact PTH was measured by an immunoradiometric assay (Diasorin Liaison, Stillwater Minnesota). Intra-assay CVs ranged from 1.3 to 2.5%, and the inter-assay CVs from 2.7 to 5.5%.

Serum IGF-1 and IGF-BP3 were measured using a competitive binding radioimmunoassay (Mediagnost, Reutlingen, Germany). The analytical ranges were 15.8 – 1010 ng/mL and 62.5 – 4000 ng/mL, respectively. The intra-assay CVs for IGF-1 and IGF-BP3 were 6.3% and 2.3% and the inter-assay CVs were 1.8% and 5.3%, respectively.

Serum cytokine (IL-1 β , IL-6, and TNF- α) concentrations were determined by enzyme-linked immunosorbent assay using MilliplexTM Multiplex kits (Millipore, Billerica, MA), and measured in duplicate using luminex technology on the Bio-PlexTM (Bio-Rad, Hercules, CA). Minimum detectable concentrations were 0.4 pg/mL for IL-1 β , 0.3 pg/mL for IL-6, and 0.1 pg/mL for TNF- α . For IL-1 β , IL-6, and TNF- α , the respective intra-assay CVs were 6.1, 8.1, and 10.5%. The respective inter-assay CVs were 7.0, 11.6, and 15.9%.

Serum aluminum was measured using Quantitative Inductively Coupled Plasma-Mass Spectrometry (ARUP laboratory, Salt Lake City). The lower limit of detection is 5 ug/L. Inter-assay CV was 9%.

Serum total bile acids were measured on IF participants by the enzymatic method at Cincinnati Children's Hospital to assess presence of cholestasis. The inter-assay CV was 4.7–6.2% and CV intra-assay was 0.6–4.5%. Criteria for cholestasis were >30 μ mol/L for 4–6 month olds, > 28 μ mol/L for 7–9 month olds, >37 μ mol/L for 10–12 month olds and >7 μ mol/L for older children.

Data Analysis

Laboratory values below the limit of detection were recoded as the mid-point between the lower limit of detection and zero for data analyses. Generalized estimating equations (GEE) method was used to compare all the variables between IF cases and controls. This approach assumes that the dependent variable is linearly related to independent variables, and that observations in different clusters are independent; observations within clusters may be correlated. In this study, the IF cases and matched controls were correlated by design. Therefore the GEE method was required for analysis to account for clustering effects. Variables that did not follow a normal distribution were log transformed prior to analyses to achieve the linearity assumption required in GEE analysis. Results were the same with untransformed and transformed variables, so only results for the untransformed variables were given. Pearson correlation coefficients were obtained between select variables. All p-values <0.05 were considered statistically significant. Statistical analyses were performed using SAS 9.2.

Results

Twenty IF and 49 control participants were enrolled. In addition, 2 IF patients awaiting small bowel transplant declined to participate and 10 IF patients were not eligible due to exclusion criteria. Of the participants, 4 IF participants had unusable DXA scans, either due to movement or to internal hardware covering the lumbar spine and 16 controls had no DXA scan available either due to failure to return for DXA scan or due to movement during the scan. Serum laboratory data from all 69 participants enrolled were included in the analyses.

The causes of intestinal failure among PN-dependent participants varied widely. Five participants had gastroschisis (1 with multiple resections, 1 with ileal atresia, 1 with midgut volvulus, jejunal atresia, and microcolon, and 1 with volvulus and jejunal atresia); 1 had midgut volvulus; 3 had ileal atresia and resections; 1 had gastric perforation and ileal resection; 1 had jejuno-ileal atresia; 3 had mitochondrial disorder; 3 had pseudoobstruction; 1 had megacystis microcolon hypoperistalsis syndrome; 1 had total Hirschsprung's; and 1 had necrotizing enterocolitis with massive resection. Eleven of 20 IF participants had small bowel resection, with amount resected ranging from 17 to 135 cm; 5 IF participants had colonic resections. The percentage of calories from parenteral nutrition, and vitamin D and calcium intakes of PN-dependent IF participants are presented in Table 1. Percent calories from enteral feeding ranged from 18–76% (median 39%). Eight PN-dependent IF participants were receiving no enteral nutrition. Of the remaining 12, 10 were receiving enteral infusions of elemental formula (e.g., Elecare, Vivonex or Neocate Jr). Two participants were on diets of their own choosing, and the content of calcium and vitamin D was not obtained. Time on PN ranged from 4 to 103 months (median 18.5 months). Number of central line infections ranged from 0 to 8 (median 1).

