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# Growth hormone secretory pattern in non-obese children and adolescents with Prader-Willi syndrome

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## Abstract

The aetiology of impaired growth hormone (GH) secretion in Prader-Willi syndrome (PWS) remains controversial due to the common occurrence of obesity. To further clarify whether suboptimal GH secretion in PWS is an artefact of excess weight, we evaluated both GH immunological activity and GH bioactivity after arginine administration in 23 non-obese PWS patients [seven females, aged 6.9±0.9 years, body mass index (BMI) SDS 0.63±0.26], in comparison with a control group of 32 healthy subjects, matched for age, gender and BMI (10 females, aged 7.9±0.3 years, BMI SDS 0.21±0.20). Serum GH concentration was measured with a time-resolved immunofluorometric assay (IFMA), while GH bioactivity was evaluated by the Nb2 cell bioassay. Serum IGF-I concentrations were measured by double-antibody RIA. GH mean peak after pharmacological stimulation was significantly lower in PWS individuals compared with controls when measured either by IFMA (6.05±1.23 μg/L vs. 23.7±1.06 μg/L, p<0.0001) or by Nb2 (6.87±0.55 μg/L vs. 12.88±0.19 μg/L, p<0.0001). Analysis of integrated GH secretion (AUC) confirmed that the PWS group differed significantly from the control subjects (387.9±76.1 μg/L/h vs. 1498.1±56.2 μg/L/h, p<0.0001); the same result was obtained when the GH rise after arginine administration was expressed as nAUC (278.2±53.3 μg/L/h vs. 1443.6±52.5 μg/L/h, p<0.0001). PWS patients had an IGF-I SDS significantly lower than those found in control subjects (p<0.0001). Subnormal IGF-I values were present in 19 PWS individuals (82.6%) and two healthy controls (6.2%). These findings are in agreement with the hypothesis that a complex derangement of hypothalamus-pituitary axis occurs in PWS.

**Keywords:** GH bioactivity; GH deficiency; obesity; Prader-Willi syndrome.

## Introduction

Prader-Willi syndrome (PWS) is the most common form of genetic obesity, affecting males and females equally. Current data suggest a population prevalence of approximately one in 50,000 (1). PWS is associated with absent expression of paternal alleles in the PWS region of chromosome 15q11-13 (2). The syndrome affects multiple body systems whose most consistent major manifestations include infantile hypotonia with feeding problems, hyperphagia leading to severe obesity in early childhood, mental retardation, behavioural problems, dysmorphic features, hypogonadism, and short stature (3). A typical pattern of growth is described, with decreased linear growth velocity in childhood and compromised final adult height. Short stature is considered the result of lack of the pubertal growth spurt and to the presence of a GH/IGF-I axis deficiency, due to hypothalamic dysfunction (4). In this respect, reduced GH response to a variety of GH secretion stimulators, as well as decreased 24 h spontaneous GH release, have been documented in a large number of studies involving about 300 affected children (5). In addition to impaired linear growth, other clinical features of PWS support the presence

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of GH deficiency (GHD), including abnormal body composition with increased body fat and decreased lean body mass, reduced muscle strength and bone mineral density, increased risk of cardiovascular disease, and psychological impairment (6). Furthermore, serum levels of IGF-I are reduced in the majority of children (7). The aetiology of the impaired GH secretory pattern in PWS, however, remains controversial due to the high frequency of obesity. In this respect, it has been suggested that the impaired GH secretory pattern in PWS may simply reflect their obesity (8). Weight excess associated with PWS is often massive and many subjects are more than twice their ideal bodyweight. Obesity is known to be associated with a decreased spontaneous GH release as well as with an impairment of stimulated GH secretion (9). Thus, permanent GHD due to hypothalamic-pituitary disease may be difficult to distinguish from the reversible blunting of GH release in obese patients. Altogether, these data suggest the importance of determining the relative contribution of weight to GH response to standardised stimulation in PWS patients, through the evaluation of GH secretory patterns in non-obese subjects. At present, data on GH secretory capacity in normal weight children with PWS are scarce and related to a small number of subjects (10, 11). Moreover, little is currently known about the biological activity of GH in these patients. To further clarify whether the suboptimal GH secretion in PWS is an artefact of obesity, this study was aimed at the evaluation of GH immunological activity and GH bioactivity after a standard provocative test in a group of non-obese children and adolescents with genetically confirmed PWS. The results were compared with those obtained in a control group of healthy subjects, matched for age, gender and body mass index (BMI).

