Bone Mineral Density and Effects of Growth Hormone Treatment in Prepubertal Children with Prader-Willi Syndrome: A Randomized Controlled Trial


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Dutch Growth Research Foundation (R.F.A.d.L.v.W., D.A.M.F., A.C.S.H.-K.), 3016 AH Rotterdam, The Netherlands; Department of Pediatrics (R.F.A.d.L.v.W., D.A.M.F., A.C.S.H.-K.), Subdivision of Endocrinology, Erasmus University Medical Center Rotterdam, Sophia Children's Hospital, 3015 GJ Rotterdam, The Netherlands; Department of Pediatrics (B.J.O.), Subdivision of Endocrinology, Radboud University Nijmegen Medical Center, 6525 GA Nijmegen, The Netherlands; Department of Pediatrics (E.G.A.H.v.M.), Hieronymus Bosch Medical Center, 5211 NL ’s-Hertogenbosch, The Netherlands; Department of Pediatrics (J.R.), Subdivision of Endocrinology, VU University Medical Center, 1081 HV Amsterdam, The Netherlands; Department of Pediatrics (R.J.O.), Catharina Hospital, 5623 EJ Eindhoven, The Netherlands; Department of Pediatrics (M.v.L.), St. Jansdal Hospital, 3844 DG Harderwijk, The Netherlands; Department of Pediatrics (D.A.J.P.H.), Diaconessenhuis, 2334 CK Leiden, The Netherlands; Department of Pediatrics (G.B.), Subdivision of Endocrinology, University Medical Center Groningen/Beatrix Children’s Hospital, 9713 GZ Groningen, The Netherlands; and Department of Pediatrics (E.C.A.M.H.), Subdivision of Endocrinology, Haga Hospitals/Juliana Children’s Hospital, 2566 MJ The Hague, The Netherlands

Background: Bone mineral density (BMD) is unknown in children with Prader-Willi syndrome (PWS), but is decreased in adults with PWS. In patients with GH deficiency, BMD increases during GH treatment.

Objectives: The aim of the study was to evaluate BMD in children with PWS and to study the effects of GH treatment.

Design: We conducted a randomized controlled GH trial. Forty-six prepubertal children were randomized into either a GH-treated group (1.0 mg/m²·d) or a control group for 2 yr. At start, 6, 12, and 24 months of study, total body and lumbar spine BMD were measured by dual-energy x-ray absorptiometry, and lumbar spine bone mineral apparent density (BMAD) was calculated.

Results: Baseline total body and lumbar spine BMD SDS score (SDS) were normal [mean (so), –0.2 SDS (1.1) and –0.4 SDS (1.2), respectively]. BMADSDS, which corrects for short stature, was also normal [mean (so), 0.40 SDS (1.1)]. Total body BMDSDS decreased during the first 6 months of GH (P < 0.0001), but increased during the second year of treatment. After 24 months of study, total body and lumbar spine BMDSDS did not significantly differ between GH-treated children and randomized controls (P = 0.30, P = 0.44, and P = 0.47, respectively). Results were similar when corrected for body mass index SDS. Repeated measurements analysis showed a significant positive association between IGFI SDS and total body and lumbar spine BMDSDS, but not with BMADSDS.

Conclusions: Our results show that prepubertal children with PWS have a normal BMD. GH treatment had no effect on BMD, except for a temporary decrease of total body BMDSDS in the first 6 months. (J Clin Endocrinol Metab 94: 3763–3771, 2009)
Prader-Willi syndrome (PWS) is characterized by hypotonia, short stature, hyperphagia, hypogonadism, scoliosis, psychomotor delay, and temper tantrums (1–7). PWS results from the lack of expression of the paternally derived chromosome 15q11-q13 (3, 6). Hypothalamic dysfunction may be responsible for many features of PWS (8, 9). GH treatment is beneficial for children with PWS because it improves height, body composition (increase in lean body mass, decrease in fat percentage), and psychomotor development (10–15).

