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Early Pituitary-Adrenal Responses and Retinopathy of Prematurity in Very Low Birth Weight Infants

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ABSTRACT

This longitudinal prospective study aimed to investigate the relationship between pituitary-adrenal responses and severity of retinopathy of prematurity (ROP) in 92 preterm, very low birth weight infants ≤ 30 wk gestation. The human corticotropin releasing hormone stimulation test was performed on these infants at D 7 and 14 of postnatal life. Univariate analysis revealed significant associations between severity of ROP and gestational age ($r = -0.53$, $p < 0.0001$), birth weight ($r = -0.56$, $p < 0.0001$), Apgar score at 1 min ($r = -0.27$, $p < 0.05$), Clinical Risk Index for Babies score ($r = 0.48$, $p < 0.0001$), duration of mechanical ventilation ($r = 0.48$, $p < 0.0001$), oxygen dependency ($r = 0.48$, $p < 0.0001$), and length of hospitalization ($r = 0.49$, $p < 0.0001$). The stage of ROP was also significantly associated with the basal and peak plasma ACTH ($r > -0.22$, $p < 0.05$) and peak serum cortisol ($r = -0.21$, $p = 0.05$) at d 7. Multivariate analysis using the classification and regression trees indicated that the two most influential risk factors affecting the development of advanced stages of ROP (≥ stage 3) were i) birth weight and ii) oxygen dependency at 28 d of life or at 36 wk postconceptional age. Our findings suggest that early endogenous or stimulated pituitary-adrenal responses are not independent risk factors associated with the development of severe ROP. Low birth weight and prolonged oxygen exposure are likely to be important factors that influence the degree of damage inflicted on the retina. **(*Pediatr Res* 55: 114–119, 2004)**

Abbreviations

$AaDO_2$, alveolar-arterial oxygen gradients
CART, classification and regression trees
CLD, chronic lung disease
CRIB, Clinical Risk Index for Babies
hCRH, human corticotropin releasing hormone
HPA, hypothalamic-pituitary-adrenal
OI, oxygenation index
ROP, retinopathy of prematurity
TAP, transient adrenocortical insufficiency of prematurity
VEGF, vascular endothelial growth factor
VLBW, very low birth weight

Recent advances in neonatal management, including the use of antenatal corticosteroids, surfactant replacement, and new forms of assisted ventilation, have paved the way for improvement in morbidity and mortality of preterm, VLBW (<1500 g) infants (1–3). ROP, however, remains a devastating disease and a significant cause of blindness and visual impairment that contributes to long-term neurologic sequelae of these vulnerable infants (4, 5). Thus far, no definitive prevention, other than judicious use of supplemental oxygen, is available for minimizing the incidence and severity of this serious condition (6, 7). As ROP is considered by some investigators to be an oxygen free radical disease (8) and occurs mainly in the most immature infants (9), treatments that can minimize the use of oxygen or enhance the maturity of body organ systems may potentially be beneficial for the management or prevention of this condition. Recent studies suggested that preterm infants, suffering from TAP, with low circulating cortisol levels, had an increased risk of developing systemic hypotension, patent ductus arteriosus, and oxygen free radical–related disease such as CLD (10–15). Corticosteroids have been shown to suppress preretinal and subretinal neovascularization in animal models (16–18), to up-regulate the expression of antioxidants such as superoxide dismutase (19, 20), and to stabilize or modulate the inflammatory response of capillary endothelium (21, 22).

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Hence, this group of drugs may be used to reduce the susceptibility of developing retinal vessels to oxidative injury, and assists in accelerating the maturation of retinal vasculature. In addition, recent reports suggest that the use of antenatal dexamethasone or a prolonged course of postnatal corticosteroids might decrease the severity of ROP (22, 23). This prospective study, therefore, aimed to investigate the relationship between endogenous or stimulated pituitary-adrenal responses and severity of ROP. Such a relationship would set the stage for clinical trials of physiologic doses of corticosteroids in the early postnatal period to prevent this disease.

