Normal Cortisol Response to High-Dose Synacthen and Insulin Tolerance Test in Children and Adults with Prader-Willi Syndrome

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Context: Prader-Willi syndrome (PWS) is a genetic disease associated with hypogonadism and partial GH insufficiency, possibly explained in part by a hypothalamic dysfunction. Partial insufficiency of the hypothalamic-pituitary-adrenal (HPA) axis has recently been suggested.

Objective: The objective of the study was to further explore the HPA axis in PWS by use of routine tests.

Design: Nonselected PWS patients were examined with a standard high-dose synacthen test or the insulin tolerance test (ITT). A random serum (s) cortisol was measured in case of acute illness.

Setting: The study was conducted at university hospitals in Denmark and Sweden.

Patients: Sixty-five PWS patients with a confirmed genetic diagnosis participated in the study.

Main Outcome Measures: A s-cortisol value above 500 nmol/liter as well as an increase of 250 nmol/liter or greater was considered a normal response.

Results: Fifty-seven PWS patients (median age 22 yr, total range 0.5–48 yr) were examined with the high-dose synacthen test. The median s-cortisol at the time of 30 min was 699 (474–1578) nmol/liter. Only one patient had a s-cortisol level below 500 nmol/liter but an increase of 359 nmol/liter. This patient subsequently showed a normal ITT response. Two patients had increases less than 250 nmol/liter but a time of 30-min s-cortisol values of 600 nmol/liter or greater. These three patients were interpreted as normal responders. Eight patients [aged 26 (16–36) yr] examined with the ITT had a median peak s-cortisol of 668 (502–822) nmol/liter. Four children admitted for acute illnesses had s-cortisol values ranging from 680 to 1372 nmol/liter.

Conclusion: In this PWS cohort, the function of the HPA axis was normal, suggesting that clinically significant adrenal insufficiency in PWS is rare. **(*J Clin Endocrinol Metab* 96: E173–E180, 2011)**

Prader-Willi syndrome (PWS) is a genetic disorder caused by lack of expression of paternal genes in region 15q11-q13. PWS is characterized by short stature, characteristic facial appearance, muscular hypotonia, global developmental delay with mild to moderate intellectual disability and behavioral/psychiatric disturbances, hypogonadism, disturbed activity of the GH-IGF-I system, and hyperphagia, potentially leading to severe obesity from early childhood (1–4).

Many of the symptoms indicate a hypothalamic dysfunction, *e.g.* hyperphagia and hormonal disturbances (*5*). Despite earlier studies testing the hypothalamic-pituitary-

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Abbreviations: AI, Adrenal insufficiency; BMI, body mass index; CAI, central adrenal insufficiency; HDST, high-dose synacthen test; HPA, hypothalamic-pituitary-adrenal; ITT, insulin tolerance test; PWS, Prader-Willi syndrome; s, serum; SDS, SD score; t^{0min}, time of 0 min; t^{30min}, time of 30 min.

adrenal (HPA) axis (6) and the adrenals at autopsy (7) in a limited number of PWS patients who did show some evidence of adrenal insufficiency (AI), the function of the HPA axis until this decade has been assumed to be normal. Recently an insufficient ACTH response to an overnight single-dose metyrapone test was reported in 15 of 25 children with PWS (60%) (8). Morning salivary cortisol and diurnal salivary cortisol profiles were normal. The clinical implications of these findings are uncertain.

The aim of the present study was to further explore the HPA axis in PWS patients by use of routine tests performed in our clinics to evaluate the cortisol reserve. The cortisol response to a standard high-dose synacthen test (HDST) was measured in a cohort of genetically verified PWS children and adults. In addition, the cortisol response to an insulin tolerance test (ITT) and a random serum (s) cortisol during acute illness were measured in small subsets of these patients and in a few other patients.

Patients and Methods

Study population

Fifty-eight children and adults with PWS were consecutively recruited from the outpatient clinic at the Centre for Rare Diseases, Aarhus University Hospital Skejby in Aarhus, Denmark. The patients were all subjected to a HDST. Two of these patients were also tested by the ITT.

During the study period, four children were admitted to the acute ward and blood samples were taken for s-cortisol analysis.

At the Department of Endocrinology, Metabolism, and Diabetology in Stockholm, Sweden, an additional six adults with PWS, in whom venous access was possible, underwent an ITT.

All patients fulfilled the clinical consensus criteria (2), and the diagnosis was confirmed in all by molecular genetic methods (Table 1).

