University of Cincinnati

Date: 10/24/2011

I, Stephanie S Appleman M.D., hereby submit this original work as part of the requirements for the degree of Master of Science in Clinical and Translational Research.

It is entitled:
Bone Disease in TPN-dependent Infants and Children with Intestinal Failure

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This work and its defense approved by:

Committee chair: Paul Succop, PhD

Committee member: James Heubi, MD

1999
Bone Disease in TPN-dependent Infants and Children with Intestinal Failure

A thesis submitted to the

Graduate School

of the University of Cincinnati

in partial fulfillment of the

requirements for the degree of

Master of Clinical and Translational Research

in the Department of Environmental Health

of the College of Medicine

by

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November 2011

Committee Chair: Paul Succop, Ph.D.
Abstract: Total parenteral nutrition (TPN) dependent infants and children with intestinal failure (IF) are at risk for reduced bone mineral content and density owing to inflammation, disturbances in the growth hormone-insulin-like growth factor (IGF)-1 axis or vitamin D and Ca metabolism, and/or aluminum toxicity. We performed a cross sectional study comparing infants and children with intestinal failure with duos of age, sex, and race matched controls. Bone mineral content (BMC) and density (BMD) of the lumbar spine was measured by dual x-ray absorptiometry, and serum cytokines, aluminum, IGF-1 and IGF-BP3, parathyroid hormone (PTH), 25(OH) Vitamin D, and 1, 25(OH)2 Vitamin D were measured. Generalized estimating equation models accounting for matching were used for comparisons. BMC and BMD were lower (15% and 12%) in IF participants than controls (p=0.0009 and p=0.004). However, group differences were attenuated (to 3% and 7%, respectfully) and did not differ (p=0.40 and p=0.07) when adjusted for length and weight. Length and weight percentiles were significantly lower in IF versus control participants (12.5% vs. 63%, p<0.0001; 29.5% vs. 54%, p=0.03). IF participants had significantly 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 significantly lower IGF-BP3 (1418 vs. 1812 ng/mL, p<0.0001) and PTH concentrations (51 vs. 98 pg/mL, p=0.0002). No significant difference between IF and control participants was seen for serum cytokines (p≥0.09). Additional investigation is needed to elucidate the cause of growth retardation in IF patients and its impact on bone mass and density, especially the role of IGF-1 resistance and aluminum toxicity.
Acknowledgements: We would like to thank Donna Buckley and Crystal Slaughter for help with participant recruitment and study management, Philippe F. Backeljauw, MD for help regarding the manuscript, staff of the CTRC, and the ENT and Urology departments for control recruitment, patients and families.
# Table of Contents

Abstract......................................................................................................................... ii

Introduction.................................................................................................................. 1

Material and Methods................................................................................................. 1

Study participants........................................................................................................ 1

Study design................................................................................................................ 2

Laboratory methods.................................................................................................... 3

Data Analysis............................................................................................................... 4

Results ......................................................................................................................... 5

Discussion.................................................................................................................... 7

References ..................................................................................................................... 16
Tables and Figures

Table 1: Descriptive Characteristics of Study Participants..........................12

Figure 1: Mean BMD – Unadjusted and Adjusted........................................13

Figure 2: Mean BMC – Unadjusted and Adjusted........................................14

Table 2: Comparison of Laboratory Values..................................................15
Introduction

Patients with intestinal failure (IF) have malabsorption due to loss of absorptive surface or intestinal dysfunction necessitating total parenteral nutrition (TPN) to sustain growth and hydration. The incidence of IF in developed countries is ~2–6.8 per 1,000,000 children. It affects infants and children of all ages, with the most common cause being short bowel syndrome. The average total cost of care per child over a 5 year period in the United States is $1,619,851[1].