Control participants were recruited from the following sources: 27 were undergoing ENT procedures (tympanostomy tube placement, adenoidectomy with and without tonsillectomy, micro laryngoscopy/ bronchoscopy); 11 were undergoing urologic procedures (treatment of hypospadias, penile adhesions, phimosis and circumcision revision); and 6 were undergoing gastrointestinal procedures for the following indications: vomiting, dysphagia, feeding problems, constipation, and GERD. Histology and endoscopic findings in all gastroenterology-related cases were normal, and all subjects had normal growth with no indications of malabsorption.

There were no differences between the IF and control participants in age and sex, but race/ethnicity differed (Table 2). Asian and White Hispanic IF participants were matched to non-Hispanic whites, and Black Hispanic IF participants were matched to non-Hispanic blacks, due to lack of available controls of these race and ethnicities. IF participants had lower length, length-for-age percentile, weight-for-age percentile, and gestational age than controls. There were no differences in weight, weight-for-length percentile, and birth weight. One participant in each group had a history of bone fracture.

Results from the GEE analyses indicated that lumbar spine BMC was 15% lower ($p=0.0009$) and BMD was 12% lower ($p=0.004$) in IF participants than control participants. There was no difference in bone area (22.39 vs. 23.60 cm², $p=0.16$). However, when length and weight

were included in the GEE models, group differences diminished to 3% for BMC and 7% for BMD and were no longer statistically significant ($p=0.40$ and 0.07 , respectively) (Figures 1 and 2).

IF participants had significantly higher serum aluminum, IGF-1, and 25(OH)D concentrations, and lower IGF-BP3 and PTH concentrations than controls (Table 3); 5% (1/20) of IF participants and 14% (7/49) of control participants had a serum 25(OH)D concentration of < 20 ng/mL. There were no significant differences in mean serum TNF- α , IL-1 β , or IL-6 concentrations between groups. Mean IGF-1 concentration was 111 ng/mL in the IF participants and 83 ng/mL in the controls, which are the 70th and 50th percentiles, respectively, for healthy 3 year olds (the mean age of participants in this study) (11). Mean IGF-BP3 concentration in the IF participants was 1418 ng/mL, and 1812 ng/mL in controls, which are the 5th and 30th percentiles, respectively, of healthy 3 year olds (11). Serum bile acid concentrations in IF patients ranged from 10–41 μ mol/L; 80% (16/20) had values indicative of mild cholestasis, all were >1 year of age.

Among IF participants, there was no association between serum aluminum concentrations and BMC or BMD ($p = 0.10$). Serum aluminum concentrations were not associated with length for age Z-score ($r = -0.15$, $p=0.53$), time on PN ($r = -0.01$, $p=0.98$), IGF-1 ($r = -0.30$, $p = 0.20$), IGF-BP3 ($r = -0.15$, $p=0.52$), PTH ($r = -0.36$, $p=0.12$), or 1,25(OH) $_2$ D ($r=0.19$, $p=0.43$).

Discussion

Metabolic bone disease diagnosed by histomorphometry or reduced bone density by DXA, has been reported in 40–100% of adult PN-dependent patients (12). Bone disease in children receiving PN has received little attention. Historically, bone disease in PN-dependent patients had been attributed to aluminum toxicity and high doses of vitamin D. We found no difference in BMC or BMD of the lumbar spine between PN-dependent infants and children with IF and healthy children of matched age, sex, and race, when adjusted for length and/or weight. IF participants were shorter and had a lower body mass compared to age, sex, and race matched control participants, thus we adjusted for length and weight to prevent size-related artifacts when comparing DXA results. Our results also show that infants and children with IF have higher IGF-1 concentrations, and are still being exposed to aluminum in PN leading to increased serum aluminum concentrations, both of which have potential effects on growth and bone.