The hCRH test was chosen to assess the pituitary-adrenal function of VLBW infants because we have previously demonstrated that the test is safe, reproducible, and capable of eliciting a consistent response similar to those of older children and adults (24). The hCRH test is also preferable to other hypothalamic-pituitary-adrenal (HPA) axis stimulation tests such as the insulin stress test, the metyrapone test, and the low-dose short-synacthen test, because these latter investigations are either unsuitable for use in preterm infants because of their potential side effects, not as sensitive as the hCRH test in demonstrating a mild degree of adrenal insufficiency, or do not assess the pituitary function of the axis (24, 25).

PATIENTS AND METHODS

Ophthalmologic examination for preterm infants. Indirect ophthalmoscopy was performed for all preterm infants in the Neonatal Intensive Care Unit at the Prince of Wales Hospital, Hong Kong, who met the screening criteria for ROP: i) infants $<$32 wk gestation or $<$1500 g at birth, and ii) preterm infants ($<$37 wk gestation) receiving supplemental oxygen for $>$2 wk. Gestation assessment was by mother's last menstrual period, early ultrasound dating, and new Ballard score assessment after birth. The infants were first examined by ophthalmologists (A.K.H.K., B.S.M.T.) at 4 wk of postnatal age. All studied infants received 0.5% tropicamide and 1.25% phenylephrine for pupil dilatation. One drop of 4% amethocaine was instilled before opening the eye with a Cook's infant speculum. The zones and stages of vascularization were recorded for each eye. The stages of ROP were classified according to the International Classification of Retinopathy of Prematurity (26). The eyes were closely monitored every 2 wk, or more frequently if indicated, until discharge or death or until retinal vascularization was complete. Indirect laser retinopexy using diode or argon laser was performed if threshold disease was present.

Management of patients. VLBW infants were looked after in a low-illumination environment with the incubators partially shaded by cloth. Eye shields were routinely used in infants receiving phototherapy. Infants requiring supplemental oxygen were continuously monitored by pulse oximetry. The target blood oxygen saturation levels were between 90% and 94%. Such levels of pulse oximetry target did not change with the identification of ROP. All studied infants received two doses of surfactant (Survanta, Abbott Laboratories, North Chicago, IL, U.S.A.) if they required mechanical ventilation but none received vitamin E supplementation.

hCRH test for preterm infants. This prospective study was performed as part of a large study for assessing the relation between pituitary-adrenal responses and systemic hypotension or CLD (10, 27). The selection criteria of preterm infants for the hCRH test have been standardized as follows: i) gestational age $<$32 wk, ii) birth weight $<$1500 g, iii) no postnatal systemic or inhaled corticosteroids treatment before the hCRH test, and iv) possession of an indwelling arterial cannulae at d 7 of life. A second hCRH test was also performed if the arterial cannulae remained *in situ* at d 14. Infants were excluded if they had concurrent hypoglycemia, systemic infection, necrotizing enterocolitis, or major surgery in the preceding week.

We chose to perform the hCRH tests on d 7 and 14 of life. The reasons for selecting this time sequence have been explained previously (24). All tests were performed between 0800 h and 1000 h. The method of performing the hCRH test in preterm infants and the laboratory hormone assays have been standardized and described in detail in our previous studies (24, 28). In brief, synthetic hCRH (Ferring Pharmaceuticals Ltd., Arzneimittal, Wittland, Germany), 1 μg/kg, was given by bolus i.v. injection. Blood samples (0.5 mL) were obtained, immediately before hCRH administration (0 min), and at 15, 30, and 60 min after the injection. The plasma ACTH and serum cortisol concentrations were measured by double-antibody RIA and solid-phase RIA, respectively (24). Plasma ACTH concentration in picomoles per liter can be converted to picograms per milliliter by multiplying by a factor of 4.5; likewise, the conversion of serum cortisol concentration from nanomoles per liter to micrograms per deciliter can be achieved by dividing by 27.6.