Metabolic bone disease associated with TPN leads to reduced bone mass in adults [2-4] with the risk of bone disease dependent upon TPN duration [5]. No studies have systematically assessed bone mineral density (BMD) in infants and children with TPN dependent IF. TPN dependent patients are at risk for bone disease owing to continued exposure to aluminum in TPN, low serum 25(OH) Vitamin D and IGF-1 and/or IGF-BP3 concentrations, and chronic inflammation.

The purposes of this study were to assess whether infants and children with IF have lower BMD compared to age, sex, and race matched controls, and to assess potential mechanisms that might contribute to low BMD. We hypothesized that infants and children with TPN-dependent IF would have lower BMD than age, sex, and race matched controls even when controlling for impaired growth. Furthermore, we hypothesized that IF patients would have higher serum cytokines (TNF-α, IL-1 ß, IL-6) and aluminum concentrations, and lower serum concentrations of IGF-1, IGF-BP3, 25(OH) Vitamin D, and 1, 25(OH)2 Vitamin D.

Materials and Methods

Study Participants

Study participants with TPN-dependent IF, defined by the inability to sustain growth without parenteral nutrition, were recruited from Cincinnati Children’s Hospital Medical Center (Cincinnati, OH), and Nationwide Children’s Hospital (Columbus, OH). Bowel dysfunction requiring TPN could be due to any cause; including, necrotizing enterocolitis, gastroschisis,
omphalocele, volvulus, intestinal atresia, intestinal pseudoobstruction, and Hirschsprung’s disease. Inclusion criteria for IF participants were age between 6 months and <18 years who had TPN-dependent IF. Exclusion criteria for IF participants were: clinically significant renal disease (<50% function for age as measured by creatinine); cerebral palsy or other disorders of the musculoskeletal system; use of medications that affect bone metabolism within the last 6 months for >1 week; antibiotic use for systemic infection <1 week from time of enrollment; any type of 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 specific ENT and urologic surgeries, and gastrointestinal endoscopic procedures, as intravenous line placement or sedation facilitated blood sample collection. Control participants were pair matched by age, sex, and race to an IF participant. The following age criteria were used to match on age: ages 6-18 months: ± 1 month; ages 19-36 months: ± 2 months; ages 37-72 months: ± 3 months; ages 73-120 months: ± 4 months; ages 121-144 months: ± 5 months; ages 145-216 months: ± 6 months. Exclusion criteria for control participants were: TPN usage for >2 weeks at any point in time; any of the following clinically significant chronic medical conditions: liver disease, cardiac disease, renal disease, endocrinopathy, cerebral palsy; disorders of the musculoskeletal system; chronic inflammatory conditions; immunodeficiency; any congenital or acquired syndromes or disorders; use of medications that affect bone metabolism within the last 6 months for >1 week; antibiotic use for systemic infection <1 week from time of enrollment; and orthopedic procedures that limit mobility. There was no restriction regarding gestational age; however, non-premature controls (>37 weeks gestation) below the 5th percentile for length (or height) or weight were excluded.

**Study Design**

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

Anthropometrics including height (ages >2y)/length (ages ≤ 2 years) 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 were determine using the CDC 2000 growth reference. Study participants provided a blood sample for measurement of serum concentrations of 25(OH) Vitamin D, parathyroid hormone, IGF-1, IGF-BP3, cytokines (IL-1 β, IL-6, TNF-α), 1,25 (OH)₂ Vitamin D, and aluminum. Laboratory results for a basic metabolic panel, liver profile, and triglycerides were performed in the clinical laboratories of CCHMC and were obtained from the medical record of IF participants.

Bone mineral density (BMD) of the lumbar spine was 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 for lumbar spine BMC and BMD is 3% and 2% for children ≤ 36 months, and 2.5% and 1% for children 6-9 years old.

This study was approved by the Institutional Review Board and the Scientific Advisory Committee of the Clinical and Translational Research Center at Cincinnati Children’s Hospital Medical Center.

*Laboratory Methods*

Serum concentration of 25(OH) Vitamin D was measured by a direct competitive chemiluminescence immunoassay (CLIA) (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 inter-assay (CV) was 9.4 ± 1.6 % with control 1 and 7.7 ± 4.2% with Control 2.

