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No central adrenal insufficiency found in patients with Prader-Willi syndrome with an overnight metyrapone test

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Abstract

Background: Individuals with Prader-Willi syndrome (PWS) have hypothalamic dysfunction and may have central adrenal insufficiency (CAI). The prevalence of CAI in PWS remains unknown.

Methods: Twenty-one subjects with PWS aged 4–53 years underwent a low dose adrenocorticotropic hormone (ACTH) stimulation test (LDAST) (1 μg/m^2, maximum 1 μg) followed by an overnight metyrapone test (OMT). Metyrapone (30 mg/kg, maximum 3 g) was administered at 2400 h. Cortisol, 11-deoxycortisol (11-DOC) and ACTH levels were collected the following morning at 0800 h. OMT was the standard test for comparison. Peak cortisol ≥15.5 μg/dL (427.6 nmol/L) on LDAST and 0800 h 11-DOC ≥7 μg/dL (200 nmol/L) on OMT were classified as adrenal sufficiency.

Results: Twenty subjects had 0800 h 11-DOC values ≥7 μg/dL on OMT indicating adrenal sufficiency. One subject had an inconclusive OMT result. Six of the 21 (29%) subjects had peak cortisol <15.5 μg/dL on LDAST.

Conclusions: We found no evidence of CAI based on OMT, yet 29% of our PWS population failed the LDAST. This suggests that the LDAST may have a high false positive rate in diagnosing CAI in individuals with PWS. OMT may be the preferred method of assessment for CAI in patients with PWS.

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Keywords: ACTH stimulation test; central adrenal insufficiency; hypothalamic dysfunction; overnight metyrapone test; Prader-Willi syndrome.

Introduction

Prader-Willi syndrome (PWS) is a multi-system genetic disorder resulting from the lack of expression of the paternal genes from chromosome 15q11.2-q13 due to paternal deletion, uniparental disomy (UPD) or an imprinting center defect [1]. The classic presentation is characterized by hypotonia, developmental delay, hyperphagia, obesity, short stature, hypogonadism and respiratory and sleep disturbances. Hypothalamic-pituitary dysfunction is believed to be responsible for many of these phenotypic features.

A startling high mortality rate of 3% per year in PWS patients has been reported [2]. Although many deaths are due to complications of morbid obesity, a substantial number of deaths are reported to occur secondary to respiratory failure associated with only mild viral illness or an upper respiratory tract infection [3–5]. Additionally, a sizable number of deaths in PWS are reported as sudden, unexpected death during sleep, and many deaths remain unexplained. It is speculated that central adrenal insufficiency (CAI) may be responsible, in part, for this high rate of excess mortality and sudden death. Furthermore, there are reports of small adrenal glands found at autopsy of PWS individuals with unexplained death [6, 7]. If CAI is present, timely diagnosis and treatment is imperative in order to prevent unnecessary and avoidable mortality in this population.

The true prevalence of CAI in PWS remains unknown as several studies, using different testing modalities, have reported strikingly differing results. A European study in 2008 reported that 60% of subjects with PWS demonstrated CAI based on the adrenocorticotropic hormone (ACTH) response to the overnight metyrapone test (OMT) [8]. However, subsequent studies using other testing methods, including insulin tolerance test (ITT),

glucagon stimulation and ACTH stimulation tests, found much lower rates of CAI ranging from 0 to 14% in subjects with PWS [9–14]. Consequently, there are no guidelines or recommendations on the appropriate evaluation and management of CAI in the PWS population. This creates a clinical dilemma for providers regarding which test is most appropriate to screen for the diagnosis of CAI in PWS, and the need for empiric glucocorticoid treatment during times of physical illness or stress.

Therefore, the aim of this study was to further examine the function of the hypothalamic-pituitary adrenal (HPA) axis and the presence of CAI in patients with PWS, and to determine the correlation of the low dose ACTH stimulation test (LDAST) compared to the OMT when used sequentially in the same patient.

Subjects and methods

Subjects

Twenty-one subjects (aged 4–53 years) (10 males, 11 females) with genetically confirmed PWS were enrolled. Subjects who were ill at the time of testing or pregnant were excluded from the study. Subjects receiving medications that are known to affect the results of the OMT or have the potential to cause toxicity in combination with metyrapone were required to undergo a 2-week washout period. These medications included phenytoin, estrogen, acetaminophen and oral antidiabetic agents. The protocol was approved by the Institutional Review Board at Nationwide Children's Hospital, The Ohio State University, Columbus, OH, USA. Informed consent was obtained from all subjects and informed assent was obtained for subjects deemed appropriate to provide assent into the study.

