Body Mass Index Differences in Pseudohypoparathyroidism Type 1a Versus Pseudopseudohypoparathyroidism May Implicate Paternal Imprinting of $G\alpha_s$ in the Development of Human Obesity

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Context: Obesity is a prominent feature of Albright hereditary osteodystrophy (AHO), a disorder caused by heterozygous GNAS mutations that disrupt the stimulatory G protein $\alpha$-subunit $G\alpha_s$. Because $G\alpha_s$ is paternally imprinted in certain hormone target tissues, maternal inheritance of AHO leads to multihormone resistance [pseudohypoparathyroidism type 1a (PHP1a)], whereas paternal inheritance leads to AHO alone [pseudopseudohypoparathyroidism (pseudoPHP)]. Classically, the obesity in AHO is described as occurring similarly in both conditions.

Setting: This observational study was conducted at the General Clinical Research Center, Johns Hopkins University School of Medicine; National Institutes of Health.

Patients: Fifty-three patients with AHO (40 with PHP1a and 13 with pseudoPHP) and two with progressive osseous heteroplasia were studied.

Main Outcome Measures: Main outcome measures were weight and height z score (SDS), body mass index (BMI) percentiles, and BMI z-scores.

Results: Patients with PHP1a had significantly greater mean weight SDS, BMI percentages, and BMI z-scores compared with patients with pseudoPHP. These differences in BMI were secondary to adipose content based on dual energy x-ray absorptiometry analysis. The mean BMI z-score $\pm$ SEM for PHP1a was 2.31 $\pm$ 0.18 compared with 0.65 $\pm$ 0.31 in pseudoPHP ($P = 0.000032$). Twenty-five of 40 (62.5%) patients with PHP1a had mean BMI z-scores greater than two SDS above the mean, whereas no patients with pseudoPHP had BMI z-scores in this range.

Conclusions: Although the AHO phenotype for PHP1a and pseudoPHP has been thought to be similar, we have found that obesity is a more prominent feature in PHP1a than in pseudoPHP and that severe obesity is characteristic of PHP1a specifically. These findings may implicate paternal imprinting of $G\alpha_s$ in the development of human obesity. (J Clin Endocrinol Metab 92: 1073–1079, 2007)

A Albright hereditary osteodystrophy (AHO) is a disorder caused by heterozygous inactivating mutations in GNAS, the gene encoding the $\alpha$-chain of the stimulatory G protein $G\alpha_s$, and is associated with short stature, obesity, brachydactyly, sc ossifications, dental abnormalities, and cognitive impairment. Patients with AHO with GNAS mutations on maternally inherited alleles often manifest resistance to multiple $G\alpha$ protein-coupled hormones (e.g. PTH, TSH, LH, FSH, GHRH), a variant termed pseudohypoparathyroidism type 1a (PHP1a). Those patients who inherit mutations on the paternal allele have the AHO developmental defects and phenotype alone without hormonal resistance, a variant termed pseudopseudohypoparathyroidism (pseudoPHP) (1–13). Patients with GNAS mutations can also manifest with a more limited disorder, progressive osseous heteroplasia (POH), which is characterized by severe heterotopic ossifications in deep connective tissue and skeletal muscle (14, 15).

The parental inheritance pattern of PHP1a vs. pseudoPHP is the result of the fact that $G\alpha_s$ is paternally imprinted (silenced) in specific hormone target tissues (3, 5, 6, 8, 9, 12). This pattern of inheritance in AHO was first observed clinically by analysis of pedigrees and ascribed to genomic imprinting (6, 12, 16, 17). Subsequent studies in murine models with targeted disruption of the Gnas gene provided evidence for tissue-specific imprinting (18–20). This tissue-specific imprinting has been found to be partial in most tissues examined with preferential expression of the maternal allele in the renal cortex, pituitary, thyroid, and gonad (3, 5, 8, 9, 19, 20). In tissues in which $G\alpha_s$ is imprinted, mutations on the active maternal allele lead to severe $G\alpha_s$ deficiency and hormone resistance,
whereas the same mutations on the relatively inactive paternal allele have little effect on Gαs expression or hormone action.

