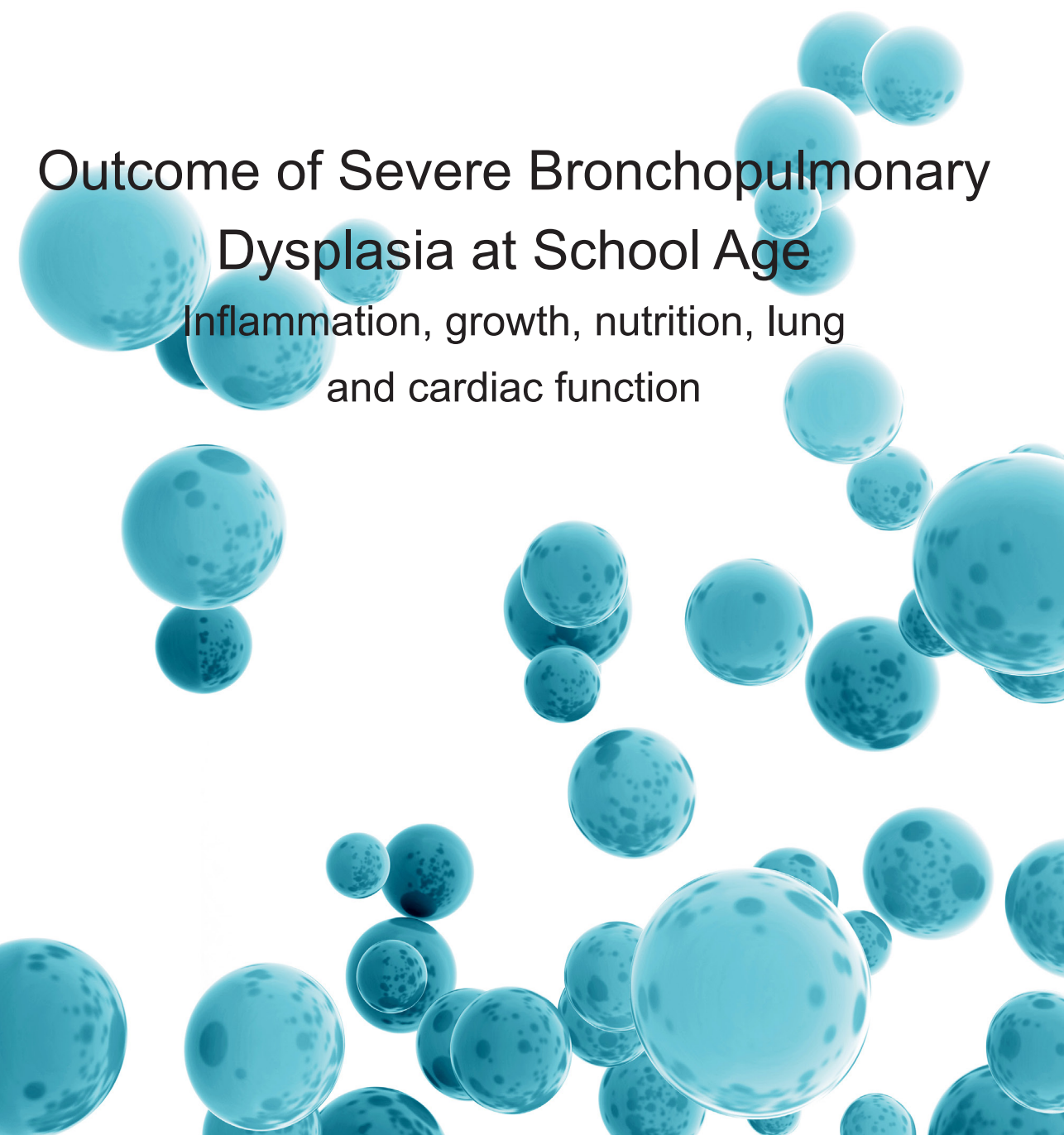


PIIA SUURSALMI

Outcome of Severe Bronchopulmonary Dysplasia at School Age

Inflammation, growth, nutrition, lung
and cardiac function





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ACADEMIC DISSERTATION

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the Board of the School of Medicine of the University of Tampere,
for public discussion in the Jarmo Visakorpi auditorium
of the Arvo building, Lääkärintäti 1, Tampere,
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UNIVERSITY OF TAMPERE

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Science is a wonderful thing if one does not have to earn one's living at it.

Albert Einstein

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List of original publications

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- I Korhonen PH, Suursalmi P, Kopeli T, Nieminen R, Lehtimäki L, Luukkaala T, Korppi M, Saari A, Moilanen E, Tammela O. Inflammatory activity at school age in very low birth weight bronchopulmonary dysplasia survivors. *Pediatric Pulmonology* 2015; 50(7):683-690. doi: 10.1002/ppul.23038.
- II Suursalmi P, Kopeli T, Korhonen P, Lehtimäki L, Nieminen R, Luukkaala T, Moilanen E, Korppi M, Paasilta M, Tammela O. Very low birthweight bronchopulmonary dysplasia survivors show no substantial association between lung function and current inflammatory markers. *Acta Paediatrica* 2015; 104(3):264-268. doi: 10.1111/apa.12837.
- III Suursalmi P, Korhonen P, Kopeli T, Nieminen R, Luukkaala T, Moilanen E, Tammela O. Severe bronchopulmonary dysplasia, growth, nutrition and adipokines at school age. *Global Pediatric Health* 2016; 16(3):2333794X16637290. doi: 10.1177/2333794X16637290.
- IV Suursalmi P, Eerola A, Poutanen T, Korhonen P, Kopeli T, Tammela O. Cardiac function in relation to lung function in school-aged bronchopulmonary dysplasia survivors. Submitted.

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Tiivistelmä

Taustaa: Tulehdusreaktio on tärkeä tekijä keskosen kroonisen keuhkosairauden bronkopulmonaalisen dysplasian (BPD) synnyssä, mutta sen merkitys myöhäisemmässä taudinkuvassa on epäselvä. Vaikka keskosten hoidon kehittyminen on lievittänyt BPD:n aiheuttamia keuhko-oireita, saattaa BPD vaikuttaa myöhempään kasvuun ja sydämen toimintaan.

Tutkimuksen tarkoitus: Tutkimuksen tarkoitus oli selvittää kouluikäisten BPD-lasten elimistön tulehdusaktiivisuutta ja sen yhteyttä keuhkojen toimintaan, kasvuun ja ravitsemukseen pienipainoisina (alle 1500g) syntyneillä keskosilla. Lisäksi tutkimme BPD-lasten sydämen toimintaa ja pienipainoisina syntyneiden lasten keuhkojen toiminnan yhteyttä sydämen toimintaan kouluikässä.

Menetelmät: Tutkimukseen osallistui 21 lasta, joilla oli vastasyntyneenä radiologisesti todettu vaikea BPD, 19 pienipainoisena syntynyttä verrokkilasta sekä 19 täysiaikaisena syntynyttä perustervettä lasta. Lasten ikä tutkimushetkellä vaihteli kuuden ja neljäntoista vuoden välillä. Taustatiedot saatiin potilasarkistoista ja vanhempien täyttämistä kyselylomakkeista. Tulehdusarvoista mittasimme plasman eosinofiilisen kationisen proteiinin, interleukiinien 6 ja 8, adiponektiinin, adipsiinin, leptiinin ja resistiinin pitoisuudet, sekä uloshengitysilman typpioksidin, leukotrieeni B4:n ja 8-isoprostaanin pitoisuudet. Seerumin immunoglobuliini E ja plasman B-tyyppin N-terminaalinen propeptidi määritettiin. Kliininen tutkimus sisälsi antropometrisia mittauksia. Ravitsemustottumuksia kartoitettiin kolmen vuorokauden ruokapäiväkirjan avulla. Keuhkojen toimintaa selvitettiin impulssioskillometrialla. Sydämen toimintaa tutkittiin perinteisen kaksikulotteisen ultraäänien, M-moodin ja Doppler-tutkimusten lisäksi kudos-Doppleria ja speckle tracking-tekniikkaa käyttäen, sekä kolmiulotteisella sydämen ultraäänitutkimuksella.

Tulokset: Kouluikäisten BPD-lasten tulehdusmerkkiainepitoisuudet olivat samaa tasoa kuin verrokeilla. Poikkeava keuhkojen toiminta todettiin 13 %:lla pienipainoisina syntyneistä lapsista. Keuhkojen toiminnan ja tulehdusmerkkiaineiden välillä ei havaittu yhteyttä kouluikäisillä BPD-lapsilla. Kouluikäisten BPD-lasten päänympäryys oli pienempi ja heillä esiintyi enemmän pienipäisyyttä verrattuna pienipainoisina syntyneisiin ja täysiaikaisiin kontrollilapsiin. Muita antropometrisia eroja ei todettu. Ravitsemus ei eronnut

ryhmien kesken, ja ravitsemussuositukset näyttivät toteutuvan huonosti kaikissa ryhmissä. Korkea painoindeksi assosioitui korkeisiin leptiini- ja adipsiini-tasoihin, mutta muita yhteyksiä kasvun ja adipokiinien välillä ei havaittu. Pienipainoisina syntyneiden adipokiinitasot korreloivat ravitsemuksen kanssa. BPD-lasten sydämen toiminta kouluiässä oli normaalia. Vastasyntyneisyyskauden keuhko-ongelmien ja kouluiässä tutkitun sydämen toiminnan välillä ei havaittu yhteyttä. Huonompi keuhkofunktio kouluiässä näytti korreloivan huonomman sydämen vasemman kammion toiminnan kanssa pienipainoisina syntyneillä.

Johtopäätökset: Vaikka tulehdusreaktiolla on tärkeä merkitys BPD:n synnyssä, kouluikäisillä BPD-lapsilla ei todettu suurentunutta tulehdusaktiivisuutta, eikä BPD-lasten keuhkojen toiminnan ja tulehdusmerkkiaineiden välillä todettu yhteyttä. BPD:n aiheuttamat keuhko-ongelmat ovat todennäköisesti seurausta rakenteellisista keuhkomuutoksista. Pienempää päänympärystä lukuun ottamatta BPD-lasten kasvu ei poikennut muiden pienipainoisina syntyneiden tai täysiaikaisten lasten kasvusta kouluiässä. Painoindeksiin liittyvä leptiini- ja adipsiini-tasojen nousu saattaa huonontaa ylipainoisten BPD-lasten keuhkoennustetta. Kouluikäisten BPD-lasten sydämen toiminta oli normaalia, mutta keuhkojen toiminta korreloi sydämen toiminnan kanssa. Pienipainoisina syntyneiden poikkeava keuhkojen toiminta saattaa altistaa myöhemmille sydämen toiminnan poikkeavuuksille.

Abstract

Background: Inflammation is inherent in the pathogenesis of bronchopulmonary dysplasia (BPD), a chronic lung disease of premature children. Less is known regarding the role of inflammation in the later sequelae of BPD. Although with advances in neonatal care the pulmonary course of BPD survivors has become milder BPD may nevertheless affect later growth and cardiac function.

Objectives: The aims of this study were, firstly, to assess the current inflammatory activity in school-aged children with BPD and to establish whether inflammation is related to lung function, growth and nutrition in school-aged very low birth weight (VLBW, birth weight < 1500g) children, and secondly, to assess the cardiac function in school-aged BPD children and to establish whether the cardiac function is related to lung function in VLBW children.

Methods: Twenty-one VLBW children with severe radiographic BPD, 19 VLBW children without radiographic BPD and 19 non-asthmatic term controls were examined. The age of the children varied between 6-14 years during the study period. Background data were gathered from the hospital records and parental questionnaire. The inflammatory markers eosinophilic cationic protein, interleukins 6 and 8, adiponectin, adipisin, leptin and resistin concentrations were measured in plasma, and nitric oxide, leukotriene B4 and 8-isoprostane in exhaled breath. In addition, serum total immunoglobulin E and plasma N-terminal pro-brain natriuretic peptide were measured. The participants underwent clinical examination with anthropometric measurements. To examine nutritional intake, a three-day food record was completed. Lung function was studied by impulse oscillometry. Echocardiographic studies included conventional echocardiography with Doppler and M-mode measurements, tissue Doppler imaging, speckle tracking echocardiography and real-time three-dimensional echocardiography.

Results: The inflammatory marker levels did not differ between children with radiographic BPD and VLBW and term controls. Abnormal lung function was found in 13% of the former VLBW children. No correlations were found between inflammatory markers and lung function in the radiographic BPD group. School-aged BPD survivors had smaller head circumferences and more microcephaly compared to VLBW and term controls; otherwise the anthropometric parameters

did not differ. No nutritional differences were found between the groups. Recommendations on daily nutrient intake were poorly met in all groups. A high body mass index standard deviation score (BMI-SDS) was associated with high leptin and adipon levels, but otherwise adipokine levels at school age seemed to have no associations with growth parameters in VLBW children. Plasma adipokine levels correlated with nutrient intake in VLBW children. Cardiac parameters did not differ between the groups. No associations were found between neonatal pulmonary morbidity and cardiac parameters in school-age. Lower lung function correlated modestly with worse LV echocardiographic parameters in VLBW children.

Conclusions: Although prominent in the pathogenesis of BPD, inflammatory activity had attenuated in BPD survivors by school age. The pulmonary function of school-aged BPD survivors was not associated with inflammatory markers. It would appear that the pulmonary consequences of BPD are caused by permanent structural damage. Apart from smaller head circumferences, the growth of BPD children was comparable with VLBW and term controls. The BMI-SDS-associated leptin and adipon levels may have a negative impact on later lung function in BPD children. Cardiac function in school-aged BPD children was normal. Lower lung function was associated with echocardiographic parameters, implicating that the impaired lung function may alter later cardiac function.

Abbreviations

3DE	three-dimensional echocardiography
A	atrial peak flow velocity
A´	late diastolic myocardial velocity
BMI-SDS	body mass index standard deviation score
BPD	bronchopulmonary dysplasia
E	early peak flow velocity
E´	early diastolic myocardial relaxation velocity
ECP	eosinophilic cationic protein
ELBW	extremely low birth weight
IgE	immunoglobulin E
IL	interleukin
IVH	intraventricular hemorrhage
MAD	mitral annular displacement
MAD-mid	mitral annular midpoint displacement
MRI	magnetic resonance imaging
NEC	necrotizing enterocolitis
NO	nitric oxide
NT-proBNP	N-terminal pro-brain natriuretic peptide
radBPD	radiographic bronchopulmonary dysplasia
RDS	respiratory distress syndrome
S´	systolic myocardial velocity
TAD	tricuspid annular displacement
TAD-mid	tricuspid annular midpoint displacement
Tmsv 12/16-Dif	maximum time difference to reach minimum systolic volume between the earliest and latest contracting segments for the 12/16 segments
Tmsv 12/16-SD	standard deviation of time to minimum systolic volume for the 12/16 segments
VLBW	very low birth weight

1 Introduction

The definition and phenotype of bronchopulmonary dysplasia (BPD) have changed in keeping with the advances in perinatal and neonatal care (Bancalari et al. 2003). The new, post-surfactant BPD affects mainly the very preterm infants (Bancalari et al. 2003). Even a minimal lung injury can initiate a pulmonary inflammatory response which may impair the growth of the immature lung and lead to BPD (Kinsella et al. 2006).

Little is known as to the role of inflammation in the later course of BPD. Deterioration of lung function during childhood in some BPD survivors suggests an ongoing disease process rather than solely a permanent structural remodeling (Filippone et al. 2009). It has been speculated that perinatal lung injury may serve as a trigger for chronic inflammation (Teig et al. 2012), but results of previous studies regarding inflammation in BPD survivors after early childhood are controversial (Baraldi et al. 2005; Carraro et al. 2015).

Adipokines, inflammatory markers secreted mainly by the adipose tissue, are involved in several chronic lung diseases (Ali Assad et al. 2012). Growth (Flexeder et al. 2014) and nutrition (Roberts et al. 2013) may affect adipokine levels. BPD impairs early growth (Natarajan et al. 2014) and prematurity is associated with altered eating habits in later life (Kaseva et al. 2013). Whether the growth and nutrition of BPD survivors affects their adipokine status and thus their lung function is not known.

Dysmorphic vascular growth and chronic respiratory disease in BPD may lead to pulmonary vascular disease, pulmonary hypertension and right ventricular dysfunction (Mourani et al. 2013). There are few studies on cardiac function in school-aged survivors of the new BPD and, to our knowledge, none comparing cardiac to lung function in very low birth weight (VLBW) survivors.

Our aim here was to evaluate the current inflammatory activity and its associations with lung function, growth and nutrition in school-aged VLBW children with and without severe BPD. Secondly, we assessed cardiac function in school-aged BPD children and the association between the cardiac and lung function in VLBW children.

2 Review of the literature

2.1 Definition of BPD

2.1.1 From classic to new BPD

Wilson and Mikity described a chronic lung disease following very preterm birth in 1960 (Wilson et al. 1960). Infants with initially little or no oxygen requirement developed cystic lung disease with a need for high supplemental oxygen concentrations at approximately two weeks of age. Chronic lung disease after mechanical ventilation due to respiratory distress syndrome (RDS) was first reported in 1964 (Shepard et al. 1964). The term BPD to describe this pulmonary disorder following mechanical ventilation and high oxygen concentrations was introduced in 1967 (Northway et al. 1967).

Advanced neonatal intensive care with antenatal glucocorticoid therapy, surfactant therapy and gentler ventilation techniques have changed the clinical course of BPD (Bancalari et al. 2003). Severe lung injury in moderate to late preterm infants is rare and current forms of BPD are related more to disrupted development of alveoli and pulmonary capillaries in very immature infants (Bancalari et al. 2003). BPD now occurs mostly in infants born before 28 weeks of gestation, who typically weigh less than 1000 g at birth and who may develop BPD without severe RDS (Kinsella et al. 2006). The spectrum of this "new BPD" may include the not so new Wilson-Mikity syndrome (Philip 2009).

2.1.2 Radiographic classification

With the abovementioned changes in the pathophysiology and clinical course, the definition of BPD has also been changed. The initial description included four radiological stages associated with histological changes (Northway et al. 1967). RDS with generalized granular pattern and air bronchograms in stage I in the first three days of life were followed by increasing opacification of the lungs with

bronchial and alveolar epithelial necrosis and early stages of lung repair in stage II. The emphysematous alveoli adjacent to atelectatic alveoli were seen as a reticular network of small rounded radiolucent areas in stage III on days 10-20, followed by cystic changes with fibrosis, peribronchial smooth muscle hypertrophy and changes in the pulmonary arterioles after 30 days in stage IV (Figure 1).

