Growth in infants with Bronchopulmonary Dysplasia, endocrine and pulmonary aspects

clinical and follow-up studies

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klinische en follow-up studies

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Aan mijn ouders

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Introduction

General introduction

In the last decades important advances in neonatal intensive care have been made leading to an increased survival of especially very preterm infants.[1] Major changes in survival rate have been attributed to the use of antenatal steroids and surfactant in the prevention and treatment of respiratory distress. In the United States survival rates of very low birth weight infants have increased between 1991 and 1996. The overall survival in VLBW infants increased slightly from 79.8% to 83% but major increases in survival have been reported in the lower weight groups of 501 to 750 g and 751 to 1000 g, respectively. Birth weight specific survival rates of infants born in 1991 and 1995/1996 have been published. Survival to discharge from the neonatal intensive care unit (NICU) increased from 42% and 81% for infants with a birth weight of 501-700 g and 751-1000 g in 1991, to 52% and 82% in 1996, respectively.[2]

Associated with the decline in mortality there was an increase in major morbidity over the 6-year interval partly due to an increase in chronic lung disease (CLD) defined as oxygen need at 36 weeks postmenstrual age. CLD increased from 19% to 23 % between 1991 and 1996 for the entire cohort of VLBW survivors. The largest increase in CLD from 41% to 56% occurred in infants born with a weight between 501-750 g.

Survival rates vary across countries as consequence of differences in obstetric and neonatal management leading to differences in major morbidity.[3] Therefore the data from the United States cannot be applied directly to the Netherlands, although a similar trend is observed.

In the Netherlands an increase in the incidence of VLBW infants between 1983 and 1993 from 8.4/1000 to 9.6/1000 live born infants has been documented, while simultaneously a decrease in mortality was observed.[4] Mortality for infants born in 1983 with gestational ages of 25, 26, 27 and 28 weeks, respectively, was 65, 62, 46 and 32%, respectively, and decreased to 49, 40, 23 and 21%, respectively for infants born in 1993. Both these trends, the increase in number of VLBW and the decrease in mortality are leading to more VLBW survivors and more infants with morbidities such as chronic lung disease/ bronchopulmonary dysplasia (BPD) or neurologic impairment. The incidence for infants surviving with BPD in the Netherlands can only be calculated from extrapolation from the incidence in the South-West region of the Netherlands. These calculations show a mean rate of surviving infants with BPD around 170/100.000 births/year.[5]

Definition/ Epidemiology BPD

Bronchopulmonary Dysplasia was first described by Northway *et al* in 1967 in a retrospective study as chronic lung injury developing after initial severe respiratory distress syndrome (RDS) in infants receiving artificial ventilation and 80-100% oxygen.[6] The clinical picture of 32 infants initially treated because of RDS was described together with the pathology of the non-surviving infants. Nine out of thirteen infants who were treated with high oxygen (80-100%) in excess of a 150 hours, survived beyond 4 weeks of age and all demonstrated chronic pulmonary disease. They had a gestational age between 30 and 39 weeks (mean 34 weeks) and a birth weight of 1474-3205 g (mean 2234 g). The clinical picture included the development of cyanosis without the use of supplemental oxygen, subcostal retractions, diffuse rales over the lungs and on X-ray examination rounded lucent areas in the lungs alternating with irregular dense strands.

This definition was changed in 1979 to the need for supplemental oxygen at 28 days after birth accompanied with radiological changes in infants who were ventilated in the first week of life for at least 3 days.[7] In 1988, as younger infants were surviving and the radiological abnormalities were not comparable with the description of Northway anymore, a new definition of BPD was proposed by Shennan stating that oxygen requirement at 36 weeks postmenstrual age, in infants with a birth weight of less than 1500 g was a better predictor of long-term respiratory outcome.[8]

The term chronic lung disease (CLD) was introduced by Koumbourlis in the 1990's to describe clinical, radiological and lung function abnormalities evolving during and persisting beyond the neonatal period in prematurely born infants.[9]

As practice on the NICU's has changed by the use of antenatal steroids, surfactant treatment and use of gentler ventilation strategies, preterm infants with a higher birth weight nowadays seldom develop BPD. (BPD: 5.6% when birth weight 1250 -1500 g, 12.2% when birth weight 1001-1250 g).[2] Presently, BPD occurs primarily in infants with a birth weight below 1000 g, in some of them even without preceding RDS.[10]

In 2001, a workshop on bronchopulmonary dysplasia initiated by the NICHD/NHLBI/ ORD reviewed the data on BPD and the epidemiological changes since the first description of BPD by Northway.[11] The consensus was to retain the name BPD instead of CLD because it is clearly distinct from the multiple chronic lung diseases of later life and a definition with classification of the severity of BPD was proposed. The BPD classification for infants born with gestational ages less than 32 weeks was defined preliminary on information from the NICHD Neonatal Network and on the data of Palta.[12] See for details Table 1.

By this definition the infants have BPD when they received oxygen treatment for at least 28 days after birth, when needed because of persistence of clinical features of respiratory failure such as tachypnea, retractions and rales. For instance, in infants born after a gestational age of less than 32 weeks needing supplemental oxygen for at least 28 days, the severity of the BPD is assessed at 36 weeks postmenstrual age or at discharge to home,

Table 1 Definition and classification of the severity of BPD.

Gestational age	**< 32 weeks**	**> 32 weeks**
Requirement	**O_2 treatment > 21% for at least 28 days**	
Time point of assessment	36 wk PMA or discharge to home, whichever comes first	> 28 d but < 56 d postnatal age or discharge to home, whichever comes first
Mild BPD	Breathing room air at 36 wk PMA or discharge, whichever comes first	Breathing room air by 56 d postnatal age or discharge, whichever comes first
Moderate BPD	Need for O_2 treatment < 30% at 36 wk PMA or discharge, whichever comes first	Need for O_2 treatment < 30% at 56 d postnatal age or discharge whichever comes first
Severe BPD	Need for O_2 treatment > 30% and/or positive pressure (PPV or NCPAP) at 36 wk PMA or discharge whichever comes first	Need for O_2 treatment > 30% and/or positive pressure (PPV or NCPAP) at 56 d postnatal age or discharge, whichever comes first

BPD: bronchopulmonary dysplasia, O2: oxygen, PMA: postmenstrual age, PPV: positive pressure ventilation, NCPAP: nasal continuous positive airway pressure.
By definition the infants have BPD when they received oxygen treatment for at least 28 days after birth and have persistence of clinical features of respiratory failure such as tachypnea, retractions and rales. Infants treated with oxygen > 21% and/or positive pressure for nonrespiratory disease do not have BPD unless they also develop parenchymal lung disease and exhibit clinical features of respiratory distress.
A day of treatment with oxygen > 21% means that the infant received oxygen > 21% for more than 12 hours on that day. Treatment with oxygen > 21% and/or positive pressure at 36 weeks PMA, or at 56 days postnatal age or discharge, should not reflect an "acute" event, but should rather reflect the infant's usual daily therapy for several days preceding and following these time points.
Adapted from NICHD/NHLB/ORD Workshop Summary 2001 Am J Respir Crit Care Med 163:1723-1729.[11]

whichever comes first. When, at that time point, the infants are breathing room air they have mild BPD, when they need oxygen treatment with a concentration less than 30% they have moderate BPD, and when they receive oxygen treatment of more than 30% or are ventilated they are classified to have severe BPD.

Although the terms BPD and CLD are both used in the literature describing persisting lung disease in preterm infants, within the framework of this thesis the term BPD is used according to the classification of the NICHD/NHLBI/ORD.[11]

Pathophysiology of BPD

Classic BPD

The classic BPD as defined by Northway, Rosan and Porter is characterized by airway injury, inflammation, fibrosis and smooth muscle hypertrophy in the airways as result of mechanical ventilation and high oxygen treatment.[6,13] Four stages were initially described. Stage 1, the acute phase lasting 2-3 days is indistinguishable from RDS with hyaline membranes, peri-

vascular and alveolar-septal edema. Stage 2 shows inflammation, eosinophilic exsudate in the airways, patchy squamous metaplasia of the epithelium of the large and segmental bronchi. Stage 3 is characterised by necrosis of bronchial and bronchiolar epithelium with inflammation, large amounts of debris within the bronchial and bronchiolar lumina, marked squamous metaplasia and hypertrophy of bronchial and bronchiolar smooth muscle, interstitial fibrosis, necrosis alveolar cells and early vascular changes. Stage 4, the most severe stage is characterised by peribronchial and peribronchiolar fibrosis, widespread squamous metaplasia, marked hypertrophy of bronchial and bronchiolar smooth muscle, large areas with atelectasis alternating with bullous emphysema, severe vascular changes with adventitial fibrosis and narrowing of arterial and arteriolar lumina.[14]

The spectrum of infants with BPD has changed in the era of surfactant treatment and after the introduction of antenatal steroids. Before the era of surfactant treatment airway injury, inflammation and parenchymal fibrosis were the prominent findings in BPD. Lungs of infants nowadays dying of BPD show less fibrosis and a more uniform inflation.[11]

New BPD

The lungs of infants nowadays dying of BPD usually only show minimal diffuse alveolar septal fibrosis and fewer, larger alveoli in both surfactant-treated and non-surfactant treated infants. There seems to be an arrest in acinar development.[15] Jobe published reviews about the new BPD describing the multifactorial pathogenesis.[16,17] Although BPD can also develop in term infants after severe ventilation because of persistent pulmonary hypertension of the newborn or meconium aspiration syndrome, most infants nowadays developing BPD are born after a gestational age of 26-28 weeks and have very immature lungs.

The human lung undergoes a transition from the canalicular stage to the saccular stage at about 22 weeks gestational age and the alveolar stage begins around 30-32 weeks (See Figure 1). Intrauterine as well as early postnatal factors have the potential of influencing lung development and lung function. Animal models of BPD show that oxygen treatment alone, but also mechanical ventilation after surfactant treatment without high oxygen treatment leads to a reduction in the number of alveoli in baboons.[18-20] A non-uniform inflation occurs with areas of atelectasis and hyperextension, inflammation and edema. In the overdistended regions the distal airspaces are dilated and appear as saccules. The walls of the terminal respiratory units are flat and straight because the units are not partitioned by alveolar secondary crests.

The baboon model demonstrates large simplified saccules with variable degrees of fibrosis and a decrease in alveolarization and vascular hypoplasia. This latter might reflect an arrest in vasculogenesis that is ongoing during the canalicular stage of lung development during which capillaries are formed in the mesenchyme and fuse with previously formed and

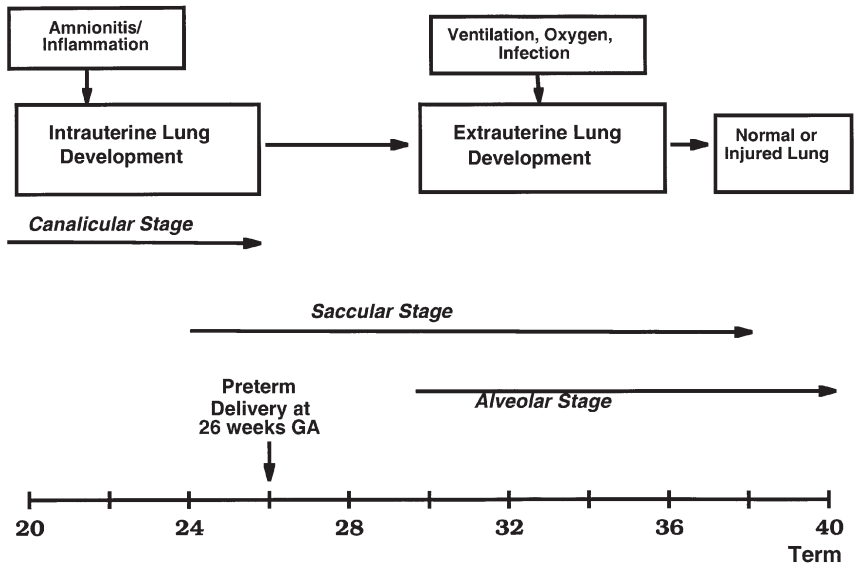

Figure 1 Stages of lung development.
Antenatal infection/ inflammation associated with chorioamnionitis can modulate lung development, as can postnatal causes of inflammation such as mechanical ventilation, supplemental oxygen or infection.
Adapted from Jobe AH and Ikegami M 2001 Resp Res 2:27-32.[17]

developing pulmonary arteries and veins.[19] Thus, there is a large decrease in surface area which is associated with a decrease in pulmonary microvasculature.

In infants dying of BPD the amount of lung elastic tissue and the diameters of saccular and ducts are increased reflecting similar pathology as demonstrated in the baboon model.[21]

The signals essential for normal regulation of alveolarization are not known. Alveolar development can be delayed by hypoxia, hyperoxia, glucocorticoids and poor nutrition and stimulated by thyroxin and retinoic acid.[22,23] Over-expression of cytokines such as tumour- necrosis-factor-α in the pulmonary epithelium results in fewer alveoli and inflammation.[24] In transgenic mice over-expression of transforming-growth-factor- α (TGF-α) results in fewer, larger alveoli and fibrosis, whereas over-expression of interleukin-11 (Il-11) in utero and in the early postnatal period results in fewer and larger alveoli. Over-expression of interleukin-6 (IL-6) induces a lymphocytic infiltrate in the lungs and fewer and larger alveoli.[25-27]

In humans elevated levels of proinflammatory cytokines like TNF-α, IL-1β, IL-6 and IL-8 in amniotic fluid within 5 days of preterm delivery are associated with an increased risk of BPD as is colonization at birth with Ureaplasma urealyticum.[28-30] Many very preterm deliveries are associated with a low-grade infection or inflammation of fetal membranes and amniotic fluid.[31] Histologic chorioamnionitis is associated with an early elevation of IL-1β in lung lavage fluid, a decrease in the incidence of respiratory distress syndrome but

with an increase in the incidence of BPD.[32] These clinical studies are consistent with the described interference of lung development by cytokines in transgenic mice.

Ventilation strategies also influence lung injury. Ventilation strategies with volumes approaching or exceeding total lung capacity, but also with volumes below a normal functional residual capacity result in edema and proinflammatory cytokines release in adult animals.[33] Prior exposure to endotoxin amplifies the ventilation-mediated injury.[34,35] Both conventional ventilation and high frequency oscillatory (HFO) ventilation interfere with septation. Although HFO results in somewhat better gas exchange, lung mechanics and lower pro-inflammatory cytokine levels, enhanced alveolarization is not observed.[36]

Glucocorticoids

Antenatal glucocorticoids

Animal studies

In the late 1960's Liggins was the first to suggest a positive effect of glucocorticoids on fetal lung maturation by enhancement of surfactant production in preterm lambs.[37,38] The effects of glucocorticoids on lung development have been reviewed by Bolt.[39] Glucocorticoids have effects on structural lung growth and development, on antioxidant enzymes, lung tissue growth factors, inflammatory mediators and the regulation of pulmonary fluid absorption. In mice glucocorticoid deficiency during pregnancy resulted in a delayed anatomic maturation of the lung parenchyma, which proceeded normally when the fetuses received supplemental glucocorticoids in utero.[40] The glucocorticoid deficiency resulted in overall hypercellularity and increased cell proliferation in the proximal and distal pulmonary epithelium and proximal mesenchyme.[41]

The effects of glucocorticoids on lung development seem to be time and dose dependent.[42] In monkeys, interstitial and epithelial lung maturation accelerated irrespective in which phase of lung development triamcinolone (weaker glucocorticoid) was administered, but alveolarization accelerated only when triamcinolone was given during the canalicular phase. Reduction in body weight and growth of the lung septa occurred when triamcinolone was administered early in gestation.

Thus, glucocorticoids have a beneficial effect on the surfactant system and on the maturation of the lung by thinning the mesenchyme, however, at the same time they can interfere with normal alveolarization.[22,43,44] The effects of glucocorticoids on the lungs and other organs seem to be gestational age and dose dependent.

Although several benefits of the use of antenatal steroids have been reported, adverse effects have been described such as diminished somatic, brain and lung growth and poorer brain function.[45] In sheep maternal betamethasone administration induces lung maturation but at the same moment growth retardation in the fetus. When, however, betamethasone

was administered directly to the fetus no growth retardation and less lung maturation occurred, despite higher plasma betamethasone levels in the fetus than after maternal treatment.[46] In monkeys, high doses of maternal betamethasone resulted in reduced length, body weight and reduced weight of brain, cerebellum, liver, pancreas, heart, adrenals, thymus, spleen, kidneys and lungs. When expressed in relative weight, the lungs, adrenals and spleen were even more reduced.[43] Apparently high doses glucocorticoids administered antenatally are capable of inducing growth disturbance in all organs, but especially in the lungs, adrenals and spleen.

Human studies

In 1972, Liggins and Howie suggested a positive effect of antenatal betamethasone on lung maturation by enhanced production or release of surfactant in preterm infants.[47] Ballard showed beneficial effects on the incidence of respiratory distress (RDS) and mortality when infants were delivered 2 to 10 days after antenatal glucocorticoid therapy and had a birth weight greater than 750 g.[48] In a review of all randomized trials on the use of antenatal glucocorticoids conducted between 1972 -1994 it was concluded that antenatal corticosteroids given between 48 hr and 7 days before preterm delivery, decreased the incidence of RDS by 40-50 % as well as intraventricular hemorrhage (IVH) and mortality.[49] However, in these trials very few pregnancies with a gestation less than 28 weeks were included. In 1995 the National Institute of Child Health and Human Development published a consensus stating that the benefits of antenatal administration of corticosteroids to fetuses between 24 and 34 weeks of gestation at risk for preterm delivery vastly outweighs the potential risks.[50] No evidence was found to prefer the use of either betamethasone or dexamethasone.[51]

In preterm infants an increase in surfactant phosphatidylcholine synthesis is observed after administration of glucocorticoids antenatally.[52] Antenatal glucocorticoids improve surfactant function in preterm infants by decreasing protein leakage in epithelial lining fluid, increasing secretion and rate of surfactant production at birth and by improving intra-alveolar metabolism of exogenous surfactant.[53-55]

The latest Cochrane review about the use of antenatal glucocorticoids included 18 trials with over 3700 infants. In these trials either 24 mg betamethasone, or 24 mg dexamethasone or 2 g hydrocortisone was administered to women expected to give preterm birth. Antenatal glucocorticoids (betamethasone or dexamethasone) decreased the incidence of RDS when infants were delivered between 48 hours and 7 days after administration, reduced the incidence of IVH and mortality, but did not have any effect on the incidence of BPD at 36 weeks or necrotising enterocolitis (NEC). No evidence was found to support or condemn the use of repeated doses of corticosteroids.[56]

Follow-up studies included in this Cochrane review, although reporting on infants born after a gestational age of 31-35 weeks, did not show any adverse effect on physical growth

or lung growth. However, hospitalization because of infectious disease in the first years was increased.[57-60]

As the use of antenatal steroids has increased to above 80-90% in mothers at risk for preterm delivery, new follow-up studies evaluating the long term effects of antenatal glucocorticoids in very preterm infants born after a gestational age of less then 28 weeks will be difficult due to the lack of a control group.

So far, in human studies the use of antenatal glucocorticoids is not associated with serious adverse outcome although the follow-up studies were performed on infants born after higher gestational ages than the infants nowadays treated at the NICU.

Postnatal glucocorticoids

Animal studies

Nowadays, most animal studies are focussed on the adverse effects of glucocorticoids. Numerous adverse effects of glucocorticoid treatment are presently known. Kamphuis reviewed the adverse effect of glucocorticoids on the cerebrum, hypothalamic-pituitary-adrenal (HPA)- axis and behaviour in the neonatal rat.[61] A permanent decrease in brain weight and DNA content in adulthood, a temporary inhibition of cell proliferation in cerebellum and dentate gyrus of the hippocampus, an effect on gliogenesis and myelination, and a delay in development of dendritic spines in the visual cortex was described in rats treated with glucocorticoids.[62-65]

The effect of glucocorticoids on the HPA -axis in rats, included a delay in maturation of the HPA-axis response to stress during development and at 20-25 days of age, thereby suggesting a long-lasting effect on the HPA-axis responsivity to stress.[66,67]

Glucocorticoids effect neurobehavioural development leading to permanent deficits in cognitive abilities, motor co-ordination, social behaviour, learning and memory processes later in life.[61]

In rats, neonatally treated with glucocorticoids a shorter lifespan was observed and this was most likely caused by end stage cardiac and renal failure as shown by histopathological examination.[68]

Human studies

Most human studies were focussed especially in the years after introduction of dexamethasone on the beneficial effects of glucocorticoids. Postnatal glucocorticoids are effective in weaning infants at risk for BPD from the ventilator. In infants developing BPD dexamethasone treatment decreases the high levels of cytokines in the broncho alveolar fluid, increases surfactant proteins and decreases albumin levels in tracheal fluid by decreasing the pulmonary microvascular permeability.[69-75] Thereby dexamethasone

improves respiratory compliance, reduces the need for oxygen supplementation and duration of mechanical ventilation.[76-79]

The effects of early (< 96 hours after birth), moderate early (7-14 days) and late (> 3 weeks) treatment with glucocorticoids in ventilated infants at risk for developing BPD have been reviewed by Halliday, Ehrenkranz and Doyle.[80-82]

Early glucocorticoid treatment (< 96 hours after birth) facilitates weaning from the ventilator, decreases the incidence of BPD at 28 days and 36 weeks as well as patency of ductus arteriosis and severe retinopathy of prematurity (ROP). It also reduces the combined outcome death or BPD at 28 days and 36 weeks and the need for later steroid treatment. No effect is observed on mortality, the incidence of air leaks, severe IVH, pulmonary hemorrhage, periventricular leucomalacia, infection, NEC, and the need for home oxygen therapy. However an increased risk is documented of gastrointestinal bleeding, intestinal perforation, hyperglycemia, hypertension, hypertrophic cardiomyopathy and growth failure. Long-term complications like an increased risk for abnormal neurological examination (RR 1.8, CI 1.33-2.47), developmental delay in one study (RR 1.68, CI1.08-2.61) and cerebral palsy (RR 1.69, CI 1.20-2.38) are observed, but no effect on the incidence of blindness, deafness or behaviour problems.[83-88]

Thus, early postnatal glucocorticoids result in short-term benefits such as earlier extubation and reduction of the incidence of BPD without an effect on mortality but with serious short tem adverse effects. The data on follow-up are limited with follow-up studies performed before school age and usually within the first 2 years of life. Regarding the serious short-term and long-term complications the benefits of early postnatal corticosteroid treatment may not outweigh the potential adverse effects. None of the reviewed studies had sufficient power to detect adverse long term neurosensory outcomes or looked for other somatic outcomes.

Moderate early dexamethasone treatment (7-14 days after birth) facilitates weaning from the ventilator, reduces mortality, decreases the incidence of BPD at 28 days and at 36 weeks as well as the combined outcome death or BPD and the need for late steroid treatment. No effect on the incidence of pneumothorax, severe IVH, NEC or severe ROP is demonstrated, but an increased risk was found for hyperglycemia, hypertension, gastrointestinal bleeding, infection, hypertrophic cardiomyopathy and infection. Four studies presented follow-up data, two by personal communication and one as article.[89] The incidence of cerebral palsy, major neurosensory disability, blindness, deafness was not increased.

Thus, the use of moderate early postnatal glucocorticoids reduces neonatal mortality and the incidence of BPD but has significant short-term adverse effects. The long-term follow-up data are very limited and so far do not show adverse effects.

Delayed dexamethasone treatment (> 3 weeks after birth) has a borderline effect on the incidence of BPD at 36 weeks, facilitates weaning from the ventilator and reduces the need for late steroid treatment and for home oxygen therapy. No effect on mortality,

gastrointestinal perforation or bleeding, NEC and infection, but an increased risk was found for glycosuria, hypertension and severe ROP.

The incidence of long-term complications as abnormal neurological examination in the total group (RR1.90, CI 1.08-3.33) and in survivors (RR1.73, CI 1.01-2.97) was increased. The importance of this finding is unclear as the incidence of cerebral palsy, blindness, deafness, major neurosensory disability and moderate to severe neurological impairment was not increased.[78,90-93] However, one study reported an increase in cerebral palsy at 1 year.[93]

Thus, delayed administration of glucocorticoids had a borderline effect on the incidence of BPD, facilitates extubation, but has no effect on mortality. Serious short-term adverse effects are found and serious long-term adverse effects are described in one follow-up study so far. Again, the long-term follow-up data are of limited quantity and quality.

Apart from the Cochrane reviews, several non-randomised studies have been published about adverse effects of glucocorticoid treatment in preterm infants. Adverse effects such as hypertension, hyperglycemia, cardiac hypertrophy, gastroduodenal perforation and catabolic effects such as increase in plasma amino acids and increased protein breakdown are reported.[76,77,94-99] Impairment of growth of length, weight but also of head-circumference has been associated with the use of glucocorticoids.[100-102] Infants treated with dexamethasone more often show nephrocalcinosis and abnormal general movements.[103,104]

In conclusion, the data of the Cochrane reviews show that glucocorticoids facilitate extubation and depending on the time of administration after birth have a beneficial effect on mortality and the incidence of BPD. Long-term follow-up data included in these reviews are very limited and mainly about neurological outcome. The non-randomised studies suggest numerous adverse effects of glucocorticoids, not evaluated in long-term follow-up studies, so far.

Adrenal gland

Fetus

During fetal life the adrenal glands grow rapidly and by term they reach an adult size weighing about 4 grams, each. Glucocorticoids are involved in fetal development and maturation and prepare various organs such as the lungs, liver and the immune system for the metabolic adaptations that are necessary for extrauterine life.[48] The fetal adrenal cortex develops into two zones, a large fetal zone compromising 80-90% of the volume and a smaller definitive zone. The cells of the fetal zone are in contrast to the definitive zone well differentiated for active steroidogenesis.

Adrenocortical cells produce cortisol, aldosterone, dehydroepiandrosterone (DHEA) and androstenedione. In these cells two branchpoints for steroidogenesis exists (See Figure 2).The first branch point is at the level of pregnenolone where the enzyme 3β-HSD

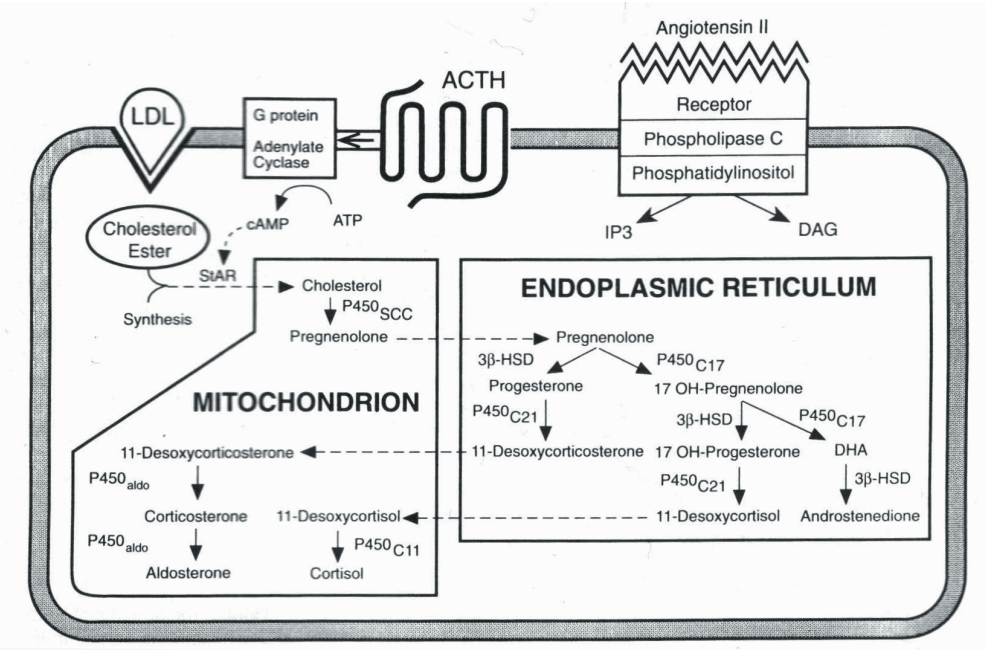

Figure 2 Pathways of steroid biosynthesis in the adrenocortical cell.
Steroidogenic enzymes: $P450_{SCC}$: cholesterol side-chain cleavage, $P450_{C17}$: 17-hydroxylase and 17,20 desmolase, $P450_{C21}$: 21-hydroxylase, $P450_{C11}$: 11β-hydroxylase, $P450_{aldo}$: 18-hydroxylase and aldosterone synthetase, 3β-HSD.
Adapted from Winter JSD 1998.[105]

converts pregnenolone to 17- desoxysteroids such as progesterone and aldosterone, while $P450_{c17}$ converts pregnenolone to 17OH-pregnenolone. The second branchpoint is at the level of 17OH-pregnenolone that can be converted to either cortisol or DHEA. The relative activities of these two enzymes determine whether 17OH-pregnenolone is converted to cortisol or to DHEA.[105]

The active fetal zone expresses abundant amounts of P_{450SCC} and P_{450C17}, but no 3β-HSD mRNA or protein. Thus the major secretion product of the fetal zone is dehydroepiandrosterone-sulphate (DHEAS). The outer definitive zone expresses small amounts of P_{450SCC} and produces little steroid in early pregnancy. Later in pregnancy some expression of P_{450SCC} and 3β-HSD occurs.

The major products of the fetal zone are several free Δ^5-3β-hydroxysteroids such as pregnenolone, 17OH - pregnenolone, DHEA and their sulfates. Lesser amounts of active Δ^4-3-ketosteroids such as cortisol are produced. The definitive zone, provides only a fraction of the total adrenal output but produces relatively more cortisol per cell.

Cord serum cortisol levels obtained at caesarian section without prior labor increase from 20 nmol/l at 13 weeks gestation to 150 nmol/l at term.[106]

Cord serum cortisol levels are influenced by input from fetal and maternal sources and by clearance through fetal and placental metabolism. About 60 - 75% of the cortisol in the fetal compartment originates directly from adrenal secretion, while the remainder derives from placental transfer of maternal cortisol or from conversion of cortisone.[107] Clearance from the fetal unit is extremely rapid with 80% being oxidized in fetal tissues and placenta to cortisone and its metabolites. About 60% of the cortisone in the fetal compartment is transferred to the mother either as cortisol or cortisone.