Bone mineral density (BMD) is the result of the equilibrium between bone formation and bone resorption. Adequate mineral acquisition during childhood is important for reaching peak BMD in late adolescence. A decreased peak BMD is a major determinant for osteoporosis and fracture risk later in life. Adequate gonadal hormone levels during pubertal development are vital for attainment of peak BMD (16, 17). In cross-sectional studies of adults with PWS, BMD was decreased in 60 to 90% of subjects (18–20). In addition, osteoporosis is considered a supportive finding in the diagnostic criteria of adult PWS (2). The decreased BMD may account for the higher risk of fractures during life and complications during spinal surgery (3, 18, 20–22). Although childhood is the critical period for bone accumulation, reports on BMD in children with PWS are very limited (12, 23, 24). Due to short stature in children with PWS, true BMD is underestimated by the standard areal measurement and should be corrected for bone size by calculating lumbar spine bone mineral apparent density (BMAD) (25–29). Because evaluation of BMD was not included in the aims of previous studies in children with PWS (12, 23, 24), a correction for short stature was never applied, and BMAD was never reported.

Patients with PWS have many symptoms resembling GH deficiency, including short stature, small hands and feet, an increased fat percentage, a decreased lean body mass, and low IGF-I levels (6, 7, 30, 31). In patients with GH deficiency, BMD is decreased but normalizes during GH treatment (27, 28). However, GH and IGF-I secretion are usually more affected in GH-deficient children than in those with PWS (7). The effects of GH treatment on BMD in children with PWS are unknown.

The aims of our study were to evaluate BMD and BMAD in prepubertal children with PWS and to investigate the effects of GH treatment. We hypothesized that the low BMD in adult patients with PWS is mainly the result of hypogonadotropic hypogonadism and is only partly due to GH insufficiency. We therefore expected BMD and BMAD to be within the low-normal range in our group of prepubertal children and expected a small effect of GH treatment. To investigate our hypotheses, we performed the present study within our randomized controlled GH trial in prepubertal children with PWS.

**Patients and Methods**

**Patients**

The study population comprised 46 prepubertal children with a genetically confirmed diagnosis of PWS by positive methylation test. At time of inclusion, girls were 4 to 12 yr old with Tanner breast stage less than 2 (32), and boys were between 4 and 14 yr old with Tanner genital stage less than 2 and a testicular volume less than 4 ml. None of the children had onset of puberty during 24 months of study. Caloric intake and activity level of all participants were standardized 3 months before inclusion. All children were naive to GH treatment at the start of study. The study protocol was approved by the Medical Ethics Committees of the Erasmus University Medical Center/Sophia Children’s Hospital in Rotterdam, The Netherlands, and of collaborating centers. Written informed consent was obtained from parents and from children over 12 yr of age. Assent was obtained from children under 12 yr of age.

**Design**

The primary objective of our study was to evaluate total body and lumbar spine BMD SD scores (SDS) and lumbar spine BMADSDS in prepubertal children with PWS. The secondary objectives were to investigate the effects of GH treatment on BMD and to study the relationship between scoliosis and BMD. After stratification for age and body mass index (BMI), all children were randomly assigned to either a GH-treated group (1.0 mg/m² · d) or a control group for 2 yr.

Biosynthetic GH (Genotropin; Pfizer Inc., New York, NY) was administered sc once daily at bedtime. The first 4 wk of GH treatment, children received 0.5 mg/m² · d to prevent fluid retention. Three-monthly, children were seen by the PWS research team of the Dutch Growth Research Foundation in collaboration with pediatric endocrinologists and pediatricians. At each visit, the GH dose was corrected to the calculated body surface area. All measurements described in this study were performed in the Erasmus University Medical Center Rotterdam/Sophia Children’s Hospital at start and at 6, 12, and 24 months of study.

**Dual-energy x-ray absorptiometry (DXA)**

In all children, bone mineral content (BMC; in grams), BMD (in grams per square centimeter) of the total body and the lumbar spine, fat mass, and lean body mass (LBM) were measured by DXA (type Lunar Prodigy; GE Healthcare, Chalfont St. Giles, UK). Quality assurance was performed daily. The coefficient of variation (CV) was 0.64% for BMC and total body BMD and 1.04% for lumbar spine BMD. The CV for lean tissue and fat tissue was 1.57 to 4.49% and 0.41 to 0.88%, respectively. In children with short stature, true BMD is underestimated by the areal presentation and should be corrected for bone size by calculating the BMAD (26). BMAD was calculated using the following model: BMAD = lumbar spine BMD · [4/(π · width)], with the width as the mean width of the second to fourth lumbar vertebral body. This model has been extensively validated by in vivo volumetric data obtained from magnetic resonance imaging of the lumbar vertebrae (26). BMADSDS was calculated according
to age- and sex-matched reference values from the Dutch population (33, 34). Fat mass was expressed as percentage of total body mass. Fat percentage SDS and LBMSDS were calculated according to sex- and height-matched reference values of the Dutch population (33, 34).

