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ORIGINAL ARTICLE

Deconvolution-based assessment of pituitary GH secretion stimulated with GHRH+arginine in Prader-Willi adults and obese controls

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Summary

Objective The assessment of GH deficiency in adult patients with Prader-Willi syndrome (PWS) has been previously assessed through the evaluation of quantitative parameters, such as the peak value of GH response to exogenous stimuli. A comprehensive description of the pattern of secretory response obtainable by deconvolution analysis is still lacking. The aim of our study was to characterize the time evolution of responses of PWS subjects compared with obese controls.

Design and subjects GH responsiveness was measured following the combined administration of GHRH+arginine to 65 PWS adults (24 males, 41 females) aged 18–41·2 years, and 17 age-, gender- and body mass index-matched obese controls. PWS subjects were analysed considering the stratification on different genotypes.

Measurements GH response to GHRH+arginine was analysed in terms of peak values, standard area under the curves (AUCs), AUCs due to the stimulus, AUCs of the Instantaneous Secretion Rate signal and Secretion Response Analysis.

Results In terms of both peak values and AUC, GH responses were statistically different between PWS UPD15 and PWS DEL15 subjects as well as between PWS UPD15 and obese controls. PWS subjects showed a lower and a more delayed GH response compared with obese controls. Moreover, PWS UPD15 subjects had the most delayed GH response.

Conclusions Our findings demonstrate that impaired GH secretion in PWS subjects compared with obese controls regards not only amplitude parameters such as peak value and AUC, but also the shape of the secretory response, which is more delayed, especially for UPD15 subjects.

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Introduction

Prader–Willi syndrome (PWS) is a complex disorder due to the lack of expression of the paternally active genes in the PWS critical region on chromosome 15.[1] In approximately 65–70% of affected individuals, there is a deletion of the paternal chromosome (15q11-q13) (DEL15), whereas 30–35% of subjects have a maternal uniparental disomy for chromosome 15 (UPD15). PWS represents the most frequent cause of syndromic obesity, occurring in 1 in 25 000 live births.[2]

The syndrome affects multiple body systems and its most consistent characteristics 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. A complex hypothalamic-pituitary dysfunction is currently thought to lie at the root of PWS phenotype: it is a recognized cause of compulsive appetite and explains sleep-related breathing disorders, body temperature instability, hypogonadism, and altered GH secretion.[4] Spontaneous GH secretion is reduced and GH peak during pharmacological stimulation test is less than 10 μg/l in 70% of children.[5] Information regarding GH secretory pattern in adult patients with PWS is beginning to emerge, indicating that GH deficiency (GHD) may be present in a significant percentage of subjects.[6–8] In addition, we have recently demonstrated a different pattern of GH secretion among PWS subjects with different genetic subtypes, higher GH responses being found in DEL15 patients in comparison with those with UPD15.[9] The

aetiology of the impaired GH secretion in PWS, however, remains controversial due to the high frequency of obesity. As far as weight excess is concerned, obesity 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.[10] Thus, permanent GHD due to hypothalamic-pituitary disease may be difficult to distinguish from the reversible blunting of GH release in obese patients.

This background suggests the importance of analysing pituitary responsiveness to standard GH stimulation tests. A first issue is the need for an adequate sample size enabling the detection of significant differences between obese controls and subpopulations of PWS subjects. Moreover, the literature on the analysis of hormonal responsiveness to stimuli has shown that the use of simple quantitative parameters as the peak value in plasma and the plasma AUC is not sufficient to capture the full complexity of glandular responses.[11–13] Indeed, due to hormonal clearance, the profile of hormone concentration in plasma provides only an indirect picture of the instantaneous secretion rate by the pituitary. To make an example, a short secretion episode would result in a sudden rise followed by a slower exponential decay. Mathematically speaking, this distortion is described by the convolution of the instantaneous secretion rate with the hormonal clearance function. To remove such distortion, one can resort to the so-called deconvolution analysis that makes it possible to recover the instantaneous secretion rate of the pituitary. In turn, this offers a precious insight into the timing of hormone prediction. Along this line, in this retrospective work, a deconvolution analysis of both PWS and obese GH responses is performed, to define quantitative and qualitative aspects of the pituitary response and to better understand the possible differences in terms of GH secretion response between PWS patients and obese subjects.