Diamanti et al (9) found that bone density in PN-dependent children was 18% lower than age matched controls. Their study participants differed from ours in several ways that may have influenced study findings. Their IF cases were receiving a larger proportion of calories from PN (median 80% vs. 61%), for a longer duration (38 mo vs. 18 mo), and were older (6.7 vs. 2.2 y). Also, they included IF patients with immune mediated and chronic inflammatory conditions, and with renal disease, which may decrease BMD, whereas we excluded patients with these conditions. Finally, they did not control for the significantly lower weight in PN-dependent patients compared to controls.

Excess urinary calcium or phosphorus losses may result bone disease in PN patients. Intravenous protein infusion as well as high glucose infusion rates increase urinary calcium losses (13, 14). Cyclic PN, administered over 12–18 hours, leads to increased urinary calcium excretion compared to continuous PN (15). Small intestinal resection in rats leads to significantly more urinary calcium loss than control rats while on PN (15, 16). It is unknown whether or not the PN-dependent patient adapts over time while on PN, and ultimately conserves more calcium (15).

Patients with short bowel syndrome (SBS) malabsorb calcium and phosphorus from intestinal feeds, potentially contributing to impaired bone accretion. The degree of malabsorption depends on amount and type of remaining bowel, underlying disease process, and amount of bowel adaptation (17–20).

Slow growth in infants and children with PN-dependent IF has been reported by others. In a study of 16 children, height and weight Z-scores were -1.5 and -0.7 respectively, after a mean 10.5y of PN (21). The investigators noted variations in growth over time related to nitrogen and energy supply in the PN. Lower height and weight has been reported in adults on home PN (22). In 40 patients (mean age 14.8 y) with infantile SBS, who were no longer receiving PN, weight for height and percent body fat were normal, but 53% of children and 78% of adults were significantly below their target height (23). Our study supports the findings of growth retardation as a significant problem for PN-dependent IF patients, whether or not metabolic bone disease is present. Reduced height-for-age and weight-for-age in PN-dependent IF participants compared to controls has also been reported in IF patients even after cessation of PN (24). Slow growth could be related to malabsorption in patients with SBS (25–27). In our study, our patients with IF were consistently above the 50th percentile for weight-for-height suggesting that caloric deficits did not play a role in poor linear growth. It similarly is unlikely there were other confounding conditions such as end stage liver disease, hemodynamically significant heart disease, renal or pulmonary disease that compromised growth, as participants with these conditions were excluded.

IGF-1 concentrations were significantly higher, and IGF-BP3 concentrations significantly lower, in IF participants in our study. IGF-1 concentrations reflect the integrated concentrations of growth hormone over 24 hours (28). Since IGF-1 concentrations were elevated compared to controls, and mean weight-for-height of IF participants was $>50^{\text{th}}$ percentile, protein malnutrition seem unlikely to contribute to the growth failure observed. Protein malnutrition decreases IGF-I production and increases its serum clearance and degradation (29). Comparable levels of cytokines between controls and IF participants in the presence of elevated serum IGF-1 do not support the notion that growth failure is related to inflammation.

Higher concentrations of serum IGF-1 in PN-dependent IF participants may indicate that IGF-1 resistance plays a role in growth retardation. Elevated serum IGF-1 concentrations with normal serum growth hormone have also been reported in patients with “idiopathic” short stature (30, 31). Among HIV and chronic renal failure patients, circulating inhibitors of serum IGF-1 have been postulated as a possible reason for IGF-1 resistance (32). Haploinsufficiency, where there is a partial resistance to IGF-1 due to either a low number

of IGF-1 receptors or a partial effect of an IGF-1 receptor mutation, resulting in decreased signal transduction, affecting only growth, has been postulated (33–35). PN-dependent patients with IF may experience a similar phenomenon, due to their underlying disease or a toxic component of PN; however, IGF-BP3 concentrations in affected patients are typically elevated or normal, and IGF-BP3 concentrations in our IF participants were lower than in controls (34–36). It is not entirely clear in humans whether IGF-BP3 is IGF-1 dependent (32). Investigating the possible role of IGF-1 resistance in growth failure of IF patients is a topic for future study.