## Subjects and methods

### Patients

Twenty-three PWS patients, 16 males and 7 females, aged 2.0–14.3 years, from nine paediatric endocrinology units were included in the study (Table 1). All subjects exhibited typical PWS clinical features (12). Cytogenetic studies were performed in all patients, 16 had interstitial deletion of the proximal long arm of chromosome 15 (15q11-q13) by fluorescent in situ hybridisation. Moreover, five subjects presented uniparental maternal disomy for chromosome 15 (UPD15), while a positive methylation test was found in two individuals, but the underlying genetic defect was not identified. Physical examination included determination of height and weight. Standing height was determined with a Harpenden Stadiometer (Holtain Limited, Crymych, Dyfed, UK), and expressed as the standard deviation score for height (HSDS), according to Tanner standards (13). Body weight was measured to the nearest 0.1 kg, by using standard equipment. BMI was defined as weight in kg divided by the height in $m^2$. The published Italian standards for sex- and age-specific BMI percentiles were used for calculating the standard deviation score (SDS) as well as for classifying PWS subjects as non-obese (BMI<2.0 SDS) or obese (BMI>2.0 SDS) (14). All PWS subjects were non-obese; BMI SDS ranged from −2.90 to 1.94. Pubertal stages were determined according to the standards of Marshall and Tanner (15, 16). Twenty-two patients were prepubertal, whereas one female was pubertal. Twenty-one patients had previously undergone GH treatment, withdrawn in all cases 2 months before starting the study protocol. The mean duration of GH therapy was 42±3 months. As a control group, we evaluated 32 healthy prepubertal children, matched to PWS subjects for gender, age and BMI SDS (Table 1). All PWS and control subjects provide normal findings on main laboratory tests, including thyroid function. The study was conducted according to the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects. The entire study protocol was approved by the ad hoc Ethical Committee of the Istituto Auxologico Italiano Foundation. Written informed consent from parents and written assent from children and adolescents (when appropriate) were obtained.

### Study protocol

After an overnight fast, all the subjects were tested at 08:30 h, 15 min after an indwelling venous catheter had been placed in an antecubital vein, to deliver a slow saline infusion. After a basal sample was obtained for GH and IGF-I assays, a solution of 30% arginine HCl (SALF, Bergamo, Italy) was infused slowly over 30 min at a dose of 0.5 g/kg body weight. Blood samples for the GH assay were taken at 30, 60, 90 and 120 min after initiating the test. Serum samples were stored at −80°C and assayed within 3 months by the laboratory at the Department of Paediatrics, University of Pavia.

### Methods

Serum GH concentration was measured by a time-resolved immunofluorimetric assay (IFMA), based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the GH molecule. The AutoDELFIA™ hGH Kit (Wallacoy, Turku, Finland) uses an automatic immunoassay system. It is highly specific (detection limit 0.039 µg/L) for the 22 kDa variant of GH and has low cross-reactivity with GH molecular variants or other pituitary hormones. The standards provided in the kit were calibrated against the First International Reference preparation of rhGH 80/505, in which 1 mg is equal to 2.6 IU of GH. The intra- and inter-assay coefficients of variation were 2.1%–5.0% and 4.2%–6.3% for a quality control range of 0.20–10.1 µg/L, respectively. GH responses were evaluated either as mean peak values (µg/L), or as the area under the curve (AUC, µg/L/h) and the AUC corrected for basal values (nAUC, µg/L/h), calculated by applying the trapezoidal method. According to current reports in the literature, a GH peak response to arginine ≥10.0 µg/L is considered normal.

