CORTICOSTEROID TREATMENT IN THE PERINATAL PERIOD

EFFICACY AND SAFETY OF ANTENATAL AND NEONATAL CORTICOSTEROIDS IN THE PREVENTION OF ACUTE AND LONG-TERM MORBIDITY AND MORTALITY IN PRETERM INFANTS

Outi-Maria Peltoniemi
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Efficacy and safety of antenatal and neonatal corticosteroids in the prevention of acute and long-term morbidity and mortality in preterm infants

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Oulu, Finland

Abstract

The aim of the study was to evaluate the efficacy and safety of antenatal and postnatal corticosteroids in the prevention for mortality and acute and long-term morbidity in preterm infants.

Altogether 109 eligible preterm infants participated in a randomized, multi-center, double-blinded controlled trial studying the efficacy of early dexamethasone (DX) treatment. The infants received either four doses of DX or placebo. DX treatment did not have a detectable influence on survival without bronchopulmonary dysplasia (BPD), severe intracranial hemorrhage, or periventricular leukomalacia.

In a meta-analysis of 15 trials, we found that early prolonged DX treatment (> 96 h, n = 1594 infants) decreased the risk of BPD (RR 0.72 95% CI 0.61–0.87), whereas early short DX course did not (n = 1069 infants). However, prolonged DX increased the risk of gastrointestinal (GI) complications (RR 1.59 95% CI 1.02–2.46).

Fifty-one very preterm infants participated in a randomized placebo-controlled trial studying early hydrocortisone (HC) started before 36 hours of age and continued for 10 days. The basal and stimulated serum cortisol levels were measured before the intervention. The study was interrupted because of GI perforations in the HC group. HC decreased the risk of patent ductus arteriosus. HC-treated infants with serum cortisol concentrations above the median had a high risk of GI perforation. HC increased survival without BPD among infants with low endogenous cortisol levels.

Altogether 45 surviving infants were enrolled in the follow-up of the early HC trial at 2 years of age. None of the study patients had died after discharge. There was no difference in the recorded rehospitalization rate, growth characteristics, or neurological development between HC and placebo-treated children.

Altogether 249 women pregnant at less than 34.0 gestational weeks participated in a randomized trial studying the efficacy of a single additional dose of betamethasone (BM). All of the 159 infants in the BM group and 167 in the placebo group were born before 36 weeks of gestation. Intact survival was comparable between the BM and placebo groups, whereas the need for surfactant therapy in RDS was increased in the BM group. According to a post hoc analysis of 206 infants delivered within 1–24 hours, the BM booster tended to increase the risk of RDS and to decrease intact survival.

Keywords: antenatal steroid, betamethasone, bronchopulmonary dysplasia, cerebral palsy, dexamethasone, glucocorticoid, hydrocortisone, intraventricular hemorrhage, postnatal steroid, preterm infant, respiratoty distress syndrome
To my beloved family
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Oulu, May 2007

Outi Peltoniemi
Abbreviations

ACTH  adrenocorticotropic hormone
BAL  bronchoalveolar fluid
BM  betamethasone
BPD  bronchopulmonary dysplasia
BSID-II  Bayley Scales of Infant Development II
CDP  continuous distending pressure
CLD  chronic lung disease
CP  cerebral palsy
CPAP  continuous positive airway pressure
CRH  corticotrophin-releasing hormone
DHEAS  dehydroepiandrosterone sulphate
DX  dexamethasone
ELBW  extremely low birthweight (< 1000 g)
ELGA  extremely low gestational age (< 28 weeks)
GBA  glucocorticoid bioactivity
GI  gastrointestinal
GR  glucocorticoid receptors
HC  hydrocortisone
HELLP  syndrome of hemolysis, elevated liver enzymes and low platelets
HPA  hypothalamic-pituitary-adrenal
11β-HSD2  11β hydroxysteroid dehydrogenase type 2
IGF  insulin-like growth factor
IGFBP-1  insulin-like growth factor binding protein 1
IPPV  intermittent positive pressure ventilation
IUGR  intrauterine growth retardation
IVH  intraventricular hemorrhage
MDI  mental development index score
MR  mineralocorticoid receptor
MRI  magnetic resonance image
MV  mechanical ventilation
NEC  necrotizing enterocolitis
NSAID  non-steroid anti-inflammatory drug
17OHP  17-hydroxyprogesterone
PDA  patent ductus arteriosus
PPROM  preterm premature rupture of membranes
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Term</th>
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<tr>
<td>PVL</td>
<td>periventricular leukomalacia</td>
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<td>RDS</td>
<td>respiratory distress syndrome</td>
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<tr>
<td>ROP</td>
<td>retinopathy of prematurity</td>
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<tr>
<td>RSV</td>
<td>respiratory syncytial virus</td>
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<tr>
<td>SP-B</td>
<td>surfactant protein B</td>
</tr>
<tr>
<td>TNF-α</td>
<td>tumor necrosis factor alpha</td>
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<tr>
<td>VLBW</td>
<td>very low birth weight</td>
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</table>
List of original papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


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1 Introduction

Although the survival of extremely low birth weight infants has improved over the past decades, preterm birth is still associated with high neonatal mortality and morbidity in developed countries (O'Shea et al. 1997, Goldenberg & Rouse 1998, Kramer et al. 2000, Tommiska et al. 2001, Horbar et al. 2002). The decrease in neonatal mortality is offset by an increase in the number of survivors with severe morbidity and neurological handicap especially among infants born at low gestational age (Goldenberg & Rouse 1998, Emsley et al. 1998, Leviton et al. 2002).

Since the mid-1990's, 60%-70% of infants with birth weight of less than 1000g have survived until discharge to home (Lemons et al. 2001, Tommiska et al. 2007). Respiratory distress syndrome (RDS) and intraventricular hemorrhage (IVH) are the most common acute diseases affecting prematurely born infants. Both RDS and IVH are associated with an increased risk of long-term morbidity. Bronchopulmonary dysplasia (BPD) is a chronic lung disease of multifactorial etiology. BPD contributes to mortality and long-term morbidity, including an increased risk of respiratory diseases and disorders in neurodevelopment in early childhood.

Corticosteroid treatment given to pregnant women is the only antenatal therapy with significant efficacy in the prevention of RDS and IVH. Preterm infants benefit from antenatal corticosteroid treatment administered more than 24 hours and less than 7 days before delivery (Liggins & Howie 1972, Crowley 1995). In practice, however, there is a notably large group of pregnant women who continue to be at high risk for premature delivery after a course of antenatal glucocorticoids given more than week ago. The common practice in such cases has been to repeat the corticosteroid treatment weekly until preterm delivery.

Postnatally, dexamethasone treatment has been shown to decrease the risk of BPD and to facilitate weaning from mechanical ventilation (Halliday et al. 2003a, Halliday et al. 2003b, Halliday et al. 2003c). Regardless of the short-term benefits, however dexamethasone treatment was associated with an increased risk of cerebral palsy (CP) in follow-up studies (Halliday et al. 2003c). Based on the reported adverse effects, routine use of steroids is not recommended in preterm infants (Committee on fetus and newborn. 2002). In a pilot study, low-dose hydrocortisone treatment showed promising efficacy in the prevention of BPD (Watterberg et al. 1999).
The present review focuses on morbidities that could be prevented by corticosteroid treatment in the perinatal period.

This thesis was designed to evaluate the efficacy and safety of corticosteroids for the prevention of acute and long-term morbidity in preterm infants.
2 Review of the literature

2.1 Mortality

The mortality of preterm infants has decreased during the last few decades (Wilson-Costello et al. 2005). In Finland, overall perinatal mortality was 6 ‰ during 1996-1997; 4‰ of the infants were stillborn and 2 ‰ died during the first 6 days (Tommiska et al. 2001). Preterm infants with extremely low birth weight (ELBW) accounted for 39% of all perinatal deaths during that period (Tommiska et al. 2001). Neonatal mortality was 38% in that cohort and did not change during the period of 1999-2000 according to a recent report (Tommiska et al. 2007). The mortality of liveborn preterm infants is highest during the early days, as 82% of such deaths occur during the first 7 days (Tommiska et al. 2001, Tommiska et al. 2007). According to a Finnish national medical birth register study, one-year mortality was 11% among liveborn preterm infants with gestational age of less than 32 weeks (Rautava et al. 2007).

Mortality decreases steadily with increasing gestational weeks and birth weight (Tommiska et al. 2001, Lemons et al. 2001, Tommiska et al. 2007). The mortality of small preterm infants (birth weight 501-1500g, VLBW) declined during the 1990’s from 18.1% to 14.8% (Horbar et al. 2002). During the same period, the use of antenatal steroids and surfactant, the deliveries of very preterm births by cesarean section, and the use of nasal CPAP and high-frequency ventilation significantly increased (Horbar et al. 2002). In a Finnish cohort collected in 1996-1997, the most common causes of death during the first 6 days of life were respiratory distress syndrome (RDS, 65%) and immaturity (51%) (Tommiska et al. 2001). During the following 3 weeks, the reported leading causes of death were necrotizing enterocolitis (NEC, 44%), RDS (22%), and severe intraventricular hemorrhage (IVH, grade 3-4, 17%). The use of antenatal corticosteroids, intubation in the delivery room, and surfactant treatment reduce mortality during the first 12 hours (Shankaran et al. 2002). Birth asphyxia, low gestational age, inappropriate fetal growth, and birth during non-office hours are associated with a higher risk of early death of preterm infants (Shankaran et al. 2002, Rautava et al. 2007). In addition, the delivery of preterm infants in level III hospitals is associated with decreased mortality during the first year of life (Rautava et al. 2007).
2.2 Neonatal morbidity

2.2.1 Respiratory distress syndrome

Respiratory distress syndrome (RDS) is defined as a need for continuous distending airway pressure and supplemental oxygen for at least 48 hours and typical chest X-ray findings or a need for surfactant therapy in cases of established respiratory failure. RDS has been the most important cause of morbidity and mortality in premature infants. RDS is still the most frequent acute pulmonary disease among ELBW infants during the neonatal period (Tommiska et al. 2001, Lemons et al. 2001). The increased risk of RDS is associated with lower gestational age and birth weight, male sex, and delivery by cesarean section (Farrell & Wood 1976, Dani et al. 1999). In twin pregnancies, the risk of RDS has been reported to be similar (Gardner et al. 1995, Friedman et al. 1997) or higher, especially at lower gestational age (Donovan et al. 1998, Marttila et al. 2004), compared to singletons of similar birth weight and gestational age. The risk of RDS is lower in first-born twins compared to singletons and second-born twins at gestational ages of higher than 29 weeks (Marttila et al. 2004). Second-born premature twins have a significantly higher risk of RDS compared to first-born twins (Hacking et al. 2001, Marttila et al. 2004). Although the heavier twin commonly has a higher risk of RDS than the smaller co-twin (Marttila et al. 2004), in twin pregnancies affected by severe growth restriction, the smaller twin has been reported to have a higher risk of RDS (Yinon et al. 2005). According to case-control and twin studies, susceptibility to RDS is partly explained by genetic variation in the surfactant proteins A, B, and C (Ramet et al. 2000, Marttila et al. 2003).

According to a Finnish population-based birth register study, the total incidence rate of RDS is 5.0 per 1000 liveborn singletons and 39.5 per 1000 first-born and 50.9 per 1000 second-born twins who are born alive (Marttila et al. 2004). The incidence of RDS is approximately 76-78% among infants with birth weight < 1000g. The length of gestation is the primary factor that influences the risk of RDS (Tommiska et al. 2001, Lemons et al. 2001, Marttila et al. 2004).

Prevention of RDS

Several randomized studies have shown that a single course of antenatal glucocorticoid treatment improves the survival of preterm infants born at 24-34
weeks gestation and reduces the incidence of RDS and the need for exogenous surfactant (Kari et al. 1994a, Crowley 1995, Ballard & Ballard 1995, Crowley 2000). The protective effect against RDS is thought to be due to the structural maturation of the lungs, increased surfactant synthesis, improved resistance to lung injury, and improved cardiovascular adaptation (Ballard & Ballard 1995). Surfactant therapy decrease the severity of RDS, pulmonary air leaks, and mortality (Jobe 1993, Schwartz et al. 1994, Soll & Morley 2000). Prophylactic surfactant given in the delivery room or during the first two hours significantly decreases the risk of pulmonary air leaks, pulmonary interstitial emphysema, and mortality compared to infants randomly given surfactant in RDS (Soll & Morley 2000, Yost & Soll 2000). Early surfactant treatment followed by early extubation within one hour and continued by NCPAP treatment decreases the need for later mechanical ventilation compared to selective surfactant treatment and continued mechanical ventilation (Stevens et al. 2004).

During the last few decades, different ventilator strategies have been developed for the treatment of respiratory problems among preterm infants. Permissive hypercapnia, permissive hypoxemia, minimal peak pressures, rapid rates, and early extubation may help to reduce ventilator-induced lung injury (Ambalavanan & Carlo 2006). According to randomized studies, prophylactic nasal CPAP commenced soon after birth had no detectable effect on neonatal outcomes among very preterm infants (Sandri et al. 2004, Subramaniam et al. 2005).

### 2.2.2 Intraventricular hemorrhage

**Definition**

Intraventricular hemorrhage (IVH) is an important cause of morbidity and mortality among very low birth weight infants. More than 50% of IVH are evident within the first 24 hours of life and 95% occur within the first 5 days of life (Vohr & Ment 1996, Heuchan et al. 2002). The incidence of IVH has decreased over time as neonatal care has improved (Philip et al. 1989). In a Finnish cohort study, the incidence varied from 4% among infants with gestational age of more than 30 weeks to 59% among infants born at 23 weeks (Tommiska et al. 2001).
IVH is graded into four groups based on the extent of damage (Table 1) (Papile et al. 1978). Subependymal and intraventricular hemorrhages without ventricular dilatation (grade I and II) have a spontaneous tendency to resolve within a few weeks, whereas IVH with ventricular dilatation and parenchymal hemorrhage (grade III and IV) cause persistent damage in surviving infants (Papile et al. 1978). The prognosis of IVH depends on the severity of parenchymal injury (Guzzetta et al. 1986). The rates of mortality and abnormal neurologic development are higher among infants with extensive periventricular intraparenchymal echodensity compared to infants with localized parenchymal injury (Guzzetta et al. 1986).

**Table 1. Diagnostic criteria of IVH (Papile et al. 1978).**

<table>
<thead>
<tr>
<th>Diagnostic criteria</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I</td>
<td>Subependymal hemorrhage with minimal or no IVH</td>
</tr>
<tr>
<td>Grade II</td>
<td>Definite IVH, but lateral ventricles not completely filled with blood</td>
</tr>
<tr>
<td>Grade III</td>
<td>IVH with at least one lateral ventricle distended</td>
</tr>
<tr>
<td>Grade IV</td>
<td>IVH with dilated ventricles and intraparenchymal echodensities</td>
</tr>
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</table>

**Risk factors**

Prevention of IVH

Preterm delivery is unavoidable in certain cases due to the clinical condition of the mother or the fetus. If preterm delivery cannot be prevented, the pregnant woman should be transported to a center with specialist-level expertise in the perinatal care of high-risk deliveries. The risk of IVH is increased among infants transported after delivery compared to in utero transported infants (Heuchan et al. 2002). The currently applied prevention strategies are listed in table 2.

In the past few years, a number of prenatal pharmacological interventions have been reported for the prevention of IVH in preterm infants. The efficacy of antenatal phenobarbital in the prevention of IVH in preterm infants was considered promising based on the initial randomized studies (Shankaran et al. 1996), but a meta-analysis revealed no difference between the phenobarbital and placebo groups (Crowther & Henderson-Smart 2003). Although preterm neonates have reduced levels of vitamin K-dependent coagulation factors, antenatal administration of vitamin K does not reduce the incidence of IVH according to a meta-analysis (Crowther & Henderson-Smart 2001). Antenatal glucocorticoids given to mothers more than 24 hours and less than 7 days before delivery have been shown to be effective in preventing IVH among preterm infants in several randomized studies and a meta-analysis (Table 5) (Crowley 2000). Both betamethasone and dexamethasone have been shown to be effective in decreasing the incidence of IVH (Crowley 2000, Lee et al. 2006).

The use of postnatal phenobarbitone does not prevent any grade of IVH, neurodevelopmental impairment, or death before hospital discharge according to a meta-analysis (Whitelaw 2001). Muscle paralysis is effective in stabilizing arterial blood pressure and cerebral blood velocity in preterm infants being ventilated for RDS. According to a meta-analysis of three trials involving 103 infants, the administration of pancuronium is associated with a decreased risk of IVH among ventilated infants with asynchronous respiratory efforts (Cools & Offringa 2005), but the treatment has been reported to involve complications, including hypotension, prolonged muscular weakness, and sensory neuronal hearing loss or even increased mortality before discharge (Mcintosh 1985, Cheung et al. 1999, Cools & Offringa 2005). Postnatal prophylactic indomethacin significantly reduces the risk of IVH according to a meta-analysis of 19 controlled trials (Fowlie & Davis 2002). However, indomethacin treatment is associated with adverse effects, such as an increased risk of gastrointestinal perforation. In follow-up studies, prophylactic treatment has not been associated with long-term

Table 2. Prevention of IVH.