Methods

The study visit was performed at Nationwide Children's Hospital during an overnight inpatient hospital admission. Subjects first underwent an LDAST followed by the OMT. The LDAST was performed at 2200 h. Baseline serum cortisol and ACTH levels were drawn followed by administration of 1 μg/m^2 (max 1 μg) of cosyntropin (synthetic ACTH). Stimulated serum cortisol levels were obtained at 20 and 40 min after cosyntropin injection. In low doses, synthetic ACTH peaks between 20 and 40 min, and then falls rapidly to undetectable levels by 60 min [15]. At 12 midnight, oral metyrapone 30 mg/kg with a maximum dose of 3 g (Laboratoire HRA Pharma, Paris, France) was administered orally. Blood pressure and pulse were monitored every 2 h from 12 midnight to 0800 h the following morning. At 0800 h, blood was drawn for determining ACTH, cortisol and 11-deoxycortisol (11-DOC) levels. Intravenous hydrocortisone was available for administration if the subjects demonstrated signs or symptoms of adrenal insufficiency during the course of the study. The subjects were discharged home at 12 noon the following day to ensure that signs or symptoms of adrenal insufficiency did not develop. Polysomnography studies were retrospectively reviewed for documentation of sleep disordered breathing for those subjects who had studies available.

Laboratory interpretation of results

The results of the LDAST were interpreted as adrenally sufficient if the stimulated peak cortisol was ≥15.5 μg/dL (427.6 nmol/L) and insufficient if the stimulated peak cortisol was <15.5 μg/dL. The peak cortisol result indicating adrenal sufficiency was chosen based on institutional normal values for a specific Cortisol was measured by immunoassay (Architect i2000SR, Abbott Laboratories, Abbott Park, IL, USA).

The OMT results were interpreted as adrenally insufficient if the 0800 h 11-DOC was measured by HPLC tandem mass spectrometry (Esoterix Inc., Calabasas Hills, CA, USA) value was <7 μg/dL (200 nmol/L) with a suppressed 0800 h cortisol <5 μg/dL (138 nmol/L), inconclusive if the 11-DOC was <7 μg/dL with a non-suppressed cortisol ≥5 μg/dL or normal (adrenally sufficient) if the 0800 h 11-DOC value was ≥7 μg/dL, regardless of cortisol value [16].

ACTH was measured by immunoassay (Exoterix Inc., Calabasas Hills, CA, USA) values at 0800 h ≥100 pg/mL (22 pmol/L) were expected in response to the single dose of oral metyrapone. Although this lab result was not used to determine the primary outcome of the OMT, a low ACTH value following metyrapone blockade would be suggestive of CAI. The results of the LDAST were compared to those of the OMT for each patient. The OMT was used as the standard test for comparison.

Statistical analysis

Statistical analysis was performed using Systat statistical software (version 13 Systat Software Inc., San Jose, CA, USA). The Shapiro-Wilk test was used to assess the parametric distribution of the variables studied. Spearman's correlation coefficient was used to examine the relationship between non-parametric data. Student's t-test and the chi-squared (χ^2) test were used to assess the differences in continuous and non-continuous variables, respectively. Differences in non-parametric data were assessed using the Kruskal-Wallis test. All data are expressed as mean ± standard deviation (SD). A p-value <0.05 was considered to indicate statistical differences in all tests.

Results

Twenty-one subjects with PWS completed the study (age 13.9 ± 10.9 years, body mass index [BMI] z-score 1.76 ± 1.1) (mean ± SD) (Table 1). Thirteen subjects (62%) had paternal deletion; six subjects (29%) had UPD, and two subjects had an unknown genetic subtype. Sixteen subjects (76%) were treated with growth hormone (GH) at the time of the study. Sleep studies were available for 15 of the 21 subjects. Five subjects (24%) were treated for sleep disordered breathing, and three subjects (14%) had documentation of mild central sleep apnea (CSA) with a central apnea index of 0.76 ± 0.24.