The severity of the AHO phenotype is extremely variable, even among members of the same family and generation (6, 12, 21). Classically, the AHO obesity phenotype has been viewed as a part of both conditions since first described by Fuller Albright in the mid-1900s (1, 2, 6, 12, 13, 22). However, with the exception of the work by Marguet et al. (22), the extent of the obesity had not been compared in PHP1a and pseudoph. On examination of our cohort of patients with AHO, we observed that the weight sd score (SDS) and body mass index (BMI) in PHP1a are significantly greater than in pseudoph. This is consistent with findings in AHO mouse models in which those mice with disruption of the maternal Gαs allele (analogous to PHP1a) have more significant obesity than those with disruption of the paternal allele (analogous to pseudoph) (18–20).

Therefore, we conducted a systematic examination of BMI in 53 patients with AHO, all with confirmed GNAS mutations verifying the diagnosis. We demonstrate that obesity is not only more common in PHP1a than in pseudoph, but it is marked by significantly greater mean weight SDS as well as BMI percentiles and z-scores. Hence, when the mutant allele is maternal in origin, like in PHP1a, obesity is pronounced and often severe. However, when the mutant allele is paternal in origin, like in pseudoph, obesity is often not present, and if present, it is not severe. In addition, we examined two patients with Poh, with presumed paternal GNAS mutations, and obesity was not present. These results implicate paternal imprinting in the development of obesity in PHP1a and are consistent with findings from AHO mouse models (18–20).

Patients and Methods

Patients

We evaluated anthropometric data on 40 patients with PHP1a and 13 patients with pseudoph, all with confirmed mutations in the GNAS gene (Table 1, A–D). All height and weight measurements were done at the most recent visit to the clinic and before the initiation of any medications or onset of any chronic illnesses that could affect these parameters. All patients had a combination of physical features typical of AHO such as short stature, brachydactyly/brachymetacarpia, obesity, round face, sc ossifications, and cognitive impairment. The diagnosis of PHP1a was differentiated from pseudoph by the presence of multihormone resistance, which included a combination of two or more of the following: PTH resistance (elevated serum intact PTH often with associated hypocalcemia and hyperphosphatemia), TSH resistance (elevated TSH with low or normal free T4), clinical symptoms of GnRH resistance, and GHRH resistance (GH deficiency). The patients were treated appropriately for the PTH and TSH resistances. Thirty-six of the 40 patients with PHP1a were documented to be euthyroid at the time of their anthropometric measurements, with the remaining four patients having unknown thyroid status at the time of these measurements. Of the 36 euthyroid patients, all but one were on thyroid hormone replacement at the time of the measurements. Calcium levels were normal for all patients, except for three in whom the levels were slightly low and for three in whom the calcium levels were not available. In patients who were found to have GH deficiency, the anthropometric measurements used for purposes of our analyses were those before the onset of GH treatment.

Two patients had the diagnosis of Poh (Table 1E), which was documented by deep heterotopic ossifications. Neither patient with Poh had hormone resistance, suggesting mutation of the paternal allele, and this was confirmed in the patient with an intron 2 mutation (23).

Twenty-three patients with PHP1a, 10 patients with pseudoph, and one patient with POH were evaluated in the General Clinical Research Center at the Johns Hopkins Hospital (Baltimore, MD), and all studies were approved by the Internal Review Board of the Joint Committee on Clinical Investigation of the Johns Hopkins University School of Medicine. Informed consent was obtained from all subjects, or a parent of each subject, before participation. In addition, 17 patients with PHP1a, three patients with pseudoph, and one patient with POH were evaluated at the Clinical Center at the National Institutes of Health (National Institutes of Health, Bethesda, MD). All studies were approved by the National Institute of Diabetes and Digestive and Kidney Diseases/National Institute of Arthritis and Musculoskeletal and Skin Diseases Institutional Review Board and informed consent was obtained from all subjects, or a parent of each subject, before participation.

GNAS mutation analyses

GNAS mutation analyses of the 13 coding exons were performed in the Johns Hopkins DNA Diagnostics Lab (CLIA-approved laboratory), a research laboratory at the Johns Hopkins University School of Medicine as described previously (4, 24), or a research laboratory at the National Institutes of Health (25).