The fetus is relatively protected against maternal glucocorticoids by the placental enzyme 11β-HSD type 2, which rapidly converts active cortisol to inactive cortisone.[108] Placental 11β-HSD type 2 activity is regulated by several factors such as thyroid hormones and related with birth weight and severe fetal distress.[109] The function of 11β-HSD type 1 is to convert cortisone back into cortisol.[110] The level of expression and activity of 11β-HSD is determined by a delicate balance between stimulatory (such as estrogens) and inhibitory (progesterone and nitric oxide) influences.[111]

In contrast to cortisol, maternal dexamethasone and betamethasone pass the placenta without being inactivated by 11β-HSD type 2. Dexamethasone binds to the glucocorticoid receptor and suppresses the secretion of ACTH and indirectly cortisol but not the secretion of DHEAS.[112]

ACTH in the fetus derives from the fetal pituitary as maternal ACTH cannot cross the placenta. Fetal immunoreactive ACTH can be detected in the pituitary by 5 to 8 weeks gestation and responds to corticotrophine releasing hormone (CRH) and arginine-vasopressine as early as 14 weeks gestation. ACTH is secreted by the anterior pituitary gland and its secretion is regulated by CRH which is synthesized in the paraventricular nucleus and released from the median eminence of the hypothalamus. Cortisol has a direct negative feedback effect on CRH and ACTH release.[113]

Neonate

After delivery the influence of the placenta on cortisol metabolism has ceased and due to the considerable excess of the adrenal secretory capacity and the stress of delivery, free cortisol levels increase on the day of birth. As a consequence plasma ACTH levels decline and the neonatal adrenal cortex rapidly involutes. In the first 4 days of life the adrenals lose 25% of their mass and by 1 month they have decreased to 50% of their fetal size.

The Δ^5- 3β- hydroxyl steroids such as DHEA, pregnenolone and 16α OH-DHEA almost disappear from the circulation. Serum cortisol levels are high at birth around 500 nmol/l, decline immediately on day 1 to 100 nmol/l, tend to increase slightly at day 4 and 5 and thereafter decrease further to 90 nmol/l at the end of the first week.[105,114,115] Serum 17-hydroxyprogesterone (17OHP) levels, one of the precursors of cortisol are also high on the first day of life, decline immediately after birth and remain stable from day 2 until day

7.[114-117] Cortisone levels are in the same range as cortisol levels and decline immediately in the first 2 hours after birth and very gradually thereafter in the first week.

The circadian rhythm of cortisol secretion, also in very low birth weight infants, develops after 3 months and is present at 6 months of age.[118,119]

Nowadays, very preterm infants admitted to the NICU show signs of systemic hypotension accompanied by oliguria and edema, hyponatremia and hyperkalemia in the first postnatal days. This hypotension is resistant to volume expansion and cardiotonic drugs, but reacts to the administration of glucocorticoids.[120]

Serum cortisol levels in sick preterm infants and in non-ventilated infants show no correlation with the degree of illness.[119,121-124] Most of these infants were treated with antenatal glucocorticoids. In the past, short and long courses of maternal glucocorticoid treatment did not result in adrenal insufficiency.[125,126] However, in those days infants were tested with high doses of ACTH and were born after a higher gestational age than the preterm infants currently admitted to the NICU.

In conclusion, in the adrenals major changes occur during the adaptation from fetal to extrauterine life. Clinical symptoms suggestive for adrenal insufficiency have been described, however these were not supported by biochemical evidence by means of ACTH testing.

Growth

Fetal growth

Fetal growth has been stated to be influenced by insulin, insulin-like growth factor-I (IGF-I) and IGF-II, placental lactogen and to a limited extent by growth hormone (GH). Studies in IGF-I and IGF-II knockout mouse demonstrated growth-retarded embryos.[127] The reduction of birth length in GH-deficient infants suggests that GH does have some effect on fetal growth.[128] The exact role of various hormones and factors such as insulin, IGF-I and IGF-II, GH and leptin in promoting growth in utero and in early postnatal life, is still unknown.[129-131]

In the fetal circulation, pituitary GH can be determined from the 12th week of gestation. Levels increase until mid-gestation when the highest levels of GH are reached (119 ± 20 ng/ml), thereafter GH levels decrease to 33 ± 4 ng/ml measured in umbilical cord blood at term age.[132] Preterm infants have elevated levels of serum GH which decrease with advancing gestational and postnatal age.[133-137] At term, GH levels are still elevated compared to childhood but there is a rapid decline of GH pulse frequency, GH amplitude and nadir GH levels within the first postnatal days.[134,135,138] The typical childhood circadian pattern of GH secretion develops after 3 months and is present at 6 months of age.[139]

GH receptors mRNA and protein are present by the 15th week of gestation in fetal chondrocytes, osteoblasts, epidermis and fibroblasts, and GH receptors can be demonstrated in fetal liver, pancreas and brain in second trimester and in the placenta throughout gestation.[140,141] However, during gestation the fetus is relatively GH insensitive due to a reduced GH receptor expression. Postnatally, GH receptor expression is gradually upregulated.[142]

IGF-I is detectable in several tissues starting at 9 weeks gestation. By mid-pregnancy IGFs are abundant in many tissues as lung, intestine, kidney, muscle and skin, with the highest levels in the lung.[143,144] Around mid-pregnancy receptors for IGF-I and-II are present in nearly all fetal tissues.[145]

Infants born small for gestational age (SGA) have elevated GH levels and lower IGF-I levels in cord blood compared to appropriate for gestational age infants.[146-148] It has been reported that SGA infants and animals have smaller kidneys compared to their size than appropriate for gestational age infants, which is supposed to increase the risk for hypertension in later life.[149]

Postnatal growth in BPD

In preterm infants, especially in those with major morbidities such as BPD, early postnatal growth retardation is reported.[101,150,151] As growth retardation in general might also reflect disturbances in growth of organs such as lungs, kidneys and the brain, the implications of such a persistent growth disturbance might have long-term effects on the development, functioning and survival of many organs. One might hypothesize that very preterm infants developing early postnatal growth retardation might suffer from comparable reduction in organ development as intra uterine growth retarded fetuses.

Especially in the 1980's and beginning of the 1990's much attention has been given to the growth of infants with BPD. These early studies report an established growth failure in infants with BPD at term age.[150,151] Follow-up studies regarding growth in preterm infants with BPD show impaired growth of weight and length throughout infancy and childhood.[101,150-154] Vrlenich and Suave reported more often stunting of growth at 8 and 10 years when compared with either preterm or term controls.[154,155] Permanent growth failure has been reported in one long-term follow-up study.[156] However, these adolescents were born and treated in the 1980's, without the use of surfactant and postnatal glucocorticoids and with ventilation strategies different from those currently used.

Many studies have focussed on etiological factors causing the retardation of growth in infants with BPD. Several factors have been proposed such as increased energy consumption for breathing, high metabolic rate, early use of glucocorticoids, undetected hypoxia during night and after feeding, and difficulty to achieve recommended feeding intake due to gastro-oesofageal reflux, fluid restriction or respiratory infection.[153,157-159]

Nowadays, glucocorticoids are frequently administered to ventilated preterm infants to facilitate weaning from the ventilator. Adverse effects of DEXA on growth in preterm infants are reported. However, most studies are limited to the short-term adverse effects of dexamethasone (DEXA) and describe an inhibiting effect on weight gain, linear growth and head circumference.[100,101,160]

Very limited data are available documenting the pattern of growth and body composition in infants with BPD after discharge from the NICU.

Long-term lung function in BPD

During the 1970's introduction of continuous positive airway pressure (CPAP) and positive end expiratory pressure (PEEP) resulted in a decrease in mortality rates from RDS. The first studies reporting on lung function in children with BPD are confined to the pre surfactant era without use of antenatal steroids.

These studies reported expiratory flow limitation in infants with BPD persisting during their second year of life.[161] Gerhardt showed a gradual improvement in lung function parameters as functional residual capacity (FRC_p), compliance and conductance towards the normal range during the first 3 years of life.[162]

The first follow-up study on adult BPD survivors from the 1960s and early 1970's performed by Northway reported pulmonary dysfunction and complaints in most adolescents and young adults (76%). In these adult BPD survivors lung function tests showed airway obstruction (68%), airway hyperreactivity (51%) and hyperinflation.[156] These BPD survivors were born after a gestational age of 33 weeks and with a mean birth weight of 1899 g. Therefore, the stage of lung development at birth in those infants is not comparable with the more immature lungs of the infants nowadays admitted to the NICU and developing BPD.

In the 1980's conventional ventilation was used without surfactant. Follow up studies on lung function on infants from this period are now available. Gross reported on preterm infants with and without BPD (oxygen need at 36 weeks) at 7 years of age. Preterm children with BPD demonstrated airway obstruction worsening with exercise, while the preterm infants without BPD had a pulmonary function similar to that of healthy control infants.[163] Half of the children with and 25% of the preterm children without BPD had asthma defined as bronchodilator responsiveness at age 7 years. Kitchen and Doyle demonstrated an association between BPD (28 days oxygen) and subsequent airway obstruction with lower FVC, FEV1 and higher residual volume even after adjustment for birth weight.[164,165] Several studies reported obstructive lung disease, air trapping and bronchial hyperreactivity at school age in preterm infants with BPD born at the end of the 1970's to mid 1980's.[166-169] Other studies reported air flow obstruction in preterm infants with and without BPD.[170,171]

In the late 1980's and beginning of the 1990's surfactant treatment has been introduced. The influence of this treatment on the population of surviving infants and the long-term prognosis for BPD-infants, have not been studied, yet.[172] The effect of high frequency oscillation described by one study, the HiFi Study showed no improvement in pulmonary mechanics at 9 months of age compared to conventional ventilation.[173]

Aim of the studies

As a result of advanced care such as surfactant treatment and the use of antenatal steroids increasing numbers of very preterm infants survive. However, despite these advances in perinatal care bronchopulmonary dysplasia (BPD) still develops, nowadays mainly in younger and smaller infants. Infants with BPD have a higher neonatal and infant mortality rate and in survivors morbidity is increased especially in relation to growth, pulmonary and neurological function. As most ventilated infants who develop BPD are treated with dexamethasone and serious concerns have been raised about possible side effects of this drug also on the long-term, we performed several studies in these infants.

The following questions were raised:

1. In very preterm infants two clinical features attract attention. First, the development of BPD is associated with an ongoing inflammation in the lungs and secondly very preterm infants are increasingly treated with glucocorticoids because of circulatory problems. Both symptoms can be related to the adrenal function. Therefore the following questions were raised:
    a. Is there biochemical evidence suggestive for adrenal insufficiency in very preterm infants?
    b. Is there a relation between adrenal function in the first days after birth and the development of BPD?

To answer these questions cortisol and 17-OH-progesterone (17OHP) levels were measured at baseline and after ACTH stimulation in very preterm ventilated and non-ventilated infants 3 days after birth. Secondly, the data were correlated with severity of illness and the outcome of BPD.

2. Diminished growth is a well-known problem in infants at risk for BPD during their stay on the neonatal intensive care unit. It is unknown if this retardation of growth is related to changes in hormonal factors. Therefore we evaluated the GH-IGF-I axis in ventilated preterm infants developing BPD and raised the following questions:

a. Are disturbances in the GH-IGF-I axis responsible for the observed growth failure?
b. Is there a relation between serum GH levels and severity of illness and nutritional intake?
c. What is the effect of dexamethasone on the GH-IGF-I axis?

The study performed to answer questions 2a and 2b was conducted in ventilated preterm infants who could not be weaned from the ventilator and were developing clinical signs of BPD. A 6-hour growth hormone (GH) profile and serum IGF-I, IGFBP-1 and-3 levels were assessed and correlated with severity of respiratory failure and nutritional intake.

The study performed to answer question 2c was conducted in 10 infants who could not be weaned from the ventilator and in whom dexamethasone treatment was started in order to wean them from the ventilator. The GH-IGF-I axis was investigated immediately before start of dexamethasone and at day 2 of the initial high doses of dexamethasone treatment.

3. As dexamethasone was demonstrated to have a significant inhibiting influence on the GH-IGF-I axis and induces growth retardation in ventilated very preterm infants, we raised the following question:

   Is it possible to overcome the adverse effect of dexamethasone on growth by simultaneous administration of recombinant human growth hormone (rhGH)?

To answer this question a randomized placebo-controlled trial with recombinant human growth hormone in ventilated very preterm infants receiving dexamethasone in order to wean them from the ventilator was performed.

4. Diminished growth in infants at risk for BPD is a well-known problem. However, it is unknown to what extent growth retardation persists during the first year of life in infants nowadays treated on the neonatal intensive care, and what pattern of growth occurs.
   Therefore the following questions were raised:
   a. To what extent does growth retardation persist in infants who developed BPD?
   b. Does growth during the first year of life in infants with BPD, result in a body composition comparable to healthy infants of the same age?

The study performed to answer this question was started in 1996/1997 and included patients who had developed BPD. Patients were included during the following 2 years and prospectively followed on the neonatal follow-up clinic.

5. In infants with BPD born in the 1980's pulmonary function tests show obstructive airways and airtrapping. However, these children were born in a period without the use of antenatal steroids and without surfactant treatment. Nowadays, treatment on the neonatal intensive care has changed and smaller preterm infants survive and develop BPD.

   Therefore the following questions were raised:

   a. What is the development of the pulmonary function in infants who developed BPD, nowadays, during their first year of life?
   b. Is the pulmonary function and growth of the airways of the infants with BPD comparable to healthy infants, during the first year of life?

The study performed to answer these questions was in collaboration with the department of paediatric pulmonology. Patients with BPD were included during 2 years and prospectively followed on the neonatal follow-up clinic.

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2

Adrenal function in sick very preterm infants

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Abstract

Some very preterm neonates admitted to the neonatal intensive care unit show circulatory and respiratory problems that improve after administration of steroids. It is unclear whether these symptoms could be caused by adrenal insufficiency.

The objective of our study was to investigate the cortisol levels and the cortisol release from the adrenals after ACTH in very preterm infants with and without severe illness and to find whether a relation exists between adrenal function and outcome. An ACTH test (0.5 μg) was performed on d 4 in 21 very preterm infants (gestational age, 25.6 –29.6 wk; birth weight, 485–1265 g). Baseline cortisol and 17-hydroxyprogesterone (17OHP) levels and the cortisol levels 30, 60, and 120 min after ACTH administration were measured. The Score for Neonatal Acute Physiology was used to measure illness severity.

All infants showed an increase in cortisol levels after ACTH, but the cortisol levels were significantly lower in the ventilated more severely ill infants. After adjusting for birth weight and gestational age, the mean baseline cortisol levels and cortisol/17OHP ratios were significantly lower and the 17OHP levels significantly higher in the ventilated infants compared with the nonventilated infants. Patients with an adverse outcome had significantly lower baseline cortisol/ 17OHP ratios and 60-min cortisol levels during ACTH testing ($p = 0.002$ and $p = 0.03$, respectively).

Conclusion: These data suggest an insufficient adrenal response to stress in sick ventilated very preterm infants with gestational ages younger than 30 wk compared with nonventilated less sick preterm infants. Further studies are required to investigate whether supplementation with physiologic doses of hydrocortisone may benefit the outcome.

Introduction

With increasing neonatal and obstetric treatment modalities, younger and smaller neonates are admitted to neonatal intensive care units (NICU). Some of these infants show not only respiratory but also circulatory problems such as systemic hypotension resistant to volume expansion and cardiotonic drugs but reactive to the administration of corticosteroids.[1] Declining cortisol levels in the first week of life in patients with ongoing respiratory problems are reported, and a higher incidence of bronchopulmonary dysplasia (BPD) in patients with an insufficient cortisol response to ACTH has been found.[2,3]

In the 1970's, the adrenal function of preterm neonates was investigated after administration of a short course of corticosteroids during gestation, and no signs of adrenal insufficiency were found.[4] These preterm neonates, however, were born after a greater gestational age, had a higher birth weight than the infants presently seen in the NICU, and were tested with a relatively high dose of ACTH (15 μg/kg) on the first day of life. In infants exposed to long courses of corticosteroids during gestation, no evidence of adrenal insufficiency was found when tested with a higher dose of ACTH (36 μg/kg).[5] These infants, however, were of greater gestational age compared with the very preterm infants now treated at the NICU.

Insufficient cortisol production may not only lead to disturbed vital signs such as low blood pressure and poor glycemic control but also may influence illness severity and outcome.

According to previous studies, cortisol and 17OHP levels are high on the first day of life. Thereafter, cortisol levels decrease immediately and tend to increase at d 4 and 5, whereas 17OHP levels decline immediately after birth but remain stable from d 2 until 7.[6–9] Recent studies show low cortisol levels in ill very preterm infants, whereas others find low cortisol levels that may be explained as physiologic as these infants no longer required mechanical ventilation.[10-14] Cortisone levels decline immediately in the first 2 h and very gradually thereafter in the first week of life. The levels between term and preterm infants are not significantly different. Corticosterone levels are much lower than cortisol levels, increase in the first 2 h, decrease until 6 h, and decrease again to 24 h, after which a slight increase is seen.

The aim of the present study was to investigate the release of cortisol and the precursor 17OHP from the adrenals of very preterm infants under conditions of stress compared with the nonstressed situation and to evaluate whether a relation exists between the cortisol release on d 4 of life and the development of BPD, severe cerebral complications, and mortality.

Methods

Patients

Twenty-one patients admitted to the NICU of the Sophia Children's Hospital were included in the present study. The inclusion criteria were immediate admission at the intensive care unit after birth, gestational age younger than 30 wk, and indwelling arterial line. Exclusion criteria were a maternal history of endocrine disorders such as diabetes, thyroid or adrenal problems, or congenital malformations. The present study was approved by the local Medical Ethics Committee, and informed consent was obtained from the parents of each child.

Methods

Infants were studied on d 4 of life. Most of the infants were tested at 0800 h, but some were tested in the afternoon. A standard dose of 0.5 μg of ACTH was given. ACTH was diluted with saline to a concentration of 1 μg/mL in a plastic syringe and immediately given i.v. by a short venous cannula and a connection close to the patient.

Baseline blood samples for cortisol, 17OHP, and C/17OHP were taken. Cortisol was also measured 30, 60, and 120 min after administration of ACTH. All blood samples were taken from an indwelling arterial line. Oxygen saturation was measured continuously by pulse oxymetry (Nellcor 3000), ventilation settings were recorded every 30 min during testing, and arterial blood pressure was measured continuously.

Illness severity was measured by the SNAP score.[15] We performed this score on d 4 to determine whether other pathophysiologic parameters such as blood pressure, frequency of bradycardia, disturbances in glucose, in serum electrolytes, and liver or kidney function, often seen in more severely ill patients and not always accompanied by higher ventilation settings, were related to the adrenal function. Although the SNAP score is designed to reflect the severity of illness on d 1, we believe that the score on d 4 also reflects the severity of illness on d 4.

BPD was defined as persisting respiratory problems with a need for oxygen at d 28 and an abnormal chest x-ray.[16] Adverse outcome was defined as mortality or severe morbidity defined by severe cerebral problems (intracranial hemorrhage grade 3 or more and severe cystic leukomalacia) and/or BPD.

Hormone Assays

Serum cortisol was determined by using a commercial chemiluminescent labeled antibody assay (Nichols Institute Diagnostics Cortisol Kit, San Juan Capistrano, CA, U.S.A.). The intraassay coefficients of variation were 3.1–4.8%, and the interassay coefficients of variation were 6.2–10.2%. Cross-reactivity with cortisone is unknown within physiologic ranges. Hyperbilirubinemia did not influence the assay.

The precursor 17OHP was determined by RIA (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA, U.S.A.) (Laboratory Internal Medicine, Academic Hospital Rotterdam, The Netherlands; Head, Prof.Dr. F. de Jong). The interassay coefficient of variation was 10% for 17OHP. The main cross-reactivity in the 17OHP assay occurred with progesterone 5%.[17]

Statistical Analyses

Statistical analyses were made by SPSS package, version 8.0 Windows, released in 1997. Results are expressed as mean (SD) unless indicated otherwise. Linear regression analysis was used to examine the relations between determinants such as birth weight, gestational age, birth weight SD-score (SDS), illness severity, and ventilation and continuous outcome variables such as cortisol, 17OHP, and C/17OHP. The relation with ventilation, BPD, and adverse outcome was adjusted for gestational age and birth weight. The baseline 17OHP data showed a skewed distribution and were log transformed for statistical analyses. A p value < 0.05 was considered significant.

Results

Twenty-one patients were enrolled in the study (14 male, seven female); the patients had a mean gestational age of 27.7 (interquartile (i.q.) range, 26.4 –28.9 wk), a mean birth weight of 887 g (i.q. range, 708–1005 g), a mean SD-score for birth weight of -1.43 (i.q. range, -2.82 to -0.30), and a mean weight at day of testing of 845 g (i.q. range, 738–933 g).

Sixteen patients received antenatal betamethasone, and 10 patients were artificially ventilated during ACTH testing. The ventilated and nonventilated group were not significantly different except for gestational age, birth weight, and SNAP score $p = 0.03$, $p = 0.03$, and $p = 0.003$, respectively). The ACTH dose expressed in µg/kg was not significantly different between the groups. Five patients died in the neonatal period.

Baseline Cortisol Levels

The mean (SD) baseline cortisol level for the total group was 277 (144) nmol/L. The baseline cortisol levels were not influenced by the mode of delivery or the presence of preeclampsia of the mother and did not correlate with gestational age, birth weight, and birth weight SD-score.

In boys, the mean baseline cortisol level was 116 nmol/L lower than in girls ($p = 0.21$). In the patients treated with antenatal steroids, the mean baseline cortisol level was not significantly lower compared with the group without use of antenatal steroids.

Cortisol Response after ACTH

The mean (SD) cortisol levels 30 and 60 min after ACTH administration were 558 (180) and 753 (250) nmol/L, respectively. Some infants continued to have high cortisol levels at 120 min, but others already showed a decline. The mean (SD) cortisol level at 120 min was 694 (251) nmol/L.

The cortisol levels at 30 and 60 min were positively correlated with gestational age ($r = 0.57$, $p = 0.008$ and $r = 0.59$, $p = 0.006$, respectively). The 60-min cortisol levels showed a positive correlation with birth weight ($r = 0.49$, $p = 0.029$). The cortisol levels at 30 and 60 min were not correlated with the ACTH dose per kilogram of body weight ($r = -0.26$, $p = 0.26$ and $r = -0.43$, $p = 0.06$, respectively) or with birth weight SD-score ($r = -0.23$, $p = 0.92$ and $r = 0.82$, $p = 0.73$).

Baseline 17OHP Levels

The baseline levels of 17OHP showed a skewed distribution and were log transformed for statistical analyses. The baseline 17OHP levels showed a negative correlation with gestational age ($r = -0.53$, $p = 0.020$) but did not correlate with birth weight or birth weight SD-score. The baseline 17OHP levels were not related with the baseline cortisol levels but were negatively correlated with the cortisol levels at 30 and 60 min ($r = -0.52$, $p = 0.025$ and $r = -0.51$, $p = 0.032$, respectively).

Cortisol and 17OHP Levels in Relation to Ventilation

The mean baseline cortisol level was not significantly lower, but the cortisol levels at 30 and 60 min after administration of ACTH were significantly lower in the ventilated infants compared with the nonventilated infants ($p < 0.001$ and $p < 0.01$, respectively) (Fig. 1). The mean baseline 17OHP level in the ventilated infants was 45.6 (34.3) nmol/L and significantly higher compared with the nonventilated infants 8.5 (6.4) nmol/L ($p = 0.014$). The mean baseline C/17OHP was significantly lower in the ventilated compared with the nonventilated infants ($p = 0.004$). After adjusting for gestational age and birth weight, the mean baseline cortisol, the cortisol level at 30 min, and the C/17OHP were significantly lower in the ventilated group ($p = 0.046$, $p = 0.013$, and $p = 0.015$, respectively).

Cortisol and 17OHP Levels in Relation to Illness Severity

The baseline cortisol levels were not related to SNAP scores, but the 30- and 60-min cortisol levels were negatively correlated with higher SNAP scores, indicating lower cortisol levels at higher SNAP scores ($r = -0.62$, $p = 0.005$ and $r = -0.61$, $p = 0.006$, respectively) (Fig. 2, A–C). For every increment of 10 points in the SNAP score, the 30-min cortisol level decreased with 215 nmol/L, and the 60-min cortisol level decreased with 272 nmol/L.

The baseline 17OHP level was positively correlated with higher illness severity as measured by SNAP ($r = 0.49$, $p = 0.039$).

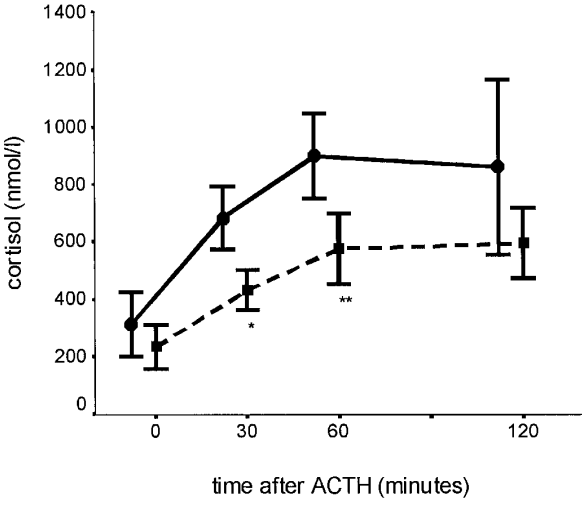

Figure 1 Cortisol levels before and after ACTH (0.5 µg) in ventilated and nonventilated infants. Results are given as mean cortisol levels with 95% confidence intervals.
● = nonventilated infants, ■ = ventilated infants.
*p < 0.001 and **p < 0.01 vs nonventilated group.

Cortisol and 17OHP Levels in Relation to Outcome

BPD. Patients who developed BPD were not significantly different with respect to gestational age, birth weight, and birth weight SD-score compared with patients who did not develop BPD.

Patients who developed BPD showed a significantly lower mean cortisol level at 60 min ($p = 0.029$) than patients who did not develop BPD. In addition, the mean baseline value for 17OHP was significantly higher and mean C/17OHP was significantly lower in infants who developed BPD ($p = 0.009$ and $p = 0.008$, respectively).

Adverse outcome. Gestational age, birth weight, and birth weight SD-score were not significantly different in patients with a poor outcome compared with patients with a good outcome.

Patients with a poor outcome showed a significantly lower mean 60-min cortisol level ($p = 0.025$), a significantly higher mean baseline 17OHP level ($p = 0.006$), and a significantly lower mean baseline C/17OHP ($p = 0.002$) compared with infants with a better outcome.

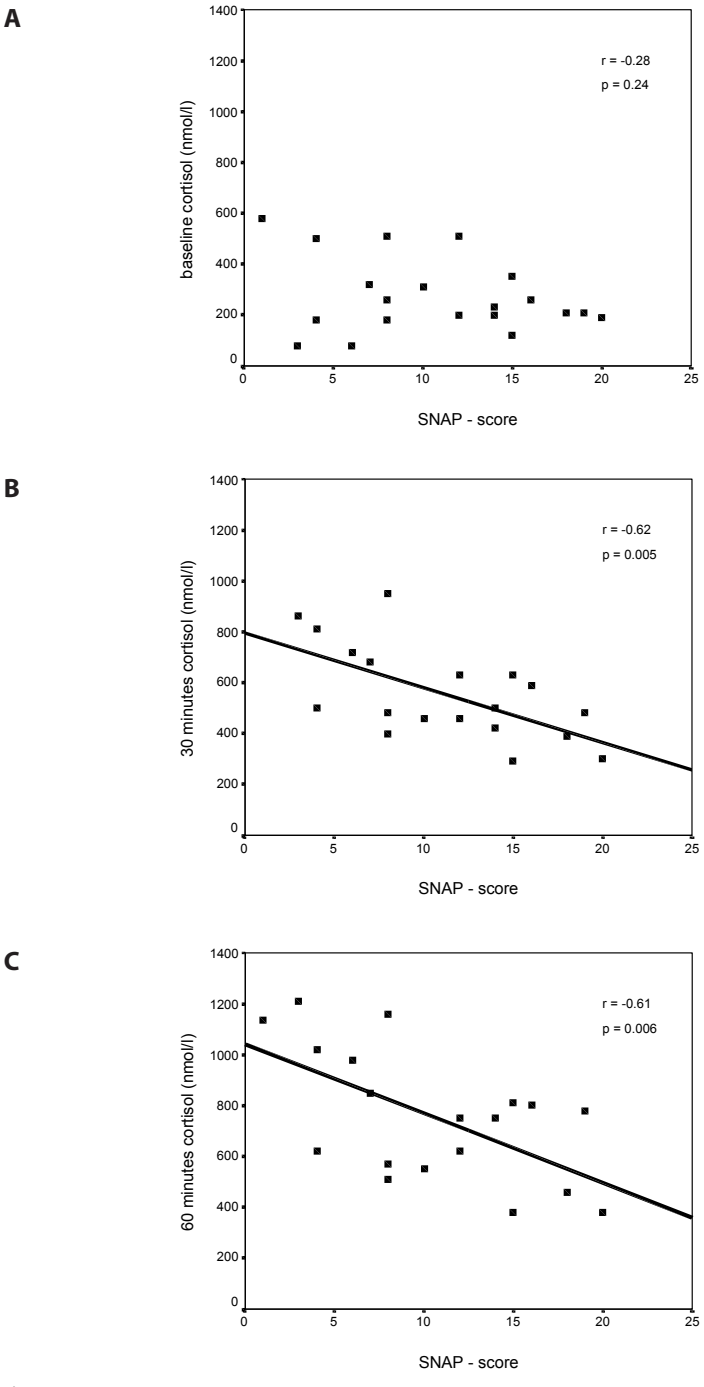

Figure 2 Cortisol levels in relation to illness severity (SNAP score).
A Baseline cortisol levels
B Thirty-minute cortisol levels after ACTH administration; regression line: C30 = 797 - 22 SNAP.
C Sixty-minute cortisol levels after ACTH administration; regression line: C60 = 1041 - 27 SNAP.

Discussion

We performed this study to evaluate the capacity of the adrenals of very preterm infants in the first week of life to produce cortisol under normal conditions and under conditions of stress.