Anthropometric methods

Physical examination included measurements of height and weight. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. BMI SD score (SDS) were obtained from Nysom *et al.* (9). In adults a BMI from 18.5 to 25 kg/m^2 was defined as normal, a BMI between 25 and 30 kg/m^2 was defined as overweight, and a BMI above 30 kg/m^2 was defined as obese according to World Health Organization criteria.

High-dose synacthen test

The HDST was performed between 1000 and 1400 h by iv administration of 0.25 mg per square meter of surface area of synthetic ACTH (Tetracosactid, Synacthen 0.25 mg/ml; Novartis, Copenhagen, Denmark). The maximum dose was 0.25 mg.

Blood samples were drawn at baseline and after 30 min. A s-cortisol value above 500 nmol/liter at a time of 30 min (t^{30min}) as well as an increase of 250 nmol/liter or greater was considered to be a normal response.

Insulin tolerance test

The ITT was performed between 0800 and 1200 h after an overnight fast. The patient was constantly monitored by a nurse and a doctor. Pulse, blood pressure, blood glucose, and consciousness were observed with shorter intervals as hypoglycemia occurred. Insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) at a dose of 0.15 U/kg was injected iv. Blood samples were drawn at 0, 30, 45, 60, 75, and 90 min to measure glucose and cortisol. A nadir plasma-glucose less than 2.0 mmol/liter was obtained in all but one, in whom it was 2.6 mmol/liter, and all patients showed hypoglycemic symptoms, *i.e.* paleness, profuse sweating, tachycardia, and somnolence, but not unconsciousness. The basal and the peak s-cortisol values were recorded in the study protocol. A peak s-cortisol value above 500 nmol/liter as well as an increase of 250 nmol/liter or greater was considered a normal response.

Cortisol measurement

Serum samples were analyzed by means of an electrochemiluminescence immunoassay (Elecsys; Roche, Mannheim, Germany) with an intra- and interassay imprecision less than 5%, and a cross-reactivity with 11-deoxycortisol of 4.1%. Units are given as nanomoles per liter. For calculation of micrograms per deciliter, the value in nanomoles per liter can be divided by the conversion factor 27.586.

Additional sampling

Blood samples were collected at time 0 min (t^{0min}) for serum analyses, *i.e.* cortisol and IGF-I. The blood was analyzed locally by routine methods, except for IGF-I, which was measured by an in-house method at the Research Laboratory M2 at Aarhus University Hospital Nørrebrogade in Denmark (10). s-IGF-I reference values for children 6 yr old or older and adults were available.

Statistics

Statistical analyses were calculated with SPSS 17.0 (SPSS Inc., Chicago, IL). Data are presented as median and total ranges. The s-cortisol values were assessed by gender, age, s-IGF-I SDS, BMI, and BMI SDS. A Student's *t* test was used to compare difference between the two groups. The Kruskal-Wallis test was used for comparison in between groups. Pearson's correlation was used for continuous variables. A covariance test with corrections was used for analysis of correlation. The statistical significance level was $P < 0.05$.

Ethics

The study was approved by the Central Denmark Region and the Karolinska University Hospital Committee on Biomedical and Research Ethics. Consent to participate was obtained from the patients and their parents or guardians before study participation, sample collection, and testing.

Results

The HDST group

During the period from September 2008 through May 2010, 58 patients (27 men and 31 women) were enrolled (Table 1). The HDST was carried out in 57 patients. One 5-month-old male baby was excluded from the calculations

TABLE 1. Characteristics of patients with PWS (n = 65) included in the study

	All	Men		Women	
n	65	33		32	
Age (yr)[a]					
Median	22	22		22	
Total range	0.42–48	0.42–42		0.5–48	
Age groups		**<17**	**≥17**	**<17**	**≥17**
n		11	22	9	23
Genetics					
Deletion	42	7	12	7	16
Uniparental disomy	13	3	5	1	4
Imprinting defect	2	1	1	0	0
Methylation pos only	8	0	4	1	3
BMI (SDS)[b]					
Median	0.92	1.1	0.9	0.79	1.1
Total range	−2.11 to 3.66	−0.81 to 3.37	−2.11 to 3.49	−1.28 to 2.77	−1.56 to 3.66
IGF-I SDS[c]					
Median	0.8	3.69	0.5	1.42	0.47
Total range	−4.7 to 7.21	1.33 to 4.91	−4.7 to 7.21	0.22 to 5.69	−1.9 to 5.36
Treatment					
GH	40	7	12	8	13
Sex steroids	17	0	9[d]	0	8[e]
Levothyroxine[f]	3	0	1	1	1
Antidiabetics[g]	4	0	2	0	2
Psychoactives[h]	10	0	4	0	6
Antiepileptics[i]	1	0	1	0	0
Antihypertensives[j]	2	0	1	0	1
Antiasthmatics[k]	3	0	2	0	1
CPAP[l]	3	1	1	0	1
Pacemaker[m]	1	0	0	0	1
Cortisol test					
HDST[n]	58	10[o]	17	9	22
ITT[p]	8	0	6	0	2
Acute illness[q]	4	3	0	1	0