The altered pathogenesis has led to changed radiographic findings in BPD, and newer radiographic (Toce et al. 1984; Hyde et al. 1989; Swischuk et al. 1996; Weinstein et al. 1994) and computed tomography (Ochiai et al. 2008) scoring systems have been developed. The majority of very preterm infants yield an abnormal chest radiograph at 36 weeks of gestational age, but cystic lesions are rare (Greenough et al. 2000). Inter-observer variation in the interpretation of chest radiographs of infants with BPD is considerable (Moya et al. 2001; Hyödynmaa et al. 2012), but better agreement has been found with cystic lesions (Moya et al. 2001). Radiographic BPD changes correlate poorly with oxygen dependency at 36 weeks of gestational age (Fitzgerald et al. 1996; Hyödynmaa et al. 2012), but may predict respiratory outcomes even better than oxygen dependency (Hirata et al. 2015).



Figure 1. Chest radiograph of a VLBW child with cystic radiographic BPD (Northway IV) taken in Tampere University Hospital in 2014.

2.1.3 Current definition

The current consensus criteria published in 2001 define BPD as supplemental oxygen dependency for at least 28 days (Jobe et al. 2001). The severity of BPD is graded according to the amount of supplemental oxygen given and the mode of respiratory support at discharge or at 36 weeks of postmenstrual age in infants born at less than 32 weeks of gestation and at 56 days of age in infants born at 32 weeks of gestation or older (Jobe et al. 2001). If a child is weaned from oxygen supplement by the time of evaluation, the BPD is considered mild. A need for supplementary oxygen $<30\%$ at time of evaluation indicates moderate and $\geq 30\%$ of oxygen or continuous positive pressure support severe BPD. A timed room-air oxygen reduction test is recommended to minimize the impact of the varying saturation thresholds for supplemental oxygen (Walsh et al. 2004).

The current consensus recommends the term BPD, rather than the previously used chronic lung disease of the newborn, to clearly distinguish the disease from chronic lung diseases of later life (Jobe et al. 2001).

2.2 Incidence of BPD

Due to the increased survival rate among the most immature infants, the incidence of BPD has not decreased (Bancalari et al. 2003). The incidence of BPD varies widely between different centers, indicating regional differences in patient characteristics and management and in the definition of BPD (Bancalari et al. 2003; Jensen et al. 2014). The incidence of BPD in VLBW infants varies between 5% and 50% (Jensen et al. 2014).

The incidence of BPD in Finnish preterm infants is presented in Figure 2. The incidence of BPD in infants born before 32 weeks of gestation in Finland in 2012-2014 was 31% (Data from Small Preterm Infants register, National Institute for Health and Welfare, Finland). At the gestational age of 42 weeks, 9.5% of these small preterms were still in hospital and 0.7% had been discharged with supplemental oxygen.

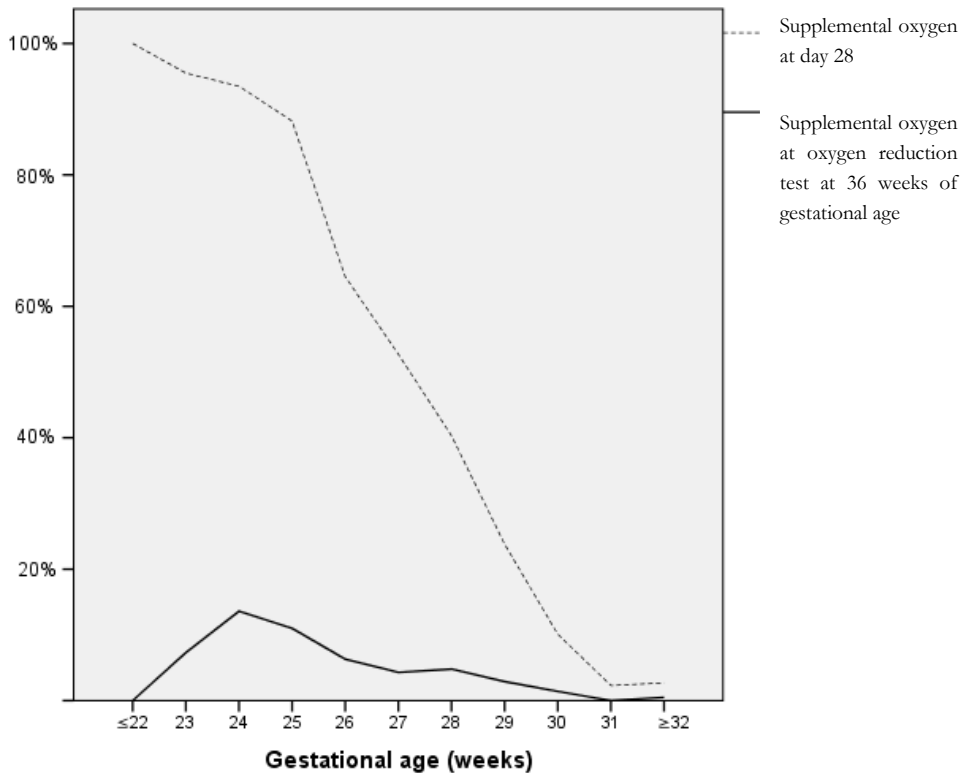


Figure 2. The need for supplemental oxygen at day 28 of life and at 36 weeks of age in Finnish live born infants in 2012-2014. Data from Small Preterm Infants register, National Institute for Health and Welfare, Finland.

2.3 Pathogenetic aspects of BPD

2.3.1 Lung development

The fetal development of the lung is illustrated in Figure 3. The conducting airways are formed mainly in embryonic and pseudoglandular stages (Joshi et al 2007). The formation of alveolar structures in the canalicular and saccular stages of lung development enables gas exchange (Joshi et al. 2007). Septation and multiplication of alveoli, the alveolar stage of lung development, extends from 36 weeks to at least three years, possibly even through the first two decades (Baker and Alvira 2014). The enlargement of terminal bronchioles and alveoli can continue up to 18-22 years (Joshi et al. 2007). Preterm birth at the canalicular or early saccular stage

disrupts normal lung development (Joshi et al. 2007; Thebaud et al. 2007). Hyperoxia, barotrauma and inflammation impair alveolization (Joshi et al. 2007). BPD is characterized by larger and fewer alveoli, i.e. alveolar simplification (Thebaud et al. 2007).

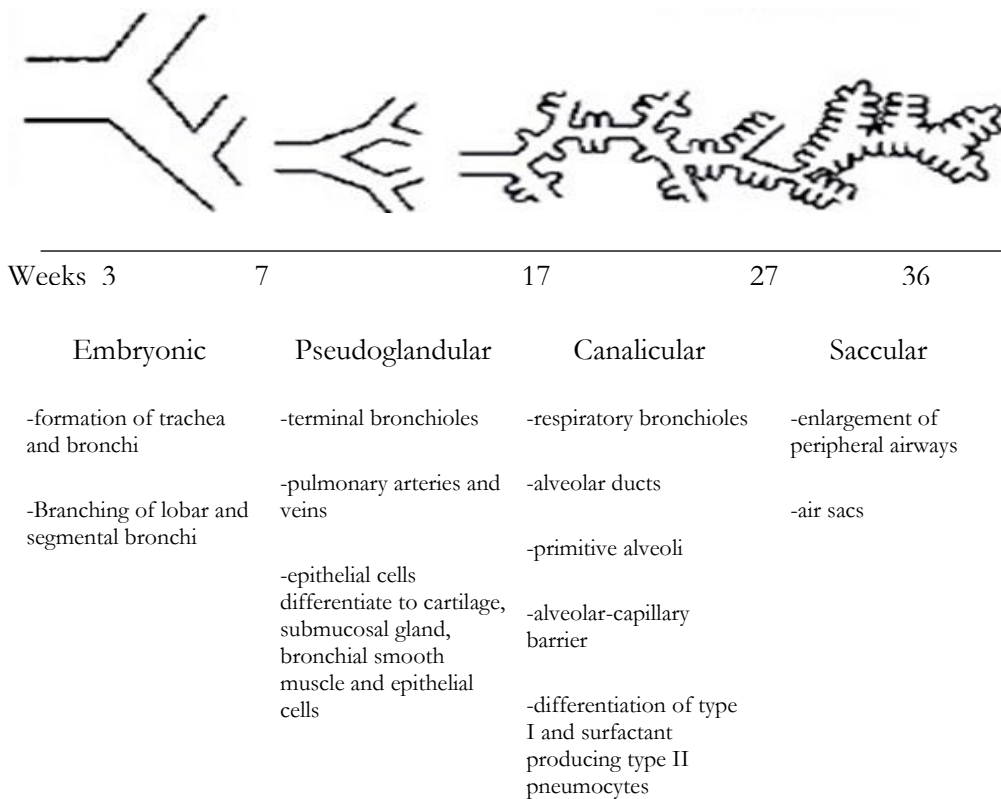


Figure 3. Fetal stages of lung development. The Figure is based on the text by Joshi et al. 2007.

The development of the pulmonary circulation consists in the formation of blood vessels from angioblasts and endothelial precursor cells and extension of existing vessels (Thebaud et al. 2007). Microvascular maturation takes place from birth to 2-3 years (Joshi et al. 2007). Insufficiency of angiogenetic growth factors such as vascular endothelial growth factor and nitric oxide (NO) may lead to impaired alveolar and vascular growth (McEvoy et al. 2014). Decreased cord blood levels in circulating angiogenic cells have been associated with BPD (Baker et al. 2012). Dysmorphic vascular growth and structural remodeling of pulmonary

vasculature in BPD includes medial hypertrophy, distal muscularization of small peripheral arteries, reduced arterial number and abnormal vasoreactivity (Thebaud et al. 2007; Mourani et al. 2013). Fetal intrapulmonary arteriovenous anastomotic vessels may persist in severe BPD (Baker et al. 2014).

Impaired alveolar and vascular growth remains a prominent finding at autopsy, with less regional heterogeneity, milder airway smooth muscle thickening and less fibrosis seen in post-surfactant compared to pre-surfactant BPD (Kinsella et al. 2006).

2.3.2 Genetic and epigenetic factors

Female gender and non-white race reduce the risk of BPD (Avery et al. 1987; Horbar et al. 1988). Twin studies demonstrate high heritability for BPD (Lavoie et al. 2008). Gene studies of first-line immune and antioxidant defences, surfactant proteins and components of vascular and lung remodeling in BPD have been limited in sample size and most of them lack confirmation (Lavoie et al. 2010). Interactions between polymorphisms may affect the risk of BPD (Huusko et al. 2014). Epigenetic alterations due to environmental stimuli reprogram cells (Hagood 2014). A whole genome expression study showed altered expression of almost 3500 genes on day 28 in children who developed BPD compared to VLBW controls (Pietrzyk et al. 2013). The majority of the under-expressed genes in the BPD children were involved in the inflammatory response (Pietrzyk et al. 2013).

2.3.3 Antenatal factors

The intrauterine environment can alter lung development and lower the capacity to protect the lung against later insults (Harding et al. 2012). Intrauterine growth restriction may lead to fewer and larger alveoli, thicker intra-alveolar septa and a thicker blood-air barrier (Harding et al. 2012) and is associated with an increased risk of BPD (Eriksson et al. 2015). The relationship between preeclampsia and BPD remains controversial (Yen et al. 2013). Maternal hypertension would appear to be a risk factor for BPD (Bi et al. 2013). The role of chorioamnionitis in the development of BPD is controversial, and is likely to depend on the pathogen, timing, duration and severity of the insult and the subsequent fetal inflammatory response (Kramer et al. 2009; Lahra et al. 2009; Adams-Chapman 2012).

The most significant risk factor for BPD is premature birth (Eriksson et al. 2015). Antenatal steroids reduce RDS and death, but have not been proved to lower the rate of BPD in survivors (McEvoy et al. 2014).

2.3.4 Inflammation during the perinatal and neonatal period

Inflammation is an essential component in the pathogenesis of the BPD (Bose et al. 2008). Pulmonary inflammation can begin antenatally or be triggered by postnatal infections or insults such as mechanical ventilation or exposure to supplemental oxygen (Bose et al. 2008).

The inflammatory cascade (Figure 4) begins with chemokines such as interleukin (IL) 8 and leukotriene B4 directing leukocytes to the site of inflammation (Charo et al. 2006). Adhesion molecules transfer the inflammatory cells from capillaries to air spaces or interstitium (Bose et al. 2008).

Pro-inflammatory cytokines such as IL-6 mediate tissue damage (Bose et al. 2008). Elevated concentrations of chemokines, adhesion molecules and pro-inflammatory cytokines have been found in tracheal aspirates of infants who develop BPD (Bose et al. 2008). Expression of cytokine IL-1 β in the lungs of fetal and newborn mice has caused a pulmonary disease resembling BPD without additional insults such as hyperoxia or mechanical ventilation (Bry et al. 2007). On the other hand, prevention of the recruitment of neutrophils to the intra-alveolar spaces has not prevented BPD in murine models (Hogmalm et al. 2012).

Inflammation (Saugstad 2005) and high concentrations of inspired oxygen lead to increased formation of reactive oxygen species (Bose et al. 2008). Oxygen radicals cause tissue damage by oxidation and by inducing pro-inflammatory cytokines (Saugstad 2005). This impairment may lead to increased vascular permeability and pulmonary edema (Perrone et al. 2012). Preterm infants have deficient antioxidant enzyme systems and low levels of antioxidants (Saugstad 2005; Kinsella et al. 2006). An imbalance between the destructive proteinase and the proteinase inhibitor system in preterm infants potentiates the inflammatory damage (Bose et al. 2008). The defense system is further compromised by under-expression of anti-inflammatory cytokines such as Clara cell protein 10 (Bose et al. 2008).

The initial injury, inflammation, and the exaggerated repair process can alter the production of growth factors responsible for lung development (Bose et al. 2008).

Over-expression of transforming growth factor β reduces production of pro-inflammatory cytokines but stimulates fibrosis (Bose et al. 2008).

The relationship between genetic factors and inflammation is bidirectional. A genetically determined inflammatory response affects the risk of developing BPD under similar external conditions (Bose et al. 2008). Antenatal inflammation may prime the pulmonary response to external stimulus (Van Marter 2005; Adams-Chapman 2012) and reprogram the responsiveness of the immune system (Kunzmann et al. 2013).

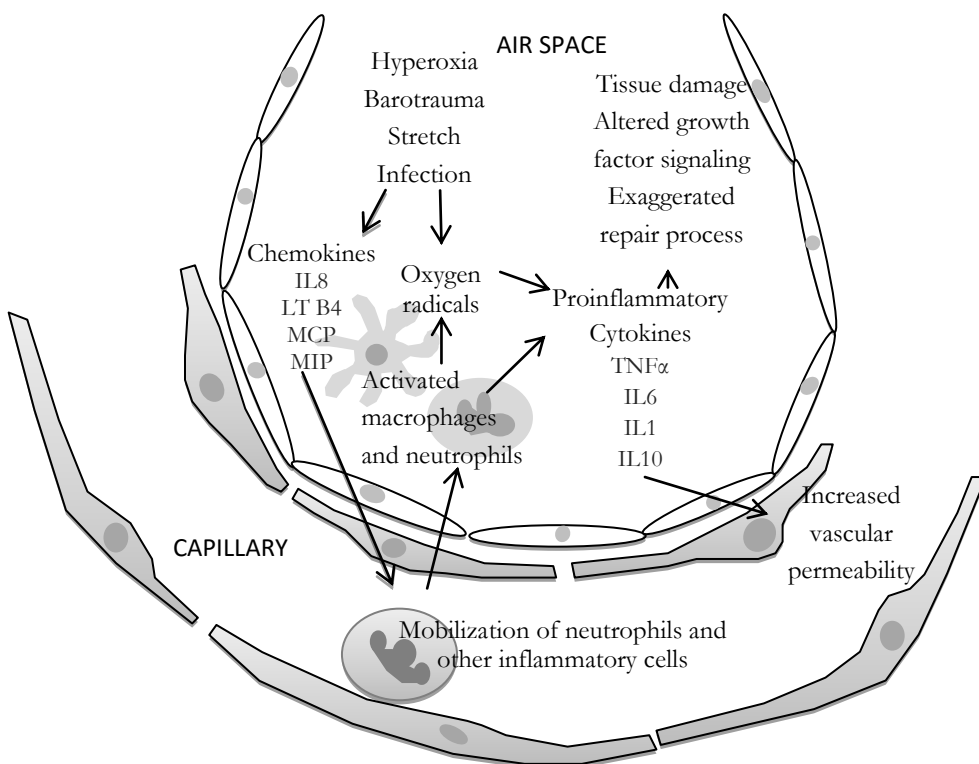


Figure 4. Inflammation in the pathogenesis of BPD.

LT B4, leukotriene B4; MCP, monocyte chemoattractant protein; MIP, monocyte inflammatory protein; TNF α , tumour necrosis factor α . Drawn by the author.

2.3.5 Infection

Both early and late neonatal sepsis are risk factors for BPD (Lahra et al. 2009; Eriksson et al. 2015). The risk varies by pathogen, candidemia entailing the greatest risk (Adams-Chapman 2012). *Ureaplasma* respiratory colonization increases the risk of BPD, but there is insufficient evidence to determine whether treatment of *Ureaplasma* has an influence on the incidence of BPD (Gancia et al. 2014).

The immature immune system of the rapidly growing lung predisposes infants to long-term changes in lung structure and function after lower respiratory tract infections (Harding et al. 2012).

2.3.6 Hyperoxia and oxidant injury

The transition from the hypoxic intrauterine environment to extra-uterine life, hyperoxia and inflammation induce oxidative stress (Perrone et al. 2012). Endothelial cells are sensitive of oxidant injury (Mourani et al. 2013; Poon et al. 2013). Hyperoxia impairs the vessel growth by inhibiting vascular endothelial growth factor (Saugstad 2005). Even transient exposure to non-physiological oxygen can result in lung injury (Adams-Chapman 2012) and arrest growth in the premature lung (McEvoy et al. 2014).