<table>
<thead>
<tr>
<th>Prevention strategies</th>
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<tr>
<td>Delivery of high-risk pregnancies in centers specializing in perinatal care</td>
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<tr>
<td>In utero transports</td>
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<tr>
<td>Antenatal pharmacological interventions</td>
</tr>
<tr>
<td>Phenobarbital?</td>
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<tr>
<td>Vitamin K?</td>
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<tr>
<td>Glucocorticoids</td>
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<tr>
<td>Betamethasone</td>
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<tr>
<td>Dexamethasone</td>
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<tr>
<td>Obstetric management</td>
</tr>
<tr>
<td>Management of labor</td>
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<tr>
<td>Delayed cord clamping</td>
</tr>
<tr>
<td>Postnatal pharmacological interventions</td>
</tr>
<tr>
<td>Phenobarbitone</td>
</tr>
<tr>
<td>Muscle paralysis;</td>
</tr>
<tr>
<td>Pancuronium (side effects?)</td>
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<tr>
<td>Prophylactic indomethacin</td>
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2.3 Complications

2.3.1 Bronchopulmonary dysplasia

Definition and diagnosis

Bronchopulmonary dysplasia (BPD) was first described by Northway in 1967 (Northway, Jr. et al. 1967a). According to the early criteria, BPD was defined as lung injury with typical radiologic changes and pathological autopsy findings due to the primary lung disease, requiring prolonged supplemental oxygen and mechanical ventilation of mostly near-term or full-term infants with acute respiratory failure. Since then, antenatal and postnatal care has improved following the introduction of antenatal corticosteroids and surfactant treatment and the development of mechanical ventilation and other treatments. As a result of this progress in the management of preterm infants, the rates of mortality and acute lung injury have decreased, leading to the emergence of a new population of
very preterm infants and to a change in the clinical presentation of BPD (Rojas et al. 1995, Charafeddine et al. 1999, Bancalari et al. 2003). Oxygen dependency at 36 weeks of postmenstrual age predicts long-term impairment of pulmonary function and has thus been chosen as a threshold for moderate to severe BPD (Shennan et al. 1988). The clinical definition of BPD at 36 weeks is associated with wide variation of incidence between centers. The physiologic definition of BPD using the room-air test among infants with a milder form of BPD has been standardized to reduce the variation between centers and to identify the infants with ongoing pulmonary disease (Walsh et al. 2004). Severe BPD is defined as a need for positive pressure support or supplemental oxygen >30% to maintain saturation values between 90% and 96%. Infants with moderate BPD require 30% or less oxygen and fail the room-air test, i.e. have oxygen saturation of 80% to 89% for 5 consecutive minutes or <80% for 15 seconds (Walsh et al. 2004). Infants who are oxygen-dependent and pass the room-air test are categorized as having mild BPD. The diagnostic criteria of BPD are shown in table 3.

<table>
<thead>
<tr>
<th>Table 3. Diagnostic criteria of BPD(Jobe &amp; Bancalari 2001).</th>
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<tr>
<td><strong>Diagnostic criteria</strong></td>
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<tr>
<td><strong>Gestational age &lt; 32 weeks</strong></td>
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<tr>
<td>Mild</td>
</tr>
<tr>
<td>Moderate</td>
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<tr>
<td>Severe</td>
</tr>
<tr>
<td><strong>Gestational age &gt; 32 weeks</strong></td>
</tr>
<tr>
<td>Mild</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>Severe</td>
</tr>
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</table>

**Risk factors**

Recent incidence figures of moderate to severe BPD vary within 29 - 42% among infants with birth weight < 1000 g (Stevenson et al. 1998, Tommiska et al. 2001, Lemons et al. 2001). Very preterm infants are most susceptible to the development of BPD (Korhonen et al. 1999, Redline et al. 2000). The rate of BPD decreases with increasing gestational age and birth weight, being approximately 40% among infants with gestational age of 24-25 weeks and less
than 10% among infants with gestational age of more than 30 weeks (Tommiska et al. 2001, Lemons et al. 2001, Bancalari et al. 2003).

According to Northway’s first description of BPD, mechanical ventilation and hyperoxia were the major risk factors (Northway, Jr. et al. 1967b). More recent evidence indicates that BPD associates with lung inflammation and uncontrolled balance between pro- and anti-inflammatory mechanisms (Merritt et al. 1983, Groneck et al. 1994, Watterberg et al. 1996, Viscardi et al. 2004). Prenatal exposure to ascending infections and chorioamnionitis elevates the level of inflammatory mediators in umbilical cord plasma and fetal blood (Watterberg et al. 1996). The fetal inflammatory response is associated with the subsequent manifestation of BPD (Groneck et al. 1994, Watterberg et al. 1996, Yoon et al. 1999, Viscardi et al. 2004). Numerous postnatal events contribute to the development of BPD. Volutrauma during neonatal resuscitation, barotrauma, lung rupture, persistent lung edema, and infections lead to poor healing of the lung. Lung growth, including early angiogenesis and formation of alveoli, are inhibited, which leads to a reduced surface available for gas exchange (Rojas et al. 1995). Infants who develop BPD have prolonged inflammation with variable, mostly elevated proinflammatory cytokine levels and simultaneous absent or irregular secretion of anti-inflammatory cytokines during the first weeks of life (Groneck et al. 1994, Watterberg et al. 1996, Jones et al. 1996, Kakker et al. 2005). Several studies have shown that the preterm infants who have low serum cortisol levels and show decreased responsiveness to adrenocorticotropic hormone (ACTH) after birth are susceptible to the development of BPD (Watterberg et al. 2000, Huysman et al. 2000, Watterberg et al. 2001, Banks et al. 2001), although this association has not been found in all studies (Merz et al. 1998). Endogenous cortisol secretion is essential for the control of inflammation, and adrenocortical dysfunction may be one contributor to the prolonged inflammation and further development of BPD among some preterm infants. There is also increasing evidence of genetic susceptibility to BPD (Bhandari et al. 2006). Polymorphisms of the surfactant protein (SP-B) (Rova et al. 2004) and tumor necrosis factor alpha (TNF-α) genes appear to influence the risk of BPD (Kazzi et al. 2004).

Prevention

According to the current view, ventilation strategies with continuous distending pressures and lower peak inspiratory pressures, tidal volumes, and oxygen concentrations diminish lung injury and improve lung growth and development.
According to a systematic review, the use of early continuous distending pressure (CDP) significantly reduced mortality and the need for intermittent positive pressure ventilation (IPPV) (Ho et al. 2002).

Based on a meta-analysis, early surfactant administration is associated with a reduced need for later mechanical ventilation (MV), but the data from randomized studies is insufficient to allow reliable evaluation of the effect on BPD (Stevens et al. 2004). Early selective surfactant treatment as prophylaxis for RDS within the first two hours of life significantly decreases neonatal mortality or BPD at the age of 36 gestational weeks (Yost & Soll 2000).

In recent years, different antioxidant agents have been widely studied for the purpose of preventing oxidative-induced lung injury. Oxygen free radicals have a role in growth and development, restricting the use of antioxidant agents (Saugstad 2003).

Inhaled nitric oxide has been shown to be effective in the treatment of pulmonary hypertension in term infants (Roberts, Jr. et al. 1997, Clark et al. 2000). In addition, inhaled nitric oxide inhibits pulmonary edema and is efficient in bronchodilation (Baveja & Christou 2006). The efficacy of inhaled nitric oxide in the prevention of BPD among preterm infants requiring ventilator support after the first week of life has been variable according to randomized trials (Schreiber et al. 2003, Ballard et al. 2006, Kinsella et al. 2006). One reported a significant increase in survival without BPD (Schreiber et al. 2003, Ballard et al. 2006), while another only reported efficacy among preterm infants with birth weight of more than 1000g (Kinsella et al. 2006). In one trial, inhaled nitric oxide was demonstrated to worsen the outcome among preterm infants with birth weight of less than 1000g (Van Meurs et al. 2005). According to one follow-up study, nitric oxide improved neurodevelopment (Mestan et al. 2005).

Vitamin A is essential for optimal cell growth and differentiation, and low plasma and tissue concentrations of vitamin A are thought to predispose ELBW infants to BPD (Baveja & Christou 2006). According to a meta-analysis, vitamin A supplementation reduces the risk of BPD at 36 weeks of postmenstrual age (Darlow & Graham 2002).

Postnatal glucocorticoid treatment is beneficial in reducing the incidence of BPD and decreases the need for MV in preterm infants with respiratory disease (Halliday et al. 2003a, Halliday et al. 2003c). However, because of the acute and long-term adverse effects associated with the use of corticosteroids among preterm infants, this commonly used treatment of established BPD has been

Table 4. Prevention of BPD.

<table>
<thead>
<tr>
<th>Prevention strategies</th>
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<tr>
<td><strong>Ventilation</strong></td>
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<tr>
<td>Continuous distending pressure</td>
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<tr>
<td>Lower peak inspiratory pressure</td>
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<tr>
<td>Lower tidal volume</td>
</tr>
<tr>
<td>Lower oxygen concentration</td>
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<tr>
<td><strong>Surfactant therapy</strong></td>
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<tr>
<td>Prophylactic surfactant in delivery room</td>
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<tr>
<td>Early selective surfactant</td>
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<tr>
<td><strong>Antioxidant agents</strong></td>
</tr>
<tr>
<td>Inhaled nitric oxide</td>
</tr>
<tr>
<td>Vitamin A</td>
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<tr>
<td><strong>Postnatal glucocorticoids</strong></td>
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<tr>
<td>Early, moderately early and delayed dexamethasone</td>
</tr>
<tr>
<td>Early hydrocortisone hydrocortisone</td>
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</table>

**Prognosis**

Preterm infants with BPD have an increased risk of rehospitalizations during the first few years of life (Furman et al. 1996, Gregoire et al. 1998, Smith et al. 2004). The rate of rehospitalizations varies from 22% to 56% at one year of age and decreases to approximately 37% during the second year (Furman et al. 1996). Increased morbidity requiring rehospitalization is still evident at 8-10 years of age among children born with very low birth weight compared to children with normal birth weight (McCormick et al. 1993). Recurrent hospitalizations are mainly due to respiratory problems such as reactive airways diseases, bronchiolitis caused by respiratory syncytial virus (RSV), and pneumonia (Furman et al. 1996, Joffe et al. 1999). In follow-up studies, children with severe BPD have still been found to have respiratory symptoms, significant airway obstruction, and persistent radiographic abnormalities at school age (Pelkonen et al. 1997, Jacob et al. 1998). According to case-control and cohort studies, very low birth weight (VLBW) infants with BPD have a high risk of impaired cognitive and motor development during the early years, which persists and manifests as problems in cognition, visual-motor perception, and performance at

### 2.3.2 Cerebral palsy and neurological development

#### Definition and prevalence

According to follow-up cohort and case-control studies, preterm infants have increased neurodevelopmental morbidity in later childhood (Doyle 2001, Hille et al. 2001, Saigal et al. 2003, Mikkola et al. 2005). The rate of cognitive impairment has been reported to be 9%-15% among ELBW and ELGA preterm infants born during the 1990’s (Doyle 2001, Mikkola et al. 2005), and even higher rates have been reported in cohorts of preterm infants of several nationalities who were born in the presurfactant era (Saigal et al. 2003). At school age, children with birth weight of less than 1000g (ELBW) also have behavioral problems, such as social, thought, and attention difficulties, regardless of cultural differences (Hille et al. 2001). Neurosensory disabilities include hearing loss and abnormal ophthalmologic findings, such as poor vision, blindness, or strabismus. Based on cohort studies of ELBW children, the rate of hearing loss with a need for a hearing aid is approximately 3-4% and the rate of abnormal ophthalmologic findings 24-30% (Marlow et al. 2005, Mikkola et al. 2005).

Cerebral palsy is defined as a group of non-progressive syndromes of often variable severity involving motor impairment secondary to lesions or anomalies of the brain arising in the early stages of development and characterized by abnormal muscle tone in at least one extremity and abnormal control of movement or posture (Mutch et al. 1992). Cerebral palsy is the most common cause of motor disability in childhood (Rumeau-Rouquette et al. 1997).

The overall prevalence of CP has remained quite stable since the 1970’s, being about 2 per 1000 live births (Rumeau-Rouquette et al. 1997, Multiple authors 2000, Himmelmann et al. 2005). The risk of CP increases with decreasing gestational age (Himmelmann et al. 2005, Mikkola et al. 2005, Vohr et al. 2005). The prevalence of CP rose among preterm infants during the 1970’s, as their survival increased, but has been followed by a decrease in the late 1990’s and early 2000’s in some cohorts (Himmelmann et al. 2005, Vohr et al. 2005, Spinillo et al. 2006, Platt et al. 2007), although the increasing trend has continued from the 1990’s to the 2000’s in another cohort (Vincer et al. 2006). The very preterm
infants account for 20-27% of all infants with CP (Himmelmann et al. 2005, Platt et al. 2007). The recent rate of CP has been reported to be 5-10% among infants with gestational age of less than 30-32 weeks (Himmelmann et al. 2005, Ancel et al. 2006, Vincer et al. 2006) and 8-14% among extremely low birth weight infants (Himmelmann et al. 2005, Mikkola et al. 2005). According to one estimate, the origin of cerebral palsy is considered to be peri/neonatal in 49% of infants born prematurely (Himmelmann et al. 2005).

**Risk factors**

The perinatal risk factors associated with a high risk of CP have been reported to be clinical or histological chorioamnionitis (Wilson-Costello et al. 1998, Wu et al. 2003), preterm premature rupture of membranes (PPROM) (Livinec et al. 2005), intrauterine growth failure (Jarvis et al. 2003, Spinillo et al. 2006), resuscitation in the delivery room (Vincer et al. 2006), and delivery in a level I hospital (Grether et al. 1996). According to cohort studies’ antenatal glucocorticoid treatment decreases the risk of CP (Gray et al. 2001, Mikkola et al. 2005, Vincer et al. 2006), but this has not been confirmed in a meta-analysis of randomized trials on antenatal glucocorticoid treatment (Roberts & Dalziel 2006). Severe, grade III-IV, intraventricular hemorrhage and periventricular leukomalacia are independent risk factors of CP (Wilson-Costello et al. 1998, Himmelmann et al. 2005, Vohr et al. 2005, Vincer et al. 2006). The other reported postnatal factors that increase the risk of CP are respiratory problems, including pneumothorax, RDS, BPD, and surgery during the primary hospitalization (Wilson-Costello et al. 1998, Doyle 2001, Vincer et al. 2006). Postnatal dexamethasone therapy is associated with an increased risk of CP in cohort studies, and this has been confirmed in follow-up studies of randomized controlled trials and in a meta-analysis (Doyle 2001, Vohr et al. 2005, Vincer et al. 2006).

### 2.4 Glucocorticoids

#### 2.4.1 Adrenocortical function

The development of the hypothalamus and the pituitary and adrenal glands is essential for fetal development and adaptation to extrauterine life. The hypothalamic-pituitary-adrenal (HPA) axis regulates the intrauterine homeostasis
and has a major role in the prenatal differentiation of the vital organs, such as the lungs, liver, intestine, and nervous system. The physiologic stress response includes activation of the HPA axis and an increase of cortisol secretion to maintain system homeostasis. The adrenocortical function of preterm infants and its association with acute illness and later outcomes has been widely studied recently.

2.4.2 Fetal adrenocortical development

The first signs of activity of the fetal HPA axis have been detected between eight and 12 weeks of gestation (Polin and Fox 2004). The development of the fetal adrenal cortex is characterized by a changing balance between maternal, placental, and fetal hormonal activity during gestation. In normal pregnancy, this interaction protects the fetus from excessive cortisol exposure and allows a limited supply of corticosteroid for the maintenance of growth and differentiation. In later gestation, increasing fetal cortisol is essential for the antepartum maturation of the organs required for extrauterine adaptation and neonatal survival (Polin and Fox 2004).

2.4.3 Regulation of the HPA axis

During pregnancy, corticotrophin-releasing hormone (CRH) is secreted from the fetal hypothalamus and the placenta. Placental CRH secretion increases toward term (McLean & Smith 2001). CRH regulates the growth of pituitary corticotrophs, adrenocortical differentiation, and steroidogenic maturation of the fetal HPA axis (Mesiano & Jaffe 1997). Anterior pituitary corticotrophs secrete ACTH, which controls fetal adrenocortical growth, differentiation, and steroidogenesis (Fig.1) (Mesiano & Jaffe 1997, Polin and Fox 2004). During pregnancy, the fetal adrenal cortex goes through a complex process of development. The function of the adrenal cortex varies at the different stages of gestation. The fetal zone of the adrenal cortex is the main source of dehydroepiandrosterone sulphate production (DHEA-S). Cortisol and mineralocorticoid are produced by the transitional and definite zone of the fetal adrenal cortex (Fig.1).

Stressful situations during pregnancy lead to secretion of CRH from the hypothalamus. CRH stimulates the production of ACTH from the pituitary, which in turn increases the secretion of cortisol from the adrenal cortex. Cortisol
prevents excessive production of CRH and ACTH (Fig.1). At mid-gestation, a small percentage of maternal cortisol is passively transmitted through the placenta into the fetal circulation, acting as negative feedback of the fetal HPA axis. In the placenta, the primary regulator of fetal exposure to maternal cortisol is 11β hydroxysteroid dehydrogenase type 2 (11 β-HSD2) enzyme, which oxidizes cortisol to inactive cortisone (Fig.1). This enzyme activity is low during early gestation, but increases during the last trimester as the placenta matures. 11 β-HSD2 activity decreases the negative feedback effects of cortisol on the fetal hypothalamic-pituitary centers and increases fetal cortisol production in late gestation (Pepe & Albrecht 1995, McTernan et al. 2001).

Although cortisol inhibits the CRH and ACTH secretion of the fetal hypothalamus-pituitary centers, it stimulates the placental release of CRH (McLean & Smith 2001). This further stimulates fetal ACTH expression and acts as a positive feedback of the fetal HPA axis, resulting in an increase of CRH, ACTH, and cortisol toward term (Fig.1) (Mesiano & Jaffe 1997, McLean & Smith 2001). CRH also stimulates the production of DHEA-S in the fetal zone of the adrenal gland, which is a key substrate for the synthesis of placental estrogens. Estrogens in turn stimulate the production of the 11 β-HSD2 enzyme in the placenta (Fig.1). This complex regulatory mechanism continues as a ‘feed-forward’ loop during the last trimester of gestation, culminating in term parturition (McLean & Smith 2001).