Statistical analysis. The descriptive statistics on demographic data were expressed as median and interquartile range. Spearman's correlation was used to assess the relationship between stages of ROP and i) clinical characteristics of the studied infants, and ii) circulating and stimulated hormone concentrations. In addition, Mann Whitney U test and χ^2 tests were used to compare the clinical characteristics of infants with stage 0–2 ROP (Group 1) and those with threshold disease or stage 3 or higher ROP (Group 2—the severe ROP group). As the absolute plasma ACTH and serum cortisol levels, and the incremental rise in hormone concentrations between the basal and peak levels [*i.e.* the difference in plasma ACTH concentration between 0 and 15 min ($\Delta ACTH_{0-15}$), and the difference in serum cortisol concentration between 0 and 30 min ($\Delta Cortisol_{0-30}$)] are important in evaluating the adequacy of pituitary-adrenal responsiveness, Mann Whitney U test was also used to assess the circulating hormone concentrations at the corresponding time points between the two groups of patients. Univariate significant results ($p < 0.05$) were further subjected to multivariate analysis using the CART method (29). CART is a nonparametric, binary recursive-partitioning algorithm developed through a two-stage process. A complex tree is initially constructed through a sequence of optimal binary splits of a set of covariates. These covariates are then partitioned recursively into two subgroups that are most different with respect to the outcome. The partitioning continues until the dependent variable is separated into homogeneous subgroups. The second stage prunes the resulting complex tree to an optimal subtree

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that minimizes the overall classification error. This error rate is then estimated by a cross-validation method and is implemented by a pruning algorithm. The statistical tests were performed by SPSS for Windows (Release 11, SPSS Inc., Chicago, IL, U.S.A.) and S-Plus 2000 (Release 3, MathSoft Inc., Seattle, WA, U.S.A.), and the statistical analysis was performed on raw and logarithmically (Ln) transformed data where appropriate to correct the skewness of the results.

Ethical approval. Ethical approval of the study was obtained from the research ethics committee of the Chinese University of Hong Kong. Informed parental consent was obtained for each case before commencement of the test.

RESULTS

Two hundred and twenty-six hCRH tests were performed on 137 VLBW infants at d 7 and 14 of postnatal age. The preliminary ACTH and cortisol results of this cohort have been compiled to provide a reference range for the hCRH test in VLBW infants (28). The findings indicated that peak plasma ACTH and serum cortisol occurred at 15 and 30 min, respectively (24, 28). The data concerning the relationship between hormones of the HPA axis and ROP have not been reported previously.

This study was carried out in the mid- and late 1990s (August 1994 to December 1996 and April 1998 to December 1999). The interruption was due to lack of funding of the program. As no patient above 30 wk gestation developed threshold disease and stage 3 or higher ROP, the analysis of data was, thus, confined to infants below this gestation and those who survived to term (*i.e.* allowing adequate time for complete ophthalmologic examination). One hundred and eighty-three VLBW infants, $\leq$ 30 wk gestation were born within the study periods. Of these infants, 25 died within the first 72 h of life, 21 were recruited to the early inhaled corticosteroid study (30), and 45 did not possess an arterial line or their parents were unwilling to take part in the study. Hence, a total of 92 infants were enrolled in the ROP study. None of the infants received postnatal corticosteroid therapy before the hCRH test. Sixty-nine and 23 infants belonged to Group 1 (stage 0–2) and Group 2 ($\geq$ stage 3), respectively. Among infants in Group 2, 11 of 23 (48%) underwent laser treatment.

Eight infants missed the first hCRH test, of whom five belonged to Group 1 and three belonged to Group 2. Twenty-seven infants were not tested on d 14. Twenty-two and five belonged to Group 1 and Group 2, respectively. The reasons for missing the hCRH test were due to systemic infection, necrotizing enterocolitis, or lack of an indwelling arterial line when due for the investigation. Six of the 27 infants not tested on d 14 were commenced on early dexamethasone because of severe lung disease. Two infants belonged to Group 1 and four belonged to Group 2. The clinical characteristics of the infants are summarized in Table 1. As expected, Group 2 infants were significantly more immature and sicker than infants of Group 1 (Table 1). There were, however, no significant differences in