The optimal oxygen saturation target for extremely preterm infants is unknown (Manja et al. 2015). A recent meta-analysis showed significantly lower mortality with an oxygen saturation target 91-95% compared to 85-89%, but no difference in the rate of BPD (Manja et al. 2015).

Home oxygen therapy for oxygen-dependent BPD children is intended to prevent hypoxic pulmonary vasoconstriction and the development of cor pulmonale and to compensate for the increased work of breathing, allowing adequate growth (Bancalari et al. 2005). A home oxygen saturation target of 92 to 94 per cent has been recommended to avoid hypoxemia and to minimize the risk of additional lung injury due to hyperoxia (Bancalari et al. 2005).

2.3.7 Mechanical ventilation

Lung immaturity, surfactant deficiency and high chest wall compliance make preterm infants susceptible to ventilator-induced lung injury and limit the ability to repair the damage (Clark et al. 2001). Excessive pressure and tidal volume causes

regional over-distension of alveoli and airways and damages pulmonary capillaries (Clark et al. 2001). An injured alveolar-capillary barrier allows fluid and protein to leak into the alveoli and lung interstitium, and inflammatory mediators and pathogens from the alveolar space gain access to the general circulation (Clark et al. 2001). Infants with RDS are prone to atelectrauma (Clark et al. 2001). Mechanical ventilation increases antiangiogenic gene expression and reduces the expression of pro-angiogenic genes (Baker et al. 2014).

The duration of mechanical ventilation is shorter in the patient-triggered ventilation modes (Greenough et al. 2008). Volume-targeted ventilation reduces BPD compared to pressure-limited ventilation (Wheeler et al. 2010). Whether neurally adjusted ventilatory assist (Stein et al. 2016) or high frequency oscillatory ventilation (Cools et al. 2015) has an impact on the incidence of BPD remains obscure.

Avoidance of endotracheal ventilation prevents volutrauma and the subsequent activation of the inflammatory process (Fischer et al. 2013). A recent meta-analysis concludes that the strategies to avoid endotracheal mechanical ventilation in preterm infants reduce the combined outcome of death or BPD (Fischer et al. 2013). Early nasal continuous positive airway pressure has reduced the need for and duration of intubation (Gittermann et al. 1997). A heated, humidified high-flow nasal cannula seems to be as safe and as effective as nasal continuous positive airway pressure (Yoder et al. 2013).

2.3.8 Nutrition

Nutrition has a supportive role in normal lung development and maturation, lung repair and defence against infections (McEvoy et al. 2014). Undernutrition has been linked to an increased risk of BPD (Moya 2014). Infants with BPD are prone to malnutrition due to fluid restriction, feeding problems and the increased energy requirement entailed in the increased work of breathing (Allen et al. 2003). Low caloric intake potentiates oxygen-induced lung damage and affects lung growth (Bancalari et al. 2005). Malnutrition is associated with more severe respiratory outcomes (Baker et al. 2012).

The fluid tolerance of infants with BPD is poor and they tend to accumulate excessive fluid in their lungs (Bancalari et al. 2005). A recent meta-analysis of randomized controlled studies found a trend toward a reduced risk of BPD with restricted water intake (Bell et al. 2014). Despite active research, repeated

intramuscular doses of vitamin A are thus far the only nutritional supplement proved to reduce the risk of BPD (Darlow et al. 2011).

2.3.9 Patent ductus arteriosus

Hemodynamically significant ductus arteriosus increases the fluid load on the lungs (Kinsella et al. 2006) and is associated with an increased risk of BPD (Gonzalez et al. 1996; Schena et al. 2015). However, medical or surgical closure of a patent ductus arteriosus has not been shown to reduce the incidence of BPD (Jensen et al. 2014). Patent ductus arteriosus has been associated with a higher rate of recurrent sepsis (Chiang et al. 2012).

2.3.10 Adrenal insufficiency

The adrenal cortex does not normally produce cortisol before 30 weeks of gestation, but insults in the intrauterine environment may enhance cortisol production (Watterberg 2004). A low cortisol level in the first week of life has been associated with an increased risk of lung inflammation and BPD (Bancalari et al. 2003). Prophylactic hydrocortisone has improved survival without BPD in extremely low birth weight (ELBW) (birth weight < 1000g) infants exposed to histologic chorioamnionitis (Watterberg et al. 2004) and in extremely preterm infants born before 28 weeks of gestation (Baud et al. 2016).

2.3.11 Comorbidity

BPD has been associated with several prematurity-related conditions such as intraventricular hemorrhage (IVH), retinopathy and necrotizing enterocolitis (NEC)(Ganapathy et al. 2013). These multifactorial relationships are complex. Hypoxia and ischemia due to respiratory problems modulate the intestinal microvascular tone (Neu et al. 2011) and corticosteroid treatment increases the risk of gastrointestinal problems (Doyle et al. 2014a; Doyle et al. 2014b), whereas NEC may induce a systemic inflammatory process affecting lung growth (Neu et al. 2011).

2.4 Pharmacological treatments in BPD

The use of antenatal corticosteroids for fetal maturation for women at risk of premature delivery reduces the neonatal mortality and RDS in premature infants, but has no impact on the incidence of BPD (Crowley 1995). Postnatal corticosteroids facilitate extubation and lower the incidence of BPD and they were widely used to prevent or treat BPD until adverse short-term and neurodevelopmental effects were seen to outweigh early benefits (Committee on Fetus and Newborn 2002). Since then the introduction of new drugs to clinics has been more considered. Although many pharmacological treatments have been studied in order to reduce the incidence of BPD, thus far only caffeine has proved both safe and effective (Schmidt et al. 2008) (Table 1). It is likely that different causal factors dominate in different patients, leading to different phenotypes of BPD (McEvoy et al. 2014). Effects of interventions are likely to depend not only on timing, dose and duration of therapy but also on the underlying pathophysiology in individual patients (McEvoy et al. 2014). Detection of genetic markers predicting the risk of BPD may help to target therapies (Lavoie et al. 2008). Stem cells have reduced inflammation and lung injury and promoted lung growth in animal and cell models, but the safety and efficacy of this therapy in BPD remain to be seen (Fung et al. 2014).

2.5 Clinical outcomes of BPD

2.5.1 Mortality

Mortality due to BPD has decreased markedly from the 67% rate in 1967 (Northway et al. 1967). A large multicenter register study showed a reduction in death rates between 2000 and 2011 among infants born before 29 weeks of gestation (Patel et al. 2015). More than half of the decrease in overall mortality was due to pulmonary causes (Patel et al. 2015). BPD accounted for 15 deaths per 1000 live births (Patel et al. 2015).

Table 1. Pharmacological treatments in BPD.

	Potential mechanism	Current evidence in BPD
<u>Corticosteroids</u>		
Systemic	Anti-inflammatory	Restricted use in ventilator-dependency or in rapidly deteriorating respiratory status (Ghanta et al. 2013)
Inhaled		Reduces BPD, but may increase mortality (Bassler et al. 2015)
<u>Surfactant</u>	Improved lung function	Current data support early (Bahadue et al. 2012), selective (Rojas-Reyes et al. 2012) and less invasive administration (Gopel et al. 2015)
<u>Macrolide antibiotics</u>		
Azithromycin	Anti-inflammatory, antimicrobial	Reduces BPD, adverse effects warrant further studies (Nair et al. 2014)
Erythromycin	against <i>Ureaplasma</i>	No impact (Mabanta et al. 2003)
Clarithromycin		Decreased incidence of BPD in a small study of <i>Ureaplasma</i> colonized preterms (Ozdemir et al. 2011)
<u>Clara cell protein</u>	Anti-inflammatory	No impact (Levine et al. 2005)
<u>N acetyl-cysteine</u>	Anti-oxidant	No impact (Ahola et al. 2003)
<u>Cromolyn sodium</u>	Anti-inflammatory	No impact (Ng & Ohlsson 2012)
<u>CuZn superoxide dismutase</u>	Anti-oxidant	No impact on BPD incidence, may ameliorate later lung symptoms (Davis et al. 2003)
<u>Methylxanthines</u>		
Caffeine	Stimulate breathing, increase successful extubation, anti-inflammatory	Reduces BPD (Schmidt et al. 2008)
Pentoxifylline	Relieve bronchospasm	No impact (Schulzke et al. 2014)
<u>Bronchodilators</u>		Limited use in infants with evidence of bronchospasm and a clinical response (Tropea et al. 2012)
<u>Diuretics</u>		
Furosemide	Increases fluid reabsorption from the lung	Can be used sparingly to treat acute pulmonary edema (Tropea et al. 2012)
Thiazide diuretics		No clear evidence supporting use in BPD (Stewart et al. 2011)
<u>NO</u>	Reduces pulmonary vasoconstriction, anti-inflammatory	Restricted use when no other option exists (Donohue et al. 2011)
<u>Sildenafil</u>	Lowers pulmonary vasoconstriction	Restricted use in BPD and pulmonary hypertension when no other option exists (Bhatt-Mehta et al. 2014)
<u>Estrogen and progesterone</u>	Improve alveolarization	No impact (Trotter et al. 2007)
<u>Erythropoietin</u>	Enhance lung repair	Controversial, may increase the risk of ROP (Ohlsson et al. 2006)

2.5.2 Respiratory symptoms

The initial pulmonary symptoms of infants who develop BPD may vary from virtually none to severe RDS (Kinsella et al. 2006). Edema, atelectasis, small airway narrowing and interstitial fibrosis reduce lung compliance (Kinsella et al. 2006). Ventilation-perfusion mismatch is common (Kinsella et al. 2006). Tachypnea and shallow breathing increase dead space ventilation (Kinsella et al. 2006). Gas trapping with hyperinflation is seen during the later stages of BPD (Kinsella et al. 2006). The initial hospital stay is longer in BPD compared to non-BPD preterms (Lodha et al. 2014). Lung growth and repair usually improve pulmonary function and most surviving infants can eventually be weaned from oxygen (Kinsella et al. 2006). The incidence of ventilator dependency due to BPD has been reported to be 4.77 per 100 000 live births (Cristea et al. 2013).

Preterm birth and BPD in particular are associated with respiratory morbidity, and asthma-like symptoms such as wheezing and coughing are common (Filippone et al. 2012; El Mazloum et al. 2014; Vom Hove et al. 2014, Edwards et al. 2016). Re-hospitalization due to respiratory symptoms, particularly respiratory infections by respiratory syncytial virus, is common during early years (Kinsella et al. 2006). Respiratory symptoms are alleviated by school age and the relationship between clinical symptoms and lung function fades (Baraldi et al. 2007; Teig et al. 2012). However, respiratory symptoms and impaired exercise tolerance may persist to adolescence (Filippone et al. 2012) and adulthood (Gough et al. 2014).

2.5.3 Lung function

Compared to term birth, prematurity and VLBW are associated with poorer lung function at school age (Malmberg et al. 2000; Korhonen et al. 2004; Filippone et al. 2009; Teig et al. 2012; Ronkainen et al. 2015; Thunqvist et al. 2016), in adolescence (Doyle et al. 2006; Filippone et al. 2009; Filippone et al. 2012) and in adulthood (Gough et al. 2014). Bronchial obstruction and airway hyperresponsiveness are common (Baraldi et al. 2007), and restriction (Choukroun et al. 2013), hyperinflation and diffusion impairment have also been described (Cazzato et al. 2013). BPD survivors have presented with lower lung function than other preterms at school age (Filippone et al. 2009; Broström et al. 2010; Vom Hove et al. 2014), in adolescence (Doyle et al. 2006; Filippone et al. 2009; Filippone et al. 2012) and as young adults (Gough et al. 2014, Vollsaeter et al. 2015). Up to 64 % of school-aged BPD survivors have evinced abnormal lung function (Vom Hove et al. 2014).

Inferior lung function has been found in adolescent BPD survivors compared to asthmatic controls (Carraro et al. 2010). Children with mild BPD have shown similar lung function compared to preterm peers, supporting the assumption that lung immaturity rather than lung injury is the cause of the initial need for supplemental oxygen in some of these children (Ronkainen et al. 2015).

Normal alveolar size in hyperpolarized helium-3 magnetic resonance found in BPD survivors aged 10-14 years indicates that catch-up alveolarization may be excellent (Narayanan et al. 2013). While BPD survivors with mild airflow limitations in early childhood have later shown improvement in lung function (Filippone et al. 2009), the reduced lung function may persist through childhood (Thunqvist et al. 2015) and into adulthood (El Mazloum et al. 2014). Persisting lung function abnormalities may lead to earlier decline in lung function with aging and predispose BPD survivors to a chronic obstructive pulmonary disease-like phenotype (El Mazloum et al. 2014). Deterioration of lung function in BPD survivors has been described in childhood and in adolescence (Doyle et al. 2006; Filippone et al. 2009), suggesting that in some individuals the respiratory disease is progressive.

2.5.4 Lung imaging

Non-specific chest radiograph findings during follow-up tend to clear with age (Kinsella et al. 2006). Abnormal findings in high-resolution computed tomography of the thorax are common in children (Broström et al. 2010) and adult survivors of BPD (Wong, Murray et al. 2011) and in extremely preterm children and young adults even without a history of BPD (Aukland et al. 2006).

2.5.5 Asthma and inhaled drugs

In addition to common risk factors such as allergic rhinitis and parental asthma, preterm birth increases the risk of asthma (Edwards et al. 2016, Jaakkola et al. 2006). In a large Norwegian study, 53% of very preterm infants with asthma diagnosed by five years of age had a history of BPD (Skromme et al. 2015) and in one Canadian study, asthma was diagnosed twice as frequently in adults with a history of BPD compared to those with neonatal RDS (Landry et al. 2012). It has been suggested that atopy is not involved in prematurity-associated wheezing (Edwards et al. 2016).

Inhaled corticosteroids are occasionally used for symptomatic BPD children, although the role of corticosteroids in BPD after the initial hospitalization is unclear. Inhaled budesonide for four months did not improve the basic lung function but reduced bronchial lability in preterm schoolchildren with abnormal lung function (Pelkonen et al. 2001). A third of these children had a history of BPD (Pelkonen et al. 2001). A four-week course of inhaled beclomethasone has reduced respiratory symptoms and increased functional residual capacity in small children born at 25-34 weeks of gestation (Yuksel et al. 1992), but had no effect on airway hyperresponsiveness, respiratory symptoms or airway function in spirometry in school-aged VLBW survivors (Chan et al. 1993). A bronchodilation response to salbutamol in older BPD survivors remains controversial (Broström et al. 2010; Cazzato et al. 2013).

2.5.6 Growth

VLBW children have been found to weigh less (Vrtenich et al. 1995) and be shorter than term controls at school age (Korhonen et al. 2004) and as young adults (Kaseva et al. 2013). VLBW females have been shown to catch up in growth by 20 years of age, whereas VLBW males remain significantly shorter and lighter than controls (Hack et al. 2003). VLBW children have been found to have smaller head circumferences than term controls at school age (Peterson et al. 2006) and at 18 years of age (Evensen et al. 2009), and more microcephaly at school age (Peterson et al. 2006). Higher subscapular-to-triceps skinfold ratios suggesting less favorable fat distribution have been found in 18-year-old VLBW survivors born before the routine use of surfactant compared to term controls (Evensen et al. 2009).

Survivors of BPD have been found to have lower weight, height and head circumference at 18-22 months of age corrected for gestational age compared to ELBW controls (Natarajan et al. 2012). Although BPD impairs early growth (Natarajan et al. 2014), growth seems to accelerate with the alleviation of respiratory symptoms even in survivors of the old BPD (Markestad et al. 1981). BPD has been linked with small head circumference at school age (Chiriboga et al. 2003), but some studies have shown no such association (Vrtenich et al. 1995; Peterson et al. 2006).

The long-term growth of preterms treated with current neonatal practices remains to be seen, since the oldest are still teenagers.

2.5.7 Cardiovascular outcomes

Altered pulmonary vascular growth, structure and function in BPD reduce the alveolar-capillary surface area for gas exchange and may lead to unfavorable pulmonary blood flow distribution, prolonged need for oxygen therapy, pulmonary vascular disease and exercise intolerance (Mourani et al. 2015). Impaired gas exchange, narrowed vessel diameter and decreased vascular compliance can lead to pulmonary hypertension (Kinsella et al. 2006). Increased pulmonary vascular resistance can cause myocardial fibrosis, sarcomeric stiffening, hypertrophy and dysfunction of the right ventricle, under-filling of the left ventricle and altered ventricular interaction (Naeije et al. 2014).

Pulmonary hypertension during the first days of life is a risk factor for BPD in preterm infants (Mirza et al. 2014). The incidence of pulmonary hypertension is between 17 and 43% in infants with BPD (Collaco et al. 2012; Berkelhamer et al. 2013). Pulmonary hypertension contributes to BPD symptoms and adds to mortality (Collaco et al. 2012). In survivors, pulmonary hypertension usually resolves with catch-up lung growth and alleviation of BPD (Collaco et al. 2012). Increased pulmonary arterial pressure and echocardiographic signs of subclinical dysfunction of both cardiac ventricles have been found up to five years of age (Kazanci et al. 2011; Koroglu et al. 2013), but not at school age (Korhonen et al. 2005; Joshi et al. 2014). Young adults born at less than 36 weeks of gestation before the routine use of surfactant therapy have been found to have increased mass, smaller size and altered function of both ventricles of the heart in cardiac magnetic resonance imaging (MRI) (Lewandowski et al. 2013a; Lewandowski et al., 2013b).

BPD has been associated with left ventricular hypertrophy (Kinsella et al. 2006). Neonatal dexamethasone treatment of BPD may cause transient myocardial hypertrophy (Wong, Digby et al. 2011; Paech et al. 2014).

Barker introduced the hypothesis that events during early development have an impact on later disease risk, suggesting that poor nutrition in early life increases the risk of ischemic heart disease (Barker et al. 1986). Preterm birth has been associated with changes in both microvasculature and macrovasculature, for example reduced dermal vascularization and stiffness of arteries (Lewandowski et al. 2014). Systolic blood pressure in preterm VLBW children, adolescents and adults is commonly higher compared with non-VLBW peers (de Jong et al. 2012). Some studies have found higher diastolic blood pressure in BPD survivors

compared to preterm controls without BPD and term children at early school age (Mieskonen et al. 2003; Korhonen et al. 2005).