Our study suggests that sick very preterm infants do not release more cortisol than less severely ill very preterm infants. Our data show significantly lower baseline cortisol levels and diminished cortisol response after ACTH in ventilated compared with nonventilated infants and a significantly lower C/17OHP in ventilated infants. A normal reaction to stress would show a higher cortisol level with increasing illness as is seen in critically ill adults.[18] Our study, however, was not designed to answer whether these infants became ill because of low cortisol production or showed adrenal insufficiency as a result of their severe illness. The results, however, do support the idea of considering supplementation of cortisol in severely ill very preterm infants with ongoing blood pressure problems and persisting hypoglycemia.

Scott and Watterberg have demonstrated that very sick infants with gestational ages younger than 28 wk have lower morning cortisol levels than less severely ill infants of the same gestational age.[19] This negative correlation between illness and morning cortisol levels, however, seems diminished in patients of greater gestational age. Other markers of illness such as ventilatory support pattern or use of surfactant were also negatively correlated with cortisol levels.

Our findings are in contrast with Hingre *et al.* who found in sick preterm and healthy full-term infants comparable baseline cortisol levels and with Thomas *et al.* who even showed higher baseline cortisol levels in sick compared with healthy preterm infants.[11,20,21] The difference with our data may be due to the fact that the patients in the Thomas study had a gestational age greater than 30 wk, whereas our patients were all younger than 30 wk. In agreement with Lee *et al.* and in contrast with other studies, we did not find a relation between baseline cortisol level and gestational age.[10,19,22] It could be that our population was too homogeneous in gestational age to detect a relationship.

Although we are not informed about the amount of cross-reactivity between baseline cortisol and cortisone, we know from other studies that the levels of cortisone can be in the same range as the cortisol levels.[8,23,24] This may have contributed to the measured baseline cortisol levels, especially when the baseline cortisol levels are low. We did find a difference in baseline cortisol levels between ventilated and nonventilated infants. As it is unlikely the levels of cortisone were higher in ventilated compared with nonventilated infants, the conclusion remains true.

The interpretation of ACTH test results is complicated by the fact that no agreement exists about the correct dose of ACTH, and different criteria for diagnosing adrenal insufficiency are used in the literature. We tested with a fixed dose of 0.5 µg, corresponding with an

ACTH dose of 0.5–1 μg/kg or 10–15 μg/1.73 m^2 and found differences between ventilated and nonventilated infants.

The baseline cortisol levels in ventilated infants are remarkably low considering the degree of illness. Ventilated infants do have significantly lower 30- and 60-min cortisol levels after ACTH compared with nonventilated infants, which can only be caused by a lower adrenal cortisol release in response to ACTH administration. It is known from the literature that increases in cortisol are much higher than increases in cortisone after ACTH.[23,25] It is only when the increase in cortisol after ACTH is very low that cortisone can still contribute to the measured cortisol level. In fact, we may have underestimated the differences in cortisol response between ventilated and nonventilated infants.

Thus, in contrast with earlier studies, we found evidence for adrenal insufficiency in very preterm ventilated infants when tested with a low-dose ACTH.[4,26,27] It seems that the adrenals in these ventilated sick infants are less capable of releasing cortisol after an additional ACTH stimulus compared with nonventilated infants. Possible explanations may be that the adrenals in these infants were already down-regulated by high circulating interleukin levels and no longer reactive to high serum ACTH levels or that the adrenals in the ventilated infants were not capable enough of recognizing higher serum ACTH levels. The ventilated infants, however, showed higher levels of the cortisol precursor 17OHP and a lower C/17OHP compared with nonventilated infants. This indicates that the adrenals are responsive to ACTH and produce precursors but, at the same time, may not be able to convert the precursor to cortisol. Hanna *et al.* reported a normal ACTH and cortisol response to ovine corticotropin releasing hormone (CRH) and a normal cortisol response to exogenous ACTH in extremely low birth weight infants, thereby suggesting that the low baseline cortisol levels were partly due to an inability of the brain to recognize the stress of illness or an inability of the hypothalamus to secrete CRH.[27] They, however, used a high dose of ACTH (62.5 μg) and 1 d after CRH testing, which by itself induces an increase in baseline cortisol levels. Ng *et al.* showed a higher ACTH response after CRH administration in ventilated infants without an increase in cortisol levels, suggesting that the pituitary is more matured than the adrenals in preterm infants.[28]

Our study showed significantly higher 17OHP levels and lower C/17OHP in ventilated infants compared with nonventilated infants adjusted for gestational age and birth weight, as well as, in more severely ill infants. Both suggest a reaction of the adrenals to stress by increasing a cortisol precursor but showing a lower capacity, especially in the severe ill and ventilated infants, to convert this precursor 17OHP to cortisol. The high levels of 17OHP in relation to cortisol can be caused by a decreased activity of the enzyme CYP21A2 or due to a lower conversion of 11-deoxycortisol to cortisol by CYP11B, which seems to be a greater problem in the sick very preterm infant. Higher 17OHP levels in sick preterm infants compared with term infants have been described earlier.[10,29,30] High baseline levels of 17OH-pregnenolone, 17OHP, and 11- desoxycortisol in sick infants with gestational ages younger

than 30 wk compared with term infants have been reported and are also consistent with a decreased CYP11B activity.[11]

In summary, our data demonstrate lower baseline cortisol levels, a lower cortisol response to ACTH, higher 17OHP levels, and lower C/17OHP in ventilated very preterm infants compared with nonventilated preterm infants and those who did not have an adverse outcome. The data suggest an insufficient adrenal response to stress in sick ventilated very preterm infants with gestational ages younger than 30 wk. Further studies are required to investigate whether supplementation with physiologic doses of hydrocortisone may benefit the outcome.

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3

Growth hormone profiles and growth factors in ventilated very preterm infants at risk for Bronchopulmonary Dysplasia

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Submitted

Abstract

Very preterm infants ventilated for a prolonged period of time and at risk for BPD frequently show an impaired growth. The potential role of GH, IGF-I and the binding proteins in the growth retardation of these infants in the neonatal period is unknown and before GH therapy is considered, it is necessary to be informed about the GH-IGF-I axis.

In twenty-four very preterm ventilated infants (mean birth weight 953 (± 330) g, mean gestational age 27.5 (± 1.7), mean postnatal age 2.8 (± 1.4) weeks) a 6- h GH profile was performed and serum levels of IGF-I, IGFBP-1 and -3, nutritional intake and severity of respiratory failure were determined. GH profiles were analysed by Pulsar.

A wide variation in serum GH levels (6.0 - 55.8 µg/l, median 19.9 µg/l) and a correlation between serum GH and IGF-I ($r = 0.42$; $p < 0.05$) was observed. Serum GH levels did not correlate with gestational age, birth weight, postnatal age, weight gain or severity of respiratory failure. IGFBP-1 correlated with IGF-I and GH levels ($r = 0.55$; $p < 0.01$ and $r = 0.48$; $p < 0.05$, respectively) and with daily caloric, fat and protein intake ($r = 0.54$, $p < 0.01$, $r = 0.53$; $p < 0.01$ and $r = 0.43$; $p < 0.05$).

Serum GH and IGF-I levels in ventilated preterm infants at risk for BPD were comparable to those found in non-ventilated preterm infants of similar postconceptional age and thus not correlated with severity of respiratory failure. Serum GH levels were related with IGF-I suggesting that GH exerts some effect through IGF-I. These findings together suggest that these infants do not have GH deficiency.

Conclusion: Our data show that poor growth in ventilated very preterm infants at risk for BPD is not the result of either low GH or IGF-I levels. For that reason our study does not provide arguments to consider GH therapy for growth-retarded ventilated preterm infants.

Introduction

Preterm infants developing a chronic lung disease in the neonatal period frequently showed an impaired growth. Their growth will further decline when steroids are started to facilitate weaning from the ventilator. The impaired growth might persist into infancy.[1-4] There are a number of definitions describing chronic lung disease in preterm infants. The term 'bronchopulmonary dysplasia' (BPD) was introduced by Northway in 1967.[5] Thereafter an increasing number of authors have used the term chronic lung disease instead of BPD.[6] Nowadays, BPD in most studies is defined as a persistent need for supplemental oxygen for at least 28 days.[7]

Due to improvement in neonatal intensive care treatment, not only the survival rate of very preterm infants, but also the incidence of surviving preterm infants with BPD has increased. The incidence of BPD in infants surviving the neonatal period increases from 12% in infants born after a gestational age of 30 weeks to 30% when born after a gestational age of 25 weeks.[8]

The early postnatal insufficient growth is a general pattern in infants at risk for developing BPD. Several factors have been considered to cause growth retardation in preterm infants with respiratory problems. Some studies showed an elevated metabolic rate in these infants, however these results have been challenged.[9,10] Caloric intake is often less than recommended due to fluid restriction whereas the absorption in the gastrointestinal tract might also be impaired. Infections, even low-grade lung infections can have a negative influence on growth. Hormonal abnormalities, particularly reduced levels of growth hormone (GH) and growth factors or the opposite, an increased GH insensitivity might play a role.

The exact role of various hormones and factors such as GH, insulin-like growth factor (IGF-I and IGF-II), leptin and insulin in promoting growth in utero and in early postnatal life, is still unknown.[11-13] Although it is widely assumed that the GH-IGF-I axis is not the major system determining foetal and early postnatal growth, the reduction of birth length in GH- deficient infants suggests that GH does have some effect on foetal growth.[14] Most studies reported a decline in serum GH levels with advancing gestational and increasing postnatal age.[15-23] The decline in GH levels has been attributed to a decrease in GH pulse frequency, GH secretory bursts amplitudes and in nadir GH levels.[16,17,24] However, no data exist about GH profiles in ventilated very preterm infants at risk for BPD.

The potential contribution of GH, IGF-I and the IGFBP's to the growth retardation of preterm infants with respiratory failure who are at risk to develop BPD in the neonatal period is unknown. Before GH therapy in these infants is considered it is necessary to be informed about the GH-IGF-I axis.

We therefore assessed serum levels of GH, IGF-I, and their binding proteins in preterm infants with respiratory failure at risk to develop BPD. In addition, we analysed if these levels were related to nutrition, medication and illness severity.

Methods

Patients

Twenty-four preterm infants, born after a gestational age of less than 33 weeks and with a persistent need for artificial ventilation (after an initial phase of respiratory distress syndrome) were studied. All patients received treatment consisting of diuretics, fluid restriction and inhalation therapy. Exclusion criteria were: steroid treatment, persistent ductus arteriosus, pulmonary infection, ongoing sepsis, grade 3 or 4 intraventricular haemorrhage, chromosomal defects or major congenital anomalies. None of the infants received blood transfusions within 2 days before the study. If the mother was being treated for an endocrine disorder with GH, hydrocortisone, thyroxin, or thyrostatic drugs, the infant was not included. All patients were admitted to the NICU of the Sophia Children's Hospital in Rotterdam, the Netherlands.

The Medical Ethics Committee of the Erasmus University Medical Center approved the study protocol and written informed consent was obtained from the parents of each child.

Methods

All patients had an indwelling arterial line. A plasma sample was taken, every 30 minutes, during a 6-hours period. A longer sampling period and more frequent blood sampling was considered unethical in this group of very small, sick, preterm infants. Studies in these preterm infants are restricted due to the small size of the infants and their severe illness. The total blood volume of these infants is 50 - 80 ml. It was considered unacceptable to withdraw more than 5% of the total blood volume for study purposes. For the present study the maximal volume was 2.5 - 4 ml, which made it impossible to sample for a longer period than 6 hours or more frequently. As these infants do not yet have a diurnal GH secretion and are being nursed under conditions that do not change significantly throughout day and night, a 6-h GH profile was considered to be sufficient.

All blood samples were stored directly on ice for no longer than 3 hours. After centrifugation, plasma samples were frozen (- 20 'C) until assayed. Serum GH was measured in all samples and serum IGF-I, IGFBP-1, and IGFBP-3 levels were determined in the first sample.

The attending neonatologist provided routine care and feeding decisions. According to the policy of the NICU, infants were started on parenteral nutrition (PN) on the second day of life and enteral feedings were initiated on day 7. Preterm infant formulas were used with

a caloric density of 80-88 kcal/100 ml and a protein content of 2.4-2.6 g/100 ml. The daily protein, caloric and fat intake was calculated from the total enteral and parenteral intake during the 48 hours period prior to the study and during the day of study.

Weight gain in the week of study was measured by performing daily weight measurements and expressed in g/kg/wk. Illness severity was assessed by calculating the oxygenation index (mean airway pressure times inspired fraction of oxygen / PaO2) and by calculating the ventilation score (mean airway pressure times inspired fraction of oxygen). Use of cardiotonic drugs because of hypotension was also recorded.

Hormone assays

All GH determinations were performed in the Endocrine Laboratory of the University Hospital Rotterdam, the Netherlands.

GH determinations in plasma were measured using a two-site immunoradiometric assay (ELSA-HGH, CIS bio international, ORIS Group, France). The assay measured the 22 kD GH and showed no cross reactivity with 20 kD GH. All samples were analysed in duplicate in the same assay. The WHO First International Reference Preparation WHO 80/505 was used as a standard. The intra-assay coefficients of variation were 2.4%, 2.8%, 2.8% and 2.3% at GH concentrations of 3.46, 7.3 17.0 and 47.4 µg/l, respectively. The inter-assay coefficients of variation were 4.2%, 3.2%, 4.4% and 4.0% at GH concentrations of 3.32, 7.10, 16.4, and 45.4 µg/l. The detection limit of the assay was 0.04 µg/l.

IGF-I was determined by RIA (SM-C-RIA-CT, Biosource Europe SA) and after acid-ethanol extraction.[25] The sensitivity was 0.25 ± 0.10 ng/ml, the intra-assay coefficients of variation were 6.1%, 4.1%, 4.7% at IGF-1 levels of 54.2, 194 and 491 ng/ml. The inter-assay precision was 9.9%, 9.6%, 9.3% at levels of 121, 251 and 494 ng/ml. Cross-reactivity with IGF-II was 0.2%, with insulin < 0.001% and with GH < 0.01%.

IGFBP-1 was determined using a two-site immunoradiometric assay principle (Total IGFBPI IRMA DSL-7800, Diagnostic Systems Laboratories, Webster, Texas, USA). The sensitivity of the assay was 0.33 ng/ml and the intra-assay coefficients of variation were between 5.2%, 4.6% and 2.7% with IGFBP-1 levels of 5.23, 50.23 and 144.60 ng/ml. The inter-assay coefficients of variation were 3.5%, 6.0% and 3.6% at IGFBP-1 levels of 5.15, 47.08 and 142.03 ng/ml. No cross-reactivity was seen with the IGFBP-2, -3, -4, -5, and -6.

IGFBP-3 was determined using a two-site immunoradiometric assay (IGF-BP3 IRMA, DSL-6600, Diagnostic Systems Laboratories Webster, Texas, USA). The sensitivity of the assay was 0.5 ng/ml, with intra-assay coefficients of variation of 1.8%, 3.2%, and 3.9% at IGFBP-3 levels of 82.72, 27.53 and 7.35 ng/ml. The inter-assay coefficients of variation were 1.9%, 0.5% and 0.6% at IGFBP-3 levels of 76.90, 21.51 and 8.03 ng/ml, respectively.

Analysis of the 6-h GH profiles

The 6-h GH profiles were analysed with the Pulsar program from Merriam and Wachter[26] and adapted for Quick Basic by Rosberg and Albertsson-Wikland (PC-Pulsar, 1987). From the Pulsar analysis, the following values were extracted: the overall mean, the baseline, the maximal GH value, and the number of GH peaks in 6 hours.

Statistical analysis

Statistical analysis was performed using SPSS 9.0 for Windows 95, SPSS software (Chicago, IL, U.S.A.). All hormone values were log transformed before analysing to obtain approximate normal distributions. Differences between two groups were tested with the Mann-Whitney test in case of deviation from a normal distribution. Correlations were evaluated with the non-parametric Spearman's rank correlation. Multiple regression analysis was used to evaluate various parameters simultaneously with regard to their relation with IGFBP-1. Results are expressed as the mean (SD), unless indicated otherwise. Two tailed p-values $\leq$ 0.05 were considered significant.

Results

Clinical characteristics

Twenty-four ventilated very preterm infants, 13 boys and 11 girls, with a mean gestational age of 27.5 (± 1.7) weeks, a mean birth weight of 935 (± 330) g and a mean postnatal age of 2.8 (1.4) weeks were included in the study.

The clinical data of the infants at day of study are shown in Table 1. The mean weight gain was 59.3 (30.1) g/kg/wk and 20 patients showed a weight gain less than 12 g/kg/day.

GH profiles

The 6-h GH profiles of the individual patients are shown in Figure 1. Serum mean GH levels varied between 6.0 and 55.8 µg/l with a median of 19.9 µ/l. The number of GH peaks within the 6-h period ranged from 0 to 4. Different types of growth hormone profile were seen. Some patients (numbers 4, 8, 14, 18, 21, 23 and 24) had serum mean GH levels below 15 µg/l without any obvious GH peaks. The other patients had serum mean GH levels above 15 µg/l with one or more GH peaks (numbers 1 - 3, 5, 7, 9 -13, 15, 17, 19, 20 and 22). Two patients had quite different GH profiles: patient 6 had relatively low GH levels, with several GH peaks and patient 16 had high serum GH levels above 50 µg/l with only slight variation. The clinical condition of these patients was not different with respect to birth weight, postnatal age, respiratory failure or need for dopamine or morphine.

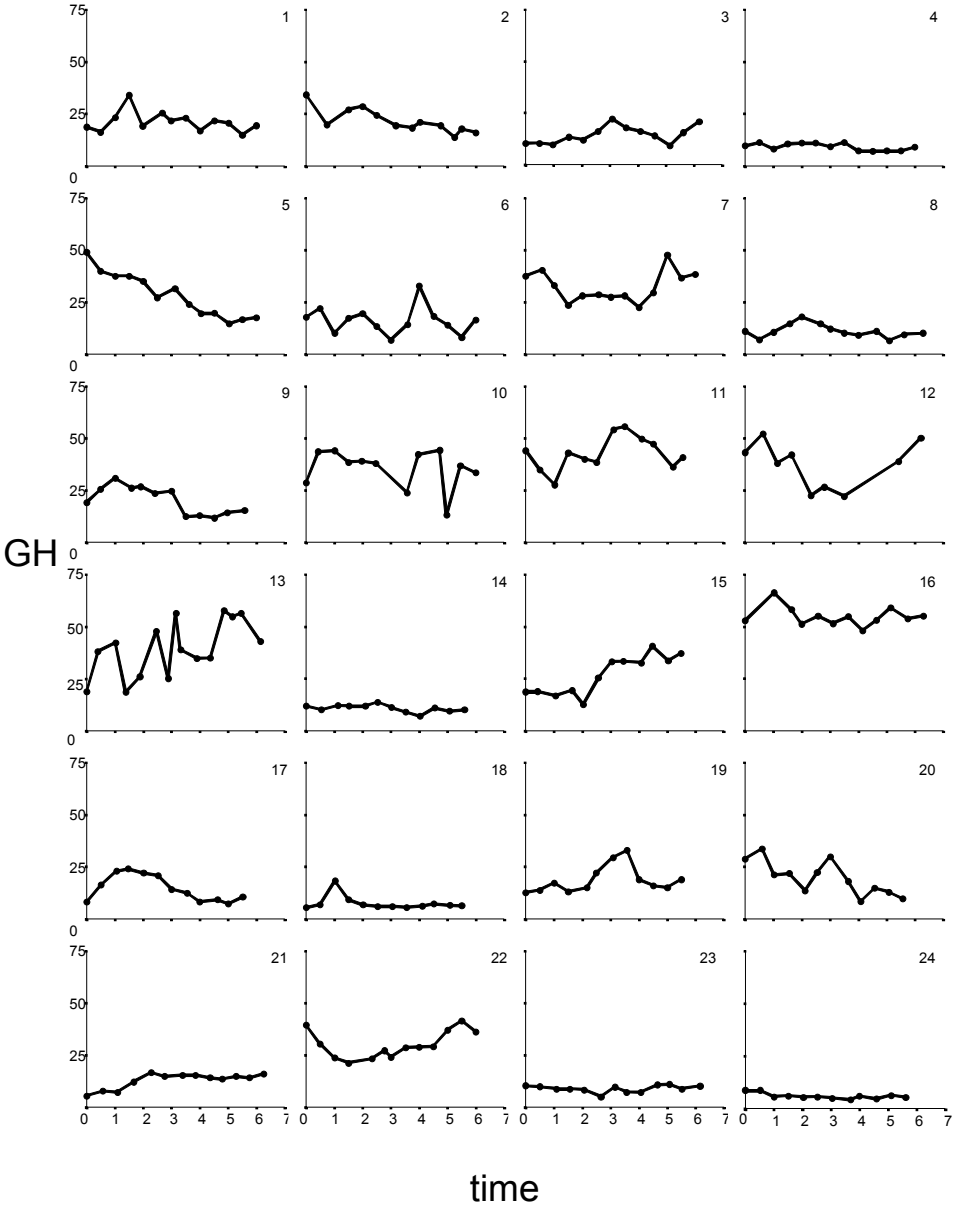

Figure 1 6-hours GH profiles in preterm infants on prolonged ventilation.
Serum GH levels expressed in µg/l, time in hours.

Table 1 Clinical data at day of study.

N = 24	Mean	SD
Weight (g)	1163	372
Postnatal age (wk)	2.8	1.4
Postconceptional age (wk)	30.2	2.4
Oxygenation Index	76	53
Ventilation score	524	273
Weight gain (g/kg/wk)	59.3	30.1
Energy intake (kcal/kg/day)	99.2	26.9
Protein intake (g/kg/day)	2.9	0.6
Fat intake (g/kg/day)	5.0	2.1
Use of PN (n)	8	
Use of PN and dopamine (n)	6	
Use of morphine (n)	6	
Use of PN, dopamine and morphine (n)	3	
No use of PN, dopamine or morphine (n)	3	

Results are given as mean SD or number of infants.

Table 2 Characteristics of 6-h GH profile and serum levels of IGF-I and IGF-binding proteins in very preterm ventilated infants.

	Median	Range
GH levels		
Mean	19.9	6.0 - 55.8
Baseline	18.1	5.7 - 55.7
Maximal	33.4	8.7 - 66.2
Peaks (n /6 hr)	2	0 - 4
IGF-I	38.6	20.7 - 117.8
IGFBP-1	108.0	7.9 - 717.4
IGFBP-3	600	200 - 3700

Serum GH levels expressed in µg/l, serum IGF-I in ng/ml, serum IGFBP-1 in ng/ml and serum IGFBP-3 in ng/ml.

Univariate analysis showed no correlation between mean, baseline or maximal GH levels and gestational age, birth weight, birth weight SD-score, weight during study, weight gain, postconceptional age or postnatal age, except for a positive correlation between gestational age and the number of GH peaks ($r = 0.44$, $p = 0.03$).

Serum IGF-I, IGFBP-1 and IGFBP-3 levels

Serum IGF-I levels ranged from 20.7 to 117.8 ng/ml with a median of 38.6 ng/ml (Table 2). Serum IGFBP -1 levels varied between 7.9 to 717.4 ng/ml, and serum IGFBP-3 levels from 200 to 3700 ng/ml. IGF-1 and IGFBP-1 levels did not correlate with gestational age, birth weight, birth weight SD-score, postnatal age, postconceptional age and weight during study. IGFBP-3 levels were only positively correlated with gestational age and birth weight ($r = 0.43$, $p = 0.04$; $r = 0.49$, $p = 0.02$, respectively). Only the IGFBP-1 levels and not IGF-I and IGFBP-3 levels were positively associated with indicators of nutritional intake (Table 3). Weight gain (g/kg/wk) was not correlated with serum IGF-I and IGFBP-1 and –3 levels.

Associations between GH, IGF-I and the binding proteins

Mean GH levels were positively correlated to baseline and maximal GH levels and negatively to the number of peaks (Table 4). IGF-I levels correlated with the mean GH levels, whereas IGFBP-1 levels correlated with mean, baseline and maximal GH levels, and IGF-I levels. No correlation was seen between IGFBP-3 and any of the other growth factors and binding proteins.

Severity of illness

The ventilation score was not correlated with serum GH, IGF-I and IGFBP levels. Infants treated with dopamine, morphine or PN during the study had a significantly higher ventilation score and oxygenation index compared to infants without such treatment ($p < 0.01$, $p < 0.05$, $p < 0.01$, respectively), indicating more severe ventilation and higher severity of illness in these infants.

The various characteristics of the GH profile were not significantly different between infants with and without use of dopamine, morphine, or PN. Infants with dopamine treatment had significantly lower serum IGF-I and IGFBP-1 levels than infants without dopamine ($p < 0.05$ and $p = 0.05$, respectively). Infants on PN had a significantly lower energy, protein- and fat intake (all $p< 0.01$) and significantly lower levels of serum IGF-I and IGFBP-1 ($p < 0.05$ and $p < 0.01$, respectively) than infants on complete enteral nutrition. However, as all patients treated with dopamine also received PN, it was impossible to distinguish between effects of dopamine and PN. Similarly, it was not possible to distinguish between the effects of PN and caloric intake, as these two factors were strongly related.

Morphine treatment was not associated with a lower nutritional intake and did not result in significantly different serum levels of IGF-I, IGFBP-1 and –3 compared to no morphine treatment. Birth weight SD-score did not have any effect in these models.

Table 3 Correlation between nutritional intake, weight gain and serum levels of GH, IGF-I and IGF-binding proteins in very preterm ventilated infants.

	Mean GH	IGF-I	IGFBP-1	IGFBP-3
Caloric intake				
48 hr before study (kcal/kg/d)	0.23	0.27	**0.52****	0.03
During study (kcal/kg/d)	0.21	0.23	**0.54***	0.23
Fat intake				
48 hr before study (g/kg/d)	0.26	0.20	**0.51****	0.27
During study (g/kg/d)	0.32	0.23	**0.53***	0.21
Protein intake				
48 hr before study (g/kg/d)	0.23	0.19	0.37	0.07
During study (g/kg/d)	0.31	0.18	**0.43****	-0.02
Weight gain (g/kg/wk)	0.01	0.21	-0.03	-0.33

*$p < 0.01$, **$p < 0.05$

Table 4 Correlation between various characteristics of 6-h GH profile and levels of IGF-I and IGF-binding proteins in very preterm ventilated infants.

	Baseline GH	Maximal GH	N of peaks	IGF-I	IGFBP-1	IGFBP-3
Mean GH	**0.99***	**0.97***	**-0.75***	**0.42*****	**0.48*****	-0.28
Baseline GH		**0.95***	**-0.79***	0.34	**0.45*****	-0.26
Max GH			**-0.71***	0.40	**0.45*****	-0.25
N of peaks				-0.21	-0.29	0.27
IGF-I					**0.55****	-0.41
IGFBP-1						0.04

*$p < 0.001$, **$p < 0.01$, ***$p < 0.05$

Discussion

This study is the first one describing 6-hours GH profiles in combination with serum IGF-I, IGFBP-1 and-3 levels in very preterm ventilated infants at risk for BPD. We found a wide range of mean GH levels comparable to GH levels previously described in non-ventilated preterm infants, thereby not providing arguments for GH deficiency in most infants. We have found a relation between GH and IGF-I suggesting that GH exerts some effect through IGF-I, even in these very preterm infants. There was no correlation between severity of disease and weight gain, and serum GH, IGF-I, and IGFBP levels, but daily nutritional intake was significantly correlated with IGFBP-1 levels.

Mean serum GH levels measured by performing 6-h GH profiles in our ventilated very preterm infants were comparable with GH levels previously reported in other groups of non-ventilated preterm infants.[16,22] However, like Ogilvy-Stuart *et al.* we observed a wide individual variance of GH levels for which no clinical explanation as nutritional factors, severity of illness, parenteral nutrition and use of cardiotonic drugs could be found.

Miller *et al.* (1992) found mean GH levels of 37.1 µg/l and 35.8 µg/l in healthy preterm boys and girls born after a gestational age of 32-33 weeks at only 40 hours of age. Ogilvy-Stuart *et al.* reported serum baseline, mean and maximal GH levels of 17.1 µg/l, 22.5 µg/l and 30.7 µg/l, respectively in preterm infants at a postnatal age of 4.4 days. These data are comparable with the GH levels we have found at a postnatal age of 2.8 weeks, suggesting that the GH levels in our ventilated preterm infants may still be in the normal range, especially when considering their younger gestational and higher postnatal age compared to those of Ogilvy-Stuart. Serum IGF-I levels were also within the normal range when compared with those reported in non-ventilated preterm infants of similar postconceptional age.[27]

In spite of these findings, however, our ventilated very preterm infants had a poor growth in terms of weight gain when compared with the described intrauterine growth rate of 14-15 g/kg/day.[28] This suggests that their poor growth is not attributable to GH deficiency but to other factors.

Serum IGFBP-1 levels were comparable with those in non-ventilated preterm infants and positively correlated with daily nutritional intake.[22] We had expected to find lower IGFBP-1 levels since our patients were continuously and adequately fed and had no signs of hypoglycaemia. It has been reported that serum IGFBP-1 levels are inversely correlated with serum insulin levels indicating that low serum insulin levels will increase serum IGFBP-1 levels.[29,30] Also undernutrition is known to up-regulate hepatic production of IGFBP-1 suggesting that elevated serum IGFBP-1 might act as glucose counterregulator and protect against hypoglycaemia during conditions of undernutrition.[31,32] We have no explanation for the positive correlation between nutritional intake and IGFBP-1 levels in our ventilated preterm infants. Further studies should measure serum insulin, glucose and

free fatty acids in various groups of preterm infants, in combination with determinations of IGFBP-1 and free IGF-I levels.

We had expected to find an association between severity of illness and serum GH and IGF-I levels. To our surprise, we did not find such a relation between severity of respiratory failure and serum GH, IGF-I, IGFBP-1 and -3 levels thereby indicating that more severely ill preterm infants do not have lower or higher levels of GH and growth factors.