Twenty subjects passed the OMT with 0800 h 11-DOC values ≥7 μg/dL (11.1 ± 3.0), indicating adrenal sufficiency

Table 1: Characteristics of PWS subjects.

Subjects with PWS	All (n=21)	Male (n=10)	Female (n=11)
Age, years	13.9±10.9	13.2±7.6	14.5±13.6
BMI	27.0±10.4	26.5±9.7	27.5±11.5
BMI z-score	1.76±1.1	1.9±1.3	1.6±0.8
del15	13[a]	7	6
UPD	6[a]	3	3
GH treatment	16	8	8
Peak cortisol, µg/dL[b]	16.9±3.4	16.3±3.8	17.6±3.0
ACTH, pg/mL[c]	333.6±441.1	243.2±141.6	415.9±529.6
11-DOC, µg/dL[c]	11.1±3.0	10.3±2.4	11.8±3.4

Data are shown as mean±SD. To convert cortisol in micrograms per deciliter to nanomoles per liter, multiply by 27.59; to convert ACTH in picograms per milliliter to picomoles per liter, multiply by 0.22; to convert 11-DOC in micrograms per deciliter to nanomoles per liter, multiply by 28.86. [a]Two subjects with an unknown genetic subtype. [b]Low dose ACTH stimulation test. [c]0800 h value on OMT.

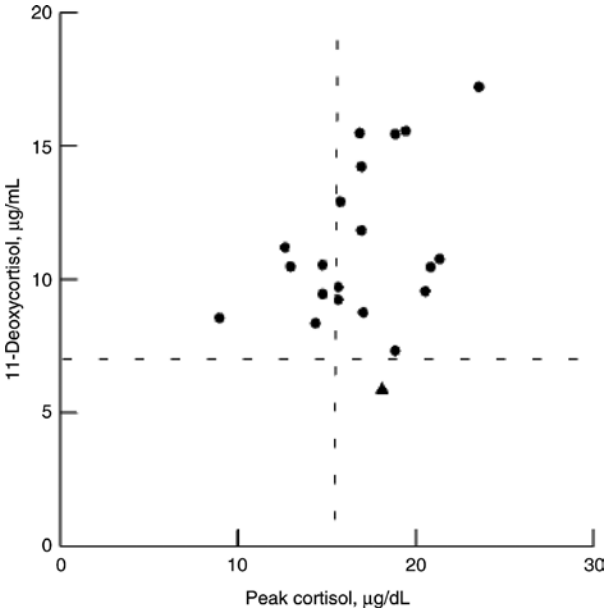

Figure 1: 0800 h 11-Deoxycortisol response after the OMT plotted against peak cortisol response to the low dose ACTH stimulation test. • Represents individual subject result of 0800 h 11-deoxycortisol response after the OMT and peak cortisol response to the low dose ACTH stimulation test. ▲ Represents subject with an inconclusive OMT result.

(Figure 1). Six of the 21 subjects (29%) failed the LDAST based on peak cortisol <15.5 µg/dL (13.1±2.2) (Figure 1, Table 2). There were no differences in age, BMI, BMI z-score, gender or genetic sub-type among those who passed and those who failed the LDAST (Table 2). There was no difference in mean peak cortisol on LDAST between those treated and those not treated with GH (16.9±3.8 vs. 16.8±1.3) (p=0.901). There was no difference in mean peak cortisol

Table 2: Characteristics of PWS subjects who passed and failed the low dose ACTH stimulation test.

	Failed low dose ACTH (n=6)	Passed low dose ACTH (n=15)	p-Value
Age, years	9.0±3.5	15.9±12.3	0.064
BMI	26.3±11.5	27.3±10.3	0.853
BMI z-score	1.8±1.2	1.7±1.1	0.885
Gender (M/F)	4/2	6/9	0.269
del15/UPD[a]	3/3	10/3	0.504
Peak cortisol, µg/dL[b]	13.1±2.2	18.5±2.4	0.001
ACTH, pg/mL[c]	235.0±61.7	373.1±520.4	0.329
11-DOC, µg/dL[c]	9.7±1.2	11.6±3.4	0.075

Data are shown as mean±SD. To convert cortisol in micrograms per deciliter to nanomoles per liter, multiply by 27.59; to convert ACTH in picograms per milliliter to picomoles per liter, multiply by 0.22; to convert 11-DOC in micrograms per deciliter to nanomoles per liter, multiply by 28.86. [a]Two subjects with an unknown genetic subtype. [b]Low dose ACTH stimulation test. [c]0800 h value on OMT.

on LDAST between those with and those without documentation of CSA (15.7±3.6 vs. 17.0±1.2) (p=0.314).