Table 1. *Clinical characteristics of the studied population*

	Group 1 (Stage 0–2; $n = 69$)	Group 2 ($\geq$ Stage 3; $n = 23$)
Gestational age (wk)*	28.3 (27.1–29.1)	26.4 (25.0–27.9)
Birth weight (g)*	1120 (975–1243)	850 (710–945)
Male sex (*n*)	38 (55%)	11 (48%)
Mode of delivery		
Cesarean section *vs* vaginal	41 (59%):28 (41%)	9 (39%):14 (61%)
Singleton *vs* multiple	56 (81%):13 (19%)	18 (78%):5 (22%)
Inborn (*n*)	66 (96%)	22 (96%)
Prolonged rupture of membrane > 24 h (*n*)	15 (22%)	3 (13%)
Pre-eclampsia (*n*)	5 (7%)	1 (4%)
Antepartum hemorrhage (*n*)	13 (19%)	3 (13%)
Apgar scores		
1 min	6 (5–7)	5 (3–7)
5 min	8 (7–9)	8 (7–9)
Antenatal dexamethasone		
Infants whose mothers received dexamethasone (n)	57 (82%)	16 (70%)
Cumulative doses (mg)	20 (10–40)	20 (0–40)
Time between last dose and delivery (h)	12 (4–51)	31 (4–49)
Respiratory parameters		
CRIB score*	2 (1–4)	7 (4–9)
OI (first 12 h)	5.7 (3.2–11.3)	7.7 (3.5–14.1)
$AaDO_2$ gradient (first 12 h)	190 (69–293)	256 (103–350)
PIE (*n*)	4 (6%)	4 (17%)
Pulmonary hemorrhage (*n*)	1 (1%)	0 (0%)
Duration of mechanical ventilation (d)	27 (11–38)	50 (38–90)
CLD requiring O_2 at 28 d (*n*)*	19 (28%)	17 (74%)
CLD requiring O_2 at 36 wk		
Postconceptional age plus death (*n*)*	16 (23%)	16 (70%)
Postnatal dexamethasone (*n*)*	20 (29%)	17 (74%)
Duration of hospitalization (d)*	96 (85–113)	136 (110–153)

PIE, pulmonary interstitial emphysema. Results are median (interquartile range) or number (%).
* $p < 0.01$

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plasma ACTH and serum cortisol at either time point or in incremental ACTH ($\Delta ACTH_{0-15}$) and cortisol ($\Delta Cortisol_{0-30}$) between the two groups (Table 2).

Univariate analysis using Spearman's correlation revealed significant associations between stages of ROP and gestational age ($r = -0.53$, $p < 0.0001$), birth weight ($r = -0.56$, $p < 0.0001$), Apgar score at 1 min ($r = -0.27$, $p < 0.05$), CRIB score ($r = 0.48$, $p < 0.0001$), duration of mechanical ventilation ($r = 0.48$, $p < 0.0001$), oxygen dependency ($r = 0.40$, $p < 0.0001$) and length of hospitalization ($r = 0.49$, $p < 0.0001$). The severity of ROP was also significantly associated with the basal and peak plasma ACTH ($r > -0.22$, $p < 0.05$) and the peak serum cortisol ($r = -0.21$, $p = 0.05$) at d 7. In contrast, none of the hormone levels at d 14 or $\Delta ACTH_{0-15}$ and $\Delta Cortisol_{0-30}$ correlated significantly with the severity of the condition. There was also no significant correlation between the severity of ROP and acute respiratory indices, including OI and $AaDO_2$, recorded within the first 14 d of life.

Univariate significant factors, including gestational age, birth weight, Apgar score at 1 min, CRIB score, duration of mechanical ventilation, oxygen dependency at 28 d of life and at 36 wk postconceptional age, use of postnatal dexamethasone, basal and peak plasma ACTH, and peak serum cortisol at d 7, were further subjected to multivariate CART analysis. Statistical significance between stages of ROP and ACTH or cortisol was lost after the analysis. The two most influential factors affecting the severity of ROP were i) birth weight and ii) oxygen dependency at 28 d of life or at 36 wk postconceptional age. The CART model accurately predicted 83% of infants (10/12 patients) <780 g for developing severe ROP (≥stage 3), whereas the accuracy for infants >918 g having a milder disease (stage 0–2) was 91% (60/66 patients). In addition, the model correctly predicted 80% of infants (4/5 patients) with birth weight ranged between 780 and 918 g who did not require oxygen supplementation at 28 d or 36 wk for having the mild stages. Conversely, the accuracy for the same group of infants who required prolonged oxygen supplementation for developing the severe stages was 67% (6/9 patients). The overall sensitivity, specificity, and positive and negative predictive values of these two factors for predicting advance stages of ROP were 70%, 93%, 76%, and 90%, respectively.