GH bioactivity was evaluated by the Nb2 cell bioassay, performed according to the method of Tanaka et al. (17), modified by Walker et al. (18), with other minor variations (19). In every assay a polyclonal antibody against hPRL (R&D systems, Minneapolis,

**Table 1** Clinical and laboratory data for Prader-Willi (PWS) patients and healthy controls (HC).

| | PWS | HC |
|---|---|---|
| Number | 23 | 32 |
| Gender, f/m | 7/16 | 10/22 |
| Age, years | 6.9±0.9 | 7.9±0.3 |
| BMI SDS | 0.63±0.26 | 0.21±0.20 |
| Height SDS | −1.09±0.28[a] | 0.42±0.15 |
| IGF-I SDS | −1.61±0.36[a] | 0.46±0.27 |

BMI, body mass index; SDS, standard deviation score. For significance: [a]$p<0.0001$ vs. HC.

MN, USA) was routinely added to each serum sample (final dilution 1:500). For every batch, the potency of the antibody anti-hPRL was checked by assaying its ability to neutralise the proliferation response of a serum sample drawn from a patient with a prolactinoma, with high serum hPRL concentration (986 µg/L). To verify the Nb2 GH-dependent cell proliferation, the cells were plated in the presence of increasing concentrations of rhGH (NIBSC, Hertfordshire, UK) with or without the addition of a mouse polyclonal anti-GH antibody (Lab Vision – Neo Markers, Fremont, CA, USA). The values obtained were transformed, by using a conversion factor, into µg/L to express the bioactivity of GH. The intra- and inter-assay coefficients of variation were 6% and 7.6%, for a quality control range of 0.05–30.0 µg/L, respectively, based on measurements of cell division in the presence of GH.

Serum IGF-I concentrations were measured by double-antibody RIA using immunochemicals and a tracer provided by Medgenix (Fleurs, Wevelgen, Belgium). The sensitivity of the assay was 150 pg/mL; the intra- and inter-assay coefficient of variation ranges were 3.9%–6% and 5.6%–7.5% for a quality control range of 30–450 µg/L, respectively. In order to avoid interference from binding proteins, single serum samples were treated with acid-ethanol. IGF-I levels were expressed as SDS.

### Statistical analysis

Data are presented as mean±SE. Statistical analysis was performed by a t-test for unpaired data, and analysis of variance for parametric and non-parametric (Mann-Whitney test) data, where appropriate. Analyses were performed using SPSS 10.0 (SPSS, Inc., Chicago, IL, USA). A p-value of <0.05 was considered significant.

## Results

At study entry, PWS patients were matched for age, gender distribution and BMI SDS with the control group (Table 1). As expected, HSDS was significantly higher in healthy children than PWS individuals.

The GH response to the arginine test in PWS subjects and control patients are reported in Figure 1. No adverse effects were noticed during or after GH testing in any of the subjects studied. GH mean peak after pharmacological stimulation were significantly lower in PWS individuals compared with controls when measured by IFMA (6.05±1.23 µg/L vs. 23.7±1.06 µg/L, p<0.0001). Analysis of integrated GH secretion (AUC) confirmed that the PWS group differed significantly from the control subjects (387.9±76.1 µg/L/h vs. 1498.1±56.2 µg/L/h, p<0.0001); the same result was obtained when the GH rise after arginine administration was expressed as nAUC (278.2±53.3 µg/L/h vs. 1443.6±52.5 µg/L/h, p<0.0001). The arginine induced GH response was similar between males and females both in the PWS group and in the control subjects (Table 2). When PWS subjects and healthy individuals were compared for gender, the GH response to the standard provocative test was lower both in males and females with PWS than in controls (p<0.0001). In the PWS group, the arginine induced GH rise was slightly higher in patients with del15q11-q13 compared with subjects with UPD15, although it did not reach statistical significance (Table 2). Considered individually, 20 of 23 PWS patients (87.0%) had reduced GH immunological activity, showing a peak GH response below the cut-off of 10 µg/L. Only two controls failed to respond to the arginine stimulus, but their HSDS and IGF-I concentration were normal.