As described above, the fetus is protected from excess cortisol until late pregnancy. Gestational disorders affecting the mother, the placenta, or the fetus may result in elevated cortisol exposure of the fetus. Excessive maternal cortisol production may be a consequence of increased maternal stress due to undernutrition or decreased 11β-HSD2 activity in the placenta. This passive cortisol exposure leads to suppression of the fetal HPA axis (Ng et al. 2002). According to animal experiments, fetal exposure to excess exogenous glucocorticoid decreases birth weight and organ weights by increasing premature maturation and hypoplasia of organs (Rudolph et al. 1999). Human and animal studies have revealed an association between intrauterine exposure to exogenous glucocorticoids and decreased birth weight (Benediktsson et al. 1993). In addition, maternal stress and decreased placental 11β-HSD2 activity have been found to be associated with intrauterine growth retardation in term and preterm infants (McTernan et al. 2001, Kajantie et al. 2003).
The fetus may actively secrete inappropriately high levels of cortisol in response to stimulatory factors, i.e. inflammatory mediators released during chorioamnionitis. The activation of the fetal HPA axis may continue after birth, as shown by elevated endogenous basal and ACTH-stimulated cortisol levels, and it is associated with a decreased risk of RDS (Watterberg et al. 1997).

IUGR as a consequence of fetal stress is associated with an increased risk of metabolic syndrome, type II diabetes, hypertension, and coronary disease (Barker et al. 1993). According to one hypothesis, intrauterine malnutrition promotes fetal hypoglycaemia and hypoinsulinism, which further stimulate the production of insulin-like growth factor binding protein 1 (IGFBP-1) in the liver. IGFBP-1 decreases the activity of insulin-like growth factor (IGF) and prevents its entry into tissues, thereby suppressing somatic growth (Wang & Chard 1992, Gluckman 1995). Approximately 15-20% of infants exposed to IUGR reach shorter final body height in adulthood than expected based on their genetic potential (Karlberg & bertsson-Wikland 1995). IUGR children with slow catch-up growth have higher serum cortisol levels at the age of 3-9 years (Cianfarani et al. 2002).
malnutrition and stress have been speculated to affect permanently the HPA axis, leading to slow or absent catch-up growth in childhood and an increased risk of cardiorespiratory diseases in adulthood.

2.4.4 Postnatal adrenocortical function

Adaptation to extrauterine life depends on the maturation of the vital organs. Adrenal development is essential for the regulation of intrauterine and postnatal homeostasis, and timely differentiation and maturation of the vital organs, including the cardiovascular, pulmonary, and central nervous systems. Preterm infants may have enough endogenous cortisol to maintain homeostasis under non-stressful conditions, but show an insufficient cortisol response due to cardiorespiratory stress.

2.4.5 Assessment of adrenocortical function

The standard ACTH stimulation test has been used to assess adrenocortical function. In the standard test, 250μg/1.73 m² of synthetic ACTH is administered as an intramuscular or intravenous bolus, and the serum cortisol response is evaluated at 60 min after the bolus (Oelkers 1996). Synthetic ACTH has been administered at variable doses to assess the function of the HPA axis among preterm infants (Hanna et al. 1993, Hingre et al. 1994, Watterberg & Scott 1995, Korte et al. 1996, Nykanen et al. 1999, Bolt et al. 2002b). The dose given in a standard ACTH test leads to a maximal cortisol response, occasionally providing misleading results and an underestimation of adrenocortical dysfunction. The low-dose ACTH test with a dose of 1μg/1.73 m² has been proposed to detect milder forms of adrenal insufficiency (Oelkers 1996). In the low-dose ACTH test, the peak cortisol concentrations reach their maximum 30 min after administration (Karlsson et al. 1999).

The Corticotropin-Releasing Hormone test (CRH test) is useful in evaluating the overall function of the HPA axis in preterm infants (Hanna et al. 1993, Karlsson et al. 2000, Ng et al. 2002, Bolt et al. 2002a). The CRH test reveals both pituitary and adrenocortical function and has been proposed to be more accurate in detecting adrenocortical suppression, especially hypothalamic depression (Karlsson et al. 2000). The commonly used dose of CRH is 1 μg/kg (Hanna et al. 1993, Karlsson et al. 2000, Ng et al. 2002), and higher doses have been speculated to cause pituitary overstimulation (Bolt et al. 2002a).
Non-invasive diagnostic techniques have been developed to determine cortisol levels to minimize confounding factors such as stress caused by the collection of blood samples and to minimize the need for blood samples especially among preterm infants. Good correlations have been obtained between plasma and salivary cortisol levels (Calixto et al. 2002), and simpler methods, such salivary cortisol collection by filter paper, have been introduced among preterm infants (Neu et al. 2007).

Adequate cortisol levels depend on the infants’ clinical status, i.e. clinically well infants have generally low cortisol levels, whereas very sick, stressed infants may suffer from adrenocortical insufficiency with equal cortisol secretion. In adults and children, a lower limit of 560 nmol/l in the ACTH test has been widely used (Jonetz-Mentzel & Wiedemann 1993, Oelkers 1998). These reference ranges may be too high for neonates and preterm infants because their cortisol secretion tends to be lower than that of older children and adults (Jonetz-Mentzel & Wiedemann 1993). In healthy preterm infants with gestational age of less than 30 weeks, the median basal cortisol levels are approximately 137 nmol/l (with the 90th percentile of 375 and the 10th percentile of 51 nmol/l) according to one cohort study (al SS et al. 1995). The intrauterine growth pattern seems to influence the postnatal cortisol levels. Higher cortisol values have been measured in preterm infants with intrauterine growth restriction compared to infants with appropriate growth (Heckmann et al. 1999). Basal and ACTH- or CRH-stimulated cortisol levels have varied between different studies investigating the adrenocortical function of preterm infants (Table 5) (Hanna et al. 1993, Hingre et al. 1994, Watterberg & Scott 1995, Nykanen et al. 1999, Ng et al. 2002, Bolt et al. 2002a, Bolt et al. 2002b).
<table>
<thead>
<tr>
<th>Study</th>
<th>Dose</th>
<th>Gestational age (weeks)</th>
<th>Number of patients</th>
<th>Age (days)</th>
<th>Basal cortisol nmol/l</th>
<th>Stimulated cortisol nmol/l (30min)</th>
<th>Stimulated cortisol nmol/l (60 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hingre</td>
<td>36 μg/kg</td>
<td>26 ± 2 mean ± SD</td>
<td>25</td>
<td>4</td>
<td>207±3.5 mean ± SD</td>
<td>504.9 ± 38.9</td>
<td></td>
</tr>
<tr>
<td>Bolt 2002</td>
<td>1 μg/kg</td>
<td>28.1 ± 1.2 mean ± SD,  &lt; 30 weeks</td>
<td>13</td>
<td>5-10</td>
<td>178±15 mean ± SD</td>
<td>391±20 mean ± SD</td>
<td>516 ± 33</td>
</tr>
<tr>
<td>Bolt 2002</td>
<td>1 μg/kg</td>
<td>30.1 ± 1.1 mean ± SD,  30-33 weeks</td>
<td>11</td>
<td>5-10</td>
<td>250±42 mean ± SD</td>
<td>542±59 mean ± SD</td>
<td>733 ± 89</td>
</tr>
<tr>
<td>Nykänen</td>
<td>0.5 μg/1.73m²</td>
<td>28 (25-30) median(range)</td>
<td>12</td>
<td>3-15</td>
<td>190(60-357) median(range)</td>
<td>(190-631)</td>
<td>391</td>
</tr>
<tr>
<td>Nykänen</td>
<td>250 μg/1.73m²</td>
<td>28 (25-30) median(range)</td>
<td>12</td>
<td>3-15</td>
<td>117(44-384) median(range)</td>
<td>(331-1445)</td>
<td>587</td>
</tr>
<tr>
<td>Walterberg</td>
<td>3.5 μg/kg</td>
<td>24-33 range</td>
<td>59</td>
<td>5-7</td>
<td>5 (2.5-7.1) μg/dL median (range)</td>
<td>(10.0-15.0)</td>
<td>13.3</td>
</tr>
<tr>
<td>Hanna</td>
<td>62.5 μg</td>
<td>24-31.5 range</td>
<td>12</td>
<td>4 (2-7)</td>
<td>603.5±130.5 mean ± SD</td>
<td>882.7 ± 136.6</td>
<td></td>
</tr>
<tr>
<td>CRH test</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Hanna</td>
<td>1μg/kg</td>
<td>24-31.5 range</td>
<td>17</td>
<td>4 (2-7)</td>
<td>349.3±58.1 mean ± SD</td>
<td>568.7 ± 60.2</td>
<td></td>
</tr>
<tr>
<td>Bolt 2002</td>
<td>1 μg/kg</td>
<td>28.9 ± 1.6 mean ± SD</td>
<td>13</td>
<td>10 (7-15)</td>
<td>350 ± 115 mean ± SD</td>
<td>582 ± 201</td>
<td></td>
</tr>
<tr>
<td>Bolt 2002</td>
<td>1 μg/kg</td>
<td>29.0 ± 1.0 mean ± SD</td>
<td>5</td>
<td>13 (8-19)</td>
<td>256 ± 120 mean ± SD</td>
<td>509 ± 167</td>
<td></td>
</tr>
<tr>
<td>Bolt 2002</td>
<td>2 μg/kg</td>
<td>29.1 ± 1.3 mean ± SD</td>
<td>6</td>
<td>12 (8-21)</td>
<td>330 ± 154 mean ± SD</td>
<td>815 ± 212</td>
<td></td>
</tr>
<tr>
<td>Ng 2002*</td>
<td>1 μg/kg</td>
<td>27.9 (26.0-30.1) median (range)</td>
<td>28</td>
<td>7</td>
<td>286 (96-847) median (10-90th percentile)</td>
<td>(257-1022)</td>
<td>513</td>
</tr>
<tr>
<td>Ng 2002#</td>
<td>1 μg/kg</td>
<td>28.6 (26.4-30.1) median (range)</td>
<td>72</td>
<td>7</td>
<td>221 (81-589) median(10-90th percentile)</td>
<td>(189-866)</td>
<td>404</td>
</tr>
</tbody>
</table>

* infants not exposed to antenatal steroids
# infants exposed to 1-2 doses of antenatal steroids
Transient adrenocortical insufficiency has been measured in some preterm infants using the reference ranges of basal (138 nmol/l) and stimulated (414 nmol/l) cortisol levels in the ACTH test (Korte et al. 1996). Low serum cortisol levels are associated with elevated levels of cortisol precursors, such as 11-deoxycortisol and 17-hydroxyprogesterone (17OHP), reflecting the deficiency of intermediate enzymes of cortisol synthesis during the early weeks of postnatal life (Hingre et al. 1994, Korte et al. 1996, Huysman et al. 2000, Bolt et al. 2002b). The levels of cortisol precursors decrease with increasing gestational age, reflecting the maturation of the adrenal gland (Bolt et al. 2002b). Low serum cortisol levels during the first postnatal week are associated with a greater need for circulatory and respiratory support and an increased risk of chronic lung disease among preterm infants (Scott & Watterberg 1995, Watterberg & Scott 1995, Korte et al. 1996, Huysman et al. 2000, Heckmann et al. 2000), although these findings have not been confirmed in all studies (Merz et al. 1998, Banks et al. 2001). In addition, a low serum cortisol/17OHP ratio is associated with higher respiratory morbidity, which is consistent with the theory of immature adrenocortical function (Watterberg et al. 2000, Huysman et al. 2000). Infants who develop persistent ductus arteriosus have been shown to have lower cortisol levels during the first week of life compared to infants with spontaneous patent ductus arteriosus (PDA) closure (Watterberg et al. 2000). Low serum cortisol levels are also associated with increased pulmonary inflammation, i.e. increased levels of interleukins and proteins in tracheal fluid (Watterberg et al. 2000).

2.4.6 Glucocorticoid effects on the fetus and newborn

Pulmonary effects

Glucocorticoids act via intracellular glucocorticoid receptors (GR), which are found in fetal tissues from early gestation onward (Bolt et al. 2001). The glucocorticoid-GR complex alters the transcription of several target genes, resulting in upregulation or downregulation of gene transcripts. The human fetal lung binds cortisol with high affinity during the second month of gestation, causing high local concentrations of glucocorticoids in the lung combined with high GR expression, suggesting that cortisol has an important regulatory role in lung development (Bolt et al. 2001). Glucocorticoids accelerate the differentiation of immature type II pneumocytes by upregulating the synthesis of surfactant.
phospholipids and proteins and by increasing the secretion of the surfactant complex (Vyas & Kotecha 1997, Bolt et al. 2001). The effects of glucocorticoid and endotoxin on the synthesis and secretion of surfactant are additive (Newnham et al. 2001). Glucocorticoids also accelerate the thinning of the alveolar walls and facilitate effective gas exchange postnatally.

According to animal studies, glucocorticoids stimulate the synthesis of antioxidant enzymes in the lungs and may thus protect the lungs against oxidative damage (Frank 1992, Keeney et al. 1993, Bolt et al. 2001). Glucocorticoids inhibit pulmonary edema after birth. In animal studies, antenatal steroids decrease the accumulation of plasma proteins in the pulmonary interstitial space and alveoli (Bolt et al. 2001). The proposed mechanisms are glucocorticoid-mediated decrease in pulmonary vascular resistance and regulation of fluid reabsorption (King et al. 1996, Bolt et al. 2001).

After birth, glucocorticoids decrease inflammation in preterm lungs. Postnatal dexamethasone was shown to decrease the concentration of inflammatory markers, i.e. chemokines and cytokines, and proteins in bronchoalveolar (BAL) fluid among preterm infants with severe RDS and at high risk to develop BPD (Groneck et al. 1993, Kari et al. 1994b). Postnatal steroids have been shown to increase SP-A and -D in BAL fluid after early initiation of treatment (Wang et al. 1996). Glucocorticoid treatment also stimulates the synthesis of antioxidant enzymes and thus prevents oxidative damage caused by inhalation of oxygen in animal models (Walther et al. 1991, Keeney et al. 1993). Glucocorticoids have undesirable effects on the developing lung, including decreased growth (Vyas & Kotecha 1997). According to animal studies, glucocorticoid treatment suppresses the formation of secondary septa and leads to a decreased number of alveoli, which are larger than normal in size (Vyas & Kotecha 1997, Bolt et al. 2001).

Effects on central nervous system

In a developing brain, the sensitivity to glucocorticoids varies in the different regions. The limbic system, especially the hippocampus, contains corticosteroid receptors and is very sensitive to glucocorticoids (Jacobson & Sapolsky 1991, Matthews 2001). The hippocampus has a notable functional role in cognition, behavior, memory, co-ordination of autonomic activity, and regulation of endocrine systems (Jacobson & Sapolsky 1991, De Kloet et al. 1998, Matthews 2001). The profile of brain development varies between different species. In primates, most of the proliferation of neurons and neuroendocrine maturation take
place before term birth, whereas in species giving birth to immature offspring, the major part of development occurs postnatally (Matthews 2001). In addition, there is a large between-species variation in glucocorticoid sensitivity. Rodents have high receptor affinity to glucocorticoids, while other species, including humans, are considered corticoreistant (Matthews 2001). These differences make it difficult to directly compare the glucocorticoid effects on developing brain in humans and animals.

Cortisol and glucocorticoids act via two types of corticosteroid receptors in the brain. The mineralocorticoid receptor (MR) is expressed mainly in the limbic structures, while the glucocorticoid receptor (GR) is diffusely distributed, being most abundant in the limbic system, hypothalamic paraventricular nucleus, and cerebral cortex (De Kloet et al. 1998, Matthews 2001). In normal conditions with basal concentrations of cortisol, MR are occupied and GR non-occupied. In stressful situations with elevated concentrations of cortisol, GR are increasingly occupied (Jacobson & Sapolsky 1991, De Kloet et al. 1998). Prenatal glucocorticoid administration is mediated through GR, because synthetic glucocorticoids, including dexamethasone and betamethasone, bind predominantly to GR (De Kloet et al. 1998). The influence of glucocorticoids on the fetal brain is dependent on the time of gestation when the drug is administrated, because MR and GR levels vary during gestation. In primates, GR and MR levels are visible after completion of 60% of gestation. In the hippocampus, GR mRNA levels increase toward term, whereas MR mRNA levels decrease, which may be due to the different regulatory roles of these receptors during pregnancy. In the hypothalamic paraventricular nucleus, GR mRNA levels decrease toward term, which decreases the glucocorticoid-mediated negative feedback in the hypothalamus and allows increased CRH, ACTH, and cortisol concentrations before birth (Matthews 2001). Increased HPA axis activity is essential for the maturation of critical tissues before term.

According to animal studies, antenatal exposure to synthetic glucocorticoid affects the behavior and motor development of adult offspring (Matthews 2001). Antenatal exposure to glucocorticoids has been shown to alter the levels of corticosteroid receptors in the brain of prepubertal guinea pigs (Dean et al. 2001). The effect seems to be sex-specific: glucocorticoid exposure increases corticosteroid receptor expression in males, but reduces it in females (Liu et al. 2001, Dean et al. 2001). In addition, glucocorticoids may alter the brain structure. Several animal and adult studies have shown an association between stressful experience or prolonged exposure to glucocorticoids and reduced volume of
hippocampus (Bremner et al. 1995, Sapolsky 1996, Stein et al. 1997, De Kloet et al. 1998). Prenatal glucocorticoid exposure may have a dose-dependent effect on the degeneration of hippocampal neurons, neuronal depletion, and the modification of neuronal structure. In a study on rhesus monkeys, fetuses exposed to multiple courses of antenatal glucocorticoids were shown to have more severely damaged brain structure compared to fetuses given a single course (Matthews 2001).