Infants receiving dopamine treatment had significantly lower serum IGF-I levels but no difference in GH levels. De Zegher *et al.* reported lower GH levels in infants treated with dopamine.[33] Some of our dopamine-treated infants also received morphine, known for its stimulatory effect on GH release.[34,35] However, no effect of concomitant morphine treatment on serum GH, IGF-I and IGFBP-3 levels was observed. Patients with dopamine treatment had the worst clinical condition and significantly lower IGFBP-1 levels than those without. This is in contrast to studies in children and adults where IGFBP-1 levels were positively correlated with disease severity and mortality risk.[36-38]

Infants receiving PN had significantly lower serum IGF-I levels as well as lower total nutritional intake than infants on total enteral feeding. The lower IGF-I levels might thus be explained by their lower nutritional intake.[39]

In conclusion, we showed that ventilated very preterm infants have poor weight gain and serum GH levels comparable to GH levels in healthy preterm infants, albeit with a wide variation. Serum IGF-I levels were also comparable with levels found in non-ventilated preterm infants of similar postconceptional age. Severity of illness was not correlated with serum GH, IGF-I, IGFBP-1 and -3 levels. Serum GH levels were related with IGF-I, suggesting that GH exerts some effect through IGF-I.

Our findings show that poor growth in most preterm infants with respiratory failure at risk for BPD is not the result of either low GH or IGF-I levels. For that reason our study does not provide arguments to consider GH therapy for growth-retarded ventilated infants at risk for developing BPD.

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Effect of Dexamethasone Treatment on Serum GH, IGF-I, and the Binding Proteins IGFBP-1 and -3 in Ventilated Very Preterm Infants

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Abstract

Very preterm infants developing bronchopulmonary dysplasia frequently show a compromised growth in the neonatal period especially when steroids are given to facilitate weaning from the ventilator.

The aim of this study was to evaluate the short-term effect of dexamethasone (DEXA) on the GH-IGF axis in ventilated very preterm infants developing bronchopulmonary dysplasia.

We studied 10 very preterm artificially ventilated infants with bronchopulmonary dysplasia [median (range) gestational age 27.5 wk (25.9 –32.0 wk), median (range) birth weight 970 g 610–2150 g)] immediately before and 2 days after the start of DEXA treatment. On both days of study, serum GH profiles were obtained, and serum IGF-I and IGF binding protein (IGFBP) -1 and -3 levels were measured. The ventilation score and the nutritional intake were calculated.

Before the start of DEXA treatment, the median serum mean GH level was 12.0 µg/L (6 –28.4 µg/L), whereas 2 days after the start of DEXA treatment the median serum mean GH level declined significantly to a value of 4.4 µg/L (1.7–11.9 µg/L). During DEXA treatment, mean, baseline, and maximal GH levels (Pulsar analysis) were significantly lower compared with pre-treatment levels ($p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively). Serum IGF-I and IGFBP-3 levels did not decline during DEXA. Serum IGFBP-1 levels were significantly lower compared with pre-treatment levels ($p < 0.01$). Serum GH levels during DEXA treatment, were correlated with neither the time interval between the administration of DEXA and the second GH profile nor the cumulative DEXA dose administered. Ventilation score and nutritional intake did not significantly correlate with serum GH, IGF-I, or IGFBP-1 or -3 levels, either before or after the start of DEXA.

Conclusions: Two days of DEXA treatment in very preterm ventilated infants has a suppressive effect on serum GH levels, without an acute decline in serum IGF-I levels. A concomitant decrease in serum IGFBP-I levels was found.

Introduction

Very preterm infants developing BPD, with a persistent need for artificial ventilation and oxygenation, frequently show a very compromised growth in the neonatal period.[1,2] Their growth will be further attenuated when DEXA treatment is given after conservative treatment, including increased ventilation settings and fluid restriction, has failed to wean them from the ventilator.[3–5] The hormonal mechanisms underlying this early postnatal growth retardation and the role of glucocorticosteroids have not been elucidated. DEXA has a catabolic effect by increasing protein breakdown and alters skeletal metabolism by inhibition of intestinal calcium absorption, stimulation of renal calcium excretion, and suppression of collagen turnover in preterm infants.[6–8]

Glucocorticosteroids have been suggested to have a suppressive effect on the pituitary GH secretion with attenuation of the spontaneous GH secretion and decreased GH responses to various stimuli.[9–11] A direct antagonizing effect of DEXA on the epiphyseal cartilage to the action of GH has been reported, probably by inhibition of the local secretion and paracrine action of IGF-I and the inhibition of the expression of the GH and IGF-I receptor.[12]

The clinical significance of GH in the perinatal period is not well understood, although GH-deficient infants show a reduction in birth length, suggesting some role for GH in regulation of fetal growth.[13] In the postnatal period in particular, insulin and IGF-I are important regulators of physical growth, and in mice disruption of genes for IGF-I, IGF-II, or their receptors results in intrauterine growth retardation.[14–16]

So far, no studies have evaluated the short-term effect of DEXA treatment on serum GH levels and growth factors in ventilated very preterm infants. To understand the effect of DEXA on spontaneous GH secretion and the GH-IGF axis, we performed a 6-h GH profile and measured serum IGF-I and IGFBPs, before and 2 days after the start of DEXA treatment in very preterm infants developing BPD.

Methods

Patients

Preterm infants born with a gestational age of ≤ 32 weeks, developing BPD, were included in the study if the attending neonatologist decided to start DEXA treatment to wean the infant from the ventilator. The indication to start DEXA treatment was a persistent need for artificial ventilation and oxygen dependency after 1 wk of age when conservative treatment such as increased ventilation settings, fluid restriction, diuretics, antibiotics, and inhalation therapy had failed to wean the infant from the ventilator. DEXA treatment was started with a dosage of 0.5 mg/kg per day, in two doses per day, during 3 days. Thereafter the dosage was tapered to 0.3 mg/kg per day for 3 days, and gradually tapered every 3 days to 0.1

mg/kg per day every other day during a 3-wk period. Infants were excluded if they had a persistent ductus arteriosus, pulmonary infection, sepsis, grade 3 or 4 intraventricular hemorrhage, chromosomal defects, or major congenital anomalies or when the mother was being treated for an endocrine disorder with GH, thyroxin, or thyreostatic drugs during pregnancy. All patients were admitted to the neonatal intensive care unit of the Sophia Children's Hospital in Rotterdam, The Netherlands.

The Medical Ethics Committee of the Erasmus University Medical Center approved the protocol, and written informed consent was obtained from the parents of each child.

Methods

All patients had an indwelling arterial catheter and were studied twice. Immediately before the start of DEXA treatment and 2 days thereafter plasma samples were taken every 30 min during a 6-h period for determination of serum GH levels and once to determine serum IGF-I, IGFBP-1, and IGFBP-3 levels. Sampling of blood in these very preterm infants was restricted because of the small size of the infants and their severe illness. The total blood volume of these infants is 50–80 mL, and it was unacceptable to withdraw more than 5% of the total blood volume for study purposes. A longer period or more frequent sampling was therefore impossible. All blood samples were stored directly on ice for no longer than 3 h. After centrifugation, plasma samples were frozen (-20°C) until assayed.

Birth weight was expressed in SD-scores according to Usher and McLean.[17] Daily weights were obtained by the nursing staff using an electronic weight scale (Digital Baby Scale, model DS-30 A; Kubota, Ltd, Japan).

The attending neonatologist provided routine care and feeding decisions. According to the policy of the neonatal intensive care unit, infants were started on parenteral nutrition on the second day of life, and enteral feedings were initiated on day 7. All infants were continuously fed, and blood glucose levels were determined every day. Preterm infant formulas were used with a caloric density of 80–88 kcal/100 mL and a protein content of 2.4 –2.6 g/100 mL. On both days of study, the protein, energy, and fat intakes were calculated.

The severity of respiratory failure was assessed by calculating the ventilation score (mean airway pressure times inspired fraction of oxygen).

Hormone assays

All hormonal determinations were performed in the Endocrine Laboratory of the University Hospital Rotterdam, The Netherlands.

GH levels in plasma were measured using a two-site immunoradiometric assay (ELSA-HGH; CIS bio international, ORIS Group, France). The assay measured the 22-kD GH and showed no cross-reactivity with 20-kD GH. All samples were analyzed in duplicate in the same assay. The WHO First International Reference Preparation WHO 80/505 was used as

a standard. The intraassay and interassay coefficients of variation were < 2.8% and 4.4%, respectively. The detection limit of the assay was 0.04 µg/L.

Serum IGF-I levels were determined by RIA (SM-C-RIACT, Biosource Europe SA), after acid-ethanol extraction.[18] The sensitivity was 0.25 ± 0.10 ng/mL, the intraassay and interassay coefficients of variation were < 6.1% and 9.9%, respectively. Cross-reactivity with IGF-II was 0.2%, with insulin < 0.001%, and with GH < 0.01%.

Serum IGFBP-1 levels were determined using a two-site immunoradiometric assay (Total IGFBP-1 IRMA DSL-7800; Diagnostic Systems Laboratories, Webster, TX, U.S.A.). The sensitivity of the assay was 0.33 ng/mL, and the intraassay and interassay coefficients of variation were < 5.2% and 6.0%, respectively. No cross-reactivity was seen with serum IGFBP-2, -3, -4, -5, and -6.

Serum IGFBP-3 levels were determined using a two-site immunoradiometric assay (IGF-BP3 IRMA, DSL-6600; Diagnostic Systems Laboratories). The sensitivity of the assay was 0.5 ng/mL, with intraassay and interassay coefficients of variation of < 3.9% and 1.9%, respectively.

Analysis of GH profiles

The GH profiles were analyzed with the Pulsar program developed by Merriam and Wachter and adapted for Quick Basic by Rosberg and Albertsson-Wikland (PC-Pulsar, 1987).[19] From the Pulsar analysis the following values were extracted: the overall mean, baseline, and maximal GH levels.

Statistical analysis

Statistical analysis was performed using SPSS 9.0 for Windows 95, SPSS software (Chicago, IL, U.S.A.). All hormone levels were logarithmically transformed in the analyses. Differences between paired samples were tested with the Wilcoxon signed ranks test. Correlations were evaluated with the nonparametric Spearman's rank correlation. Results are expressed as median (range), unless indicated otherwise. Two tailed p values ≤ 0.05 were considered significant.

Results

Clinical characteristics

Ten very preterm infants, seven boys and three girls, with a median (range) gestational age of 27.5 wk (25.9–32.0 wk) and a median (range) birth weight of 970 g (610–2150 g), were included in the study. Six infants were treated with antenatal steroids.

The clinical characteristics of the infants on both days of study, before and 2 days after start of DEXA treatment, are shown in Table 1. The infants were studied when they

Table 1 Clinical data before and during DEXA treatment in 10 very preterm infants developing BPD.

	Pre-treatment		During dexamethasone	
	Median	Range	Median	Range
Weight (g)	1092	750 - 2265	1125	695 - 2195
Postconceptional age (wk)	29.6	27.6 - 34.6	30.5	2.1
Postnatal age (wk)	2.1	1.0 - 3.6	2.4	1.4 - 4.0
Doses of dexamethasone (n)	-		5	3 - 7
Cum. dexamethasone dose (mg/kg)	-		1.23	0.76 - 1.7
Ventilation score	454*	240 - 1700	210	105 - 1024
Energy intake (kcal/kg/d)	97.3	64.2 - 144.3	118.7	43.6 - 144
Protein intake (kcal/kg/d)	2.6	1.9 - 3.9	2.7	1.9 - 3.9
Fat intake (g/kg/d)	4.9	1.4 - 8.6	5.2	0.6 - 9.6

Results are given as median and range.
* $p<0.05$

had received at least three doses of DEXA. The nutritional intake was not significantly different between both days of study. Three infants were receiving parenteral nutrition. The ventilation score, however, had decreased significantly on the second day of study compared with the day before the start of DEXA ($p < 0.05$).

GH profiles

The GH profiles of the individual patients before and during DEXA treatment are shown in Figure 1. Mean pre-treatment serum GH levels varied between 6 and 28.4 µg/L with a median of 12.0 µg/L, whereas 2 days after the start of DEXA treatment the mean GH level had declined to a median of 4.4 µg/L.

Two days after start of DEXA treatment the overall mean, baseline, and maximal serum GH levels were significantly lower compared with pre-treatment levels ($p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively; Table 2). In all patients, except one, the GH levels declined during DEXA treatment. The percentage decline in serum mean GH levels was on average 54%, in serum baseline GH levels 56%, and in serum maximal GH levels 35%, and these levels were not correlated with gestational or postnatal age. Serum GH levels were not significantly different between patients receiving parenteral or enteral nutrition.

The time interval between the administration of DEXA and the start of the second GH profile varied. In the first five patients DEXA was administered between 6 and 0 h before the start of the second profile, whereas in the other five patients DEXA was also given during the sampling period. No significant correlation was found between the time interval between administration of DEXA and the serum GH levels during the second profile.

The cumulative dose of DEXA (in mg/kg of body weight) administered before the second GH profile did not correlate with mean, baseline, and maximal GH levels, nor with the

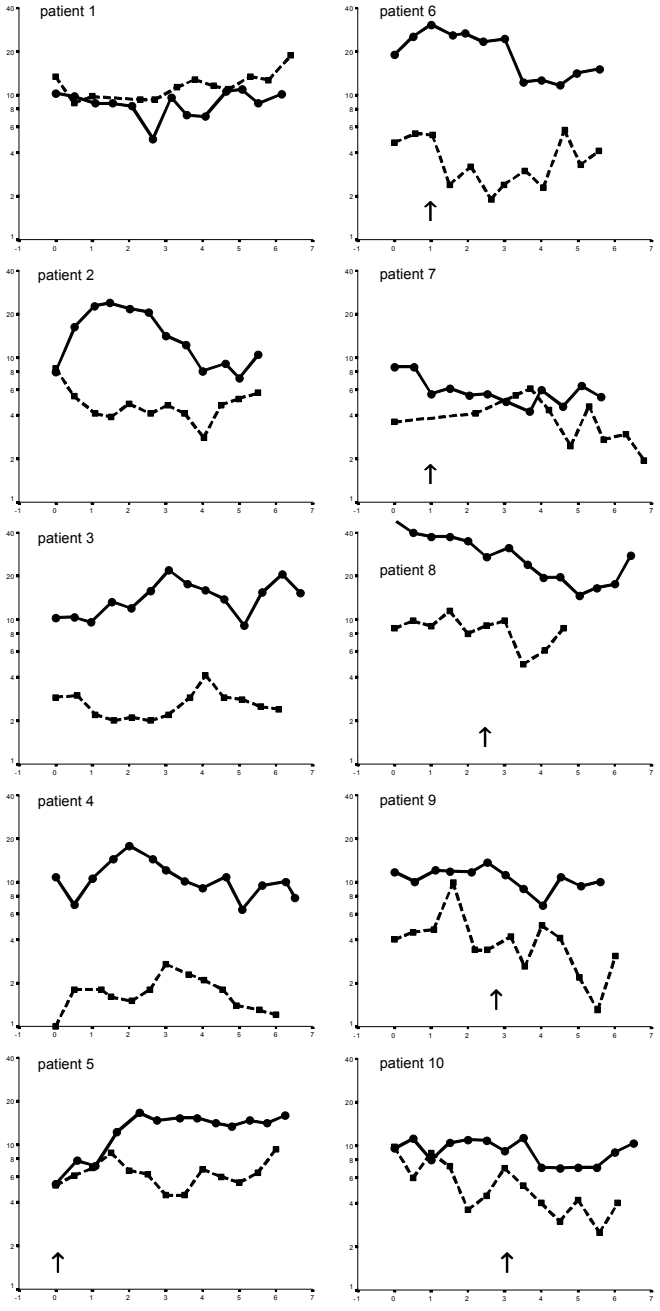

Figure 1 6-hours GH profiles in ventilated preterm infants before (●—●) and during (■---■) DEXA treatment.
GH levels are expressed in µg/l on a logarithmic scale against time in hours.
In patients 1–5, time t = 0 corresponds to 6, 5, 2.5, 0.5, and 0 h after DEXA administration, respectively. In patients 6–10, ↑ corresponds with time of DEXA administration.

Table 2 Characteristics of 6-h GH profile, IGF-I and binding proteins before and during DEXA in 10 very preterm infants with BPD.

	Pre-treatment		During treatment	
	Median	Range	Median	Range
GH profile				
Baseline GH	11.3	5.7 - 28.6	3.8*	1.7 - 11.8
Mean GH	12.0	6.0 - 28.4	4.4*	1.7 - 11.9
Max GH	17.3	8.7 - 48.9	8.9**	2.7 - 18.8
IGF-I	32.9	22.2 - 89.5	36.3	19.9 - 65.8
IGFBP-1	69.6	23.2 - 341.4	13.5*	8.2 - 62.6
IGFBP-3	0.6	0.4 - 1.2	0.6	0.4 - 1.0

Results are given as median and range.
Serum GH levels calculated by Pulsar analysis and expressed in µg/l, serum IGF-I and IGFBP-1 in ng/ml and serum IGFBP-3 in mg/l.
* $p < 0.01$ compared to levels before DEXA.
** $p < 0.05$ compared to levels before DEXA.

percentage decline in serum GH levels. The same applied for the number of DEXA doses given before the second profile.

Serum IGF-I and IGFBP-1 and -3 levels

During DEXA treatment, serum IGFBP-1 levels significantly declined from a median of 69.9 to 13.5 ng/mL ($p < 0.01$), whereas serum IGF-I and IGFBP-3 levels did not change (Fig. 2). The number of doses of DEXA administered before the start of the second profile did not significantly correlate with serum levels of IGF-I, IGFBP-1, and IGFBP-3. The cumulative dosage of DEXA (in mg/kg of body weight) given before the start of the second profile showed a trend toward lower IGF-I levels, although this did not reach significance (r =

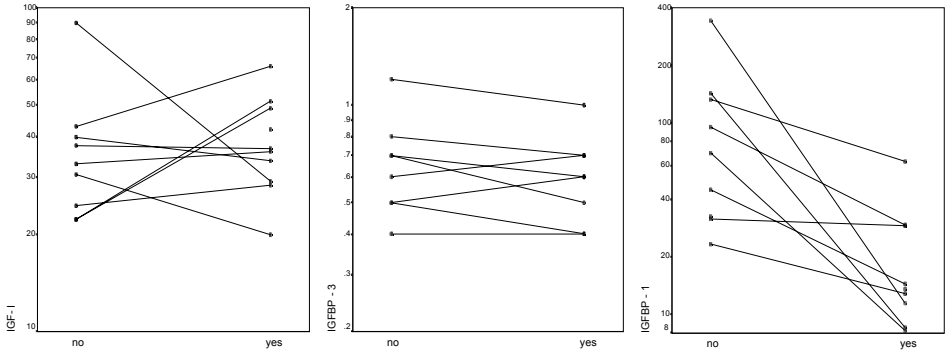

Figure 2 Effect of DEXA treatment on serum IGF-I, IGFBP-3, and IGFBP-1 levels in ventilated preterm infants.
Serum IGF-I is expressed in ng/ml, serum IGFBP-3 in mg/ml, and serum IGFBP-1 in ng/ml.

-0.6; p = 0.08). Serum IFG-I, IGFBP-1, and IGFBP-3 levels were not significantly different between patients receiving parenteral or enteral nutrition.

Correlations between serum GH and IGF-I, IGFBP-1, and IGFBP-3 levels

Before the start of DEXA, no correlation was found between gestational age, birth weight, birth weight SD-score, and serum levels of IGF-I, IGFBP-1, and IGFBP-3. Mean, baseline, and maximal serum GH levels also did not correlate with serum IGF-I, IGFBP-1, and IGFBP-3 levels. No correlation was found between serum levels of IGF-I and IGFBP-1 or IGFBP-3 levels.

During DEXA treatment, however, serum IGFBP-1 levels were significantly correlated with mean and baseline GH levels (r = 0.71, p = 0.03 and r = 0.70, p = 0.04, respectively), but not with serum IGF-I levels. Ventilation score and nutritional intake did not significantly correlate with serum GH, IGF-I, IGFBP-1, and IGFBP-3 levels, neither before nor after the start of DEXA treatment.

Discussion

In this study we demonstrate a significant decline in serum baseline, mean, and maximal GH levels and serum IGFBP-1 levels in ventilated very preterm infants 2 days after the start of DEXA treatment. Two days of DEXA treatment had no significant acute effect on serum IGF-I and IGFBP-3 levels. So far, no studies have described the effect of 2 days of high-dose DEXA administration on GH secretion, serum IGF-I levels, and serum IGFBPs in ventilated very preterm infants.

It has been reported that DEXA treatment in preterm infants results in reductions in weight gain, growth in occipitofrontal head circumference, linear growth, and lower leg growth; has a catabolic effect by increased protein breakdown; and alters skeletal metabolism.[3–8,20–22] Despite substantial concerns questioning the benefits of the use of postnatal steroids and concerns about long-term neurologic outcome raised by the American Academy of Pediatrics and Canadian Paediatric Society, DEXA is still administered to preterm infants to wean them from the ventilator when clinical and radiologic signs of BPD develop and conservative treatment, such as increased ventilation settings, fluid restriction, diuretics, antibiotics, and inhalation therapy, has failed.[23] The starting dose of DEXA usually given to very preterm infants in most neonatal intensive care units is 0.5 mg/kg per day for 3 days, which is equivalent to 13.3 mg/kg per day of hydrocortisone or 3.3 mg/kg per day of prednisone. Thus, compared with other corticosteroid treatments, the DEXA dose used in preterm infants is 11- to 22-fold higher than in children with kidney transplants (prednisone, 0.15 – 0.25 mg/kg per day) or in physiologic replacement in relative adrenal insufficiency in very preterm infants (1.2 mg of hydrocortisone in a 1000-g infant).

Besides, the biologic half-time of DEXA is much longer than that of prednisone (36–72 h compared with 8–12 h).[24]

Inasmuch as we present the first data of an acute DEXA effect on serum GH levels in preterm infants, we cannot compare our results with other studies in preterm infants. In older individuals the effects of glucocorticoids on spontaneous and stimulated GH secretion appear contradictory.[25–27] Chronic hypercortisolism inhibits spontaneous and stimulated GH secretion in children and adults.[9–11,28] Hokken-Koelega *et al.* demonstrated a significant decrease in mean GH levels during chronic prednisone administration in pediatric growth-retarded renal allograft recipients.[29] In contrast, acute administration of DEXA increases spontaneous GH secretion in children and adults.[30–33] In rats, spontaneous GH levels were significantly decreased and the GH-releasing hormone–induced GH response was blunted after 4 days of DEXA treatment. Subsequent immunologic neutralization of somatostatin, however, resulted in a significantly enhanced GH response, suggesting that steroids inhibit the GH response to GH releasing hormone by increasing the hypothalamic somatostatin secretion.[27] Miell *et al.*, however, found in healthy volunteers a persistent but attenuating rise in serum GH levels after 4 days of DEXA treatment.[34] This different effect of DEXA administration compared with our data might be caused by the 10-fold lower dosage of DEXA (0.05 mg/kg per day) used by Miell *et al.* than the dosage used in our preterm infants.[34] Thus, the potentiating or blocking effect of DEXA on GH secretion seems to be dependent on the duration and dose of administration of DEXA.

We could not demonstrate a relationship between the serum GH levels and the cumulative dose or the number of doses of DEXA given before the second profile. This might be because of the small variation in the cumulative dose in our patients. The suppressive effect of DEXA on serum GH levels in most infants was still present 12 h after the last dose, suggesting a GH-suppressive effect of at least 12 h in most infants. In children undergoing renal transplantations, the suppressive effect of prednisone on GH secretion was similar whether given as daily or alternate day prednisone therapy, suggesting a prolonged effect of prednisone administration. As the biologic half-life of DEXA is much longer and the dose of DEXA given to our patients much higher compared with the prednisone dose in the renal allograft patients, we speculate that the suppressive effect on serum GH levels might be at least 24 h.

After 2 days of high-dose DEXA treatment, we could not demonstrate a decline in serum immunoreactive IGF-I and IGFBP-3 levels whereas serum GH levels significantly declined. We had expected to find the decline in serum GH levels being accompanied by a decline in serum IGF-I levels. However, it might be that 2 days of DEXA treatment was too short to observe an acute decline in serum IGF-I or IGFBP-3 levels. It was not possible to perform the second GH profile at a later moment during DEXA treatment as most infants did not retain their arterial catheter once they were extubated. Pre-treatment IGF-I levels were low already, with high serum GH levels, suggesting a relatively GH-resistant state; this might

also explain the apparent lack of effect of DEXA on circulating IGF-I. Serum IGF-I levels might also be more influenced by nutritional intake.[35] Data on the effect of DEXA on serum IGF-I and IGFBP-3 levels in preterm infants are limited. Serum IGF-I and IGFBP-3 levels have been reported to rise in preterm infants after cessation of DEXA treatment.[5,20] Bloomfield *et al.* showed increasingly higher levels of IGF-I and IGFBP-3 with reducing dosages of DEXA, suggesting a dose-dependent effect of DEXA on IGF-I and IGFBP-3 levels.[22] However, in these studies, at all time points during and after DEXA treatment, serum IGF-I and IGFBP-3 levels were higher than before the start of DEXA and therefore not lower than baseline values. Our study showed that there was no decline in serum IGF-I and IGFBP-3 levels at least after 2 days of DEXA treatment. Because none of the studies had an untreated control group, no data are available about the IGF-I and IGFBP-3 levels from patients with similar respiratory problems when no DEXA has been started. The higher levels of IGF-I and IGFBP-3 observed after stopping DEXA might not be merely caused by cessation of steroids but also by other factors such as improvement in the clinical condition and nutritional intake of the infants.[35]

In this study we did not observe a relationship between serum IGF-I and IGFBP-3, in contrast to other studies in older children. Whether this is related to the developmental range of the infants or other factors needs further study.

The high pre-treatment IGFBP-1 levels observed in our patients might be related to their severe illness as has been described in older children.[36] High IGFBP-1 levels are also found in conditions such as prolonged fasting, anorexia nervosa, and fetal hypoxemia.[37,38] The high IGFBP-1 levels in our study were not caused by fasting as all our preterm infants were continuously fed and never showed low glucose levels. We cannot rule out a possible additional effect of short-term hypoxemia on serum IGFBP-1 levels immediately before starting DEXA treatment. The observed decline in serum IGFBP-1 levels during DEXA treatment in our patients agrees with Miell *et al.*, who described an increase in serum insulin levels accompanied by a decrease in serum IGFBP-1 after short-term DEXA treatment in adults.[39] As it has been demonstrated that insulin and not glucose levels are regulating serum IGFBP-1 levels *in vivo*, we speculate that high insulin levels during DEXA might have resulted in a decline in serum IGFBP-1 as observed in our patients.[40,41] However, as some of our patients were extubated after 2 days of DEXA, thereby showing clinical improvement, this might also have contributed to the decrease in serum IGFBP-1 levels.

The GH-IGF axis in childhood is important for growth, but the precise role of GH and IGF-I in preterm infants is as yet unknown. DEXA treatment is known to reduce physical growth and to induce catabolism. Although our study showed a significant decline in serum GH levels after 2 days of DEXA, no acute decline in serum immunoreactive IGF-I levels was seen. This is in agreement with other studies.[5,20,22] It therefore remains a question whether the low GH levels observed after the start of DEXA are responsible for the decline in growth rate in these preterm infants. It might well be that the decline in growth is rather the result

of a direct effect of DEXA on the epiphyseal cartilage by inhibition of the local secretion and paracrine action of IGF-I.[12] Prednisone causes *in vitro* a 46% fall in IGF bioactivity and has a suppressive effect on IGF-I mRNA in the tibia, liver, lung, and kidney in the rat, without causing significant changes in serum IGF-I levels.[42,43] Thus, DEXA-induced inhibition of local IGF-I gene expression might be one of the mechanisms of growth retardation as well. In addition the low serum GH levels might result in a diminished direct effect on the epiphyseal plate as well.[12]

In conclusion, our data show a suppressive effect of 2 days of DEXA treatment on serum GH levels, without an acute decline in serum IGF-I levels. A concomitant decline in serum IGFBP- 1 levels was found.

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5

Randomised, placebo-controlled GH trial in very preterm infants at risk for bronchopulmonary dysplasia (BPD): rhGH treatment does not prevent growth failure caused by dexamethasone

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Submitted

Abstract

Preterm infants developing bronchopulmonary dysplasia (BPD) are frequently treated with dexamethasone (DEXA) to facilitate weaning from the ventilator. DEXA has a growth suppressive effect which might have long-term consequences on growth and development.

The aim of this study was to evaluate if treatment with recombinant human growth hormone (rhGH) is able to overcome the growth inhibiting effect of DEXA treatment in preterm infants.

Thirty preterm artificially ventilated infants median (range) gestational age 27.0 (24.6-30.6) wks, birth weight 852 (501-1335 g) were randomly assigned to receive either DEXA and placebo (n = 15) or DEXA and GH (n = 15) treatment. DEXA was given during a 24-day period, starting with a dose of 0.5 mg/kg/day and tapering off every third day. Simultaneously rhGH (0.3 mg/kg/day) or placebo was administered during 6 weeks. Weight, length (CHL), knee-heel length (KHL) and head-circumference (OFC) were measured, as well as, serum GH, IGF-I and IGFBP-1 and -3 levels. Adverse effects were recorded and echocardiography was performed.

In the first week of DEXA treatment (dose 0.5-0.3 mg/kg/day) no gain in weight, CHL, KHL and OFC occurred in both groups. In week 2 and 3 on DEXA, as well as in the 3-week period after discontinuation of DEXA, growth was not significantly different between the GH and the placebo group. Growth during week 2 and 3 on DEXA was not different from growth after discontinuation of DEXA.

Serum IGF-I levels did not increase during the 6-week period, while serum IGFBP-3 levels increased significantly ($p < 0.02$) during DEXA, in both groups. In the placebo group two patients died. The frequency and severity of hyperglycemia, hypertension, retinopathy and cardiac hypertrophy was not different between the groups.

Conclusion: Two major conclusions arise from this study. First rhGH treatment has no effect in preventing impairment of growth in DEXA-treated preterm infants when a rhGH dose of 0.3 mg/kg/day is given for a 6 - week period. Secondly, the impairment of growth is particularly associated with high-dose DEXA treatment during the first week. Follow-up studies are needed to evaluate possible effects of rhGH on growth, lung function and neurodevelopment on the longterm.

We suggest that future research could be directed at the use of lower doses of glucocorticoids, aiming at both, maintenance of the beneficial effect of DEXA of weaning from the ventilator, and prevention of adverse effects.