**Anthropometrics**

Standing height was measured with a Harpenden Stadiometer (Holtain Ltd., Crosswell, UK). Weight was assessed on an accurate scale (Servo Balance KA-20-150S; Servo Berkel Prior, Katwijk, The Netherlands). HeightSDS and BMISDS were calculated with Growth Analyser 3.0 (available at www.growthanalyser.org), according to age- and sex-matched reference values from the Dutch population (35).

**Scoliosis**

At the start and subsequently each year, standardized posteroanterior x-rays were taken in standing position. Scoliosis is defined as a spinal curve with a Cobb angle of more than 10° on a posteroanterior radiograph. The Cobb angle is the angle between the two steepest vertebrae, i.e. the upper border of the upper vertebra in the curve and the lower border of the lower vertebra. As previously reported (36, 37), Cobb angles were measured independently by two observers with minimal intra- and interobserver variance (intraclass correlation coefficient = 0.998 and 0.97, respectively).

**Assay**

Serum IGF-I levels were measured using an immunometric technique on an Advantage Automatic Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, CA). The intraassay CV was 4%, and the interassay CV was 6%. IGF-I SDS was calculated according to age- and sex-matched reference values of the Dutch population (38).

**Data analysis**

Because reference values for BMD parameters were not available for children under 4 yr of age, four children between 3 and 4 yr old were omitted from analyses, rendering a total number of 46 patients. All patients participating for at least 12 months were included in our analyses. Five children had not yet completed the 24-month protocol, but were not excluded from 12-month analyses because they were not considered dropouts. Therefore, the total number of patients at 24 months of study was five less than at 12 months of study.

Statistical analyses were performed with SPSS 15.0 (SPSS Inc., Chicago, IL) and with SAS 9.1 (SAS Institute Inc., Cary, NC). All data had a normal distribution, based on a P value above 0.05 with Levene’s test for normal distribution. Therefore, all data were expressed as means with SD values. To correct for multiple testing, the changes over time and differences between GH-treated children and randomized controls were analyzed using repeated measures of variance (39). Repeated measurements analysis was also performed to investigate the effects of different variables on BMD during study. Additional analyses were performed, correcting for any effect of BMISDS on BMD parameters. Effects are presented as β with 95% confidence intervals (CI). SDS were compared with −2, 0, and +2 SDS using Student’s one-sample t-test. P values less than 0.05 were considered statistically significant.

**Results**

**Baseline**

**Clinical data**

Forty-six prepubertal children with PWS (21 boys, 25 girls) were included in our randomized controlled GH trial (Table 1). At start of study, the mean (SD) age was 7.1 (2.2) yr. The genotype was specified in 40 children: 18 (45%) had a deletion of chromosome 15q11-q13, 17 (43%) had a maternal uniparental disomy, and five (13%) had an imprinting center defect. Positive methylation test was demonstrated in the remaining six patients, but the underlying genetic defect was not identified. Children had short stature, with a baseline height significantly below −1 SD (P < 0.0001). Baseline BMI was high but was still significantly lower than 2 SD (P < 0.0001). Body composition showed an increased fat mass and decreased lean body mass. Twenty-two children had a baseline IGF-I SDS below −2 SD.

**BMD**

Bones in the total body consist of 80% cortical bone, whereas the lumbar spine mainly consists of trabecular bone. Therefore, BMD was divided into total body and lumbar spine BMD. At baseline, the mean ± SD total body BMD was −0.23 SD ± 1.1, which did not significantly differ from zero SDS (Table 2 and Fig. 1). The mean ± SD baseline lumbar spine BMD was −0.44 SD ± 1.2. Baseline lumbar spine BMDSDS was significantly lower than zero SDS (P = 0.01) but was still significantly higher than −2 SD (P < 0.0001). Lumbar spine BMDSDS correlated significantly with age (r = 0.38; P = 0.01; Fig. 1). In children with PWS, BMD should be corrected for height by calculating the lumbar spine BMAD. The mean ± SD baseline BMAD was 0.40 SD ± 1.1, which was even significantly higher than zero SDS (P = 0.02) but was still significantly lower than 2 SD (P < 0.0001).