2.5.8 Neurodevelopmental outcomes

BPD is an additional risk factor for disrupted brain development in already vulnerable immature infants (Anderson et al. 2006). BPD has been associated with an increased risk of cerebral palsy, movement disorders and impaired motor skills (Anderson et al. 2006). The rate of visual and hearing disabilities may be increased by BPD (Anderson et al. 2006; Jensen et al. 2014). Compared with preterm peers, children with BPD have been found to evince lower cognitive performance and poorer verbal skills in early childhood (Natarajan et al. 2012) and at school-age (Gray et al. 2004). Impairments of spatial memory, executive skills and learning skills described in very preterm children may be even further compromised by BPD (Anderson et al. 2006). BPD survivors are more likely to need physiotherapy, speech-language therapy and educational assistance compared with preterm children without BPD (Anderson et al. 2006). Children discharged with supplemental oxygen seem to have similar neurodevelopmental outcomes compared with other BPD survivors at three years of age (Lodha et al. 2014).

2.5.9 Psychosocial outcomes

Preterm birth and BPD in particular are associated with behavioral problems and an increased risk of attention deficit hyperactivity disorder (Anderson et al. 2006). BPD children display more internalizing behavior and have more social and attention problems at school-age compared to preterm peers (Gray et al. 2008). BPD adults have more deficits in executive functioning compared with preterm controls (Gough et al. 2015).

Quality of life does not seem to be adversely affected in school-aged BPD children compared to preterm controls (Gray et al. 2008). In ELBW adults, BPD has been associated with a better self-reported mental health status (Natalucci et al. 2013).

Parents of oxygen-dependent BPD infants are prone to anxiety upon hospital discharge and the anxiety decreases as the child's condition improves (Zanardo et al. 2001). In a longitudinal study from birth to 14 years of age, mothers of BPD children experienced greater parenting stress in the first years of the child's life

compared to VLBW peers without BPD and term controls, but differences narrowed by school-age (Singer et al. 2010). Financial stress decreased over time but was greater for mothers of BPD children under conditions of low social support (Singer et al. 2010). The mothers of BPD children expressed the highest levels of parenting satisfaction at 14 years among VLBW survivors (Singer et al. 2010).

2.6 Inflammatory markers

2.6.1 Eosinophilic inflammation

Eosinophilic inflammation produces several measurable markers. Immunoglobulin E (IgE) activates mast cells and basophils and mediates allergic inflammation (Busse et al. 2001). Eosinophilic cationic protein (ECP), produced and stored in eosinophils, has various cytotoxic and non-toxic effects (Venge et al. 1999). NO is produced by epithelial cells, airway nerve cells, macrophages, neutrophils, mast cells and vascular cells (Ricciardolo 2003). Asthma, the most common lung disease in children, can be divided into eosinophilic and non-eosinophilic phenotypes according to the airway inflammation (Bel 2004).

2.6.2 Cytokines

Cytokines are responsible for intercellular interactions and communications (Zhang et al. 2007). Higher tracheal fluid levels of pro-inflammatory cytokines such as IL-6 and IL-8 have been found in infants who develop BPD (Hsiao et al. 2016, Su et al. 2005). Cytokine profiles may differ between infants developing classical and new BPD (D'Angio et al. 2016).

2.6.3 Neutrophilic inflammation

Increased neutrophilic infiltration occurs in many acute and chronic lung diseases (Stockley 2006). Leukotriene B₄, a marker of neutrophilic inflammation produced by activated neutrophils, has been associated with lung inflammation and pulmonary hypertension in an experimental BPD model (Ee et al. 2016).

2.6.4 Oxidative stress

Oxidative stress reflects the imbalance between reactive oxygen species and antioxidant defences. Isoprostanes, produced by free radical-induced peroxidation of membrane lipids, are considered reliable markers of oxidative stress (Janssen 2001). Elevated plasma 8-isoprostane levels have been found in ELBW neonates who later develop BPD (Ahola et al. 2004).

2.6.5 Adipokines

Adipokines are bioactive peptides secreted mainly by adipocytes (Ronti et al. 2006). They regulate metabolic homeostasis and affect inflammatory processes (Ronti et al. 2006) (Table 2). Adipokines contribute to chronic inflammatory diseases (Ali Assad et al. 2012). Lower resistin levels have been found in atopic asthmatic schoolchildren compared to non-atopic asthmatic and healthy controls (Kim et al. 2008). Serum leptin and adiponectin levels have correlated with lung function in spirometry (Kim et al. 2008) and with exercise-induced bronchoconstriction in

Table 2. Inflammatory and metabolic effects of adipokines.

	<u>Inflammatory effect</u>	<u>Metabolic effect</u>
Leptin	Proinflammatory	Controls satiety and food intake Stimulates fatty acid oxidation Increases insulin sensitivity Inhibits lipogenesis and stimulates lipolysis Promotes increased energy expenditure Proatherogenic
Adiponectin	Both pro- and anti-inflammatory	Increases insulin sensitivity Stimulates fatty acid oxidation Antiatherogenic
Resistin	Proinflammatory	Reduces insulin sensitivity Promotes endothelial dysfunction
Adipsin	Proinflammatory	Stimulates triglyceride storage Inhibits lipolysis

school-aged asthmatic children (Baek et al. 2011), and leptin has been associated with greater asthma prevalence and severity in children (Ali Assad et al. 2012). Birth weight and early growth pattern have been linked to adipokine status in later life (Flexeder et al. 2014).

2.6.6 Inflammatory markers in BPD after infancy

It is not clear whether the long-term respiratory consequences of prematurity and BPD are associated with chronic inflammation or result solely from structural remodeling. It has been speculated that perinatal lung injury may serve as a trigger for chronic inflammation (Teig et al. 2012).

Differences in metabolomics analysis of exhaled breath condensate in adolescent BPD survivors compared to healthy term controls indicate metabolic abnormalities in the lung and suggest differences in inflammatory and anti-inflammatory processes (Carraro et al. 2015). Lower percentages of CD4⁺ T lymphocytes and a lower CD4:CD8 ratio in peripheral blood has been found in school-aged children born VLBW or under 30 weeks of gestation compared to term controls (Pelkonen et al. 1999).

School-aged (Baraldi et al. 2005) and adolescent BPD survivors have not evinced higher fractional exhaled NO concentrations compared with preterm and term controls (Carraro et al. 2010; Filippone et al. 2012; Malmberg et al. 2013). Airway hyperresponsiveness has been associated with bronchial NO flux only in atopic VLBW children at school-age (Malmberg et al. 2013). Higher blood ECP concentrations have been found in school-aged children born VLBW or under 30 weeks of gestation compared to term children (Pelkonen et al. 1999), whereas no difference have been found between preterm schoolchildren with and without BPD (Broström et al. 2010). Exhaled air temperature as a potential marker of inflammation has been significantly elevated in clinically stable asthmatic adolescents but not in survivors of BPD (Carraro et al. 2010).

Higher urinary leukotriene E4 concentrations (Halvorsen et al. 2005) and higher sputum neutrophil proportions and IL-8/protein values reflecting lower airway inflammation have been found in preterm compared to term children at school-age (Teig et al. 2012). Blood levels of myeloperoxidase secreted by activated neutrophils have not been elevated in school-aged children born VLBW or under 30 weeks of gestation (Pelkonen et al. 1999).

Preterm adolescents have had higher 8-isoprostane levels in exhaled breath condensate compared to term controls regardless of history of BPD (Filippone et al. 2012).

2.7 Pulmonary function tests

Two of the most common pulmonary function tests used in children are impulse oscillometry and spirometry. Impulse oscillometry allows evaluation of lung function during spontaneous breathing with minimal co-operation (Smith et al. 2005). It is sensitive to obstruction of the small airways. Impedance, the sum of the forces opposing the pressure impulses, can be divided into resistance and reactance (Smith et al. 2005). Resistance rises in airway obstruction (Smith et al. 2005). Reactance includes the inertive forces of the moving air and the elastic properties of the lungs and decreases in restriction, hyperinflation and obstruction (Smith et al. 2005). Resonance frequency, the frequency where the inertive and elastic forces equal, increases in obstructive and restrictive conditions (Smith et al. 2005).

In spirometry, forced maximal exhalation is performed after a maximal inspiration (Miller et al. 2005). Due to forced exhalation, spirometry is more sensitive to minor changes compared to impulse oscillometry, but is also more prone to the effects of malacia of the large airways. Forced expired volume in one second is the most frequently used value in spirometry, but other variables such as vital capacity are also used (Miller et al. 2005). Spirometry has been the most common method of measuring pulmonary function in BPD.

3 Aims of the study

The aim of this study was to assess the outcomes of severe radiographic BPD (radBPD) in school age with special focus on two aspects so far but little investigated: the role of inflammation and cardiac function. The specific aims were as follows:

1. To establish whether school-aged children with radBPD evince higher inflammatory activity in plasma and exhaled air than VLBW survivors without BPD and term controls (I).
2. To assess whether the lung function in school-aged VLBW children with radBPD is poorer than in VLBW children without BPD and in term controls and whether inflammatory marker levels are associated with poor lung function in VLBW children (II).
3. To establish whether the growth and nutrition of children with radBPD at school-age differ from VLBW children without BPD and term controls and whether the adipokines are associated with growth and nutrition in VLBW children at school age (III).
4. To ascertain whether abnormalities in cardiac function can be detected in school-aged radBPD children with modern echocardiographic methods and whether cardiac function is associated with lung function in school-aged VLBW children (IV).

4 Patients and methods

4.1 Patients (Studies I-IV)

This study was a single-center case-control cohort study. The hospital records of VLBW infants treated in Tampere University Hospital, Finland between January 1st 1995 and April 13th 2003 were abstracted for BPD diagnosis. The timeline was chosen in order to ensure a sufficiently large cohort. Infants with severe congenital anomalies were excluded. We used radiographic criteria to identify the most severe BPD cases. Children with severe, Northway grade III-IV cystic radBPD confirmed by a pediatric radiologist were contacted. Twenty-one out of 31 eligible children agreed to participate in the study. There was no significant difference in birth weight or gestational age between the ten non-participants and the 21 participating children.

VLBW control group was formed by nineteen age-matched children without radBPD findings with birth weight matching as well as possible. Nineteen term-born age- and sex-matched non-asthmatic children of the families of the staff working in Tampere University Hospital participated as term controls.

Three radBPD and two VLBW controls refused to participate in cardiac examinations. One term control had a suboptimal echocardiographic acoustic window. Thus, 18 radBPD children, 17 VLBW controls and 18 term controls participated in the cardiac studies.

The examinations were carried out in 2009-2010. The ages of the children varied between 6-14 years at the time of the examinations.

4.2 Methods

4.2.1 Neonatal data (Studies I-IV)

Perinatal and neonatal data were gathered from the hospital records of Tampere University Hospital and from the central hospitals responsible for the children after the initial treatment.

Chorioamnionitis was an obstetric clinical diagnosis including elevated C-reactive protein and maternal fever. Prenatal corticosteroid therapy included a two-day course of betamethasone or dexamethasone, repeated in selected cases.

Birth weight, length and head circumference were compared to old population-based references in study I (Pihkala et al. 1989) and to new population-based references in the other studies (Sankilampi et al. 2013). Birth weight for gestational age $<-2SD$ was considered small for gestational age (Pihkala et al. 1989; Sankilampi et al. 2013). Head circumference $<-2SD$ was considered to constitute microcephaly and $>+2SD$ macrocephaly (Sankilampi et al. 2013).

Blood culture positive neonatal sepsis and Bell stage II or III (Bell et al. 1978) NEC were recorded. A significant patent ductus arteriosus was recorded when treated with indomethacin or surgical ligation. All VLBW infants underwent serial cranial ultrasound screening during their primary hospitalization. IVH seen in cranial ultrasound were graded by Papile classification (Papile et al. 1978). Surfactant for RDS and intravenous corticosteroids, mainly dexamethasone, in weaning from the ventilator were administered based on the clinical decision of the neonatologist. Dexamethasone was administered in doses descending from 0.2-0.5 mg/kg/day. The oxygen saturation target was normally 90-94% and 95-97% in pulmonary hypertension.

4.2.2 Clinical examination (Studies I-IV)

The physical examination was performed by a pediatrician. Pubertal status was assessed by Tanner stages (Tanner et al. 1976). Height, weight and head circumference were measured and body mass index standard deviation scores (BMI-SDS) calculated and compared to national age- and sex-specific references (Sorva et al. 1984; Saari et al. 2011). Overweight was defined as a BMI-SDS of more than 1.16 in girls and more than 0.78 in boys and underweight as less than -

1.65 in girls and less than -1.83 in boys (Saari et al. 2011). Height was also compared to that predicted based on the height of the parents. Body surface area was calculated. Thigh, hip, waist and middle upper arm circumferences were measured with a plastic tape measure and an average of two measurements was recorded. Biceps, triceps, subscapular and suprailiacal skinfold thicknesses were measured with a Harpenden skinfold caliper according to the guidelines of the manufacturer (Baty International, West Sussex, UK), and an average of three measurements was recorded. Reported parental heights and weights were recorded and body mass index was calculated. Parental overweight was defined as body mass index $>25\text{kg}/\text{m}^2$.

Blood pressure was measured non-invasively (Dinamap) from the right arm in supine position. Values over the 90th but under the 95th percentile of the age- and sex-specific references were considered pre-hypertensive and $\geq 95^{\text{th}}$ percentile hypertensive (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents 2004). Oxygen saturations by pulse oximetry and 12-lead electrocardiograms were obtained from the VLBW children by a pediatric nurse.

4.2.3 Parental questionnaire (Studies I-III)

The parents of participants completed a modified International Study of Asthma and Allergies in Childhood questionnaire (Csonka et al. 2000) on the family background and medical history of the child. The highest socio-economic status of the family was assessed according to parental occupation (Central Statistical Office of Finland 1989).

4.2.4 Food records (Study III)

The families completed a food record of everything the children ate and drank over three consecutive days. A trained nutritionist interviewed the parents and the participants to ensure that food records were properly filled. Nutrient intakes were calculated with a dietary analysis program (Diet32, Aivo AB Sverige, Solna, Sweden) based on the national database (Finnish Food Composition Database, Helsinki, Finland) and compared with national age-specific recommended daily doses and the upper level of tolerable intake for adults (Fogelholm et al. 2014). Energy intake variation up to 200 kcal below or above the age- and sex-adjusted

estimated energy requirement was considered normal in order to accommodate for different levels of physical activity.

4.2.5 Laboratory tests (Studies I-IV)

4.2.5.1 Blood samples (Studies I-IV)

Plasma samples were stored at -70°C until analyzed. Plasma IL-6, IL-8, ECP, adiponectin, adipsin, resistin and leptin concentrations were determined by enzyme immunoassay using commercial reagents (IL-6: PeliPair ELISA, Sanquin, Amsterdam, the Netherlands; IL-8: Opt EIA BD Biosciences, Erembodegem, Belgium, ECP: ECP ELISA kit, MBL International, Woburn, MA, USA; adiponectin, adipsin, and leptin: DuoSet ELISA, R&D Systems Europe Ltd, Abingdon, U.K).

Serum total IgE was determined nephelometrically using the Siemens N Latex IgE mono assay (Marburg, Germany) up to November 2009, and thereafter by an electrochemiluminescence immunoassay ECLIA IgE II test (Roche Diagnostics, Mannheim, Germany) using a Roche Cobas e601 analyzer. IgE exceeding 90 IU/L in children 6-9 years of age and 200 IU/L in children 10-15 years of age were considered elevated according to the reference values of the hospital laboratory.

Plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) was determined by an electrochemiluminescence immunoassay method using Cobas 6000 immunoanalyzer, an e601 module and the proBNP II kits (Roche Diagnostics, Mannheim, Germany). Concentrations <160 pg/ml were considered normal according to the age-related reference values (Nir et al. 2009).

4.2.5.2 Exhaled breath condensate (Studies I, II)

Exhaled breath condensate was collected during 15 minutes of tidal breathing with an Ecoscreen condenser (Jaeger, Würzburg, Germany) while wearing a nose clip. The samples were stored at -70°C until assayed. Leukotriene B₄ and 8-isoprostane concentrations in the condensate were measured by EIA (Cayman Chemical Company, Ann Arbor, MI, USA).

4.2.5.3 Exhaled NO measurement (Studies I, II)

NO concentrations in exhaled air were measured with a Sievers NOA 280 analyzer (Sievers Instruments, Boulder, CO, USA) at four exhalation rates (50, 100, 200 and 300ml/s). NO output was calculated and plotted against the exhalation flow rate. A regression line was set to correlate these variates. The alveolar NO concentration reflecting NO dynamics in the peripheral lung is the slope and the bronchial NO concentration reflecting NO dynamics in central conducting airways is the intercept of this regression line (Tsoukias et al. 1998). In addition, fractional exhaled NO at 50 ml/s reflecting mainly the NO production of large bronchi was reported.

4.2.6 Impulse oscillometry (Studies II, IV)

Impulse oscillometry (Master Screen IOS; Jaeger, Höchberg, Germany) was applied before and 15 minutes after inhalation of 0.3 mg salbutamol (Ventoline®, GlaxoSmithKline, UK) in sitting position. A nose clip was used and the cheeks were supported by the hands of the investigator to minimize the pressure lost due to upper airway shunt. Impulse oscillometry was repeated until three acceptable curves free of artifacts were obtained and the curve with the highest resistance value at 5 Hz and 20 Hz levels was selected.

Pre- and post-bronchodilator impedance, resistance, reactance and resonance frequency were determined. Lung function was considered abnormal if baseline resistance or reactance at 5Hz was lower than $-1.96SD$ or higher than $+1.96SD$ or if the post-salbutamol decrease was $>36\%$ in impedance, $>37\%$ in resistance or $>45\%$ in resonance frequency (Dencker et al. 2006; Malmberg et al. 2002, Malmberg et al. 2011).

The children had been free of any respiratory infection for at least six weeks before the impulse oscillometry.

4.2.7 Echocardiographic examinations (Study IV)

Transthoracic echocardiographic examinations were made by a pediatric cardiologist using the iE33 ultrasound machine (Royal Philips Electronics, Philips Healthcare, Bothell, Washington) and S8-3, S5-1, X5-1 or X7-2 transducer. An electrocardiogram was recorded simultaneously. The children lay supine or in left

lateral semirecumbent position. An average of the measurements of three cardiac cycles was recorded.