Materials and methods

Patients

Sixty-five PWS patients, 24 males and 41 females, aged 18–41·2 years, consecutively recruited between July and December 2005 from Istituto Auxologico Italiano and Ospedale Pediatrico Bambino Gesù, were included in the study (Table 1). All patients showed the typical PWS clinical phenotype.[3] Cytogenetic analysis was performed in all subjects, and 49 of them had DEL15, while UPD15 was found in the remaining 16 individuals. Standing height was determined by a Harpenden Stadiometer (Holtain Ltd, Dyfed, UK) and expressed as standard deviation score for height (HSDS), according to the published Italian standards.[14] In our population, HSDS ranged from −0·63 to −5·64 (mean ±SD: −2·63 ± 1·02). Body weight was measured to the nearest 0·1 kg, by using standard equipment. BMI was defined as weight in kilograms divided by the square of height in metres. Mean BMI was 41·9 ± 11·2 (range: 20·0–70·8). At the time of the study, 17 of the women were undergoing sex steroid substitution. Twenty-one PWS subjects had previously undergone GH treatment, withdrawn in all cases at least 2 years before starting the study protocol.

As controls, we evaluated 17 patients (7 males, 10 females) with essential obesity, matched for age, gender- and BMI (Table 1). Their individual characteristics have been previously described.[7] As expected, HSDS of obese controls was significantly higher than that observed in PWS patients. All PWS and control obese subjects provided normal findings in main laboratory tests, including thyroid, liver and kidney function.

Endocrine protocol

All subjects underwent a standard GH Releasing Hormone (GHRH)+arginine (ARG) test. Tests started at 8:30 am after overnight fasting, with the patients recumbent. Fifteen minutes after an indwelling catheter had been placed in an antecubital vein, each subject received GHRH (1–29) injection (GHRH, Ferring GmbH, Kiel, Germany; 1 μg/kg as i.v. bolus at 0 min). From 0 to 30 min after GHRH administration, 0·5 g/kg (maximum dose 30 g) of ARG hydrochloride (SALF, Bergamo, Italy) was infused. Blood samples for GH determination were drawn at −15, 0, 30, 45, 60, 90 and 120 min after the i.v. bolus of GHRH. No patient underwent caloric restrictions before the test was performed.

Hormonal dosages were centralized in the laboratory of the Istituto Auxologico Italiano. GH levels were measured by chemiluminescence (Immulite 2000 Analyser, DPC, Los Angeles, CA, USA) calibrated against World Health Organization International

Table 1. Clinical and laboratory data of Prader-Willi (PWS) patients and obese controls

	PWS DEL15	PWS UPD15	PWS (all)	Obese controls
Number	49	16	65	17
Gender (M:F)	18:31	6:10	24:41	7:10
Age	26·0 ± 5·9	26·1 ± 7·4	26·0 ± 6·2	28·0 ± 5·8
BMI	41·2 ± 11·8	44·1 ± 9·4	41·9 ± 11·2	43·4 ± 4·4
HSDS	−2·62 ± 1·08	−2·84 ± 1·05	−2·63 ± 1·02	−0·05 ± 0·92*
IGF-I (μg/l)	134·2 ± 75·3	116·9 ± 54·7	130·0 ± 70·8	206·9 ± 100·7†

Data are reported as mean ± SD. DEL15, interstitial deletion of the proximal long arm of chromosome 15; UPD15, uniparental maternal disomy for chromosome 15; BMI, body mass index (kg/m2); HSDS = Height Standard Deviation Score. *$P < 0·0001$ *vs* PWS DEL15, PWS UPD15 and all PWS; $P < 0·0001$ *vs* all PWS; †$P < 0·005$ *vs* PWS DEL15 and PWS UPD15.

Reference Preparation (WHO 1st IRP) 80/505, having a sensitivity of 0·01 μg/l and intra- and interassay coefficients of variation (CVs) of 2·9–4·2% and 4·2–6·5% respectively. All measurements were performed in a single run.

Serum IGF-I concentrations were determined by chemiluminescence IGF-I immunoassay by Liaison (Nichols Advantage, San Juan Capistrano, CA, USA), with intra- and interassay CVs of 4·8% and 6·7% respectively. All samples were measured in the same batch.