2.5 Antenatal glucocorticoids

2.5.1 Single treatment

The first randomized, controlled trial of antenatal glucocorticoid treatment of pregnant women with imminent preterm delivery was published in 1972 by Liggins and Howie (Liggins & Howie 1972). Betamethasone given as a mixture of 6 mg betamethasone phosphate and 6 mg betamethasone acetate twice at a 24-hour interval decreased the risk of RDS and intracerebral haemorrhage among preterm infants with gestational age of less than 32 weeks. Since then, the efficacy of antenatal glucocorticoids in decreasing acute morbidity, i.e. RDS, IVH, and mortality of preterm infants has been demonstrated in various controlled trials and meta-analyses (Table 6) (Halac et al. 1990, Kari et al. 1994a, Crowley 1995, Roberts & Dalziel 2006). According to the results of a recent randomized trial, antenatal steroids given more than 48 hours before delivery even decrease the risk of RDS and transient tachypnoea among term infants delivered by elective caesarean section (Stutchfield et al. 2005). The treatment does not have any effect on the incidence of BPD (Crowley 1995, Crowley 2000). The risk of NEC is significantly decreased among infants exposed to antenatal steroids (Halac et al. 1990). As reported in a recently published cohort study, antenatal betamethasone may decrease the incidence of white matter lesions among infants with gestational age of less than 31 weeks (Agarwal et al. 2002). The efficacy of antenatal steroids in decreasing periventricular leukomalacia (PVL) was not reported in a meta-analysis (Crowley 2000), although a significant decreasing effect on the risk of a combined outcome of IVH and PVL was found in a randomized trial (Kari et al. 1994a). Antenatal steroids are most beneficial in reducing the incidence of RDS when given to infants with gestational ages between 26 and 34+6 weeks (Roberts & Dalziel 2006). Antenatal steroid
treatment does not increase the maternal risk of infections, hypertension, or death, although steroid-exposed mothers have been shown to have glucose intolerance more likely than placebo controls (Roberts & Dalziel 2006).

**Timing of single treatment**

The timing of the administration of glucocorticoids has been shown to influence the outcome. Treatment is most effective when administrated more than 24 hours and less than 7 days before delivery (Liggins & Howie 1972, Crowley 2000, Peaceman *et al.* 2005), although the influence of this interval on the outcome is not evident in all studies (Vermillion *et al.* 2001b, Sehdev *et al.* 2004). In a retrospective study, infants exposed to steroids 7-14 days before birth benefited from the treatment (Peaceman *et al.* 2005). According to a meta-analysis, RDS is significantly reduced among infants exposed to steroids between 24 hours and 7 days, but not among infants exposed to steroids less than 24 hours before birth or more than 7 days before birth, respectively (Roberts & Dalziel 2006). According to a randomized trial, antenatal steroids may protect against intracerebral complications when given less than 24 hours before delivery (Kari *et al.* 1994a). In a meta-analysis, their protective effect against intracerebral complications was evident when they were administered 24 - 48 hours before delivery (Roberts & Dalziel 2006). However, the increased risk of RDS has been reported in a randomized trial among preterm infants exposed to corticosteroid shortly before birth (Schutte *et al.* 1980).

Almost all trials of single-dose antenatal glucocorticoid were performed before the era of surfactant therapy. Antenatal steroids decrease the risk of RDS in PPROM when the rupture of membranes is evident less than 48 hours before delivery. However, the risk of IVH was not decreased 24 hours after the rupture of membranes (Roberts & Dalziel 2006). Infants born from pregnancies complicated by hypertension have a reduced risk of RDS, neonatal death, and IVH when exposed to antenatal steroids (Roberts & Dalziel 2006).

**Choice of drug**

Betamethasone and dexamethasone have been commonly used for the prevention of neonatal morbidity. Both drugs have similar biological activity and are associated with a significant reduction in the risks of RDS, IVH, and neonatal death, but dexamethasone significantly increases the risk of puerperal sepsis.
Glucocorticoid metabolism after a single course of antenatal steroids

Betamethasone is detectable in cord blood one hour after administration to the mother (Ballard et al. 1975). The betamethasone concentration declines after six hours and reaches a minimum approximately 6 to 20 hours after a single 12 mg dose of betamethasone. At the same time, endogenous cortisol levels are suppressed up to 24-36 hours after the administration and reach their minimum at 36-48 hours (Ballard et al. 1980). Betamethasone was not detectable in cord blood 62 to 72 h after the first dose (Ballard et al. 1975). After the administration of a second dose of betamethasone 24 hours after the first dose, the levels were somewhat higher at 1-10 hours compared to the levels after a single dose (Ballard et al. 1975). After a complete course of betamethasone (12 mg twice at 24-hour interval), the unbound glucocorticoid levels were comparable to the total cortisol levels of the stressed preterm infants, who were born after PPROM or developed RDS shortly after birth (Ballard et al. 1975). The circulating glucocorticoid bioactivity (GBA) after birth is dependent on the interval between the steroid administration and the delivery. Excess GBA (not caused by endogenous cortisol) is highest when the delivery occurs within 12 hours after betamethasone treatment and declines rapidly thereafter, being similar to that in untreated infants 7 days after the treatment (Kajantie et al. 2004). Serum cortisol levels remain depressed for at least 3 days after the treatment and reach the baseline within 7 days (Ballard et al. 1975, Ballard et al. 1980, Ng et al. 1997).

2.5.2 Multiple courses of antenatal corticosteroid treatment

Multiple courses of antenatal corticosteroids versus placebo

The first randomized study on the effect of multiple courses of betamethasone in reducing the morbidity of preterm infants was published in the 1970’s (Papageorgiou et al. 1979). Weekly repeated courses of betamethasone (an initial
A dose of 12 mg intramuscularly was repeated after 24 hours and then weekly until delivery) significantly decreased the incidence and severity of RDS and the incidence of death among preterm infants with gestational age of less than 34 weeks. Infants exposed to premature rupture of membranes more than 24 hours before birth (PPROM) also significantly benefited from betamethasone in the prevention of RDS. In this trial, there was no difference in the growth characteristics at birth between the study groups, but the treated infants had higher gestational age compared to the placebo group. The beneficial effect of weekly repeated betamethasone versus placebo in the prevention of RDS, IVH, PDA, neonatal infections, and death was confirmed in a randomized trial published in the 1990’s (Table 6) (Garite et al. 1992, Lewis et al. 1996, Silver et al. 1996, Amorim et al. 1999). The efficacy of repeated courses of antenatal corticosteroid using dexamethasone was evaluated in a small group of pregnant women with the syndrome of hemolysis, elevated liver enzymes, and low platelet counts (HELLP) (Magann et al. 1994). The women who received high doses of dexamethasone (10 mg intravenously every 12 hours until delivery) had significantly better laboratory and clinical parameters associated with the HELLP syndrome compared to the placebo group. However, infants exposed to high doses of dexamethasone (DX) had no detectable decrease in the risk of RDS (Magann et al. 1994).

Multiple courses of antenatal corticosteroid versus single treatment

Because the antenatal steroid effect has been shown to diminish approximately 7 days after administration, there have been randomized and non-randomized studies comparing the effects of weekly repeated and single course of antenatal steroids (Banks et al. 1999, Abbasi et al. 2000, Vermillion et al. 2000, Guinn et al. 2001, Shelton et al. 2001, McEvoy et al. 2002, Agarwal et al. 2002, Celik et al. 2002, Wijnberger et al. 2002). According to non-randomized studies, weekly repeated betamethasone or dexamethasone may decrease the risk of RDS and improve respiratory compliance and functional residual capacity compared to infants not exposed to corticosteroid treatment or with a single treatment more than 7 days before birth (Abbasi et al. 2000, McEvoy et al. 2000), although this association was not found in all studies (Banks et al. 1999, Vermillion et al. 2000, McEvoy et al. 2000). According to a randomized trial, weekly repeated corticosteroid significantly decreased the risk of severe RDS (Table 6) (Guinn et al. 2001), although repeated treatment did not improve pulmonary function
compared to placebo in a small study of 37 infants (McEvoy et al. 2002). Weekly treatment did not prevent intraventricular hemorrhage and did not have any effect on the incidence of BPD or other neonatal morbidity, either (Guinn et al. 2001). In a subgroup of women with PPROM, weekly treatment did not have any effect on composite morbidity, i.e. severe RDS, BPD, severe IVH, PVL, proven NEC, and sepsis, or morbidity before discharge, although the treatment significantly decreased the incidence of severe RDS (Lee et al. 2004).

The efficacy of one rescue course of corticosteroids in postponing imminent preterm delivery was evaluated in a cohort study, where the rescue dose of betamethasone given more than 24 hours before delivery was associated with a decreased risk of RDS among preterm infants with gestational ages between 28 and 34 weeks (Vermillion et al. 2001a). In this study, infants exposed to treatment for less than 24 hours before birth were excluded from the analysis.

Multiple courses of antenatal corticosteroids may influence intrauterine growth. An association between exposure to multiple courses of corticosteroids and a reduction of head circumference or birth weight at birth has been found in some retrospective studies (Banks et al. 1999, Abbasi et al. 2000), but not in others (Shelton et al. 2001).

An increased risk of early-onset neonatal sepsis, maternal endometritis, or even neonatal death has been associated with multiple courses of antenatal steroids in retrospective studies (Banks et al. 1999, Abbasi et al. 2000, Vermillion et al. 2000). These associations were not confirmed in a randomized trial (Guinn et al. 2001). However, weekly courses of corticosteroids given in PPROM associated with an increased risk of clinical chorioamnionitis (Lee et al. 2004).
Table 6. Randomized, placebo-controlled trials on antenatal glucocorticoid treatment during the surfactant era.

<table>
<thead>
<tr>
<th>Study</th>
<th>Intervention</th>
<th>Number of infants treatment/placebo</th>
<th>Outcomes RR (95% CI)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Neonatal mortality</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RDS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IVH</td>
</tr>
<tr>
<td>Single treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kari 1994</td>
<td>6 mg DX 4 doses at 12 h interval</td>
<td>95/95</td>
<td>0.64 (0.19-2.21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.73 (0.52-1.02)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.38 (0.18-0.82)</td>
</tr>
<tr>
<td>Weekly repeated vs. placebo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amorim 1999</td>
<td>12 mg BM twice at 24 h interval</td>
<td>110/110</td>
<td>0.50 (0.28-0.89)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.53 (0.35-0.82)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.35 (0.15-0.86)</td>
</tr>
<tr>
<td>Garite 1992</td>
<td>12 mg BM twice at 24 h intervals</td>
<td>49/42</td>
<td>0.99 (0.47-2.10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.91 (0.65-1.26)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.64 (0.35-1.18)</td>
</tr>
<tr>
<td>Lewis 1996</td>
<td>12 mg BM twice at 24 h interval</td>
<td>39/40</td>
<td>1.03 (0.07-15.82)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.42 (0.20-0.90)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.15 (0.01-2.74)</td>
</tr>
<tr>
<td>Silver 1996</td>
<td>5 mg DX 4 doses at 12 h intervals</td>
<td>54/42</td>
<td>0.68 (0.27-1.73)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.98 (0.81-1.20)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1.14 (0.72-1.82)</td>
</tr>
<tr>
<td>Weekly repeated vs. single treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinn 2001</td>
<td>12 mg BM twice at 24 h interval</td>
<td>256/246</td>
<td>0.80 (0.59-1.10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.95 (0.71-1.25)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.03 (0.65-1.63)</td>
</tr>
<tr>
<td>Crowther 2006</td>
<td>11.4 mg BM one dose</td>
<td>567/577</td>
<td>0.82 (0.71-0.95)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.86 (0.55-1.35)</td>
</tr>
<tr>
<td>Wapner 2006</td>
<td>12 mg BM twice at 24 h interval</td>
<td>252/243</td>
<td>0.73 (0.44-1.20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.83 (0.43-1.61)</td>
</tr>
</tbody>
</table>

**Timing of repeated treatment**

The optimal timing of antenatal corticosteroids has been widely investigated in randomized and non-randomized studies. According to case-control studies, weekly repeated treatment may be effective in the prevention of RDS when administered more than 24 hours and less than a week before delivery (Abbasi et al. 2000, Celik et al. 2002), but the optimal timing has not been confirmed in all studies (Banks et al. 1999). Infants exposed to corticosteroids less than 24 hours before delivery do not seem to benefit from the treatment according to case-control studies (Celik et al. 2002).
Effects on the HPA axis

The influence of weekly repeated corticosteroid treatment on serum cortisol levels after birth may be more pronounced than that of a single treatment (Banks et al. 1999). Adrenocortical suppression has been found to persist in infants delivered 73-240 hours after the last dose of steroids (Banks et al. 1999), although another study of infants exposed to more than 3 courses of steroids did not show HPA axis suppression at the age of 24 hours compared to infants exposed to a single course or no treatment (Terrone et al. 1997). No suppression of HPA axis has been found at the age of 7 to 14 days after treatment with multiple courses of steroids (Ng et al. 1997, Ng et al. 1999).

Follow-up

In follow-up studies at the ages of two, three, and 10-12 years, no differences have been found in psychological and physical development or in the incidence of cerebral palsy between the treatment and placebo groups (Multiple authors 1984, Doyle et al. 1986, Smolders-de et al. 1990, Schmand et al. 1990, Salokorpi et al. 1997). According to randomized trials, infants exposed to steroids have similar growth characteristics at the ages of two to 12 years and in young adulthood compared to infants given placebo (Multiple authors 1984, Smolders-de et al. 1990, Salokorpi et al. 1997, Dessens et al. 2000). The treatment did not influence respiratory morbidity during the early years (Doyle et al. 1986) or pulmonary function at school age (Smolders-de et al. 1990). Although one follow-up study revealed a significant delay in the onset of puberty among boys exposed to steroids (Smolders-de et al. 1990), no difference was evident in the incidence of sexual development or genital problems at 20-year follow-up (Dessens et al. 2000). No differences have been reported in learning disabilities and behavioral disturbances between steroid and placebo groups at school age (Schmand et al. 1990) or in socioeconomic status and cognitive function in young adulthood (Dessens et al. 2000). Recently, the 30-year follow-up results of the first randomized trial were reported (Liggins & Howie 1972, Dalziel et al. 2005a, Dalziel et al. 2005b, Dalziel et al. 2006). The participants did not have any difference in the cardiovascular risk factors, including blood lipids, blood pressure, plasma cortisol, or the prevalence of diabetes or cardiovascular diseases. However, individuals exposed to betamethasone antenatally had higher plasma insulin concentrations at 30 min and lower glucose concentrations at 120 min.
after an oral glucose test, suggesting that antenatal steroid exposure may have long-term effects on glucose metabolism (Dalziel et al. 2005b). There was no difference in the incidence of asthma or other respiratory morbidity between the study groups (Dalziel et al. 2006). The treatment did not influence cognitive function, working memory and attention, or psychiatric morbidity in adulthood (Dalziel et al. 2005a).

No follow-up of randomized trials on multiple courses of antenatal steroids in preventing neonatal morbidity have been published so far. A retrospective follow-up study of infants during their first 4 years of life revealed no difference in growth, neuromotor development, or cognitive development or in the incidence of respiratory morbidity between infants exposed to more than 5 courses of antenatal steroids and controls (exposed to a single course or no antenatal steroids) (Hasbargen et al. 2001).