Serum 1, 25 (OH)₂ Vitamin D 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. Intact PTH was measured by an immunoradiometric (IRMA) assay (Diasorin Liaison, Stillwater Minnesota). Intra-assay CVs with Control 1 and Control 2 were 2.5% and 1.3%, respectively. The inter-assay CVs were 5.5% and 2.7% CV, respectively.

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

Serum cytokine (IL-1β, IL-6, and TNF-α) concentrations were determined by enzyme-linked immunosorbent assay (ELISA) using MilliplexTM Multiplex kits (Millipore, Billerica, MA). Samples were measured in duplicate. Plates were read using luminex technology on the Bio-PlexTM (Bio-Rad, Hercules, CA). Minimum detectable concentrations were 0.4 pg/mL for IL-1β, 0.3 pg/ml for IL-6, and 0.1 pg/ml for TNF-α. For IL-1β, 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) with a reference interval of 0-15ug/L, but <5 ug/L was the lower limit of detection. Inter-assay CV was 9%.

Data Analysis

All continuous variables were described using mean, median, standard deviation, and range. Categorical variables were described using frequencies and proportions. When lab values were reported as below the limit of detection for the assay, we used the mid-point between the lower limit of detection and zero in data analyses. All variables were compared between IF cases and matched controls using generalized estimating equations (GEE), which
accounted for clustering/matching effects. Variables that did not follow the normal distribution were log transformed for analyses. Results were the same with untransformed and transformed variables, so only the 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 done 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: 1 had significant heart disease requiring diuretics, 2 had cerebral palsy, 1 had panhypopituitarism, 1 had adrenal insufficiency and hypothyroidism, 3 had immunodeficiency, 1 was > age 18 years, 1 was receiving pamidronate. 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.

There were no differences between the IF and control participants on 2 matching criteria (age and sex), but race differed (Table 1). Asian and Hispanic White IF participants were matched to Caucasians, and Hispanic Black IF participants were matched to Blacks, due to lack of available controls of these race and ethnicities. Significant differences were seen between the two groups in length, length-for-age percentile, weight-for-age percentile, and gestational age. There were no differences in weight, weight-for-length, and birth weight. One participant in each group had a history of bone fracture.

Among IF participants, the cause of intestinal failure varied widely. Five participants had gastroschisis (1 with 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 (1
with perforation and massive resection, 1 with massive resection, and 1 with bowel perforation; 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 Hirschsprung’s of the small and large intestine; and 1 had necrotizing enterocolitis with massive resection. Eleven of 20 IF participants had a small bowel resection, with amount resected ranging from 17 cm to 135 cm; 5 IF participants had colonic resections. Percent of calories from enteral feeding ranged from 18-76% (median 39%). Eight IF participants were receiving no enteral nutrition. Time on TPN ranged from 4 to 103 months (median 18.5 months). Number of central line infections ranged from 0 to 8 (median 1).

Results from the GEE analyses indicated that participants with IF had lower lumbar spine BMC (9.11 vs. 10.70 gm, p=0.0009) and BMD (0.38 vs. 0.43 g/cm², p=0.004) than controls. 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, the magnitude of difference in BMC and BMD between groups was reduced and was no longer statistically significant (p=0.40 and 0.07 respectively) (Figures 1 and 2). BMC and BMD were 15% and 12% lower in IF participants than controls, prior to adjustment for weight and length, and 3% and 7% lower after adjustment.