Anthropometric evaluations

Standing height was obtained using a Harpendon stadiometer (Holtain Ltd., Crymych, Dyfed, UK), and weight was measured using a Detecto scale (Detecto Scale Co., Webb City, MO). Height and weight measurements were calculated as sd values from the mean compared with age- and gender-matched controls and expressed as height and weight SDS. BMI was calculated from height and weight measurements and expressed in kilograms per square meter. The National Institutes of Health definitions for obesity of BMI 30.0 kg/m² or more and extreme obesity of 40.0 kg/m² or more were used for adults (26). For children, BMI percentiles were used to determine obesity, because absolute BMI cannot be compared when analyzing children of different ages. For the purposes of this study, we defined children as overweight if their BMI was more than 85% and less than 95% and obese if their BMI was 95% or higher. Classification was according to the Centers for Disease Control and Prevention/National Center for Health Statistics (27). BMI percentage values were all age- and gender-matched (27). To enable us to compare a mixed population of adults and children, BMI z-scores were calculated and compared. BMI percentiles and z-scores were calculated using www.bcm.edu/bodycomplab/Applications/bmirecalc.htm (Baylor College of Medicine, 2006).

Dual energy x-ray absorptiometry (DEXA) scan analyses for adiposity

DEXA scan (QDR 4500; Hologic, Bedford, MA) of the total body for percent adiposity was performed on 18 of the patients with PHP1a. (Values were also provided for the head, trunk, and each extremity.) The coefficient of variation of these analyses was ± 1%.

Statistical analyses

SEM was calculated for each mean value. Differences in height SDS, weight SDS, BMI percentage, and BMI z-scores between subjects were analyzed by unpaired two-tailed Student t tests. P values < 0.05 were considered significant.

Results

Patients

We evaluated the anthropometric data on 53 patients with AHO, which included 40 patients with PHP1a and 13 subjects with pseudoph, all with confirmed mutations in the GNAS gene (Table 1, A–D). The age range of these patients was age 2 to 82 yr. We defined children as all patients less
than 18 yr of age. The children included 28 with PHP1a (12 males, 16 females) and three with pseudoPHP (two males, one female). The adults included 12 with PHP1a (two males, 10 females) and 10 with pseudoPHP (two males, eight females). The two patients with POH were adults (one male, one female; Table 1E).

### Anthropometric evaluation

**Height and weight analyses.** Height and weight SDS, BMI, BMI percentage, and BMI z-score for patients with PHP1a, pseudoPHP, and POH are shown in Table 1, A–E, respectively. When comparing mean height SDS, we limited our evaluation to height SDS

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**TABLE 1. Anthropometric data**