We found elevated serum aluminum concentrations in IF participants, all exceeded the normal plasma aluminum concentration of $<10 \mu\text{g/L}$ (37). It is not known if the degree of elevation in our IF participants is clinically meaningful. Serum aluminum was not associated with BMC, BMD, or $1,25(\text{OH})_2\text{D}$ concentrations. Aluminum toxicity to bone, including osteomalacia and adynamic bone disease, is observed in dialysis patients when serum aluminum concentrations are $>135 \mu\text{g/mL}$ (38). In bone biopsies performed on patients with high serum aluminum concentrations, there is a negative correlation between the amount of bone surface staining for aluminum and indices of bone resorption and formation (38). In a study of PN-dependent adults and 2 children receiving PN, bone formation rate was inversely related to plasma aluminum concentrations and bone surface staining for aluminum. Plasma aluminum concentrations in a subset of these study participants, receiving PN protein constituents similar to our IF participants, were $15 \pm 9 \mu\text{g/L}$ (39). De Vernejoul found that elevated plasma aluminum alone, without surface stainable aluminum or increased total bone aluminum, was associated with reduced bone formation in PN patients (40). Information regarding bone histology in patients with lower serum aluminum concentrations would be helpful to ascertain the lower limit of serum aluminum causing bone disease.

Serum PTH was lower in the IF compared to control participants in our study, which may lead to lower bone turnover and bone mass. Patients receiving PN have suppressed PTH, which could lead to abnormalities in bone turnover, as has been seen by bone biopsy in patients receiving almost exclusive PN (41). The lower PTH may be due to the continuous infusion of calcium in the PN provided to the IF patients (42) or to the higher serum aluminum. Aluminum has been shown to affect PTH synthesis and secretion in vitro (43–46). It is also possible that the higher $25(\text{OH})\text{D}$ concentrations in the IF participants led their lower PTH. Higher serum $25(\text{OH})\text{D}$ concentrations in IF participants probably reflected their supplementation through PN, with intakes uniformly $> 400 \text{ IU/d}$.

Our study has limitations. Small sample size and lack of statistical power limited our ability to detect a 3% lower BMC and a 7% lower BMD in IF compared to control participants after adjustment for weight and length. A sample size of 150 (50 cases, 100 controls) would be required to identify a 7% difference. The small sample size also prevented further regression modeling beyond length and weight, to examine effects of significantly different serum laboratory measures on BMC and BMD. Due to the cross-sectional nature of our study, BMC, BMD and serum analytes were measured at one time point. IF patients have adjustments made to their PN constituents over time based on enteral feeding tolerance and growth, and these variations could alter bone accrual at a given point in time. Also, we did

not collect information on physical activity, stool or urinary losses of calcium and phosphate, all of which may affect bone mass. We did not have information on dietary calcium intake of our control participants, preventing us from comparing the calcium intake between groups, and determining whether control participants were consuming sufficient calcium to optimize bone health.

Given the difficulty of recruiting healthy young children to serve as control subjects when a blood sample is needed, we recruited healthy controls that were having blood drawn for a minor surgical procedure that required placement of an intravenous catheter. Blood samples in controls were obtained under fasted conditions whereas this was not necessarily so for IF participants. The difference in fasting status between cases and controls is not likely to affect IGF-1 (29, 47) or PTH. There is evidence that PTH decreases with prolonged fasting; however, not the short term fasting that our controls were subjected to pre-operatively (48). It is unlikely that fasting status affected serum aluminum, cytokine, or 25(OH)D concentrations.

A potential limitation of our control group is that they may not have been receiving sufficient vitamin D and calcium to support optimal bone formation. The vitamin D status of our control participants was worse than that of cases. Thus it is possible that this difference may have biased our study findings towards the null. The Institute of Medicine specified a cutoff for serum 25(OH)D concentration of > 20 ng/mL (50 nmol/L) as vitamin D sufficient for nearly all children. However, we recognize that there is controversy regarding optimal serum 25(OH)D concentrations and that others have advocated for a higher cutoff (49). The median 25(OH)D concentrations of our control participants was 29.6 ng/mL, comparable to that of children in the US of similar age (50).