[a] Six children younger than 2 yr of age; five children 2–5 yr of age.

[b] Patients 2 yr old or older (n = 59 of 59). The reference values cover individuals ≥2 yr of age or older [Nysom *et al.* (9)].

[c] Patients 6 yr old or older (n = 51 of 54). The reference values cover individuals 6 yr of age or older [Frystyk *et al.* (10)].

[d] Testosterone given orally (n = 6) or as im injection (n = 3).

[e] Estrogen given as adhesive (n = 1), Trisequens (n = 3), or contraceptive pill (n = 4). Two women had spontaneously regular menses.

[f] All patients were euthyroid; however, one child and two adults had hypothyreosis and were substituted with levothyroxine.

[g] Four adults had diabetes (two type 1). All had normal glycosylated hemoglobin (HbA1c) values, except one 42-yr-old man with type 2 diabetes treated with oral antidiabetics (HbA1c 10.1).

[h] Eleven adults were treated with psychoactive drugs: selective serotonin reuptake inhibitor (n = 6), benzodiazepine (n = 3), antipsychotics (n = 7), and carbamazepine (n = 2).

[i] One teenager had epilepsy treated with topiramate.

[j] Two adults had hypertension and hypercholesterolemia treated with angiotensin-converting enzyme inhibitor, hydrochlorothiazide, and simvastatine.

[k] Two children had asthmatic bronchitis and one adult had asthma; all were treated with inhaled budesonide and a β2-agonist.

[l] Three adults had sleep apnea treated with continuous positive airway pressure (CPAP) during the night.

[m] One 44-yr-old woman had a pacemaker due to a previously diagnosed sick sinus node syndrome. She had no cardiac symptoms.

[n] Two persons were also tested with the ITT. Three children were also examined during acute illness.

[o] One 5-month-old boy was excluded due to difficulties in getting venous access. His basal s-cortisol was 656 nmol/liter.

[p] Two persons were also tested with the HDST.

[q] Three children were also tested by HDST. See text for case stories.

because the HDST had to be given up due to difficulties in getting venous access. His basal s-cortisol value was 656 nmol/liter. At the age of 8 months, he was tested during an acute illness (case 1).

The median age was 22 (0.58–48) yr. Eighteen were children younger than 17 yr of age. All patients were DNA methylation analysis positive, and 54 of 57 patients were further genetically characterized (Table 1).

TABLE 2. Serum cortisol response (nanomoles per liter) to HDST in patients with PWS (n = 57)

	Men		Women	
Age groups (yr)	**<17**	**≥17**	**<17**	**≥17**
n	9	17	9	22
Age (yr)	7 (0.6–16)[a]	31 (17–42)	5 (0.5–15)	27.5 (18–48)
s-cortisol, t^{0min}	220 (147–554)	194 (58–427)	133 (78–851)	196 (86–1020)
s-cortisol, t^{30min}	802 (702–1066)	617 (474–881)[b]	807 (599–996)	668 (555–1578)
Delta s-cortisol	553 (468–720)	436 (261–585)	572 (128–842)[c]	483 (237–613)[c]

[a] Numbers are presented as median and total range.

[b] One 32-yr-old man had an increase to only 474 nmol/liter. The ITT was normal.

[c] Two women (0.5 and 20 yr old) had increases less than 250 nmol/liter, but 30-min values 600 nmol/liter or greater. See text.

The median BMI SDS of patients 2 yr old or older (n = 52) was 0.87 (−2.11–3.37) (9). The BMI SDS did not change with age (Pearson's correlation coefficient −0.163, $P = 0.25$). Among the 39 persons aged 17 yr or older, the median BMI was 25.1 (18.0–42.7) kg/m^2. One was underweight, 18 were normal weight, 12 were overweight, and eight were obese.