BMISDS was associated with total body BMDSDS [β (95% CI) = 0.35 (0.06–0.65); P = 0.02], lumbar spine BMDSDS [β (95% CI) = 0.73 (0.46–0.99); P < 0.0001], and BMADSDS [β (95% CI) = 0.33 (0.02–0.64); P = 0.04]. The normal BMD in prepubertal children could be related to the high-normal BMISDS. We therefore calculated the BMD parameters after correction for BMISDS. Corrected for BMISDS, the total body and lumbar spine BMDSDS were within normal range with a mean ± SD of −0.70 ± 1.0 and −1.40 ± 0.9 SD, respectively. The BMAD was −0.04 ± 1.1 SD when corrected for BMISDS and did not significantly differ from zero SDS (P = 0.88). This indicates that prepubertal children with PWS have a normal BMD, independently from BMISDS. Total body and lumbar spine BMDSDS, as well as BMADSDS did not significantly differ between boys and
Define as a Cobb’s angle of 10° or above. The mean IGF-I SDS.

Those with a level above those without.

Increased during the first year of GH treatment, but significantly lower than baseline after 24 months of study. LBMSDS significantly higher than in randomized controls at 6, 12, and 24 months of study (Table 1). In contrast to the control group, BMISDS decreased in GH-treated children during the first year and was still significantly lower than baseline after 24 months of study. LBMSDS of the control group declined continuously during 24 months of study. Fat percentage SDS significantly decreased during GH treatment, but did not significantly change in the control group. Also, IGF-I SDS significantly increased in GH-treated children, but did not change in randomized controls.

**Effects of GH treatment on total body BMD**

Total body BMDSDS fluctuated in GH-treated children, whereas it did not significantly change in randomized controls (Table 2 and Fig. 2). In the GH-treated group, total body BMDSDS significantly decreased during the first 6 months of treatment \( P < 0.0001 \) but did not significantly change during the second 6 months of treatment. Total body BMDSDS increased during the second year of GH treatment \( P = 0.04 \) compared with randomized controls. After 24 months of study, total body BMDSDS did not significantly differ between GH-treated children and randomized controls.

The fluctuation in total body BMDSDS may be the result of a disturbed equilibrium between bone expansion and mineral acquisition. Concurrently with the temporary decrease in total body BMDSDS during the first 6 months,