Although we had a relatively small number of IF participants, they reflected a broad range of patients in terms of etiology of IF and percent enteral nutrition. Participants were only excluded for conditions that would likely have a large effect on their bone density. Given the heterogeneity of IF, multicenter studies will be required to investigate disease specific consequences to bone accrual. Future research should longitudinally monitor the growth and bone health in larger samples of IF patients to better assess changes over time. Further investigation of the role of IGF-1 resistance and aluminum excess in the growth retardation of IF patients could be insightful.

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References

1. Spencer AU, Kovacevich D, McKinney-Barnett M, et al. Pediatric short-bowel syndrome: the cost of comprehensive care. *Am J Clin Nutr.* 2008; 88(6):1552–1559. [PubMed: 19064515]
2. Nousia-Arvanitakis S, Angelopoulou-Sakadami N, Metroliou K. Complications associated with total parenteral nutrition in infants with short bowel syndrome. *Hepatogastroenterology.* 1992; 39(2): 169–172. [PubMed: 1353049]
3. Raman M, Gramlich L, Whittaker S, et al. Canadian home total parenteral nutrition registry: preliminary data on the patient population. *Can J Gastroenterol.* 2007; 21(10):643–648. [PubMed: 17948134]
4. Ukleja A, Romano MM. Complications of parenteral nutrition. *Gastroenterol Clin North Am.* 2007; 36(1):23–46. v. [PubMed: 17472873]
5. Foldes J, Rimon B, Muggia-Sullam M, et al. Progressive bone loss during long-term home total parenteral nutrition. *JPEN J Parenter Enteral Nutr.* 1990; 14(2):139–142. [PubMed: 2112620]
6. Burnes JU, O'Keefe SJD, Fleming CR, et al. Home parenteral nutrition—a 3-year analysis of clinical and laboratory monitoring. *J Parenteral Enteral Nutr.* 1992; 16(4):327–332.
7. Compheer C, Pazianas M, Benedict S, et al. Systemic inflammatory mediators and bone homeostasis in intestinal failure. *J Parenteral Enteral Nutr.* 2007; 31(2):142–147.
8. Ling PR, Khaodhiar L, Bistrrian BR, et al. Inflammatory mediators in patients receiving long-term home parenteral nutrition. *Digestive Diseases Sciences.* 2001; 46(11):2484–2489. [PubMed: 11713958]
9. Diamanti A, Bizzarri C, Basso MS, et al. How does long-term parenteral nutrition impact the bone mineral status of children with intestinal failure? *J Bone Mineral Metab.* 2010; 28(3):351–358.