The entire study protocol was approved by the *ad hoc* Ethical Committee of Istituto Auxologico Italiano. Written informed consent was obtained from patients, or from their parents when necessary.

Response assessment

Peak value. The gland response to the stimulus can be easily assessed through the evaluation of the highest plasma concentration, i.e. the response peak value, among the measurements following GHRH+ARG stimulation. However, the true peak value of the concentration profile might not be coincident with the highest collected sample. Furthermore, the peak value is rather sensitive to the measurement noise, because such a value is based only on a single sample.

Standard AUC. A more reliable assessment of the global effect of the stimulus is usually obtained by computing the AUC of the plasma concentration samples through the trapezoidal rule. The AUC should be calculated from the instant when the stimulus is given to the time when its effect ceased. However, spontaneous secretions may occur before and/or after the stimulation. In particular, standard AUC may be affected by several artefacts: spontaneous prestimulus secretion may interfere with the AUC of interest, the stimulation effect may last beyond the last observed sample and a poststimulus secretion episode may interfere with the GH response to the stimulus.

AUC of the ISR. A way to prevent possible artefacts is to assess gland responsiveness through the evaluation of the AUC of the Instantaneous Secretion Rate (ISR) signal. The glandular ISR cannot be directly measured. However, the GH plasma concentration, the glandular ISR and the clearance are linked by a convolution integral that can be leveraged to reconstruct the ISR profile by so-called deconvolution analysis.[11,15] Previous works showed that the AUC of the ISR can be assessed by a linear combination of plasma hormone concentration samples.[13,16] By applying this method to our sampling schedule, the following formula is obtained:

$$\begin{aligned}\mathrm{AUC}_{\mathrm{ISR}} = {} & 0.2537y_1 + 1.9011y_2 + 0.6998y_3 \\ & + 1.9032y_4 + 2.5714y_5 + 2.0189y_6\end{aligned} \quad (1)$$

where y_i, $i = 1,\ldots, 6$, are the serum GH concentration observations at the sampling times $t = 0$, 30, 45, 60, 90, 120. This approach should reduce the artefacts due to possible spontaneous secretion events. Moreover, this technique is less sensitive to measurement errors compared with the evaluation of the peak value alone.

AUC due to the stimulus. According to the usual linear model of GH kinetics, the GH concentration profile is given by the superposition of the response to the GHRH+ARG stimulus and possible pre- and post-stimulus spontaneous secretion. Accordingly, the AUC of the plasma GH can be seen as the sum of a term $\mathrm{AUC}_{\mathrm{GH}}$ due to the GHRH+ARG stimulus and other terms due to spontaneous secretion. Note that $\mathrm{AUC}_{\mathrm{GH}}$ and $\mathrm{AUC}_{\mathrm{ISR}}$ are linked through the fractional hormone clearance (FCR):

$$\mathrm{AUC}_{\mathrm{GH}} = \frac{\mathrm{AUC}_{\mathrm{ISR}}}{\mathrm{FCR}} \quad (2)$$

The FCR can be regarded as the inverse of the AUC of the plasma concentration $g(t)$ obtained through a unitary-per-volume hormone bolus. For GH, $g(t)$ is usually approximated by an exponential function, that is

$$g(t) = e^{-\alpha t}\ t \geq 0 \quad (3)$$

where FCR = α = 0·0779[11] is the population value of FCR.

Hence, by means of equations (1) and (2),

$$\begin{aligned}\mathrm{AUC}_{\mathrm{GH}} = {} & 3.2567y_1 + 24.4044y_2 + 8.9833y_3 \\ & + 24.4313y_4 + 33.008986y_5 + 25.9166y_6\end{aligned} \quad (4)$$

where the coefficients are just obtained by dividing the weights in equation (1) by the FCR.

Secretion response analysis

To evaluate the shape of the GH secretion response, a Secretion Response Analysis (SCR) was introduced. Given the ISR profile of a subject (Fig. 1, upper panel), the normalized unit-area ISR curve is obtained (Fig. 1, middle panel), so as to make the response profiles comparable among different subjects. From that, the Cumulative Secretion Rate (CSR) (Fig. 1, lower panel) can be obtained. The half-secretion time is defined as the time when the CSR curve reaches 0·5, corresponding to 50% of secretion. The half-secretion time measures the response delay. In particular, slower responses yield longer half-secretion times.