Forty patients with a median age of 20 (0.83–42) yr were on GH treatment. At the time of testing, they had been treated for a period of 4 months to 16 yr (median 3 yr), with daily doses ranging from 0.15 to 1.8 mg. The median s-IGF-I SDS value (n = 34) was 2.04 (−0.6 to 7.4). For those not treated (n = 17), the median age was 24 (0.58–48) yr, and the median s-IGF-I SDS value (n = 11) was −0.45 (−2.45 to 0.81). The s-IGF-I SDS differed significantly between these groups (Student's *t* test, $P < 0.000$). The s-IGF-I SDS did not change with age (Pearson's correlation coefficient −0.222, $P = 0.147$).

None of the patients were treated with systemic hydrocortisone.

None of the patients had ever shown any signs or symptoms of AI, *e.g.* uncontrolled weight loss, episodes of hypoglycemia, and circulatory instability during acute illness.

For the total group of patients, the median s-cortisol in the HDST at t^{0min} was 194 (58–1020) nmol/liter, and at t^{30min} was 699 (474–1578) nmol/liter (Table 2). Responses to the HDST are shown in Fig. 1. Only one 32-yr-old male patient showed a t^{30min} cortisol of less than 500 nmol/liter (474 nmol/liter); however, the increase was 359 nmol/liter. In this patient, the ITT showed a normal peak s-cortisol of 583 nmol/liter. The median increase or delta value for the total group was 483 (128–842) nmol/liter. A 0.5-yr-old female baby had a normal baseline cortisol but a rather modest increase. The s-cortisol value rose from 851 nmol/liter to 979 nmol/liter (delta value 128 nmol/liter). A 20-yr-old female patient had a marginally low delta value of 237 nmol/liter, but a t^{30min} value of 600 nmol/liter. All three patients were interpreted as normal responders. Individual responses are shown in Fig. 2.

No gender difference was found [Student's *t* test, $P = 0.584$ (t^{0min}), $P = 0.120$ (t^{30min}) and $P = 0.277$ (delta), respectively]. The basal s-cortisol did not change with age, but the t^{30min} s-cortisol and the delta s-cortisol decreased with increasing age. These findings were explained by changes in infants and children as the t^{30min} and the delta s-cortisol values did not change with age among those 17 yr of age or older (see Fig. 2).

The s-cortisol values tended to be higher the higher BMI SDS for those aged 2 yr or more and for those aged 17 yr or more when divided into normal weight, overweight, and obese groups. However, the findings were not significant (Pearson' correlation and Kruskal-Wallis test, data not shown).

Eight women aged 21–40 yr were treated with estrogen in different formulas (Table 1). Their basal (Student's *t* test, $P = 0.003$) and 30-min (Student's *t* test, $P = 0.002$) s-cortisol values, but not their delta value (Student's *t* test, $P = 0.204$), were significantly higher compared with those not treated.

The data showed that the higher s-IGF-I SDS (n = 44 of 48 patients ≥6 yr of age), the lower basal s-cortisol value (Pearson's correlation coefficient −0.407, $P = 0.006$). The t^{30min} and delta s-cortisol values did not show signif-

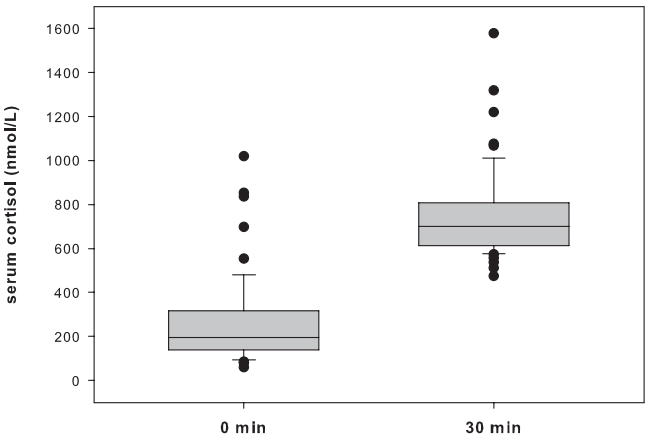

FIG. 1. Serum cortisol response to HDST in patients with PWS (n = 57): s-cortisol values at 0 and 30 min, respectively, shown as box plot (median and interquartile ranges with 10 and 90% whiskers).