### TABLE 1. Data at baseline and during GH treatment in prepubertal children with PWS

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6 months</th>
<th>12 months</th>
<th>24 months</th>
<th>6 months vs. start</th>
<th>12 months vs. start</th>
<th>24 months vs. start</th>
</tr>
</thead>
<tbody>
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<td>GH-treated (n)</td>
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<td>27</td>
<td>27</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n)</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH-treated</td>
<td>7.37 ± 2.3</td>
<td>7.87 ± 2.3</td>
<td>8.37 ± 2.3</td>
<td>9.61 ± 2.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>6.81 ± 2.0</td>
<td>7.32 ± 2.1</td>
<td>7.82 ± 2.1</td>
<td>8.88 ± 2.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>0.41</td>
<td>0.41</td>
<td>0.41</td>
<td>0.31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HeightSDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH-treated</td>
<td>-1.95 ± 1.5</td>
<td>-1.46 ± 1.5</td>
<td>-1.07 ± 1.3</td>
<td>-0.46 ± 1.1</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Controls</td>
<td>-2.35 ± 1.1</td>
<td>-2.38 ± 1.1</td>
<td>-2.38 ± 1.2</td>
<td>-2.51 ± 1.2</td>
<td>0.61</td>
<td>0.74</td>
<td>0.36</td>
</tr>
<tr>
<td>LBMSDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH-treated</td>
<td>1.28 ± 1.2</td>
<td>0.82 ± 1.3</td>
<td>0.74 ± 1.2</td>
<td>0.85 ± 1.3</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Controls</td>
<td>1.37 ± 0.7</td>
<td>1.39 ± 0.7</td>
<td>1.47 ± 0.6</td>
<td>1.41 ± 0.6</td>
<td>0.84</td>
<td>0.44</td>
<td>0.82</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>0.74</td>
<td>0.02</td>
<td>0.02</td>
<td>0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMISDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH-treated</td>
<td>-2.44 ± 1.8</td>
<td>-1.79 ± 1.3</td>
<td>-1.90 ± 1.1</td>
<td>-2.30 ± 1.3</td>
<td>&lt;0.0001</td>
<td>0.004</td>
<td>0.37</td>
</tr>
<tr>
<td>Controls</td>
<td>-1.70 ± 2.2</td>
<td>-2.02 ± 2.1</td>
<td>-2.19 ± 1.7</td>
<td>-2.74 ± 1.6</td>
<td>0.045</td>
<td>0.03</td>
<td>0.0002</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>0.22</td>
<td>0.64</td>
<td>0.56</td>
<td>0.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat% SDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH-treated</td>
<td>2.80 ± 0.4</td>
<td>2.09 ± 0.7</td>
<td>1.93 ± 0.7</td>
<td>2.05 ± 0.6</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Controls</td>
<td>2.81 ± 0.6</td>
<td>2.81 ± 0.5</td>
<td>2.89 ± 0.5</td>
<td>2.89 ± 0.4</td>
<td>0.90</td>
<td>0.56</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>0.98</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-I SDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH-treated</td>
<td>-1.89 ± 1.1</td>
<td>1.81 ± 0.9</td>
<td>2.49 ± 1.3</td>
<td>2.26 ± 1.0</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Controls</td>
<td>-1.87 ± 1.0</td>
<td>-1.51 ± 1.3</td>
<td>-1.63 ± 2.0</td>
<td>-1.58 ± 1.4</td>
<td>0.38</td>
<td>0.30</td>
<td>0.41</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>0.97</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± sd. Significant P values are in bold. Fat%, Fat mass expressed as percentage of total body mass.

\(^a\) P values for differences between GH-treated children and randomized controls.

\(^b\) P values for results compared to baseline (both with repeated measurements analysis).

Girls or between children with different genotypes. Furthermore, these parameters did not differ significantly between children with an IGF-I level below -2 SDS and those with a level above -2 SDS and did not correlate with IGF-I SDS.

Twenty children (44%) had scoliosis at start of study, defined as a Cobb’s angle of 10° or above. The mean ± sd baseline Cobb angle was 22.6 ± 12.5°. Total body and lumbar spine BMDSDS, as well as BMADSDS, did not significantly differ between children with scoliosis and those without.

**GH treatment**

**Effects on height, BMI, body composition, and IGF-I levels**

HeightSDS increased in GH-treated children and was significantly higher than in randomized controls at 6, 12, and 24 months of study (Table 1). In contrast to the control group, BMISDS decreased in GH-treated children during the first year and was still significantly lower than baseline after 24 months of study. LBMSDS significantly increased during the first year of GH treatment, but returned to baseline values thereafter. In contrast, the
the total body bone area increased significantly more in GH-treated children than in randomized controls ($P = 0.001$). Although the increase in total body bone area during the second 6 months and the second year also was significantly greater in GH-treated children compared with randomized controls ($P = 0.02$ and $P = 0.001$, respectively), the total body BMDSDS did not significantly differ between GH-treated children and randomized controls after 24 months of study. This indicates that the equilibrium between bone expansion and mineral acquisition had restored.

Results in Table 2 were similar when corrected for BMISDS. Repeated measurements analysis showed a significant positive association between IGFI SDS and total body BMDSDS at 24 months of study [$\beta$ (95% CI) = 0.17 (0.06–0.29); $P = 0.004$]. LBM and fat mass corrected for age and gender were both significantly associated with total body BMDSDS[$\beta$ (95% CI) = 0.05 (0.01–0.09), $P = 0.01$; and 0.05 (0.02–0.07), $P = 0.0001$, respectively].

### Effects of GH treatment on lumbar spine BMD

Lumbar spine BMDSDS did not significantly change during study, both in GH-treated children and in randomized controls (Table 2 and Fig. 2). Thus, lumbar spine BMDSDS did not significantly differ between GH-treated children and randomized controls after 24 months of study, nor did the change in lumbar spine BMDSDS.