Introduction

In very preterm infants the development of bronchopulmonary dysplasia (BPD) is a major problem. According to the latest consensus BPD is defined as a persistent need for supplemental oxygen for at least 28 days and respiratory features such as retractions, tachypnea and rales.[1] The incidence of BPD increases from 12% in infants with a gestational age of 30 weeks to 30 - 40% in those born after a gestational age of 25-26 weeks.[2,3]

In the neonatal period systemic corticosteroids are frequently given to reduce the duration of artificial ventilation, although they do not always effect mortality.[4,5,6] Most infants at risk for development of BPD will be treated with dexamethasone (DEXA) to facilitate weaning from the ventilator. DEXA treatment in preterm infants is known for its inhibiting effect on weight gain as well as linear growth and head circumference.[7-11] Some follow-up studies suggest impairment in brain growth and persisting growth retardation into childhood.[12-15] In addition, DEXA has a catabolic effect by increasing protein breakdown whereas skeletal metabolism is altered by inhibition of intestinal calcium absorption, stimulation of renal calcium excretion and suppression of collagen turnover in preterm infants.[16-18]

Corticosteroids have a suppressive effect on pituitary GH secretion in children.[19] In a previous study we evaluated the effect of high dose DEXA (0.5 mg/kg/day) on serum GH levels in ventilated preterm infants and found a significant DEXA-induced decline in serum GH levels.[20] Klaus *et al* suggested that the growth - inhibiting effect of DEXA might be overcome by high dose GH treatment.[21] Several studies have demonstrated a beneficial effect of GH treatment on linear growth and on neurodevelopment in steroid - treated older children.[22]

As the growth suppressive effects of DEXA in preterm infants might have long-term consequences on longitudinal growth and development, we performed a randomised double-blind GH-controlled trial in ventilated preterm infants treated with DEXA in order to evaluate if recombinant human GH (rhGH) overcomes the growth inhibiting effects of DEXA treatment.

Methods

Patients

Thirty ventilated preterm infants born after a gestational age ≤ 32 weeks at risk for developing BPD were included in the study at the moment DEXA treatment was initiated in order to wean them from the ventilator. The indication to start DEXA treatment was made by the attending neonatologist and based on a persistent need for artificial ventilation and oxygen dependency accompanied by an abnormal chest X-ray. Patients were excluded when they had a persistent ductus arteriosus, pulmonary infection or sepsis at

randomisation or when they had a grade 3 or 4 intraventricular hemorrhage, chromosomal defect or major congenital anomalies. Patients were also excluded when the mother was treated for an endocrine disorder with rhGH, thyroxin or thyreostatic drugs. All patients were admitted to the Neonatal Intensive Care Unit (NICU) of the Sophia Children's Hospital in Rotterdam, The Netherlands.

The Medical Ethics Committee of the Erasmus University Medical Center approved the study protocol and written informed consent was obtained from the parents of each child.

Methods

Directly after inclusion all patients started with DEXA treatment and were randomly and blindly assigned to receive recombinant human GH (rhGH) 0.3 mg/kg/day (Norditropin R, Novo Nordisk A/S) or placebo treatment (Novo Nordisk A/S) administered once daily by subcutaneous injection for a period of 6 weeks. GH or placebo treatment was started within 4- 6 hours after start of DEXA. DEXA treatment was started with a dosage of 0.5 mg/kg/day, in two doses per day, during 3 days. Thereafter the dosage was tapered to 0.3 mg/kg/day for three days and gradually tapered every three days to 0.1 mg/kg/day every other day over a 24 - day period. When extubation did not succeed a second course or prolonged course of DEXA was given, while GH or placebo treatment was continued. The day and time of starting DEXA treatment was t = 0 and the number of days in study were calculated from the start of DEXA.

All patients underwent a physical examination before start of DEXA. Attending neonatologists, investigators and parents were unaware of the treatment allocation due to the double-blind-design.

The attending neonatologist provided routine care and feeding decisions. According to the policy of the NICU, infants were started on parenteral nutrition on the second day of life and enteral feedings were initiated on day 7. Premature infant formulas were used with a caloric density of 80-88 kcal/100 ml and a protein content of 2.4-2.6 g/100 ml.

Serum GH levels were measured weekly and in infants with an arterial line a 6-hours GH profile was performed. Serum IGF-I and IGF- binding protein -3 (IGFBP- 3) and -1 (IGFBP-1) levels were measured before as well as 3 and 6 weeks after start of DEXA.

Severity of respiratory failure at start of DEXA was assessed by calculating the ventilation score (mean airway pressure times inspired fraction of oxygen). Possible neonatal side effects of DEXA and of GH treatment such as hypertension requiring treatment with antihypertensive drugs, hyperglycaemia treated with insulin therapy, cardiac hypertrophy, and incidence and severity of retinopathy of prematurity (ROP) were recorded.

Nutritional intake was calculated from the daily nutritional intake during the 6 week study period.

Cardiologic evaluation occurred in all patients before, during and after stopping DEXA treatment by the same investigator. M-Mode echocardiography was used to measure the

dimensions of the left ventricular wall and internal dimensions at systole and diastole according to the recommendations of the American Society of Echocardiography.[23] The occurrence of left ventricular hypertrophy was assessed by calculating the relative wall thickness of the left ventricle. Relative wall thickness (RWTh) was calculated by dividing the posterior wall thickness of the left ventricle by the left ventricle internal dimension measured at end- diastole. Left ventricular hypertrophy was defined as a RWTh > 0.2.

Anthropometric measurements

Anthropometric measurements as weight, recumbent crown-heel length (CHL), knee-heel length (KHL) and occipito-frontal circumference (OFC) were performed. All anthropometric measurements were done by two experienced observers. Baseline measurements were performed before starting DEXA. Weight was measured daily, OFC and CHL weekly. KHL was measured twice a week. The time of measurements was recorded and calculated as hours from the start of DEXA administration.

CHL was measured using a neonatometer in the incubator, with full extension of the hips and knees and with both heels pressing the end of the neonatometer. CHL was measured three times and recorded to the nearest millimeter and the mean was used for analysis. Weight was measured on an electronic baby scale (TEC, Tokyo Electronic Co) to the nearest gram, every day on the same time. OFC was measured three times using a standard plastic measuring tape (1 cm wide) and recorded to the nearest millimetre. The mean of three measurements was used for analysis. KHL was measured according to Gibson with a hand-held knemometer.[7] Each measurement consisted of 8 readings; the mean of these measurements was used for analysis. The investigator performed the measurements and a second observer performed the readings.

Birth weight was expressed as SD-scores for gestational age according to the standards of Usher and McLean.[24]

Hormone assays

All hormone determinations were performed in the Endocrine Laboratory of the Erasmus University Hospital Rotterdam, the Netherlands.

Serum GH levels were measured using a two-site immunoradiometric assay (ELSA-HGH, CIS bio international, ORIS Group, France). The assay measured the 22 kD GH and showed no cross reactivity with 20 kD GH. All samples were analysed in duplicate in the same assay. The WHO First International Reference Preparation WHO 80/505 was used as a

standard. The intra-assay and inter-assay coefficients of variation were less then 2.8% and less then 4.4%, respectively. The detection limit of the assay was 0.04 µg/l.

Serum IGF-I levels were determined by RIA (SM-C-RIA-CT, Biosource Europe SA) after acid- ethanol extraction. The sensitivity was 0.25 ± 0.10 ng/ml, the intra-assay coefficients of variation were less than 6.1%. The inter-assay precision was less than 9.9%. Cross-reactivity with IGF-II was 0.2%, with insulin < 0.001% and with GH < 0.01%.

Serum IGFBP-1 levels were determined using a two-site immunoradiometric assay principle (Total IGFBPI IRMA DSL-7800, Diagnostic Systems Laboratories, Webster, Texas, USA). The sensitivity of the assay was 0.33 ng/ml and the intra-assay coefficients of variation were less than 5.2%. The inter-assay coefficients of variation were less than 6.0%. No cross-reactivity was seen with the IGFBP-2, -3, -4, -5, and -6.

Serum IGFBP-3 levels were determined using a two-site immunoradiometric assay (IGF-BP3 IRMA, DSL-6600, Diagnostic Systems Laboratories Webster, Texas, USA). The sensitivity of the assay was 0.5 ng/ml, with intra-assay coefficients of variation of less than 3.9%.The inter- assay coefficients of variation were less than 1.9%.

Statistical analyses

Statistical analyses were performed using SPSS 9.0 for Windows 95, SPSS software, Chicago (IL) or SAS version 6.12 for Windows, SAS Institute Inc., Cary (NC), U.S.A. Power calculations had led to a required number of 2 x 25 patients. However, as recruitment was slower than expected, the inclusion period was stopped after 3 years when a total of 30 patients was included. The decision to stop was made when the trial was still blind and without consideration of the accumulated data.

Visual inspection of scatterplots of anthropometric data showed more or less stable values during the first week for all parameters, but an increase thereafter. Therefore these data were analysed using peacewise linear regression, also called the "broken-stick" method, with a breakpoint at day 7.[25] In a secondary analysis, the effect of stopping DEXA was investigated using the same method. This was done by allowing for a second breakpoint at the day of stopping DEXA. These analyses were done using repeated measurements analysis of variance, and calculations for this particular analysis were done with SAS software (random coefficients model from SAS PROC MIXED).

Weight and the hormone values were logarithmically transformed before analysis to obtain approximate normal distributions. Percentages were compared using Fisher's exact test. Two-sided p-values ≤ 0.05 were considered significant.

Results

Clinical characteristics

Thirty patients were included in the study. Fifteen patients received DEXA and GH and 15 patients were treated with DEXA and placebo. The clinical data of the patients before start of the study are shown in Table 1. The groups were comparable regarding all characteristics.

The clinical data during the study are shown in Table 2. The baseline data at randomisation were comparable between groups. During the 6 - week study period no difference in daily nutritional intake, number of days on artificial ventilation, on supplemental oxygen and on DEXA treatment between the GH and the placebo group was observed.

Anthropometric measurements

First, we analysed the data for the total study period of 6 weeks in both treatment groups. In the secondary analysis we evaluated within group differences comparing the period on DEXA treatment with the period after discontinuation of DEXA (Table 3).

Table 1 Perinatal data of the 30 study patients.

	DEXA-GH N = 15	**DEXA-placebo N = 15**
Male / female (n)	7 / 8	11 / 4
Gestational age (wk)	27.2 (24.6 - 30.6)	27.3 (24.9 - 29.9)
Birth weight (g)	889 (580 - 1335)	847 (501 - 1335)
Antenatal steroids (n)	14	12
Premature rupture of the membranes (n)	2	4
Preeclampsia (n)	3	4
Caesarian Section (n)	6	7
Twin pregnancy (n)	1	4
Apgar 1 min	4.6 (2.2)	5.3 (2.4)
Apgar 5 min	7.3 (1.4)	8.0 (1.3)
Respiratory distress syndrome (n)	9	14
Surfactant use (n)	10	14
Patent ductus arteriosus (n)	9	14
Intraventricular haemorrhage grade 1-2 (n)	6	6
Intraventricular haemorrhage grade 3-4 (n)	0	0

Results are given as median (range) or number of infants.

Table 2 Clinical data of the study patients.

	DEXA-GH N = 15	DEXA-placebo N = 15
At randomisation		
Postnatal age (wk)	3.1 (1.1 - 4.6)	2.6 (1.0 - 5.4)
Postconceptional age (wk)	30.3 (27.6 - 35.1)	29.0 (27.4 - 33.9)
Ventilation score	429 (255 - 880)	450 (250 - 1800)
Weight (g)	1101 (750 - 1510)	1035 (720 - 1630)
Crown- heel length (cm)	36.5 (31.9 - 40.7)	36.1 (31.1 - 41.2)
Knee-heel length (mm)	87.7 (73.9 - 98.1)	87.6 (69.4 - 99.2)
Occipito-frontal headcircumference (cm)	26.5 (23.0 - 29.7)	26.3 (22.5 - 29.6)
During 6- weeks study period		
Caloric intake (kcal/kg/day)	133.6 (17.1)	128.7 (21.1)
Protein intake (g/kg/day)	3.4 (0.2)	3.5 (0.3)
Number of days on DEXA (days)	24 (22 - 42)	24 (3 - 36)
Number of days on artificial ventilation (days) [a]	3 (1 - 31)	4 (1 - 30)
Number of days O2 (days) [a]	36 (2 - 42)	42 (4 - 42)
Use of antihypertensive drugs (Y/N)	0 / 15	1 / 14
Insulin treatment (Y/N)	3 / 12	2 / 13
LVH 3 wks after start DEXA (Y/N) [b]	12 / 14	13 / 14
Follow-up at 36 wk PCA (after study period)		
Need for supplemental O2 at 36 wk PCA (Y/N)	11 / 4	9 / 4
ROP grade 3-4 (Y/N)	2 / 13	3 / 11
Cystic PVL (Y/N)	0 / 15	1 / 13
Death (Y/N)	0 / 15	2[#] / 15

Results are given as median (range) or number of infants.
PCA: post conceptional age (wks), O2: oxygen, LVH: left ventricular hypertrophy, ROP: retinopathy of prematurity, PVL: periventricular leucomalacia.
[a] after start of DEXA,
[b] defined as Relative Wall Thickness of the left ventricle (posterior wall thickness left ventricle / left ventricle internal dimension) > 0.2
[#] 2 patients died: one patient due to progressive respiratory insufficiency (study day 3) and one patient due to cystic PVL (study day 46)

Weight. In the first week on DEXA no increase in weight was observed in both the GH and the placebo group. The mean change in weight in the first week was –2.7 and –3.1 g /kg /day in the GH and placebo group, respectively. In both treatment groups a significant increase in weight occurred ($p < 0.001$) during the 2 to 6 week period after start of DEXA. The mean increase in weight in the GH group was 15.8 g/kg/day versus 15.6 g/kg/day in the placebo group (Figure 1).

Table 3 Anthropometric measurements during the 6 - week study period.

	DEXA-GH				DEXA-placebo			
	Week 1	Week 2 – 6			Week 1	Week 2 – 6		
		During DEXA[1]	After DEXA[2]	Total		during DEXA[1]	after DEXA[2]	Total
Weight g/kg/day	-2.7 (-7.8; 2.4)	16.2 (14.6; 17.8)	15.8 (14.2; 17.4)	15.8 (12.5; 19.1)	-3.1 (-7.8; 1.6)	15.3 (13.5; 17.1)	16.0 (14.2; 17.8)	15.6 (12.3; 18.9)
CHL cm/wk	-0.5 (-1.1; 0.2)	0.8 (0.5; 1.0)	1.2[#] (1.0; 1.4)	1.0 (0.8; 1.1)	-0.5 (-0.9; 0.0)	0.9 (0.7; 1.1)	1.1 (0.9; 1.3)	1.0 (0.8; 1.1)
KHL mm/wk	0.1 (-1.3; 1.5)	2.6 (2.1; 3.2)	3.7[##] (3.2; 4.2)	3.1 (2.8; 3.5)	0.0 (-1.3; 1.3)	2.7 (2.2; 3.4)	3.5 (3.0; 3.9)	3.1 (2.7; 3.4)
OFC cm/wk	-0.0 (-0.3; 0.2)	1.0 (0.9; 1.2)	1.0 (0.9; 1.1)	1.0 (0.9; 1.1)	-0.3 (-0.6; 0.1)	1.0 (0.8; 1.1)	0.9 (0.8; 1.0)	0.9 (0.8; 1.0)

Results are given as mean (95% CI) increase per day or week.
CHL: crown-heel length, KHL: knee-heel length, OFC: occipito-frontal head circumference.
[1]period from week 2 until stop DEXA, [2]period after stop of DEXA.
[#]p=0.028, [##]p=0.022, significant increase versus during DEXA.

Occipito Frontal Circumference (OFC). In the first week on DEXA treatment no growth in OFC was observed in both the GH and the placebo group. In both treatment groups OFC increased significantly during week 2 to 6 after start of DEXA (both groups, $p < 0.001$). The increase in OFC in the GH group was 1.0 cm/wk versus 0.9 cm/wk in the placebo group and not significantly different between the groups ($p = 0.32$).

Crown Heel Length (CHL). In the first week on DEXA no significant increase in mean CHL occurred in both the GH and the placebo group. The mean change in CHL was –0.5 cm/wk in both groups. In both treatment groups a significant increase in CHL occurred ($p < 0.0001$) during week 2 to 6 after start of DEXA. In the GH as well as in the placebo group CHL increased with 1.0 cm/wk (Figure 2).

Knee Heel Length (KHL). In the first week on DEXA treatment no increase in mean KHL was observed in both the GH and the placebo group. In both treatment groups an identical significant increase in KHL ($p < 0.001$) was observed during week 2 to 6 after start of DEXA. In both treatment groups the increase in KHL was 3.1 mm/wk.

Serum GH levels

Serum GH profiles were performed in 6/15 infants during treatment with rhGH (Figure 3). Serum GH levels varied between 1.4 and 518 µg/l. In all patients high GH levels after administration of rhGH were followed by a rapid decline. In 3 patients serum GH half-life could be calculated and these were 1.9, 5.2 and 5.5 hours, respectively. Serum GH levels measured just before administration of the next dose GH were lower than 5 µg/l.

Table 4 Serum IGF-I, IGFBP-3 and -1 levels in DEXA-GH and DEXA-placebo treated infants.

	DEXA- GH			DEXA – placebo		
	Baseline	**Day 21**	**Day 42**	**Baseline**	**Day 21**	**Day 42**
IGF-I	39.8 (20.7 - 96.7)	53.2 (9.9 - 95.6)	36.0 (9.9 - 222.6)	42.1 (7.7 - 234.8)	30.6 (6.1 - 65.8)	37.5 (17.6 - 73.4)
IGFBP-1	108.0 (7.9 - 485.0)	48.4*,# (5.9 - 138.0)	35.9 (6.4 - 183.0)	64.1 (32.4 - 421.0)	105.7 (16.2 - 385.0)	102.2 (14.4 - 997.0)
IGFBP-3	0.7# (0.3 - 3.7)	1.3* (0.7 - 2.2)	1.2** (0.8 - 3.2)	0.5 (0.2 - 0.9)	1.1*** (0.4 - 1.6)	1.2*** (0.8 - 1.5)

Results are given as median (range).
Serum levels of IGF-I expressed in ng/ml, IGFBP-1 in ng/ml and IGFBP-3 in mg/l.
* $p < 0.01$, ** $p < 0.02$, *** $p < 0.001$, versus baseline.
# $p < 0.05$ between both groups.

Serum IGF- I, IGFBP- 3 and –1 levels

Serum IGF-I levels did not increase during the 6- week study period in both the GH and placebo group (Table 4). Serum IGFBP-3 levels increased significantly during DEXA in both groups ($p < 0.02$), but the increase in IGFBP-3 was not significantly different between the groups ($p = 0.15$).

Baseline serum IGFBP-3 levels were significantly higher in the GH than in the placebo group ($p < 0.05$). Serum IGFBP-1 levels at day 21 were significantly lower in the GH compared to the placebo group ($p < 0.05$) and to baseline levels ($p < 0.01$). The change from baseline was not significantly different between the groups.

Adverse effects

One patient received antihypertensive treatment and 2 patients received insulin treatment in the placebo group, while 3 patients in the GH group received insulin treatment. Two patients in the placebo group died; one patient because of progressive respiratory insufficiency at study day 3 and one patient died because of severe cystic leucomalacia at study day 54 during the follow up period. None of the infants in the GH group died.

At follow-up until 36 weeks postconceptional age 11/15 infants in the GH group and 9/13 in the placebo group had developed bronchopulmonary dysplasia (BPD). Severe retinopathy had developed in 2 patients in the GH group and 3 patients in the placebo group.

Left ventricular hypertrophy measured by RWTh of the left ventricle occurred in the majority of infants in both groups during DEXA treatment. The mean RWTh of the left ventricle 3 weeks after start DEXA was not significantly different between the groups.

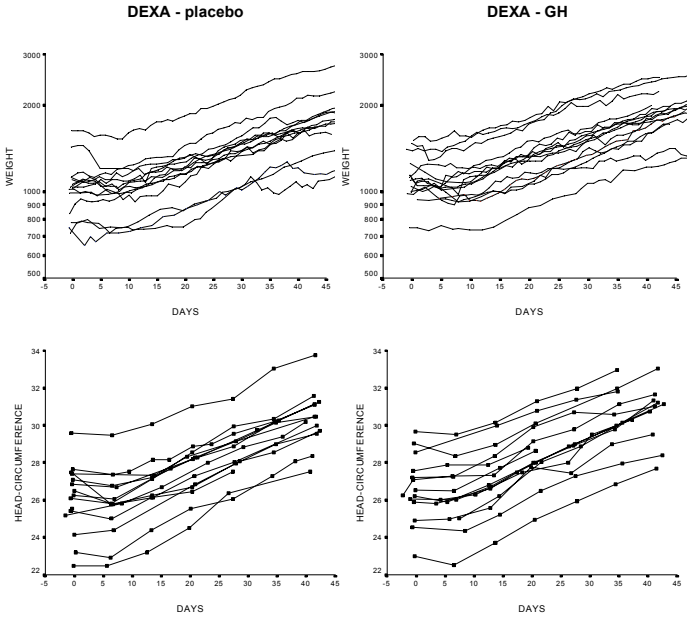

Figure 1 Weight and occipito-frontal headcircumference in DEXA-GH and DEXA-placebo treated infants.
Weight on a logaritmic scale and expressed in grams. Head-circumference expressed in cm. DAYS meaning the number of days after starting of DEXA treatment, calculated from t = 0 (hour of first DEXA dose).

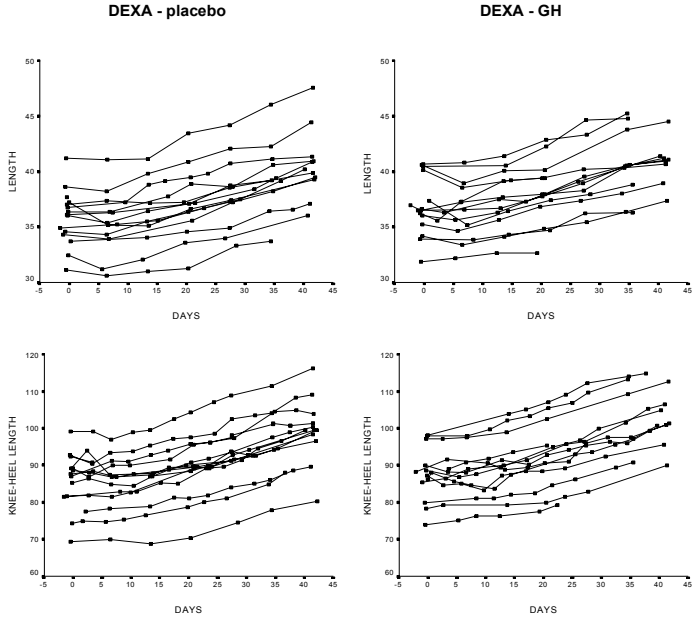

Figure 2 Crown-heel length and Knee-heel length in DEXA-GH and DEXA-placebo treated infants.
Crown-heel length expressed in cm and knee-heel length in mm. DAYS meaning the number of days after starting of DEXA treatment, calculated from t = 0 (hour of first DEXA dose).

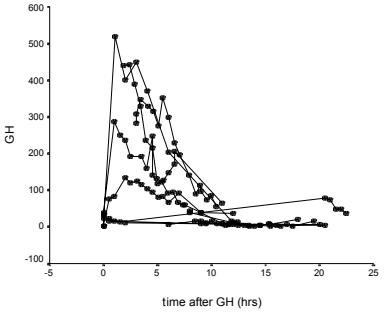

Figure 3 Serum GH levels in 6 patients after administration of rhGH.
Serum GH levels expressed in µg/l versus time expressed in hours after administration of rhGH.

DEXA effects on growth and serum IGF-I, IGFBP-1 and -3 levels

In both treatment groups the increase in weight, CHL, KHL and OFC was significantly lower during the first week than during the next weeks on DEXA (both groups, $p < 0.001$). Interestingly, the increase in weight and OFC during week 2 and 3 on DEXA, was not significantly different from the increase in weight and OFC after discontinuation of DEXA (Table 3).

In the placebo group the increase in CHL and KHL after discontinuation of DEXA was not significantly different from the increase during week 2 and 3 on DEXA ($p = 0.26$ and $p = 0.059$, respectively). In the GH group the increase in CHL and KHL after discontinuation of DEXA was significantly higher than during week 2 and 3 on DEXA ($p = 0.028$ and $p = 0.022$, respectively). This increase in CHL and KHL during GH treatment was not different from the corresponding increase in the placebo group ($p = 0.37$ and $p = 0.55$, respectively).

In both treatment groups start and discontinuation of DEXA did not result in a change in serum IGF-I levels from baseline. Serum IGFBP-3 levels increased during DEXA, but discontinuation of DEXA did not result in a further increase in serum IGFB-3 levels in both groups ($p = 0.46$).

Discussion

In this double-blind GH-controlled trial we showed that 6 weeks of treatment with rhGH is not able to overcome the growth failure caused by DEXA in very preterm infants who are at risk for BPD. No gain in weight, CHL, KHL, and OFC was observed during the first week of DEXA treatment in both the GH and placebo group. In the second and third week on DEXA as well as during the 3 – week period after discontinuation of DEXA the gain in weight, CHL, KHL and OFC was not significantly different between the groups.

Interestingly, in the placebo group, the growth during DEXA treatment with reduced doses of DEXA during the second and third week was not different from the growth observed after discontinuation of DEXA. In the GH group the growth in CHL and KHL was significantly higher after discontinuation of DEXA. This increase, however, was not different from the increase observed in the placebo group.

GH treatment did not result in an increase in serum IGF-I levels and higher serum IGFBP-3 levels.

Nowadays, DEXA treatment in very preterm infants in order to facilitate extubation is an area of great concern especially when high doses of glucocorticoids are given. DEXA treatment in very preterm infants has been associated with reduced cerebral cortical gray matter, an arrest of alveolarisation of the lungs, catabolic effects, alteration of skeletal metabolism and cessation of growth.[7-18] Despite these substantial concerns, DEXA is still administered to preterm infants in order to wean them from the ventilator when clinical and radiological signs of BPD develop and conservative treatment such as increased ventilation settings, fluid restriction, diuretics, antibiotics and inhalation therapy has failed.[26] The starting dose of DEXA usually given to very preterm infants in most neonatal intensive care units is 0.5 mg/kg/day for 3 days, which is equivalent to 13.3 mg/kg/day hydrocortisone and to 3.3 mg/kg/day prednisone, and thus much higher than the physiological hydrocortisone production of approximately 1 mg/kg/day.

This is the first GH - controlled study measuring the effect of rhGH versus placebo in combination with DEXA on growth in preterm infants. By accurately measuring weight, CHL, KHL and OFC we were able to minimize intra- and inter-observer variations and obtained narrow confidence intervals, allowing evaluation of growth on a weekly base. Two major conclusions arise from this study. First rhGH treatment has no effect in preventing impairment of growth in DEXA-treated preterm infants when a rhGH dose of 0.3 mg/kg/day is given for a 6 - week period. Secondly, the impairment of growth is particularly associated with high-dose DEXA treatment during the first week.

During the first week, a high dose of DEXA 0.5 mg/kg for 3 days is given, followed by a dose of 0.3 mg/kg for 3 days and 0.25 mg/kg for 3 days, respectively. It is of importance, that when the daily DEXA dose was decreased to less than 0.25 or 0.20 mg/kg/day, which is equivalent to 5 mg/kg/day of hydrocortisone, the rate of growth in these preterm infants was similar to the growth rate during the first 3 weeks after discontinuation of DEXA. This applied to growth of weight, crown-heel length and knee-heel length as well as for occipito-frontal head-circumference. Apparently there is a dose- effect relationship as has also been described in older children. One study evaluating the catabolic effect of DEXA treatment showed a higher nitrogen excretion, leucine turnover and breakdown at day 4 with a DEXA dose of 0.35 mg/kg/day, compared to day 19 when a dose of 0.10 mg/kg/day was adminstered.[17] In addition, increases in plasma amino acid levels have been reported during high doses of DEXA, whereas Crofton *et al.* reported a dose–dependent

decrease in collagen markers during DEXA and a significant increase after discontinuation of DEXA.[18,27]

Several other studies reported a decline in growth during DEXA treatment in preterm infants.[7,9,28,29] Shrivasta *et al*. described in a small study a decline in growth of OFC, weight and KHL during the first week of DEXA, but thereafter growth was unclear. Gibson *et al* found a decline in KHL velocity during 10 days of DEXA treatment and an even higher KHL velocity after discontinuation of DEXA compared with pre-treatment velocity. No relation with dosage of DEXA was described. Skinner *et al*. reported in 10 infants a lower weight gain, smaller increase in OFC and lower increase in CHL during DEXA and lower weight gain during the first 2 weeks on DEXA compared with week 3 and 4. Although there was a great variation in duration of DEXA treatment (10 - 70 days) these data suggest that the dose of DEXA was negatively correlated with growth. In another study, comparing the effect of a long course of DEXA with a 3-day pulse of DEXA every 10 days, a lower KHL velocity was found during the first 2 weeks of DEXA in infants receiving the long course. Thus, all studies point to a possible dose- effect of DEXA on growth.

The mechanisms by which DEXA induces growth retardation in preterm infants are as yet poorly understood. In an earlier study we showed that two days of DEXA in a dose of 0.5 mg/kg/day induced a significant decrease of serum GH levels in ventilated preterm infants.[20] Although preterm infants are known to be GH insensitive, the low GH levels we have found during DEXA might cause the growth retardation occurring in preterm infants during DEXA treatment. These findings formed the reason to initiate the present randomized double-blind GH-placebo controlled trial.

Very limited data are available about GH treatment in the neonatal period in either term or preterm infants. Only one study evaluated the effects of recombinant GH treatment in preterm infants born small for gestational age (SGA) showing that rhGH did not have any effect on growth and body composition during the neonatal period.[30] It is known that the GH/ IGF-I ratio in SGA infants is higher than in AGA infants during the neonatal period, suggesting a higher GH insensitivity in SGA compared to AGA infants, at least directly after birth. Therefore the results of the GH study in SGA infants cannot be directly applied to other preterm infants.