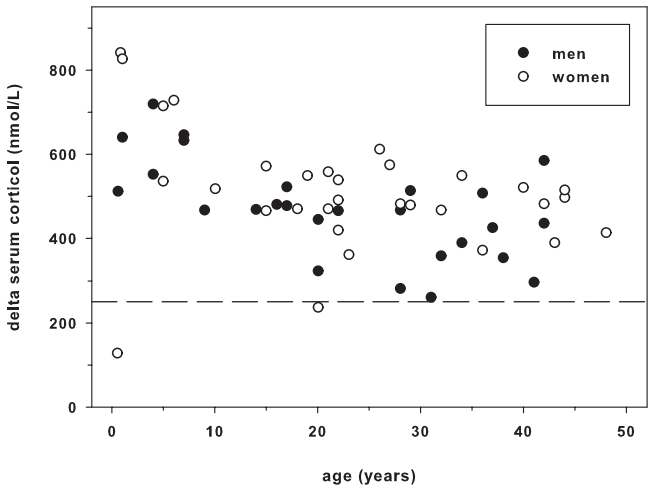

FIG. 2. Change in serum cortisol value as response to HDST in patients with PWS (n = 57) distributed according to age and gender. The *broken line* shows the lower limit of a normal response (250 nmol/liter). Basal s-cortisol values did not change with age (Pearson's correlation coefficient −0.045, $P = 0.739$). Thirty-minute and delta s-cortisol decreased with increasing age (Pearson's correlation coefficient −0.268, $P = 0.044$ and −0.424, $P = 0.001$, respectively). Thirty-minute and delta s-cortisol did not change with age among those 17 yr of age or older (Pearson's correlation efficiency −0.041, $P = 0.753$ and −0.055 $P = 0.738$, respectively).

icant correlation with s-IGF-I SDS [Pearson's correlation coefficient −0.284 ($P = 0.061$) and 0.191 ($P = 0.214$), respectively]. In a covariance test with corrections made for gender, age, BMI SDS, and estrogen treatment, GH treatment did not change the basal s-cortisol values significantly ($P = 0.397$).

The ITT group

Six patients plus two patients from the HDST group were tested using the ITT. All patients were DNA methylation analysis positive. Of those, three patients were further genetically characterized as having either deletion (n = 2) or maternal UPD (n = 1).

The medium BMI SDS was 1.29 (−0.42 to 3.66). Four patients were obese. None had diabetes and all were otherwise healthy. Two patients were treated with GH, and none were treated with sex steroids or systemic hydrocortisone.

None of the patients had ever shown any signs or symptoms of AI.

The median s-cortisol value at t^{0min} was 188 (175–281) nmol/liter, and the median peak s-cortisol value was 668 (502–822) nmol/liter. The median duration to obtain the peak value was 60 (45–120) min, depending on the time for hypoglycemia to occur. The median change in the s-cortisol value between t^{0min} and the time at peak was 447 (302–641) nmol/liter. Patients and cortisol data are presented in Table 3. Seven of the patients, including a 25-yr-old male with a low normal response of 502 nmol/liter (delta cortisol 308 nmol/liter), were tested 10 yr ago, and during this observation period, none of these patients have presented with clinical signs of AI.

TABLE 3. Serum cortisol (nanomoles per liter) response to ITT in patients with PWS (n = 8)

	Men	Women
n	6	2
Age (yr)	26 (20–33)[a]	23.5 (16–31)
s-cortisol, t^{0min}	188 (175–281)	188 (186–190)
s-cortisol, peak	668 (502–822)	681 (620–742)
Delta s-cortisol	447 (302–641)	493 (434–552)

[a] Numbers are presented as median and total range.

s-cortisol in acute illness

During the HDST study period, four children were admitted acutely. Three had febrile illness and one had a first episode of status asthmaticus. The random s-cortisol values were 1372, 775, 1080, and 680 nmol/liter, respectively.

Case 1

Case 1 was an 8-month-old boy. He had a first episode of febrile illness with rhinitis and cough. A thoracic x-ray was normal. His general condition was relatively good. His maximum temperature of 39.7 C was on the third day when a s-cortisol of 1372 nmol/liter was measured. He became asymptomatic after a few days without any treatment.