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Gender</th>
<th>Height SDS</th>
<th>Weight SDS</th>
<th>BMI</th>
<th>BMI %</th>
<th>BMI z-score</th>
<th>Mutation</th>
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<td>A. PHP1a: children</td>
<td>11.00 F</td>
<td>-0.60</td>
<td>-1.33</td>
<td>14.8</td>
<td>7.3</td>
<td>1.45</td>
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<td></td>
<td>14.00 F</td>
<td>-1.61</td>
<td>-1.13</td>
<td>18.8</td>
<td>13.6</td>
<td>1.10</td>
<td>ex.1c:85C&gt;T (Q289X)*</td>
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<tr>
<td></td>
<td>16.50 F</td>
<td>-2.22</td>
<td>0.66</td>
<td>28.6</td>
<td>90.8</td>
<td>1.33</td>
<td>ex.10c:772C&gt;T (R258N)*</td>
</tr>
<tr>
<td>B. pseudoPHP: children</td>
<td>18 F</td>
<td>-2.17</td>
<td>2.19</td>
<td>44.8</td>
<td>99.2</td>
<td>2.42</td>
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<tr>
<td></td>
<td>18 F</td>
<td>-1.46</td>
<td>0.96</td>
<td>28.8</td>
<td>92.6</td>
<td>1.45</td>
<td>ex.13c:1174G&gt;A (E392K)</td>
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<td>19 F</td>
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<td>2.18</td>
<td>42.4</td>
<td>98.8</td>
<td>2.26</td>
<td>ex.1c:103C&gt;T (Q35X)</td>
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<td>21 F</td>
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<td>1.70</td>
<td>39.1</td>
<td>98.1</td>
<td>2.09</td>
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<td>21 F</td>
<td>-3.13</td>
<td>1.06</td>
<td>35.7</td>
<td>98.7</td>
<td>2.24</td>
<td>ex.1c:21insT</td>
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<tr>
<td></td>
<td>25 F</td>
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<td>0.31</td>
<td>28.1</td>
<td>89.7</td>
<td>1.57</td>
<td>intron10:IVS10+1G&gt;C</td>
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<td>26 F</td>
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<td>1.28</td>
<td>35.4</td>
<td>97.1</td>
<td>1.90</td>
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<td></td>
<td>27 F</td>
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<td>-0.13</td>
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<td>51.8</td>
<td>0.05</td>
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<td>-2.84</td>
<td>0.83</td>
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<td>C. PHP1a: adults</td>
<td>18 F</td>
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<td>-2.46</td>
<td>19.0</td>
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<td>-1.03</td>
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<tr>
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<td>33 F</td>
<td>-2.43</td>
<td>-0.63</td>
<td>24.3</td>
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<td>-3.23</td>
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<td>93.5</td>
<td>1.51</td>
<td>ex.7c:575C&gt;T (P192L)*</td>
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<td></td>
<td>42 F</td>
<td>-2.33</td>
<td>-0.31</td>
<td>26.3</td>
<td>80.8</td>
<td>0.87</td>
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<td></td>
<td>44 F</td>
<td>-2.97</td>
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<td>26.4</td>
<td>84.6</td>
<td>1.02</td>
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<td>50 F</td>
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<td>0.10</td>
<td>27.4</td>
<td>87.9</td>
<td>1.17</td>
<td>ex.1c:814Cdel</td>
</tr>
<tr>
<td></td>
<td>54 F</td>
<td>-2.98</td>
<td>-0.33</td>
<td>26.7</td>
<td>85.6</td>
<td>1.06</td>
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<tr>
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<td>0.15</td>
<td>25.2</td>
<td>79.3</td>
<td>0.82</td>
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<tr>
<td></td>
<td>82 F</td>
<td>-1.98</td>
<td>0.84</td>
<td>31.0</td>
<td>95.5</td>
<td>1.70</td>
<td>ex.1c:85C&gt;T (Q289X)*</td>
</tr>
<tr>
<td>E. POH</td>
<td>26 M</td>
<td>-1.82</td>
<td>-1.69</td>
<td>20.6</td>
<td>18.4</td>
<td>-0.90</td>
<td>intron2.c:212+3delAAGT</td>
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<tr>
<td></td>
<td>61 F</td>
<td>-1.68</td>
<td>-0.71</td>
<td>22.5</td>
<td>58.4</td>
<td>0.21</td>
<td>ex.1c:1007insC*</td>
</tr>
</tbody>
</table>

del, Deletion; ins, insertion; * unpublished mutation; M, male; F, female. Superscript letters (a–m) indicate patients from the same kindred.
measurements in adults, because most children with PHP have normal stature until they undergo premature closure of the epiphyses that results in short final heights (4, 24, 28, 29). Analysis of the height SDS for the PHP1a subjects in this study is shown in Fig. 1. There was no statistical difference between mean adult height SDS in PHP1a (n = 12) and pseudoPHP (n = 10), respectively, in our patient population (−2.51 ± 0.30 vs. −2.26 ± 0.18, P = 0.49).