DISCUSSION

To our knowledge, this is the largest study using the hCRH stimulation test to assess the HPA axis response in preterm infants, and the first report to investigate the relationship between endogenous or stimulated pituitary-adrenal hormone responses and ROP. The 61% incidence of ROP in the current study is similar to those reported in previous trials (23, 31). Our results suggest that there is a negative but weak association between advanced stages of ROP and peak plasma ACTH or serum cortisol concentrations. However, it has also been shown that a correlation exists between gestational age and serum cortisol due to progressive maturation of the HPA axis (32). Multivariate CART analysis was, therefore, used to identify the relative importance of different potential risk factors. The findings indicated that other factors such as gestational age, birth weight, CRIB score, and CLD (oxygen dependency >28 d of life or >36 wk of postconceptional age) are more influential than endogenous or stimulated pituitary-adrenal hormonal responses in correlating with the evolution of ROP. In fact, the two most important risk factors predisposing to the development of stage 3 or higher ROP are birth weight and prolonged requirement for oxygen supplementation. These results closely correspond to the findings observed by Hussain *et al.* (9), which demonstrate that gestational age and days on supplemental oxygen therapy are strongly associated with the development of ROP. The latter study also indicates that infants <28 wk gestation or with birth weight <1000 g are at increased risk of requiring retinal surgical intervention (9). VEGF, an oxygen-regulated factor, and IGF-I, a somatic growth factor, are important mediators associated with the pathogenesis of ROP (33). Preterm infants with low birth weight tend to have low circulating IGF-I level (34). VEGF, in the absence of IGF-I, is unable to stimulate normal retinal vascular development, and thereby increases the likelihood of developing clinical ROP (35). In addition, there was no significant difference in hormone concentrations at any corresponding time points or incremental rise in hormone concentrations between Group 1 and Group 2 infants. There were also no significant differences in plasma ACTH and serum cortisol even when the above comparisons were limited to infants <27 wk gestation [*i.e.* when there was no statistical difference in gestational age between the two groups; median peak plasma ACTH were 10.3 and 8.6 pmol/L, and median peak serum cortisol were 425 and 414 nmol/L for Groups 1 ($n = 18$) and 2 ($n = 15$), respectively]. Thus, the overall results suggest that the endocrine factor *per se* probably plays a minor or secondary role in predisposition to severe ROP.

It is known that the etiology of ROP is likely to be complex and multifactorial, and many etiologic factors are interrelated. However, the influence of corticosteroid treatment on ROP is

Table 2. *Hormone concentrations of Group 1 (stage 0–2) and Group 2 infants (≥ stage 3) on d 7 and 14*

	ACTH (pmol/L)			Cortisol (nmol/L)		
	0 min	15 min	$\Delta ACTH_{0-15}$	0 min	30 min	$\Delta Cort_{0-30}$
d 7						
Group 1 ($n = 64$)	5.9 (4.3–7.4)	11.4 (8.0–14.7)	4.7 (2.2–9.3)	251 (167–430)	473 (333–664)	183 (101–275)
Group 2 ($n = 20$)	4.8 (3.4–6.6)	8.6 (7.1–13.6)	4.2 (2.3–5.9)	190 (128–336)	406 (257–502)	157 (129–222)
d 14						
Group 1 ($n = 47$)	8.8 (5.1–10.9)	13.7 (9.3–17.8)	4.3 (2.2–7.6)	255 (171–398)	490 (377–645)	185 (111–307)
Group 2 ($n = 18$)	6.4 (4.4–9.1)	11.8 (7.7–17.2)	4.9 (1.8–7.4)	288 (161–625)	524 (342–869)	198 (155–304)

Results are median (interquartile range).