Case 2

Case 2 was a 19-month-old girl. She had her second febrile episode with rhinitis. Her general condition was relatively good. After 10 h of illness, her temperature was 39.6 C, and the s-cortisol was 775 nmol/liter. She became asymptomatic in 2 d without any treatment.

Case 3

Case 3 was a 4-yr-old boy with no history of asthma. He had a first episode of status asthmaticus. On d 1 he had nasopharyngitis. On the second day, he began coughing and became increasingly dyspneic. On the third day, he was admitted with severe dyspnea, prolonged expiration phase, and a silent chest on auscultation. The percutaneous oxygen saturation was 0.81 and the temperature was 37.6 C. He rapidly improved on inhaled salbutamol supplemented with iv terbutaline and methylprednisolone. On admission the s-cortisol was 1080 nmol/liter. The chest x-ray was normal. After 3 d he was discharged and asymptomatic on treatment with inhaled terbutaline and orally administered montelukast.

Case 4

Case 4 was an 11-month-old boy. He had his third febrile episode. On admission he was whimpering and uncomfortable with no signs of sepsis or meningitis. He had a culture-confirmed *Escherichia coli* urinary tract infection. After 28 h of illness and before start of oral treatment with amoxicillin and clavulanic acid, his temperature was 39.6 C and the s-cortisol 680 nmol/liter. He became asymptomatic within 2 d.

Discussion

In the present study, we investigated a cohort of 65 children and adults with a clinically and genetically confirmed diagnosis of PWS. Sixty-three of 63 patients showed a normal s-cortisol response to accepted routine clinical tests and four children a normal s-cortisol value during acute illness.

Many of the signs and symptoms seen in PWS are thought to be caused by a hypothalamic dysfunction, although the pathophysiology is largely unknown. From an endocrine perspective, partial GH insufficiency (1, 11, 12) and hypogonadism have been described (3, 4). The latter has recently been attributed to a combined central and peripheral origin pointing to the possibility of a complicated hormonal dysfunction in PWS not solely involving the hypothalamus (13–15). An increased frequency of hypothyroidism of central or peripheral origin has still not been documented (16).

Annual mortality rates in PWS have been reported to be as high as 3% (17). Many deaths are caused by infectious diseases or diseases related to obesity (18, 19). However, a substantial number of deaths were unexpected and unexplained, and an undiscovered dysfunction of the HPA axis could be a possible etiology.

In the 1960s before genetic testing for PWS became available, Rudd *et al.* (6) found an inadequate adrenal cortisol response to ACTH after a single dose (n = 5) or a 3-d stimulation (n = 2) in six obese children with clinically PWS when compared with three groups of normal, short stature, or obese children, respectively. Small adrenal glands have been found on autopsy in four of four examined PWS children. One of one examined adult had normally appearing adrenals (7). Small volumes of the hypothalamic paraventricular nuclei with a decreased cell number have been found in five adults with PWS (5). Whether these findings influence the function of the HPA axis is unknown. Despite the fact that these data add some supportive observations to a possible AI in PWS, a normal function of the HPA axis has been generally anticipated until the recent findings by de Lind van Wijngaarden *et al.* (8). In this study, a partial central insufficiency of the HPA axis was suggested on the basis of an insufficient ACTH response to an overnight single-dose metyrapone test in 15 of 25 children (60%) (median age 9.7 yr) with PWS. Because normal morning and diurnal salivary cortisol profiles were found, the authors concluded that the central adrenal insufficiency (CAI) becomes apparent only during stressful conditions.

Compared with the de Lind van Wijngaarden (8) population, our patients were older with a median age of 22 yr. One explanation of the different results could be an improved function of the HPA axis in PWS with increasing age. Our data did not support that because we found higher increases in stimulated s-cortisol in infants and children when compared with adults.

Another explanation of our different findings may the methods used. Metyrapone acts on 11β-hydroxylase and thereby blocks the cortisol synthesis in the adrenals. The feedback by the following decreased cortisol level leads to increased release of ACTH by the pituitary gland (20). It can be argued that the metyrapone test has a higher sensitivity and specificity in identifying individuals with CAI because the metyrapone test has been shown to identify more individuals with decreased reactivity as determined by the ACTH level compared with the ITT (21, 22), the low-dose (1 μg) synacthen test (23–25), and the HDST (24, 25). To the extent of clinical relevance, or clinical specificity, of these findings, testing populations at risk of AI without overt signs and symptoms of AI is debated (21, 22) because lower ACTH levels may be sufficient to give normal cortisol responses (22). Moreover, data on the metyrapone test in normal populations are scarce.

Recently Butler *et al.* (26) found no difference between morning plasma-cortisol level in 63 PWS patients aged 2.5 months to 50 yr when compared with a group of obese people (n = 30). One subject in each group had low values. One of four PWS infants had low measures on repeated testing during an unknown time span. Information on symptoms or reaction to stress stimuli was not given. Butler *et al.* also described significantly lower cortisol values in the group of four infants 3 yr old or younger compared with 59 subjects older than 3 yr. This age dependency may at least partly be explained by obesity because our data supported, although not significantly, that the higher the BMI, the higher the cortisol level. In our study the basal cortisol values did not differ at all with the age of the patient.

In our experience clinically significant AI in PWS is rare. None of the PWS patients visiting our clinics, some of whom we have followed up for more than 2 decades, have ever had symptoms of hypocortisolism, not even during episodes of stressful conditions such as infections and operations.

In an attempt to further explore the prevalence of AI in PWS, we used our routine evaluation methods for AI, HDST, and ITT in the present study. The ITT is considered to be the gold standard in testing AI (20). The hypoglycemia induced by the ITT stimulates the whole HPA axis, whereas the HDST stimulates the adrenal cortex. Both tests are considered valuable in testing for primary as well as secondary AI (20, 27, 28). The sensitivity and specificity of both methods in diagnosing CAI are debated. The sensitivity of the HDST in detecting primary AI is as high as 97% (20, 29), whereas the sensitivity of the HDST in detecting secondary AI is lower, even as low as 57%, partly depending on the cutoff cortisol value (23, 24, 29, 30), the methods used for comparison (24, 25, 28), the populations studied, and the duration of the insufficiency (27, 31, 32). The HDST may miss identifying cases of CAI (33), especially those with a recent onset (31, 32). On the other hand, the specificity of HDST is high (24, 25).

In an extensive review of diagnosis and investigation of AI, Wallace *et al.* (20) pointed out that the HDST cannot be used in acute pituitary insufficiency; this is not the case in our present study. Bearing in mind that the adrenal atrophy caused by secondary or tertiary AI of a certain duration, longstanding clinically significant CAI is likely to be disclosed by a HDST (20, 27, 32). In view of our present findings of a normal t^{30min} s-cortisol response to the HDST in 56 of 57 PWS patients and to the ITT in eight of eight patients (including one who just failed the HDST), as well as during acute illness in four children, we found a prevalence of CAI of 60% to be very high.

We found no significant gender effect on s-cortisol levels.

Estrogen treatment leads to an increased level of s-cortisol-binding globulin and total s-cortisol (34). We also found an increased level of basal and t^{30min} s-cortisol in eight women on estradiol treatment when compared with those not treated. The increase in s-cortisol, or delta value, did not differ from the total patient population.

GH treatment is an integrated part of the medical care in children with PWS. Because IGF-I inhibits the activity of 11β-hydroxysteroid dehydrogenase 1 in adipose tissue (35, 36) and the liver (35) and thereby reduces the peripheral conversion of cortisone to cortisol, GH treatment may precipitate AI, at least in susceptible hypopituitary patients (35, 37, 38). Because the effect of the enzyme is largest in visceral fat, compared with sc fat, patients with PWS may be less susceptible (11, 12, 39). Some authors (37, 40) have found a significantly lower basal s-cortisol level in GH-treated patients when compared with those patients who are not treated. We found lower basal s-cortisol values, but normal responses, the higher the s-IGF-I SDS. On the other hand, we found no differences in the s-cortisol levels and responses when comparing patients with or without GH treatment when corrections were made for age, gender, BMI SDS, and estrogen treatment.

de Lind van Wijngaarden *et al.* (8) suggested considering hydrocortisone treatment during acute illness in PWS patients unless CAI has recently been ruled out. In our opinion, routine prophylactic treatment with hydrocortisone during acute illness in PWS is of concern because of the well-known side effects such as obesity, hypertension, dysmetabolic features, and changes in behavior. We would rather recommend treating if it is clinically indicated.

Because CAI, if part of the PWS, must be considered on a long-term basis, our results suggest this condition is rare.

In conclusion, our study including a cohort of 65 children and adults with PWS did not reveal insufficient responses to either generally accepted tests of adrenal cortisol secretion or during acute illness. Thus, clinically important AI is rare in children and adults with PWS.

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