2.6 Postnatal glucocorticoid therapy

2.6.1 Early postnatal (<96 hours) dexamethasone for the prevention of BPD

Postnatal corticosteroid treatment in the prevention and treatment of BPD has been widely studied over the past few years. The efficacy of early treatment started within 3 days of life has been evaluated in 19 randomized trials reviewed in a meta-analysis (Halliday et al. 2003c). According to these randomized trials and the meta-analysis, early treatment significantly decreases the risk of BPD and/or death at the ages of 28 days and BPD or death at 36 gestational weeks (Table 7) (Rastogi et al. 1996, Yeh et al. 1997, Tapia et al. 1998, Garland et al. 1999, Romagnoli et al. 1999a, Halliday et al. 2003c). In addition, early treatment decreased incidence and treatment of PDA and severe retinopathy of prematurity (ROP) (Suske et al. 1996, Yeh et al. 1997, Kopelman et al. 1999, Garland et al. 1999, Multiple authors 2001, Halliday et al. 2003c).

effect on the incidence of IVH, but there was a non-significant association with an
increased risk of PVL among dexamethasone-treated infants (Halliday et al. 2003c). Early dexamethasone treatment significantly decreased the need for late
steroid treatment in established BPD (Rastogi et al. 1996, Romagnoli et al. 1999a,

2.6.2 Moderately early (7-14 days) and delayed (<3 weeks) postnatal
corticosteroids for the prevention and treatment of BPD

According to 7 randomized trials and a meta-analysis, postnatal steroids started
during the second week of life significantly reduce mortality and BPD at the ages
of 28 days and 36 gestational weeks (Durand et al. 1995, Papile et al. 1998,
Romagnoli et al. 1999b, Halliday et al. 2003a). The acute side effects
significantly associated with dexamethasone treatment were hypertension,
hyperglycaemia, hypertrophic cardiomyopathy, adrenocortical suppression,
infections, and gastrointestinal bleeding (Kari et al. 1993, Brozanski et al. 1995,

Delayed dexamethasone treatment decreased the risk of BPD or death at the
age of 36 gestational weeks and discharge home on oxygen therapy (Kothadia et
al. 1999, Halliday et al. 2003b). It also reduced failures in extubation by 7 and 28
days (Multiple authors 1991, Ohlsson et al. 1992, Halliday et al. 2003b). Delayed
treatment increased the risk of hypertension and suppression of the HPA axis, but
it did not increase the risk of gastrointestinal complications or infections (Wilson

2.6.3 Follow-up

According to follow-up studies of randomized trials, early postnatal
dexamethasone treatment is significantly associated with adverse effects on
neuromotor development, including an increased risk of cerebral palsy (Yeh et al.
dexamethasone exposure on respiratory morbidity in childhood is contradictory,
with some studies revealing no effect on the rate of respiratory infections or
pulmonary function during early childhood (Yeh et al. 1998, Lin et al. 2005) and
others reporting a significantly reduced risk of severe repeated respiratory
morbidity among dexamethasone-treated infants (Romagnoli et al. 2002).
Dexamethasone exposure during the neonatal period may have long-term
consequences on somatic growth. In a follow-up study dexamethasone-treated boys had significantly lower body weight and shorter height than control boys (Yeh et al. 1998), though no such association was evident in another study (Romagnoli et al. 2002). In a follow-up study of a randomized trial of a 42-day tapering course of dexamethasone, the infants in the treatment group had a significantly higher incidence of CP (O'Shea et al. 1999), although meta-analyses of trials on moderately early and delayed corticosteroid treatment revealed no difference in the incidence rates of CP or death in early childhood between the study groups (Halliday et al. 2003a, Halliday et al. 2003b). In a follow-up study of infants participating in a trial on early dexamethasone treatment, those exposed to early steroid had significantly poorer motor skills, motor coordination, and visiomotor integration at school age (Yeh et al. 2004). In addition, dexamethasone-exposed children had significantly smaller head circumference and shorter stature than children on placebo (Yeh et al. 2004). Postnatal corticosteroid treatment has not been found to be associated with neurosensory impairment (Halliday et al. 2003a, Halliday et al. 2003b, Halliday et al. 2003c). Although prematurely born children have high respiratory morbidity at school age, no difference was found in pulmonary or cardiac function between 8-year-old children given dexamethasone or placebo (Mieskonen et al. 2003). Recently, a small follow-up study was conducted on 15-year-old children who participated in a controlled trial of a 42-day course of dexamethasone treatment beginning at the age of 2 postnatal weeks and were dependent on mechanical ventilation at that time (Gross et al. 2005). According to this study, the children who were randomized to the 42-day course had significantly increased survival without severe neurologic, cognitive, or academic handicap when compared to children randomized to an 18-day course or placebo. In another follow-up study of delayed dexamethasone administration, the growth, respiratory, and neurologic outcomes were unaffected at teen age, although the overall rates of disabilities and educational problems were high in the study population (Jones 2005a, Jones 2005b). The effect of postnatal corticosteroids on a combined outcome of death or CP may be influenced by the initial risk of BPD during the neonatal period. In a meta-regression of 14 randomized studies, a significant negative relationship was found between the risk for death or CP and the rate of BPD in the control group (Doyle et al. 2005). Thus, postnatal corticosteroid treatment for the prevention or treatment of BPD significantly increased the risk of death or CP when the rate of BPD was less than 35% in the control group. On the other hand, the treatment
significantly improved the long-term outcome when the rate of BPD was more than 65% in the control group (Doyle et al. 2005).

### 2.6.4 Postnatal hydrocortisone

The first placebo-controlled trial on early hydrocortisone (HC) treatment in infants with RDS was published in 1972. The purpose of this study was to evaluate the effect of hydrocortisone in altering the course and outcome of RDS when started under the age of 24 hours with doses of 15 mg/kg twice at 12-hour intervals. There were no significant effects on short-term pulmonary morbidity, the need for mechanical ventilation, or mortality, although the mean PaO₂ tended to be higher and the mean PaCO₂ lower in the HC-treated group during the first 72 hours (Baden et al. 1972).

Since then, postnatal hydrocortisone has been used for the treatment of hypotension among preterm infants. In a randomized trial, hydrocortisone started with an initial dose of 2.5 mg/kg and continued with tapering doses for 6 days was equally efficient as dopamine in the treatment of volume-resistant hypotension among preterm infants with birth weight of less than 1500g (Bourchier & Weston 1997). The treatment was started at a mean age of 11 hours in the hydrocortisone group and 14 hours in the dopamine group. Three infants in the hydrocortisone group became hypertensive, and their medication was curtailed earlier than initially intended. No difference was seen in the incidence of BPD or symptomatic PDA between the hydrocortisone and dopamine groups. Four infants on hydrocortisone vs. one on dopamine had NEC, but this difference was not statistically significant (P>0.05) (Bourchier & Weston 1997). According to a retrospective review of 21 preterm infants with volume- and inotrope-resistant hypotension, hydrocortisone at doses varying from 2 mg/kg/d to 6 mg/kg/d administered for 3 days increased the mean blood pressure, decreased the use of volume expanders and inotropes, and simultaneously increased urine output approximately 6 hours after the start of the treatment (Seri et al. 2001).

Scientific interest in early hydrocortisone treatment as a way to prevent BPD among preterm infants was revived after widely published reports of the increased risk of adverse effects in dexamethasone treatment. The efficacy of early hydrocortisone treatment in the prevention of BPD among ELBW infants with birth weight under 1000 g was first evaluated in a pilot study published in 1999 (Watterberg et al. 1999). The study course was started before the age of 48 hours with hydrocortisone doses of 1 mg/kg/d for 9 days followed by 0.5 mg/kg/d for 3
days. The early hydrocortisone treatment increased survival without BPD at 36 weeks’ postmenstrual age. The results were also promising in that the infants treated with hydrocortisone had significantly fewer days on mechanical ventilation and supplemental oxygen (Watterberg et al. 1999).
Table 7. Randomized placebo-controlled trials on early corticosteroid treatment during the surfactant era.

<table>
<thead>
<tr>
<th>Study</th>
<th>Duration</th>
<th>Number of infants</th>
<th>RR</th>
<th>95% CI</th>
<th>RR</th>
<th>95% CI</th>
<th>RR</th>
<th>95% CI</th>
<th>RR</th>
<th>95% CI</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>steroid/placebo</td>
<td>Neonatal mortality</td>
<td></td>
<td>BPD at 36 weeks</td>
<td>Severe IVH</td>
<td>GI perforation</td>
<td>CP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early short DX treatment</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garland 1999</td>
<td>3 days</td>
<td>118/123</td>
<td>0.79</td>
<td>(0.46-1.36)</td>
<td>0.62</td>
<td>(0.35-1.09)</td>
<td>0.68</td>
<td>(0.39-1.19)</td>
<td>1.79</td>
<td>(0.73-4.38)</td>
<td>-</td>
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</tr>
<tr>
<td>Kopelman 1999</td>
<td>one dose</td>
<td>37/33</td>
<td>1.78</td>
<td>(0.68-4.68)</td>
<td>1.07</td>
<td>(0.36-3.18)</td>
<td>1.78</td>
<td>(0.59-5.38)</td>
<td>6.26</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sanders 1994</td>
<td>1 day</td>
<td>19/21</td>
<td>0.32</td>
<td>(0.07-1.34)</td>
<td>0.88</td>
<td>(0.28-2.82)</td>
<td>1.33</td>
<td>(0.48-3.65)</td>
<td>3.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shinwell 1996</td>
<td>3 days</td>
<td>132/116</td>
<td>1.24</td>
<td>(0.76-2.01)</td>
<td>1.20</td>
<td>(0.57-2.50)</td>
<td>0.62</td>
<td>(0.24-1.56)</td>
<td>2.78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sinkin 2000</td>
<td>1 day</td>
<td>189/195</td>
<td>1.25</td>
<td>(0.83-1.89)</td>
<td>0.82</td>
<td>(0.56-1.19)</td>
<td>1.08</td>
<td>(0.62-1.90)</td>
<td>1.55</td>
<td>(0.26-9.16)</td>
<td>3.38</td>
<td>(0.40-28.4)</td>
</tr>
<tr>
<td>Early prolonged DX treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lin 1999</td>
<td>28 days</td>
<td>20/20</td>
<td>1.25</td>
<td>(0.39-3.99)</td>
<td>0.33</td>
<td>(0.11-1.05)</td>
<td>1.33</td>
<td>(0.34-5.21)</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rastogi 1996</td>
<td>12 days</td>
<td>36/34</td>
<td>1.89</td>
<td>(0.37-9.65)</td>
<td>0.07</td>
<td>(0.00-1.24)</td>
<td>0.47</td>
<td>(0.09-2.41)</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soll 2001</td>
<td>12 days</td>
<td>273/269</td>
<td>1.21</td>
<td>(0.90-1.61)</td>
<td>0.73</td>
<td>(0.55-0.96)</td>
<td>0.75</td>
<td>(0.51-1.11)</td>
<td>1.53</td>
<td>(0.89-2.61)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Stark 2001</td>
<td>10 days</td>
<td>111/109</td>
<td>0.81</td>
<td>(0.50-1.31)</td>
<td>0.94</td>
<td>(0.70-1.27)</td>
<td>0.91</td>
<td>(0.56-1.48)</td>
<td>1.84</td>
<td>(0.89-2.61)</td>
<td>0.90</td>
<td>(0.41-1.95)</td>
</tr>
<tr>
<td>Suske 1996</td>
<td>5 days</td>
<td>14/12</td>
<td>0.86</td>
<td>(0.06-12.3)</td>
<td>0.29</td>
<td>(0.01-6.50)</td>
<td>-</td>
<td>(0.06-12.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tapia 1998</td>
<td>12 days</td>
<td>55/54</td>
<td>0.93</td>
<td>(0.54-1.60)</td>
<td>0.25</td>
<td>(0.07-0.82)</td>
<td>0.74</td>
<td>(0.27-1.98)</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yeh 1997</td>
<td>28 days</td>
<td>132/130</td>
<td>1.08</td>
<td>(0.77-1.52)</td>
<td>0.53</td>
<td>(0.33-0.87)</td>
<td>1.23</td>
<td>(0.72-2.10)</td>
<td>0.98</td>
<td>(0.06-15.6)</td>
<td>1.79</td>
<td>(0.89-3.59)</td>
</tr>
<tr>
<td>Early hydrocortisone treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watterberg 1999</td>
<td>12 days</td>
<td>20/20</td>
<td>1.00</td>
<td>(0.23-4.37)</td>
<td>0.50</td>
<td>(0.21-1.20)</td>
<td>2.00</td>
<td>(0.20-20.3)</td>
<td>1.00</td>
<td>(0.07-14.9)</td>
<td>0.50</td>
<td>(0.05-4.86)</td>
</tr>
<tr>
<td>Watterberg 2004</td>
<td>15 days</td>
<td>180/180</td>
<td>0.89</td>
<td>(0.48-1.63)</td>
<td>0.94</td>
<td>(0.61-1.45)</td>
<td>1.23</td>
<td>(0.69-2.09)</td>
<td>4.59</td>
<td>(1.51-13.9)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
3 Aims of the research

The purpose of the present work was to evaluate specific aspects of the efficacy and safety of antenatal and postnatal corticosteroid treatment given to very high-risk preterm infants or fetuses. We were particularly interested in steroid treatments given shortly after or shortly before birth. The specific aims were:

1. in a randomized trial, to study whether a short course of dexamethasone (DX) treatment given to extremely low birth weight infants increases survival without BPD, PVL, or severe intracranial hemorrhage (I);
2. in a meta-analysis, to evaluate whether the duration of early DX therapy given to very premature infants influences outcome or adverse effects (I);
3. in a randomized trial, to study whether the early low-dose hydrocortisone treatment of very low birth weight infants with respiratory failure improves survival without BPD (II);
4. to investigate whether the serum cortisol concentrations recorded before the initiation of hydrocortisone treatment predict the therapeutic response (II);
5. to evaluate the long-term safety of early low-dose hydrocortisone treatment, including its effects on growth and neurologic and neurosensory development in early childhood (III);
6. in a randomized trial, to investigate whether a single additional dose of betamethasone (BM) given in the case of imminent preterm birth before 34 weeks of pregnancy, and at least seven days after a full course of betamethasone treatment, increases survival without RDS or without severe IVH (IV).
4 Subjects and methods

The subjects, materials, and methods included in the studies I-IV are shown summarized in Table 8. These studies included three randomized, placebo-controlled, multicenter trials including one follow-up study (III) and a meta-analysis (I). The ethics committee of Oulu University Hospital, Finland, and Finland’s National Agency for Medicines approved the protocols of the trials II and IV, and the ethics committees of all participating hospitals approved the protocol of the multicenter study including university hospitals in four European countries (I). Written informed consent was obtained from the mothers and/or fathers of the infants. Participation in only one trial was allowed.

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Setting</th>
<th>Intervention</th>
<th>Sample size</th>
<th>Primary outcomes</th>
<th>Number of subjects</th>
<th>Reason for interruption</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Double-blind randomized, controlled trial in six neonatal units</td>
<td>Clinical trial</td>
<td>4 doses of DX 0.25 mg/kg at 12 hr intervals</td>
<td>192</td>
<td>Survival without severe IVH, PVL or BPD</td>
<td>109 infants</td>
<td>Other studies reported neurodevelopmental problems in DX-treated infants</td>
</tr>
<tr>
<td>I</td>
<td>Double-blind, randomized controlled</td>
<td>Meta-analysis</td>
<td>DX treatment initiated within 3 days after birth</td>
<td>Mortality and incidence of BPD</td>
<td>16 trials</td>
<td>2685 infants</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Double-blind randomized, controlled trial in 3 national centers</td>
<td>Clinical trial</td>
<td>Hydrocortisone 2.0 mg/kg i.v. 2 days, 1.5 mg/kg i.v. 2 days, 0.75 mg/kg, i.v. 6 days</td>
<td>320</td>
<td>Survival without BPD</td>
<td>51 infants</td>
<td>Increased risk of GI perforation in HC-treated infants</td>
</tr>
<tr>
<td>III</td>
<td>Double-blind randomized, controlled</td>
<td>Follow-up of study II</td>
<td></td>
<td>45 children</td>
<td>98% of surviving children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Double-blind randomized controlled trial in 8 national centers</td>
<td>Clinical trial</td>
<td>12 mg dose of betamethasone i.m.</td>
<td>440 mothers</td>
<td>Survival without BPD or severe IVH</td>
<td>249 mothers</td>
<td>Proposed by Committee for Safety Concerns</td>
</tr>
</tbody>
</table>
Study I. This randomized, blinded, placebo-controlled trial involved 109 preterm infants recruited from six neonatal units in four European countries. Written informed consent was obtained from the parents before enrolment. The entry criteria were birth weight between 500 and 999 g, gestational age of less than 31.0 weeks, and need for mechanical ventilation and supplemental oxygen by the age of four hours. The infants with life-threatening congenital anomalies or known chromosomal anomaly were excluded. Randomization and preparation of the coded vials was performed blindly by the study investigators in the pharmacy of the coordinating center. After stratification into two weight groups (500-749 g and 750-999 g), altogether 109 infants were recruited during the study period. The study was interrupted by the decision of the Steering Committee. The decision was based on the published studies reporting late neurodevelopmental problems in infants who had received postnatal DX, although the Safety Monitoring Committee recommended continuation of the trial, as no difference in early adverse events between the study groups was seen.

The treatment was started before the age of six hours and given from identical vials as four doses (DX 0.25 mg/kg) at 12 hr intervals or normal saline as placebo. The use of open-label postnatal DX was discouraged, but allowed when considered necessary by the attending physician.

Meta-analysis: The relevant articles were identified by searching for randomized, controlled studies from the MEDLINE and Cochrane databases, including the Controlled Trials Register. The search was performed using the following terms: steroids and preterm infants or dexamethasone and BPD, or CLD and dexamethasone limited to children aged <23 months. In addition, a manual search was done from the proceedings of American Pediatric Society/Society of Pediatric Research during the years 2000-2003 and the articles recently published in Pediatrics, The Journal of Pediatrics, European Journal of Pediatrics, Acta Pediatrica, Archives of Disease of Children, and Pediatric Pulmonology and from the reference lists of the publications. The search included randomized, placebo-controlled trials on premature infants with birth weight ≤ 2000 g who were blindly given DX or placebo before the postnatal age of 3 days. The principal investigators of the included studies were contacted for further information, if necessary. If the data was unavailable, the study was excluded from the meta-analysis of secondary outcomes. We found 2160 articles by using the aforesaid key words. The 15 articles of placebo-controlled randomized trials studying the efficacy of DX treatment starting before three days of age were selected. Early prolonged (>96 h duration) DX treatment was used in ten trials,
and early short (< 96 h duration) DX treatment in five studies, including the present report. The dosage varied from a single dose to six doses at 12-hour intervals in the early short DX trials, with the total dose of DX ranging from 0.2 to 1.5 mg/kg. In the early prolonged DX studies, the duration of treatment was 5 to 28 days and the total dosage 0.9 to 14.0 mg/kg.

Study II. This randomized, blinded, placebo-controlled trial involved three university hospitals in Finland (Oulu, Helsinki, and Kuopio). The entry criteria were birth weight of 501-1250 g, gestational age between 23 + 0 and 30 + 0 weeks, and need for mechanical ventilation before the age of 24 hours. The subgroup of infants with birth weight of 1000-1250 g had the additional criterion of supplemental oxygen and mechanical ventilation beyond 24 hours despite surfactant therapy. The exclusion criteria were lethal malformation or suspected chromosomal abnormalities. The infants received either HC (2.0 mg/kg i.v. divided into three doses at 8-hour intervals for 2 days, 1.5 mg/kg i.v. divided into three doses at 8-hour intervals for 2 days, and 0.75 mg/kg i.v. divided into two doses at 12-hour intervals for 6 days) or isotonic saline as placebo from identical syringes. Fifty-one infants were enrolled before the Steering Committee decided to discontinue enrolment based on a recommendation by the Safety Committee (Oulu 26 infants, Helsinki 20 infants, and Kuopio 5 infants). The decision to interrupt recruitment was made after the risk of gastrointestinal (GI) perforation in the HC group became evident in the present study and in another similar trial (Watterberg et al. 2004). The flow chart of the study design is shown in Figure 2.
Fig. 2. Study design and the main results of Study II. Randomization was performed in 6 groups according to birth weight. The rates of survival without bronchopulmonary dysplasia (primary outcome) and cerebral palsy are shown in the figure (n = affected infants/children and N = total number of infants/children).