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controversial. A recent animal study suggests that rodent pups exposed to dexamethasone and oxygen have significantly lower retinopathy scores compared with pups exposed to oxygen alone (36). The use of corticosteroid therapy in preterm infants has been reported to have protective (22, 23), harmful (37–40), and no effect (41–43) on ROP. Similarly, early (<96 h), moderately early (7–14 d), and delayed postnatal corticosteroid treatment (>3 wk), has been associated with a reduction, no effect, and an increased risk of developing severe ROP, respectively (2, 44, 45). We postulate that the inconsistency and conflicting observations may be related to i) the retrospective nature (22, 37–43), ii) the relatively small sample size (46, 47), iii) the timing of corticosteroids administration (46–50), iv) a weak association between HPA axis hormones and ROP, and v) an interdependent relationship between various risk factors. Suppression of VEGF expression by hyperoxia and low IGF-1 level in premature low birth weight infants can prevent normal vascularization of the retina in the early postnatal period (phase 1 of ROP). Conversely, inhibition of these factors during the neovascular phase (phase 2 of ROP) may minimize the destructive retinal neovascularization process (33). Hence, the timing of therapeutic interventions, such as the use of corticosteroids or replacement of IGF-I, may be critical in affecting the disease process (35). Although this study series failed to show a direct association between TAP and CLD or prolonged oxygen requirement (27), other investigators have suggested a connection between these factors (11–13). Thus, a plausible integrated mechanism could be that premature and extremely low birth weight infants with a higher incidence of TAP are more prone to require prolonged oxygen supplementation (11–13), which ultimately increases the risk of oxidative damage to the premature retina. Our findings suggest that low birth weight (closely associated with prematurity) and prolonged oxygen exposure are likely to be the primary and essential risk factors involved in the development of severe ROP, and low circulating serum cortisol may probably act as a secondary link for progression to chronic lung damage.

The results also indicate that the severity of respiratory distress syndrome *per se*, as reflected by OI and $AaDO_2$ gradients, does not contribute significantly to the development of advanced stages of retinal disease. These findings suggest that an acute or transient increase in mechanical ventilation or oxygen requirement is probably not the major risk factor predisposing to severe ROP. In contrast, chronic oxygen exposure appears to be more damaging to the retina, as cessation of normal retinal vascular growth and subsequent retinal neovascularization are dynamic but slow processes that would not be completed within the first few weeks of postnatal life.

Similar to previous studies investigating the pathogenesis and etiologic factors of ROP, the current exercise also suffers from its own intrinsic limitations. First, although the current series represents one of the largest studies of pituitary and adrenal responses in preterm infants, the moderate sample size may still not have sufficient statistical power to detect a small difference in the risk factors between the two groups. Subgroup analysis, such as excluding infants who received antenatal or postnatal corticosteroids, is not feasible as the number of untreated infants would be too few to be considered for a meaningful analysis. Second, the multifactorial etiology of the disease and the interdependent relationship between various risks factors further complicates the issue. We have, however, tried to minimize some of the important confounders by standardizing the time schedule of performing the hCRH tests and using a sophisticated mathematical model (CART) to identify the relative importance of individual factors. Third, despite evaluating a comprehensive list of risk factors, some less recognized or unidentified factors might not have been included in the statistical model. Other factors such as perinatal and postnatal sepsis, systemic fungal infection, and use of bronchodilators and diuretics have not been studied. Nonetheless, the inclusion of such factors would not have altered the findings of the multivariate analysis, as we did not find a significant association between early serum cortisol level and ROP. The importance of these risk factors, in particular sepsis, in causing ROP could not be assessed by the current study. Fourth, it is arguable that the studied infants might represent a sicker category of patients, as all required arterial line monitoring soon after birth. The relatively liberal use of indwelling arterial lines (10) and the balanced distribution of normotensive and hypotensive infants in our cohort (10), suggest that a wide spectrum of VLBW infants with different severity of TAP and disease conditions have been studied in this series.

CONCLUSIONS

In summary, circulating plasma ACTH and serum cortisol levels show only a weak correlation with the severity of ROP, suggesting at best a minor contributory but not a substantive effect. Multivariate CART analysis identifies birth weight and CLD to be the two most important risk factors associate with advanced stages of ROP. Further, the severity of the initial lung disease, as reflected by OI and $AaDO_2$ gradients within the first 14 d of life, does not appear to play a major role in the pathogenic process, whereas prolonged oxygen exposure greater than 28 d or more than 36 wk postconceptional age is associated with significant damage to the retina. Although, at first glance, our results suggest that corticosteroids replacement in the early postnatal period may not be particularly useful for prophylaxis of ROP, there is some preliminary evidence to suggest their beneficial role in prevention of CLD (2, 51). Whether physiologic doses of corticosteroids administered in the early postnatal period would translate into a reduced requirement for supplemental oxygen and a subsequent decrease in the incidence of ROP will require a large, multi-center, randomized control study to determine their effects. Our present data suggest that endogenous or stimulated pituitary-adrenal responses are not independent risk factors associated with the development of severe ROP.

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