

High Prevalence of Central Adrenal Insufficiency in Patients with Prader-Willi Syndrome

Roderick F. A. de Lind van Wijngaarden, Barto J. Otten, Dederieke A. M. Festen, Koen F. M. Joosten, Frank H. de Jong, Fred C. G. J. Sweep, and Anita C. S. Hokken-Koelega

Dutch Growth Research Foundation (R.F.A.d.L.v.W., D.A.M.F., A.C.S.H.-K.), 3016 AH Rotterdam, The Netherlands; Departments of Pediatric Endocrinology (R.F.A.d.L.v.W., D.A.M.F., A.C.S.H.-K.) and Pediatric Intensive Care (K.F.M.J.), Erasmus University Medical Center/Sophia Children's Hospital, 3015 GJ Rotterdam, The Netherlands; Department of Pediatric Endocrinology (B.J.O.) and Chemical Endocrinology (F.C.G.J.S.), Radboud University Nijmegen Medical Center, 6525 GA Nijmegen, The Netherlands; and Department of Internal Medicine (F.H.d.J.), Laboratory of Endocrinology, Erasmus University Medical Center, 3015 GE Rotterdam, The Netherlands

Context: The annual death rate of Prader-Willi syndrome (PWS) patients is very high (3%). Many of these deaths are sudden and unexplained.

Objective: Because most deaths occur during moderate infections and PWS patients suffer from various hypothalamic insufficiencies, we investigated whether PWS patients suffer from central adrenal insufficiency (CAI) during stressful conditions.

Design: Overnight single-dose metyrapone tests were performed. Metyrapone (30 mg/kg) was administered at 2330 h. At 0400, 0600, and 0730 h, ACTH, 11-deoxycortisol, cortisol, and glucose levels were measured. Diurnal salivary cortisol profiles were assessed on a different day at wake-up, 30 min after wake-up, at 1400 h, and at 2000 h.

Setting: The study was conducted in a pediatric intensive care unit.

Patients: Patients included 25 randomly selected PWS patients.

Main Outcome Measure: Patients were considered as having CAI when ACTH levels remained below 33 pmol/liter at 0730 h.

Results: Median (interquartile range) age was 9.7 (6.8–13.6) yr. Fifteen patients (60%) had an insufficient ACTH response (CAI, $P < 0.001$). There was no significant difference in age, gender, genotype, and body mass index $_{SD}$ score between patients with CAI and those without. Morning salivary cortisol levels and diurnal profiles were normal in all children, suggesting that CAI becomes apparent only during stressful conditions.

Conclusions: Strikingly, 60% of our PWS patients had CAI. The high percentage of CAI in PWS patients might explain the high rate of sudden death in these patients, particularly during infection-related stress. Based on our data, one should consider treatment with hydrocortisone during acute illness in PWS patients unless CAI has recently been ruled out with a metyrapone test. (*J Clin Endocrinol Metab* 93: 1649–1654, 2008)

Prader-Willi syndrome (PWS) is characterized by hypotonia, short stature, hyperphagia, obesity, hypogonadism, psychomotor delay, and sleep-related breathing disorders (1–4). PWS results from the lack of paternal expression of the q11-q13

region of chromosome 15 caused by deletion, uniparental disomy, imprinting center defect, or balanced translocation (1, 5).

The annual death rate in PWS patients is very high (3%) (6). Among the causes of death in older children and adults are cor

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Abbreviations: BMI, Body mass index; CAI, central adrenal insufficiency; HPA, hypothalamus-pituitary-adrenal; iqr, interquartile range; PWS, Prader-Willi syndrome; SDS, $_{SD}$ score; URTI, upper respiratory tract infection.

pulmonale (7, 8), fatal apneas (9), unexpected bathtub drownings (10), and gastric necrosis (11). In some patients, low adrenal weight was reported (12, 13). Deaths in younger children are mostly related to only mild or moderate upper respiratory tract infections (URTIs) (9, 10). Many of the sudden deaths in PWS children are still unexplained.