Study III. The infants who initially participated in a randomized, controlled, blinded, multicenter trial studying the efficacy of early hydrocortisone in prevention of BPD (Study I) were recruited for a follow-up study. Altogether 45 surviving infants were enrolled in the follow-up at 2 years of postnatal age counted from the postmenstrual age of 40 weeks. The study patients were examined by a single paediatrician and/or paediatric neurologist in each of the three participating centers. Their overall development was evaluated using the Griffiths Developmental Score (Griffiths R. 1954). The psychometric evaluations were performed by neuropsychologists using the Bayley Scales of Infant Development II (BSID-II) (Bayley N. 1993), and speech was evaluated by a speech therapist. The growth characteristics, i.e. weight, height, and head circumference, were recorded. The additional questionnaire included data on
respiratory problems, infections, and medical history during the first two years of life.

Study IV. 249 pregnant women were recruited during May 2001 – March 2005 in the five Finnish university hospitals and in three central hospitals. Pregnant women with imminent preterm birth before 34.0 weeks were eligible if they had had their first complete course of antenatal corticosteroid (12 mg betamethasone given twice) at least 7 days before the trial entry. The specific criteria for the second course of betamethasone/placebo were: elective delivery within 48 hours as indicated by the clinical status of the mother and/or the fetus or a very high risk of spontaneous delivery within 48 hours. Exclusion criteria were maternal long-term systemic corticosteroid therapy, clinical chorioamnionitis, or lethal disease of the fetus. Randomization was performed centrally and stratified by the center. The study medication and placebo were prepared into masked identical syringes. The patients were stratified according to gestational age (less than 28 weeks + 0 days or between 28 weeks + 0 days and 34 weeks + 0 days) and the number of fetuses (singleton or multiple pregnancies). The eligible mothers received one 12 mg dose of either betamethasone or isotonic saline intramuscularly. The flow chart of the study design is shown in Figure 3.
Interim safety analysis was performed after recruitment had been going on for 3 years 8 months. Altogether 79% of the infant population were born within 24 hours after the intervention and 65% at 30 weeks of pregnancy or later. Based on the recommendation of the Safety Committee, an efficacy analysis was performed and the population was divided into the following strata: gestation at birth (<30 wk and ≥30 weeks) and treatment-to-delivery interval (<24 hours and ≥24 hours). According to the interim analysis, short exposure to BM associated with a decrease in intact survival. In addition, recruitment was slower than anticipated, and neonatal management changed during the enrolment period, including the increased use of prophylactic surfactant in the delivery room. There were additional concerns about the long-term side effects of the glucocorticoid. As proposed by the Safety Committee, the Board decided to terminate recruitment.
prematurely primarily on the basis of the safety concern. Altogether 159 infants were born in the betamethasone group and 167 in the placebo group, and they were all born before 36 weeks’ gestation.

4.1 Outcomes

*Primary outcomes (I-II, IV).* The primary outcome of the randomized trial in study I was survival at 36 weeks post-conception without IVH, grade 3-4, PVL, or BPD. In the meta-analysis included in study I, the primary outcomes were mortality and BPD. In the randomized trial on early hydrocortisone treatment (II), the primary outcome was survival without BPD. BPD was defined as need for supplemental oxygen consistent with signs of respiratory disease at 36 weeks’ gestation. PVL was defined as the presence of periventricular echodensities after the first week or periventricular cysts on a cranial ultrasound (de Vries et al. 1992), and severe intraventricular hemorrhage, grade 3-4, was defined as intraventricular bleeding with ventricular dilatation (grade 3) or parenchymal hemorrhage (grade 4) (Papile et al. 1978). The primary outcome of the trial studying the effect of an additional antenatal betamethasone dose in imminent delivery (IV) was survival without RDS or severe IVH during the first hospitalization episode (i.e. intact survival). RDS was defined as typical chest X-ray findings, need for continuous distending airway pressure and supplemental oxygen for at least 48 hours, or need for surfactant therapy in cases of established respiratory failure. The administration of surfactant in the delivery room was recorded but not included in the diagnosis of RDS. The infants were examined by cranial ultrasound between the days 4 and 8 and at 36 weeks of postmenstrual age in all these three trials.

*Secondary outcomes (I-II, IV).* The number of days on assisted ventilation, the use of supplemental oxygen, the length of open-label corticosteroid treatment, and the length of hospital stay were recorded. Nosocomial sepsis was defined as a positive blood or cerebrospinal fluid culture (I, II and III) or a strong suspicion of sepsis with clinical deterioration and increased infection markers (C-reactive protein, leucocytes, Study I) after day 3 of life. Diagnostic data on hyperglycemia requiring insulin treatment, hypo- or hypertension requiring therapy, PDA requiring prostaglandin inhibitor therapy or surgery, gastrointestinal (GI) bleeding, and GI perforations were recorded. Necrotizing enterocolitis was defined according to the criteria of Bell et al. (Bell et al. 1978) For the diagnosis
of retinopathy of prematurity (Multiple authors 1984), the first ophthalmoscopic examination was performed 4 to 7 weeks after birth or before 32 weeks’ postmenstrual age and thereafter until the retinas were mature or reached the ROP endpoint. The growth characteristics were measured at 7 and 28 days and at 36 weeks post-conception.

In study II, adrenocortical function was evaluated by the ACTH test, which was performed before the study intervention (i.e. before 36 hours’ age) and controlled on the day following the end of the intervention (day 11). Serum samples for cortisol measurements were collected immediately before (basal level) and 30 min after the i.v. administration of ACTH (Synachten, Novartis, Basel, Switzerland). The ACTH dose (0.1 μg/kg) was based on previous experience, and slightly lower (0.5-1 μg/1.73 m²) ACTH doses were given to premature neonates (Karlsson et al. 1999, Nykanen et al. 1999). Cortisol assays were performed after the interruption of the study.

4.2 Sample size calculations

Study I. Randomized trial. Sample size calculation was based on data from the Vermont-Oxford Neonatal Network database and on data from the Finnish pilot study performed at Oulu University Hospital. According to prospective analyses of the non-randomized data from Oulu, Finland, early DX seemed to increase the percentage of good outcomes from 40 to 65 percent. The present study was powered to show evidence of the improvement in favorable outcome from 45 to 65 % among all infants. Given the errors (α = 0.05 and β = 0.2), 96 patients in each group were required.

Study II. Sample size justification was based on unpublished data from Finland. HC treatment was expected to increase survival without BPD from 55% to 70% among the very high-risk preterm infants, which required 160 patients in each arm (α error 0.05 and β error 0.2).

Study IV. Sample size analysis was based on the Finnish perinatal morbidity and mortality statistics (National Research and Development Centre for Welfare and Health). The baseline rate of survival without RDS or severe IVH was 50 percent. The present trial was designed to detect an improvement in intact survival from 50 to 62.5%, which required a sample size of 220 women in each arm to reject the null hypothesis with α of 0.05 and a power of 80 percent (two-sided).
4.3 Statistical analysis

Randomized trials in the studies I-IV. Baseline data for the infants enrolled in the study were compared by unpaired *t*-tests for continuous variables and by chi-square tests for categorical data (I-IV). In outcome analysis, we calculated the odds ratios with 95% confidence intervals and also compared the differences between the treatment and placebo groups by chi-square tests for categorical data (I-IV). The correlations between the timing of antenatal steroids and cortisol values were tested using Spearman's correlation coefficient (II). The single continuous variables were compared by unpaired *t*-tests between the two groups, and the repeated measurements were analyzed by repeated measurements of ANOVA (I-IV) and by using summary measures (Matthews et al. 1990) (I). The Mann-Whitney U test was used when the data were not normally distributed (I-IV). Statistical analyses in all papers were performed using SPSS 12.0.1 for Windows (SPSS Inc, Chicago, IL).

Meta-analysis (I). The pooled risk ratios for event reduction with 95% confidence intervals for the outcome were calculated using the random-effect model (Mantel-Haenszel). Testing of within-study and between-studies variability was included in the analysis. The data were analyzed using Comprehensive Meta-analysis, version 1.0.25 (BioStat).

4.4 Authors’ role in the studies I-IV

The author performed the meta-analysis included in study I. She also participated in the statistical analysis and writing of the article on study I. The author was the primary investigator in the studies II-IV. The primary investigator closely participated in planning of the study protocols including the study design, randomization and data collection. She performed training of the other investigators, nurses, and doctors in the participating centers and served as a coordinating investigator between centers. The author recruited and examined the study patients, including follow-up examination in one center (Oulu), and collected the study data from all participating centers into a SPSS database for further statistical analysis. The author analyzed the results from the studies II-IV and was the primary author of these reports.
5 Results

5.1 Randomized trial of early dexamethasone treatment in the prevention of BPD among very preterm infants

The study was interrupted because of safety concerns about the long-term side effects on neurologic development. Altogether 109 infants were enrolled in the trial, which was 57% of the planned sample size. 53 infants received dexamethasone, and 56 were randomized into the placebo group. The baseline characteristics were comparable between the study groups, including gestational age and type of delivery. The incidence of survival without BPD, PVL, or severe IVH was 57% in the DX group and 45% in the placebo group, but this difference was not statistically significant (OR 1.27 with 95% CI 0.87-1.85). The mortality rate and the incidence rates of PDA and ROP did not differ between the study groups. The risk of gastrointestinal complications, i.e. NEC, GI perforation, or hemorrhage, was 15% in the DX group vs. 9% in the placebo group (RR 1.7, 95% CI 0.59 to 4.84, P = 0.4). The infants in the DX group had significantly higher blood pressure values during the first week (P=0.015), although the use of inotropes was similar between the study groups (P=0.81). Although early use of surfactant was common (91%) in both study groups, a significantly smaller proportion of infants in the DX group (42%) required more than one dose of surfactant compared to the infants in the placebo group (69%, P=0.006). Growth measured at birth and at the age of 36 weeks of gestation did not differ between the groups. Open-label glucocorticoid treatment was commonly used in both groups, being administered to 66% in the DX and 73% in the placebo group (RR 0.90 with 95% CI 0.70-1.2).

5.1.1 Meta-analysis

Our selection criteria for placebo-controlled randomized trials studying the efficacy of early (≤ 72h of age) DX treatment identified 15 articles. Early prolonged (>96 h duration) DX treatment was used in ten trials and early short (< 96 h duration) DX treatment in five studies, including the above report. In the early short DX trials, the dosage of DX varied from a single dose to six doses, and the total dose of DX varied from 0.2 to 1.5 mg/kg. In the early prolonged DX
studies, the treatment lasted for 5 to 28 days, and the total dosage varied from 0.9 to 14.0 mg/kg.

Early prolonged DX therapy significantly reduced the incidence of BPD at 36 weeks (Fig. 4), whereas the difference was not statistically significant between the DX and placebo groups in the meta-analysis concerning early short DX treatment (Fig. 5). There was no significant difference in neonatal mortality in the meta-analysis regardless of the duration of the treatment.

Both early short and prolonged DX therapy significantly reduced the risk of PDA. The risk of severe ROP was reduced in the early prolonged DX group, but there was no difference in the rate of total ROP. Hyperglycemia requiring insulin treatment increased in the DX arm regardless of the duration of DX therapy. The
incidence rates of gastrointestinal perforation were comparable between the study groups in the meta-analysis of early short DX therapy (4% in the DX and 2% in the placebo group, RR 1.83 with 95% CI 0.82-4.09). Early prolonged DX therapy was significantly associated with GI perforations (9% in the DX and 5% in the placebo group, RR 1.59 with 95% CI 1.02-2.46) and gastrointestinal bleedings (10% in the DX and 6% in the placebo group, RR 1.72 with 95% CI 1.17-2.53).

5.2 Randomized trial of early low-dose hydrocortisone treatment in the prevention of BPD among VLBW infants

Fifty-one infants were enrolled in this study; 25 infants randomly received hydrocortisone and 26 placebo. The demographic characteristics and respiratory status were similar at the time of enrolment (Table 8). The study was discontinued when 16% of the intended sample size had been recruited because of the increased risk of GI perforation in the HC group that became evident in the present study and in another similar trial (Watterberg & PROPHET Study Group 2004).

Table 9. Baseline characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hydrocortisone group</th>
<th>Placebo group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight – g, mean ± SD</td>
<td>888 ± 204</td>
<td>903 ± 220</td>
</tr>
<tr>
<td>Gestational age – wk, mean ± SD</td>
<td>26.7 ± 1.6</td>
<td>26.5 ± 2.8</td>
</tr>
<tr>
<td>Male sex – n (%)</td>
<td>16 (64)</td>
<td>14 (54)</td>
</tr>
<tr>
<td>Antenatal glucocorticoid therapy * – n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h-7 d before birth</td>
<td>12 (48)</td>
<td>11 (42)</td>
</tr>
<tr>
<td>&gt; 7 d before birth</td>
<td>2 (8)</td>
<td>4 (16)</td>
</tr>
<tr>
<td>Chorioamnionitis – n (%)</td>
<td>9 (36)</td>
<td>9 (35)</td>
</tr>
<tr>
<td>Pre-eclampsia, n (%)</td>
<td>4 (16)</td>
<td>6 (23)</td>
</tr>
<tr>
<td>PROM, n (%)</td>
<td>5 (20)</td>
<td>7 (27)</td>
</tr>
<tr>
<td>Multiple birth</td>
<td>5 (20)</td>
<td>7 (27)</td>
</tr>
<tr>
<td>Vaginal delivery - n (%)</td>
<td>14 (56)</td>
<td>13 (50)</td>
</tr>
<tr>
<td>Apgar scores – median (interquartile)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 1 min</td>
<td>4.0 (1.5)</td>
<td>4.5 (4.5)</td>
</tr>
<tr>
<td>At 5 min</td>
<td>7.0 (5.0)</td>
<td>6.5 (6.0)</td>
</tr>
<tr>
<td>Cord blood pH, mean ± SD</td>
<td>7.30 ± 0.08</td>
<td>7.23 ± 0.18</td>
</tr>
<tr>
<td>Surfactant, n (%)</td>
<td>24 (96)</td>
<td>23 (89)</td>
</tr>
</tbody>
</table>
Hydrocortisone-treated infants did not show a detectable increase in survival without BPD (64% vs. placebo 54%, OR 1.52, 95% CI 0.50-4.69), nor was there a decrease in BPD among the survivors (OR 0.53, 95% CI 0.17-1.71). However, the results should be evaluated with caution because of the possible type II error due to a small sample size. The overall incidence of survival without BPD was 60% in Oulu (51% of study patients) and 68% in Helsinki (39% of study patients). Two infants died in the HC group; one had a multiorgan failure after surgery for a GI perforation, and the other had neonatal sepsis. Two infants in the placebo group died of severe BPD and a third of pulmonary hemorrhage and sepsis. The risk of PDA was significantly lower in the HC group; 36% in the HC and 73% in the placebo group (OR 0.21 with 95% CI 0.06-0.68). In addition, HC treatment significantly shortened the duration of oxygen treatment (median 34 days in HC vs. 62 days in placebo group, P=0.02) and the mean airway pressure during the first 7 days (P=0.03, Fig.6). There was an increased risk of GI perforation in the HC group; four (16%) in the HC group and none in the placebo group (P=0.05). Three infants with GI perforation had PDA (2 treated with indomethacin, 1 with ibuprofen), and the fourth had spontaneous closure of the ductus. There was no difference in gastrointestinal complications between the study groups. Nor was there any detectable difference in hyperglycemia requiring insulin (36% vs. 27%, OR 1.53 95% CI 0.46-5.02). The lowest glucose concentrations on each day were similar between the study groups during the first week (P = 0.80).
5.2.1 Adrenocortical function

The ACTH test was performed on 41 infants at the baseline of the trial and on 46 infants after the HC treatment. The first ACTH test was incomplete in 3 patients. There was a non-significant correlation between the timing of the antenatal corticosteroids and basal (P = 0.06) or stimulated (P = 0.11) cortisol values soon after birth. The basal and ACTH-stimulated cortisol levels were similar in the study groups at the baseline and after the intervention.

The incidence of BPD or death at 36 weeks of postmenstrual age was lower in the HC-treated group among the infants with basal cortisol below the median (P = 0.02, Table 10). Similarly, the HC treatment decreased the incidence of BPD/death among the infants with ACTH-stimulated cortisol below the median compared to the placebo-treated ones (P = 0.04, Table 10). No difference was seen in the incidence of BPD or death among the patients with basal or stimulated cortisol values above the median.
Table 10. Risks of BPD or death and GI perforation in association with adrenocortical function before the intervention.

<table>
<thead>
<tr>
<th>ACTH response</th>
<th>BPD or death at 36 GW</th>
<th>GI-perforation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>Placebo</td>
</tr>
<tr>
<td>Basal cortisol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Below median</td>
<td>2/10†</td>
<td>7/10</td>
</tr>
<tr>
<td>(&lt;140 nmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Above median</td>
<td>5/10‡</td>
<td>3/10</td>
</tr>
<tr>
<td>(≥140 nmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min cortisol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Below median</td>
<td>2/9</td>
<td>7/10</td>
</tr>
<tr>
<td>(&lt;270 nmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Above median</td>
<td>3/10*</td>
<td>3/10</td>
</tr>
<tr>
<td>(≥270 nmol/l)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The stimulated cortisol value was missed in two patients; † one with GI perforation (later died of sepsis); basal cortisol 120 nmol/l and ‡ another with BPD at 36 GW; basal cortisol 160 nmol/l. The basal cortisol value was missed in * one patient with good outcome; stimulated cortisol 790 nmol/l.