On analysis of all patients regardless of age (Table 2A), the mean weight SDS ± SEM was statistically different between the two groups: 1.84 ± 0.22 for patients with PHP1a (n = 40) compared with −0.18 ± 0.29 for patients with pseudoPHP (n = 13, P = 0.000015). The difference in mean weight SDS between patients with PHP1a and those with pseudoPHP remained significant when categorized by age: mean weight SDS for children with PHP1a was 2.23 ± 0.26 (n = 28) vs. −0.60 ± 0.63 (n = 3) for children with pseudoPHP (P = 0.0019) and 0.93 ± 0.27 (n = 12) vs. −0.06 ± 0.34 (n = 10) for adults with PHP1a and pseudoPHP, respectively (P = 0.033). The differences in weight SDS in PHP1a and pseudoPHP were more striking in children. The weight in patients with PHP1a appeared to reach its maximum SDS in early childhood and then plateaued with age such that the weight SDS was not as markedly elevated in adulthood (Fig. 1). Therefore, although two thirds of the adult patients with PHP1a fulfilled the criteria of obesity, the overall severity was not as great as in childhood in which almost 90% of patients are obese (see below).

BMI analyses. Our evaluation of adult patients revealed that eight of 12 (66.7%) adult patients with PHP1a vs. three of 10 (30%) patients with pseudoPHP met the definition for obesity with a BMI 30.0 kg/m² or higher (27). Two of the eight (25%) obese patients with PHP1a had extreme obesity (BMI ≥ 40.0 kg/m²). None of the patients with pseudoPHP were extremely obese.

According to the latest data on the prevalence of overweight and obesity in the United States [National Health and Nutrition Examination Surveys (26)], 32.2% of adults were obese. Therefore, in our population of adults with PHP1a, the prevalence was significantly higher (66.7%). Only one patient had a normal BMI of less than 25 kg/m². In pseudoPHP, the prevalence of obesity was approximately the same as the general population (30%). Two of 10 patients with pseudoPHP (20%) had BMIs less than 25 kg/m², and five of them (50%) had BMIs between 25 and 27 kg/m² and were, therefore, very close to the normal range.

When analyzing extreme obesity (BMI ≥ 40.0), 4.8% of adults in the general population fit this criterion. In patients with PHP1a, however, the prevalence of extreme obesity was much greater at 16.7%, whereas in pseudoPHP, there were no patients who were extremely obese.

For children and adolescents in the National Health and Nutrition Examination Surveys study (defined by their study as ages 2–19 yr) (26), the terms “at risk for overweight” (>85th and <95th percentile) and “overweight” (≥95th percentile) were used in place of the terminology we have used in the present study, i.e. “overweight” and “obese,” respectively [from prior Centers for Disease Control and Prevention criteria (27)]. The mean BMI percentage for children with PHP1a was 95.4 ± 2.7 compared with 37.2 ± 26.8 for those with pseudoPHP (P = 0.000016). The prevalence of obesity in children and adolescents with PHP1a was 89.3% (25 of 28 total patients) compared with 17.1% in the general population based on the National Health and Nutrition Examination Surveys data. On examination of all children and adolescents inclusively who were either overweight or obese, 96.4% of those with PHP1a fulfilled these criteria vs. 33.6% of the general population. None of the children with pseudoPHP were obese, and one of three was overweight.

To be able to compare BMI values for patients of all ages, we analyzed BMI z-scores, because BMI percentage is not used in the assessment of adults to determine obesity status. The mean BMI z-scores (Table 2B) for patients with PHP1a (n = 40) and pseudoPHP (n = 13) were significantly different at 2.31 ± 0.18 vs. 0.65 ± 0.31, respectively (P = 0.000032). Children with PHP1a (n = 28) had higher mean BMI z-scores than children with pseudoPHP (n = 3) with mean z-scores of 2.58 ± 0.23 compared with −0.41 ± 0.87 (P = 0.00045). Similarly, adults with PHP1a (n = 12) had significantly higher BMI z-scores than adults with pseudoPHP (n = 10) with mean z-scores of 1.69 ± 0.18 and 0.97 ± 0.26, respectively (P = 0.031). The greater significance in P values for children compared with adults is most likely secondary to