PWS patients have hypothalamic dysregulations and show no or few signs of illness. Often they do not get fever, cannot vomit, and have a higher pain threshold. Also during URTIs, PWS patients can appear less ill than they actually are.

Because hypothalamic dysfunction is responsible for many other endocrine deficiencies in PWS patients (14, 15), we hypothesized that PWS patients suffer from central adrenal insufficiency (CAI) during stressful conditions.

We used the overnight single-dose metyrapone test because this is the best dynamic test for the diagnosis of CAI (16–19). Metyrapone blocks the synthesis of cortisol by inhibiting 11- β -hydroxylase type 1, which converts 11-deoxycortisol (compound-S) to cortisol. The decline in plasma cortisol stimulates ACTH production. The cutoff level for an appropriate ACTH response is 33 pmol/liter at 0730 h (19). Those failing to achieve such an ACTH response are considered as having CAI (16, 17, 19–21).

To investigate the basal cortisol secretion and in the search for other parameters for the identification of patients at risk of CAI, diurnal salivary cortisol profiles were studied.

Patients and Methods

Patients

Twenty-five children with genetically confirmed PWS were randomly selected from our outpatient clinics. Patients underwent a metyrapone test during an overnight stay at the Pediatric Intensive Care Unit of the Erasmus University Medical Center/Sophia Children's Hospital (Rotterdam, The Netherlands). Twelve patients had paternal deletion (63%), six had maternal disomy (32%), and one an imprinting center mutation (5%). All were treated with GH, Genotropin 1.0 mg/m²·d (Pfizer Inc., New York, NY), with a median (interquartile range) duration of 33 (20–43) months. The protocol was approved by the Medical Ethics Committee of the Erasmus University Medical Center (International Standard Randomized Clinical Trial registration no. 49726762). Informed consent was obtained from parents and children above 12 yr of age.

Overnight single-dose metyrapone test

Metyrapone (30 mg/kg, Metopiron; Novartis Pharma BV, Arnhem, The Netherlands) was administered at 2330 h. The maximal cortisol suppression has been reported to occur at 0400 h (18, 22). The decline in plasma cortisol stimulates ACTH production, which causes 11-deoxycortisol before the enzyme blockade to accumulate. Maximal levels of ACTH and 11-deoxycortisol are found at 0730 h (16–19).

In 25 children, fasting blood samples were taken for the analysis of ACTH, 11-deoxycortisol, cortisol, and glucose at 0730 h (16, 17, 19) and in 16 children also at 2330, 0400, and 0600 h.

During the metyrapone test, heart rate and oxygen saturation were measured continuously and blood pressure was measured every 30 min. If a rise in heart rate (30% or more) or a decrease in blood pressure (10% or more) occurred, blood pressure was measured every 15 min until normalization. After the last fasting blood samples were taken at 0730 h, a single dose of hydrocortisone (25 mg) was administered.

Diurnal cortisol profile

Diurnal salivary cortisol profiles were assessed in 16 of the 25 PWS patients on a different day and during health, using Salivettes (Sarstedt, Nümbrecht, Germany), at spontaneous wake-up (0600–0900 h), 30 min after wake-up, at 1400, and at 2000 h. Maximal morning salivary cortisol levels were defined as the highest cortisol level in the morning (wake-up or 30 min thereafter) and compared with those established in 237 healthy schoolchildren (same laboratory with same assay).

Assays

To rule out cross-reactivity of steroids (16, 23, 24), cortisol was measured by RIA after extraction with dichloromethane and subsequent paper chromatography, according to the method described earlier for cortisol measurement in plasma and saliva (24). The minimal detection level was 10 nmol/liter for serum cortisol, 0.30 nmol/liter for salivary cortisol, and 0.17 nmol/liter for serum 11-deoxycortisol. Plasma ACTH levels were measured with an immunoradiometric assay (BioInternational, Gif sur Yvette, France) with a minimal detection level of 1.1 pmol/liter. Glucose levels were measured with the Hitachi 917 (Hitachi Device Development Center, Tokyo, Japan), detecting glucose levels between 0 and 42 mmol/liter.