Results

In our study, the peak values of the GH response, the AUC due to the stimulus of GHRH+ARG and the half-secretion times were considered, see Table 2.

The differences in terms of peak values, $\mathrm{AUC}_{\mathrm{GH}}$ and half-secretion times were evaluated by means of the *t*-test (see Table 2). Concerning peak values and $\mathrm{AUC}_{\mathrm{GH}}$, the differences were statistically significant (*P*-value <0·05) between PWS DEL15 and PWS UPD15, as well as between PWS UPD15 and

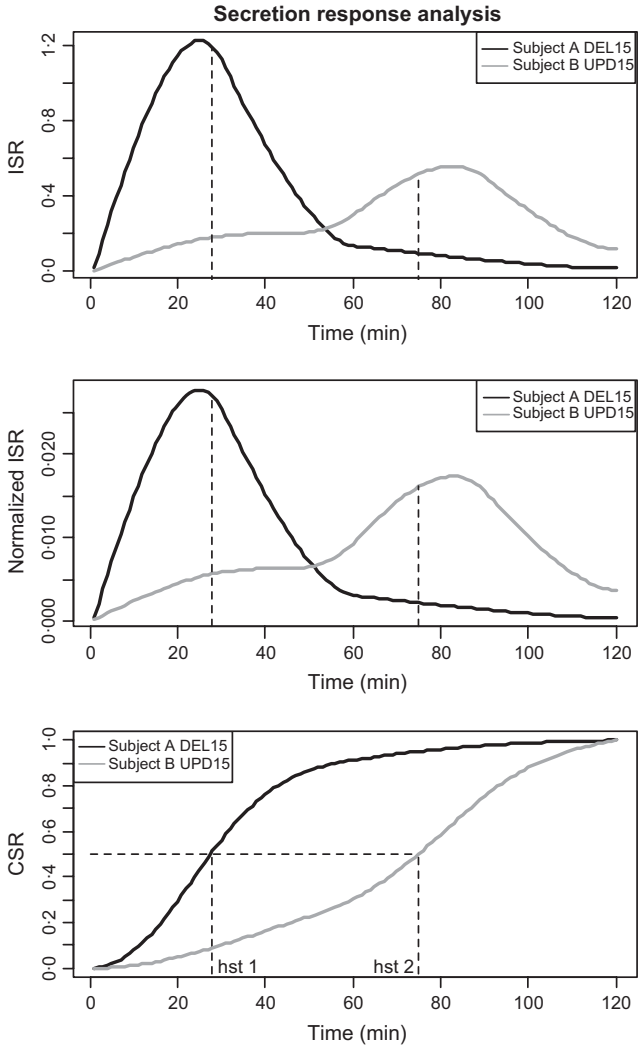

Fig. 1 Secretion Response Analysis (SRA) of two subjects with different karyotypes (Subject A with DEL15 and Subject B with UPD15). The ISR profiles of the two subjects are shown in the upper panel. These curves are then normalized to have unit area (middle panel), to make them comparable. The Cumulative Secretion Rate (CSR) plot is performed to assess the half-secretion times of the two subjects. Subject A has a shorter half-secretion time (hst1), that is the response of subject B is more delayed than that of subject A.

obese controls, also when the populations were stratified with respect to the gender, the only exception being the comparison of AUC_{GH} between UPD15 males and obese males that was not significant (data not shown).

Furthermore, considering half-secretion times, statistically significant differences were observed between the whole populations of PWS adults and obese controls (*P*-value: 0·0012), as well as between the same female populations (*P*-value: 0·0042). Moreover, significant *P*-values were obtained for female subjects when PWS adults were stratified by karyotype and compared with obese controls (*P*-values: 0·018 and 0·005, when considering UPD15 and DEL15 populations, respectively).