In our study, we showed that rhGH treatment versus placebo did not result in an improved growth in very preterm infants during DEXA treatment in the neonatal period. The effect on growth was not different between the GH and the placebo group during the whole period of DEXA treatment. Therefore, we conclude that rhGH treatment was not effective, neither during the high doses nor during the lower doses of DEXA to enhance growth in this period. This is in contrast to studies performed in newborn piglets where adjunctive treatment with either GH or GH plus IGF-I in DEXA-treated animals resulted in an improvement of growth.[31] Treatment with GH plus IGF-I did enhance protein synthesis but did not result in a further improvement of growth compared to GH treatment alone.[32]

Possible explanations for this discrepancy in results between very preterm infants and animals might be a significantly higher dose of glucocorticoids and/ or GH insensitivity in very preterm infants. The dose of DEXA used in our preterm infants is much higher than the dosages used in older children in which GH treatment is effective. Improvements of weight and height velocity with GH treatment have been reported in children receiving glucocorticoids dosages equivalent to 0.5 mg/kg day of prednisone in chronic diseases as asthma, lupus, autoimmune colitis and to 0.1-0.2 mg/kg/day of prednisone in children after renal transplantation.[33,34,22] The effect of GH treatment on linear growth has been suggested to be dependent on the dose of glucocorticoids used.[35] Another explanation might be that very preterm infants still have a GH insensitivity as shown by the high serum GH and low serum IGF-I levels. Three weeks after start of DEXA no change from baseline levels of serum immunoreactive IGF-I was observed, neither in the placebo nor in the GH group. In our earlier study we did not find an effect on serum IGF-I levels two days after starting DEXA.[20] Therefore it might well be that serum IGF-I levels do not decline during DEXA treatment and that the growth reducing effect of DEXA is not mediated by IGF-I, but due to a direct effect of DEXA on the epiphyseal cartilage by inhibition of the local secretion and paracrine action of IGF-I.[36] Prednisone causes in vitro a 46% fall in IGF bioactivity and has a suppressive effect on IGF mRNA in the tibia, liver, lung and kidney in the rat, without causing significant changes in serum immunoreactive IGF-I levels.[37,38] We and others did not observe a decline in serum IGF-I levels after administration of DEXA.[29,39,40] Thus DEXA-induced inhibition of local IGF-I gene expression might be one of the mechanisms of growth retardation as well.

We carefully evaluated adverse effects such as cardiac hypertrophy, hyperglycemia, hypertension and retinopathy and mortality. We did not find any significant difference between the GH and the placebo group. Two patients in the placebo group died. Insulin requirement because of hyperglycemia occurred in both groups and was most likely caused by insulin resistance due to DEXA. GH treatment did not increase the need for insulin. Similarly, we could not detect an increase in cardiac hypertrophy in the GH group compared to the placebo group. In adults, an excess of GH is associated with cardiac hypertrophy and even a low dose of GH is capable of increasing left ventricular mass.[41,42] In our GH- treated patients we did not find an increase in severe retinopathy (ROP), although recent studies suggest an association between the persistence of high GH levels in preterm infants and the development of severe retinopathy.[43]

Although treatment with rhGH did not result in a positive effect on growth on the short-term, it might be that GH treatment is able to diminish the reported adverse effects of DEXA on neurodevelopment and lung function. For that reason, we will follow these patients on the long-term.

In conclusion, treatment with recombinant human GH during 6 weeks is not able to overcome the growth failure induced by DEXA when dosages of 0.5 to 0.3 mg/kg/day

are used in very preterm infants at risk for BPD. No gain in weight, CHL, KHL, and OFC was observed during the first week of high- dose DEXA treatment despite GH treatment. Therefore the use of rhGH in the neonatal period cannot be recommended to prevent growth failure when treating very preterm infants with DEXA. Secondly, the lower gain in weight, CHL, KHL and OFC was restricted to the first week of DEXA when a high dose of DEXA was used. Growth in week 2 and 3 was comparable to the growth in the first weeks after discontinuation of DEXA, indicating that the growth- limiting effect of DEXA might be dose-related. We, therefore suggest that future research should be directed at the use of lower doses of glucocorticoids, aiming at both, maintenance of the beneficial effect of DEXA of weaning from the ventilator, and prevention of adverse effects.

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Growth and body composition in preterm infants with bronchopulmonary dysplasia

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Abstract

In this study we compared growth and body composition in preterm infants with bronchopulmonary dysplasia (BPD) with normal healthy term infants during the first year of life.

Twenty nine preterm infants with BPD with a mean (SD) gestational age 27.1 (1.6) weeks and birth weight 852 (173) g) were followed prospectively. Anthropometry and body composition determined by total body electrical conductivity were measured and compared with those of healthy term infants at the same post-term age.

In infants with BPD, the mean weight standard deviation scores (SD-scores) at 6 weeks post-term age were significantly lower (-1.44 and -2.68, boys and girls respectively) than in healthy term infants of the same age and did not improve during the first year. The mean length SD-score in infants with BPD at 6 weeks post-term age was significantly lower than in healthy term infants of the same age, and, although it improved significantly during the first year, the mean length SD-score in girls with BPD remained significantly below zero at 12 months post-term age.

In infants with BPD, the mean free fat mass (FFM) SD-score and the mean total body fat (TBF) SD-score at 6 weeks post-term age were significantly below zero. The mean FFM SD-scores (-1.01 and -2.56, boys and girls respectively) and the mean TBF SD-scores (-1.14 and -2.40, boys and girls respectively) at 12 months post-term age were significantly lower than in healthy term infants of the same age.

Conclusions: Preterm infants with BPD have impaired growth, with a deficit in TBF and FFM already at 6 weeks post-term age; FFM and TBF remain low compared with healthy term infants during the first year of life. Nutritional intervention studies in infants with BPD are needed to evaluate if nutrition is the major determinant of growth and body composition or that this observed pattern of growth is the result of disturbed endocrine control.

Introduction

Preterm infants who develop bronchopulmonary dysplasia (BPD) show impaired growth during early infancy compared with term and preterm infants who do not.[1,2,3] The impaired growth sometimes extends over the first few years of life.[4] Different explanations for the slow growth in infants with BPD have been proposed, such as the increased work required to breathe, early use of glucocorticoids, intrauterine growth retardation, early postnatal growth retardation and feeding problems related to inadequate intake or high metabolic rate.[5-7]

In rats, early postnatal starvation results in reduced growth in infancy.[8] Barker *et al.* showed that intrauterine malnutrition is associated with adult morbidity, especially when the malnutrition is combined with accelerated catch up growth during childhood.[9-11] Therefore, both under-nutrition and over-nutrition during early postnatal life should be avoided. Nutritional intervention should aim to obtain not only an increase in weight and length, but also a body composition comparable to that of healthy term infants.

Body composition can be measured by total body electric conductivity (TOBEC), which has emerged as an accurate, precise, and reproducible method for estimating fat free mass (FFM) and total body fat (TBF).[12–14] Reference standards for healthy term Caucasian Dutch infants for TBF and FFM have been developed by De Bruin *et al.* as well as the normal pattern of TBF and FFM accretion in infants in the first year of life.[15]

No data exist on the pattern of body composition in preterm infants with BPD during the first year of life, and without these data it is impossible to design an appropriate nutritional intervention for infants with BPD and to know whether to recommend supplemental energy or protein or both.

Therefore, the aim of our study was to collect data on growth and body composition in preterm infants with BPD, after discharge from the neonatal intensive care unit, and to compare them with reference data in healthy term infants.

Methods

Patients

Patients were enrolled in the study between January 1997 and January 1999, had a gestational age < 30 weeks, and had developed BPD according to the criteria of Northway *et al.*[16] All patients had been admitted to the neonatal intensive care unit of Sophia Children's Hospital. The study was approved by the local Medical Ethics Committee, and informed consent was obtained from the parents of each child.

All patients were prospectively followed at the neonatal follow-up clinic at the post-term ages of 6 weeks, 3, 6, 9, and 12 months. During these follow-up visits, recumbent crown-heel length, weight, and head circumference were measured. TOBEC measurements were

only performed if the infants weighed more than 3 kg, because such measurements are only validated for infants with a body weight of 3–10 kg.[13] Neonatal risk factors such as intrauterine growth retardation, use of antenatal steroids, duration of ventilation, duration of supplemental oxygen, use of dexamethasone, sepsis, necrotising enterocolitis, and intraventricular haemorrhage were determined. None of the infants showed signs of fluid retention or was artificially ventilated during the period of study.

We calculated dietary intake by recording a 48 hour feeding history during each visit at the neonatal follow-up clinic. All infants received formula feeding according to the policy on the unit, and were exclusively formula fed until at least 3 months post-term age. Solid food was introduced at a time determined by the paediatrician (current Dutch recommendations for term infants are to introduce solids at 4 – 6 months of age).

Anthropometric measurements

Anthropometric measurements were carried out by one observer (WH) at each visit. Recumbent crown-heel length was measured to the nearest millimetre on a length board. Weight was measured on an electronic baby scale (TEC, Tokyo Electronic Co, Tokyo, Japan) to the nearest gram. Head circumference (fronto-occipital) was measured using a standard plastic measuring tape (1 cm wide) to the nearest millimetre. All anthropometric measurements were performed three times, and the mean was used for analysis. Birth weight was expressed as SD-scores for gestational age according to the standards of Usher and McLean.[17] The anthropometric measurements after discharge were expressed as SD-scores for post-term age, according to the Dutch reference.[18]

Measurement of body composition

The TOBEC measurements (Body Composition Analyser, model HP-2; EM-Scan Inc, Springfield, Illinois, USA) were performed by GH and MH. The principle underlying the TOBEC technique is that lean tissue or FFM is far more electrically conductive than fat, because of its greater content of electrolytes. When a conductive mass passes through the electromagnetic field, a small amount of energy is produced within the conductive mass. This energy is distracted from the magnetic field, detected as a phase change in coil impedance, and serves as an index of the amount of conductive mass—that is, the infant's FFM. The amount of TBF is calculated by subtracting FFM from body weight.

The TOBEC procedure was carried out as described by De Bruin *et al*.[14] Before each measurement, background noise and a cylindrical reference phantom were measured. All measurements were performed in the peak mode. The infants were undressed and carefully swaddled in a fully extended position in a large blanket. Care was taken that the limbs did not touch each other or the trunk. The infants were not fed for two hours before the measurement and they did not have a fever. When the infant had urinated, it was swaddled again in a dry blanket and measured again.

One TOBEC reading took about 10 seconds, and a complete TOBEC measurement consisted of 8–10 of these readings. The mean of the readings was taken for further body composition calculations. FFM, TBF, and TBF as a percentage of weight (%TBF) were calculated. The SD-scores for FFM, TBF, and %TBF were expressed for post-term age using the reference centiles for body composition constructed by De Bruin *et al*.[15] Hydration is known to influence the conductance of the FFM, and is a potential measurement error in non-stable infants. As all our infants were stable with supplemental oxygen without a need for additional diuretic treatment, we believe that the calculation of the FFM was not influenced by their hydration.

Statistical analysis

Statistical analysis was performed using SPSS 9.0 for Windows 95 (SPSS Software, Chicago, Illinois, USA) and SAS version 8.0 for Windows (SAS Institute Inc, Cary, North Carolina, USA). Differences in baseline data between boys and girls were tested with Student's *t* test.

All statistical analysis requiring repeated measurement analyses were carried out using SAS Proc mixed. Repeated measurement analyses were performed because of incomplete data for some of the infants. We used a model for repeated measurements with a random intercept and a random slope (age) for the subjects. The models for anthropometric data included age and sex as fixed effects, and the interaction between age and sex if significant. The models for body composition data also included weight, to correct for the fact that infants weighing less than 3 kg could not be measured by TOBEC. Using these models, we tested the differences in absolute values between boys and girls with BPD, the differences between infants with BPD and the reference population by measuring the difference from zero for the mean SD-scores, and we measured the differences in mean SD-scores at different ages in the infants with BPD.

To compare data at different ages, the means given in the Results section were calculated (estimated) means from the repeated measurement models. A p value < 0.05 was considered significant. Pearson correlation was used to evaluate the relation between nutritional intake and growth.

Results

Twenty nine patients with BPD (15 boys) were prospectively followed up. Table 1 shows the baseline characteristics and neonatal morbidity of these infants. The mean (SD) gestational age was 27.1 (1.6) weeks. Boys with BPD had a significantly higher mean birth weight 955 (165) g and mean birth weight SD-score -0.56 (1.0) than girls with BPD (743

Table 1 Neonatal data for 29 infants with bronchopulmonary dysplasia.

N = 29	Mean	SD		N
Gestational age (wk)	27.1	1.6	Antenatal steroids	25
Birth weight (g)	852	173	HELLP	12
Birth weight SD score	-1.11	1.17	PROM	5
Head circumference	-0.21	1.10	RDS treated with surfactant	23
Apgar 1 min	5.0	2.5	Ductus treated with indocid and/or ligation	22
Apgar 5 min	7.8	1.7	Sepsis	19
Dexamethasone treatment (days)	32.5	34.9	Intraventricular hemorrhage > grade 2	6
Ventilation (days)	29.1	15.8		
Extra O_2 need (days)	201	221		

HELLP: haemolysis elevated liver enzymes low platelet syndrome, PROM: premature rupture of the membranes, RDS: respiratory distress syndrome

(102) g and -1.69 (1.0) respectively; $p < 0.001$ and $p < 0.01$, respectively). Also the mean head circumference SD-score in boys was significantly higher than in girls ($p < 0.01$). The boys and girls with BPD were not significantly different with regard to their perinatal morbidity, particularly the number of days receiving dexamethasone, artificial ventilation, or supplemental oxygen. All infants developed BPD as defined by Northway *et al*. [16], and 21 infants had a requirement for supplemental oxygen at 36 weeks postmenstrual age. Twenty six infants were treated with dexamethasone in order to be weaned from the ventilator.

Feeding history could be accurately collected until 6 months post-term age. Weaning was started at 3 months post-term age in some of the infants, but most were predominantly formula fed until 6 months post-term age. Table 2 shows the calculated energy, protein, and fat intake during the first half year after term.

Table 2 Daily nutritional intake in preterm infants with BPD during the first half year of life.

	6 weeks	3 months	6 months
Volume intake (ml/kg/day)	138 (28)	142 (30)	115 (17)
Energy intake (kcal/kg/day)	119 (32)	109 (26)	100 (21)
Protein intake (g/kg/day)	2.7 (0.7)	2.4 (0.5)	2.2 (0.4)
Fat intake (g/kg/day)	6.7 (2.5)	5.7 (1.5)	4.1 (1.0)

Results are given as mean (SD) at given post-term age.

Anthropometric measurements

Table 3 gives the mean weight, length, and head circumference during the first year of life. As in healthy infants, a clear sex difference was seen. Boys with BPD had a significantly higher mean weight, length, and head circumference at all ages than girls with BPD ($p < 0.001$). In boys and girls with BPD, a similar growth pattern for weight and length was observed.

Infants with BPD had significantly lower mean weight SD-scores at 6 weeks post-term age than healthy term infants of the same age (-1.44 and -2.68, boys and girls respectively; $p < 0.0001$). The mean weight SD-score improved significantly from 6 weeks to 6 months post-term age ($p < 0.0001$), but worsened again from 6 to 12 months post-term age ($p < 0.0001$). At 12 months post-term, the mean weight SD-score in infants with BPD was not improved compared with the mean weight SD-score at 6 weeks post-term and remained significantly lower than in healthy term infants of the same age ($p < 0.0001$) (Fig. 1).

Infants with BPD had significantly lower mean length SD-scores at 6 weeks post-term age than healthy infants of the same age (-2.31 and -2.99, boys and girls respectively; $p < 0.0001$). The mean length SD-scores improved significantly from 6 weeks to 12 months post-term age ($p < 0.0001$). In girls with BPD, the mean length SD-score was significantly lower than in healthy term infants at 12 months post-term age ($p < 0.001$) (Fig. 2).

Table 3 Anthropometric measurements in preterm infants with BPD during the first year of life.

	6 weeks	3 months	6 months	9 months	12 months
Boys					
Weight (kg)	3.863(0.128)	5.156(0.127)	7.038(0.178)	8.209(0.259)	8.670(0.364)
Length (cm)	51.4(0.6)	56.7(0.6)	64.8(0.6)	70.5(0.7)	73.9(0.9)
Headcircumference (cm)	37.9(0.3)	40.2(0.3)	43.5(0.3)	45.4(0.4)	46.0(0.4)
Girls					
Weight (kg)	3.134(0.134)	4.276(0.132)	5.874(0.176)	6.761(0.246)	6.938(0.337)
Length (cm)	48.8(0.6)	54.1(0.6)	62.3(0.7)	67.9(0.7)	71.3(0.9)
Headcircumference (cm)	36.0(0.4)	38.3(0.4)	41.6(0.4)	43.5(0.4)	44.1(0.4)

Results are given as estimated mean (SE) at given post-term ages. At all ages, weight, length, and head circumference were significantly greater for boys than for girls with BPD ($p < 0.001$).

Measurements of body composition

Total FFM increased gradually during the first year of life in infants with BPD (Table 4). TBF increased during the first 9 months after term, but decreased between 9 and 12 months after term. FFM and TBF in the infants with BPD were compared with sex specific body composition data for healthy term Dutch infants of the same post-term age and expressed

Table 4 Body composition in preterm infants with BPD during the first year of life.

	6 weeks	3 months	6 months	9 months	12 months
Boys					
FFM (kg)	3.300 (0.070) **	4.127 (0.069) **	5.393 (0.078)**	6.297 (0.089)**	6.838 (0.105)**
TBF (kg)	0.560 (0.070)	1.033 (0.069)	1.647 (0.078)*	1.913 (0.089)**	1.831 (0.105)**
%TBF	15.3 (1.6)	19.0 (1.5)	23.2 (1.5)	24.0 (1.6)	21.3 (1.7)
Girls					
FFM (kg)	2.637 (0.078)	3.387 (0.076)	4.506 (0.079)	5.266 (0.089)	5.665 (0.110)
TBF (kg)	0.493 (0.078)	0.893 (0.075)	1.364 (0.079)	1.493 (0.089)	1.274 (0.110)
%TBF	17.0 (1.7)	20.0 (1.6)	22.8 (1.5)	22.2 (1.5)	18.2 (1.7)

Results are given as estimated mean (SE) at given post-term ages.
FFM: Fat Free Mass, TBF: Total Body Fat, %TBF: percentage Total Body Fat.
* $p < 0.01$, ** $p < 0.001$, significantly different from girls with BPD.

in SD-scores (Fig. 3). Although variability is seen between patients, the pattern for each individual patient is quite constant.

In infants with BPD, the mean FFM SD-scores were significantly below zero at 6 weeks post-term age (-2.39 and -3.11, boys and girls respectively; $p < 0.0001$). They increased slightly during the first year, but were significantly lower than in healthy infants at 12 months post-term age (-1.01 and -2.56, boys and girls respectively; $p < 0.001$).

The mean TBF SD-scores in infants with BPD were significantly below zero at 6 weeks post-term age (-0.91 and -0.73 for boys and girls respectively; $p < 0.05$). The mean TBF SD-scores at 12 months post-term age were significantly lower than in healthy infants of the same age (-1.14 and -2.40, boys and girls respectively; $p < 0.005$).

The mean %TBF SD-score in infants with BPD was not significantly below zero at 6 weeks post-term age. However, at 12 months post-term age, the mean %TBF in girls with BPD was -2.05 and significantly lower than in healthy term infants of the same age ($p < 0.0001$).

Nutritional intake

The relation between nutritional intake at the start of a period (from 6 weeks to 3 months, from 3 to 6 months, and from 6 to 9 months post-term age) and the increase in absolute weight, length, FFM, and TBF at the end of that period was evaluated. No correlation was found between the energy and protein intake at 6 weeks, 3 and 6 months post-term age and the absolute gain in weight, length, FFM, and TBF. The relation between nutritional intake at the start of a period and the change in SD-score during that period was evaluated. No correlation was found between energy and protein intake at 6 weeks, 3 and 6 months post-term age and the change in SD-score for weight, length, FFM, and TBF.

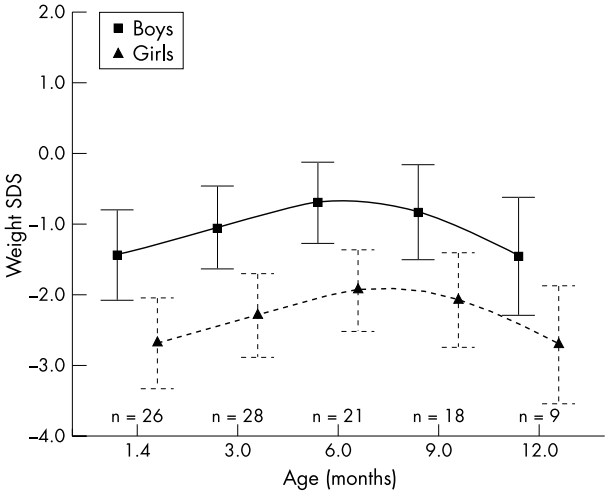

Figure 1 Weight SD-scores in infants with bronchopulmonary dysplasia in the first year of life.
Weight SD-scores (SDS) plotted against post-term age in the first year of life. The mean and the 25th and 75th centiles are shown.

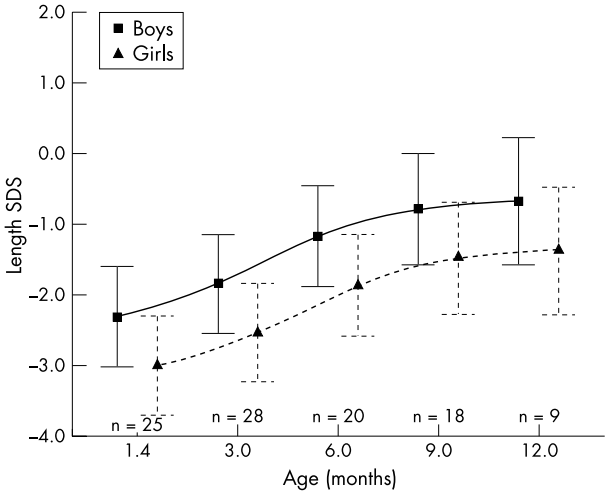

Figure 2 Length SD-scores in infants with bronchopulmonary dysplasia in the first year of life.
Length SD-scores (SDS) plotted against post-term age in the first year of life. The mean and the 25th and 75th centiles are shown.

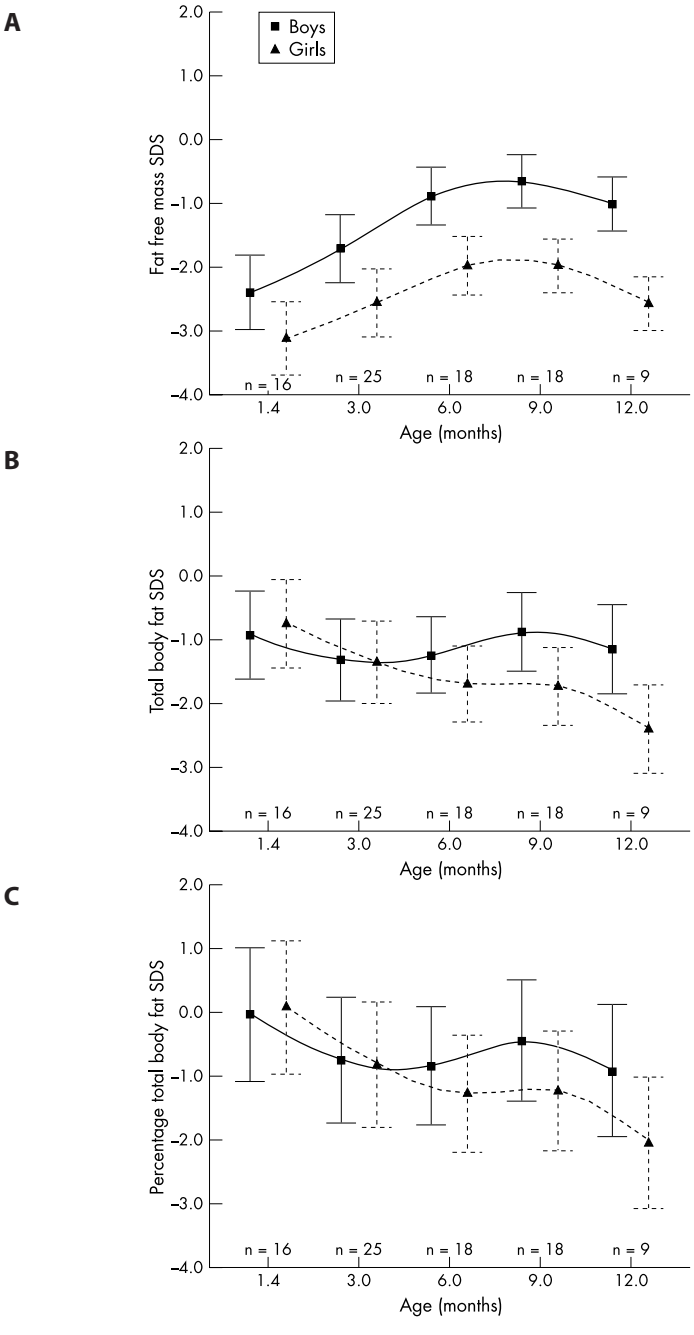

Figure 3 Fat free mass - , total body fat - , and % total body fat SD-scores in infants with bronchopulmonary dysplasia in the first year of life.
A: Fat free mass SD-score,
B: Total body fat SD-score,
C: % total body fat SD-score, plotted against post-term age in the first year of life. The mean and the 25th and 75th centiles are shown.

Discussion

This study was designed primarily to measure the length, weight, and body composition of preterm infants with BPD during their first year of life and to compare these with data obtained for healthy term infants.

The first result of interest is that weight, length, FFM, and TBF in infants with BPD were already low at the first measurement at 6 weeks post-term age, indicating that the growth pattern was already abnormal in early life and that a deficit in FFM and TBF had built up in the first months of life. Interestingly, both FFM and TBF were low, resulting in a proportionately normal body composition. Several other studies already showed early postnatal growth retardation in preterm infants especially in those with major morbidities such as BPD.[5,6] We report for the first time that this growth retardation has an effect on both TBF and FFM. Prenatal growth retardation has a similar effect on body composition, resulting in lower FFM and TBF.[19]

The second result of interest is that growth during the first year in infants with BPD was not sufficient to catch up. Infants with BPD have a significantly lower weight, length, FFM, and TBF than healthy term infants at 12 months post-term age. This was despite the fact that the energy and protein intake during the first six months after term were at, or even above, the recommendations for healthy term infants.[20,21]

There are several possible explanations for the reduced growth in preterm infants with BPD. In the early neonatal period, major morbidities resulting in a delay in starting and achieving full enteral feeding may be a major cause.[6] Subsequently, when enteral feeding is established, difficulties in sucking and swallowing or gastro-oesophageal reflux may lead to a lower intake of energy and protein than calculated.[22-24] Furthermore, frequent pulmonary infections may lead to episodes of decreased intake with consequently lower growth rates. It has also been argued that the energy and protein requirements of infants with BPD are higher because of an elevated resting metabolic rate, caused by a high respiratory rate due to lower lung compliance.[23,25,26] Recurrent episodes of hypoxemia during feeding and sleep may result in inadequate oxygenation for metabolic demands, with subsequent insufficient use of dietary substrates.[27,28] However, we can extract from our data that the nutritional intake of the preterm infants with BPD in this study was insufficient to achieve catch up growth. This may indicate that preterm infants with BPD need a higher intake of energy and protein after discharge than recommended for healthy term infants. Another possible explanation is that the growth disturbance in the perinatal period resulted in disturbance of the endocrine control of growth with long term effects on the growth pattern.

It is important to emphasise three aspects of the study. Firstly, the number of subjects is relatively small because we decided to include cases encountered over a period of two years only. We did not want to prolong the inclusion period, because the changes in medical and nutritional treatments that are likely to occur over a long period may have influenced

the outcome. Secondly, the food intake of the infants was not controlled, so as to collect growth and body composition data in the situation present in most paediatric departments. Thirdly, we were only able to compare our patients with healthy term infants and not with a healthy preterm control group. The results obtained can be explained by the presence of BPD in these preterm infants, but may also be explained by prematurity itself.

TOBEC is a reliable and validated method for measuring body composition. We were therefore able to compare the data in preterm infants with BPD with data from healthy term Dutch infants. At the end of the first year, TBF and FFM in these preterm infants with BPD were still low compared with that in controls.[15] Also, when compared with the reference infants described by Fomon *et al.* the infants with BPD have lower FFM and TBF at the end of the first year.[29]

Other data on body composition in (pre) term infants during the first year of life have recently been published. Butte *et al.* combining several techniques to measure body composition, showed that FFM was slightly higher in healthy term infants and TBF was much higher than in our children with BPD.[30] Rawlings *et al.* found a similar pattern of accretion of FFM and TBF in preterm infants to that in our study, with an increase in FFM during the first year, while TBF increased from birth till 6 months, but not between 6 and 12 months.[31] Both in the study of Rawlings *et al.* and in our study, FFM was significantly lower than the reference data. Interestingly, the absolute amount of TBF was higher in their study, and not different between boys and girls. This may partly be explained by the method used, as Rawlings *et al.* used the dual energy *x* ray absorptiometry method, which may have overestimated TBF, or it may be due to differences between the groups of infants studied. The number of girls included in our study who were small for gestational age may have influenced our results.

The fact that FFM and TBF in our preterm infants with BPD were lower than healthy term reference values, measured by the same method, may have different explanations. It may well be that BPD itself causes less deposition of FFM and TBF because of inadequate absorption of nutrients during the acute phase of lung disease, the medical treatment of the infants (with fluid restriction, steroids, and diuretics), elevated metabolic rate, and the increased work of breathing. The data of Rawlings *et al.* suggest that prematurity itself may also explain at least some of our results.[31] As we did not have a control group of healthy preterm infants, it is not possible to conclude whether the effect on FFM and TBF is due to BPD or prematurity itself. Finally, growth retardation at birth or during the first few weeks of life in preterm infants, especially those with major morbidities as observed by Ehrenkranz *et al.* may have caused a persisting abnormal body composition.[6]

Dietary manipulation of growth rates and body composition in preterm infants seems reasonable.[32-37] Increases in weight, TBF, and FFM have been obtained in preterm boys, but not girls, who were fed a preterm formula (higher in energy, protein, calcium, and phosphorus) from discharge for 6 months. The authors attributed the changes in growth

and body composition to the higher protein intake.[34] Recent intervention studies have shown a positive effect on weight, length, and head circumference, especially in boys.[36-38] So far, only one study, by Brunton *et al.* has described the effect of nutritional intervention in infants with BPD.[32] Feeding an enriched formula (2.5 g protein/kg/day compared with 2.0 g protein/kg/day) resulted in a significantly greater gain in length and FFM, but not in weight, in infants with BPD fed the enriched formula at 3 months of age. From this study, it seems to be possible to increase FFM and linear growth by giving a high protein/high mineral formula. Interestingly, the intake for our infants was comparable to the enriched formula, but both weight and FFM were lower in our children than in the infants studied by Brunton *et al.*

Interpretation of body composition data depends on whether post-term age or body weight is regarded as the appropriate reference. The weights of our preterm infants with BPD at 6 weeks post-term age are comparable to the weights of healthy newborn infants, and the %TBF is comparable to the %TBF found by TOBEC and dual energy *x* ray absorptiometry in healthy term neonates.[39-41] Also, the FFM in infants with BPD is comparable to that of infants of equal weight.