4.2.7.1 Conventional echocardiography (Study IV)

Pulsed wave Doppler was used to assess main pulmonary artery flow from the short axis view, the flow in the ascending aorta from the apical long axis and suprasternal views, and the peak velocities of the early (E) and atrial (A) filling of the mitral and tricuspid inflow between the tips of the valve leaflets in the apical four-chamber view.

M-mode analysis was made from the parasternal long axis view. The end-diastolic and end-systolic dimensions of the left ventricle, intraventricular septum and posterior wall were measured according to the recommendations of the American Society of Echocardiography (Lang et al. 2005) and compared to reference values (Colan 2009; Sluysmans et al. 2009).

4.2.7.2 Tissue Doppler imaging (Study IV)

Tissue Doppler imaging was undertaken from the apical four-chamber view with the highest possible frame rate. Peak systolic (S'), early diastolic (E') and late diastolic (A') myocardial velocities were measured along the annulus of the right ventricular free wall, intraventricular septum and left ventricle free wall.

4.2.7.3 Tissue motion annular displacement analysis (Study IV)

Tissue motion annular displacement analysis was made from two-dimensional images of both left and right ventricles obtained from the apical four-chamber view. The data were analyzed off-line using Q-lab software (Philips Q-lab, version 10.0; 3DQA; Philips Healthcare, Bothell, Washington, USA). The lateral and septal edges of the mitral and tricuspid valve leaflets and the apex of the corresponding ventricle were marked in a diastolic frame and tracked automatically throughout the cardiac cycle. The software calculated the longitudinal displacement of the marked points and their imaginary midpoint towards the apex of the ventricle. Mitral (MAD) and tricuspid (TAD) annular displacement were expressed in millimeters. The total midpoint displacement (MAD-mid and TAD-mid) was

expressed in millimeters and, in order to normalize for the ventricular length, as a ratio of the longitudinal ventricle length at end-diastole.

4.2.7.4 Real-time three-dimensional echocardiography (Study IV)

Real-time three-dimensional echocardiography (3DE) was applied from the apical four-chamber view. A pediatric cardiologist carried out the off-line analysis with the Q-lab software. The edges of the mitral valve in the four-chamber and the two-chamber views and the apex of the left ventricle in either view were set in end-diastole (the frame before full closure of the mitral valve) and end-systole (the frame with the smallest left ventricle cavity with the mitral valve closed). The endocardial border was traced by semiautomatic detection and manual corrections were made when necessary. The software created a three-dimensional cast of the left ventricle cavity for the entire cardiac cycle.

The left ventricle was divided into 16 standard myocardial segments with the apex excluded, as defined by the American Society of Echocardiography (Cerqueira et al. 2002). The dyssynchrony indexes calculated were standard deviation of the time to reach the minimum regional volume for each segment (Tmsv 16-SD) and the difference between the segments reaching the minimum regional volume earliest and latest (Tmsv 16-Dif) presented as a percentage of the cardiac cycle. Similar calculations were made for the 12 (six basal and six middle) segments.

4.2.8 Statistical methods (Studies I-IV)

Statistical analyses were made with SPSS version 18.0 (SPSS Inc. Chicago, Illinois, USA). The Shapiro-Wilk test for normality was used to assess the distribution of continuous variables. Differences between the groups were tested by independent-samples t-test or one-way analysis of variance for normally distributed and with Mann-Whitney U or Kruskal-Wallis test for non-normally distributed continuous variables. In study IV, non-parametrical analyses were used for all continuous variables. Chi-square or Fisher's exact tests were used for categorized variables.

Correlations were assessed with Pearson's correlation test (r) for normally distributed and Spearman's correlation test (ρ) for non-normally distributed variables. A correlation coefficient of -0.3 - 0.3 suggested low correlation, -0.6 - -0.3 or 0.3 - 0.6 modest correlation and <-0.6 or >0.6 strong correlation.

Logistic regression analysis (Enter) was used to analyze risk factors and confounding variables in VLBW children. After univariate analysis, selected variables were entered simultaneously to the multivariate model. The results were given as odds ratios (OR) and 95% confidence intervals (CI). P-value <0.05 was considered statistically significant.

4.3 Ethics (Studies I-IV)

The study complied with the 1964 declaration of Helsinki and its later amendments. The study protocol was approved by the institutional review board of Tampere University Hospital, the former Ethics Committee of the hospital district. Parents of the children gave their written informed consent. The participating children and their care-givers were fully informed before and during the study.

5 Results

5.1 Characteristics of the patients (Studies I-IV)

5.1.1 Neonatal characteristics (Studies I-IV)

The prenatal and neonatal characteristics of the VLBW children are presented in Table 3. Prenatal inflammatory history in terms of antibiotic and steroid treatments and chorioamnionitis was similar in radBPD children and VLBW controls (Table 3). The radBPD children had shorter gestational age and smaller birth weight than VLBW controls (Table 3). One radBPD child was born small for gestational age. The initial weight gain was poorer in radBPD children compared with VLBW controls (Table 3). At term age, two radBPD children and one VLBW control weighed under -2 SD. The mean gestational age of term control children was 39.5 weeks (SD 1.7) and the mean birth weight 3.499 g (SD 610). None of the term controls needed neonatal intensive care.

RadBPD children had longer ventilator and oxygen therapies and needed postnatal corticosteroids more often than VLBW controls (Table 3). Four radBPD children had been weaned from oxygen supplementation before 36 weeks of gestational age, representing mild BPD, and two children in the VLBW control group needed supplemental oxygen until gestational age 36+2 and 36+5 weeks, representing moderate BPD according to the current classification. None was discharged with home oxygen.

Neonatal sepsis and NEC were more common in radBPD children compared with VLBW controls (Table 3).

5.1.2 Characteristics at school age (Studies I-IV)

The median (range) age during the study period was 11.8 (6.8 - 14.2) years in radBPD children, 11.0 (6.3-13.5) years in VLBW controls and 11.3 (6.4 - 14.1)

years in term children ($p = 0.976$). The number of males was 14 in radBPD, 11 in VLBW controls and 13 in term children ($p = 0.767$).

Table 3. Prenatal and neonatal characteristics of the VLBW infants with and without radBPD.

	radBPD children (n=21)	VLBW controls (n=19)	P
Multiple pregnancy, n (%)	2 (10)	7 (37)	0.060
Chorioamnionitis, n (%)	11 (52)	13 (68)	0.514
Prenatal corticosteroid, n (%)	17 (81)	18 (95)	1.000
Prenatal antibiotics, n (%)	15 (71)	14 (74)	1.000
Cesarian section, n (%)	11 (52)	10 (53)	0.618
Smoking during pregnancy, n (%)	9 (43)	3 (16)	0.062
Male sex, n (%)	14 (67%)	11 (58%)	0.567
Gestational age (weeks), mean (SD)	26.6 (1.6)	28.9 (1.8)	<0.001
Birth weight			
g, mean (SD)	919 (252)	1.198 (222)	<0.001
SD, median (range)	0.7 (-2.1-1.8)	0.2 (-1.9-2.1)	0.655
Birth length			
cm, mean (SD)	34.9 (3.4)	38.2 (2.8)	0.002
SD, mean (SD)	0.3 (1.2)	0.4 (1.1)	0.732
Head circumference at birth			
cm, mean (SD)	23.7 (2.4)	26.8 (1.8)	0.001
SD, median (range)	0.2 (-1.8-1.1)	0.5 (-0.5-1.2)	0.224
Weight gain in hospital* (g/day), mean (SD)	19.1 (4.8)	23.1 (4.6)	0.031
Surfactant therapy, n (%)	14 (67)	9 (47)	<0.001
Ventilator therapy, n (%)	19 (90)	11 (58)	0.017
Duration of ventilator therapy (days), MD (range)	24 (0-271)	1 (0-5)	<0.001
Duration of oxygen therapy (days), MD (range)	73 (50-730)	11 (1-74)	<0.001
Postnatal systemic corticosteroids, n (%)	14 (67)	2 (11)	<0.001
Neonatal sepsis, n (%)	8 (38)	1 (5)	0.021
Fungal sepsis, n (%)	2 (10)	0 (0)	0.488
Necrotizing enterocolitis, n (%)	8 (38)	1 (5)	0.020
Patent ductus arteriosus treated, n (%)	6 (29)	5 (26)	0.873
IVH gradus \geq III or PVL, n (%)	5 (24)	0 (0)	0.047

PVL, periventricular leukomalacia

*n=18 in radBPD children and n=12 in VLBW controls

RadBPD children were most and term children least exposed to tobacco smoke. Hairy pets had been more common in the families of the VLBW (75%) than term children (37%). Atopy or asthma in the family was equally common in every group. High socio-economic status was seen in 33% of the radBPD children, 53% of the VLBW controls and 89% of the term children. Age, height and body mass index of the parents did not differ between the groups. A majority (80%) of the children had at least one overweight parent.

5.2 Growth at school age (Study III)

The anthropometric parameters of the school-aged children are presented in Table 4. One of the overweight children was obese. One radBPD and one VLBW control child were severely underweight. One child in each group had macrocephaly. The pubertal stage did not differ between the groups. Children with radBPD had smaller age-adjusted head circumferences and had microcephaly more often compared with VLBW and term controls; otherwise the anthropometric parameters did not differ between the groups (Table 4). BMI-SDS and length for age were similar in VLBW children with and without microcephaly.

5.2.1 Neonatal history and growth at school age in VLBW children (Study III)

Eight children with microcephaly at school age showed poorer weight gain during the initial hospitalization [median (range) 14.8 g/day (14.3 - 20.4) vs. 22.8 g/day (7.0 - 28.5), $p = 0.011$] compared with the other VLBW children. The head circumference of the school-aged NEC survivors was smaller compared with the non-NEC children [mean (SD) -1.6 (2.8) vs. -0.4 (1.3), $p = 0.033$]; the other anthropometric parameters did not differ. One of the five children with severe, grade \geq III IVH or periventricular leukomalacia and half of the children with cerebral palsy had microcephaly at school age. Thirteen VLBW children with long-term illness had smaller waist-to-hip ratios compared with the healthy children [median (range) 0.88 (0.72 -1.16) vs 0.92 (0.86 -1.07), $p = 0.031$]. The other anthropometric parameters did not differ.

RadBPD (OR 9.69, 95% CI 1.06 - 88.65, $p = 0.044$) and neonatal sepsis (OR 11.25, 95% CI 1.91 - 66.39, $p = 0.008$) were associated with an increased risk of microcephaly in univariate logistic regression analysis. In multivariate logistic

Table 4. Growth statistics of the school-aged VLBW children with and without radBPD and term controls.

	radBPD children (n=21)	VLBW controls (n=19)	Term controls (n=19)	p
Age (years), median (range)	11.8 (6.8-14.2)	11.0 (6.3-13.5)	11.3 (6.4-14.1)	0.976
Length (cm), mean (SD)	141.7 (19.7)	143.9 (14.4)	144.8 (18.9)	0.857
Length for age (SD), mean (SD)	-0.76 (1.74)	-0.19 (1.71)	-0.21 (1.49)	0.496
Deviation from predicted length (SD), mean (SD)	-0.40 (1.54)	0.06 (0.76)	0.38 (1.20)	0.395
Weight (kg), mean (SD)	37.3 (15.8)	36.8 (11.9)	38.2 (13.5)	0.949
BMI-SDS, median (range)	-0.69 (-2.72-2.48)	-0.48 (-2.36-1.52)	-0.34 (-1.59-1.18)	0.594
Overweight, n (%)	3 (14)	3 (16)	2 (11)	0.887
Underweight, n (%)	2 (10)	2 (11)	0 (0)	0.358
HC for age (SD), mean (SD)	-1.3 (2.1)	-0.1 (1.3)	0.5* (1.1)	0.014
Microcephaly, n (%)	7 (33)	1 (5)	0 (0)	0.001
MUAC (cm), mean (SD)	20 (4)	21 (3,5)	21 (3,1)	0.976
Waist circumference (cm), mean (SD)	61.5 (14.4)	60.9 (10.1)	59.7 (8.3)	0.887
Hip circumference (cm), mean (SD)	68.0 (13.7)	70.0 (10.3)	70.0 (9.9)	0.825
Waist-to-hip ratio, mean (SD)	0.91 (0.10)	0.87 (0.08)	0.86 (0.07)	0.217
Thigh circumference (cm), mean (SD)	36.4 (6.9)	37.3 (5.3)	37.8 (5.0)	0.773
Biceps SFT (mm), median (range)	5.0 (2.6-21.8)	6.2 (2.6-15.0)	5.6 (3.0-14.3)	0.686
Triceps SFT (mm), mean (SD)	10.2 (4.3)	10.6 (4.1)	10.0 (3.4)	0.907
Subscapular SFT (mm), median (range)	6.0 (3.6-27.9)	5.8 (4.4-16.6)	6.3 (3.9-16.4)	0.945
Suprailiacal SFT (mm), median (range)	7.0 (3.5-39.3)	8.7 (3.4-37.4)	5.9 (3.7-21.3)	0.726

HC, head circumference; MUAC, middle-upper-arm circumference; SFT, skinfold thickness

*n=17

regression analysis adjusted for birth weight, radBPD, neonatal sepsis, grade \geq III IVH or periventricular leukomalacia and NEC, neonatal sepsis seemed to predict length for age in the lowest quartile at school-age (OR 11.57, 95% CI 1.01 -132.40, $p = 0.049$); no other associations were found. Neonatal dexamethasone treatment was associated with microcephaly at school-age in the univariate analysis (OR 8.63, 95%CI 1.44 - 51.72, $p = 0.018$), but the association faded when added to the multivariate analysis.

5.3 Nutrition (Study III)

One radBPD, three VLBW controls and one term child did not return a food record. There were no differences between the groups in the reported intake of energy, water, protein, fat, carbohydrates or micronutrients (see Study III, Table 3, Appendix). Recommendations regarding daily nutrient intake were poorly met in all groups (see study III, Table 4, Appendix). Most children reported excessive intake of protein and saturated fatty acids and lower than recommended fiber intake was common.

5.3.1 Nutrition and growth in school age in VLBW children (Study III)

In a multivariate logistic regression analysis adjusted for intake of energy, fat, protein and carbohydrates, short stature was associated negatively with intake of protein (OR 0.25, 95% CI 0.07 - 0.93, $p = 0.038$), fat (OR 0.66, 95% CI 0.00 - 0.96, $p = 0.046$) and carbohydrates (OR 0.27, 95% CI 0.08 - 0.98, $p = 0.046$) and positively with energy intake (OR 1.38, 95% CI 1.03 - 1.58, $p = 0.026$). No other associations between unfavorable growth and nutrition intake were found in logistic regression analysis. Adjusting for socioeconomic status did not significantly alter the results.

5.4 Pulmonary outcomes (Study II)

5.4.1 Medical history (Studies I-III)

Table 5 shows the respiratory symptoms of the children. The radBPD children needed inhaled medication and were hospitalized due to respiratory symptoms more often than the control children during the early years of life, but not during the past year (Table 5). Two radBPD children had used inhaled steroid for a few weeks during the past year. Regular physical exercise was reported by 71% of the radBPD, 74% of the VLBW control and 84% of the term control children. None reported respiratory symptoms during exercise. Atopic eczema and allergic symptoms were equally common in all groups.

One radBPD child had short bowel syndrome. Three radBPD children and one VLBW control child had cerebral palsy.

5.4.2 Impulse oscillometry (Study II)

Two radBPD children were not able to perform the impulse oscillometry. The results are presented in Table 6. The baseline resistance was highest in radBPD children, next in VLBW controls and lowest in term control children (Table 6). Bronchodilator responses were most marked in VLBW controls, next in radBPD children and lowest in term children (Table 6). The results remained similar when the current definition of BPD was used and when the seven children with physician-diagnosed asthma were excluded.

Two radBPD children and three VLBW controls (with no BPD according to the current criteria) evinced abnormal lung function in impulse oscillometry. Baseline bronchial obstruction in terms of elevated resistance was seen in one radBPD child and two VLBW controls. A significant bronchodilation response to salbutamol was presented by one radBPD child and three VLBW control children, including the two with baseline obstruction. Thus, only one radBPD child presented with irreversible obstruction.

Seven VLBW children with physician-diagnosed asthma showed higher resistance at 5 Hz compared to 31 non-asthmatic VLBW children [median (range) 99% (63 - 144) vs. 81% (57 - 132), $p = 0.047$]. Other lung function parameters did not differ between the asthmatics and non-asthmatics. Lung function in atopic and

Table 5. Respiratory symptoms.

	radBPD children (n=21)	VLBW controls (n=19)	Term controls (n=19)	P
Asthma diagnosed by a physician, n (%)	5 (24)	2 (11)	0 (0)	0.058
Inhaled corticosteroid				
After hospital discharge, n (%)	11 (52)	6 (32)	0 (0)	0.001
In the past 12 months, n (%)	2 (10)	0 (0)	0 (0)	0.323
Inhaled β -agonist				
After hospital discharge, n (%)	16 (76)	8 (42)	0 (0)	<0.001
In the past 12 months, n (%)	1 (5)	0 (0)	0 (0)	1.000
Hospitalizations due to respiratory symptoms				
After hospital discharge, n (%)	8 (38)	4 (21)	1 (5)	0.030
In the past 12 months, n (%)	0 (0)	0 (0)	0 (0)	1.000
Pneumonia in the past 12 months, n (%)	1 (5)	0 (0)	1 (5)	0.609
Respiratory symptoms in the past 12 months				
Wheezing, n (%)	2 (10)	0 (0)	1 (5)	0.766
Shortness of breath, n (%)	3 (14)	3 (16)	0 (0)	0.202
Cough at night without infection, n (%)	5 (24)	3 (16)	1 (5)	0.293

non-atopic VLBW children did not differ.

The lung function of eight overweight children was comparable with that of 49 normal or underweight children.