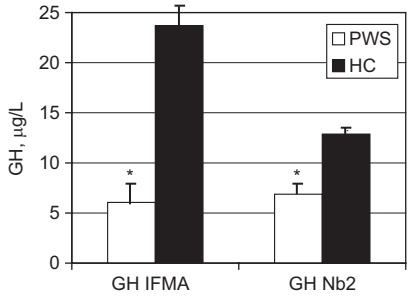


**Figure 1** Growth hormone peak levels measured by IFMA and Nb2 cell assay in children and adolescents with Prader-Willi syndrome (PWS) and in healthy controls (HC). For significance: *p<0.0001 vs. HC.

Evaluation of the GH peak following arginine administration by the Nb2 assay showed lower GH bioactivity in PWS children and adolescents in comparison with healthy subjects (6.87±0.55 µg/L vs. 12.88±0.19 µg/L, p<0.0001) (Figure 1).

Finally, PWS patients had an IGF-I SDS significantly lower than those found in control subjects (p<0.0001) (Table 1). Subnormal IGF-I values were present in 19 PWS individuals (82.6%) and two healthy controls (6.2%).

## Discussion

PWS, in the absence of intervention, has a characteristic phenotype including short stature for genetic background. Spontaneous GH secretion is reduced and the GH peak during the pharmacological stimulation test is <10 µg/L in the majority of PWS children (5). Nevertheless, the prevalence of severe GHD is unclear because blunted GH levels may be attributed to the effects of obesity alone. In this respect, a decrease in

**Table 2** Stimulated growth hormone levels in Prader-Willi patients (PWS) and healthy controls (HC): comparison of gender (PWS and HC) and different genetic subtypes (PWS del15 vs. PWS UPD15). GH secretion is expressed either as GH mean peak (GHp) response (µg/L), area under the curve (AUC, µg/L/h) or AUC corrected for basal values (nAUC, µg/L/h) after arginine administration.

| | GHp | GH AUC | GH nAUC |
|---|---|---|---|
| HC males | 24.4±1.1[a] | 1532.7±54.5[a] | 1475.8±50.6[a] |
| HC females | 22.1±1.3[b] | 1458.3±60.7[b] | 1416.8±58.5[b] |
| PWS males | 6.0±1.6 | 362.8±96.4 | 238.6±52.0 |
| PWS females | 6.3±1.7 | 445.2±126.7 | 368.9±129.8 |
| PWS del15 | 6.6±1.5 | 406.2±92.1 | 322.3±171.8 |
| PWS UPD15 | 5.4±2.6 | 359.5±186.2 | 260.9±53.0 |

del15, interstitial deletion of the proximal long arm of chromosome 15 (15q11-q13); UPD15, uniparental maternal disomy for chromosome 15. For significance: [a]p<0.0001 vs. PWS males; [b]p<0.0001 vs. PWS females.

spontaneous GH release as well as an altered response to all provocative stimuli in obesity have been well described (9). In spite of this, it is evident that GH administration in children and adolescents with PWS is efficacious in improving growth, body composition and neurodevelopment (20, 21). Consequently, GH therapy has been approved by the member states of the European Union for the long-term treatment of growth failure in children with PWS, even in the absence of GH testing. However, experts agree on the potential importance of knowing the GH status to evaluate differential effects of GH administration, depending on GH status (6). In this context, it seems useful to evaluate PWS patients who are of normal weight, in order to better understand whether suboptimal GH secretion in these individuals is related to obese status. Nevertheless, there is little information regarding the GH/IGF-I axis physiology in PWS patients with normal BMI (10, 11).