One subject had inadequate suppression of 0800 h cortisol of 10.7 µg/dL (295.2 nmol/L) and a subsequent insufficient rise in 11-DOC to 5.3 µg/dL which was interpreted as an inconclusive OMT result. This subject had a peak cortisol of 18.1 µg/dL (500 nmol/L) on LDAST.

In all subjects, post OMT, the 0800 h ACTH and 11-DOC were 333.6±441.1 pg/mL and 11.1±3.0 µg/dL, respectively, (Table 1). There was a positive relationship between 0800 h ACTH and 11-DOC values following metyrapone administration (r=0.712, p<0.05).

In a separate *post hoc* analysis, subjects were divided into those with 0800 h ACTH <150 pg/mL (33 pmol/L) (n=5) and those with 0800 h ACTH ≥150 pg/mL (n=15) post metyrapone (Table 3). There was a significant difference in 11-DOC levels between the two groups (9.5±0.8 vs. 11.9±3.1 µg/dL) (p=0.013), but there were no differences in age or BMI z-score. Four of the five subjects who had a rise in ACTH <150 pg/mL passed the LDAST. No subject displayed signs or symptoms of adrenal insufficiency.

Table 3: Sub-group analysis of PWS subjects with 0800 h ACTH <150 pg/mL and ≥150 pg/mL on OMT.

	ACTH <150 (n=5)	ACTH ≥150 (n=15)	p-Value
ACTH, pg/mL	117.4±8.1	424.1±497.1	0.032
11-DOC, µg/dL	9.5±8.0	11.9±3.1	0.013

Data are shown as mean±SD. To convert ACTH in picograms per milliliter to picomoles per liter, multiply by 0.22; to convert 11-DOC in micrograms per deciliter to nanomoles per liter, multiply by 28.86.

Discussion

In the present study, we found no clinically significant cases of CAI in a cohort of 21 children and adults with PWS based on OMT, yet 29% of this cohort failed the LDAST. The discrepancy in our results using different testing methods, and therefore, diagnosis of CAI in subjects with PWS has been demonstrated in previous studies [9–14]. However, this is the first study that we are aware of to compare the results of the LDAST and OMT sequentially in the same patient.

Optimal evaluation of the HPA axis for the assessment of CAI is dubious and remains an area of controversy within the field of Endocrinology. The ITT is considered the 'gold standard' for the assessment of CAI [17] as it stimulates the entire HPA axis; however, this test carries inherent risks, including severe hypoglycemia. Alternatively, the single-dose OMT has been well validated against the ITT with good sensitivity and specificity [18–20]. Metyrapone inhibits the conversion of 11-DOC to cortisol, causing a fall in circulating cortisol, stimulating a rise in ACTH secretion and a subsequent rise in 11-DOC. This test may be best performed in the inpatient setting given the theoretical risk of adrenal crisis. Like the ITT, the OMT directly assesses the response of the entire HPA axis and therefore is considered ideal to assess for CAI and ACTH reserve. Practically, many centers utilize the LDAST for the diagnosis of CAI. In contrast to the ITT and OMT, it relies on indirect assessment of adrenal gland function. While some studies have shown that the LDAST has a reasonable correlation to the ITT and OMT [21–25], others have shown the reliability of the LDAST in comparison to the OMT to be poor [26].

The results of our study contrast significantly with a prior study by de Lind van Wijngaarden et al. [8] who found that 60% of a cohort of individuals with PWS had CAI based on the OMT. It is important to note that de Lind van Wijngaarden et al. used different criteria for the diagnosis of CAI than those used in our present study. A diagnosis of CAI was made based on insufficient post-OMT rise in ACTH values <150 pg/mL (33 pmol/L), rather than 11-DOC as in our study. A review of post-OMT 0800 h ACTH values in our study revealed that all values were >100 pg/mL (22 pmol/L). Using a similar ACTH cut-off of 150 pg/mL (33 pmol/L) as used in de Lind van Wijngaarden et al.'s study, we found that all but five subjects had a rise in ACTH >150 pg/mL. Four of these five subjects passed the LDAST. We performed a *post hoc* analysis, dividing subjects into those who had a rise in ACTH <150 pg/mL (n = 5) and those who had a rise in ACTH ≥150 pg/mL (n = 15). Although there was a significant difference between the rise in 11-DOC between the groups, none of our subjects displayed signs or symptoms of adrenal insufficiency or required treatment with hydrocortisone. We believe this observation is clinically significant.