Table 6. Impulse oscillometry results.

	radBPD children (n=19)	VLBW controls (n=19)	Term controls (n=19)	p
Rrs5Hz of predicted (%), median (range)	92 (65-144)	79 (63-132)	70 (57-120)	0,011
Xrs5Hz of predicted (%), median (range)	92 (50-197)	80 (41-229)	69 (40-150)	0,137
Rrs5Hz br response (%), mean (SD)	-17 (9)	-21 (10)	-11 (11)	0,010
Zrs5Hz br response (%), mean (SD)	-18 (9)	-22 (11)	-12 (11)	0,013
Fr br response (%), median (range)	-22 (-55 to -3)	-24 (-39 to 3)	-14 (-47 to 50)	0,267

Rrs, resistance; Xrs, reactance; br, bronchodilator; Zrs, impedance; Fr, resonance frequency

5.4.3 Lung function and neonatal factors (Study II)

No associations were found between lung function and BPD (defined radiologically or according to the current criteria), gestational age, prenatal antibiotics, ventilator therapy, postnatal corticosteroids or neonatal sepsis.

5.4.4 Lung function and environmental factors (Study II)

In the multivariate logistic regression analysis adjusted for atopy in the family, hairy pets at home, exposure to tobacco smoke and BMI-SDS, hairy pets at home predicted a significant risk of having resistance at 5 Hz in the highest quartile (OR 7.15, 95% CI 1.04 – 49.00, $p = 0.045$) in VLBW children.

5.5 Inflammation (Studies I-III)

5.5.1 Inflammatory activity in school age (Study I)

Exhaled breath condensate could not be collected from two radBPD children. Fractional exhaled NO concentrations could be measured from 16 radBPD children, 18 VLBW controls and 18 term children and alveolar and bronchial NO concentrations could be determined from 14 radBPD, 16 VLBW and 18 term control children.

Serum IgE was elevated in 6 (29%) radBPD children, 3 (16%) VLBW controls and 3 (16%) term children ($p = 0.556$). The concentrations of inflammatory markers in plasma, exhaled breath condensate or exhaled breath did not differ between the groups (see Study I, Table 3, Appendix). The result did not alter when the current definition of BPD was used.

5.5.2 Inflammatory markers and neonatal factors (Studies I, III)

In the multivariate logistic regression analysis adjusted for birth weight, male gender, chorioamnionitis, neonatal sepsis, ventilator therapy, neonatal systemic corticosteroid therapy and radBPD, postnatal systemic corticosteroid therapy was associated with an increased risk of having adiponectin concentrations in the highest quartile (OR 32.0, 95% CI 1.3 - 793.0, $p = 0.034$) and with a reduced risk of having adiponectin in the lowest quartile (OR 0.01, 95% CI 0.00 - 0.45, $p = 0.016$). Chorioamnionitis was associated with an increased risk of having IL-8 concentrations in the lowest quartile (OR 29.0, 95% CI 3.3 - 258.0, $p = 0.003$) and with a reduced risk of having adiponectin (OR 0.09, 95% CI 0.01 - 0.67, $p = 0.019$) or alveolar NO (OR 0.03, 95% CI 0.00 - 0.82, $p = 0.038$) in the highest quartile.

Birth length for gestational age correlated negatively with resistin levels ($\rho -0.32$, $p = 0.049$) and head circumference for gestational age with adiponectin levels at school age ($\rho -0.47$, $p = 0.009$).

5.5.3 Inflammatory markers and environmental factors (Study I)

In the multivariate logistic regression analysis adjusted for atopy or asthma in the family, hairy pets, exposure to tobacco smoke at home and BMI-SDS, atopy in the family was associated with a lesser risk of having IL-6 concentrations in the lowest quartile (OR 0.07, 95% CI 0.01 - 0.62, $p = 0.017$), hairy pets at home lowered the risk of having ECP (OR 0.14, 95% CI 0.02 - 0.89, $p = 0.037$) or IL-8 (OR 0.11, 95% CI 0.01 - 0.83, $p = 0.033$) in the highest quartile. Exposure to tobacco smoke was associated with an increased risk of having 8-isoprostane in the lowest quartile (OR 7.11, 95% CI 1.2 - 41.0, $p = 0.028$).

5.5.4 Lung function and inflammation (Studies I, II)

Of the children with available exhaled breath condensate, the six VLBW children with physician-diagnosed asthma had lower 8-isoprostane levels in exhaled breath condensate than the 51 non-asthmatic children [median (range) 0.2 (0.2 –3.6) vs. 2.0 (0.2 – 28.6) pg/ml, $p = 0.042$]. Other inflammatory markers were similar between asthmatics and non-asthmatics.

Adiponectin correlated modestly (ρ 0.45, $p = 0.005$) with resistance at 5 Hz in VLBW children. No other correlations were found between the inflammatory markers and baseline lung function. In the bronchodilation test, negative correlations were seen between adiponectin and resistance (r -0.38, $p = 0.020$), impedance (r -0.41, $p = 0.010$) and resonance frequency (ρ -0.47, $p = 0.003$) responses, between IL-6 and resonance frequency response (ρ -0.42, $p = 0.009$) and between alveolar NO concentrations and resistance (r -0.41, $p = 0.023$) and impedance (r -0.44, $p = 0.012$) responses in VLBW children. When correlation coefficients were calculated for the radBPD children only, no correlations were found.

No associations were found between lung function and inflammatory markers in the logistic regression analysis.

5.5.5 Adipokines in relation to growth and nutrition (Studies I, III)

Pre-pubertal and pubertal children had similar adipokine concentrations. Girls had higher adipisin [median (range) 916 ng/ml (598 - 1264) vs 787 ng/ml (483 -1409), $p = 0.034$] and leptin [median (range) 7.1 ng/ml (2.1 - 37.6) vs 1.8ng/ml (0.5 - 50.0), $p < 0.001$] levels compared with boys, whereas adiponectin and resistin levels were similar.

Plasma leptin concentrations correlated positively with BMI-SDS (ρ 0.57, $p < 0.001$) and negatively with the intake of energy (ρ -0.55, $p = 0.001$) total fat (ρ -0.36, $p = 0.030$), saturated fatty acids (ρ -0.45, $p = 0.006$) and monounsaturated fatty acids (ρ -0.38, $p = 0.027$). Adiponectin (ρ -0.36, $p = 0.031$) and adipisin levels (r -0.33, $p = 0.048$) correlated negatively with energy intake, as did resistin levels with carbohydrate intake (ρ -0.36, $p = 0.031$). In the multivariate logistic regression analysis adjusted for atopy or asthma in the family, hairy pets, exposure to tobacco smoke at home and BMI-SDS, higher BMI-SDS was associated with having adipisin (OR 2.47, 95% CI 1.07 - 5.75, $p = 0.035$) and leptin (OR 5.76, 95% CI 1.8 - 18.2, $p = 0.003$) concentrations in the highest quartile.

5.6 Cardiac findings (Study IV)

Two radBPD children had oxygen saturation levels of 93-95% at rest. All other VLBW children had oxygen saturation $\geq 97\%$.

5.6.1 Blood pressure (Study IV)

The median (range) blood pressure did not differ between radBPD children [109/57 mmHg (96 - 128)/(49 - 82)], VLBW control children [108/58 mmHg (91 - 135)/(46 - 70)] and term children [108/57 mmHg (90 - 132)/(49 - 73)]. One radBPD child had a hypertensive systolic blood pressure and three radBPD children, two VLBW controls and two term children had pre-hypertensive systolic blood pressure.

5.6.2 Electrocardiogram (Study IV)

Apart from a partial right bundle branch block in one radBPD child and a first-degree atrioventricular block in one VLBW control child, the electrocardiograms of all children were considered normal. However, radBPD children had deeper SV6 [median (range) 2 mm (0 - 34) vs. 1 mm (0 - 13), $p = 0.028$] and more rightward electrical axis [median (range) 80° (34 - 181) vs. 58° (17 - 80), $p = 0.002$] compared to VLBW controls.

5.6.3 Echocardiography (Study IV)

Minimal mitral leak was found in one VLBW control, and another VLBW control had both minimal mitral and aortic insufficiency. One radBPD child had left superior vena cava. One radBPD child had an ejection fraction $<50\%$ in 3DE, but normal fractional shortening. Otherwise the echocardiographic findings were considered normal. No signs of pulmonary hypertension were found. Results of conventional echocardiography are shown in Table 7, tissue Doppler imaging and tissue motion annular displacement analysis in Table 8 and results of 3DE in Table 9. The children with radBPD evinced no abnormalities in cardiac function according to any echocardiographic parameter compared with VLBW and term

control children (Tables 7-9). The results did not alter when current criteria based on oxygen supplementation were used.

Echocardiographic parameters in the seventeen children with birth weight <1000g were comparable with the other eighteen VLBW children. Higher tricuspid E/E' was the only echocardiographic parameter to differ between the thirteen children with neonatal dexamethasone treatment compared to 22 VLBW children without dexamethasone treatment [median (range) 4.18 (2.95 - 5.28) vs. 3.14 (1.86 - 5.51), p = 0.028]. Children with a duration of neonatal oxygen or ventilator therapy in the highest quartile evinced no poorer cardiac function than the other school-aged VLBW children.

Table 7. Results of 2D echocardiography, Doppler and M-mode examinations shown as medians (ranges). The M-mode measurements are given as z-scores of the reference values.

	radBPD children		VLBW controls		Term controls		
	(n=18)		(n=17)		(n=18)		p
LVED (SD)	-0.9	(-2.8-1.2)	-0.6	(-1.2-1.0)	-0.3	(-2.0-1.4)	0.145
LVES (SD)	-0.1	(-2.2-1.1)	0.5	(-1.6-1.4)	0.3	(-1.1-2.0)	0.190
LVPWd (SD)	0.5	(-0.7-3.5)	0.9	(-0.8-1.8)	0.6	(-0.5-2.0)	0.969
IVSd (SD)	0.4	(-0.8-1.3)	0.5	(-0.8-2.0)	0.6	(-0.3-2.9)	0.280
RVED (SD)	-1.4	(-0.2- -0.4)	-0.8	(-2.2-0.5)	-0.8	(-2-0.5)	0.059
FS (%)	37	(28-47)	36	(31-48)	34	(28-40)	0.099
Mitral E (cm/s)	98	(80-123)	107	(82-131)	100	(74-123)	0.825
Mitral A (cm/s)	46	(32-73)	43	(29-68)	42.0	(34-63)	0.239
Mitral E:A	2.1	(1.5-2.7)	2.2	(1.5-3.7)	2.3	(1.7-3.0)	0.341
Tricuspid E (cm/s)	61	(46-93)	61	(50-76)	56	(41-72)	0.163
Tricuspid A (cm/s)	35	(28-55)	32	(23-47)	33	(23-46)	0.394

LVED, left ventricular end-diastolic dimension; LVES, left ventricular end-systolic dimension; LVPWd, left ventricular posterior wall diastolic diameter; IVSd, interventricular septum diastolic diameter, RVED, right ventricular end-diastolic dimension; FS, fractional shortening

Table 8. Results of the tissue Doppler (TDI) and speckle tracking (STE) echocardiography studies shown as medians (ranges).

	radBPD children (n=18)	VLBW controls (n=17)	Term controls (n=18)	p
<u>TDI</u>				
Mitral annulus (lateral)				
E´ (cm/s)	17.9 (11.9-28.7)	19.1 (14.9-33.6)	21.4 (15.9-35.0)	0.145
A´ (cm/s)	5.2 (4.2-7.8)	4.9 (3.2-6.8)	5.6 (4.0-9.6)	0.083
S´ (cm/s)	9.5 (6.2-15.1)	8.4 (6.6-18.4)	10.1 (6.6-15.7)	0.241
E/E´	4.9 (3.9-10.1)	5.2 (3.9-7.7)	4.7 (3.0-7.4)	0.164
Mitral annulus (septal)				
E´ (cm/s)	13.9 (11.9-21.2)	13.8 (10.6-15.5)	14.1 (10.2-22.2)	0.301
A´ (cm/s)	5.4 (3.9-8.6)	5.0 (3.1-7.0)	5.2 (4.2-14.3)	0.285
S´ (cm/s)	8.2 (6.9-21.8)	8.1 (6.0-10.6)	7.6 (6.1-14.1)	0.281
E/E´	7.5 (4.7-8.6)	7.7 (5.6-10.7)	6.4 (4.8-8.4)	0.121
Tricuspid annulus				
E´ (cm/s)	19.5 (8.4-29.0)	16.6 (10.7-21.0)	16.3 (8.1-21.3)	0.257
A´ (cm/s)	7.7 (2.7-14.1)	7.1 (4.5-10.6)	7.8 (4.1-13.0)	0.675
S´ (cm/s)	17.1 (11.7-23.0)	14.2 (10.0-19.2)	13.8 (10.3-23.1)	0.211
E/E´	3.1 (1.9-4.8)	3.8 (2.5-5.5)	3.6 (2.0-5.1)	0.511
<u>STE</u>				
TAD lateral (mm)	17.3 (8.3-21.9)	17.3 (11.4-27.4)	16.3 (13.0-23.0)	0.650
TAD septal (mm)	12.0 (6.5-15.4)	13.7 (8.5-17.1)	12.0 (8.3-19.1)	0.164
TAD-mid (mm)	15.5 (8.3-19.2)	16.5 (11.4-22.0)	14.8 (11.6-20.9)	0.400
TAD-mid (%)	26.4 (18.2-33.4)	24.3 (20.0-31.8)	23.0 (18.3-30.0)	0.142
MAD lateral (mm)	10.2 (7.1-14.2)	11.8 (4.9-16.3)	9.8 (5.7-14.6)	0.462
MAD septal (mm)	11.0 (6.8-15.4)	12.5 (7.4-15.0)	10.4 (8.3-13.5)	0.114
MAD-mid (mm)	11.3 (7.5-15.2)	12.6 (7.3-16.5)	10.7 (7.9-14.7)	0.125
MAD-mid (%)	16.2 (11.2-19.9)	16.2 (11.8-18.8)	15.2 (10.6-19.1)	0.180

Table 9. Results of the 3DE studies shown as medians (ranges).

	radBPD children		VLBW controls		Term controls		p
	(n=18)		(n=17)		(n=18)		
LV EDV (ml/m ²)	52.8	(34.7-73.3)	58.4	(36.3-88.9)	59.0	(46.7-84.8)	0.177
LV ESV (ml/m ²)	23.7	(16.1-34.5)	27.6	(18.3-37.0)	26.8	(20.1-45.5)	0.145
LV SV (ml/m ²)	29.4	(19.6-38.8)	32.0	(18.0-53.1)	31.5	(22.7-45.4)	0.190
EF (%)	55	(49-61)	55	(50-65)	54	(50-60)	0.654
Tmsv 16-SD (%)	2.6	(1.6-4.4)	2.5	(1.1-4.0)	2.7	(1.9-5.0)	0.813
Tmsv 16-Dif (%)	8.4	(5.4-11.9)	8.8	(3.7-14.5)	8.5	(6.1-14.9)	0.711
Tmsv 12-SD (%)	2.5	(1.1-4.0)	2.4	(0.8-3.4)	2.5	(1.5-5.0)	0.614
Tmsv 12-Dif (%)	8.7	(3.6-14.8)	8.0	(2.2-13.7)	9.8	(3.9-17.4)	0.360

LV, left ventricular; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; EF, ejection fraction

5.6.4 NT-proBNP (Study IV)

NT-proBNP values did not differ between the groups. Elevated NT-proBNP concentrations were seen in four radBPD children and one term control ($p = 0.081$). NT-proBNP values did not differ between children with normal and abnormal lung function.

Higher NT-proBNP correlated with thicker left ventricle posterior wall ($\rho = 0.35$, $p = 0.039$) and with lower TAD lateral ($\rho = -0.32$, $p = 0.031$) and TAD-mid (mm) ($\rho = -0.47$, $p = 0.005$) in VLBW children.

5.6.5 Cardiac parameters in relation to lung function in VLBW children (Studies II, IV)

Smaller mitral A' was the only echocardiographic parameter which differed between the five children with abnormal lung function compared to term controls [median (range) 3.8 cm/s (3.2 - 5.3) vs. 5.6 cm/s (4.0 - 9.6), $p = 0.002$].

Higher resistance correlated with 3DE dyssynchrony indices Tmsv 16-SD ($\rho = 0.48$, $p = 0.005$), Tmsv 12-SD ($\rho = 0.38$, $p = 0.027$) and Tmsv 12-Dif ($\rho = 0.37$, $p = 0.048$). Poorer reactance correlated with greater left ventricular end-systolic ($\rho = 0.45$, $p = 0.009$) and left ventricular end-diastolic volumes ($\rho = -0.50$, $p = 0.003$).

Greater bronchodilation responses in resistance and impedance correlated with higher tricuspid A (ρ 0.40, $p = 0.028$ and ρ 0.42, $p = 0.017$, respectively) and tricuspid E' (ρ 0.41, $p = 0.022$ and ρ 0.39, $p = 0.030$) and bronchodilation response in resonance frequency correlated with tricuspid A (ρ 0.46, $p = 0.010$) and mitral E' (ρ 0.42, $p = 0.033$).

No significant associations with the cardiac parameters were found in multivariate logistic regression analysis adjusted for radBPD, dexamethasone treatment and duration of oxygen and ventilator therapies. No associations were found between the poorest quartiles of the cardiac parameters and lung function among VLBW children.

6 Discussion

6.1 Growth (Study III)

The head growth of the school-aged radBPD children was impaired compared to VLBW and term controls. No other differences were found in anthropometric parameters.

The relationship of BPD to head growth is controversial (Vrlichen et al. 1995; Chiriboga et al. 2003; Peterson et al. 2006). Since BPD is associated with increased morbidity, there are many confounding factors. Compared to VLBW controls, the radBPD children had more risk factors for poor growth, for example lower gestational age, smaller birth weight, and more need for postnatal corticosteroid treatment. Although children with radBPD had more microcephaly than VLBW controls, radBPD did not remain a significant predictor for microcephaly in the multivariate logistic regression analysis adjusted for confounding neonatal factors. The children with microcephaly had poorer neonatal weight gain compared with normocephalic VLBW children. Although weight gain before discharge is associated with the neurodevelopmental outcomes of preterm children (Ehrenkranz et al. 2006), it has been suggested that poor postnatal growth itself does not alter the later head growth (Ehrenkranz et al. 2006). On the other hand, the sickest infants appear to have the slowest growth velocity (Ehrenkranz et al. 2006).

Although children with microcephaly at school age had smaller age-adjusted birth head circumferences than VLBW children without microcephaly, none had microcephaly at birth. The head circumference measurements between the birth and the study date were not available in our study. Previous studies have estimated that the critical period of brain growth extends to late infancy and after the first year of life a small head circumference tend to persist (Brandt et al. 2003). Head circumference was not measured in the parents, which is a major limitation. The heritability of head size is marked, even 88% in childhood (Smit et al. 2010), and can explain microcephaly.