10. Ceriotti F, Boyd JC, Klein G, et al. Reference intervals for serum creatinine concentrations: assessment of available data for global application. *Clin Chem.* 2008; 54(3):559–566. [PubMed: 18202155]
11. Blum WF, Breier BH. Radioimmunoassays for IGFs and IGFBPs. *Growth Regul.* 1994; 4(Suppl 1):11–19. [PubMed: 7515738]
12. Hurley DL, McMahon MM. Long-term parenteral nutrition and metabolic bone disease. *Endocrinol Metab Clin North Am.* 1990; 19(1):113–131. [PubMed: 2113469]
13. Bengoa JM, Sitrin MD, Wood RJ, et al. Acid-induced hypercalciuria in patients on total parenteral nutrition. *Am J Clin Nutr.* 1983; 38(2):264–269. [PubMed: 6410898]
14. Berkelhammer CH, Wood RJ, Sitrin MD. Acetate and hypercalciuria during total parenteral nutrition. *Am J Clin Nutr.* 1988; 48(6):1482–1489. [PubMed: 3144173]
15. Wood RJ, Bengoa JM, Sitrin MD, et al. Calciuretic effect of cyclic versus continuous total parenteral nutrition. *Am J Clin Nutr.* 1985; 41(3):614–619. [PubMed: 3919560]
16. Chu R, Barkowski S, Buhac J. Small bowel resection-associated urinary calcium loss in rats on long-term total parenteral nutrition. *J Parenteral Enteral Nutr.* 1990; 14(1):64–67.
17. Gouttebel M, Saint Aubert B, Colette C, et al. Intestinal adaptation in patients with short bowel syndrome. *Digestive Dis Sci.* 1989; 34(5):709–715.
18. Hylander E, Ladefoged K, Jarnum S. The importance of the colon in calcium absorption following small-intestinal resection. *Scand J Gastroenterol.* 1980; 15(1):55–60. [PubMed: 7367822]
19. Hylander E, Ladefoged K, Jarnum S. Calcium absorption after intestinal resection: the importance of a preserved colon. *Scand J Gastroenterol.* 1990; 25(7):705–710. [PubMed: 2396084]
20. Matarese LE, Steiger E. Dietary and medical management of short bowel syndrome in adult patients. *J Clin Gastroenterology.* 2006; 40(Suppl 2):S85.
21. Colomb V, Dabbas M, Goulet O, et al. Prepubertal growth in children with long-term parenteral nutrition. *Hormone Res Paediatrics.* 2002; 58(1):2–6.
22. Tjellessen L, Staun M, Rannem T, et al. Body composition in patients on home parenteral nutrition. *Scand J Clin Lab Invest.* 1996; 56(4):295–303. [PubMed: 8837235]
23. Olieman JF, Penning C, Spoel M, et al. Long-term impact of infantile short bowel syndrome on nutritional status and growth. *Br J Nutr.* 2012; 107(10):1489–1497. [PubMed: 21917192]