These data, together with the anthropometric data, indicate that preterm infants with BPD are growth retarded, with less body fat and lean mass than healthy infants of the same post-term age, and they have body proportions regarding fat free mass and fat according to their weight. As this growth restriction may have permanent effects, interventions are needed to improve growth in these preterm infants. Long lasting nutritional intervention studies with both increased protein and energy are needed to determine whether this will improve growth and body composition at a later age. As growth disturbance in the perinatal period may result in disturbance of the endocrine control of growth, these studies should include hormonal evaluations. Measurements of body composition are essential in these studies to evaluate the FFM and TBF and to prevent over-nutrition in some infants.

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Worsening of V'_{MAXFRC} in infants with bronchopulmonary dysplasia in the first year of life: a more favorable outcome after High-Frequency Oscillation ventilation

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Abstract

Little is known about the development of maximal flow at functional residual capacity, a measure of airway patency, in infants with bronchopulmonary dysplasia (BPD). In a follow-up study, we evaluated V'_{MAXFRC} in very low birth weight infants with BPD, treated with high-frequency oscillation ventilation (HFO) or conventional mechanical ventilation.

In 36 infants with BPD, V'_{MAXFRC} was evaluated at 6 and/or 12 months post-term age, and the relationship between perinatal factors and lung function was studied. Mean (SD) birth weight and gestational age were 837 (152) g and 26.8 (1.7) weeks, respectively. At 6 and 12 months, mean V'_{MAXFRC} was significantly below normal. Between 6 and 12 months, there was a mean (95% confidence interval) reduction in V'_{MAXFRC} (SD-score) of 0.5 (0.2–0.7) ($p < 0.001$). At 12 months, the mean V'_{MAXFRC} (SD-score) was higher for children initially treated with HFO ($n = 15$), as compared with children treated with conventional mechanical ventilation ($n = 16$): mean (95% confidence interval) difference was 0.6 (0.2–1.0) ($p = 0.008$).

Conclusion: We conclude that very low birth weight infants with BPD have decreased V'_{MAXFRC} that worsens during the first year of life. Initial treatment with HFO was associated with a more favourable outcome of V'_{MAXFRC} at 12 months post-term age.

Introduction

Bronchopulmonary dysplasia (BPD) is a common sequel of mechanical ventilation and oxygen therapy in prematurely born infants.[1] Despite advances in prenatal and neonatal care, including antenatal and postnatal steroids, surfactant treatment, and high-frequency oscillation ventilation (HFO), BPD is still one of the major complications in mechanically ventilated premature infants.[2] The overall incidence of BPD has remained high as a result of the increased survival of extremely premature infants, who are most likely to develop BPD.[2] Long-term studies show that survivors of BPD have abnormal pulmonary function tests at school age, whereas infants who received initial HFO showed normal lung function at school age.[3,4,5] Only a few studies evaluated lung function during the first years of life in children with BPD. In young children with BPD, lung function parameters, such as functional residual capacity (FRCp), compliance, resistance, and conductance, show a gradual improvement toward the normal range during the first 3 years of life.[6–8] Nevertheless, maximal flow at FRC (V'_{MAXFRC}), used as a measure of airway patency, is known to be decreased during the first 2 years of life.[6,8,9] Due to advances in prenatal and neonatal care, results obtained in the past may not be valid for infants who develop BPD nowadays. There are no recent studies that evaluated V'_{MAXFRC} during the first year of life in very low birth weight (VLBW) infants with BPD, in the era of surfactant therapy and HFO.

Therefore, we aimed at evaluating V'_{MAXFRC} at 6 and 12 months post-term age, in a group of VLBW infants with BPD. Furthermore, we studied the relationship between lung function and perinatal patient characteristics.

Methods

Patients

A follow-up study was conducted in neonates who developed BPD, born between January 1998 and September 1999. All infants were born in or transferred immediately after birth to the Neonatal Intensive Care Unit of the Sophia Children's Hospital. The inclusion criteria were birth weight of 1250 g or less, need for mechanical ventilation from day 1 for at least 7 days, need for continuous supplemental oxygen for at least 28 days and/or at 36 weeks gestational age, and chest radiogram at 1 month of age typical for BPD. The exclusion criteria were major congenital anomalies, meconium aspiration, or suspected hypoplasia of the lungs. Artificial ventilation in the Neonatal Intensive Care Unit was administered by conventional mechanical ventilation (CMV) or HFO. Initial ventilation strategy was not randomized in our study. Preferably, initial HFO was started in the youngest and smallest infants. This was not always feasible due to the limited availability of HFO equipment, and

hence, initial ventilation strategy was partly determined by chance. When infants developed hyaline membrane disease, surfactant (Survanta, 100 mg/kg/dose) was administered.

Neonates with severe hyaline membrane disease received additional doses. When infants developed a persistent need for artificial ventilation, treatment also included fluid restriction and diuretics. To wean them off the ventilator, most infants were treated with dexamethasone, administered in a 3-week course starting with a dose of 0.5 mg/kg/day that was gradually tapered. All infants were age-corrected to a gestational age of 40 weeks. The study was approved by the Medical Ethics Committee of the Erasmus University Medical Center. All parents gave informed consent.

Lung Function

Lung function measurements were performed at 6 and 12 months post-term age, when the infants were free from acute respiratory symptoms. To prevent the infants from waking up during the measurements, they were sedated with choral hydrate (50–75 mg/kg). FRCp was measured by means of a modified whole body plethysmograph (Jaeger, Wurzburg, Germany). Equipment and procedures were in accordance with recently published guidelines, in which the FRCp measurement is described in detail.[10] The mean FRCp of three to five technically acceptable measurements was expressed as SD-score. V'_{MAXFRC} was assessed using the end-tidal rapid thoracoabdominal compression technique (RTC) (Custom-made equipment; Department for Experimental Medical Instrumentation, Erasmus University Medical Center, Rotterdam, The Netherlands). Equipment and procedures were in accordance with recently published guidelines, in which the rapid thoracoabdominal compression technique is described in detail.[11] The mean V'_{MAXFRC} of three to five technically acceptable measurements was expressed as SD-score according to Sly *et al.* and Tepper and Reister.[11,12]

Statistical analysis

Lung function at the first and second measurements was compared using mixed-model analysis of variance (SAS, PROC MIXED). Between the groups initially treated with HFO or CMV, lung function and anthropometric data were compared using independent-samples *t* tests. Comparison of percentages was done using Fisher's exact test. Where applicable, the difference in lung function was evaluated using paired Student's *t* test. The influence of various perinatal variables on the level of lung function was evaluated by multiple regression analyses. The significance level was set at a p value of less than 0.05.

Results

A cohort of 36 white infants was enrolled. Lung function was measured in 28 infants at 6 months and in 31 infants at 12 months post-term age. In 23 infants, lung function was measured both at 6 and 12 months post-term age. Reasons for not completing both measurements were failure to sleep during the procedure (n = 6), airway infections (n = 5), and loss to follow-up (n = 2). Anthropometric data of the total cohort of 36 infants and of the subgroups of 28 infants measured at 6 months and 31 infants measured at 12 months are shown in Table 1.

The first and second lung function measurements were performed at mean (SD) post-term ages of 6.2 (0.9) months and 12.6 (1.1) months, respectively. The results of the FRCp and the V'_{MAXFRC} measurements are shown in Table 2. Mean (SEM) FRCp values in SD-score at the first and second measurements were -1.2 (0.3) and -0.6 (0.2), respectively. Mean (SEM) V'_{MAXFRC} in SD-score was significantly below zero (normal value) at the first and second measurements: -1.7 (0.1) and -2.2 (0.1), respectively (Table 2, Figure 1). Between

Table 1 Anthropometric data.

	Total group N = 36	6 months N = 28	12 months N = 31
Gestational age (wk)	26.8 (1.7)	26.9 (1.7)	26.9 (1.7)
Birth weight (g)	837 (152)	826 (156)	852 (156)
Birth weight SD score[a]	-1.2 (1.3)	-1.4 (1.1)	-1.2 (1.4)
Males	22	18	19
Antenatal steroids	28	22	24
Tocolyses	24	19	21
PROM	7	7	5
PDA	27	19	24
Surfactant treated newborns	29	23	24
Dexamethasone treated newborns	32	25	27
Dexamethasone treatment (days)	23 (15)	21 (10)	25 (16)
Initial HFO ventilation	18	15	15
Ventilation (days)	27 (13)	25 (10)	27 (14)
Extra Oxygen requirement (days)	151 (161)	150 (174)	166 (169)
Oxygen dependence at 28 days	35	27	30
Oxygen dependence at GA 36 weeks	30	22	26

Results are given as mean (SD) or number of infants. Shown are the total group and the subgroups of infants measured at 6 months and at 12 months post-term age. Twenty-three infants completed both measurements.
PROM: premature rupture of membranes. PDA: persistent ductus arteriosus. HFO ventilation: high-frequency oscillatory ventilation. GA: gestational age.
[a]: Reference values by Usher and McLean [20].

Table 2 Lung function during first year of life in infants with BPD.

	Measurement 1 N = 28	Measurement 2 N = 31	Mean difference (95% CI)
FRC_p (ml/kg)	23.6 ± 1.3	25.5 ± 1.0	1.9 (-1.3 to 5.0)
FRC_p (SD - score) [a]	-1.2 ± 0.3	-0.6 ± 0.2	0.6 (-0.2 to 1.4)
V'_{maxFRC} (ml/s)	70.3 ± 9.4	119.0 ± 9.0	48.7 (26.9 to 72.4) [**]
V'_{maxFRC} (SD-score) [b]	-1.7 ± 0.1	-2.2 ± 0.1	-0.5 (-0.7 to –0.2) [**]

Results are given as mean ± SEM.
FRC_p: functional residual capacity, V'_{maxFRC}: forced expiratory flow at FRC
At measurement 1 and 2, the mean (SD) corrected age was 6.2 ± 0.9 and 12.6 ± 1.1 months, respectively.
[a]: Reference equation by Stocks *et al.*[10].
[b]: Reference equation by Sly *et al.*[11].
[**]: p<0.001.

the two measurements, there was a mean (95% confidence interval) change of V'_{MAXFRC} in SD-score of -0.5 (-0.7 to -0.2) ($p < 0.001$). When V'_{MAXFRC} in SD-score was calculated using normative data by Tepper and Reister [12], similar results were seen: the mean (SEM) V'_{MAXFRC} SD-scores at the first and second measurements were -1.6 (0.1) and -2.0 (0.1), respectively. The mean (95% CI) change of V'_{MAXFRC} in SD-score was -0.4 (-0.7 to -0.1) ($p = 0.006$).

At 12 months, the mean (SEM) V'_{MAXFRC} in SD-score was better in the group that received initial HFO (n = 15) as compared with the group that initially received CMV (n = 16): -1.9 (0.2) and -2.5 (0.1), respectively. Mean (95% CI) of the difference between the groups was: 0.6 (0.2 - 1.0), ($p = 0.008$) (Table 3). The distributions of perinatal factors did not differ between these two groups, except for birth weight (in grams) and requirement of surfactant therapy (Table 4). After allowing for the potential confounders (days on ventilation, gestational age, and birth weight) using multiple regression analyses, this difference remained significant ($p = 0.038$). However, when both ventilation groups were

Table 3 Lung function measurements at 12 months post-term age in infants with BPD, after initial high-frequency oscillation or conventional mechanical ventilation.

	HFO N = 15	CMV N = 16	Mean difference (95% CI)
FRC_p (ml/kg)	25.5 ± 1.3	25.5 ± 1.4	n.s.
FRC_p (SD-score) [a]	-0.6 ± 0.3	-0.6 ± 0.4	n.s.
V'_{maxFRC} (ml/s)	148.1 ± 11.9	89.7 ± 11.3	58.5 (24.9 to 92.0)[‡]
V'_{maxFRC} (SD-score) [b]	-1.9 ± 0.2	-2.5 ± 0.1	0.6 (0.2 to 1.0)[§]

Results are given as mean ± SEM or mean (95% confidence interval).
FRC_p: functional residual capacity. V'_{maxFRC}: forced expiratory flow at FRC.
[a]: Reference equation by Stocks *et al.*[10].
[b]: Reference equation by Sly *et al.*[11].
[‡]: p=0.001. [§]: p=0.008

Table 4 Anthropometric data of 31 infants with BPD measured at 12 months post-term age, subgrouped by initial ventilation treatment.

	Initial HFO N = 15	CMV N = 16
Gestational age (wk)	26.5 (1.7)	27.2 (1.8)
Birth weight (g)	778 (135) †	921 (146) †
Birth weight SD-score [a]	-1.5 (1.6)	-0.9 (1.1)
Males	12	7
Maternal steroids	14	10
Tocolyses	12	9
PROM	3	2
PDA	10	14
Surfactant treated newborns	9	15
Number of times of surfactant therapy	1 (0-2) ‡	2 (0-4)‡
Dexamethasone treated newborns	14	13
Duration dexamethasone treatment (days)	24 (11)	25 (20)
Duration of ventilation (days)	28 (10)	26 (17)
Duration of oxygen dependence (days)	127 (128)	202 (197)
Oxygen dependence at 28 days	14	16
Oxygen dependence at GA 36 weeks	13	13

Results are given as mean (SD), or median (range), or number of infants.
HFO ventilation: high-frequency oscillatory ventilation, CMV: conventional mechanical ventilation, PROM: premature rupture of membranes, PDA: persistent ductus arteriosus, GA: gestational age.
[a]: Reference values by Usher and McLean [20].
†: p=0.009.
‡: p=0.002.

compared with adjustment for number of surfactant dosages, the difference in mean V'_{MAXFRC} (SD- score) at 12 months lost significance (p = 0.085).

Similar results were seen within the subgroup of 23 infants who completed both measurements. To study the difference between 6 and 12 months precisely, individual mean V'_{MAXFRC} values were inter- or extrapolated linearly to values at exactly 6 and 12 months post-term age. At 6 and 12 months, the mean (SEM) of these adjusted V'_{MAXFRC} SD-scores were -1.7 (0.1) and -2.1 (0.1), respectively (mean [95% CI] change: -0.4 [-0.7 to -0.1], p = 0.006). At 12 months, the mean (SEM) of the adjusted V'_{MAXFRC} in SD-score was better in the group that received initial HFO (n = 12) as compared with the group that received CMV (n = 11): -1.9 (0.2) and -2.4 (0.1), respectively (mean [95% CI] difference: 0.6 [0.1–1.0], p = 0.014) (Figure 2).

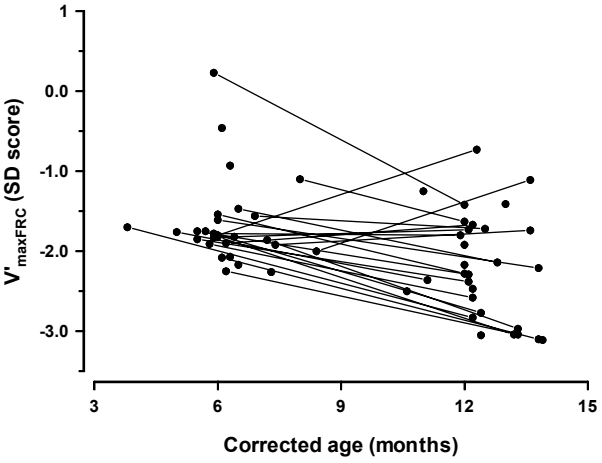

Figure 1 Lung function data of 36 infants with BPD in the first year.
Mean V'_{MAXFRC} is expressed in SD-score according to Sly *et al*.[11] The first (n=28) and second (n=31) measurements were done at mean (SD) post-term ages of 6.2 (0.9) months and 12.6 (1.1) months, respectively. Twenty-three infants completed both measurements (connected data points).

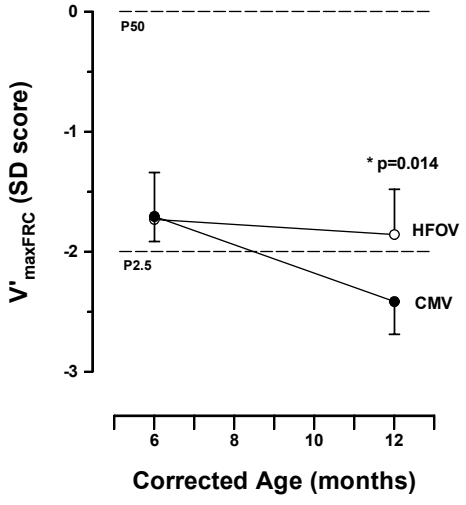

Figure 2 Effect of first intention HFO on V'_{MAXFRC} in 23 infants with BPD during the first year.
Mean V'_{MAXFRC} in SD-score[11] in the subgroup of 23 infants who completed both lung function measurements at 6 and 12 months. Error bars represent SEM. Individual mean V'_{MAXFRC} values were inter- or extrapolated to values at exactly 6 and 12 months post-term age.
○ = infants treated with first intention HFO (n=12)
● = infants treated with CMV (n=11).

Discussion

In a follow-up study, we evaluated lung function in a cohort of 36 VLBW infants with BPD, during the first year of life. Furthermore, we studied the relationship between lung function and perinatal patient characteristics. During the first year of life, mean V'_{MAXFRC} was below reference values and showed a significant worsening between 6 and 12 months post-term age. At 12 months, mean V'_{MAXFRC} was significantly better in the initial HFO-treated group, as compared with the group treated with CMV.

To our knowledge, this is the first study on growth of airway function during the first year of life in VLBW infants with BPD, which also addresses a possible relationship with HFO. Tepper *et al.* and Iles and Edmunds also found decreased V'_{MAXFRC} during the first year of life.[6,9]. However, due to the survival of younger and smaller infants and differences in treatment modalities, our study population cannot be compared with the population studied by Tepper *et al.*[6] Iles and Edmunds studied a population more comparable to our population, but no information about treatment modalities was provided.[9] The decreasing V'_{MAXFRC} may reflect abnormal functional or anatomic development of the airways, which is consistent with pathologic findings.[6,13] This could explain the abnormal pulmonary function tests in preterm-born children with BPD at school age.[3,4] Alternatively, worsening of airway patency may be due to airway damage and dysfunction of peripheral airways, and central airway damage and collapse during dynamic compression.[14,15] Factors such as thickened airway walls, increased smooth muscle layer, disturbed development of airway size and/or airway compliance, or altered alveolar architecture may also play a role here.[16,17] Furthermore, the relative decline of V'_{MAXFRC} during the first year of life was irrespective of the reference equation used.[11,12] The FRCp was within the normal range at 6 months and demonstrated a trend to normalization at 12 months of age. This is consistent with previous reports.[6–8,18] With no apparent decline of the mean FRCp between 6 and 12 months, the change in FRCp cannot explain the reduction in $V'_{MAXFRC.}$[19]

First intention HFO is associated with a shorter time of ventilator dependency and oxygen dependency in VLBW infants with respiratory distress syndrome.[20] Furthermore, it is speculated that early HFO used with a lung recruitment strategy in combination with surfactant therapy ameliorates acute neonatal lung injury that predisposes some preterm infants to develop BPD.[5] The HIFI study group found that the use of HFO, in comparison with CMV, did not improve V'_{MAXFRC} at 9 months post-term age.[21] In our study, the V'_{MAXFRC} at 12 months was significantly better in the group initially treated with HFO, compared with the group initially managed with CMV. This discrepancy could be explained by the difference in timing of measurement or by the fact that in our study, HFO was used as initial therapy. Our data suggest that, in VLBW infants, initial treatment with HFO is associated with a more favourable development of V'_{MAXFRC} at 12 months post-term age. This finding provides further suggestive evidence that initial HFO combined with surfactant therapy reduces

acute neonatal lung injury.[5] Initial ventilation treatment was not intentionally randomized in our study, and therefore this association cannot be considered causal. Nevertheless, the HFO and CMV groups were not different by any perinatal patient characteristic, except for small difference in birth weight in grams, but not in SD-score, and number of surfactant doses. The difference in birth weight does not explain our finding, as the lower birth weight of the HFO group would unfavourably affect lung function, whereas we found better results after HFO. Fewer doses of surfactant were given to the infants who were initially ventilated with HFO, as compared with CMV. This may reflect reduced respiratory distress after HFO. We regard the number of surfactant doses not as a confounder but as a possible first positive outcome of HFO.

In summary, VLBW infants with BPD, born in the era of surfactant therapy and HFO, show a worsening of decreased V'_{MAXFRC} during the first year of life. Initial treatment with HFO was associated with a more favourable development of V'_{MAXFRC} at 12 months post-term age. This finding supports the hypothesis that initial treatment with HFO in premature neonates prone to develop BPD leads to less airway damage and better medium term outcome.

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General discussion and future perspectives

Infants who develop BPD often show retarded growth in the first years of life. It is unknown if this growth retardation is due to their prematurity, the lung disease they have developed or is induced by drugs like glucocorticoids, or due to other factors or a combination.

Glucocorticoids are used frequently in preterm infants. They are given antenatally to mothers who are at risk to deliver prematurely in order to induce lung maturation. Glucocorticoids are also given postnatally to preterm infants with persisting respiratory problems to improve lung function and promote weaning from the ventilator.

Adrenal function

Glucocorticoids in preterm infants are frequently given either in the first postnatal week because of circulatory problems not reacting on volume expansion and cardiotonic drugs, or later because of persisting respiratory problems in order to wean the infants off the ventilator. In the latter case the dose is much higher and the duration of glucocorticoid treatment much longer. In the past no evidence for adrenal insufficiency was found in preterm infants with gestational age of 34 - 37 weeks when the infants were tested with high doses of ACTH.[1] However, recent studies show that serum cortisol levels in preterm infants are not correlated with clinical illness.[2] These observations seemed to be contradictory, questioning the adrenal function in very preterm infants, as they are treated nowadays.

Therefore we investigated the adrenal function in very preterm infants with and without severe illness on day 4. We have shown by performing a low dose ACTH test in very preterm infants with a gestational age of less than 30 weeks that baseline serum cortisol levels and cortisol levels after ACTH administration were significantly lower in ventilated infants, while baseline 17-OHprogesterone levels were significantly higher. Interestingly, the infants who developed BPD had lower cortisol levels after ACTH administration. These data show an impaired adrenal response to stress in sick ventilated very preterm infants born after gestational ages less than 30 weeks. Other studies, by measuring either cortisol levels or performing an ACTH test also suggested a relative adrenal insufficiency in sick preterm infants, especially in the very young infants, suggesting a gestational age and thereby maturational effect.[3,4]

Although, it is not quite clear whether the adrenals in sick ventilated very preterm infants are down-regulated by high interleukin levels or have a decreased activity of the enzymes CYP21A2 or CYP11B, the fact remains that these infants have relative low cortisol levels when submitted to additional stress such as an ACTH test. This impaired adrenal function is related to adverse outcome like BPD, major cerebral morbidity and mortality.

Glucocorticoids effect many aspects of lung development including growth and differentiation and have anti-inflammatory characteristics. As the development of BPD is associated with chorioamnionitis, high interleukin levels, and ongoing inflammation it

may be worthwhile to dampen this response by the use of physiologic or stress doses of hydrocortisone to compensate for the relative adrenal insufficiency in order to prevent BPD and major morbidity.[5] Double-blind, placebo-controlled trials starting with physiologic doses of glucocorticoids within the first week might give an answer to the question if it is possible with a physiological dose of glucocorticoids to reduce the incidence of BPD and, may be of even greater importance, the need for later glucocorticoid treatment. A recent study suggests a beneficial effect of prophylactic use of hydrocortisone on the incidence of BPD in very preterm infants.[6] More trials are needed to confirm this observation and to evaluate potential adverse effects.

Growth

Disturbance of growth in preterm infants with BPD can be due to their prematurity, the lung disease they have developed or induced by drugs like glucocorticoids given to the infants, or due to endocrine changes in the growth hormone- insulin-like growth factor-I (GH-IGF-I axis) or to other factors or a combination.

Glucocorticoids are known to have a number of adverse effects in preterm infants, reduced growth being one of them. How glucocorticoids effect growth is not very clear. Studies have shown a higher protein breakdown in infants receiving glucocorticoids. Other studies indicate that the impaired growth in infants with BPD might be due to an increased metabolic rate, which is still debated. Another aspect regarding growth is the nutritional intake. Infants treated for BPD often have a restricted fluid and thereby restricted energy and protein intake. So far no study has clearly shown that the growth retardation in infants with BPD can be overcome by increasing the nutritional intake, which can be expected when the reduced growth is due to either higher energy requirements or a less than optimal intake. Studies on growth and body composition of preterm infants with BPD might help to answer this question.

The potential contribution of GH, IGF-I and the IGFBP's to the impairment of growth in sick very preterm infants at risk for BPD is unknown. Also the effect of glucocorticoids on the GH-IGF-I axis in these very preterm infants is not known. In this study we therefore measured levels of GH, IGF and IGF-binding proteins before and during the administration of glucocorticoids, which were given to facilitate weaning from the ventilator. Secondly, we investigated if the administration of recombinant human growth hormone rhGH during glucocorticoid treatment in very preterm infants might improve growth.

GH-IGF-I axis

In our study in ventilated very preterm infants we have found serum GH and IGF-I levels comparable to those found in non-ventilated preterm infants.[7,8] Serum GH and IGF-I levels were not correlated with severity of respiratory failure. Serum GH levels were related with IGF-I levels suggesting that GH exerts some effect through IGF-I production. Based on these results no evidence was found for GH deficiency in sick ventilated very preterm infants. Therefore we conclude that the reduced growth found in sick very preterm infants cannot be explained by GH deficiency.

Effect of glucocorticoids

We have shown that the use of DEXA results in low levels of serum GH after two days, without an acute decline in serum immunoreactive IGF-I levels. Therefore the question remained whether these low GH levels observed after start of DEXA were responsible for the decline in growth in these infants.

In the randomised-placebo-controlled trial with rhGH in ventilated preterm infants being treated with DEXA, two major observations were obtained. Concerning the effect of recombinant human growth hormone, no beneficial effect was observed of simultaneous administration of rhGH during and three weeks after discontinuation of DEXA treatment on growth. The stagnation in increase of length, weight, knee-heel length and head circumference occurred in the group receiving DEXA/placebo as well as in the group receiving rhGH next to DEXA, and was limited to the first week of DEXA treatment. Simultaneous treatment with rhGH and DEXA did not result in an improvement in growth in the rGH treated group. Therefore the use of rhGH in order to prevent the adverse-effects of high dose DEXA in preterm infants cannot be recommended.

Concerning the effect of glucocorticoids on growth (weight, length, knee-heel length and head-circumference), we observed that the impairment in growth was confined to the first week of DEXA treatment suggesting a dose-related growth inhibiting effect of DEXA. In most NICU's (and also in our study) DEXA treatment is given in a tapering dose with the highest doses of 0.5, 0.3 and 0.25 mg/kg, respectively in the first week. Several other studies, by comparing growth during and after discontinuation of DEXA, reported a decline in growth during DEXA treatment in preterm infants.[9] One, small, study suggested a possible dose- effect of DEXA on growth.[10]

The use of glucocorticoids is associated with an extensive list of adverse effects. Evidence for its beneficial effects as the decreased incidence of mortality and incidence of BPD justify the use of glucocortocoids in very selected cases only. Because of the described serious adverse effects, future research might be directed at:

a. finding alternative treatments in order to avoid the use of systemic glucocorticoids
b. restriction of the indication for systemic glucocorticoids to infants developing signs of BPD in whom all other treatment options aimed at weaning them from the ventilator have failed

c. finding the lowest safe dose, optimal time and duration of administration of glucocorticoids for those infants where systemic glucocorticoids seem to be the only option to wean them from the ventilator.

The fact, we did not see stunting of growth after the first week of DEXA, suggests that dosages of DEXA less than 0.25 mg/kg/day may have less side effects as far as growth of weight, length and head-circumference is concerned. A dose of 0.25 mg/kg/day DEXA is approximately six times the basal cortisol production in a preterm infant.

Several treatment options instead of high dose DEXA are currently and will be in the coming years under investigation. Lower dose of DEXA as suggested in our study results in less impairment of growth. A recently published trial with low dose DEXA treatment shows promising results with a similar beneficial effect on dynamic pulmonary mechanics and subsequent oxygen requirement in infants either treated with high or low dose DEXA during one week.[11] Pulse treatment with glucocorticoids in one study produced less side effects but was found to be less effective in preventing BPD.[9] The use of other glucocorticoids such as solumedrol or hydrocortisone might, according to recent studies, also be an alternative, although the latter study was retrospectively performed in different NICU's.[12,13]

Outcome of infants with BPD

Growth and body composition.

We have shown that most of the infants with BPD are growth retarded at 6 weeks post-term age implicating that the infants were discharged from the neonatal intensive care unit with retarded growth. Secondly, our study shows that with a nutritional intake equal to or even above current recommendations we are not able to achieve a catch-up growth in infants with BPD and the infants remain proportionately low in total body fat and fat free mass.

Many infants with major morbidities develop growth retardation during their stay on the NICU possibly because it takes longer to begin with and convert to full enteral feeding.[14] Dietary strategies should focus on achieving early sufficient protein and energy intake in order to prevent the severity of this growth retardation. This might be achieved by introduction of early enteral feeding with the intention to achieve an adequate nutritional intake earlier. This strategy could also affect the incidence of BPD in another way as sepsis often occurs in patient with parenteral nutrition. By decreasing the incidence of catheter-related sepsis it might be possible to influence the incidence of BPD positively.[15,16]

Our study indicates that growth retarded infants with BPD might have a need for a higher caloric and protein intake after discharge in order to achieve catch-up growth. Intervention studies with postdischarge feeding in preterm infants without BPD show an increase in length, weight and fat free mass.[17-19] Therefore, it might be worthwhile to

perform a postdischarge intervention study in infants with BPD who have developed a deficit in protein and energy during their stay on the NICU. Remarkably, the greatest effect of a postdischarge feeding in otherwise healthy preterm infants was observed in infants with a birth weight below 1250 g and in boys.[18,19] No good explanation has been found for these results.