Furthermore, in de Lind van Wijngaarden et al.'s study, while adequate cortisol blockade was demonstrated mid-way through the OMT, the morning cortisol at 0730 h was not adequately suppressed <5 μg/dL (138 nmol/L) in subjects labeled as CAI (mean cortisol 7.79 μg/dL, 214.9 nmol/L). Inadequate cortisol suppression may have led to insufficient negative feedback to stimulate ACTH secretion and the subsequent rise in 11-DOC, further accounting for their high rates of CAI. This difference in the interpretation of the OMT results between our two different studies may account for our vastly differing rates of CAI. We believe that this study may have overstated the true incidence of CAI in patients with PWS.

The results of our LDAST indicate a higher rate of CAI compared to previous studies in the PWS population (29% vs. 0–14%) [9, 10, 12, 13]. These prior studies used a more stringent cut-off value of 18.1 μg/dL (500 nmol/L) for the normal cortisol response to ACTH stimulation. However, cortisol assays are not standardized across institutions, interpretation of cortisol values are assay dependent [27, 28] and optimal peak cortisol value on LDAST varies across studies [29, 30]. Our institution employs a cut-off value of 15.5 μg/dL for the normal cortisol response to ACTH stimulation based on normal values for our specific cortisol assay. Even though 29% of our cohort failed the LDAST, with a normal response to OMT, no subject was determined to have CAI. We speculate that poor reliability and reproducibility inherent of the LDAST is to blame for the inconsistencies in our results. We do not believe that the timing of the LDAST affected our results, as peak cortisol response to synthetic ACTH has been shown to be constant and unrelated to the time of day [27, 28]. Although the LDAST is an acceptable alternative to traditional gold standards in otherwise normal patients with pituitary dysfunction, it is possible that the PWS population may be inherently different from the populations previously studied that validated the LDAST given their unique physiology.

Whether or not there is an association between GH treatment or CSA and clinically significant CAI in individuals with PWS is currently unknown. Treatment with GH may theoretically impair the conversion of cortisone to the active hormone cortisol. However, Tauber et al. found no differences in causes of death between those PWS children who received GH and those who did not [5]. The majority of our subjects (76%) were receiving GH at the time of study evaluation. We found no difference in mean peak cortisol on the LDAST between those treated and those not treated with GH, further discrediting

this potential concern. Insufficient ACTH response during stress in combination with CSA has also been suggested to be responsible for the increased risk of sudden death. We found that peak cortisol on LDAST did not differ between those with and those without mild CSA. These findings are supported by other studies examining the relationships between cortisol and the central apnea index on polysomnography [14]. This is an area of study that has not been well explored and warrants further investigation.

In conclusion, we demonstrate dramatically differing test results of adrenal sufficiency in PWS individuals when comparing two widely accepted methods to test for CAI: LDAST and OMT. We believe that clinically significant CAI in patients with PWS is rare. Our OMT results confirm our suspicion, with no subjects demonstrating CAI based on this test. OMT may be the preferred method of assessment for CAI in patients with PWS in order to avoid overdiagnosis and unnecessary treatment with glucocorticoids in a population already at risk for obesity. We recognize that there may be limitations to this recommendation and to the findings in our study. Metyrapone may not be readily available in many hospital centers. Additionally, it requires close observation or overnight hospital stay to monitor for potential side effects of transient adrenal insufficiency due to cortisol blockade, making this test somewhat precarious. It may, however, more accurately identify those PWS patients who necessitate treatment of CAI with glucocorticoid therapy. Lastly, it is possible that hypothalamic dysfunction is progressive or develops over the lifetime, and the need for repeat testing for CAI in PWS individuals is unclear. Longitudinal studies are needed to better characterize the function of the HPA axis in PWS individuals over the lifetime, and to form official guidelines and recommendations regarding the diagnosis and management of CAI in PWS individuals.

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