24. Dellert SF, Farrell MK, Specker BL, et al. Bone mineral content in children with short bowel syndrome after discontinuation of parenteral nutrition. *J Pediatrics*. 1998; 132(3):516–519.
25. Atia A, Girard-Pipau F, Hébuterne X, et al. Macronutrient Absorption Characteristics in Humans With Short Bowel Syndrome and Jejunocolonic Anastomosis: Starch Is the Most Important Carbohydrate Substrate, Although Pectin Supplementation May Modestly Enhance Short Chain Fatty Acid Production and Fluid Absorption. *J Parenteral Enteral Nutr*. 2011; 35(2):229–240.
26. Mziray-Andrew CH, Sentongo TA. Nutritional Deficiencies in Intestinal Failure. *Pediatric Clinics North America*. 2009; 56(5):1185.
27. Nordgaard I, Hansen B, Mortensen P. Importance of colonic support for energy absorption as small-bowel failure proceeds. *Am J Clin Nutr*. 1996; 64(2):222–231. [PubMed: 8694024]
28. Bope E. *Conn's Current Therapy*. Elsevier Inc. 2011
29. Ketelslegers JM, Maiter D, Maes M, et al. Nutritional regulation of insulin-like growth factor-I. *Metabolism Clin Experimental*. 1995; 44(10):50–57.
30. Lanes R, Plotnick LP, Spencer EM, et al. Dwarfism associated with normal serum growth hormone and increased bioassayable, receptorassayable, and immunoassayable somatomedin. *J Clin Endocrinol Metab*. 1980; 50(3):485–488. [PubMed: 6987254]
31. Momoi T, Yamanaka C, Kobayashi M, et al. Short stature with normal growth hormone and elevated IGF-I. *Eur J Pediatr*. 1992; 151(5):321–325. [PubMed: 1396882]
32. Jain S, Golde DW, Bailey R, et al. Insulin-like growth factor-I resistance. *Endocr Rev*. 1998; 19(5):625–646. [PubMed: 9793761]
33. Veenma DC, Eussen HJ, Govaerts LC, et al. Phenotype-genotype correlation in a familial IGF1R microdeletion case. *J Med Genet*. 2010; 47(7):492–498. [PubMed: 19955558]
34. Ester WA, van Duyvenvoorde HA, de Wit CC, et al. Two short children born small for gestational age with insulin-like growth factor 1 receptor haploinsufficiency illustrate the heterogeneity of its phenotype. *J Clin Endocrinol Metab*. 2009; 94(12):4717–4727. [PubMed: 19864454]
35. Walenkamp MJ, van der Kamp HJ, Pereira AM, et al. A variable degree of intrauterine and postnatal growth retardation in a family with a missense mutation in the insulin-like growth factor I receptor. *J Clin Endocrinol Metab*. 2006; 91(8):3062–3070. [PubMed: 16757531]
36. Inagaki K, Tiulpakov A, Rubtsov P, et al. A familial insulin-like growth factor-I receptor mutant leads to short stature: clinical and biochemical characterization. *J Clin Endocrinol Metab*. 2007; 92(4):1542–1548. [PubMed: 17264177]
37. Klein GL, Alfrey AC, Miller NL, et al. Aluminum loading during total parenteral nutrition. *Am J Clin Nutr*. 1982; 35(6):1425–1429. [PubMed: 6805302]
38. Hodsman AB, Steer BM. Serum aluminum levels as a reflection of renal osteodystrophy status and bone surface aluminum staining. *J Am Soc Nephrol*. 1992; 2(8):1318–1327. [PubMed: 1378308]
39. Vargas JH, Klein GL, Ament ME, et al. Metabolic bone disease of total parenteral nutrition: course after changing from casein to amino acids in parenteral solutions with reduced aluminum content. *Am J Clin Nutr*. 1988; 48(4):1070–1078. [PubMed: 3138907]
40. de Vernejoul MC, Messing B, Modrowski D, et al. Multifactorial low remodeling bone disease during cyclic total parenteral nutrition. *J Clin Endocrinol Metab*. 1985; 60(1):109–113. [PubMed: 3917265]
41. Ament ME. Bone mineral content in patients with short bowel syndrome: the impact of parenteral nutrition. *J Pediatr*. 1998; 132(3):386–388. [PubMed: 9544887]
42. Ledger GA, Burritt MF, Kao PC, et al. Role of parathyroid hormone in mediating nocturnal and age-related increases in bone resorption. *J Clin Endocrinol Metab*. 1995; 80(11):3304–3310. [PubMed: 7593443]
43. Morrissey J, Rothstein M, Mayor G, et al. Suppression of parathyroid hormone secretion by aluminum. *Kidney Int*. 1983; 23(5):699–704. [PubMed: 6308327]
44. Lajeunesse D, Moreau R, Hobbs W, et al. Influence of aluminum on the regulation of PTH- and 1,25(OH)2D3-dependent pathways in the rat osteosarcoma cell line ROS 17/2.8. *J Bone Miner Res*. 1998; 13(6):962–969. [PubMed: 9626627]
45. Diaz-Corte C, Fernandez-Martin JL, Barreto S, et al. Effect of aluminium load on parathyroid hormone synthesis. *Nephrol Dial Transplant*. 2001; 16(4):742–745. [PubMed: 11274267]