Postnatal steroid treatment was associated with microcephaly at school-age in the univariate, but not in the multivariate analysis. Steroids were administered to

infants with severe pulmonary problems, making it difficult to differentiate between the effects of BPD and steroids. Dexamethasone treatment of premature infants has been associated with reduced brain tissue volumes in MRI at term (Parikh et al. 2007) and at 18 years of age (Cheong et al. 2014).

Subnormal head growth in VLBW school-children has been associated with a poorer intelligence quotient equivalent, perceptual motor skills, academic achievement and adaptive behavior (Peterson et al. 2006). As we did not measure neurodevelopmental outcomes, the clinical relevance of the impaired head growth in our radBPD children remains unclear.

Neonatal sepsis was associated with short stature at school age and the survivors of NEC had a smaller head circumferences compared with the other VLBW children. The systemic inflammatory response can cause direct cytotoxic injury to neurons and activate a local inflammatory response, leading to white matter damage, adverse neurodevelopmental outcomes and impaired head growth (Adams-Chapman 2012). Inflammation affects metabolism, nutritional requirements and growth in the neonatal period (Ramel et al. 2014). It would appear that early inflammation may have a long-term effect on the growth of VLBW children.

6.2 Nutrition (Study III)

No differences were found between the groups in reported nutrient intake, but the nutrient recommendations were poorly met in all groups. Plasma adipokine levels correlated with nutrient intake.

Although prematurity is associated with oral motor difficulties and behavioral eating problems at school age (Samara et al. 2010) and prenatal nutrition and growth may affect dietary preferences in later life (Lussana et al. 2008; Barbieri et al. 2009), we found no differences in diet in school-aged VLBW children compared to term-born controls. Parents affect the eating habits of children by providing the food at home and acting as role models in eating behavior (Salvy et al. 2011). Finnish VLBW young adults less dependent on their parents than our school-aged subjects have been shown to exhibit differences in diet compared to term controls (Kaseva et al. 2013).

The nutrient recommendations were poorly met in all study groups: all children reported excessive intake of saturated fatty acids, many had a higher than recommended protein intake and low intakes of fiber and micronutrients were

common. Excessive intake of saturated fatty acids and a low intake of fiber and micronutrients have also been established in young VLBW adults (Kaseva et al. 2013). Preterms are prone to metabolic and cardiovascular disease (Sipola-Leppänen et al. 2015), and an unhealthy diet may thus be more harmful to them than to term children. Counselling as regards healthier eating from childhood could provide an important tool in preventing later disease.

Recommended intakes of micronutrients are set to the average requirement +2 SD to meet the nutrient requirement of nearly all healthy individuals, and the upper level of tolerable intake is the highest safe level of continuing daily nutrient intake (Fogelholm et al. 2014). Lower than recommended intake does not mean an inadequate food supply, and occasional excessive intake is not necessarily harmful (Fogelholm et al. 2014). We did not measure blood vitamin or mineral levels.

Most of the children here reported a lower than recommended daily energy intake, but only four were underweight. We did not record physical activity. The amount of physical exercise may have been low in our VLBW children, this explaining adequate weight gain despite low energy intake. Underestimation of energy intake in food records is common (Bandini et al. 1990) and cannot be ruled out in our study, although our food diaries were checked with the nutritionist. Food diaries give an estimate of only short-term intakes and rarely used food items might be heavily under- or overestimated (Vyas et al. 1999; Kaseva et al. 2013). On the other hand, the reported energy intake has been shown to be highly reproducible (Bandini et al. 1990).

Short stature was associated with higher energy intake. A previous study among Finnish children has shown that short children consume relatively more calories than their normal-sized peers (Niinikoski et al. 1997).

6.3 Pulmonary outcomes (Study II)

Survivors of radBPD had more impaired baseline lung function with less bronchial reversibility than VLBW children without a history of BPD. Preterm birth is associated with wheezing disorders (Been et al. 2014) and decreased forced expiratory volume in 1 second, indicating small airway obstruction (Ronkainen et al. 2015). Previously, both increased (Kotecha et al. 2015) and similar (Ronkainen et al. 2015) bronchodilator responses have been reported in school-aged BPD survivors compared to preterm peers. Bronchial hyperresponsiveness may be related more to prematurity than to the permanent airway damage in BPD.

Abnormal lung function was found in 13% of our VLBW children who were able to undergo impulse oscillometry. Most of the children evinced normal baseline lung function, in line with a previous Finnish study of school-aged VLBW survivors with and without BPD (Malmberg et al. 2013). However, another study from the surfactant era has reported 58% of very preterm children to present with abnormal lung function at school age (Choukroun et al. 2013). While bronchial obstruction has been the most common finding, pulmonary restriction has been found in 11% of school-aged children born very preterm (Choukroun et al. 2013). Since impulse oscillometry is not sensitive to study pulmonary restriction, we might have missed some cases (Smith et al. 2005). On the other hand, reactance at 5 Hz was lowest in term children and highest in children with radBPD, suggesting that the elastic properties of the lungs in our BPD survivors had in fact recovered well by school age.

6.4 Inflammation (Studies I-III)

We found no evidence of excessive inflammatory activity in school-aged VLBW children with severe radBPD. Inflammation has a critical role in the pathogenesis of BPD, but the inflammatory activity seems to normalize by school age.

The origin of the plasma inflammatory markers cannot be established and the absence of systemic inflammation does not rule out local inflammation of the lung. Inflammatory markers from exhaled air showed no evidence of airway inflammation. Neutrophil activation in terms of leukotriene B₄, oxidative stress in terms of 8-isoprostane and NO measurements reflecting eosinophilic inflammation of both central and peripheral lung did not differ between the school-aged VLBW children with and without radBPD and term controls. Our NO results are in line with previous studies showing normal (Lum et al. 2011; Malmberg et al. 2013b) or low (Baraldi et al. 2005) exhaled NO levels in school-aged BPD children. Some previous studies showing elevated inflammatory activity in school-aged and adolescent preterms regardless of history of BPD have included survivors from the pre-surfactant era and the results may reflect the long-term effects of different antenatal treatments compared to modern-era preterms (Halvorsen et al. 2005; Filippone et al. 2012; Teig et al. 2012). Elevated levels of several inflammatory markers such as exhaled breath condensate 8-isoprostane and leukotriene B₄ reflect sustained airway inflammation in children with asthma (Barreto et al. 2009;

Trischler et al. 2015). In contrast, respiratory problems of the school-aged BPD survivors appear to be mainly structural.

We found no evidence of chronic inflammation in school-aged radBPD survivors free of infections. However, the inflammatory response to an acute infection might differ between BPD survivors and preterm and term peers.

6.4.1 Inflammatory markers and neonatal factors (Studies I, III)

Chorioamnionitis and postnatal corticosteroid therapy were associated with decreased inflammation. Chorioamnionitis was associated with low plasma IL-8 concentrations, which may be a consequence of immunologic hypo-responsiveness induced by antenatal inflammation (Kunzmann et al. 2013). Postnatal systemic corticosteroid was associated with high concentrations of adiponectin, which is mainly anti-inflammatory. Since postnatal corticosteroids were administered only to ventilator-dependent patients, our finding may be associated with a direct anti-inflammatory influence of corticosteroids, or with early severe lung damage itself.

In our VLBW children, head circumference at birth correlated negatively with adiponectin levels, and birth length with resistin levels at school age. Previous studies have associated low birth weight with low adiponectin levels at school age (Giapros et al. 2007) and high leptin levels in adults (Lissner et al. 1999). Neonatal weight gain has been associated positively with leptin concentrations at school age (Flexeder et al. 2014) and negatively with adiponectin levels in adolescence (Larnkjaer et al. 2010). It has been suggested that adipokine secretion programmed by the intrauterine environment may modify the risk of metabolic diseases (Cianfarani et al. 2004).

6.4.2 Inflammatory markers and environmental factors (Study I)

Tobacco smoke exposure may contribute to chronic airway inflammation and is a risk factor for asthma (Goksor et al. 2006). In our study exposure to tobacco smoke at home was associated with low 8-isoprostane levels in VLBW children. A previous study has found reduced leukotriene B4 production by alveolar macrophages exposed to tobacco smoke (Tardif et al. 1990).

6.4.3 Lung function and inflammation (Study II)

No correlations were found between the inflammatory markers and lung function in the school-aged radBPD children, although they had lower baseline lung function than VLBW children without a history of radBPD. Our findings support the conception that the reduced lung function in school-aged BPD survivors is not associated with ongoing inflammation.

No association was found between radBPD and adipokines. In VLBW children, higher adiponectin levels correlated with poorer baseline lung function in terms of higher resistance, and with bronchodilation response. Adiponectin receptors are expressed in human airway smooth muscle cells (Shin et al. 2008) and adiponectin has been reported to maintain the contractile phenotype of smooth muscle cells (Ding et al. 2012); adiponectin may thus contribute to airway obstruction. The role of adiponectin in airway inflammation is controversial (Ali Assad et al. 2012). Although mainly anti-inflammatory, the pro-inflammatory effects of adiponectin may dominate under certain physiologic conditions (Ali Assad et al. 2012). It is unclear whether alterations in adipokine secretion precede pulmonary disease or are a consequence of it (Ali Assadet al. 2012).

Plasma IL-6 concentrations correlated with the bronchodilator response assessed by resonance frequency in VLBW children, but no correlations were found with bronchodilation responses assessed by resistance, reactance or impedance. Higher plasma IL-6 has been found in asthmatic compared to non-asthmatic adults and IL-6 concentrations have been associated with lower forced expiratory volume in 1 second (Wood et al. 2012).

Alveolar NO, but not fractional exhaled NO or bronchial NO, correlated with a greater bronchodilator response. In a previous Finnish study, airway hyper-responsiveness in a histamine challenge test was associated with bronchial nitric oxide flux in atopic school-aged VLBW children, showing that atopic airway inflammation may modify airway hyper-responsiveness in VLBW children (Malmberg et al. 2013). Correlations between various inflammatory markers and bronchodilation response indicate that some VLBW children may benefit from anti-inflammatory treatment, as previously suggested (Malmberg et al. 2013).

6.4.4 Adipokines in relation to growth and nutrition (Studies I, III)

High BMI-SDS was associated with high adipisin and leptin levels. No other associations were found between adipokine concentrations and anthropometric

parameters. Greater weight has been associated with higher leptin and lower adiponectin levels in term school-aged children (Baek et al. 2011). School-aged VLBW survivors do not seem to evince any more marked association between inflammatory marker levels and growth parameters compared with other children, but the overproduction of pro-inflammatory adipokines with increasing adiposity may induce pulmonary problems originating from the neonatal period.

Contradictory findings of leptin concentrations correlating positively with BMI but negatively with the intake of energy have been reported previously (Bienertova-Vasku et al. 2014). A study of obese adults found a positive correlation between leptin and weight, a negative correlation between leptin receptor levels and weight and negative correlations between both leptin and leptin receptor levels and total energy intake (Bienertova-Vasku et al. 2014). The leptin/leptin receptor levels seem to be essential in controlling satiety and total energy and macronutrient intake (Bienertova-Vasku et al. 2014). Adipokines are also likely to have synergistic interrelationships which escalate energy intake and fat accumulation in obese (Bienertova-Vasku et al. 2014). The total daily energy expenditure and energy requirements may be reduced in the heaviest individuals (Bienertova-Vasku et al. 2014) and they may be the subjects with the lowest physical activity levels.

Plasma concentrations of adiponectin and adipsin correlated negatively with the intake of energy, and resistin levels with the intake of carbohydrates in our VLBW children. Adiponectin is linked to insulin sensitization, and adipsin to triglyceride storage, and resistin has controversial effects on glucose metabolism (Ronti et al. 2006). A decrease in energy intake and weight has been associated with an increase in adiponectin levels in adults (Boas Huguenin et al. 2014; Cassani et al. 2015) and a high-fiber diet has lowered resistin levels in children (Roberts et al. 2013). It would appear that adipokine concentrations, and thus the risk of metabolic diseases, can be modified by diet.

6.5 Cardiac findings (Study IV)

Children with radBPD evinced normal cardiac function at the age of 6-14 years compared to VLBW controls without a history of BPD and term controls assessed by conventional and modern echocardiographic methods.

No signs of pulmonary hypertension were found in echocardiography in school-aged radBPD children despite lower baseline lung function. In the few studies available from the surfactant era, BPD survivors have had increased

pulmonary arterial pressure until the end of the first year of life (Subhedar et al. 2000), but not at school age (Korhonen et al. 2005; Joshi et al. 2014).

Tissue Doppler imaging can be used to study the systolic and diastolic function of both ventricles (Mori et al. 2000). An increasing right ventricle E/E' ratio reflecting increased end diastolic pressure has correlated with the clinical severity of BPD in infants (Yates et al. 2008). Tissue Doppler imaging-derived left and right ventricular myocardial performance indices have been higher in BPD survivors compared with control children of preschool age, suggesting subclinical dysfunction of both ventricles (Kazanci et al. 2011; Koroglu et al. 2013). A recent study evaluating left and right ventricular function by conventional echocardiography and tissue Doppler imaging in 28 school-aged BPD children found normal cardiac function and no evidence of increased pulmonary artery pressure (Joshi et al. 2014), in line with our findings.

Tissue motion annular displacement analysis is an easy, objective speckle tracking-based means of measuring longitudinal function in both left and right ventricles (Tsang et al. 2010; Ahmad et al. 2012) not previously used to study school-children with primary lung disease. Impaired longitudinal myocardial function of the right ventricle has been found in BPD children at four years of age (Xie et al. 2016). The longitudinal myocardial function of right and left ventricle in our school-aged radBPD survivors was normal compared to VLBW and term controls.

3DE provides reliable non-invasive measurements of left ventricular volumes (Poutanen et al. 2001) and enables quantification of ventricular dysfunction (Kapetanakis et al. 2005). Dysmorphic pulmonary vasculature and chronic lung disease in BPD may increase the pressure load on the right side of the heart and lead to right ventricular dysfunction (Mourani et al. 2013). Right ventricular function affects the left ventricle due to ventricular interdependence (Haddad et al. 2008), and impaired left ventricular function may follow right ventricular dysfunction (Mourani et al. 2013). 3DE has not been used in follow-up studies of BPD or VLBW survivors before. We found normal left ventricular function in 3DE, but unfortunately the acoustic window for the right ventricle was inaccurate. Echocardiographic imaging of the geometrically complex right ventricle is challenging (Haddad et al. 2008). Cardiac magnetic resonance imaging would provide a more accurate noninvasive means of evaluating the right ventricle (Haddad et al. 2008). There are, to our knowledge, no studies of cardiac magnetic resonance imaging in BPD survivors.

The electrocardiograms of the radBPD children were more right-biased, which could be an indirect sign of an abnormal right ventricular load. However, none of the echocardiographic parameters differed between radBPD children and term controls and no signs of elevated right ventricular pressure were found in echocardiography. Normal plasma NT-proBNP concentrations support the normal echocardiographic findings. NT-proBNP is secreted in response to cardiomyocyte stretch (Lammers et al. 2009). NT-proBNP is used in the diagnosis and follow-up of pulmonary hypertension in children and the concentrations correlate with the severity of pulmonary hypertension (Lammers et al. 2009). Higher NT-proBNP levels have been found in infants who developed BPD compared to preterm controls (Joseph et al. 2010), but at the age of 6-12 months NT-proBNP levels have been within the normal limits (Akcan et al. 2013). Low NT-proBNP practically excludes pulmonary hypertension in adults with chronic lung disease (Andersen et al. 2013). In our study higher NT-proBNP levels correlated with left ventricular posterior wall thickness and with low TAD.

6.5.1 Cardiac parameters in relation to lung function in VLBW children (Studies II, IV)

The relation of current lung function and modern echocardiographic findings in school-aged VLBW survivors has not previously been studied. In our study lower lung function was modestly related to lower echocardiographic parameters. We found modest correlations between the elastic properties of the lungs in terms of reactance and left ventricular end-systolic and end-diastolic volumes, and between bronchodilation responses and tricuspid A and myocardial velocities in VLBW children. Baseline obstruction in terms of resistance was associated with greater left ventricular dyssynchrony indices. Associations between lung function and cardiovascular physiology have been inadequately studied in preterm survivors after early childhood. In a previous study of school-aged children born ≤ 25 weeks of gestation increased arterial stiffness was inversely related to baseline lung function and directly related to the bronchodilation response as assessed by spirometry (Bolton et al. 2012). Reduced lung function is an independent risk factor for cardiac mortality in adults (Hole et al. 1996).

We found no associations between cardiac function and neonatal dexamethasone exposure, or duration of oxygen or ventilator therapy. In a previous Finnish study of 7-9-year-old VLBW survivors with or without neonatal

dexamethasone treatment, no signs of hypertrophic cardiomyopathy were found in conventional echocardiography (Mieskonen et al. 2003). Blood pressure, intima-media thickness, and left ventricular function at school-age have been comparable between prematurely born children with antenatal corticosteroid exposure, neonatal corticosteroid treatment or without any glucocorticoid therapy (de Vries et al. 2008). If there were dexamethasone-induced cardiac changes in the neonatal period, they have apparently resolved in children who survived up to school-age.

6.6 Strengths and limitations of the study

The data on the role of inflammation in school-aged BPD survivors are limited, as are data on their cardiac function. As our study is a single-center study, all VLBW children were treated with unified neonatal protocols, limiting the confounding factors consequent upon variability between centers. The oldest study patients were born in 1995. Although neonatal intensive care practices in the late 1990s were not so different from today, more restricted postnatal steroid treatment and more gentle surfactant administration and ventilator support are likely to have an impact on the long-term outcomes of the current preterm survivors. All available patients with severe radiographic BPD treated in our hospital during an eight-year period were studied. The number of eligible patients was limited and the small sample size is the main limitation of our study weakening the power of analyses. Due to under-powering, some associations, though in fact present, may not have reached statistical significance. Multiple testing may result in incidental findings, which we tried to control by means of multivariate analyses. Our results should be interpreted with caution. However, small studies add cumulative information and may give directions for future research.

Ten out of 31 eligible children born in the chosen eight-year period refused to participate. Although the gestational ages and birth weights of participants and non-participants did not differ, there is a risk of selection bias.