IF participants had significantly higher serum aluminum, IGF-1, and 25(OH) Vitamin D concentrations, and significantly lower IGF-BP3 and PTH concentrations (Table 2). There was no significant difference in serum TNF-α, IL-1 β, or IL-6 concentrations between groups. Mean IGF-1 concentration in the IF participants was 111 ng/mL, and in the control participants was 83ng/mL which are the 70th and 50 th percentiles, respectively, for a larger population of normal 3 year olds (the mean age of participants in this study). Mean IGF-BP3 concentration in the IF participants was 1418 ng/mL, and in the control participants was 1812 ng/ml, which are the 5th and 30th percentiles, respectively, for a larger population of normal 3 year olds.
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), length of time on TPN (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 Vitamin D (r=0.19, p=0.43).

**Discussion**

Metabolic bone disease may complicate the management of children and adults on long term TPN. Metabolic bone disease diagnosed by histomorphometry or reduced bone density on a DXA scan, has been reported in 40-100% of adult TPN dependent patients [6]. Bone disease in children receiving TPN has not been studied previously. Historically, bone disease in TPN dependent patients had been attributed to aluminum toxicity and high doses of vitamin D in TPN. We found no difference in bone mineral content or density of the lumbar spine between infants and children receiving TPN 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 TPN solutions leading to increased serum aluminum concentrations, both of which have potential effects on growth and bone.

Slow growth in infants and children with TPN dependent IF has been reported by others. In a study of 16 children, height and weight Z-scores were -1.5 ± 1.3 SD and -0.7 ± 0.8 SD respectively, after a mean 10.5y of TPN [7]. Over the total duration of TPN use, Z-scores were -0.75 ± 1.4 SD for height and 0.2 ± 1.5 SD for weight. The investigators noted periods of slow growth over the time examined, and that the nitrogen and energy supply in the TPN was higher during the periods of normal growth compared to slow growth, suggesting that increasing calories and protein in TPN at the first sign of poor growth might attenuate the problem.
Tjellesen et al found significantly lower height and weight in 37 adult Scandinavian patients on home parenteral nutrition for 6-216 months, but found that they had a relatively normal body composition [8]. In a recently published cross sectional analysis of 40 patients (mean age 14.8 ± 6.8 years) with infantile short bowel syndrome, who were no longer receiving TPN, patients had normal weight for height, and percent body fat, but 53% of children and 78% of adults were significantly below their target height [9]. Our study supports the findings of growth retardation as a significant problem for TPN dependent patients, whether or not metabolic bone disease is present. Our finding of reduced height-for-age and weight-for-age in TPN dependent IF participants compared to controls has also been reported in IF patients even after cessation of TPN[10].

IGF-1 concentrations were significantly higher, and IGF-BP3 concentrations significantly lower, in the IF participants studied compared to control participants. IGF-1 is responsible for most of the trophic and growth-promoting effects of growth hormone, and is the most important factor in the negative feedback for growth hormone at both the pituitary and the hypothalamus. IGF-1 concentrations reflect the integrated concentrations of growth hormone over 24 hours [11]. Since IGF-1 concentrations were elevated compared to controls, and weight for height was >50\textsuperscript{th} percentile in 15 of 20 of the IF participants, protein and energy malnutrition seem unlikely to contribute to the growth failure observed. In addition, 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 ongoing inflammation.

Higher concentrations of serum IGF-1 in TPN dependent IF participants may indicate IGF-1 resistance, which may play a role in growth retardation. The IGF-1 receptor plays an important role in intrauterine and postnatal growth. Six receptor mutations that lead to IGF-resistance, with elevations of serum IGF-1 to varying degrees, have been described in humans who exhibit growth retardation of varying severity [12]. Elevated serum IGF-1 concentrations, with normal serum growth hormone, have also been reported in patients with "idiopathic" short
stature, with one author suggesting that IGF-1 resistance may be tissue specific [13, 14]. Among HIV and chronic renal failure patients, circulating inhibitors of serum IGF-1, have been postulated as a possible reason for IGF-1 resistance [15]. 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, with resulting decrease in signal transduction, affecting only growth has been postulated [16-18]. TPN dependent IF failure patients may experience a similar phenomenon, due to their underlying disease or a toxic component of TPN. Against this hypothesis is that IGF-BP3 concentrations in these patients are typically elevated or normal, and IGF-BP3 concentrations in our IF participants were lower than in control participants [17-19]. However, it is not entirely clear in humans as to whether IGF-BP3 is IGF-1 dependent [15]. Investigating the role of IGF-1 resistance on growth failure in IF participants was beyond the scope of the current study, but should be a topic for future studies.