The lumbar spine mainly consists of trabecular bone, which has a higher rate of bone turnover than cortical bone (25). The equilibrium between bone formation and bone expansion is therefore earlier restored in trabecular bone. We found no significant fluctuation in lumbar spine BMDSDS during 24 months of study, whereas the increase in lumbar spine bone area was significantly greater in GH-treated children than in randomized controls.

BMISDS was significantly associated with lumbar spine BMDSDS during 24 months of study [$\beta$ (95% CI) = 0.36 (0.19–0.53); $P < 0.0001$]. After correction for BMISDS, the lumbar spine BMDSDS at 24 months of study was significantly greater compared with baseline in GH-treated children, but not in randomized controls ($P = 0.009$ and $P = 0.55$, respectively). However, corrected for BMISDS, there was no significant difference in lumbar spine BMDSDS at 24 months of study between GH-treated children and randomized controls ($P = 0.19$).

### Table 2. BMD in 46 prepubertal children with PWS

<table>
<thead>
<tr>
<th></th>
<th>Start</th>
<th>6 months</th>
<th>12 months</th>
<th>24 months</th>
<th>6 months vs. start$^b$</th>
<th>12 months vs. start$^b$</th>
<th>24 months vs. start$^b$</th>
<th>24 months vs. start$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total body BMDSDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH-treated</td>
<td>-0.37 ± 1.1</td>
<td>-0.71 ± 1.1</td>
<td>-0.73 ± 1.2</td>
<td>-0.49 ± 1.1</td>
<td>&lt;0.0001</td>
<td>0.001</td>
<td>0.23</td>
<td>0.70</td>
</tr>
<tr>
<td>Controls</td>
<td>-0.04 ± 1.0</td>
<td>-0.07 ± 1.1</td>
<td>-0.05 ± 0.9</td>
<td>-0.15 ± 0.9</td>
<td>0.72</td>
<td>0.96</td>
<td>0.51</td>
<td>0.47</td>
</tr>
<tr>
<td>$P$ value$^a$</td>
<td>0.31</td>
<td><strong>0.04</strong></td>
<td><strong>0.03</strong></td>
<td>0.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total body area (dm$^2$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH-treated</td>
<td>8.92 ± 3.5</td>
<td>9.92 ± 3.8</td>
<td>11.15 ± 4.1</td>
<td>12.86 ± 3.8</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Controls</td>
<td>8.33 ± 2.4</td>
<td>8.72 ± 2.4</td>
<td>9.09 ± 2.4</td>
<td>10.10 ± 2.6</td>
<td>0.007</td>
<td>0.0003</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$P$ value$^a$</td>
<td>0.35</td>
<td>0.13</td>
<td>0.05</td>
<td><strong>0.009</strong></td>
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<tr>
<td>Lumbar spine BMDSDS</td>
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</tr>
<tr>
<td>GH-treated</td>
<td>-0.41 ± 1.3</td>
<td>-0.52 ± 1.3</td>
<td>-0.19 ± 1.1</td>
<td>-0.08 ± 1.0</td>
<td>0.28</td>
<td>0.11</td>
<td>0.07</td>
<td><strong>0.009</strong></td>
</tr>
<tr>
<td>Controls</td>
<td>-0.49 ± 0.9</td>
<td>-0.26 ± 0.9</td>
<td>-0.27 ± 1.1</td>
<td>-0.34 ± 1.2</td>
<td>0.05</td>
<td>0.18</td>
<td>0.51</td>
<td>0.55</td>
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<tr>
<td>$P$ value$^a$</td>
<td>0.83</td>
<td>0.43</td>
<td>0.82</td>
<td>0.46</td>
<td></td>
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<tr>
<td>Lumbar spine area (dm$^2$)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>GH-treated</td>
<td>11.07 ± 3.0</td>
<td>12.10 ± 3.4</td>
<td>12.93 ± 3.7</td>
<td>14.91 ± 4.0</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<tr>
<td>Controls</td>
<td>10.67 ± 2.9</td>
<td>10.87 ± 3.1</td>
<td>11.29 ± 3.1</td>
<td>12.29 ± 3.2</td>
<td>0.31</td>
<td><strong>0.02</strong></td>
<td><strong>&lt;0.0001</strong></td>
<td><strong>&lt;0.0001</strong></td>
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<tr>
<td>$P$ value$^a$</td>
<td>0.49</td>
<td>0.14</td>
<td>0.11</td>
<td><strong>0.01</strong></td>
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<td>BMADDS</td>
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<tr>
<td>GH-treated</td>
<td>0.39 ± 1.2</td>
<td>0.23 ± 1.4</td>
<td>0.35 ± 1.1</td>
<td>0.37 ± 1.2</td>
<td>0.27</td>
<td>0.82</td>
<td>0.85</td>
<td>0.64</td>
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<tr>
<td>Controls</td>
<td>0.40 ± 1.0</td>
<td>0.66 ± 1.2</td>
<td>0.48 ± 1.2</td>
<td>0.67 ± 1.4</td>
<td>0.14</td>
<td>0.73</td>
<td>0.38</td>
<td>0.39</td>
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<tr>
<td>$P$ value$^a$</td>
<td>0.97</td>
<td>0.24</td>
<td>0.70</td>
<td>0.57</td>
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<tr>
<td>BMCSDS</td>
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<tr>
<td>GH-treated</td>
<td>-2.06 ± 1.6</td>
<td>-1.78 ± 1.5</td>
<td>-1.41 ± 1.4</td>
<td>-0.97 ± 1.3</td>
<td><strong>&lt;0.0001</strong></td>
<td><strong>&lt;0.0001</strong></td>
<td><strong>&lt;0.0001</strong></td>
<td><strong>&lt;0.0001</strong></td>
</tr>
<tr>
<td>Controls</td>
<td>-2.03 ± 0.8</td>
<td>-1.97 ± 0.8</td>
<td>-1.92 ± 0.7</td>
<td>-1.83 ± 0.8</td>
<td>0.44</td>
<td>0.29</td>
<td>0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>$P$ value$^a$</td>
<td>0.93</td>
<td>0.46</td>
<td>0.17</td>
<td><strong>0.03</strong></td>
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</tbody>
</table>