Studies aimed at both the prevention of diminished growth in infants developing BPD in the neonatal period as well as studies on achieving catch-up growth thereafter are needed.

Lung function.

We have shown an abnormal development or function of the airways in infants with BPD during the first year. The lung function even worsened, in spite of the fact that the pulmonary complaints of the infants clinically improved during the first year. Infants initially treated with HFO showed a less impaired lung function compared to the infants initially treated with conventional ventilation. If this can be confirmed in other trials, then HFO might be the initial choice for infants with RDS who need ventilation. Recently a beneficial effect of HFO on pulmonary outcome was published.[20]

Longterm follow-up studies are needed to evaluate the development of lung function in infants who develop BPD these days.

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Summary
Samenvatting

General introduction

In the last decades important advances in neonatal intensive care have been made leading to an increased survival of especially very preterm infants. Associated with the decline in mortality there is an increase in morbidity partly because of an increase in chronic lung disease (CLD) defined as oxygen need at 36 weeks postmenstrual age. The incidence of CLD is 56% in infants born with a weight between 500-750 g. The terms bronchopulmonary dysplasia (BPD) and CLD are both used in the literature describing persisting lung disease in preterm infants. Within the framework of this thesis the term BPD is used according to the classification of the NICHD/NHLBI/ORD. By this definition infants have BPD when they received supplemental oxygen for at least 28 days after birth because of persistence of clinical features of respiratory failure such as tachypnea, retraction and rales.

Many infants who develop BPD show retarded growth during infancy and childhood. It is unknown if this growth retardation is due to prematurity, the lung disease the infants have developed or is induced by drugs like glucocorticoids, or due to endocrine disturbances or other factors. As retarded growth in general might also reflect disturbances in growth of organs such as lungs, kidneys and the brain, the implications of such a persistent growth disturbance might have long-term effects on the development, functioning and survival of many organs. In addition, glucocorticoids have a number of adverse effects, reduced growth being one of them.

In **Chapter 1** an introduction to the epidemiology and pathofysiology of BPD is given. Nowadays, many infants who might develop BPD are being treated with glucocorticoids either antenatally, postnatally or both. The benefits and the potential adverse effects of treatment with glucocorticoids in human and animal studies are described. Glucocorticoids influence lung development, disturb growth and endocrine function. Therefore we performed studies in infants with BPD investigating the adrenal function, the GH-IGF-I axis, growth and lung function.

In **Chapter 2** the adrenal function in very preterm infants born after a gestational age of less than 30 weeks is described. The aim of the study was to investigate whether there was a difference in adrenal function in very preterm infants with and without severe illness and if there was a relation between adrenal function and outcome.

In 21 infants a low dose ACTH test with a standard dose of 0.5 µg iv. (corresponding to 5 µg/m^2 in these small infants) was performed on day 4 of life. Baseline cortisol and 17 OH-progesterone levels, and cortisol levels 30 and 60 minutes after ACTH administration were determined. Illness severity was measured using the SNAP score.

In ventilated infants baseline serum cortisol levels and cortisol/17OH-progesterone ratios were low while at the same moment baseline serum 17OH-progesterone levels

were high compared to non-ventilated infants. The increase in serum cortisol after ACTH administration was significantly lower in ventilated than in non-ventilated infants. Infants who developed BPD showed lower baseline cortisol/17OH-progesterone ratio's and 60 minutes cortisol levels after ACTH administration.

These data suggest an impaired adrenal response to stress in sick ventilated very preterm infants with gestational ages younger than 30 weeks, which is associated with a more adverse outcome as BPD. The high levels of the precursor 17OH-progesterone suggest a diminished activity of the enzyme CYP21A2 or a lower conversion of 11-deoxycortisol to cortisol by CYP11B. Further studies are required to investigate if supplementation with physiological doses of hydrocortisone in the first week of life might reduce the incidence of BPD.

In **Chapter 3** the GH-IGF-I axis is described in sick ventilated very preterm infants. The aim of the study was to evaluate the potential contribution of GH, IGF-I and the IGFBP's to the growth retardation of preterm infants with respiratory failure at risk for BPD. In 24 very preterm ventilated infants 6- hour GH profiles were performed and serum levels of IGF-I, IGFBP-1 and -3, nutritional intake and severity of respiratory failure were determined. The 6-hour GH profiles were analysed using the Pulsar program resulting in mean, baseline and maximal GH values and the number of GH peaks.

A wide variation was found in serum mean GH levels ranging from 6.0 to 55.8 μg/l. Serum IGF-I levels varied from 20.7 to 117.8 ng/ml and were correlated with serum GH levels. Unexpectedly, serum GH levels did not correlate with birth weight, gestational and postnatal age, weight gain and severity of respiratory failure. Serum GH and IGF-I levels in these ventilated very preterm infants were comparable to those found in non-ventilated preterm infants and thus not correlated with severity of respiratory failure. GH levels were related with IGF-I suggesting GH exerts some effect through IGF-I production.

Based on these results no evidence for GH deficiency in sick ventilated very preterm infants was found. Therefore we conclude that the reduced growth found in sick very preterm infants cannot be explained by GH deficiency.

In **Chapter 4** the effect of dexamethasone (DEXA) administration is described on the GH-IGF-I axis in ventilated very preterm infants developing BPD. The aim of the study was to evaluate the short-term effect of DEXA on the GH-IGF axis in ventilated very preterm infants at risk for developing BPD. In 10 very preterm ventilated infants 6-hours GH profiles were performed and serum IGF-I, IGFBP-1 and -3 levels were measured immediately before and two days after start of dexamethasone treatment.

Serum GH levels declined significantly during DEXA treatment, however without a decline in serum IGF-I and IGFBP-3 levels. A concomitant decline in serum IGFBP-1 was found. Serum GH levels during DEXA correlated neither with the time interval between the

administration of DEXA and the second GH profile nor with the cumulative DEXA dose administered.

In conclusion, two days of DEXA treatment in very preterm ventilated infants has a suppressive effect on serum GH levels, without an acute decline in serum immunoreactive IGF-I levels. Therefore, the question remains whether the low GH levels observed after start of DEXA are responsible for the decline in growth rate in these infants.

In **Chapter 5** a randomised-placebo-controlled trial with recombinant human GH (rhGH) in ventilated preterm infants being treated with DEXA is described. The aim of this study was to investigate if treatment with rhGH is able to overcome the growth inhibiting effect of DEXA treatment in very preterm infants. Ventilated very preterm infants (n = 30) were included in the study after the attending neonatologist had decided to initiate DEXA treatment to facilitate weaning from the ventilator. The infants were randomly assigned to receive either DEXA/ placebo or DEXA/ GH treatment. DEXA was administered during a 24 days period, in a tapering scheme. Simultaneously rhGH or placebo was administered during 42 days. Fifteen infants were treated with DEXA/GH. Weight, length, knee-heel length and head-circumference were measured. Serum GH, IGF-I and IGFBP-1 and -3 levels were determined.

The growth in weight, length, knee-heel length and head-circumference was not significantly different between the two treatment groups over a period of 42 days. In the first week on DEXA no growth in weight, length, knee-heel length and head-circumference occurred in both treatment groups. In the second and third week of DEXA treatment as well as in the 3- week period after discontinuation of DEXA, growth was not significantly different between the GH and the placebo group. Interestingly growth during week two and three of DEXA was not different from the growth after discontinuation of DEXA.

Serum IGF-I levels did not increase during the 42-days study period, while serum IGFBP-3 increased during DEXA, in both groups.

We conclude that treatment with rhGH is not able to overcome the DEXA-induced growth failure in this 6-week period in very preterm infants at risk for BPD. However, long-term follow-up is needed to evaluate the effect on growth, neurodevelopment and lung function. Secondly, the negative effects on growth by DEXA are limited to the first week of DEXA administration with high doses of DEXA, thereby suggesting a dose-related growth inhibiting effect of DEXA. We therefore suggest that future research should be directed at the use of lower doses of glucocorticoids, aiming at both, maintenance of the beneficial effect of DEXA of weaning from the ventilator, and prevention of adverse effects.

In **Chapter 6** the growth and body composition in preterm infants with BPD during the first year is described. The aim of the study was to evaluate the pattern of growth and body composition in infants who had developed BPD, in their first year of life. Preterm infants

with BPD were prospectively followed at the neonatal follow-up clinic at 6 weeks, 3, 6, 9 and 12 months post-term age. Anthropometry and body composition determined by total body electric conductivity (TOBEC) were measured and compared with those of healthy infants at the same post-term age. Nutritional intake was calculated.

The infants with BPD had a significantly lower weight and length at 6 weeks post-term age compared to healthy term infants. A transient catch-up growth in weight, but an insufficient catch-up growth in length occurred during the first year. At 12 months post-term age the weight in infants with BPD was significantly lower than in healthy infants, as was the length in girls with BPD. The amount of fat free mass was significantly lower at 6 weeks post-term age, increased, but remained lower than in healthy term infants at the end of the first year, while the amount of total body fat remained lower during the whole first year compared to healthy controls.

Thus, although the nutritional intake during the first 6 months was normal and the protein intake even above recommendations made by the WHO for healthy infants, an insufficient catch-up in length and fat free mass and no catch-up in weight and total body fat occurred.

This study indicates that infants with BPD have a retarded growth already at 6 weeks post-term age, while their body composition at 12 months shows lower amounts of fat free mass and fat compared to healthy controls. Despite an adequate nutritional intake for these infants, levels of protein and fat remain lower than normal during the first year. Nutritional intervention studies are needed to evaluate if nutrition is the major determinant of growth and body composition in these preterm infants and to find out whether enriched formulas will improve growth and body composition at later age.

In **Chapter 7** the development of airway function in preterm infants with BPD is described either treated with conventional ventilation or with high frequency oscillation (HFO). The aim of the study was to evaluate the V'_{MAXFRC} (measure of airway patency) in preterm infants after they had developed BPD, during their first year. In 36 preterm infants with BPD lung function measurements were performed at 6 and/ or 12 months post-term age. Functional residual capacity (FRC_p) and maximal flow at FRC (V'_{MAXFRC}) was determined and related with perinatal factors.

The FRC_p was within the normal range at 6 and 12 months post-term age. In infants with BPD the V'_{MAXFRC} at 6 and 12 months was significantly lower than in reference infants and even worsened between 6 and 12 months post-term age. In infants initially treated with HFO the mean V'_{MAXFRC} at 12 months was better than in infants initially treated with conventional ventilation.

This study shows that infants with BPD have decreased V'_{MAXFRC} that worsens during the first year of life. Initial treatment with HFO was associated with a more favourable outcome at 12 months post-term age.

Groei bij zuigelingen met bronchopulmonale dysplasie, hormonale en pulmonale aspecten

klinische en follow-up studies

Inleiding

In de afgelopen decennia hebben belangrijke ontwikkelingen in de behandeling van pasgeboren zuigelingen (neonatologie) plaats gevonden. Hierdoor is de kans op overleving voor met name de zeer veel te vroeg geboren (premature) pasgeborene duidelijk toegenomen. Deze daling in sterfte gaat echter gepaard met een grotere kans op restverschijnselen, zoals chronische longziekte (CLD). CLD wordt gedefinieerd als de noodzaak tot extra zuurstoftoediening bij premature pasgeborenen op de leeftijd van 36 weken na de laatste menstruatie. Bij ongeveer 56% van de pasgeborenen met een geboortegewicht van 500-750 gram onstaat CLD. Naast de term CLD wordt ook de term BPD (bronchopulmonale dysplasie) gehanteerd om de chronische longbeschadiging bij premature pasgeborenen te beschrijven. In dit proefschrift wordt de term BPD gehanteerd volgens de laatste classificatie van de NICHD/NHLBI/ORD. In deze classificatie is er sprake van BPD als pasgeborenen vanaf de geboorte gedurende minstens 28 dagen zuurstofbehoefte hebben en daarbij klachten van de luchtwegen zoals een versnelde ademhaling, intrekkingen van de borstkas en abnormaal ademgeruis bij het beluisteren van de longen.

Veel zuigelingen met BPD vertonen een verminderde groei, zowel op de zuigelingen- als op de kinderleeftijd. Het is onduidelijk waardoor deze groeiachterstand wordt veroorzaakt: door de vroeggeboorte, de longbeschadiging, medicatie zoals het gebruik van glucocorticoiden, door veranderingen in de hormoon-assen of door andere factoren. Een verminderde groei kan een uiting zijn van veranderingen in de groei van organen zoals de longen, de nieren en de hersenen, waardoor deze groeiachterstand mogelijk langdurige effecten heeft op de ontwikkeling, functie en overleving van diverse organen. Veel premature pasgeborenen krijgen, voor of na de geboorte, glucocorticoiden toegediend. Enerzijds, om voor de geboorte de longrijping te bevorderen, anderzijds om bij beademde pasgeborenen de ernst van BPD te verminderen. Het gebruik van glucocorticoiden is geassocieerd met verminderde groei.

In **hoofdstuk 1** wordt een inleiding gegeven over de epidemiologie en pathofysiologie van BPD. De pasgeborenen die in de huidige tijd BPD ontwikkelen zijn veelal behandeld met glucocorticoiden. De voor- en nadelen van behandeling met glucocorticoiden gevonden

bij studies in mensen en dieren worden besproken. Glucocorticoiden beinvloeden de longontwikkeling, de groei en de functie van de hormoon-assen. Bij zuigelingen met BPD hebben wij dan ook de bijnier- en de groeihormoonas, de groei en longfunctie onderzocht.

In **hoofdstuk 2** wordt de functie van de bijnier van premature zuigelingen geboren na een zwangerschapsduur van minder dan 30 weken beschreven. Het doel van de studie was om te onderzoeken of er een verschil was in bijnierfunctie van zeer jonge premature pasgeborenen die wel of niet ernstig ziek waren na de geboorte. Tevens onderzochten wij of er een relatie bestond tussen de bijnierfunctie en de outcome van deze kinderen.

Bij 21 pasgeborenen werd op de 4e levensdag een bijnierstimulatietest met een standaarddosis van 0.5 µg ACTH (lage dosis) verricht. Uitgangswaarden van cortisol, en de voorloper 17OH-progesteron werden bepaald evenals cortisol spiegels 30 en 60 minuten na toediening van ACTH. De ernst van ziekte werd bepaald met behulp van de SNAP-score.

Bij beademde pasgeborenen bleken de basale waarden van cortisol en de ratio cortisol/10OH-progesteron laag en de 17OH-progesteron waarden hoog in vergelijking met niet beademde pasgeborenen. De stijging in cortisol na toediening van ACTH was significant lager in de beademde groep. Bij pasgeborenen die later BPD onwikkelden, was op dag 4 de basale cortisol/17OH-progesteron ratio lager evenals de cortisol waarde 60 minuten na toediening van ACTH vergeleken met beademde pasgeborenen die geen BPD ontwikkelden.

Deze data suggereren een veminderde bijnierfunctie in reactie op stress bij ernstig zieke beademde pasgeborenen geboren na een zwangerschapsduur van minder dan 30 weken. Deze verminderde functie is geassocieerd met een slechtere prognose zoals het krijgen van BPD.

De hoge waarden van de voorloper van cortisol, 7OH-progesteron suggereert een verminderde activiteit van het enzym CYP21A2 of een verminderde omzetting van 11-deoxycortisol naar cortisol door CYP11B. Nieuwe studies zijn nodig om aan te tonen of suppletie met fysiologische doseringen hydrocortison in de 1e levensweek zinvol is om de incidentie van BPD te verminderen.

In **hoofdstuk 3** wordt de activiteit van de groeihormoon- IGF as beschreven bij ernstig zieke beademde premature pasgeborenen. Het doel van de studie was om na te gaan wat de activiteit van de GH-IGF-I as bijdraagt aan de groeiachterstand die ontstaat bij pasgeborenen die BPD ontwikkelen. In totaal werd bij 24 kinderen een GH profiel verricht, waarbij gedurende 6 uur GH spiegels en eenmalig IGF-I, IGFBP-1 en IGFBP-3 spiegels werden bepaald. De gemeten GH waarden werden geanalyseerd met het Pulsar programma waardoor de gemiddelde, de baseline en de maximale GH waarden werden berekend.

De gemiddelde GH waarden varieerden van 6.0 tot 55.8 µg/l. De IGF-I waarden varieerden van 20.7 tot 117.8 ng/ml en correleerden met de GH waarden. Er bleek geen relatie te bestaan tussen de serum GH waarden en zwangerschapsduur, geboortegewicht, postnatale leeftijd, gewichtstoename en ernst van ziekte. De gemeten GH en IGF-I waarden bij zieke beademde premature pasgeborenen bleken overeen te komen met serum GH waarden gevonden bij gezonde premature pasgeborenen. De gevonden correlatie tussen serum GH en IGF-I suggereert dat er mogelijk sprake is van enige invloed van GH op de IGF-I produktie.

Op basis van deze gegevens concludeerden wij dat er geen sprake is van GH deficientie bij beademde premature pasgeborenen. De groei-achterstand die ontstaat bij zieke premature pasgeborenen kan dan ook niet door GH deficientie verklaard worden.

In **hoofdstuk 4** wordt het effect van dexamethason (DEXA) op de GH-IGF-I as beschreven bij zeer premature beademde pasgeborenen die BPD onwikkelen. Het doel van de studie was om het korte-termijn effect van DEXA op de GH-IGF-I as bij beademde premature pasgeborenen die BPD onwikkelen te onderzoeken.

Bij 10 beademde premature pasgeborenen werd een 6-uur GH profiel verricht en serum IGF-I, IGFBP-1 en -3 waarden bepaald, direct voor start van de dexamethason behandeling en 2 dagen daarna. Tijdens DEXA behandeling daalden de GH waarden, echter zonder een gelijktijdige daling van IGF-I en IGFBP-3. Zowel het tijdsinterval tussen de toediening van DEXA en het 2^{e} GH profiel als de cumulatieve dosis DEXA die was toegediend, waren niet gecorreleerd met de gemeten GH waarden tijdens DEXA behandeling.

Concluderend, 2 dagen behandeling met DEXA bij zeer premature pasgeborenen veroorzaakt een daling van de serum GH waarden, zonder gelijktijdige daling van de serum IGF-I waarden. Het blijft dan ook de vraag of de lage GH waarden gemeten na toediening van DEXA verantwoordelijk zijn voor de afname in groei tijdens behandeling met dexamethason bij deze pasgeborenen.

In **hoofdstuk 5** wordt een gerandomiseerde-placebo-gecontroleerde studie beschreven met recombinant humaan GH (rhGH) in beademde premature pasgeborenen die behandeld worden met dexamethason. Het doel van de studie was te onderzoeken of gelijktijdige behandeling met rhGH het groeiremmende effect veroorzaakt door behandeling met dexamethason kan voorkomen. In deze studie werden 30 zeer premature beademde pasgeborenen geïncludeerd, nadat de behandelend neonatoloog had besloten om dexamethason behandeling te starten om de kinderen van de beademing te krijgen. De kinderen werden gerandomiseerd en behandeld met de combinatie DEXA en placebo of met DEXA en rhGH. DEXA werd gedurende 24 dagen, in een afbouwschema gegeven. Tegelijkertijd werd gedurende 42 dagen rhGH of placebo behandeling gegeven. Vijftien kinderen werden behandeld met DEXA en GH, 15 kinderen met DEXA en placebo.

Gewicht, lengte, knie-hiel lengte en schedelomvang werden gemeten. Serum GH, IGF-I en IGFBP-1 en -3 waarden werden bepaald.

De groei van zowel gewicht, lengte, knie-hiel lengte en schedelomvang was niet verschillend tussen beide behandelingsgroepen in deze periode van 6 weken. In de 1e week van de DEXA behandeling vond geen groei plaats, niet in de GH en niet in de placebogroep. Opmerkelijk was het feit dat de groei in de 2e en 3e week van de DEXA behandeling gelijk was aan de groei in de eerste 3 weken na staken van DEXA. Serum IGF-I waarden veranderden niet in deze 42 dagen, terwijl serum IGFBP-3 waarden toenamen tijdens DEXA behandeling.

Wij concluderen dat behandeling met rhGH, in een periode van 6 weken, de negatieve effecten van DEXA op de groei bij zeer premature pasgeborenen die BPD ontwikkelen, niet kan compenseren. Lange termijn vervolgstudies zijn nodig om eventuele latere effecten op groei, longfunctie en ontwikkeling te evalueren. Als tweede wordt geconcludeerd dat de negatieve effecten van DEXA op de groei beperkt zijn tot de 1e week van de DEXA behandeling, tijdens de hoge doseringen. Dit suggereert een dosis-afhankelijk, groei-remmend effect van DEXA. Wij suggereren dat toekomstige studies vooral het gebruik van DEXA in een lagere dosis dan thans gebruikelijk evalueren. Hierdoor kunnen wellicht andere ernstige bijwerkingen voorkomen worden.

In **hoofdstuk 6** wordt de groei en de lichaamssamenstelling van zuigelingen met BPD beschreven. Het doel van deze studie was de groei en verandering in lichaamssamenstelling van zuigelingen met BPD gedurende het eerste levensjaar te beschrijven. In dit onderzoek werden 29 zuigelingen met BPD op de neonatale follow-up polikliniek op de leeftijden van 6 weken, 3, 6, 9 en 12 maanden na de uitgerekende datum vervolgd. Tijdens deze follow-up periode werden lengte, gewicht en lichaamssamenstelling met behulp van de TOBEC methode gemeten en de voedingsintake van de kinderen berekend. Alle kinderen werden in de neonatale periode langdurig beademd (gemiddeld 29 dagen) en behandeld met dexamethason.

In vergelijking met gezonde Nederlandse kinderen hadden de kinderen met BPD een achterstand in lengte en gewicht op de leeftijd van 6 weken na de uitgerekende datum. Gedurende het eerste jaar is er sprake van enige inhaalgroei van lengte, maar vindt geen inhaalgroei van het gewicht plaats. Aan het eind van het eerste jaar zijn de kinderen met BPD lichter en de meisjes met BPD ook kleiner in vergelijking met hun leeftijdsgenoten.

Met behulp van de TOBEC metingen kon de hoeveelheid vetweefsel en vetvrije massa van de kinderen berekend worden. De zuigelingen met BPD hadden op 6 weken na de uitgerekende datum een duidelijk verminderde hoeveelheid vetweefsel en vetvrije massa in vergelijking met gezonde Nederlandse kinderen. In het eerste levensjaar vindt zowel bij de jongens als bij de meisjes enige inhaalgroei van de hoeveelheid vetvrije massa plaats, maar deze blijft te laag in vergelijking met gezonde kinderen. De hoeveelheid vetweefsel blijft

lager gedurende het eerste levensjaar en bij meisjes is er tevens een duidelijke afname van de procentuele hoeveelheid vetweefsel.

De voedingsintake werd gedurende de eerste 6 maanden berekend. Ondanks een normale energie intake en een eiwitintake hoger dan geadviseerd door de WHO voor gezonde zuigelingen, vond een onvolledige inhaalgroei van lengte en vetvrije massa plaats en geen inhaalgroei van gewicht en hoeveelheid vetweefsel.

Deze resultaten suggereren dat premature pasgeborenen met BPD waarschijnlijk een hogere energie- en eiwitbehoefte hebben dan gezonde pasgeborenen om een goede inhaalgroei te kunnen bewerkstelligen. Interventie studies met eiwit- en energieverrijkte voeding zullen in de toekomst laten zien of het mogelijk is bij deze kinderen een normale groei en lichaamssamenstelling te bewerkstelligen.

In **hoofdstuk 7** wordt de ontwikkeling van de luchtwegen beschreven bij premature zuigelingen met BPD, die in de neonatale periode beademd werden met conventionele beademing of hoog-frequente beademing (HFO). Het doel van de studie was de lucht wegdoorgankelijkheid (V'_{maxFRC}) bij zuigelingen met BPD gedurende het 1e levensjaar te onderzoeken.

Bij 36 ex-premature zuigelingen met BPD werd op 6 en /of 12 maanden na de uitgerekende datum longfunctie onderzoek verricht. De functionele residuale capaciteit en de V'_{maxFRC} werden bepaald. Bij de zuigelingen met BPD was de V'_{maxFRC} op 6 en 12 maanden duidelijk lager en verslechterde in deze periode ten opzichte van gezonde kinderen. De zuigelingen met BPD, initieel behandeld met HFO hadden een betere luchtwegdoorgankelijkheid op 12 maanden dan de zuigelingen initieel behandeld met conventionele beademing.

De resultaten van deze studie tonen een verminderde en verdere verslechtering van de luchtweg-doorgankelijkheid bij zuigelingen met BPD gedurende het 1e levensjaar. Initiele behandeling met hoog-frequente beademing resulteerde in een betere longfunctie op 12 maanden.

In conclusie: Zuigelingen met BPD tonen een verminderde groei in het eerste levensjaar met te weinig vetweefsel en vetvrije massa. De luchtwegdoorgankelijk van zuigelingen met BPD is verminderd in vergelijking met gezonde zuigelingen van dezelfde leeftijd en lengte. De groei van de luchtwegen is vertraagd.

De achterstand in de groei bij premature pasgeborenen die BPD ontwikkelen ontstaat reeds tijdens het verblijf op de NICU, maar wordt niet veroorzaakt door groeihormoon deficientie. Dexamethason veroorzaakt een daling van de serum GH waarden, zonder daling van IGF-I. GH behandeling tijdens gebruik van dexamethason heeft geen positieve invloed op de groei. Het groeiremmend effect van DEXA lijkt beperkt tot de 1e week tijdens de hoge doseringen DEXA. Zieke premature pasgeborenen hebben een relatieve bijnierinsufficientie in de eerste levensweek. Pasgeborenen die later BPD ontwikkelen

waren minder goed in staat hun cortisol productie te vergroten dan pasgeborenen die geen BPD ontwikkelden. Mogelijk leidt behandeling van deze bijnierinsufficientie tot voorkoming van BPD en is daarmee samenhangend ook de groeiachterstand te voorkomen. Tevens zal blijken of verbeterde groei eveneens resulteert in een verbeterde groei van de luchtwegen.

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Curriculum vitae

De auteur van dit proefschrift werd in 1958 te Benthuizen geboren. In 1976 behaalde zij het VWO diploma aan het Erasmus College te Zoetermeer. In datzelfde jaar werd de studie geneeskunde aangevangen. Zij behaalde in 1983 het artsexamen. In 1983 werd zij aangesteld als arts-assistent in de kinderkliniek te Leiden en vanaf 1984 was zij werkzaam op de afdeling kinderchirurgie van het Erasmus MC, locatie Sophia. In 1986 werd aangevangen met de opleiding tot kinderarts op de afdeling kindergeneeskunde van het Erasmus MC (opleider prof.dr. H.K.A. Visser). De niet-academische stage vond plaats in het Medisch Centrum Rijnmond Zuid, locatie Zuider (opleider dr. R.N. Sukhai). Registratie tot kinderarts volgde in april 1990.

Vanaf januari 1990 was zij werkzaam als chef de clinique van de medium care van het Erasmus MC, locatie Sophia. Tevens was zij werkzaam bij de subafdeling nefrologie (subhoofd dr. E.D. Wolff). In augustus 1992 werd gestart met de opleiding neonatologie (opleider prof.dr. P.J.J.Sauer). Sedertdien is zij werkzaam als staflid op de afdeling neonatologie (afdelingshoofd dr. L.J.I. Zimmermann).

Sedert 1992 is zij betrokken bij de neonatale follow-up en vanaf 1996 met name bij de follow-up van kinderen met bronchopulmonale dysplasie (BPD). In 2001 is zij gestart met follow-up onderzoek naar de morbiditeit en hospitalisatie van kinderen met BPD (subsidie: ZonMW en NAF)

De auteur is lid van de sectie neonatologie van de Nederlandse Vereniging voor Kindergeneeskunde en van de werkgroep Landelijke Neonatale Follow-up. Binnen deze werkgroep is zij betrokken bij de opzet van een uniforme landelijke neonatale follow-up registratie. Tevens is zij lid van de Medisch Ethische Commissie, patientenzorg van het Erasmus MC, locatie Sophia.

List of abbreviations

ACTH	adrenocorticotropic hormone
AGA	appropriate for gestational age
BPD	bronchopulmonary dysplasia
BW	birth weight
CHL	crown-heel length
CI	confidence interval
CLD	chronic lung disease
CMV	conventional mechanical ventilation
C/ 17OHP	cortisol/ 17-hydroxyprogesterone ratio
CPAP	continuous positive airway pressure
CRH	corticotrophin-releasing hormone
CYP11B	11ß-hydroxylase
CYP21A2	21-hydroxylase
DEXA	dexamethasone
DHEA	dehydroepiandrostenedione
FEV1	forced expiratory volume 1 sec
FFM	fat free mass
FRC_p	functional residual capacity
FVC	forced vital capacity
GA	gestational age
GH	growth hormone
HFO	high frequency oscillation
HPA-axis	hypothalamic-pituitary- adrenal axis
IGF-I	insulin-like growth factor-I
IGFBP-1	IGF-binding protein-1
IGFBP-3	IGF-binding protein-3
IVH	intraventricular hemorrhage
KHL	knee-heel length
MAP	mean airway pressure
NCPAP	nasal continuous positive airway pressure
NEC	necrotising enterocolitis
NICU	neonatal intensive care unit
O_2	oxygen
OFC	occipito-frontal headcircumference
17 OHP	17-hydroxyprogesterone
PDA	patent ductus arteriosus
PEEP	positive end expiratory pressure

PMA	postmentrual age
PPV	positive pressure ventilation
PROM	premature rupture of membranes
RDS	respiratory distress syndrome
rhGH	recombinant human growth hormone
ROP	retinopathy of prematurity
SDS	standard deviation score
SGA	small for gestational age
SNAP	Score for Neonatal Acute Physiology
TBF	total body fat
TOBEC	total body electric conductivity
VLBW	very low birth weight
$\mathbf{V'_{MAXFRC}}$	maximal flow at FRC