### Table 2. Weight and BMI data for PHP1a and pseudoPHP

<table>
<thead>
<tr>
<th></th>
<th>PHP1a</th>
<th>pseudoPHP</th>
<th>P value</th>
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<tr>
<td>A. Weight SDS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.84 ± 0.22</td>
<td>−0.18 ± 0.29</td>
<td>0.000015</td>
</tr>
<tr>
<td>Children</td>
<td>2.23 ± 0.26</td>
<td>−0.60 ± 0.63</td>
<td>0.0019</td>
</tr>
<tr>
<td>Adults</td>
<td>0.93 ± 0.27</td>
<td>−0.06 ± 0.34</td>
<td>0.033</td>
</tr>
<tr>
<td>B. BMI z-score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2.31 ± 0.18</td>
<td>0.65 ± 0.31</td>
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<tr>
<td>Adults</td>
<td>1.69 ± 0.18</td>
<td>0.97 ± 0.26</td>
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</tbody>
</table>

Data are expressed as mean ± SEM.
the declining BMI with age in PHP 1a along with a rising BMI with age in pseudoPHP and in the normal population.

BMI data were obtained on 20 mothers and 14 fathers who represent parents of 27 patients with PHP1a. Twelve parents had BMIs in the normal range (<25 kg/m²); 13 with BMIs from 25 up to 30 kg/m²; eight with BMIs 30 kg/m² or higher but less than 40 kg/m² (with the highest BMI in this group being 35.5 kg/m²); and one with a BMI 40 kg/m² or higher. Therefore, eight of 34 (23.5%) were obese, and only one of 34 (2.9%) was extremely obese. This is significantly lower than the prevalences found in the patients with PHP1a (66.7% obese and 25% extremely obese) and is consistent with the national prevalence of obesity of 32.2%, thereby providing further confirmation that the obesity does not have a familial distribution. When analyzing members of the same kindred who all have PHP1a, they are often obese even in the face of nonobese parents.

Presence of a GNAS mutation can also lead to POH. In the two patients we examined, one was confirmed to have a mutation on the paternal allele [intron 2 mutation (23)] and the other is assumed to have a paternal allele mutation because she had no evidence of hormonal resistance. The two patients with POH were not obese. The mean BMI z-score for the patients with POH was −0.35 ± 0.56, which was significantly different from the mean BMI z-score of 2.31 ± 0.18 in PHP1a (P = 0.0026). Comparison of mean BMI z-scores in pseudoPHP and POH showed no statistical difference (P = 0.255).

Evaluation of percent adiposity

In patients with PHP1a (n = 18) in whom percent adiposity by DEXA scan analyses were performed, a significant correlation was present between percent total adiposity and BMI (P = 0.017) indicating that the differences in BMI were the result of differences in adiposity. When evaluating children only, a significant correlation was also present between trunk and total adiposity and BMI (P = 0.036 and P = 0.003, respectively).

GNAS mutation correlation (Table 1, A–D)

The two largest clusters of mutations in AHO occurred in exon 1 and exon 7. There was no statistical difference between the mean BMI percentiles and mean BMI z-scores when comparing the patients with mutations in exon 1 vs. exon 7. In addition, there were no significant differences in these parameters in patients with exon 1 mutations compared with the cohort as a whole. Patients with missense mutations were not less affected overall compared with those with nonsense mutations, deletions, or mutations that resulted in frameshifts or premature termination codons. Therefore, we were unable to identify a correlation between genotype and obesity phenotype in this study.

Discussion

Our results show that obesity is more common and more severe in PHP1a than in pseudoPHP, with significantly greater mean weight SDS, BMI percentage, and BMI z-scores in PHP1a despite the fact that the AHO obesity phenotype has classically been described as similar in both disorders (1–12). These differences in BMI were secondary to adipose content based on DEXA analyses done on a subgroup of these patients (4, 24). In addition, the significant differences in BMI were observed in both children and adults when analyzed separately. Although the comparisons in children were significant, the analyses were limited because of the small number of children with pseudoPHP in this study. These children rarely come to medical attention secondary to the lack of biochemical abnormalities and possibly because of the lack of short stature and obesity in childhood, which most physicians have viewed as an expected part of the phenotype. By far, the majority of patients with pseudoPHP come to medical attention as adults because they have a child with PHP1a.

Compared with the general population, our data indicate that adults with PHP1a have an approximately 2-fold greater prevalence of obesity and a 3.5-fold greater prevalence of extreme obesity. Furthermore, there is an approximately 5.2-fold increase in obesity prevalence in children with PHP1a. The same is not true for patients with pseudoPHP for whom the prevalence of obesity in adult patients is the same as for the general population and for whom none of them had extreme obesity. In addition, none of the children with pseudoPHP were overweight or obese.