Data are presented as mean ± SD. Significant $P$ values are in bold. BMAD, Lumbar spine bone mineral apparent density (volumetric BMD).

$^a$ $P$ values for differences between GH-treated children and randomized controls.

$^b$ $P$ values for results compared to baseline.

$^c$ Corrected for BMISDS (all with repeated measurements analysis).
IGF-I SDS was significantly associated with lumbar spine BMDSDS at 24 months of study \( \beta (95\% \text{ CI}) = 0.19 (0.05–0.35), P = 0.01 \). LBM and fat mass corrected for age and gender were both significantly associated with lumbar spine BMDSDS \( \beta (95\% \text{ CI}) = 0.08 (0.03–0.12), P = 0.002 \) and \( 0.05 (0.02–0.08), P = 0.002 \), respectively.

**Effects of GH treatment on BMAD**

BMAD is the BMD corrected for height. BMADSDS did not significantly change during study, both in GH-treated children and in randomized controls (Table 2 and Fig. 2). Therefore, BMADSDS did not significantly differ between GH-treated children and randomized controls after 24 months of study. Results in Table 2 were similar when corrected for BMISDS.

IGF-I SDS was not associated with BMADSDS during 24 months of study \( P = 0.62 \). Furthermore, LBM and fat mass corrected for age and gender were not significantly associated with BMADSDS.

**Discussion**

Our study shows that total body and lumbar spine BMD, as well as lumbar spine BMAD, are normal in prepubertal children with PWS. BMADSDS, which corrects for short stature, was even significantly above zero SDS, indicating that BMD is not decreased in prepubertal children with PWS.

The normal BMADSDS is in contrast to results from cross-sectional studies in adults, showing osteoporosis in...
with PWS, our results show no effect of gender on BMD. In prepubertal children toward a low BMADSDS in adults. We postulate that this decrease results from delayed and abnormal pubertal development due to hypogonadotropic hypogonadism in many patients with PWS, which will lead to a lack of increase in BMD and a lower peak bone mass. Because hypogonadotropic hypogonadism more often affects men than women with PWS, it would be interesting to investigate whether the prevalence of a decreased BMD in adults with PWS varies with gender. Unfortunately, no data are available regarding gender-specific BMD in adults with PWS. In prepubertal children with PWS, our results show no effect of gender on BMD.