IGF-I levels were significantly higher in the obese controls when compared with all PWS as well as to the different genetic

Table 2. Population statistics (mean ± SD) of PWS adults, stratified by karyotype, and obese controls

Population	Peak value (μg/l)	AUC_{GH} (μg/l/h)	Half-secretion time (min)
Obese controls	15·7 ± 12·0	966·4 ± 750·8	39·3 ± 13·2
PWS (all)	10·1 ± 11·3	744·0 ± 950·1	52·4 ± 12·6**
PWS DEL15	11·9 ± 12·1†	879·2 ± 1038·6†	51·7 ± 12·3*
PWS UPD15	4·8 ± 6·4*	329·8 ± 396·6*	54·5 ± 13·6*

**P*-value <1% between obese control adults and PWS subjects;
***P*-value <0·1% between obese control adults and PWS subjects;
†*P*-value <1% between PWS adults with different karyotype).

subtypes of PWS (Table 1). By means of deconvolution analysis,[12] the instantaneous secretion rate profiles of all the subjects of the two populations were reconstructed. Figure 2 shows an example of the deconvolution and reconvolution profiles of a subset of subjects. In accordance with Table 2, it can be seen that obese controls have a higher GH response than PWS adults (Fig. 2).

The response delay was assessed through the comparison of the half-secretion time of the subpopulations. Figure 3 shows the mean cumulative secretion rates of each subpopulation. In particular, obese controls have the lowest half-secretion time, whereas the PWS UPD15 have the most delayed response, probably due to the higher severity of the syndrome. In general, PWS subjects show a more delayed response than the obese control subjects. However, PWS DEL15 have a slightly faster GH response compared with PWS UPD15 adults (Fig. 3).

Concerning BMI, no statistically significant differences were found between PWS adults (considering both the whole population and the two karyotypes) and obese controls, also when stratifying subjects by gender. The possible correlation between AUC_{GH} and the available covariates, i.e. age, BMI, HSDS and IGF-I, was evaluated for each subpopulation. Furthermore, PWS subjects were stratified by karyotype. Figure 4 shows that AUC_{GH} is negatively correlated with BMI in the entire group of PWS adults (correlation coefficient: −0·584, *P*-value: <0·01%) as well as in PWS DEL15 (correlation coefficient: −0·626, *P*-value: <0·01%). Concerning the obese controls, AUC_{GH} is positively correlated with IGF-I (correlation coefficient: 0·579, *P*-value: 0·015) (Fig. 4).

Discussion

The aetiology of impaired GH secretory pattern in PWS is still controversial, because the cause of reduced GH levels may be attributed to the effect of obesity alone. However, the clinical picture of PWS in adulthood strongly supports the presence of GHD. Apart from short stature, both PWS and GHD are characterized by impaired physical strength. Decreased left ventricle mass and lower chronotropic response to an adrenergic stimulus have been demonstrated.[17] Furthermore, adults with PWS showed both a reduced bone mineral density and an abnormal

