0031-3998/05/5703-0412
PEDIATRIC RESEARCH
Vol. 57, No. 3, 2005
Printed in U.S.A.

Assessing Cortisol Production in Preterm Infants: Do Not Dispose of the Nappies

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ABSTRACT

The aim of this study was to develop a practical approach allowing a reliable and noninvasive assessment of cortisol production rates in premature infants. To measure daily urinary excretion rates of glucocorticoids, we developed a procedure using a hydraulic compression method to collect urine from cellulose nappies (diapers). Glucocorticoid metabolites were profiled by quantitative gas chromatography–mass spectrometry. Recovery of steroids after the process of hydraulic extraction from the nappy was approximately 100%. Consecutively, urinary excretion rates of glucocorticoids could be determined in nine healthy preterm infants. The median urinary excretion rate of glucocorticoids increased significantly during the first 5 d of life and remained between 566 μg/kg/d at d 5 and 302 μg/kg/d at 4 wk of age. However, this increase of urinary excretion rates of glucocorticoids in the first days of life was no longer significant when corrected for creatinine excretion. When calculated per square meter body surface area, the median urinary excretion rates of glucocorticoids were 5.1, 4.2, 4.1, and 3.7 mg/m^2/d on d 5, and at wk, 2, 3, and 4, respectively. Urinary excretion rates of glucocorticoids constitute approximately 70% of the natural cortisol production rate as determined by stable isotope dilution technique in older children. Additionally, low cortisol production was detected in two of five preterm infants with arterial hypotension requiring treatment with catecholamines. In conclusion, 24-h urine collection using disposable nappies in combination with gas chromatography–mass spectrometry steroid profiling proved to be a reliable, noninvasive, nonstressful procedure to assess cortisol production and metabolism in premature infants. **(*Pediatr Res* 57: 412–418, 2005)**

Abbreviation

GC-MS, gas chromatography–mass spectrometry

The important question, whether early adrenal insufficiency—possibly associated with increased pulmonary and circulatory morbidity—might be present in premature babies, has not yet been answered (1–3). Studies addressing this problem have been based on single plasma cortisol determinations by immunoassays. However, a single plasma cortisol concentration does not present a timely integral parameter and therefore does not allow assessment of the level of the cortisol production rate. Furthermore, steroid determinations by immunoassays can be associated with severe problems arising from cross-reactivity of steroids, especially in premature infants. This is due to the peculiarity of fetal and neonatal steroid metabolism: large amounts of potentially cross-reactive steroid sulfates retaining a 3β-hydroxy-5-ene structure are produced in the unique fetal zone of the adrenals (4). As a result, no commercially available cortisol immunoassays have been developed that are appropriate for use in measuring steroids in plasma from premature infants or neonates.

A suitable scientific method allowing assessment of cortisol production rates in premature infants is still lacking. Such a procedure should ideally comprise measurement of the correct metabolites of glucocorticoid metabolism and should, for ethical reasons, be as noninvasive and gentle as possible with respect to the fragile premature infant.

Steroid determination in 24-h urinary specimens enables noninvasive assessment of hormonal production rates (5,6). However, such an approach primarily requires a reliable method of collecting 24-h urinary specimens, a challenging task with regard to premature babies. A second hurdle consists in the premature baby's metabolism of cortisol, which is very different from the metabolism during the later periods of life. In particular, highly polar metabolites bearing additional hydroxyl groups at positions 1β and 6α are produced. Furthermore, the high activity of 11β-hydroxysteroid dehydrogenase in many tissues of the fetus and neonate lead to a dominance of 11-keto over 11β-hydroxy compounds (7,8). A third hindrance

Received January 5, 2004; accepted August 16, 2004.
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Supported by a grant (HE 3557/1–1) of the Deutsche Forschungsgemeinschaft to M.H. and S.A.W.

DOI: 10.1203/01.PDR.0000153947.51642.C1

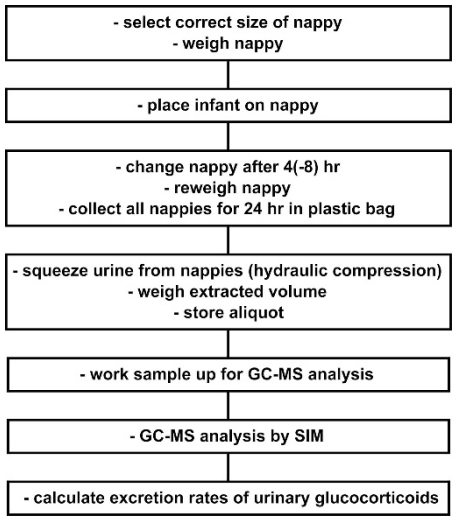

Figure 1. Assessment of glucocorticoid excretion rates in premature infants: flowchart of the procedure.

is the choice of the correct analytical method. Apart from the problem arising from cross-reactivity, there is no commercially available immunoassay for measuring these typical neonatal steroids.

The aim of this study was to develop a practical approach allowing reliable and noninvasive assessment of cortisol production rates in premature infants. To measure urinary excretion rates of glucocorticoids, we developed a special method for careful collection of 24-h urinary specimens in premature infants. To circumvent analytical problems arising from immunoassay detection, we chose the highly specific and nonselective analytical technique of GC-MS for profiling of urinary glucocorticoid metabolites in premature infants.

METHODS

The procedure of assessment of urinary excretion rates of glucocorticoids in premature infants is depicted in Figure 1.

Urine collection procedure. Urine was collected for 24 h using disposable nappies (diapers) (9,10). We used two sizes of disposable nappies (Pampers, Procter & Gamble, Schwalbach, Germany). The small size derived from a serial production, which was used for infants weighing <2.3 kg (Pampers P). For infants weighing 2.3–6 kg, medium-sized nappies were manufactured exclusively for us. The nappies were composed of pure cellulose.

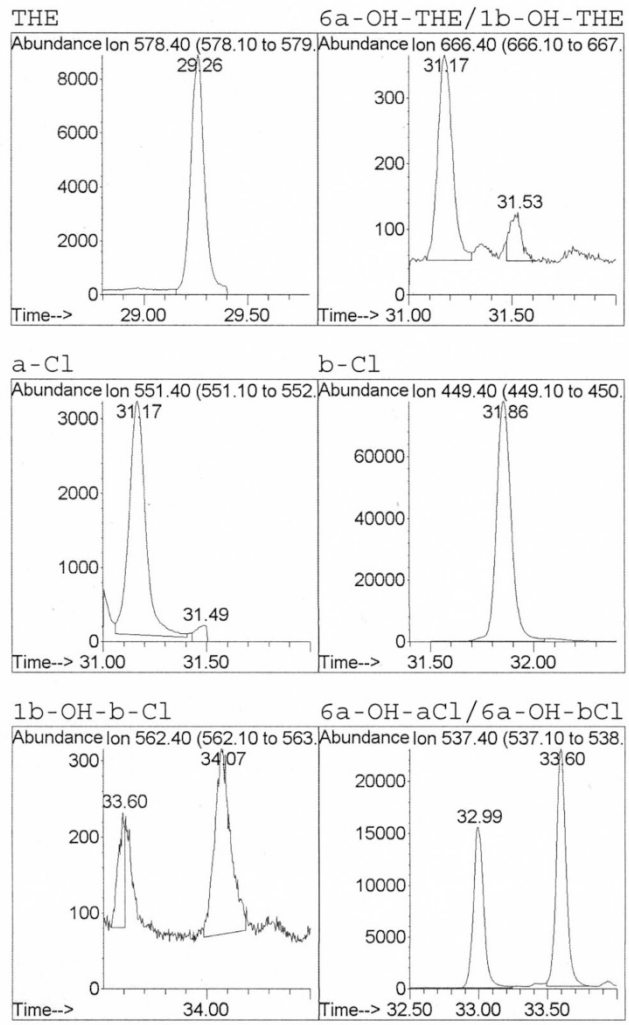

Figure 2. Portions of ion chromatograms of a urinary steroid profile of a premature infant (26 wk of gestation; birth weight, 1100 g) on d 3 of life. The steroid profile was recorded with the mass spectrometer run in the selected ion monitoring (SIM) mode (Agilent 6890 series GC coupled with an Agilent 5973 mass selective detector). The peaks at typical retention times (t_r) represent the following compounds: THE (m/z 578.40, t_r 29.26 min), 6α-OH-THE (m/z 666.40, t_r 31.17 min), 1β-OH-THE (m/z 666.40, t_r 31.53 min), α-CL (m/z 551.40, t_r 31.17 min), β-CL (m/z 449.40, t_r 31.86 min), 1β-OH-β-CL (m/z 562.40, t_r 34.07 min), 6α-OH-α-CL (m/z 537.40, t_r 32.99 min), and 6α-OH-β-CL (m/z 537.40, t_r 33.60 min).

Table 1. *Evaluation of the urine collection procedure I: recovery of urinary volumes and total urinary glucocorticoid concentrations (sum of glucocorticoid metabolites) after hydraulic compression of small and medium nappies*

Pool urine (control specimens)		Urine recovered from nappies			
Volume (mL)	Total glucocorticoids (μg)	Total volume recovered (mL)	Relative volume recovery (%)	Total glucocorticoid content (μg)	Relative glucocorticoid recovery (%)
Small nappies (n = 5 for each different volume)					
15	79 ± 1	5.6 ± 1.6	37	82 ± 4	104
30	158 ± 2	17.6 ± 3.1	59	167 ± 9	105
45	237 ± 3	32.6 ± 1.0	72	256 ± 11	108
Medium nappies (n = 5 for each different volume)					
30	158 ± 2	15.8 ± 1.0	53	164 ± 7	104
50	264 ± 3	36.4 ± 2.2	73	282 ± 8	107
70	369 ± 4	55.2 ± 2.5	79	398 ± 12	108

To correct for the difference between inoculated and extracted volume, amounts of recovered steroids were calculated by multiplying the concentration of the steroid with the respective inoculated volumes. Data are given as mean ± SD.

Preterm infants were placed on a nappy of the appropriate size. Nappies were closed properly to limit evaporation, even when the infant underwent phototherapy. It was shown that phototherapy was associated with slight but significant changes in evaporation (11). The common interval for changing the nappies was 4 h. However, to be able to collect a sufficient volume of urine for steroid analysis in the smallest infants, the nappies were changed every 8 h. Weighing the nappies before and after urine collections allowed exact calculation of 24-h urine output (10). To reduce contamination with meconium or stool to a minimum, a thin gauze was placed between the baby's skin and the surface of the inner side of the nappy, thus, allowing urine to pass through the gauze and, at the same time, withholding meconium or stool, which then could easily be separated from the nappy. Samples from infants with diarrhea were omitted.

Nappies were collected in a plastic bag at room temperature until completion of a 24-h collection period and then stored at −20°C. At weekly intervals, nappies were defrosted, folded inside out, and placed in a plastic bag (Melitta, Minden, Germany) before extraction of the urine using a specially constructed hydraulic press applying a maximum of 120 kPa/cm^2. The extracted urine from a 24-h urine collection was pooled. After centrifugation, the collected urinary specimens were stored at −80°C until analysis by GC-MS was performed (Fig. 1).

To calculate the 24-h excretion rates of urinary glucocorticoids, the amount of urine that could not be completely squeezed out of the nappy, had to be taken into account. Thus, steroid concentrations were adjusted for the 24-h urine output, which was determined by weighing the nappies.

Laboratory analyses. Urinary steroid profiles were determined using quantitative data produced by GC-MS analysis according to the method of Shackleton and colleagues (12,13). In brief, free and conjugated urinary steroids were extracted by solid phase extraction (Sep-Pak C18 cartridges; Waters, Milford, MA) from a 5-mL aliquot of a 24-h urinary specimen. The conjugates were enzymatically hydrolyzed (type H-1 sulfatase from helix pomatia; Sigma-Aldrich Chemie GmbH, Taufkirchen bei München, Germany), followed by recovery of the hydrolyzed steroids by a second solid phase extraction step. Known amounts of three internal standards (5α-androstane-3α, 17α-diol; stigmasterol; and cholesteryl butyrate) were added to a portion of each extract before formation of methyloxime-trimethylsilyl ethers. The gas chromatograph (6890 series GC; Agilent Technologies GmbH, Böblingen, Germany), housing a OV-1 fused silica column (Optima-1-MS, 25 m × 0.2 mm; film 0.1 μm; Macherey-Nagel, Düren, Germany) and equipped with an Agilent 7683 series injector, was directly interfaced to a mass selective detector (Agilent 5973N MSD) operated in selected ion monitoring mode. Helium was used as carrier gas. The injections took place with the GC oven at 80°C for 2 min; the temperature was gradually increased by 20°C/min up to 190°C (1 min). For separation of steroids, the temperature was increased by 2.5°C/min to 272°C.

The following C21 steroids, all cortisol metabolites, were determined by selected ion monitoring: tetrahydrocortisone (THE; 5β-pregnan-3α,17α,21-triol-11,20-dione; m/z 578); α-cortolone (α-CL; 5β-pregnan-3α,17α,20α, 21-tetrol-11-one; m/z 551); β-cortolone (β-CL; 5β-pregnan-3α,17α,20β, 21-tetrol-11-one; m/z 449); 6α-OH-tetrahydrocortisone (6α-OH-THE; 5β-pregnan-3α,6α,17α,21-tetrol-11,20-dione; m/z 666); 1β-OH-tetrahydrocortisone (1β-OH-THE; 5β-pregnan-1β,3α,17α,21-tretrol-11,20-dione; m/z 666); 6α-OH-α-cortolone (6α-OH-α-CL; 5β-pregnan-3α,6α,17α,20α,21-pentol-11-one; m/z 537); 6α-OH-β-cortolone (6α-OH-β-CL; 5β-pregnan-3α,6α,17α,20β,21-pentol-11-one; m/z 537); 1β-OH-β-cortolone (1β-OH-β-CL; 5β-pregnan-1β,3α,17α,20β,21-pentol-11-one; m/z 562); tetrahydrocortisol (THF; 5β-pregnan-3α,11β,17α,21-tetrol-20-one; m/z 652); allotetrahydrocortisol (5α-pregnan-3α,11β,17α,21-tetrol-20-one; m/z 652); α-cortol (α-C; 5β-pregnan-3α,11β ,17α,20α,21-pentol; m/z 523); β-cortol (β-C; 5β-pregnan-3α,11β,17α, 20β,21-pentol; m/z 523); cortisol (F; 4-pregnene-11β,17α,21-triol-3,20-dione; m/z 605); and 6β-OH-cortisol (6β-OH-F; 4-pregnene-6β,11β,17α,21-tetrol-3,20-dione; m/z 693). In addition to these target ions, a second ion (qualifier ion) was monitored for each analyte to ensure specificity. Calibration plots showed excellent linearity (r^2 = 0.982–1.000). To assess overall cortisol secretion, the 14 major urinary glucocorticoid metabolites were quantified (peak area integration) and summed. As usually done with GC-MS steroid analysis, single measurements were performed for each urine sample. Intra-assay precision (n = 8) varied between 1.3% (for 6α-OH-β-CL) and 7.0% (for 1β-OH-β-CL), and interassay precision (n = 6) varied between 0.5% (for THE) and 5.3% (for 1β-OH-β-CL). Creatinine was measured by the Jaffé method. Daily urinary excretion rates of glucocorticoids were corrected for body weight and per micromole creatinine.

Evaluation of urine collection procedure. Pooled neonatal urine was used for testing our urine collection procedure. To simulate a normal 24-h collection procedure, the nappies were inoculated with aliquots of pooled urine and stored for 36 h in plastic bags at room temperature; small nappies were inoculated with aliquots of 15, 30, and 45 mL of urine and medium nappies with 30, 50, and 70 mL, respectively. Each nappy was weighed before and after inoculation with urine. After incubation, nappies were stored at −20°C until urine was extracted as mentioned above.

Determination of steroid excretion rates in preterm infants. Nine preterm infants (five females) with a median gestational age of 28.7 wk (range, 25.0–30.9) and a median birth weight of 1080 g (range, 640–1690) were investigated. Six of nine infants were delivered by cesarean section. All infants were classified as being healthy according to recently described criteria (14). They had no signs of infection and did not receive treatment with surfactant or inotropes. A postnatal steroid therapy was an exclusion criterion. All mothers received a complete course of prenatal betamethasone therapy. Twenty-four-hour urine collections were made on d 1, 2, 3, and 5 of life and at weekly intervals thereafter up to the age of 4 wk.

Additionally, steroid excretion rates of five preterm infants with arterial hypotension as a potential sign of adrenal insufficiency were determined. The Score for Neonatal Acute Physiology (SNAP) served as an indicator of the severity of illness (15).

Table 2. *Evaluation of urine collection procedure II: relative recovery of individual urinary glucocorticoid metabolites after extraction from pure cellulose nappies*

	Small nappies, volume incubated (mL)			Medium nappies, volume incubated (mL)		
	15 (n = 5)	30 (n = 5)	45 (n = 5)	30 (n = 5)	50 (n = 5)	70 (n = 5)
Recovery of 11-hydroxy-metabolites (%)						
F	85	93	97	90	97	101
6β-OH-F	59	70	90	66	70	93
THF	112	112	112	101	99	101
αTHF	115	117	119	100	102	104
β-C	104	101	109	96	101	102
Total 11-hydroxy-metabolites	90	96	104	87	91	100
Recovery of 11-keto-metabolites (%)						
THE	101	100	105	97	99	101
1β-OH-THE	108	109	121	109	107	108
6α-OH-THE	115	116	118	111	111	112
α-CL	110	112	116	103	115	111
6α-OH-α-CL	108	109	110	107	111	112
β-CL	109	109	110	102	107	104
1β-OH-β-CL	93	99	105	96	105	102
6α-OH-β-CL	107	110	106	114	118	114
Total 11-keto-metabolites	107	107	109	107	111	110

Various sizes of nappies were employed and different amounts of aliquots of pool urine were used for incubation. To correct for the difference between inoculated and extracted volume, amounts of recovered steroids were calculated by multiplying the concentration of the steroid with the respective inoculated volumes. Then, the recovery rate of the individual steroid is presented as percentage of the inoculated amount of the metabolite.

The parents gave written informed consent. The study was approved by the local ethics committee.

Statistics. All data were analyzed in the Institute of Medical Statistics using the SAS V8 statistical package (SAS Institute Inc., Cary, NC). Intragroup changes in excretion rates of urinary glucocorticoids were compared over time by paired Wilcoxon test.

RESULTS

Evaluation of the urine collection procedure. Urinary volumes and amounts of total glucocorticoid metabolites recovered after hydraulic compression of nappies are given in Table 1. The amounts of total glucocorticoid metabolites in native urine were compared with those determined in urine recovered from nappies. For the correction of the difference between inoculated and extracted volume, amounts of recovered steroids were calculated by multiplying the concentration of the steroid with the respective inoculated volumes.

Fourteen urinary glucocorticoid metabolites were identified by GC-MS. Portions of a typical urinary steroid profile (selected ion monitoring data) of a preterm infant of 25 wk gestational age are shown in Figure 2.

Relative recovery rates of individual glucocorticoid metabolites after extraction from nappies are given in Table 2. Recovery rates were excellent with a mean (SD) of 105% (12.3%). No relevant influences were noted with regard to nappy size and urine volume.

Determination of steroid excretion rates in preterm infants. Results of urinary excretion rates of glucocorticoids in healthy preterm infants as a function of postnatal age are shown in Figure 3. When calculated per kilogram body weight, the median urinary excretion rates of glucocorticoids increased during the first 5 d of life ($p < 0.015$; Fig. 3*A*). Thereafter, there was no change in urinary excretion rates of glucocorticoids.

When calculated per micromole creatinine, the progression of the curve was smoothed and only a slight, nonsignificant increase of the urinary excretion rates of glucocorticoids from d 1 to d 3 was noted (Fig. 3*B*). The following urinary excretion rates of glucocorticoids corrected for creatinine declined at d 3 from 5.3 μg/kg/d per micromole creatinine to 2.8 μg/kg/d per micromole creatinine at wk 4 ($p < 0.02$).

When calculated per square meter of body surface area, the median urinary excretion rates of glucocorticoids were 5.1, 4.2, 4.1, and 3.7 mg/m^2/d at d 5 and wk 2, 3, and 4, respectively.

Table 3 shows the excretion rates of individual glucocorticoid metabolites to describe their contribution to total glucocorticoid excretion. 11-keto-metabolites (cortisone metabolites) represented 80–96% of the total glucocorticoids excreted.

Finally, the urinary excretion rates of glucocorticoids were determined in five preterm infants with arterial hypotension requiring treatment with catecholamines. The severity of illness was high during the first week of life, as indicated by high SNAP in all five infants (Fig. 4*A*). In two of the five infants with hypotension and severe illness, the urinary excretion rates of glucocorticoids did not increase (Fig. 4*B*). Table 4 shows the clinical characteristics of these infants.

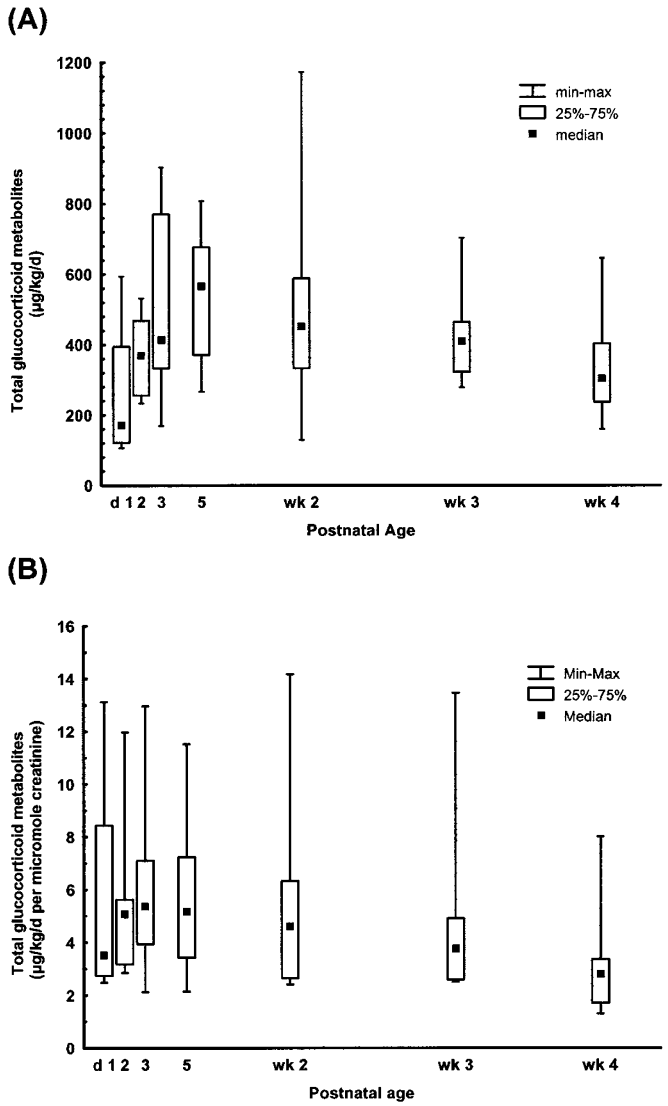

Figure 3. (*A*) Changes in urinary excretion rates of total glucocorticoid metabolites in healthy preterm infants ($n = 9$) as a function of postnatal age. The increase during the first 5 d of life was significant ($p < 0.015$). (*B*) Excretion rates corrected per micromole creatinine to take immediately postnatal changes of renal function into account. There was a significant decline from the d 3 to wk 4 ($p < 0.02$).

DISCUSSION

When trying to assess cortisol production by measuring urinary excretion rates of glucocorticoids, a reliable method ensuring complete collection of urine is of crucial importance. The use of adhesive plastic collection bags on the perineum is problematic for several reasons. First, removing the bag from the perineum can result in abrasions and tears in the extremely vulnerable skin of the premature infant. Secondly, especially in female neonates, it is not easy and always reliable to achieve a complete urine collection, due to leakage of the system. Alternatively, a bed can be used, wherein the baby is placed on a nylon sheet through which urine can pass and be channeled to a collection point (16,17). The disadvantages of this system include skin care in immobilized, extremely ill infants, and impracticality in more mobile older infants. Bladder catheterization is invasive and not acceptable for study reasons.

Table 3. *Twenty-four-hour urinary excretion rates of individual glucocorticoid metabolites (μg/kg/d) in healthy preterm infants (n = 9)*

	d 1	d 2	d 3	d 5	wk 2	wk 3	wk 4
11-hydroxy-metabolites							
F	10.6 (5.1–53.9)	10 (6.7–102.8)	12.4 (4.5–192.2)	9.9 (4.5–47.4)	5.8 (0–19.8)	5.5 (2.3–12.3)	4.8 (4–10.1)
6β-OH-F	8.3 (0–61.5)	17.8 (0–47.5)	18.3 (0–65.6)	19.7 (0–167.2)	12.4 (0–99.4)	11.4 (0–16.3)	6.7 (0–15.2)
THF	1.7 (0–11.8)	3.2 (0–7.9)	2.2 (0–6.4)	0 (0–7.2)	3 (0–7.9)	2.3 (0–3.5)	2.5 (0–6.4)
αTHF	0 (0–4.5)	1.3 (0–5.3)	0 (0–5.7)	0 (0–6.7)	0 (0–3.4)	0 (0–2.3)	0 (0–9.1)
α-C	0 (0–1.3)	0 (0–2.1)	0 (0–1.4)	0 (0–1.3)	0 (0–1.5)	0 (0–1.3)	0 (0–0.4)
β-C	0.2 (0–6.1)	0 (0–5.3)	0 (0–9.8)	0 (0–5.1)	0	0	0 (0–1.8)
11-keto-metabolites							
THE	25.4 (13.2–75.8)	36.8 (18.1–62.3)	37.5 (26.6–73.9)	52.6 (15.7–110.8)	50.2 (19.4–118.3)	66.2 (47–107.6)	51.6 (33.2–174.1)
1β-OH-THE	0.3 (0–5.1)	0.9 (0–3.4)	1.1 (0–4.3)	2.4 (0–4.3)	2.2 (1.1–5.3)	2 (1.1–4.5)	2.1 (1.1–4.2)
6α-OH-THE	13.2 (3.5–43.3)	14.1 (3–69.9)	17.2 (2.7–60.2)	36 (5.8–59.9)	60 (17.4–107.7)	52.6 (24–138)	47.1 (25.3–150.1)
α-CL	20.4 (0–48.2)	19.9 (0–33.7)	18.7 (0–64.3)	11.5 (4.9–36.4)	6.5 (3.6–17.6)	3.5 (0–6.9)	3.4 (0–8)
6α-OH-α-CL	21.6 (9.7–119.9)	42.6 (13.2–134.3)	43.1 (7.9–248.2)	44.6 (16.3–188.8)	32 (7.9–160.6)	35.7 (21.3–95.2)	30.5 (11.8–57.2)
β-CL	6.5 (1.8–90.8)	8.3 (3.5–118.4)	10.6 (2.3–167)	14.7 (6–144.9)	9.5 (2–37.6)	13.5 (7.2–22.2)	15.8 (3.2–31.9)
1β-OH-β-CL	0.8 (0–3.8)	2.2 (1.5–5.3)	4.5 (1.6–5.3)	4.3 (0–6.9)	6.0 (0–24.5)	5.9 (0–10.2)	4.4 (0–8.2)
6α-OH-β-CL	74.9 (27.6–250.2)	131.7 (108.1–196.2)	224 (75.6–307.4)	230.6 (114.8–436)	258.7 (69.3–623.1)	232.1 (125.9–326.3)	122.4 (71.6–211.9)

Data are given as median (minimum–maximum). Individual steroids were quantified by GC-MS in a pooled 24-h urine sample and excretion rates were calculated by adjusting steroid concentration to the 24-h urine output, which was determined by weighing the nappies of a 24-h collection period.

In contrast, collecting urine samples from compressible nappies, taking care to minimize evaporation (11) and correcting for losses by weighing is a suitable alternative for measuring accurately 24-h urine output and determining several constituents of urine (9,10). This method does not interfere with the nursing care and is completely noninvasive and nonstressful. A nonstressful approach is necessary when assessing the function of the permanent adrenal zone, as it has been shown in healthy term infants that plasma cortisol rose up to 11-fold half an hour after a simple venous puncture (18). Furthermore, this method allows urine collection over long periods of time, making longitudinal studies possible for delineation of the change from the neonatal to the adult pattern of cortisol metabolism.

Evaluation of our urine collection procedure showed that application of a hydraulic press allowed sufficient recovery, even when nappies were incubated with small volumes of urine (Table 1). This is of particular importance, especially during the first days of life of the extremely low birth weight infant when urine output is low. The amounts of steroids determined in the urinary specimens recovered after hydraulic pressing of nappies reveal that no relevant bacterial decomposition or absorption of steroids occurs in the filling material of the nappies used. In fact, we observed a negligible "concentration effect" (Table 1), explaining recovery rates slightly more than 100%, which perhaps is due to evaporation or absorption of water by the nappy. We conclude from the high recovery rate of the individual steroids that hardly any absorption or degradation of steroids occurred in case when the nappies were left at room temperature for 36 h (Table 2).

The nappies used in this study were composed of pure cellulose, whereas the commonly used ultra-absorbent types of nappies were unsuitable for squeezing, because extracted samples always contained the gel-like material of the nappy. Our results demonstrate that glucocorticoid metabolites deriving from the adrenal permanent zone can be identified and quantified by GC-MS from urine collection using disposable nappies with a high recovery rate.

Nonselective multicomponent methods such as gas chromatography or GC-MS are required for profiling steroids in complex steroids mixtures like neonatal urine. The use of GC-MS permits the highest specificity due to its capacity for providing structural information of the analytes. Urinary steroid profiling by GC-MS is a nonselective procedure of highest diagnostic potential concerning delineation of disorders of human steroid metabolism (19). The high specificity of our GC-MS method can be assessed by the clear shapes of the chromatographic peaks (Fig. 2). In contrast to studies in which quantification of neonatal urinary glucocorticoids relied on gas chromatography, determination by GC-MS and selected ion monitoring did not require separation of steroid sulphates from the free and glucuronide conjugates by chromatography with Sephadex LH-20 columns, a time-consuming, laborious, and expensive preparative step (13). The panel of our analytes comprised all major neonatal neutral cortisol metabolites that were either excreted free or as glucuronides, carrying additional hydroxyl groups at positions 1β and 6α. We furthermore included all major representative metabolites of the adult pattern of cortisol catabolism. Unlike studies based on quantitative gas chromatography (20), we were able to further determine 11-hydroxy-steroids such as THF and 5α-THF, although in minor concentrations, in preterm infants after birth.

In our cohort of healthy preterm infants, the median urinary excretion rates of glucocorticoids tripled during the first 5 d of life. This may be explained by the increase in glomerular filtration rates directly after birth. Because the immediate postnatal period is of great interest regarding adrenal function in the premature infant, renal function has to be taken into account. Therefore, glucocorticoid excretion rates were corrected for creatinine values. It has already been demonstrated that the determination of creatinine in urinary samples from extracted nappies is reliable and accurate (9,10). Creatinine excretion rates in our population were in the previously reported range for premature infants (21). In our case, excretion rates corrected for creatinine increased only by 50% during the first 5 d of life, and thus were not significant. We speculate that glucocorticoid excretion rates corrected for creatinine better reflect adrenal function in the immediate postnatal period of the premature infant.

The median excretion rates of glucocorticoid metabolites in our infants ranged between 170 and 566 μg/kg/d and were 4 to

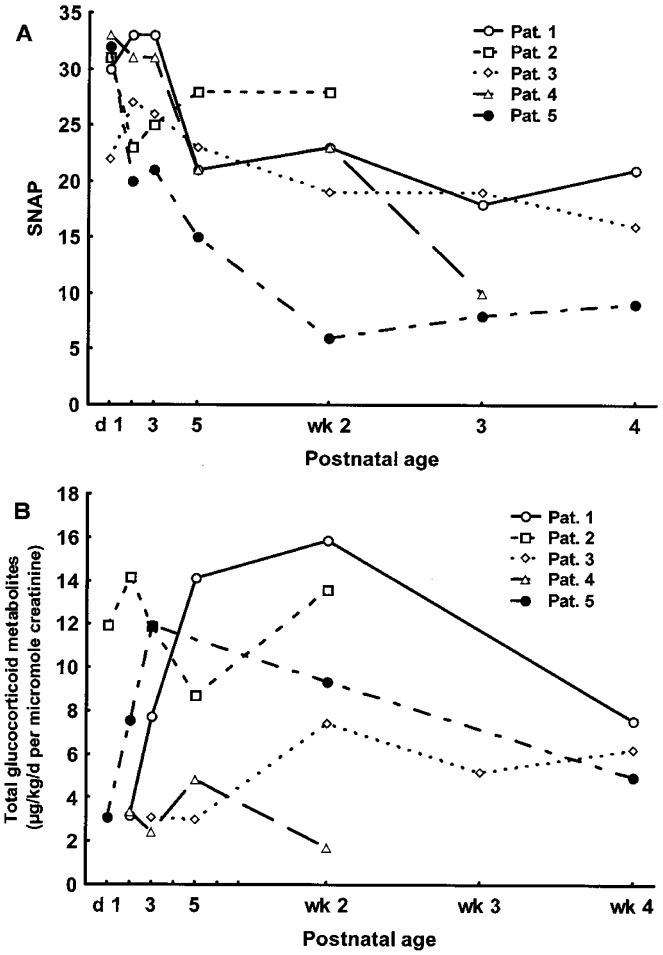

Figure 4. Severity of illness measured by the Score for Neonatal Acute Physiology (SNAP) (*A*) and urinary excretion rates of glucocorticoids as a function of postnatal age in 5 preterm infants with arterial hypotension requiring treatment with catecholamines (*B*). Excretion rates were corrected per micromole creatinine.

6 times higher than those reported by Midgley *et al.* (20). It has been shown that determination of total glucocorticoid metabolites in 24-h urinary specimens reflects 75% of cortisol production rates in adults (5). It has also been convincingly shown that the urinary excretion rate of free cortisol does not correlate with cortisol production rate (22,23).

Cortisol production rates were reported for moderately premature infants (6.6–8.8 mg/m^2/d) using deconvolution analysis in plasma (24) and for older children (6.8 ± 1.9 mg/m^2/d) (23) or adults (7.7–11.4 mg/m^2/d) (5) using a stable isotope dilution technique. The median urinary excretion rates of glucocorticoids in our infants reached up to 70% of these reported cortisol production rates.

Using our method in ill preterm infants, low cortisol production was detected in two of five infants with arterial hypotension requiring treatment with catecholamines. Hypotension in these two male neonates might be a symptom of adrenal insufficiency. In contrast, there was an increase in cortisol production in the three remaining female infants during severe illness. The number of preterm infants investigated in our study was too small to draw any conclusions regarding gender differences in cortisol production in preterm infants. These preliminary data warrant further evaluation in a greater number of infants, because several other factors might influence the adrenal response like gestational age, administration of prenatal steroids, mode of birth, labor, and the nature of neonatal disease. However, our findings indicate that adrenal insufficiency may be not a unique finding in the group of ill preterm infants and steroid treatment may require an individual approach.

In conclusion, 24-h urine collection using disposable nappies in combination with GC-MS steroid profiling proved to be a reliable, noninvasive, and nonstressful procedure in premature infants. We suggest its application as a tool to assess cortisol production and metabolism in these babies.

Acknowledgments. The authors thank Professor Dr. F. Manz (Research Institute of Child Nutrition, Dortmund, Germany), who provided the hydraulic press; Professor Dr. J Homoki (Department of Pediatrics, University of Ulm, Germany) for providing reference steroids; Dr. M. Lazaro for editing the manuscript; and Procter & Gamble for providing the medium-sized nappies.

Table 4. *Clinical characteristics of five preterm infants with arterial hypotension as a potential sign of adrenal insufficiency*

	Patient no.				
	1	2	3	4	5
Gestational age (wk)	27.6	25.1	25.9	25.9	28.3
Birth weight (g)	1120	700	850	700	1100
Gender, male			x	x	
Prenatal steroids (complete course)					x
Prenatal steroids (incomplete course)		x	x	x	
Cesarean section	x	x	x	x	x
Umbilical cord arterial pH	7.33	7.12	7.39	7.26	7.37
Apgar scores at 5 min	8	7	8	5	9
RDS treated with surfactant	x	x	x	x	x
PDA requiring treatment	x	x			x
Intraventricular hemorrhage > II°	x	x		x	x
NEC	x		x		
Infection at birth	x		x	x	x
Death before discharge	x	x	x		

RDS, respiratory distress syndrome; PDA, patent ductus arteriosus; NEC, necrotizing enterocolitis.

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