46. Fuleihan G, Brown E. Parathyroid hormone secretion and action. <http://www.uptodate.com/contents/parathyroid-hormone-secretion-and-action>.
47. Clemmons DR, Klibanski A, Underwood LE, et al. Reduction of plasma immunoreactive somatomedin C during fasting in humans. *J Clin Endocrinol Metab*. 1981; 53(6):1247–1250. [PubMed: 7197688]
48. Schlemmer A, Hassager C. Acute fasting diminishes the circadian rhythm of biochemical markers of bone resorption. *European journal of endocrinology*. 1999; 140(4):332–337. [PubMed: 10097253]
49. Vieth R. Why the minimum desirable serum 25-hydroxyvitamin D level should be 75 nmol/L (30 ng/ml). *Best Practice & Research Clinical Endocrinology & Metabolism*. 2011; 25(4):681–691. [PubMed: 21872808]
50. Looker, AC.; Johnson, CL.; Lacher, DA., et al. Vitamin D status: United States, 2001–2006. In: Statistics, NCHS, editor. NCHS Data Brief. Hyattsville, MD: 2011.

Figure 1. Mean bone mineral content (BMC) of the lumbar spine of IF and control participants with and without adjusting for length and weight. Means were compared using GEE modeling, and all regression models included age, sex, and race/ethnicity. Error bars are standard errors.

Figure 2.

Mean bone mineral density (BMD) of the lumbar spine of IF and control participants with and without adjusting for length and weight. Means were compared using GEE modeling, and all regression models included age, sex, and race/ethnicity. Error bars are standard errors.

Table 1

Calcium and vitamin D intakes of participants with PN-dependent intestinal failure

Participant	Age (y)	% Parenteral Nutrition	Total calcium (mg/d)	Total vitamin D (IU/d)
T001	0.6	82	657	672
T002	2.2	49	764	692
T003	6.4	41	192*	400*
T004	2.2	62	796	689
T005	5.9	50	181*	3600*
T006	4.2	50	1018	745
T007	1.2	100	243	400
T008	3.1	100	340	400
T009	1.9	42	1075	1925
T010	4.0	24	896	680
T011	1.6	28	1219	928
T012	2.5	100	144	400
T013	10.7	100	180	400
T015	7.3	100	563	400
T016	1.3	100	180	400
T017	3.5	57	885	1163
T018	0.8	33	896	760
T019	1.5	100	401	400
T020	0.8	100	381	400
T021	0.7	60	530	595
Median Excluding *	2.2	61	465 546	633 633

Table 2

Descriptive Characteristics of Study Participants

	Intestinal Failure	Control	p-value
N	20	49	
Age (mo)²	26 (6–127)	25 (7–127)	
Sex (% male)	45% (9/20)	45% (22/49)	
Race/ethnicity[*]			
White	12	37	
Black	4	12	
Asian	1	0	
Hispanic White	2	0	
Hispanic Black	1	0	
Length or height (cm)³	89.3 (20.8)	95.4 (20.1)	0.0011
Length-for-age percentile²	12.5 (3.8, 42.0)	63.0 (35.0–85.0)	<0.0001
Height Z-score	−0.96 (1.19)	0.14 (1.00)	0.0002
Weight (kg)³	14.1 (6.6)	16.0 (7.7)	0.08
Weight-for-age percentile²	29.5 (11.0–58.0)	54.0 (30.0–77.0)	0.029
Weight Z-score	−0.54 (1.09)	0.05 (1.15)	0.07
Weight for height or BMI percentile¹	0.62 (0.24)	0.58 (0.33)	0.61
Birth weight (kg)³	2.91 (0.94)	3.12 (0.75)	0.32
Gestational age (weeks)³	36.3 (3.6)	38.0 (3.0)	0.03

^{*} Asian and Hispanic participants were matched to White participants due to lack of an available match for their race/ethnicity.

¹ <2 years weight for height; >2 years BMI percentile.

² Median (inter-quartile range)

³ Mean (standard deviation)

Table 3

Comparison of Laboratory Values using Generalized Estimating Equations (GEE) Modeling

	Intestinal Failure (IF) Participants	Control Participants	p-value
N	20	49	
Aluminum ($\mu\text{g/L}$) ¹	22.5 (8.8)	7.1 (3.6)	<0.0001
TNF- α (pg/mL) ¹	18.4 (8.5)	21.6 (8.5)	0.09
IL-6 (pg/mL) ²	0.32 (0.32–0.32)	0.32 (0.32–0.32)	0.42
IL-1 β (pg/mL) ²	0.32 (0.32–4.12)	0.32 (0.32–1.56)	0.36
IGF-BP3 (ng/mL) ¹	1418.4 (447.7)	1812.3 (568.8)	<0.0001
IGF-1 (ng/mL) ²	97.2 (85.9–127.1)	64.2 (43.9–109.1)	0.036
PTH (pg/mL) ²	51.2 (38.4–79.0)	98.1 (72.2–124.0)	0.0002
25(OH) D (ng/mL) ¹	39.5 (11.5)	29.6 (8.0)	0.0005
1,25(OH) ₂ D (ng/mL) ²	55.5 (30.0–197.0)	--	

¹ Mean (standard deviation)

² Median (inter-quartile range)