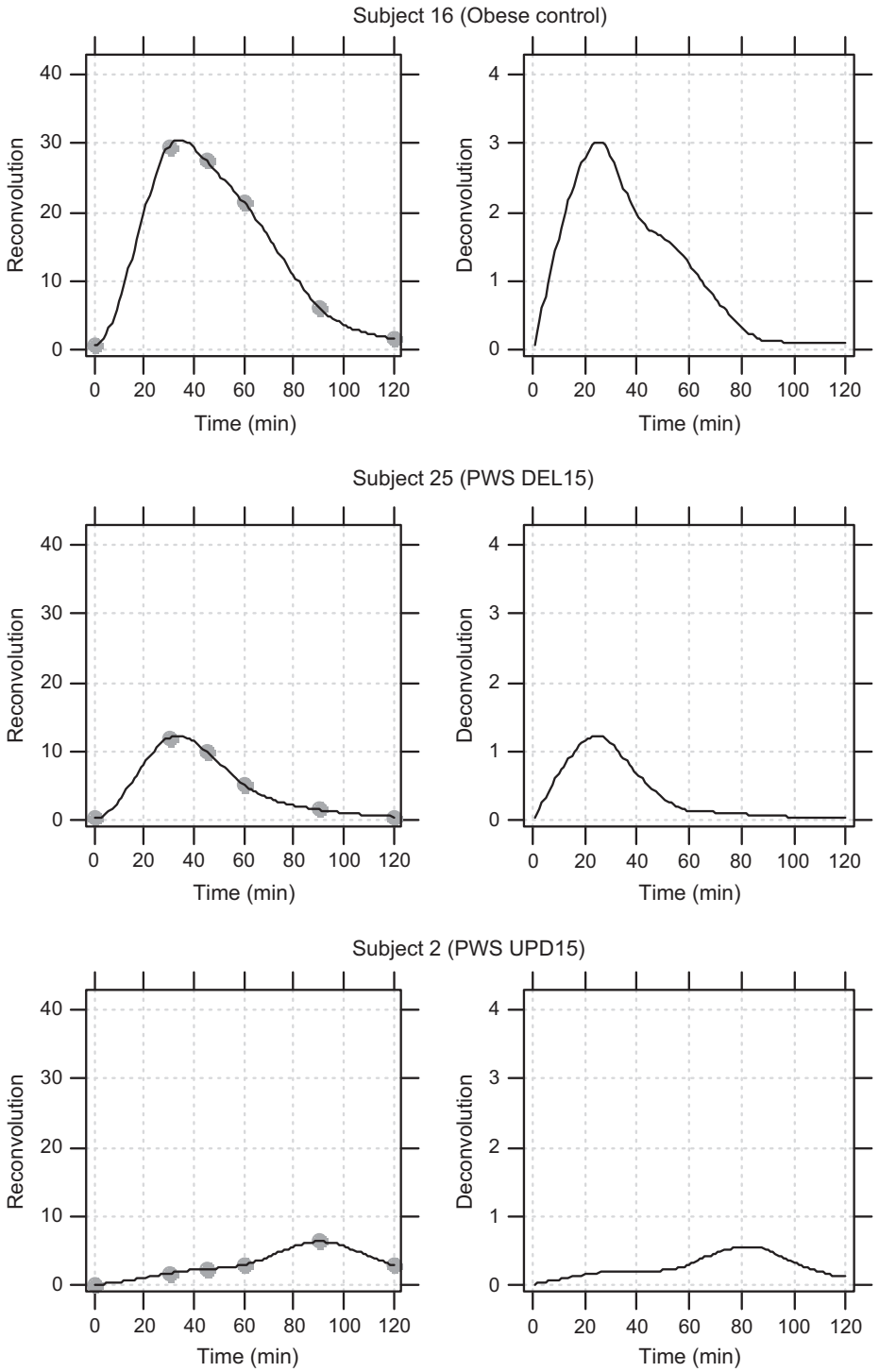

Fig. 2 Example of deconvolution (right panels) and reconvolution (left panels) profiles for some representative subjects. In the upper panel, deconvolution and reconvolution profiles are shown for an obese control. The middle and bottom panels display the reconvolution and deconvolution profiles relative to a PWS DEL15 subject and a PWS UPD15 one, respectively. In all cases, the response profile is well reconstructed.

body composition, with increased fat to lean mass ratio and decreased lean body mass.[18] In this context, it has been found that stimulated GH levels are different in PWS adults when compared with patients with similar BMI[7] as well as with obese subjects having similar fat mass percentage.[19] In addition, reduced GH stimulated levels are present in a significant proportion of PWS adults.[6–8] Nevertheless, all previous studies have adopted a quantitative assessment of gland responsiveness to exogenous stimuli, using the peak value of the hormone concentrations in plasma, or the AUC. However, none of these meth-