Data analysis

Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS 15.0, Chicago, IL). Data are expressed as medians and interquartile ranges (iqr). SD scores (SDS) for height, weight, and body mass index (BMI) were calculated based on Dutch reference data by Growth Analyzer 3.0 (www.growthanalyser.org) (25). Blood pressure SDS was calculated according to reference values reported by Rosner *et al.* (26). Spearman's rho was calculated for the analysis of correlations.

Results

Median age of the PWS patients was 9.7 yr (range 3.7–18.6 yr). Median (iqr) BMI SDS was 0.8 (0.2–1.3); weight-for-height SDS, 1.1 (0.3–1.6); and height SDS, –0.9 (–1.8 to 0.5).

At start of the metyrapone test (2330 h), hormone levels were not significantly different between patients later diagnosed with CAI and those who were not. In all children, metyrapone maximally suppressed cortisol concentrations at 0400 h ($P = 0.005$, compared with baseline; Table 1). After 0400 h, cortisol levels increased as the effect of metyrapone declined. ACTH and 11-deoxycortisol levels increased during the entire test, with a maximum increase between 0400 and 0600 h (Table 1 and Fig. 1).

Adrenal insufficiency

Fifteen patients (60%, $P < 0.001$) showed an insufficient ACTH response at the end of the metyrapone test (0730 h; Table 1 and Fig. 1). Because metyrapone blocks cortisol synthesis, it causes a sudden increased demand for ACTH production, a situation mimicking stress. Patients with an insufficient ACTH response during the metyrapone test are therefore considered as having CAI during stressful conditions. Directly from start of the enzyme blockade, patients with CAI had a significantly lower increase in ACTH levels than those without CAI (Fig. 1).

ACTH levels correlated significantly with 11-deoxycortisol levels ($r = 0.5$, $P = 0.03$). Children with CAI had lower 11-deoxycortisol levels than those without, but this did not reach statistical significance ($P = 0.08$ at 0730 h; Table 1). All children

TABLE 1. Results of the metyrapone test in PWS children

	2330 h			0400 h			0730 h		
	ACTH (pmol/liter)	11-DOC (nmol/liter)	Cortisol (nmol/liter)	ACTH (pmol/liter) ^a	11-DOC (nmol/liter) ^b	Cortisol (nmol/liter) ^c	ACTH (pmol/liter) ^d	11-DOC (nmol/liter) ^e	Cortisol (nmol/liter)
CAI	3 (3–4)	0.4 (0–4.0)	75 (28–193)	9 (7–10) ^f	167 (160–281)	30 (10–61)	19 (14–26) ^g	172 (139–320)	215 (130–301)
Non-CAI	4 (4–5)	0.5 (0.2–1.2)	150 (100–333)	32 (12–180) ^f	206 (162–320)	40 (26–95)	63 (49–76) ^g	269 (230–313)	130 (70–190)

ACTH, 11-deoxycortisol, and cortisol levels at different time points in PWS children with CAI and those without (non-CAI). At 2330 and 0400 h, $n = 16$; at 0730 h, $n = 25$. To convert ACTH in picomoles per liter to picograms per milliliter, divide by 0.22; to convert 11-deoxycortisol in nanomoles per liter to micrograms per deciliter, divide by 28.99; to convert cortisol in nanomoles per liter to micrograms per deciliter, divide by 27.59. 11-DOC, 11-Deoxycortisol.

^a ACTH levels increased significantly over time, compared with baseline (total group: $P = 0.003$).

^b 11-Deoxycortisol levels increased significantly over time, compared with baseline values (total group: $P = 0.012$).

^c Maximal cortisol suppression at 0400 h occurred in both groups (total group: $P = 0.005$).