Elevated serum Al concentrations were found in our IF participants with all 20 exceeding normal plasma aluminum concentrations of <10ug/L [20]. It is not known if the elevated serum aluminum concentrations in our IF participants are clinically meaningful. Serum aluminum concentrations were not associated with BMC, BMD, or 1, 25 (OH)2 vitamin D concentrations; however, our sample size was small so a type 2 error is possible. Aluminum toxicity to bone, including osteomalacia and aplastic bone disease, is observed in dialysis patients when serum aluminum concentrations are >135 ug/mL[21]. In bone biopsies performed on patients with high serum aluminum concentrations, there was a negative correlation between the amount of stained surface aluminum deposits and indices of resorption and bone formation [21]. In an adult study of IF patients receiving TPN, that included 2 children, bone formation rate was inversely related to plasma aluminum concentrations and bone surface staining for aluminum. Serum aluminum concentrations in a subset of these study participants, who received TPN protein constituents similar to our IF participants, were 15 ± 9 ug/L. Their rate of bone mineralization was still abnormal [22]. Information regarding bone histology in patients with
lower serum aluminum concentrations would be helpful to ascertain the lower limit of serum aluminum that results in bone disease.

Aluminum may lead to bone disease indirectly, potentially through IGF-1 or PTH. De Vernejoul found that elevated plasma aluminum alone, without surface stainable aluminum or increased total bone aluminum, was associated with reduced bone formation in TPN patients [23]. In animal studies, elevated serum aluminum concentrations have been associated with a decrease in IGF-1 [24, 25], although that was not seen in this study. Species differences may explain this finding. Serum parathyroid hormone was lower in the IF group compared to controls in our study. This is potentially due to the continuous infusion of calcium in the TPN provided to the IF patients, which could suppress PTH secretion [26]. It is also possible that the lower 25 (OH) Vitamin D concentrations in the control participants led to this finding. However, aluminum has also been shown to affect PTH synthesis and secretion in vitro [27-30]. Lower PTH concentrations in the IF participants may potentially lead to abnormally low bone turnover and compromised bone mass. Higher serum 25(OH) Vitamin D concentrations in the IF participants probably reflected their supplementation through TPN.

Our study had some limitations. A sample size and lack of power limited our ability to detect a 3% difference in BMC and a 7% difference in BMD between IF and control participants after adjustment for weight and length. The adjusted p-value for BMD was 0.07, which is very close to significance. A sample size of 150 (50 cases, 100 controls) would be required to determine if this effect would be significant. The small sample size also prevented further regression modeling beyond length and weight, to examine effects of serum aluminum and IGF-BP3 on BMC and BMD. These laboratory measures were different between groups, and have biologically plausible effects on BMD; therefore, they should be investigated in future studies of larger populations of patients.
Due to the cross sectional nature of our study, bone mineral content and density, as well as serum analytes, were measured at one point in time. IF patients requiring TPN have adjustments made to their TPN constituents, rate, and volume over time based on enteral feeding tolerance and growth. One could speculate that any of these variations over time could alter serum analytes leading to alterations in accrual of bone mass.

Our intestinal failure participants may not have been representative of all infants and children with intestinal failure, but they reflect a broad range of patients. As CCHMC is a transplant center, a wide range of IF patients were included, in terms of etiology of IF and percent enteral nutrition. Participants were only excluded for conditions which would likely have a large effect on their bone density.