Our study analysed the GH secretory pattern after arginine administration in a group of non-obese children and adolescents with PWS in comparison with a control group of healthy subjects. We have demonstrated that GH immunological activity after the standard provocative test was significantly lower in PWS patients than in controls with similar BMI SDS. These results were associated with reduced IGF-I levels in more than 80% of PWS subjects.

Concerning the pathophysiology of the GH/IGF-I system in PWS, no data are currently available on the biological activity of GH. To our knowledge, this study is the first to document reduced GH bioactivity in a representative sample of children and adolescents with PWS, in comparison with a group of healthy controls. The potential interest of this finding is related to the small proportion of individuals with PWS who exhibit normal GH responses with standard testing. Considering that in many of these cases the clinical picture and the IGF-I levels are characteristic for PWS and GHD, our results seem to suggest that growth failure in these patients may be due, at least in part, to the presence of low GH bioactivity.

Altogether, our data support the view that an impaired GH response may occur in a significant percentage of PWS patients regardless of their weight status. However, this finding cannot be further substantiated with this study since body composition was not available in our subjects. In this context, the weight excess index adopted in our investigation is widely used as a simple measure of body weight in both children and adolescents. Nevertheless, it is known that BMI is not an exact measure of adiposity in PWS, because it underestimates the percentage of body fat (22). In fact, PWS harbour a higher fat mass than simple obese subjects, with the same degree of weight excess (23). In this light, dual-energy X-ray photon absorptiometry (DXA) seems to be the best available technique to evaluate body composition. However, we did not perform DXA in our healthy children and adolescents for ethical reasons. Consequently, it may be said that our study groups were not fully matched for body composition and that the different GH responses may be related to the different amounts of fat tissue. This is consistent with a study reporting an increased percentage of fat mass in young underweight PWS children (24). Nevertheless, it is of note that 21 of 23 PWS patients were undergoing GH therapy for up to 2 months before starting the study. As mentioned above, a sustained improvement in body composition by increasing lean body mass and reducing fat mass is observed during prolonged GH treatment in PWS patients, both in children (25) and in adults (26). Based on these observations, we may hypothesise that the body fat percentage of our PWS subjects with normal BMI SDS, who have undergone GH therapy, is lower than expected. Moreover, it is important to keep in mind that GH deficiency is associated with increased body fat and a lower lean body mass. Therefore, body composition abnormalities observed in young PWS subjects may be related, at least in part, to the presence of a primary derangement of the GH/IGF-I axis (4). In addition, we have previously reported that GHRH+arginine administration elicits a lower GH response in PWS adults when compared with a group of unaffected obese patients, matched both for BMI and fat mass percentage (27). Altogether, these data seem to confirm that GHD in PWS is consistent with a true GHD, and is not an artefact of abnormal body composition.

In conclusion, this is the first study evaluating both GH immunological activity and GH bioactivity in non-obese PWS patients. We have found that arginine administration elicits a lower GH response in PWS patients when compared with healthy subjects, both in terms of immunoreactive and bioactive levels. Evaluation of the GH peak following pharmacological stimulus showed a pathological response in the great majority of PWS children and adolescents. In addition, PWS individuals showed mean IGF-I levels significantly lower than controls. Interestingly, reduced IGF-I values were present in all but one PWS subjects with GH peak <10 μg/L. We are inclined to interpret these data as supportive of the hypothesis that a complex derangement of the hypothalamus-pituitary axis occurs in PWS, indicating that a true GHD may be present in a significant percentage of patients with PWS. Although it is unlikely that GH/IGF-I axis impairment is the only cause of growth failure in PWS, our results suggest that GH insufficiency may be a significant contributing factor.

The relevance of our findings, however, remain to be fully confirmed in a large number of patients as well as an evaluation of body composition is needed to better determine the impact of fat mass on the GH secretory pattern of non-obese children and adolescents with PWS.

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