^d ACTH levels increased significantly over time, compared with baseline (total group: $P = 0.001$).

^e 11-Deoxycortisol levels increased significantly over time, compared with baseline values (total group: $P = 0.008$).

^f Differences in ACTH levels between CAI and non-CAI were significant at 0400 h ($P = 0.04$).

^g Differences in ACTH levels between CAI and non-CAI were significant at 0730 h ($P < 0.001$).

without CAI had 11-deoxycortisol levels above the classical cut-off level of 200 nmol/liter at 0730 h. Seven of 11 children with CAI had 11-deoxycortisol levels less than 200 nmol/liter, suggesting adrenal atrophy.

At 0730 h, cortisol levels were still low in all children. Children with CAI had higher levels of cortisol at 0730 h than children without CAI ($P = 0.08$), as was previously reported (19). Levels did not differ at 2330, 0400, and 0600 h ($P = 0.2$, $P = 0.4$, and $P = 0.2$, respectively). Cortisol suppression therefore did not differ between children with CAI and those without.

There was no significant difference in age, gender, BMI SDS,

weight-for-height SDS, height SDS, and genotype between patients with CAI and those without (data not shown).

Diurnal cortisol profile

Figure 2 shows that all PWS patients had normal morning cortisol levels and a normal diurnal rhythm, with high levels in the morning and decreasing during the day. This indicates that CAI occurs only during stressful conditions. Patients with an insufficient ACTH response during the metyrapone test had lower salivary cortisol levels at wake-up [2.7 (1.8–5.1) *vs.* 6.8 (3.5–12.3) nmol/liter, $P = 0.05$]. Cortisol levels at wake-up correlated with ACTH levels at 0730 h during the metyrapone test, although this did not reach statistical significance ($\rho = 0.5$ with $P = 0.1$). Salivary cortisol levels were not useful for identifying PWS patients at risk of CAI due to low sensitivity. The highest accuracy was obtained at a cutoff of 2.8 nmol/liter, with 99.2% specificity, but only 55.6% sensitivity. Levels at wake-up of 12 nmol/liter or above seemed indicative for absence of CAI (100% sensitivity), but such high levels were found in only one patient.

Side effects

None of the patients reported side effects during the metyrapone test, such as nausea and headache. Diastolic and systolic blood pressure SDS decreased significantly between 0030 and 0730 h (Fig. 3, $P = 0.002$ – 0.03 and $P = 0.004$ – 0.05 , respectively). The overall median systolic blood pressure SDS was lower in patients with CAI than those without [-0.5 (-1.6 to 0.5) *vs.* 0.2 (-0.5 to 1.3), $P = 0.07$]. The overall diastolic blood pressure SDS correlated with cortisol (diastolic: $\rho = 0.5$, $P = 0.004$; systolic: $\rho = 0.3$, $P = 0.07$). Blood pressure SDS did not significantly correlate with ACTH or 11-deoxycortisol.

All other vital parameters remained within the normal range: pulse 49/min or greater, breathing 13/min or greater, saturation 94% or greater. Naturally, while

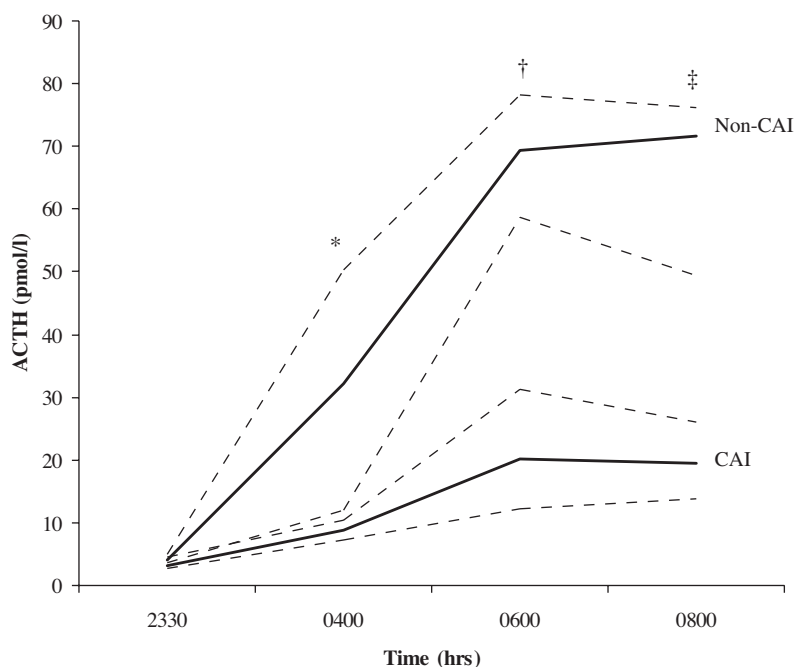


FIG. 1. ACTH response in PWS patients during a metyrapone test. Median (iqr) ACTH levels during the metyrapone test in children with CAI and those without (non-CAI). The cutoff level for a normal ACTH level is 33 pmol/liter at 0730 h. In 16 children, ACTH levels were measured at 2330, 0400, and 0600 h. *, $P = 0.048$, †, $P = 0.01$, ‡, $P < 0.001$, all CAI *vs.* non-CAI. To convert ACTH in picomoles per liter to picograms per milliliter, divide by 0.22.

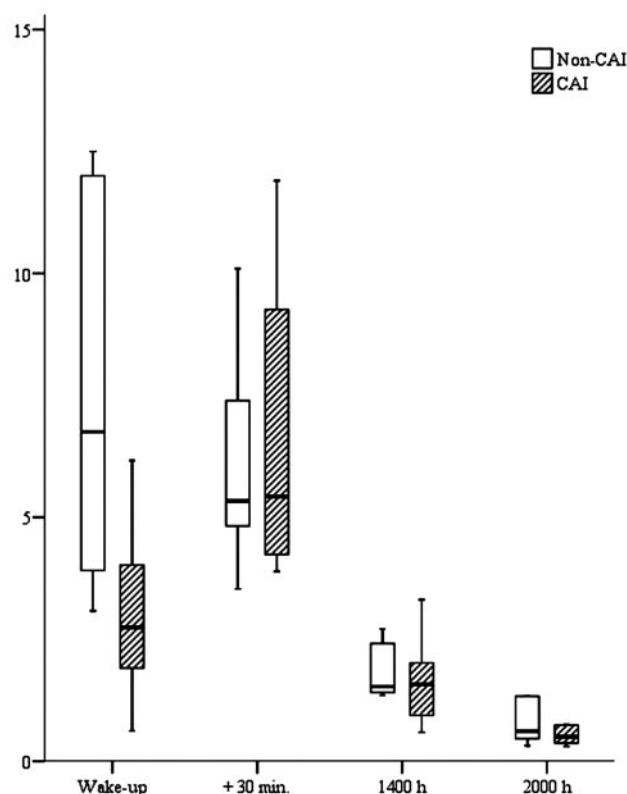


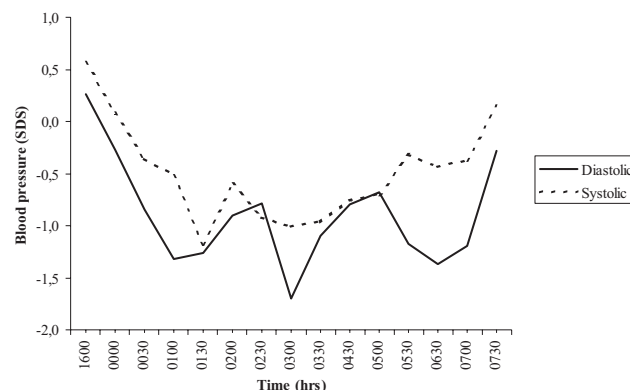
FIG. 2. Diurnal salivary cortisol levels in PWS patients. Box-whisker plot of salivary cortisol levels at wake-up, 30 min after wake-up (+30 min), at 1400 h, and at 2000 h in PWS children with CAI and without (non-CAI), expressed as median (iqr). Differences at wake-up did not reach statistical significance ($P = 0.053$). Median (iqr) reference values for morning maximal cortisol levels (either at wake-up or 30 min thereafter): 5.7 (1.9–16.2) nmol/liter. To convert cortisol in nanomoles per liter to micrograms per deciliter, divide by 27.59.

fasting, glucose levels decreased significantly during the night but were never less than 3.9 mmol/liter in any patient.

Discussion

Strikingly, 60% of PWS patients had central adrenal insufficiency, with ACTH levels failing to increase sufficiently during a metyrapone test. Because metyrapone blocks cortisol synthesis, it causes a sudden increased demand for ACTH production, a situation mimicking stress. Patients with an insufficient ACTH response during the metyrapone test are therefore considered as having CAI during stressful conditions. The insufficient ACTH response was apparent directly from start of the metyrapone test. The diagnosis of CAI is in line with the presence of other hypothalamic insufficiencies and oral reports about hypoglycemia during surgery in PWS patients.

We are the first to report an inappropriate ACTH response during a metyrapone test in a large percentage of PWS children. In view of the importance of an adequate function of the hypothalamus-pituitary-adrenal (HPA) axis for survival, the high prevalence of CAI may be an explanation for the high death rate in PWS patients (3%) (6). In addition to CAI, the condition of acutely ill PWS patients is further compromised by an increase in



Median diastolic and systolic blood pressure SDS decreased during the metyrapone test. Blood pressures at 2330 h 0400 h and 0600 h were excluded, because patients were awake.

FIG. 3. Blood pressure SDS during the metyrapone test. Median diastolic and systolic blood pressure SDS decreased during the metyrapone test. Blood pressures at 2330, 0400, and 0600 h were excluded because patients were awake.

the number of sleep apneas during upper URTIs (2) and a vague clinical presentation because PWS patients often have an increased pain threshold and do not vomit or develop fever.

During a metyrapone test, only the ACTH response is measured. During illness, factors other than ACTH will influence the HPA axis, such as cytokines and vasopressin (23, 27). Nevertheless, an adequate increase of ACTH is a prerequisite for an appropriate response to stress.

Naturally the metyrapone test was performed only in healthy PWS children, and no signs of shock were present during the test. Despite the absence of acute illness, blood pressure was low in the early morning hours in all patients, as previously reported (20) and correlated significantly with serum cortisol levels. The early-morning hours might be the critical period during which PWS patients with CAI are at risk of dying. This hypothesis is in line with the fact that most PWS patients with mild infections (mostly URTIs) die during the early-morning hours (9).

Interestingly, Stevenson *et al.* (12) reported autopsies in four children in whom the condition rapidly worsened after mild or moderate infections. Adrenal weight was low in one and severely low in the other three patients. Other reports also show low adrenal weight in deceased patients (13). Adrenal size is related to the cause and duration of the various disease states leading to adrenal insufficiency (28).

In 11 of 15 children with CAI (ACTH < 33 pmol/liter), enough blood was available for measurement of 11-deoxycortisol levels. Of these 11 children with CAI, seven had 11-deoxycortisol levels below the classical cutoff level of 200 nmol/liter (16, 17, 19, 21), suggesting that these children have CAI with adrenal atrophy. Four children had CAI with 11-deoxycortisol levels above 200 nmol/liter, suggesting that these patients have partial or no adrenal atrophy. This is in line with reports showing that some but not all patients who died after mild or moderate infections had low adrenal weight during autopsy (12, 13).

All patients received GH treatment. We do not believe that this influenced the outcome of our study. Data on relationships between the GH-IGF-I system and the HPA axis are contradictory (29, 30). The prevalence of CAI is not increased in GH-

treated children with isolated GH deficiency, Turner syndrome, or other disorders (29). l'Allemand *et al.* (31) reported a decrease in random morning cortisol levels at 12 and 42 months of GH treatment. However, as stated by the authors, their study was not designed to examine the effects of GH treatment on the HPA axis, and the statistical power for such an analysis was too low. Importantly, all cortisol levels remained within the normal range, as was also shown by our normal diurnal salivary cortisol profiles during health. l'Allemand *et al.* (31) found no correlation between IGF-I and cortisol levels. Interestingly, there was a tendency toward a negative correlation of cortisol levels with fat mass ($r = 0.48$, $P = 0.082$). Because GH treatment is known to reduce fat mass in PWS patients (15, 32), GH could indirectly increase cortisol levels. Possibly in patients with PWS, any direct negative effect of GH and/or IGF-I on cortisol levels may be outweighed by the positive effect of the reduction of fat mass on cortisol levels. This may explain why a correlation between GH/IGF-I and cortisol was not found.

Moreover, the similar death rate in PWS patient with and without GH treatment (9) suggests an intrinsic rather than an extrinsic cause of CAI.

Morning salivary cortisol levels were normal in all PWS children, as has been previously reported (31), indicating that CAI occurs only during stressful conditions (17). Therefore, diurnal salivary cortisol profiles are not useful in identifying children at risk. In our study, salivary cortisol levels at wake-up (≥ 12 nmol/liter) provided a sensitivity of 100%, but only one patient met this criterion.

Based on our data, 60% of PWS patients worldwide may be at risk of CAI. There are two possibilities for the therapeutical approach to this problem. The first option is that all PWS patients undergo a metyrapone test and should, in case of an impaired ACTH response, receive hydrocortisone treatment during stressful conditions. However, PWS patients suffer from (hypothalamic) hormonal insufficiencies of varying severity, not only between patients (33), but also within patients (34). In non-PWS patients, CAI may become more severe over time (19). Due to these inconsistencies, even an appropriate ACTH response during a single metyrapone test will not rule out future development of CAI. More research needs to be performed on this matter.

The second option is to treat all PWS patients with hydrocortisone during stress. In our study, 40% of PWS patients would have been treated unnecessarily. However, the prevalence of CAI, a life-threatening illness, is very high in PWS patients (60%). During moderate stress, patients should take hydrocortisone capsules, in total 30–50 mg/m²·d in two to four times. During severe stress, parents should always contact a pediatrician or pediatric-endocrinologist, and a higher dose of hydrocortisone should be administered (in total 75 mg/m²·d in two to four times). For young children, suppositories are available. If the route of administration is compromised and/or during severe stress, the physician may decide to administer hydrocortisone im or iv. PWS patients show few signs of illness. Often they cannot vomit and do not develop fever. The severity of stress can therefore not be read from these classical symptoms. In our experience, parents of children with PWS often know how to classify the severity of stress of their child (mild, moderate, or severe). To

guard the safety of PWS patients, a good collaboration between parents and physicians is crucial.

In our opinion, PWS patients should be considered to have CAI during stress until proven otherwise with a metyrapone test.

Conclusion

Strikingly, 60% of our PWS patients have CAI. We expect that the combination of an insufficient ACTH response during stress with an increased number of sleep apneas during illness, in the presence of reduced clinical symptoms, might lead to an increased risk of sudden death in PWS patients. More research is required, but at this moment, it is important to consider hydrocortisone treatment for PWS patients during stressful conditions, including mild URTIs.

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Address all correspondence and requests for reprints to: Roderick de Lind van Wijngaarden, Clinical Research Fellow, Dutch Growth Research Foundation and Erasmus University Medical Center/Sophia Children's Hospital, Westzeedijk 106, 3016 AH Rotterdam, The Netherlands. E-mail: r.delindvanwijngaarden@erasmusmc.nl, r.delind@kindengroei.nl.

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