Future research should follow the growth and bone health in larger populations (multicenter studies) of IF patients longitudinally to better assess changes over time. Further investigation as to whether IGF-1 resistance plays a role in the growth retardation of IF patients could be insightful, as well as the role that aluminum might play, as the etiology of their poor growth is not clear.
Table 1 Descriptive Characteristics of Study Participants

<table>
<thead>
<tr>
<th></th>
<th>IF Participants</th>
<th>Control Participants</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>20</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Age (months)$^2$</td>
<td>26 (6-127)</td>
<td>25(7-127)</td>
<td>0.95</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>45% (9/20)</td>
<td>45% (22/49)</td>
<td>0.99</td>
</tr>
<tr>
<td>Race/ethnicity*</td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>White</td>
<td>12</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>4</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hispanic White</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hispanic Black</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Length/height (cm)$^3$</td>
<td>89.3 ± 20.8</td>
<td>95.4 ± 20.1</td>
<td>0.0011</td>
</tr>
<tr>
<td>Length-for-age percentile$^2$</td>
<td>12.5 (0.1-81.0)</td>
<td>63.0 (1.0-99.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Weight (kg)$^3$</td>
<td>14.1 ± 6.6</td>
<td>16.0 ± 7.7</td>
<td>0.077</td>
</tr>
<tr>
<td>Weight-for-age percentile$^2$</td>
<td>29.5 (0.7-94.0)</td>
<td>54.0 (0.05-99.9)</td>
<td>0.029</td>
</tr>
<tr>
<td>Weight for Height or BMI percentile$^1$</td>
<td>0.62 ± 0.24</td>
<td>0.58 ± 0.33</td>
<td>0.61</td>
</tr>
<tr>
<td>Birth weight (kg)$^3$</td>
<td>2.91 ± 0.94</td>
<td>3.12 ± 0.75</td>
<td>0.32</td>
</tr>
<tr>
<td>Gestational Age (weeks)$^3$</td>
<td>36.3 ± 3.6</td>
<td>38.0 ± 3.0</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*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 and range.  $^3$Mean and standard deviation.
Unadjusted compares the BMD of IF and control participants. Adjusted compares the BMD of IF and control participants after taking into account the effect of length and weight on BMD. Generalized estimating equation modeling was used.
Unadjusted compares the BMC of IF and control participants. Adjusted compares the BMC of IF and control participants after taking into account the effect of length and weight on BMC. Generalized estimating equation modeling was used.
Table 2 Comparison of Laboratory Values

<table>
<thead>
<tr>
<th></th>
<th>IF Participants</th>
<th>Control Participants</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>20</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Aluminum (mcg/L)(^1)</td>
<td>23 ± 9</td>
<td>7 ± 4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TNF-α (pg/mL)(^1)</td>
<td>18.37 ± 8.51</td>
<td>21.65 ± 8.47</td>
<td>0.09</td>
</tr>
<tr>
<td>IL-6 (pg/mL)(^2)</td>
<td>0.32 (0.32-21.90)</td>
<td>0.32 (0.32-13.52)</td>
<td>0.42</td>
</tr>
<tr>
<td>IL-1 β (pg/mL)(^2)</td>
<td>0.32 (0.32-37.33)</td>
<td>0.32 (0.32-4.66)</td>
<td>0.36</td>
</tr>
<tr>
<td>IGF-BP3 (ng/mL)(^1)</td>
<td>1418.4 ± 447.7</td>
<td>1812.3 ± 568.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IGF-1 (ng/mL)(^2)</td>
<td>97.1 (53.5-266.1)</td>
<td>64.1 (7.9-312.4)</td>
<td>0.036</td>
</tr>
<tr>
<td>PTH (pg/mL)(^2)</td>
<td>51.1 (23.4-185.0)</td>
<td>98.1 (25.1-353.0)</td>
<td>0.0002</td>
</tr>
<tr>
<td>25 (OH) D (ng/mL)(^1)</td>
<td>39.5 ± 11.5</td>
<td>29.6 ± 8.0</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

\(^1\)Mean and standard deviation. \(^2\)Median and range.
References:


