CLINICAL STUDY

Impaired adrenocortical function in very low birth weight infants after multiple pregnancies

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Abstract

Objective: To evaluate the differences in adrenal function between very low birth weight (VLBW) infants from singleton and multiple pregnancies.

Design and methods: Forty infants of birth weights less than 1500 g underwent an ACTH test. Thirty infants born from singleton pregnancies (singleton group) and ten born from multiple pregnancies (multiple group) were enrolled. A baseline blood sample was drawn for cortisol measurement and thereafter serum cortisol was measured 1 and 2 h after an i.v. injection of ACTH.

Results: In multiple pregnancies, the median basal cortisol level of the infants was significantly lower than that in the singletons. The median cortisol level at 1 and 2 h after administration of ACTH was significantly lower in infants from multiple gestations than in singletons. Of infants from the multiple gestation group six, and of the singleton infants 12, had baseline cortisol levels lower than the reference values ($P = 0.48$). One hour after ACTH stimulation all multiple and 53% of the singleton group infants showed a subnormal (< 500 nmol/l, $P = 0.007$) cortisol response. Two hours after ACTH, nine multiple group patients and 43% of the singletons had subnormal (< 500 nmol/l, $P = 0.01$) stimulated cortisol levels.

Conclusions: We have concluded that VLBW infants from multiple gestations seem to be at an increased risk of insufficient postnatal adrenocortical function. In the future, specific attention should be paid to evaluate further newborn infants from multiple pregnancies with regard to a possible benefit of hydrocortisone substitution in stressful clinical situations.

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Introduction

Adequate hypothalamo–pituitary–adrenal (HPA) axis response to stress is critical for survival. In very low birth weight (VLBW) infants, adrenal insufficiency and inappropriately low basal cortisol levels have been reported, these being connected with risks of hypotension, respiratory distress syndrome (RDS), chronic lung disease, fluid retention and an increased incidence of patent ductus arteriosus (PDA) (1–4). The HPA axis has been shown to be intact as early as 26 weeks of gestation (5), but precise criteria for sufficient adrenal function have not been established. Earlier studies have demonstrated low cortisol values in the first week of life with no difference between sick and well premature infants (5, 6). In contrast, an insufficient adrenal response to stress has been observed in sick ventilated VLBW infants of gestational ages of less than 30 weeks (7), and about 70% of infants of less than 30 weeks of gestational age with severe hypotension had significantly lower cortisol concentrations compared with those in well preterm infants (8).

The condition has been associated with endocrine dysfunction at the adrenal level and corticosteroid treatment in severely hypotensive preterm infants could be life saving (9).

To our knowledge there are no previous studies comparing adrenal function among VLBW infants after singleton and multiple pregnancies, such as have been reported with twin fetal sheep (10). A different development in cortisol response to corticotropin (ACTH) stimulation *in vivo* between twin fetal sheep was suggested as the mechanism responsible for the asynchronous increase in resting cortisol concentration between the twins (10, 11). We sought here to establish differences in adrenal function in VLBW infants between infants from multiple and singleton gestations.

Subjects and methods

Infants admitted to the neonatal intensive care unit at Tampere University Hospital were considered for the study if their birth weight was less than 1500 g and

indwelling access was available for blood sampling. Exclusion criteria included major congenital anomalies and gestational age of less than 24 weeks. The Ethics Committee of Tampere University Hospital had approved the study and written informed consent was obtained from the parents.

Thirty infants born from singleton pregnancies (singleton group) and ten born from multiple pregnancies (multiple group) were enrolled. Infants in the multiple group underwent an ACTH test at a mean (S.D.) 3.8 (1.2) days and those in the singleton group at a mean 3.6 (1.7) days of age ($P = 0.675$). All tests were performed between 0800 and 1200 h via an indwelling catheter. A baseline blood sample was drawn for cortisol measurement and thereafter a standard dose of 0.15 mg/m^2 ($250 \text{ } \mu\text{g}/1.73 \text{ m}^2$; Synacthen, 0.25 mg/ml ; Novartis, Basel, Switzerland) was administered intravenously. Serum cortisol was also measured 1 and 2 h after the injection. Four infants received hydrocortisone with a maximum dosage of 5 mg/kg per day as rescue therapy for severe hypotension during the study, but hydrocortisone was withheld for at least 8 h preceding the tests.

For the evaluation of adrenal function we used reference values according to criteria from Grinspoon & Biller (12), Alkalay *et al.* (13) and Korte *et al.* (14). Cases with basal cortisol values $<138 \text{ nmol/l}$ were regarded as having adrenocortical insufficiency and ill premature patients with basal or stimulated cortisol values $>414 \text{ nmol/l}$ were considered to have adequate adrenocortical function. A peak cortisol value $>500 \text{ nmol/l}$ at any time during the ACTH test in acutely ill adult patients represents normal adrenal function (12–14).

Statistical analyses were performed on the SPSS package, version 10.0 for Windows. The Mann–Whitney U test was used for non-parametric comparisons and differences between the groups in terms of categorical variables were analysed using χ^2 statistics. A P value <0.05 was considered significant. To evaluate factors (antenatal use of steroids, maternal chorionamnionitis, gestational age (week), birth weight (g), small birth weight for gestational age (SGA), sex, assisted ventilation, treatment with surfactant, use of inotropics or multiple vs singleton pregnancy) possibly affecting adrenal cortisol production (sufficient vs insufficient) logistic regression analyses with backward stepwise method were performed.

SGA was determined at a birth weight less than the 10th percentile (The UK 1990 growth reference birth weight centiles (g), Child Growth Foundation 1995). Chorionamnionitis was defined clinically by the occurrence of preterm labor in association with at least two of the following criteria: febrile mother, uterine tenderness, foul-smelling amniotic fluid or fetal or maternal tachycardia. Surfactant was used as rescue therapy for RDS at the discretion of the clinician. Hypotension was defined as a need for inotropics. Intracranial

hemorrhages (IVH) were diagnosed and classified according to Papile *et al.* (15). A significant PDA was diagnosed according to clinical findings and echocardiography.

Results

Characteristics of study infants

The singleton group was comprised of 30 and the multiple group of ten infants, of whom seven were twins and three were triplets from the same pregnancy. The characteristics of the singleton and multiple group infants, including gestational age, birth weight, sex, antenatal use of steroids, mode of delivery, need for surfactant or inotropics, and occurrence of PDA or sepsis were similar, except for statistically significantly more frequent occurrence of maternal chorionamnionitis and a need for assisted ventilation in the singleton in comparison with the multiple group (Table 1).

Basal cortisol levels after birth

The mean (S.D.) basal cortisol level in the twin and triplet patients ($n = 10$) was significantly lower than that of the singletons ($111 (73) \text{ nmol/l}$ vs $230 (220) \text{ nmol/l}$, $P = 0.03$). Six (60%) patients in the multiple and 12 (40%) in the singleton group had insufficient basal cortisol production of less than 138 nmol/l ($P = 0.48$). The mean basal cortisol level was similar in 13 cases with maternal chorionamnionitis and in those ($n = 27$) without ($276 (301)$ vs $161 (114) \text{ nmol/l}$), but significantly higher ($225 (215) \text{ nmol/l}$) among infants who needed ventilator therapy ($n = 31$), compared with those who did not ($n = 9$).

Table 1 Clinical characteristics of the study groups. *Values are presented as means $\pm$ S.D.

	Singleton ($n = 30$)	Multiple ($n = 10$)	<i>P</i> value
Birth weight (g)*	1084 ± 262	1142 ± 174	NS
Gestational age (week)*	28.3 ± 2	29.2 ± 1.5	NS
Male/female	19/11	8/2	NS
Chorionamnionitis (%)	13 (43)	0 (0)	0.016
PROM; $>24 \text{ t}$ (%)	10 (33)	2 (20)	NS
Antenatal use of steroids (%)	25 (83)	9 (90)	NS
Need for surfactant (%)	16 (53)	4 (40)	NS
Mode of delivery:			
Cesarean section (%)	17 (57)	3 (30)	NS
PDA (%)	8 (27)	2 (20)	NS
Hypotension (%)	17 (57)	5 (50)	NS
Ventilation therapy (%)	26 (87)	5 (50)	0.029
Sepsis (culture +) (%)	4 (13)	0 (0)	NS
IVH (%)	9 (30)	2 (20)	NS
SGA	4 (13)	1 (10)	NS

PROM, preterm rupture of membrane; $>24 \text{ t}$, rupture of amniotic membranes more than 24 hours before delivery; culture +, positive bacterial culture. NS, not significant.

Table 2 Cortisol responses before, and 1 and 2 h after ACTH stimulation test. Patients with basal cortisol values <138 nmol/l, and stimulated cortisol values <414 nmol/l or <500 nmol/l were considered to have an inadequate adrenocortical function (see references 12–14).

	No. of infants	Basal cortisol	Stimulated cortisol			
		0 h	1 h		2 h	
		<138 nmol/l (%)	<414 nmol/l (%)	<500 nmol/l (%)	<414 nmol/l (%)	<500 nmol/l (%)
All	40	19 (48)	22 (55)	27 (68)	12 (30)	21 (53)
Single	30	12 (40)	12 (40)	16 (53)	6 (20)	13 (43)
Infants from multiple gestation	10	6 (60)	10 (100)	10 (100)	7 (70)	9 (90)
Chorionamnionitis	13	6 (46)	4 (31)	6 (50)	1 (8)	4 (31)
Need for respiratory treatment	31	12 (39)	17 (55)	17 (55)	11 (35)	14 (45)
Need for inotropics	22	7 (32)	11 (50)	11 (50)	5 (23)	8 (36)

(115 (103) nmol/l, $P = 0.03$). Likewise, among patients who needed inotropics the mean basal cortisol level was significantly higher than among those who did not (254 (246) vs 133 (91) nmol/l, $P = 0.03$ respectively). The percentage of patients whose baseline cortisol level was insufficient did not differ significantly between infants with or without maternal chorionamnionitis (6 (46%) vs 12 (44%), $P = 1.000$), ventilator therapy (12 (39%) vs 6 (67%), $P = 0.27$) or treatment with or without inotropics (7 (32%) vs 11 (61%), $P = 0.103$) (Table 2). Of the covariates identified in the logistic regression analysis, birth weight (odds ratio (OR) 1.004, 95% confidence interval (CI) 1.001; 1.008, $P = 0.014$) and gestational age (OR 1.71, 95% CI 1.07; 2.72, $P = 0.025$) correlated with inadequate basal cortisol production.

Cortisol response after ACTH

The mean cortisol level at 1 h after the administration of ACTH was significantly lower in infants from multiple gestations than in the singletons (243 (106) vs 584 (332) nmol/l, $P < 0.001$) and after 2 h (316 (150) vs 822 (515), $P < 0.001$) respectively (Fig. 1). Figure 2 shows the basal and stimulated levels of

cortisol in the ten preterm infants from multiple pregnancies participating in the study. The basal cortisol values and those 1 and 2 h after ACTH did not differ significantly between the time-points observed. Infants whose mothers had chorionamnionitis had significantly higher mean cortisol values at 1 and 2 h after the ACTH injection (718 (405) and 1070 (631) nmol/l) compared with those whose mothers did not (383 (214) nmol/l and 500 (286) nmol/l, $P = 0.005$).

In the multiple gestation group, the stimulated cortisol level of all ten infants was less than 414 nmol/l 1 h after ACTH administration, whereas 16 (53%) of the singleton infants had corresponding stimulated cortisol levels less than 500 nmol/l ($P = 0.007$), determined as insufficient by Grinspoon & Biller (12), and 12 (40%) less than 414 nmol/l ($P = 0.002$). Both of these differences were statistically significant. After 2 h, nine (90%) multiple group and 13 (43%) singleton patients had an insufficient (<500 nmol/l) peak cortisol level ($P = 0.01$) and seven (70%) of the multiple group and six (20%) of the singletons a subnormal (<414 nmol/l) stimulated cortisol level ($P = 0.006$) (Table 2).

The percentages of study patients whose peak cortisol levels 1 and 2 h after ACTH were 414 nmol/l or more did not differ significantly between groups

Figure 1 Cortisol response (means ± S.D.) to ACTH stimulation in preterm infants. The cortisol levels for 30 single are compared with values for ten infants from multiple gestations. * $P < 0.03$, ** $P < 0.001$. **Figure 2** Cortisol response to ACTH stimulation in preterm infants from multiple pregnancies. Numerals indicate sets of infants from multiple pregnancies.

with or without ventilator treatment (14 (45%) vs 2 (22%), $P = 0.248$ and 20 (65%) vs 5 (56%), $P = 1.000$ respectively). One hour after ACTH injection, the peak cortisol levels were similar regardless of the presence or absence of a history of maternal chorionamnionitis or need for inotropics, but there were significantly more cases with an adequate stimulated cortisol response after 2 h (> 500 nmol/l) among infants whose mothers had suffered chorionamnionitis (9 (69%)) compared with those without such a history (8 (30%), $P = 0.03$). In the analysis of risk factors associated with abnormal cortisol production evaluated by means of a 2-h ACTH test, multiple pregnancy (OR 10.3, 95% CI 1.94; 54.3, $P = 0.006$) was the only significant factor, regardless of the limit for adequate cortisol secretion that was used.

Of the infants from the groups ($n = 18$) with baseline cortisol levels lower than the reference value, five (28%) showed a normal response 1 h after ACTH stimulation. When more than 414 nmol/l was considered to be a normal stimulated cortisol level, there were nine (50%) infants showing adequate cortisol production 2 h after stimulation. This constituted a significantly smaller proportion of patients compared with infants with normal baseline cortisol production ($P = 0.04$) before ACTH stimulation. When the normal response was determined as a level of more than 500 nmol/l, there were three (17%) patients with a normal response 1 h after the ACTH test and after 2 h there were four (22%) infants with a normal stimulated cortisol level. There were significantly fewer patients in both groups ($P = 0.04$) and ($P = 0.01$) respectively who showed a normal response after ACTH compared with infants with normal baseline cortisol levels before stimulation with ACTH.

Discussion

This study was undertaken to evaluate adrenocortical function in VLBW infants and specifically to detect differences in sufficiency of cortisol response to ACTH between infants from singleton and multiple gestations. Judging from our findings it would appear that adrenocortical function in infants from multiple pregnancies is significantly poorer than that in infants from singleton gestations. To our knowledge such a finding in humans has not been described in the literature hitherto.

In this study, we used a standard dose (SD) ACTH test. In the recent literature there has been much discussion regarding the correct method of evaluating adrenal insufficiency. Nowadays, the trend is to use the low-dose (LD) ACTH test, since it is capable of detecting mild adrenal insufficiency. This LD ACTH test has therefore been introduced for adults (16, 17) and for premature infants (18, 19). In our study population, significant amount of patients showed inadequate cortisol production after ACTH stimulation. Earlier studies have confirmed a significant incidence of

adrenocortical insufficiency in VLBW infants (14, 18, 19). It is possible that we might have found more infants with adrenal insufficiency by the LD ACTH test, but its clinical usefulness in premature infants remains controversial. Recent studies have shown that the LD and SD ACTH tests are equal in sensitivity and there has been concern regarding the reliability to detect subtle degrees of secondary adrenocortical insufficiency (20–22). It has also been speculated that since HPA axis suppression after dexamethasone treatment cannot be reliably determined by the ACTH test, a corticotropin-releasing hormone (CRH) test should be considered (23). It has been demonstrated in previous studies of preterm infants that the CRH test is safe and reliable for eliciting consistent pituitary–adrenal responses (23, 24).

In the lamb fetus late in gestation there is an increase in the resting cortisol concentration in plasma. In singleton gestations and in sheep this increase in resting levels appears to be connected with an increase in response to exogenous ACTH, which occurs during gestation (25). Schwartz & Rose (10) have demonstrated that in fetal sheep twins the cortisol concentration in one sibling rises and remains at a higher level than that in the other. It has been speculated that this activation of the HPA axis in one twin triggers the mechanisms which lead to delivery. There were identical cortisol responses to ACTH in both twins before the separation of basal cortisol values, but subsequent ACTH challenge showed higher cortisol production in the sibling with an increased fetal cortisol level (10, 11). In our study, we had too few multiple gestation infants to compare the co-twins' cortisol responses with each other. This issue needs further evaluation in future studies.

A pulsatility of cortisol production prevails (26), although newborn infants lack a circadian rhythm for cortisol secretion (27) and only small differences in basal cortisol levels in sick, very preterm infants were detected by Jett *et al.* (28). In our study, the infants were tested in the morning. Although the reference values for cortisol concentration have been established at a gestational age of less than 30 weeks in both ill and well appropriate for gestational age preterm infants (8, 29), in our opinion a single random basal cortisol level does not adequately reflect the cortisol production capacity of a VLBW infant. The time at which the samples were obtained might not be the crucial point. SGA (30, 31), arterial hypotension (1, 8, 9), respiratory problems (4) and antenatal exposure to corticosteroids have been reported to affect basal cortisol production in preterm infants (32–34). The condition has been associated with endocrine dysfunction at the adrenal level or the mechanism might be connected with the inability of the hypothalamus to recognize the stimulatory signal (5, 9, 34, 35). High birth weight and high gestational age emerged as the only statistically significant covariates related to insufficient basal serum cortisol levels in our logistic regression analysis.

The discrepancy between the previous reports and ours is most probably due to differences in study populations and study designs. Appropriate serum cortisol reference values for the smallest and most premature infants are difficult to determine, because the general condition after birth in such cases is almost always poor and it is not possible to find 'healthy' control cases of similar birth weights and gestational ages.

In conclusion, VLBW infants from multiple gestations seem to be more prone to adrenal insufficiency after birth than VLBW singleton infants when evaluated by means of an ACTH test. The number of cases in our study was small and further trials are required to confirm our finding and the possible mechanisms behind the phenomena observed. In the future, there is a need to evaluate whether newborn infants from multiple pregnancies would benefit from hydrocortisone substitution in concomitant severe clinical problems including hypotension, need for surgical treatment, serious infection or other stress factors.

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