In the subgroup with pretreatment cortisol levels below the median, the infants were intubated for a median of 3 days in HC group vs. 27 days in the placebo group (P = 0.08). The infants with higher cortisol levels generally required a shorter period of intubation without any difference between the HC and placebo groups (median 6 vs. 4 days, P = 1.0). HC decreased the mean airway pressures during the first week (P = 0.02) in the group of infants with endogenous cortisol levels below the median, whereas the infants with endogenous cortisol levels above the median had similar mean airway pressures in both study groups (P=0.58). There was no difference in systolic blood pressure during the first week between the HC and placebo groups with high (P = 0.89) or low serum cortisol levels (P = 0.90). Diastolic blood pressure revealed no difference between the study groups, either.

The infants with and without GI perforation were analyzed separately regardless of the study treatment. The median basal cortisol levels at the onset of the study were not different between the infants who developed GI perforation compared to those with no GI perforation. There was no significant difference in stimulated cortisol levels between the infants with later GI perforation and the ones with no GI perforation (median 419 nmol/l vs. 240 nmol/l, P = 0.126). Three of the 4 GI perforations occurred in the group of patients with basal cortisol above the median at the baseline. Three GI perforations occurred in the HC group with stimulated cortisol levels above the median; the 4th case of GI perforation had no stimulated cortisol value available.
5.3 Trial of early hydrocortisone treatment: 2-year follow-up

Forty-five of the 47 surviving infants were recruited into the follow-up study at the age of 2 years of postnatal age. The infants in the HC group were examined at a mean age of 24.1 ± 0.5 months and the placebo-treated infants at 23.7 ± 2.09 months. Growth characteristics are presented in Table 10. 50% of infants in the HC and 63% in the placebo group had been rehospitalized during the first two years. The incidence of severe respiratory disease, i.e. pneumonia, obstructive bronchitis, or stridor/asthma, was 52% in the HC vs. 47% in the placebo group. One patient in the HC group was followed up repeatedly because of aortal coarctation, as were two patients in the placebo group for congenital deformation of the larynx and urethral valve, respectively. In addition, one patient in each group had inguinal hernia after the first episode of hospitalization.

Two patients in the HC group developed cerebral palsy versus none in the placebo group (P=0.49). Both of these infants were born after severe birth asphyxia, and one of them had additionally been exposed to clinical chorioamnionitis antenataly.

The mean IQ score evaluated by the Griffiths developmental scale was 95 (± 21 SD) in the HC group and 98 (± 13) in the placebo group (P=0.54). The mean mental development index score (MDI) was 94 ± 19 for the HC infants and 100 ± 17 for the controls (P=0.26). The infants were divided into subgroups according to adrenocortical function soon after birth. Among the infants with serum cortisol levels below the median, the IQ score was 97 ± 7 in the HC and 96 ± 15 in the placebo group (P=0.82), and the respective MDI values were 98 ± 13 and 100 ± 15 (P=0.88). The HC-exposed infants in the subgroup with endogenous cortisol levels above the median had a mean IQ (92 ± 27) comparable to that of the placebo group (100 ± 13, P=0.35), and corresponding results were obtained for MDI levels (91 ± 22 in the HC vs. 100 ± 19 in the placebo group, P=0.29).
Table 11. Growth characteristics at follow-up.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Treatment group</th>
<th>Control group</th>
<th>OR or difference between means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at follow-up (months), mean ± SD</td>
<td>24.1 ± 0.5</td>
<td>23.7 ± 2.09</td>
<td>0.42 (-0.59 – 1.42)</td>
</tr>
<tr>
<td>Mean body weight (kg), mean ± SD</td>
<td>12.0 ± 1.8</td>
<td>11.9 ± 1.7</td>
<td>0.08 (-1.03 – 1.20)</td>
</tr>
<tr>
<td>Relative weight</td>
<td>-6.5 ± 9.06</td>
<td>-6.2 ± 9.01</td>
<td>-1.31 (-6.5 – 5.8)</td>
</tr>
<tr>
<td>Mean body height (cm), mean ± SD</td>
<td>86.6 ± 4.4</td>
<td>86.1 ± 3.5</td>
<td>0.55 (-2.02 – 3.12)</td>
</tr>
<tr>
<td>&lt;2.5 percentile, n (%)</td>
<td>2 (9)</td>
<td>2 (10)</td>
<td></td>
</tr>
<tr>
<td>2.5-97.5 percentiles, n (%)</td>
<td>20 (91)</td>
<td>18 (90)</td>
<td></td>
</tr>
<tr>
<td>Mean head circumference (cm), mean ± SD</td>
<td>48.5 ± 1.7</td>
<td>49.2 ± 1.5</td>
<td>0.70 (-1.76 – 0.35)</td>
</tr>
<tr>
<td>&lt;2.5 percentile, n (%)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>2.5-97.5 percentiles, n (%)</td>
<td>21 (95)</td>
<td>19 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation

5.4 Randomized trial of treatment with a single additional dose of antenatal betamethasone in imminent preterm birth: acute neonatal morbidity

A total of 249 mothers were enrolled: 125 into the BM and 124 into the placebo group. Altogether 159 infants were born in the betamethasone group and 167 in the placebo group.

The maternal and pregnancy characteristics were comparable between the study groups, including the length of gestation at the intervention and the time from the intervention to birth (Table 11). The infants in the BM group were delivered at a mean gestational age of 30.7 weeks vs. 31.0 weeks in the placebo group. All mothers delivered before 36 weeks of gestation. The birth characteristics of the infants were similar in the BM and placebo groups (Table 12).
Table 12. Maternal and pregnancy characteristics.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Betamethasone (N = 125)</th>
<th>Placebo (N = 124)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years), mean ± SD</td>
<td>32.3 ± 5.8</td>
<td>30.0 ± 5.5</td>
<td>P = 0.001</td>
<td></td>
</tr>
<tr>
<td>PROM, no. (%)</td>
<td>46 (37)</td>
<td>49 (40)</td>
<td>0.88</td>
<td>0.53 - 1.47</td>
</tr>
<tr>
<td>Pre-eclampsia, no. (%)</td>
<td>30 (24)</td>
<td>25 (20)</td>
<td>1.24</td>
<td>0.68 - 2.26</td>
</tr>
<tr>
<td>Vaginal delivery, no. (%)</td>
<td>48 (38)</td>
<td>44 (36)</td>
<td>1.11</td>
<td>0.66 - 1.85</td>
</tr>
<tr>
<td>Multiple pregnancy, no. (%)</td>
<td>32 (26)</td>
<td>39 (32)</td>
<td>1.33</td>
<td>0.77 - 2.32</td>
</tr>
<tr>
<td>Triplets</td>
<td>4 (3)</td>
<td>6 (5)</td>
<td>0.65</td>
<td>0.18 - 2.34</td>
</tr>
</tbody>
</table>

Days from 1st ANC to intervention median (interquartiles) 16 (10-27) 17 (10-26) P = 0.43

Weeks of gestation at the intervention, mean ± SD 30.3 ± 2.6 30.7 ± 2.5 P = 0.18

Hours from intervention to birth median (interquartiles) 9 (3-23) 7 (3-23) P = 1.0

* PROM denotes premature rupture of fetal membranes for >24 hours, DM diabetes mellitus, and ANC antenatal glucocorticoid

Table 13. Infant characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Betamethasone (N = 159)</th>
<th>Placebo (N = 167)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks, mean ± SD)</td>
<td>30.7 ± 2.7</td>
<td>31.0 ± 2.5</td>
<td>0.24</td>
</tr>
<tr>
<td>Birth weight (g, mean ± SD)</td>
<td>1460 ± 500</td>
<td>1558 ± 487</td>
<td>0.08</td>
</tr>
<tr>
<td>SGA (BW &lt; -2 SD)</td>
<td>47 (30)</td>
<td>37 (22)</td>
<td>0.13</td>
</tr>
<tr>
<td>Head circumference (cm, mean ± SD)</td>
<td>28.1 ± 2.9</td>
<td>28.6 ± 2.8</td>
<td>0.12</td>
</tr>
<tr>
<td>Length</td>
<td>39.4 ± 4.1</td>
<td>40.2 ± 3.7</td>
<td>0.09</td>
</tr>
<tr>
<td>Male sex, no. (%)</td>
<td>82 (52)</td>
<td>100 (60)</td>
<td>0.18</td>
</tr>
<tr>
<td>Apgar at 1 min [md (iq)]</td>
<td>8 (6-9)</td>
<td>8 (6-9)</td>
<td>0.86</td>
</tr>
<tr>
<td>Apgar at 5 min [md (iq)] †</td>
<td>8 (7-9)</td>
<td>8 (7-9)</td>
<td>0.87</td>
</tr>
<tr>
<td>Cord blood pH (mean ± SD) ‡</td>
<td>7.31 ±0.08</td>
<td>7.32 ± 0.07</td>
<td>0.40</td>
</tr>
</tbody>
</table>

* SGA denotes birth weight <-2 SD of the mean for given gestation. † 122 infants in the BM and 123 in the placebo group.‡ 149 infants in the BM and 159 in the placebo group.

Intact survival, i.e. survival without RDS or severe IVH, was 49 percent in the BM and 52 percent in the placebo group (OR 0.84 with 95% CI 0.55 – 1.30). Among all infants born at 28 weeks or later, the intact survival rates were 55 percent in the BM group and 62 percent in the placebo group (OR 0.73 with 95% CI 0.42 – 1.28) (Table 13). The overall rate of intact survival was 59-68% in the three centers which recruited 85% of the study patients (Helsinki recruited 53%, Oulu 19%, and Tampere 13%).
### Table 14. Intact survival.

<table>
<thead>
<tr>
<th></th>
<th>BM</th>
<th>Placebo</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/N</td>
<td>n/N</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Intention to treat analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>76/159</td>
<td>87/167</td>
<td>0.84</td>
<td>0.55 – 1.30</td>
</tr>
<tr>
<td><strong>Gestation at birth</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 28 weeks</td>
<td>2/25</td>
<td>0/26</td>
<td>2.50</td>
<td>0.73 – 8.58</td>
</tr>
<tr>
<td>≥ 28 weeks</td>
<td>74/134</td>
<td>87/141</td>
<td>0.73</td>
<td>0.42 – 1.28</td>
</tr>
<tr>
<td>Singletons</td>
<td>40/93</td>
<td>40/85</td>
<td>0.85</td>
<td>0.47-1.53</td>
</tr>
<tr>
<td>Twins and triples</td>
<td>36/66</td>
<td>47/82</td>
<td>0.89</td>
<td>0.47-1.72</td>
</tr>
<tr>
<td><strong>Post hoc analysis: intervention to delivery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-24 hours†</td>
<td>40/97</td>
<td>60/110</td>
<td>0.59</td>
<td>0.34-1.02</td>
</tr>
<tr>
<td>≥ 24 hours‡</td>
<td>20/34</td>
<td>15/33</td>
<td>1.71</td>
<td>0.65-4.51</td>
</tr>
</tbody>
</table>

† GA at birth (mean ± SD): BM 30.6 ± 2.7 weeks, Placebo 31.1 ± 2.4 weeks  
‡ GA at birth (mean ± SD): BM 30.8 ± 2.6 weeks, Placebo 30.9 ± 2.6 weeks. 18 infants were exposed to the intervention more than seven days before birth (8 infants in the BM and 10 infants in the placebo group)

The rates of mortality and RDS or severe IVH were similar between the BM and placebo groups. Of the 11 infants who died, 6 were multiples (4 cases of twin-to-twin transfusion), 1 had a lethal malformation, and 3 weighed <600 g. There was one fetal death in each treatment group, both representing twin pregnancies. In addition, one twin fetus had died before the intervention.

The growth characteristics, i.e. height (P=0.55), weight (P=0.65), and head circumference (P=0.76), were comparable between the BM and placebo groups during the first 28 days. Growth did not differ between the study groups when girls and boys were analyzed separately, either.

Other neonatal morbidity was comparable between the study groups. The 206 infants delivered 1 to 24 hours after the drug were subjected to a post hoc analysis. The intact survival rate was lower after BM when the intervention took place 1-24 h before birth (41 vs. 55 percent, P=0.05). In this subgroup BM increased the need for surfactant in RDS. The BM- and placebo-treated subjects born within 1 – 24 hours after the intervention were very similar to the rest of the study population with regard to both the pregnancy complications and the infant characteristics.
6 Discussion

6.1 Efficacy of early dexamethasone in the prevention of BPD among ELBW infants and influence of the duration of dexamethasone on outcome

The randomized trial on early dexamethasone treatment failed to demonstrate any detectable improvement in survival without major neonatal morbidity, i.e. BPD, severe IVH, and PVL, although the severity of acute respiratory disease was significantly milder in the DX group. The study was underpowered because it was interrupted at the point when 57% of the planned sample size had been recruited following published concerns about potential long-term side effects reported in the follow-up of randomized trials (Yeh et al. 1998, O'Shea et al. 1999, Shinwell et al. 2000). The high frequency of open steroid treatment in both study arms also contaminated the study and made the interpretation of the results complicated.

The previously published controlled trials and meta-analyses have shown that, despite the beneficial effects of postnatal dexamethasone treatment on the incidence of BPD (Rastogi et al. 1996, Yeh et al. 1997, Garland et al. 1999, Halliday et al. 2003a, Halliday et al. 2003b, Halliday et al. 2003c), the treatment has acute side effects, including hyperglycemia, hypertension, intestinal hemorrhage, and perforation (Yeh et al. 1990, Shinwell et al. 1996, Garland et al. 1999, Halliday et al. 2001, Stark et al. 2001, Halliday et al. 2003a, Halliday et al. 2003b, Halliday et al. 2003c). Although exogenous glucocorticoid given ante- and postnatally has been shown to have beneficial effects on preterm infants, the treatment should be evaluated critically for efficacy and safety, especially concerning dosage and duration. We approached this issue by conducting a randomized trial and making a meta-analysis of the early neonatal DX trials with the hypothesis that a short course would be beneficial and have minimal side effects. Since the dosage and duration of dexamethasone treatment in the prevention of BPD have varied widely in controlled trials, the meta-analysis focused on the duration of early DX treatment.

Previous meta-analyses have grouped studies according to the initiation of the intervention (Halliday et al. 2003a, Halliday et al. 2003b, Halliday et al. 2003c). In these meta-analyses, the course of DX treatment varied from one day to four weeks and was started within 2 hours to 15 days after birth (Halliday et al. 2003a, Halliday et al. 2003b, Halliday et al. 2003c). In our meta-analysis, we divided the
trials starting within 3 days after birth on the basis of the duration of DX into short (<96 h) and prolonged (>96 h) therapies. Based on this division, we could further evaluate the benefits and risks of the duration of early dexamethasone treatment. According to our meta-analysis, an early short course of DX therapy did not significantly decrease the risk of BPD or postnatal mortality at the latest reported age. On the other hand, early prolonged DX therapy had a significant beneficial effect by reducing the incidence of BPD, but no significant effect on mortality, which is consistent with the results of a previous meta-analysis (Halliday et al. 2003c).

Both short and prolonged DX treatments increased spontaneous closure of PDA, as has been shown in a previous meta-analysis (Halliday et al. 2003c). The risk of hyperglycemia was significantly increased in the DX group, regardless of the duration of the therapy.

Another well-known side effect of postnatal DX treatment is the increased risk of GI perforations (Halliday et al. 2003a, Halliday et al. 2003c). According to the present meta-analysis, this tendency was significant only among the infants on early prolonged DX therapy. Spontaneous GI perforation may have a non-inflammatory pathogenesis. The interference of steroid with rapid proliferation of gastrointestinal tissue could serve as a factor leading to intestinal perforation (Brownlee et al. 1992, Yeung & Smyth 2002). Another risk factor is the simultaneous use of NSAID drugs for the treatment of PDA, which increases the risk of gastrointestinal perforation (Nagaraj et al. 1981, Kuhl et al. 1985). Preterm infants exposed to prolonged DX treatment are more likely to be predisposed to both steroid and NSAID drugs during the corticosteroid treatment, which may increase the risk of gastrointestinal perforation.

There are concerns about neurodevelopmental complications in very preterm infants due to DX therapy in the perinatal period. Although antenatal DX has been shown to protect against IVH (Crowley 1995, Roberts & Dalziel 2006), the corresponding effect on the risk of severe or any grade of IVH was not confirmed in the present meta-analysis of postnatal DX. The incidence of PVL has been poorly presented in the previous randomized trials, as only 3 individual trials (including the present one) on early short DX therapy had data available for the evaluation of the risk of PVL (Shinwell et al. 1996, Garland et al. 1999). There was a non-significant tendency toward an increased risk of PVL, which was comparable to the findings of a meta-analysis where all the early DX trials were pooled together (Halliday et al. 2003c). The present results are consistent with the
possibility that even a short course of DX after very premature birth increases the susceptibility to a brain insult in a very premature infant.

6.2 Efficacy and safety of early low-dose hydrocortisone in the prevention of BPD and influence of early adrenocortical function on outcome

This study showed that hydrocortisone supplementation shortly after birth promoted spontaneous closure of PDA, shortened the duration of oxygen therapy and intubation, and decreased the severity of respiratory failure during the first week. The study was underpowered because of the early discontinuation of the trial for safety reasons. However, the results are comparable with those of a meta-analysis concerning early corticosteroid treatment (Halliday et al. 2003c). In a previously published pilot study of 40 infants, hydrocortisone supplementation appeared to reduce the development of BPD (Watterberg et al. 1999). According to the results of a larger multicenter, placebo-controlled, randomized trial, infants exposed to histologic chorioamnionitis significantly benefited from hydrocortisone in the prevention of BPD, but this effect was not seen in the whole study population (Watterberg et al. 2004). In a further analysis, hydrocortisone treatment was beneficial among infants exposed to antenatal inflammation compared to infants without fetal inflammation. In our study, chorioamnionitis was not associated with a favorable response to hydrocortisone treatment, but the small number of infants exposed to chorioamnionitis makes further speculation on this aspect difficult.