BMI comparisons between PHP1a and pseudoPHP have not been previously reported. However, on our analysis of data reported by Marguet et al. (22), we noticed that the mean weight/height SD ratio was significantly different in 33 children with PHP1a compared with seven children with pseudoPHP (2.97 ± 0.24 vs. 1.45 ± 0.32, P = 0.008), which is consistent with our findings. We have shown that BMI is a useful parameter in distinguishing the PHP1a phenotype from that of pseudoPHP in cases in which hormonal resistance has not yet manifested. Unlike the previous report (22), we have mutation confirmation on each patient thereby verifying the diagnosis of AHO. The increased BMI observed in the patients with PHP1a in our study was not present at birth based on birth weights and lengths in the 26 of 40 investigated patients for whom we had these measurements. The weight dramatically increased in these patients during the first few years of life implicating that the development of obesity is triggered after birth.

Our findings are consistent with previous observations in AHO mouse models in which exon 1 has been disrupted (18, 19). The mice that inherit a disrupted maternal allele (analogous to PHP1a) are more obese than those that inherit a disrupted paternal allele (analogous to pseudoPHP). In addition, our two patients with POH in which the affected allele is paternally inherited (experimentally confirmed in one patient) were not obese. Collectively, the mouse and human data may implicate paternal imprinting in the development of obesity. The more pronounced decreased basal metabolic rate in the mice that inherit the affected maternal allele (18) could contribute to this obesity. It has been demonstrated that there is no imprinting in adipose tissue in mice with targeted disruption of exon 1 (19) or in normal human adipose tissue (11), thereby making imprinting in other tissues suspect for the etiology of the severe obesity observed in PHP1a.

Ga₂ plays an important role in lipid metabolism. Several
studies have examined adipocyte responses in PHP1a. Resistance to the lipolytic action of epinephrine by G\textsubscript{\alpha}\text{\textsubscript{S}}-coupled \(\beta\)-adrenergic receptors has been shown in patients with PHP1a (30). In addition, defective \(\beta\)-adrenergic receptor stimulation of adipocyte adenyl cyclase with isopropenol has been shown in patients with PHP1a (31). Reduced levels of G\textsubscript{\alpha}\text{\textsubscript{S}} expression influence fat metabolism, because decreased cAMP levels result in decreased lipolysis (30, 31). Based on these results, one would expect patients with PHP1a and those with pseudoPHP to be at equal risk for obesity as a result of G\textsubscript{\alpha}\text{\textsubscript{S}} deficiency in adipocytes, a cell type in which GNAS is biallelically expressed (11, 19). Hence, other mechanism(s) seem responsible. A potential role for a central (sympathetic) mechanism is suggested by the finding of low norepinephrine levels in patients with PHP1a compared with levels in obese children and lean controls (30).

Another possibility for the difference observed in BMI between PHP1a and pseudoPHP could be related to hypothalamic imprinting and the melanocortin-4 receptor as has been suggested by Chen et al. (18) and Ong et al. (32). Melanocortin-4 receptor is a hypothalamic G\textsubscript{\alpha}\text{\textsubscript{S}}-coupled receptor that binds the neuropeptide MSH and is thought to mediate the central effects of lepin on satiety. Mice with \(M\text{\textsubscript{ecd}}\) deletions and mice that express a competitive antagonist to MSH have severe obesity and hyperphagia (33). Spontaneous and inherited melanocortin-4 receptor mutations have also been described in humans and result in morbid obesity starting in infancy as well as elevated leptin levels (34). Imprinting of GNAS has not yet been shown in the hypothalamus. It is possible that paternal imprinting at this location could lead to obesity in patients with PHP1a secondary to reduced melanocortin-G\textsubscript{\alpha}\text{\textsubscript{S}} signaling. Paternal imprinting of GNAS in other tissues involved in metabolism is also possible.