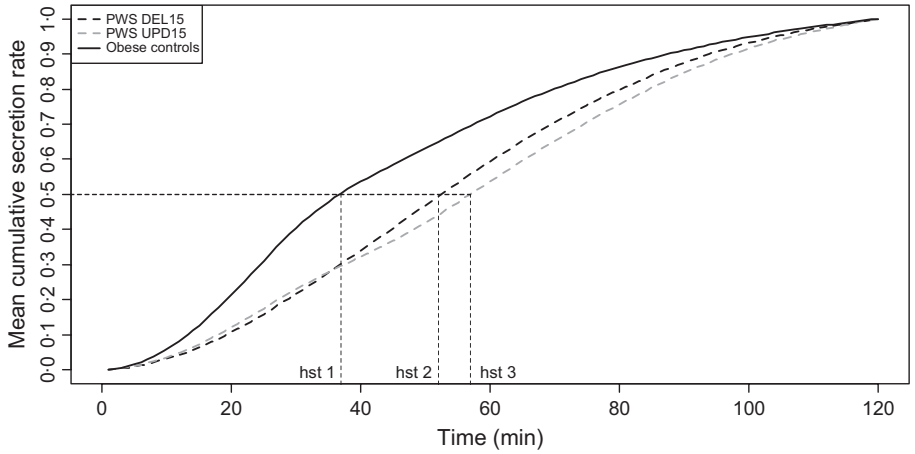

Fig. 3 Mean CSR of the subpopulations, namely obese controls, PWS DEL15 and PWS UPD15 subjects. The three half-secretion times are denoted by hst1, hst2 and hst3. Obese controls have the fastest response, whereas PWS UPD15 have the highest half-secretion time, corresponding to the most delayed secretion response.

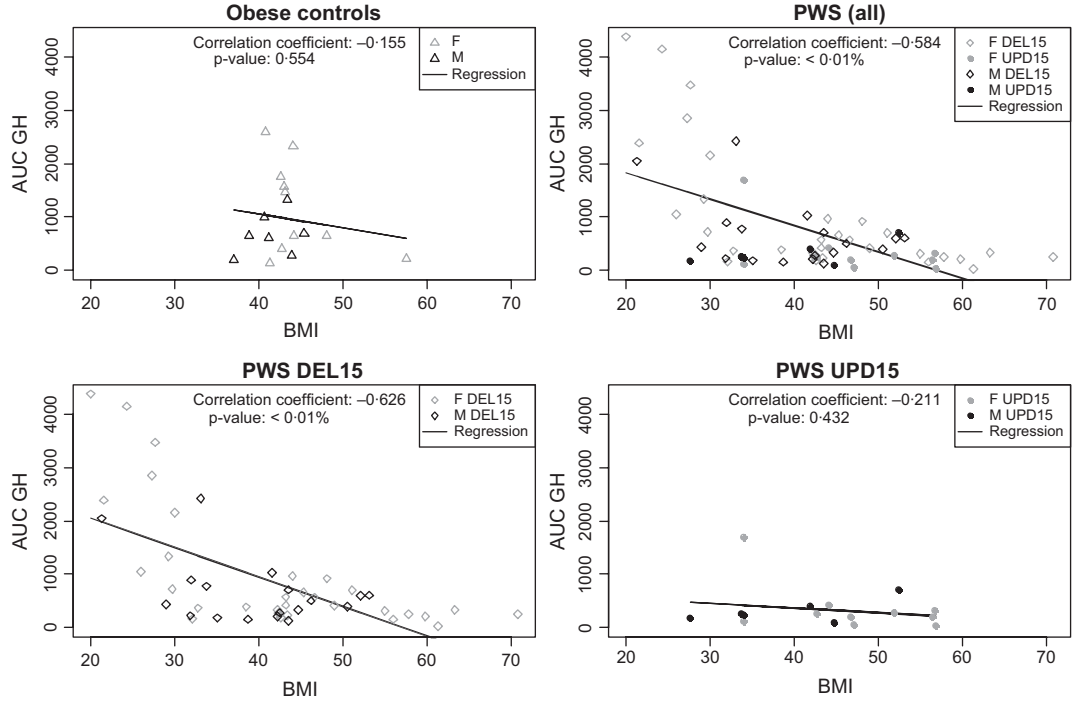

Fig. 4 Correlations between AUC_{GH} and BMI for obese controls and PWS subjects (upper panels) and PWS DEL15 and PWS UPD15 (bottom panels); F, females, M, males. A negative correlation is observed for all PWS (top-right panel, correlation coefficient: −0·584, *P*-value <0·01%) and for PWS DEL15 patients (bottom-left panel, correlation coefficient: −0·626, *P*-value <0·01%). No significant correlation is observed for obese controls (correlation coefficient: −0·155, *P*-value: 0·554) and for UPD15 patients (correlation coefficient: −0·211, *P*-value: 0·432).

ods is satisfactory, due to either sensitivity to measurement errors or various sources of bias.