The mean BMISDS in our group of children was within normal range, but was higher than zero SDS. Although BMISDS was significantly associated with BMD parameters, BMAD did not significantly differ from zero SDS after correction for BMISDS. This indicates that prepubertal children with PWS have a normal BMD, independently from BMISDS. We did not measure dehydroepiandrosterone sulfate (DHEAS) levels in our patients. Other studies have demonstrated higher serum levels of dehydroepiandrosterone sulfate (DHEAS) in children with PWS (40, 41), which may contribute to the normal BMD demonstrated in our patients (42). In another report (47), we showed that bone age is only delayed in PWS children under 5 yr of age. Our current study population consisted of only five children younger than 5 yr. Therefore, we do not believe that BMADSDS according to bone age would yield different results than when calculated according to calendar age. Of note, a DXA scan provides information with regard to BMD, but future research may focus on bone strength as well as bone structure to account for the higher risk of fractures and complications during scoliosis surgery (3, 18, 20–22).

We evaluated the effects of GH treatment on BMD. Total body BMADSDS significantly decreased during the first 6 months of treatment, whereas the total body bone area increased significantly more in GH-treated children than in randomized controls. This finding suggests that the rate of bone expansion is higher than the rate of mineral acquisition. This has also been suggested in studies of other patient populations (27, 28). In contrast to bones in the total body, which consist of 80% cortical bone, the lumbar spine mainly consists of trabecular bone, which has a higher rate of bone turnover (25). Therefore, the equilibrium between bone expansion and mineral acquisition is earlier restored, which most likely explains why no significant decrease in lumbar spine BMADSDS was found during the initial phase of GH treatment despite a significantly higher increase of lumbar spine bone area in GH-treated children. The decrease in total body BMADSDS in our study was temporary, and we found no significant difference in total body BMADSDS between GH-treated children and randomized controls after 24 months of study. The fluctuation in total body BMADSDS during GH treatment was not described by Carrel et al. (12) and Myers et al. (23), who studied a group of infants and toddlers with PWS. Their data suggested a normal total body BMD and no effect of GH treatment on total body BMD in young children with PWS. However, because measurements were not performed at 6 months of study, a fluctuation in total body BMD could not be detected. Additionally, our results suggest that long-term GH treatment might increase total body BMADSDS as well as lumbar spine BMD, suggested by the significant positive association with IGF-I SDS at 24 months of study. We have evaluated BMAD as the best presentation of true BMD in children with PWS (29). Our study showed that BMADSDS did not significantly change during GH treatment and was not significantly associated with IGF-I SDS. Children with GH deficiency have a low BMD, which normalizes during GH treatment. In contrast, baseline BMD is not decreased in prepubertal children with PWS. Therefore, it is not unexpected to find no effect of GH treatment on BMD in this patient population. Because BMADSDS did not significantly change during the study, we conclude that GH treatment has no significant effect on BMD in prepubertal children with PWS during 24 months of study. Future research evaluating the long-term effect of GH treatment on BMD in children with PWS is warranted, particularly with regard to the influence of hypogonadotropic hypogonadism.

In line with other reports (43, 44), LBM and fat mass corrected for age and gender were significantly associated with total body BMADSDS and lumbar spine BMADSDS. In contrast to reports on adolescents with idiopathic scoliosis (45), we found no significant difference in BMD between children with scoliosis and those without. Thus, our study shows that the high prevalence of scoliosis in children with PWS (36, 37, 46) is not caused by a decreased BMD.

In conclusion, our study shows that prepubertal children with PWS have a normal BMADSDS of the total body, the lumbar spine, and corrected for short stature (BMADSDS). Total body BMADSDS temporarily decreased during the first 6 months of GH treatment. After 24 months of study, total body and lumbar spine BMADSDS and lumbar spine
BMADSDS did not significantly differ between GH-treated children and randomized controls.

Acknowledgments

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Address all correspondence and requests for reprints to:
Roderick de Lind van Wijngaarden, Clinical Research Fellow, Dutch Growth Research Foundation, Erasmus University Medical Center/Sophia Children’s Hospital, Westzeedijk 106, 3016 AH Rotterdam, The Netherlands. E-mail: r.delindvanwijngaarden@erasmusmc.nl.

Trial registration number: ISRCTN 49726762.

Disclosure Summary: The authors have nothing to disclose.

References