GH deficiency may also contribute to the difference in BMI between patients with PHP1a and those with pseudoPHP. Recently, we found that GH deficiency in PHP1a is common (69%) (4), as did other investigators (10), most likely secondary to GHRH resistance. GNAS is paternally imprinted in the pituitary (5), thereby making it likely that a defective maternal GNAS allele could lead to G\textsubscript{\alpha}\text{\textsubscript{S}} deficiency in somatotrophs and resultant reduced GH responsiveness to GHRH. It is also possible that the GH deficiency could be secondary to imprinting in the hypothalamus with a direct reduction of GHRH expression itself. Thus far, we have found significantly greater mean BMI z-scores in GH-deficient patients with PHP1a compared with GH-sufficient patients with PHP1a (\(P = 0.0004\)) and also significantly greater BMI z-scores for GH-deficient patients with PHP1a compared with those with pseudoPHP (\(P = 0.00006\)) (Refs. 4 and 24, and Long, D. N., and E. L. Germain-Lee, unpublished data). There was no significant difference between the BMI of GH-sufficient patients with PHP1a and those with pseudoPHP (\(P = 0.486\)). All obese patients with PHP1a were GH-deficient; those who were not GH-deficient were not obese. The truncal and total percent adiposity in each group correlated significantly to BMI implicating adipose in the etiology of this difference (Ref. 4 and 24, and Long, D.N., and E.L. German-Lee, our unpublished data).

It is possible that hypothyroidism could play a role in the obesity in PHP1a. Although 36 of the 40 patients with PHP1a were euthyroid at the time of the anthropometric measurements, with all but one on thyroid hormone replacement therapy and the status unknown in the remaining four of the 40 patients, we cannot rule out that these patients may have had intermittent hypothyroidism that influenced anthropometric measurements. However, in Gnas mice with targeted disruption of exon 1, the mice inheriting the mutant maternal allele were obese with normal thyroxine levels, thereby implicating that factors other than hypothyroidism are involved in the obesity (18, 19). Therefore, although we cannot rule out hypothyroidism as contributing to the obesity, it is unlikely to be the only factor.

In those patients with GNAS mutations on the paternal allele in exons 2 to 13, one would expect decreased expression of XL\text{\textsubscript{a}s}, an alternative G\textsubscript{\alpha}\text{\textsubscript{S}} isoform expressed only from the paternal allele, which is encoded by an alternative first exon and exons 2 to 13 of G\textsubscript{\alpha}\text{\textsubscript{S}} (35, 36). XL\text{\textsubscript{a}s} deficiency has been shown to lead to opposite effects on obesity and insulin sensitivity in mice compared with G\textsubscript{\alpha}\text{\textsubscript{S}} deficiency (20, 37–40). Based on the data from these mouse models, one would predict patients with pseudoPHP with mutations in exons 2 to 13 to be hypermetabolic and lean. However, this is not evident in our group of patients with pseudoPHP as a whole. Three of the patients with pseudoPHP had BMI z-scores less than −1.0, whereas the remaining 10 had BMI z-scores between 0.64 and 1.92. In addition, the patients with exon 1 mutations did not have higher BMI z-scores overall than the patients with mutations in exons 2 to 13. The difference between the patients and the mice in this regard may be secondary to effects of XL\text{\textsubscript{a}s} that are unique to certain species.

Based on the results in the Gnas exon 1 knockout mouse (18), one would predict that patients with PHP1a have greater alterations in energy balance as well as more pronounced insulin resistance than patients with pseudoPHP, but this remains to be studied. As mentioned previously, one potential mechanism for these differences is paternal imprinting of G\textsubscript{\alpha}\text{\textsubscript{S}} in the hypothalamus such that maternal, but not paternal, G\textsubscript{\alpha}\text{\textsubscript{S}} mutations lead to loss of melanocortin signaling, a pathway known to be an important regulator of energy balance. The data reported here provide another unique metabolic distinction between PHP1a and pseudoPHP and, even more importantly, may implicate a role for the paternal imprinting of G\textsubscript{\alpha}\text{\textsubscript{S}} in the development of human obesity. In this regard, PHP1a can be considered a potentially important model in which to evaluate the usefulness of antiobesity agents that influence levels of cAMP in the central nervous system or other organ systems.

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