This is the first study to provide a thorough investigation of the impairment of GH secretory response in PWS subjects. In particular, both the quantitative and qualitative features of responses to GH stimuli have been investigated.

In its basic version, the study of pituitary responsiveness may rely on the quantitative assessment of few phenomenological parameters, such as the peak value of the GH concentration and the area under its curve (AUC). However, as shown in previous studies,[11–13,16] a better description of the characteristics of the glandular secretory response is obtained by taking into account that plasma GH concentration provides just an incomplete and indirect picture of pituitary responsiveness: incomplete because only few blood samples are collected at prespecified instants in time and indirect because we do not have direct access to the

glandular secretion rate. Indeed, the plasma concentration of GH is the convolution of the Instantaneous Secretion Rate (ISR) with the hormone clearance function, meaning that deconvolution analysis is needed to recover the ISR, which best reflects the quantitative and qualitative aspects of pituitary response.[11]

The use of deconvolution analysis has been shown to overcome several limitations of clinical responsiveness studies characterized by sparse and limited sampling. In particular, rather than relying on a single peak value that is rather sensitive to measurement errors, the quantitative response can be measured by the AUC of the ISR.[13,16] In fact, such a secretory AUC is less prone to biases due to the short duration of the sampling schedule that rarely exceeds few hours.

By using deconvolution methodology previously developed and validated on a variety of pituitary hormones,[11,13,15] in the present study, the responsiveness of PWS subjects has been characterized. First of all, the quantitative impairment compared with obese group was quantified. In this respect, it turned out that the secretory response of PWS UPD15 subjects, measured through both the peak value of the plasma GH concentration and AUC of the ISR, was significantly lower than that of both essential obese controls and PWS DEL15 subjects. For peak values, but not for AUC, the finding was maintained also if comparisons were performed between gender-matched groups. It is noteworthy that statistically significant differences were not found between PWS DEL15 and obese controls, thus highlighting that, as far as GH impairment is concerned, the karyotype identifies two distinct subpopulations of PWS subjects, accordingly to our previous findings.[9]

A second relevant aspect is the qualitative secretion response analysis of the pattern of GH secretion. This analysis was performed by using a new parameter (the half-secretion time), representing the time needed to deliver half of the total hormone secretory response. To enable comparison, the ISR is normalized to obtain a unit-area curve from which the Cumulative Secretion Rate and the half-secretion time are obtained. In particular, the longer the half-secretion time, the more delayed the gland response.

A significant difference between the half-secretion time of obese control subjects and the whole set of PWS adults was observed, significance being maintained if the comparisons were performed between PWS DEL15 and obese control subjects or between PWS UPD15 and obese control subjects.

It is noteworthy that the PWS DEL15 subpopulation, which is not significantly different from obese controls in terms of response amplitude (either peak value of GH response or AUC), is conversely significantly different in terms of response pattern, as characterized by the half-secretion time. In view of these findings, it can be conjectured that the common trait shared by the two subpopulations of PWS subjects is more related to the pattern of secretory profiles rather than the mere amplitude.

The delayed GH response in all PWS subjects, compared with obese controls, is suggestive of an impairment of GH hypothalamic regulation network, involving the interplay of both GHRH and somatostatin tone. Concerning this issue, hypothalamic anomalies are well proven in PWS,[20,21] consistent with deficiency of many pituitary hormones[22,23] and brain imaging[24] and histological abnormalities.[25] The presence of a GH/IGF-I axis impairment in PWS seems to be supported by our data of lower IGF-I levels in respect of obese controls.

One limitation of this study includes the lack of a control group of normal-weight adults. In fact, knowing how much and in which way the obese group differed from the normal BMI population would render the study more complete. Further research is needed to better discriminate the impact of fat mass on the pattern of GH secretion.

In conclusion, this study demonstrates that quantitative and qualitative analyses complement each other to provide a comprehensive description of GH responsiveness, highlighting the key differences between PWS adults and obese controls, which would not be fully characterized by a purely quantitative analysis. Moreover, our results support the view that the degree of GH impairment in PWS depends on the genetic subtypes, with a lower GH secretion ability in the PWS UPD15 subjects in respect of PWS DEL15 patients.

Disclosure summary

All authors have nothing to disclose.

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