Regional differences in neonatal mortality and morbidity were detected in a Finnish nationwide cohort of ELBW infants during 1996-1997 (Tommiska et al. 2001). According to a repeated investigation in 1999-2000, differences in mortality were detectable between centers, although no regional differences in morbidity were seen later (Tommiska et al. 2007). In the present study, the influence of regional differences in the treatment strategies on outcomes was controlled by separate randomization in each participating center. The overall distribution of primary outcomes was similar between the centers, although the small number of study patients makes the comparison of outcomes to national cohorts difficult.

An association between postnatal corticosteroid treatment and an increased risk of gastrointestinal perforation has been reported in meta-analyses of early postnatal DX treatment, but not in randomized studies or meta-analyses of
moderately early and delayed corticosteroids (Kari et al. 1993, Durand et al. 1995, Brozanski et al. 1995, Kovacs et al. 1998, Halliday et al. 2003a, Halliday et al. 2003b, Halliday et al. 2003c). According to the present study and another independent study, early HC treatment increased the risk of GI perforation with a consequence that our project as well as Watterberg’s (Watterberg et al. 2004) study were interrupted as soon as the GI complications became evident. In two randomized trials studying the efficacy of hydrocortisone in vasopressor-resistant hypotension, no difference was found in the incidence of GI perforation between the study groups (Efird et al. 2005, Ng et al. 2006). The treatment of PDA with NSAID has been demonstrated to increase the risk of GI perforation among preterm infants (Nagaraj et al. 1981, Kuhl et al. 1985). Although HC decreased the risk of PDA and consequently reduced the need for NSAID, the combination of HC and NSAID was apparently responsible for the GI perforations. In adult patients, on the other hand, the combination of corticosteroids and NSAID increased the risk of GI bleeding more than twofold compared to the use of corticosteroid alone (Nielsen et al. 2001). The administration of DX simultaneously with indomethacin for the treatment of PDA was also associated with an increased risk of GI perforation among very preterm infants (Stark et al. 2001). In our trial, three of the four patients with GI perforation had been treated with HC and NSAID. On the other hand, preterm infants experience the most stressful situations during their first week of life and may thus be especially susceptible to spontaneous GI perforation.

The relationship between postnatal adrenocortical function and morbidity among preterm infants has been evaluated and discussed during the last few years, but no definite conclusion has been reached about adequate adrenocortical function among infants born before term. Low serum basal and/or stimulated cortisol levels during the first week have been demonstrated to associate with an increased risk of BPD (Watterberg et al. 2001), although the association has not been confirmed in all studies (Merz et al. 1998). Low adrenocortical function is also associated with severe hypotension and an increased risk of PDA (Watterberg et al. 2000, Ng et al. 2001, Ng et al. 2004). In our trial, the infants with low serum cortisol levels soon after birth benefited from HC treatment in the prevention of BPD. In the subgroup of infants with cortisol levels below the median, the HC-treated infants had significantly lower mean airway pressure during the first week and tended to have shorter intubation times compared to the infants on placebo. On the other hand, in the subgroup of infants with cortisol levels above the median, the incidence of BPD was apparently not different.
between the HC and placebo groups. This subgroup generally had a short intubation time with no difference between the study groups. Blood pressure levels were similar between the study groups regardless of the cortisol levels after birth, although the HC-treated infants tended to require vasopressors for shorter periods than the placebo-treated ones in the subgroups with low cortisol levels. However, this speculation is only hypothetical because differences were mostly nonsignificant between the HC and the placebo groups in these subgroups.

Randomized trials have been carried out on postnatal hydrocortisone as a treatment of refractory hypotension and adrenocortical insufficiency among preterm infants (Efird et al. 2005, Ng et al. 2006). In one of these studies, preterm infants benefited from a stress dose of hydrocortisone (3mg/kg/d) for 5 days for hypotension that did not respond to volume expanders and dopamine infusion (Ng et al. 2006). The rates of other morbidities were comparable between the study groups. In another randomized trial of 34 infants, early hydrocortisone treatment, started within 3 hours of life and lasting for 5 days, reduced the incidence of hypotension, i.e. the need for vasopressors (Efird et al. 2005). In this study, the treatment had no detectable effect on other outcomes, including BPD, PDA, IVH, and PVL. One infant in the HC group had gastrointestinal perforation within the first week vs. none in the placebo group. In the observational study, hydrocortisone treatment improved blood pressure without any adverse effects on cardiac function, systemic perfusion, or cerebral and renal blood flow among preterm and term infants with vasopressor-resistant hypotension (Noori et al. 2006). However, too few patients have been enrolled in these studies in order to allow evaluation of whether the treatment improves the outcome rather than just elevates blood pressure.

The follow-up studies of randomized trials on early dexamethasone treatment have revealed an increased risk of abnormal neurologic development in dexamethasone-treated infants, including an increased risk of CP (Yeh et al. 1998, Shinwell et al. 2000, Halliday et al. 2003c). In the present study of very preterm infants treated with either HC or placebo, no detectable difference was seen in neurological or overall development at the age of two years. Although two patients had cerebral palsy in the HC group and none in the placebo group, both of these infants had suffered from severe birth asphyxia, and the one with severe tetraplegia had additionally been exposed to clinical chorioamnionitis before birth. Both clinical chorioamnionitis and severe birth asphyxia are independent risk factors of CP (Wilson-Costello et al. 1998, Wu et al. 2003). No other follow-up results of randomized studies concerning postnatal hydrocortisone treatment
have been published so far. In an observational follow-up study of 60 preterm infants, postnatal hydrocortisone exposure was not associated with abnormal cerebral MRI findings or disorders in neurodevelopment compared to prematurely born children without steroid exposure (Lodygensky et al. 2005). The preterm infants as a group had a reduced volume of cerebral gray matter and an increased volume of cerebrospinal fluid compared to their term controls. According to another report of the same cohort, postnatal delayed hydrocortisone treatment lasting for a median of 26 days did not have any effect on memory or hippocampal metabolism at school age compared to children with no exposure to postnatal steroids (Rademaker et al. 2006).

In the present study, the rate of rehospitalization during the first two years of life corrected for gestational age was high in both study groups (50% in the HC and 63% in the placebo group). This finding is consistent with the earlier follow-up results. (Cunningham et al. 1991, Yeh et al. 1998, Chien et al. 2002, Halliday et al. 2003c) Although the incidence of respiratory morbidity did not significantly differ between the study groups, a higher proportion of infants with BPD (77%) had respiratory tract symptoms during the first two years of life (i.e. wheezing, pneumonia), whereas only 37% of the infants without BPD had respiratory symptoms. This is in accordance with the previous evidence showing that BPD increases the incidence of respiratory diseases in childhood. (Cunningham et al. 1991, Furman et al. 1996)

6.3 Efficacy and safety of a single repeat dose of antenatal betamethasone in the prevention of RDS and severe IVH in preterm infants

The original purpose of the present trial was to study whether a single repeat dose of BM given more than 7 days after the first course of corticosteroid improves the neonatal outcome of preterm fetuses. According to the results, a single repeat dose of antenatal betamethasone did not increase survival without RDS or severe IVH among preterm infants. In addition, the infants exposed to a repeat dose of betamethasone tended to require more surfactant for established RDS compared to the control group. This lack of efficacy is consistent with the earlier published randomized trials of weekly repeated courses of betamethasone (Guinn et al. 2001, McEvoy et al. 2002), but partly discrepant with the recently published randomized trials, in which weekly repeated betamethasone treatment significantly reduced neonatal morbidity (Crowther et al. 2006) or severe
respiratory disease (Wapner et al. 2006). It was noteworthy in present study that all mothers delivered before 36 weeks of gestation. In some other randomized trials studying weekly courses, the proportion of infants delivered after 37 weeks of gestation ranged from 16% to 37% (Crowther et al. 2006, Wapner et al. 2006), and up to half of the infants were delivered after 34 weeks of gestation in one study (Guinn et al. 2001).

The overall rate of survival without RDS or severe IVH was comparable between the three centers that recruited 85% of the study patients. As mentioned earlier, a Finnish cohort of ELBW infants recruited during 1996-1997 revealed regional differences in the morbidity and mortality of preterm infants (Tommiska et al. 2001). In the present trial, randomization was performed separately in each center to avoid the influence of regional treatment strategies on outcomes. On the other hand, the recruitment of study patients was performed during 2001-2005, and according to a later nationwide cohort study, no regional differences were detectable in the morbidity of ELBW infants in 1999-2000 (Tommiska et al. 2007).

The time from corticosteroid treatment to delivery clearly influences neonatal outcome. The most powerful evidence of benefit was obtained when the complete course was given more than 24 hours and less than 7 days before delivery (Liggins & Howie 1972, Roberts & Dalziel 2006). According to previous studies, a shorter interval after the first corticosteroid dose has been associated with decreased morbidity, as it may protect against cerebral complications (Kari et al. 1994a, Wright et al. 1995, Elimian et al. 2003). At the onset of the present trial, we presumed that the beneficial effects of a single repeat dose of antenatal BM would become evident even earlier, i.e. within 24 hours before birth. However, in a post hoc analysis of the present data, the infants exposed to a repeat dose of BM 1-24 hours before birth had a lower rate of intact survival, i.e. survival without RDS or severe IVH, and a trend toward a higher incidence of RDS than those born shortly after the placebo treatment. Corresponding results were reported from a previous study showing a higher risk of RDS among infants who received a single dose of BM less than 24 hours before birth (Schutte et al. 1980), although this finding is not unchallenged (Kari et al. 1994a). In the present study, on the other hand, the infants who received BM more than 24 hours before delivery tended to have a higher rate of intact survival and a lower incidence of RDS than the placebo-treated infants. This finding is consistent with two recent reports showing a significant reduction in RDS or the need for ventilatory support among infants exposed to weekly repeated BM (Crowther et al. 2006, Wapner et al. 2006).
In one of these studies, approximately 65% of the infants were exposed to one to two additional doses of BM antenatally (Crowther et al. 2006), whereas in the other study, 64% of the infants were exposed to 4 or more courses (Wapner et al. 2006). In this latter study, the group receiving one course remained in utero for a median of 5 days. Although the other trial did not report the time from the latest course to delivery, the infants were born on an average of 4 weeks after the study entry. Since, in the present study, the mean treatment-to-delivery interval was only 9 hours, it is likely that most of the infants in Crowther’s trial remained undelivered for more than 24 hours after the intervention.

Corticosteroids have important anti-inflammatory and cardiopulmonary effects during early adaptation (Scott & Watterberg 1995, Huysman et al. 2000). Corticosteroids regulate the inflammatory pathways by modulating the transcription of genes (Kallapur et al. 2003, Nie et al. 2005). Antenatal glucocorticoid causes acute suppression of the lung inflammation induced by intra-amniotic injections of endotoxin in pregnant ewes (Kallapur et al. 2003). Although this suppression is visible as early as one day after the treatment, the inflammation is paradoxically amplified 5 to 15 days after the treatment (Kallapur et al. 2003). Similarly, maternal betamethasone suppresses the monocyte function of preterm lambs 15 hours after the treatment, which is followed by augmented function 7 days after the steroid administration (Kramer et al. 2004). Maternal corticosteroid treatment has been shown to have a time-dependent effect on the inflammatory reactions of preterm infants, which may influence postnatal adaptation. In the present study, the subgroup of infants exposed to clinical chorioamnionitis tended to benefit from betamethasone given 1-24 hours before birth, whereas the infants without exposure to chorioamnionitis had a significantly lower incidence of intact survival without RDS or severe IVH (data not shown). The first dose of glucocorticoid given at least a week before the booster dose of BM may have increased inflammatory responsiveness. A repeat dose of BM shortly before birth may possibly inhibit the anti-inflammatory endocrine response that takes place shortly after birth.

Antenatal corticosteroids also influence the adrenocortical function of preterm infants. Fetal serum cortisol begins to decrease 6 hours after the treatment and reaches its lowest level 10-15 hours after a single dose of betamethasone (Ballard et al. 1980). Adrenocortical function begins to be restored approximately 24 hours after a single injection and is fully restored within 7 days after a complete course (Ballard et al. 1975, Ballard et al. 1980, Ng et al. 1997). Betamethasone is detectable in cord blood one hour after the injection and
continues to increase until 6 hours after the injection. Betamethasone is nearly undetectable approximately 20 hours after a single 12 mg dose (Ballard et al. 1975). However, despite accurate analysis, these measurements do not reveal the actual corticosteroid activity in the fetus.

Preterm infants are able to respond to stress (i.e. asphyxia, RDS, inflammation), and their serum cortisol levels increase shortly after preterm birth (Huysman et al. 2000, Banks et al. 2001). However, corticosteroid given shortly before birth may transiently suppress the HPA axis during early adaptation (Ballard et al. 1980, Ervin et al. 2000). The acute activation of the HPA axis shortly after birth may both moderate the acute inflammatory response to microbes and augment the adaptational changes required for neonatal transition.

Animal studies have revealed that repeated antenatal corticosteroid exposure results in growth restriction (Stewart et al. 1998, Jobe et al. 1998), and that, furthermore, the effect of corticosteroids appears to be dose-dependent (Bruschettini et al. 2006). In the present trial, the single repeat dose of BM did not affect growth during 28 days after birth. On the contrary, the infants exposed to weekly courses had lower birth weight or head circumference values compared to the placebo-treated infants (Kramer et al. 2004, Crowther et al. 2006). The proportion of growth-restricted infants was especially large in the subgroup exposed to more than 3 courses of corticosteroids (Wapner et al. 2006). According to the present evidence, a single repeat dose of BM given within 1 to 7 days before very preterm birth may decrease serious acute neonatal morbidity.
7 Conclusions

1. Is early steroid therapy of very high-risk VLBW infants effective and safe?

   a) Prevention of BPD

   The timing and duration of postnatal corticosteroid appears to be critical in the prevention of BPD. Early dexamethasone treatment was shown to be efficient in the prevention of BPD when the treatment lasted more than 4 days. The randomized trial concerning a 10-day course of early hydrocortisone showed a decreased risk of BPD in the risk groups of preterm infants who had low endogenous cortisol secretion soon after birth. Low serum cortisol levels were evident both among electively delivered infants and among those born after spontaneous labor with chorioamnionitis. The identification of infants with high HPA activity shortly after birth requires serum cortisol determination. The duration of treatment may be critical in the prevention of BPD. In the meta-analysis, short DX treatment failed to prevent BPD. Similarly, a 5-day early course of hydrocortisone may have no effect on the risk of BPD according to preliminary evidence from randomized trials of early hydrocortisone in the treatment of severe hypotension.

   b) Side effects

   An early 10- to 15-day course of hydrocortisone treatment was associated with an increased risk of gastrointestinal perforation especially when NSAID treatment was used during the hydrocortisone course. In contrast, the risk of GI perforation was not increased when the course of HC was limited to 5 days or less. In meta-analyses of early dexamethasone trials, the risk of GI perforation was significantly increased among infants exposed to early DX for more than 4 days. In the follow-up studies, early dexamethasone treatment was significantly associated with an increased risk of abnormal neurodevelopment, including cerebral palsy and abnormal cognitive development. The present small follow-up study of early hydrocortisone treatment did not reveal any detectable difference in neurologic or somatic development between the study groups. However, the present evidence is insufficient to warrant decisions about the efficacy and safety of early hydrocortisone treatment in the prevention of BPD. According to the present results, it is possible that preterm infants with low adrenocortical function after birth are at high risk of BPD and may benefit from early hydrocortisone
without acute or long-term side effects. However, the evaluation and determination of this risk group of preterm infants should be done in further randomized trials with sufficiently large samples and adequate follow-up.

2. Does repeat dosage of antenatal betamethasone prevent early neonatal morbidity?

The dosage of antenatal corticosteroids has been widely studied over the past few years in cases with imminent preterm delivery at least one week after the first complete course of corticosteroids. A single repeat dose of betamethasone (12mg i.m.) given in imminent threatened preterm delivery was not efficient in preventing RDS or severe IVH. The treatment was associated with an increased risk of acute respiratory morbidity when given less than 24 hours before delivery. The efficacy of a longer interval (>24 hours) could not been adequately evaluated due to the small sample size, although the trend toward benefit was evident. Weekly repeated courses of antenatal betamethasone tended to decrease the risk of severe RDS according to some randomized studies. However, repeated BM courses appear to have a negative influence on growth, especially among infants exposed to more than 3 courses of BM. A single repeated course of BM did not modify growth during 28 days after birth. The effect of antenatal corticosteroid on growth is probably dose-dependent. Repeat doses of BM may have additional adverse long-term consequences, programming the growth pattern or increasing the risk of metabolic syndrome, or even cause a risk of abnormal neurological outcome. In spite of the possible benefit of repeated steroid courses in the prevention of acute severe respiratory morbidity, the postnatal growth restriction associated with repeated courses is of concern. More accurate indication and timing of antenatal BM diminishes unnecessary antenatal glucocorticoid exposure of very high-risk fetuses. Limiting the single repeat dose of BM to a select group of very preterm pregnancies very likely to end in delivery within 1 to 7 days may deserve a further study. The possible long-term effects of repeated treatment should be evaluated in follow-up studies before any conclusions about the safety of repeated antenatal corticosteroids can be made.


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Outi-Maria Peltoniemi

CORTICOSTEROID TREATMENT IN THE PERINATAL PERIOD

EFFICACY AND SAFETY OF ANTENATAL AND NEONATAL CORTICOSTEROIDS IN THE PREVENTION OF ACUTE AND LONG-TERM MORBIDITY AND MORTALITY IN PRETERM INFANTS

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