Having both VLBW and term age-matched controls is a strength, although due to the small number of eligible patients and over-representation of BPD cases in the most premature newborns, matching for gestational age and birth weight was not optimal between the BPD and nonBPD cases.

BPD is a heterogeneous disease and other rare lung diseases may mimic it (Lord et al. 2014). We used radiographic BPD criteria to identify the most severe BPD cases before introduction of the oxygen reduction test. In the VLBW control

group, two children needed supplemental oxygen at 36 weeks of gestational age and four children with severe radiological changes had only mild BPD according to current consensus criteria. However, our results did not change significantly when BPD was defined by the current criteria.

Some of the data were collected retrospectively and from the questionnaire. Chorioamnionitis was a clinical diagnosis without placental histology. The growth statistics at discharge and the neonatal echocardiographic studies were incomprehensive. To overcome the challenges of the wide age range, age- or size-dependent reference values were used when available. All of the children studied were Caucasians.

Individual inflammatory marker levels may vary greatly even without infection. To rule out the effects of acute infection, the children were studied when they were free of infections. The role of inflammation during exacerbation was not studied. We studied different types of inflammation and both systemic and lung inflammation, although inflammatory markers from exhaled air could not be obtained from all of the children.

The anthropometric measurements were taken by a single pediatrician. Food records were obtained from three consecutive days and do not necessarily represent the regular eating habits of the participants. An experienced trained nutritionist interviewed the families to ensure that food records were properly completed. However, food records can be biased by tendency to underestimate intake and adjust responses towards socially more favorable answers (Kaseva et al. 2013). Five children did not complete food diaries.

We used impulse oscillometry instead of spirometry to study lung function. Impulse oscillometry has been shown to yield information concordant with spirometry of lung function in BPD survivors near or at school age (Malmberg et al. 2000). Impulse oscillometry allows evaluation of lung function during spontaneous breathing and with less cooperation compared to spirometry. Two radBPD children could not undergo impulse oscillometry. Impulse oscillometry detects obstruction, but is not sensitive to pulmonary restriction (Smith et al. 2005). We evaluated the bronchodilation response to salbutamol, but an exercise challenge test was not applied. National reference values were used to define whether lung function was normal or abnormal, whereas predicted values were used for correlation analysis.

Five children refused echocardiographic examinations. All echocardiographic examinations were made by a pediatric cardiologist. Echocardiographic parameters were presented as averages of three cardiac cycles. The adequate interobserver

reliability of our echocardiographic study group has been previously reported (Ylänen et al. 2014). The echocardiographic studies were comprehensive, although we could not use 3DE to study the right ventricle.

6.7 Future considerations

We found no evidence of excessive inflammatory activity in school-aged VLBW children with and without BPD. However, the inflammatory response to an acute infection might differ between BPD survivors and preterm and term peers, and measuring inflammatory marker levels during an acute infection might reveal different inflammatory profiles.

Although the baseline lung function of BPD children was poorer compared with VLBW children without BPD and term controls, no correlations were found between the current inflammatory markers and lung function in school-aged BPD children. The reduced lung function is thus apparently due to permanent structural damage.

Nutritional requirements were poorly met in our VLBW survivors. Healthier eating may modulate the later disease risk. It would be worth studying whether dietary counselling of families from early childhood affects dietary habits, adipokine concentrations and later incidence of metabolic disease in VLBW survivors. The role of excessive inflammatory marker levels associated with increased BMI in the later lung function of BPD survivors remains to be seen.

We found normal cardiac function in school-aged BPD survivors undergoing comprehensive echocardiographic studies. Cardiac magnetic resonance imaging could provide a more accurate noninvasive method to evaluate the right ventricle and assess whether BPD survivors have fibrotic changes in the heart. Spiroergometry could provide more information on cardiac function in relation to lung function. Age-related deterioration in lung function may be faster in BPD survivors, which may affect their cardiac function. Studies with longer follow-up into adulthood are needed.

7 Conclusions

The following conclusions can be drawn on the basis of our studies:

1. School-aged children with severe radiographic BPD do not evince higher levels of inflammatory markers than VLBW or term controls. Other perinatal factors, including chorioamnionitis and neonatal sepsis, may modulate inflammatory responsiveness in VLBW children up to school age.
2. Poorer lung function in school-aged BPD survivors is not associated with inflammation.
3. Plasma adipokine levels correlate with nutrient intake and body mass index standard deviation score in school-aged VLBW children. Thus, VLBW children are likely to benefit from healthy eating habits.
4. School-aged BPD children evince normal cardiac function compared with VLBW and term peers. Poorer lung function may affect echocardiographic parameters.

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Original publications

Inflammatory Activity at School Age in Very Low Birth Weight Bronchopulmonary Dysplasia Survivors

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Summary. Objective: Airway inflammation is involved in the pathogenesis of bronchopulmonary dysplasia (BPD). The aim of the study was to evaluate the inflammatory activity in plasma and exhaled air in very low birth weight (VLBW) BPD survivors at school age. Methods: Twenty-one 6–14-year-old former VLBW (birth weight $\leq 1,500$ g) children with severe radiographic BPD (radBPD), 19 without radBPD (nonBPD group) and 19 non-asthmatic term controls underwent measurement of eosinophil cationic protein, IL-6, IL-8, adiponectin, adipsin, leptin, and resistin in plasma, leukotriene B₄ and 8-isoprostane in exhaled breath condensate, and NO in exhaled breath. Background data were obtained from patient records, clinical examination and parental questionnaire. Both univariate and multivariate models were applied in the statistical analysis. Results: There were no significant differences between the groups in any of the inflammatory markers measured. Five (25%) radBPD and 2 (11%) nonBPD children reported asthma ($P=0.058$). In logistic regression analysis, exposure to chorioamnionitis was associated with low IL-8 (OR 29.0, 95% CI 3.27–258) and postnatal corticosteroid therapy with high adiponectin (OR 32.0, 95% CI 1.29–793). High body mass index standard deviation score (BMI-SDS) was associated with high plasma adipsin (OR 2.47, 95% CI 1.07–5.75) and leptin (OR 5.76, 95% CI 1.83–18.2) levels. Conclusions: The inflammatory activity seems to decrease by school age in VLBW BPD survivors. Chorioamnionitis and postnatal corticosteroid treatment may modulate the inflammatory responsiveness in VLBW subjects even up to school age. The respiratory outcome in VLBW infants might be improved by preventing excessive weight gain. **Pediatr Pulmonol.**

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Key words: adipokine; body mass index; exhaled breath; interleukin; nitric oxide; premature infant.

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Conflict of interest: None.

Preliminary results of the study have been presented in the Pediatric Academic Societies Meeting in Washington D.C. May 7th 2013.

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INTRODUCTION

Airway inflammation is one of the pathogenic factors in bronchopulmonary dysplasia (BPD). Previous studies have reported increased levels of inflammatory markers in the serum,¹ tracheobronchial fluid² or exhaled breath^{3,4} in neonates who have later developed BPD. At school age, former BPD patients present with an increased risk of respiratory problems such as asthma and asthma-suggestive symptoms.^{5,6} However, the response to inhaled corticosteroids in former BPD children is not as favorable as in other children with asthma.⁷ In BPD, in contrast to asthma, the early mostly antenatal inflammation evolves into chronic inflammation and airway remodeling during childhood.⁸ Antenatal inflammation can also affect normal lung growth and development.⁹

Cytokines such as interleukines (IL) modulate inflammatory and immune responses through pro- and anti-inflammatory properties. T-helper (Th) lymphocytes are the major source of cytokines, and can be subdivided into Th1 and Th2 subgroups. IL-6 and IL-8 are regarded as Th1-type cytokines. The Th2-type cytokines include IL-4, IL-5, and IL-13, which are associated with the promotion of immunoglobulin (Ig)E-mediated and eosinophilic responses.¹⁰ Eosinophil cationic protein (ECP) is secreted by activated eosinophil granulocytes. Increased ECP in serum in infancy predicts childhood asthma.¹¹

Adipokines such as leptin, adiponectin, adipisin, and resistin are protein mediators secreted by adipocytes and macrophages. Leptin, adipisin, and resistin have pro-inflammatory but adiponectin has mainly anti-inflammatory properties. Leptin production is elevated in obese subjects and obesity has many features of a low-level chronic inflammatory condition.¹² Accordingly, adipokines may have a role in asthma. In children, high serum leptin and low serum adiponectin have been associated with exercise-induced bronchial hyperreactivity¹³ and asthma.¹⁴

Analysis of exhaled breath condensate (EBC) seems a promising noninvasive means of evaluating airway inflammation. Increased levels of leukotrienes (LT) and 8-isoprostane have been reported in the EBC of asthmatic children.¹⁰

Exhaled nitric oxide (eNO) is a marker of eosinophilic airway inflammation in asthma. At school age, eNO levels have been similar^{15,16} or even lower¹⁷ in former VLBW children with BPD than in controls. Currently available methods allow differentiation of bronchial and alveolar NO output and thereby peripheral and central airway inflammation.¹⁸

The aim of the present study was to evaluate the inflammatory status in school-aged BPD survivors by measuring inflammatory mediators in plasma and exhaled breath in former VLBW (birth weight $\leq 1,500$ g) infants with and without severe radiological BPD. Our hypothe-

sis was that the inflammation present in infancy when BPD is active, attenuates by school age in BPD survivors.

MATERIALS AND METHODS

The hospital records of VLBW infants treated in Tampere University Hospital between January 1st, 1995, and April 13th, 2003, were abstracted for BPD diagnosis, defined by radiographic findings in chest X-rays and need for O₂ supplementation at 36 weeks' corrected gestational age. Cases with severe cystic radiographic BPD, Northway grade III–IV,¹⁹ confirmed by a pediatric radiologist, were eligible to the study. Infants with severe congenital anomalies were excluded. Ten of the 31 eligible children refused to participate. Thus, 21 cases with cystic radiographic findings constituted the radiological BPD (radBPD) group. Nineteen former VLBW infants without radiological BPD findings, matched for sex and year of birth, and for birth weight as well as possible formed the nonBPD group, and 19 term-born age- and sex-matched non-asthmatic children the term control group. There was no significant difference in birth weight or gestational age between the children who participated and those who did not. The birth weights of the non-participants ranged from 605 to 1,440 grams and the gestational ages from 26 to 30 weeks.

The children were recruited at 6–14 years of age. Perinatal and neonatal data were obtained from hospital records. Chorioamnionitis was an obstetric clinical diagnosis including elevated C-reactive protein and fever in the mother. Prenatal corticosteroid therapy included a 2-day course of betamethasone or dexamethasone, followed by weekly repeated doses in selected cases. Infants with a birth weight more than 2 SD below the average for gestational age and sex were considered as small for gestational age (SGA).²⁰ Patent ductus arteriosus was recorded if treated with indomethacin or surgical ligation. Surfactant was administered as rescue therapy for respiratory distress syndrome (RDS). Intravenous corticosteroids were administered based on the decision of the neonatologist in order to wean the infant from the ventilator. The oxygen (O₂) saturation targets were set at 90–94%, and in the case of pulmonary hypertension at 95–97%.

During the study visit, a pediatrician (TK) performed a clinical examination. Weight and height were measured and body mass index standard deviation scores (BMI-SDS) calculated using Finnish age- and sex-specific population-based references.²¹ Overweight and obesity were defined as BMI-SDS >1.16 and >2.10 in girls, and as BMI-SDS >0.78 and >1.70 in boys.²¹ The parents completed a questionnaire on the family background and medical history of the child, modified from the International Study of Asthma and Allergies in Childhood questionnaire.²² Socioeconomic status (SES) was assessed according to the occupations of the parents.²³

The Ethics Committee of the hospital district approved the study. Informed written consent was obtained from the parents.

Plasma Samples

Plasma samples were stored at -70°C until analyzed. Plasma concentrations of IL-6, IL-8, ECP, adiponectin, adipin, resistin, and leptin were determined by enzyme immunoassay (EIA) using commercial reagents (IL-6: PeliPair ELISA, Sanquin, Amsterdam, the Netherlands; IL-8: Opt EIA BD Biosciences, Erembodegem, Belgium, ECP: ECP ELISA kit, MBL International, Woburn, MA; adiponectin, adipin, and leptin: DuoSet ELISA, R&D Systems Europe Ltd, Abingdon, UK). Serum total IgE was determined up to November 2009 nephelometrically using the Siemens N Latex IgE mono assay (Marburg, Germany), and thereafter by an electrochemiluminescence immunoassay ECLIA IgE II test (Roche Diagnostics, Mannheim, Germany) using a Roche Cobas e601 analyzer. According to the reference values of the hospital laboratory, serum total IgE exceeding 90 IU/L in children 6–9 years of age and 200 IU/L in children 10–15 years of age was regarded as elevated.

Exhaled Breath Condensate

Exhaled breath condensate was collected during 15 min of tidal breathing with an Ecoscreen condenser (Jaeger, Würzburg, Germany), the subject wearing a nose clip. The samples were stored at -70°C until LTB₄ and 8-isoprostane concentrations were measured by EIA (Cayman Chemical Company, Ann Arbor, MI).

Exhaled NO (eNO) Measurement

NO concentrations in exhaled air were measured with a Sievers NOA 280 analyzer (Sievers Instruments, Boulder, CO) at four exhalation flow rates (50, 100, 200, and 300 ml/sec), as previously described.¹⁸ NO output (product of eNO concentration and exhalation flow rate) was calculated and plotted against the exhalation flow rate. A regression line was set to correlate these variables, alveolar NO concentration and bronchial NO flux constituting the slope and intercept of the regression line, respectively.²⁴ The alveolar NO concentration reflects NO dynamics in the peripheral lung (from respiratory bronchioles to alveoli), and bronchial NO flux the NO dynamics in central conducting airways.

Statistical Analyses

Statistical analyses were made using SPSS for Windows statistical software version 18.0. *P* values less than 0.05 were considered statistically significant.

T-test was used for normally distributed, Mann–Whitney *U*-test or Kruskal–Wallis test for non-normally

distributed, and Chi-square test or Fisher's exact test for categorical variables. Logistic regression (Enter) analysis was used to find predictors for high (highest quartile) and low (lowest quartile) concentrations of inflammatory markers among VLBW children. First, a univariate analysis was made. To optimize the number of variables, adjusting variables were then entered into the analysis in two categories: (1) male gender, birth weight, chorioamnionitis, postnatal systemic corticosteroids, sepsis, ventilator therapy, and radBPD, and (2) BMI-SDS, hairy pets, tobacco smoke exposure at home and atopic tendency in the family. The results were expressed as odds ratios (OR) and 95% confidence intervals (CI).

RESULTS

The radBPD group presented with a shorter gestational age, lower birth weight, more RDS, and longer postnatal corticosteroid therapy than the nonBPD group (Table 1). Among term controls the mean (SD) gestational age was 39.5 (1.7) weeks and the mean (SD) birth weight 3,499 (610) g.

After the discharge from hospital, use of inhaled corticosteroids and hospital admissions due to respiratory symptoms were most common in the radBPD group (Table 2). The radBPD children were most exposed to tobacco smoke at home and had lowest SES, whereas the non-asthmatic term controls had least hairy pets at home, were least exposed to tobacco smoke and had highest SES (Table 2). BMI-SDS did not differ between the radBPD, nonBPD and term groups [median (range) -0.69 (-2.72 – 2.48), -0.48 (-2.36 – 1.52), and -0.34 (-1.59 – 1.18), respectively]. Overweight was equally common in the radBPD, nonBPD, and term groups [3 (14%), 3 (16%), and 2 (14%)]. One of the eight overweight children was obese. Serum IgE exceeded the upper normal limit in 6 (29%) radBPD, in 3 (16%) nonBPD and in 3 (16%) term children ($P = 0.556$).

The concentrations of inflammatory markers in plasma, EBC or exhaled breath did not differ between the radBPD, nonBPD, and term groups (Table 3). The six subjects with physician-diagnosed asthma had lower 8-isoprostane concentrations in EBC than those 50 without asthma [median (range) 0.2 (0.2–3.6) vs. 2.0 (0.2–28.6) pg/ml, $P = 0.042$]. Asthmatics and non-asthmatics did not differ with respect to any of the other inflammatory markers.

The statistically significant results in logistic regression analysis of the high inflammatory marker levels (highest quartile) among VLBW children are presented in Table 4. Postnatal systemic corticosteroid therapy was associated with high adiponectin. Higher BMI-SDS was associated with high adipin and leptin, and tended to increase the risk of high bronchial NO. In addition, having hairy pets at home was associated with reduced risk of high ECP and IL-8, and chorionamnionitis with reduced risk of high

TABLE 1— Characteristics of Very Low Birth Weight Infants With and Without Radiological BPD During First Hospitalization

	RadBPD (n = 21)	NonBPD (n = 19)	P-value
Gestational age (weeks), mean (SD)	26.6 (1.6)	28.9 (1.8)	<0.001
Birth weight (g), mean (SD)	919 (252)	1,198 (222)	<0.001
SGA, n (%)	2 (10)	4 (21)	0.106
Male sex, n (%)	14 (67)	11 (58)	0.567
Preeclampsia, n (%)	2 (10)	4 (21)	0.398
Chorioamnionitis, n (%)	11 (55)	13 (68)	0.514
Smoking during pregnancy, n (%)	9 (43)	3 (16)	0.062
Prenatal corticosteroid, n (%)	17 (94)	18 (100)	1.000
Number of prenatal corticosteroid doses, MD (range)	2 (0–5), n = 16	2 (1–5), n = 18	0.297
Prenatal antibiotics, n (%)	15 (79)	14 (74)	1.000
Sepsis, n (%)	8 (38)	1 (5)	0.021
Ventilator therapy, n (%)	19 (91)	11 (58)	0.017
Duration of ventilator therapy (d), MD (range)	24 (0–271)	1 (0–5)	<0.001
Surfactant, n (%)	14 (67)	9 (47)	<0.001
Postnatal systemic corticosteroids, n (%)	14 (67)	2 (11)	<0.001
Duration of corticosteroid therapy (days), MD (range)	9 (0–29), n = 19	0 (0–4)	0.002
Diuretic therapy at least for 7 days, n (%)	15 (75)	2 (11)	<0.001
PDA treatment, n (%)	6 (29)	5 (26)	0.873
Duration of O ₂ therapy (d), MD (range)	73 (50–730)	11 (1–74)	<0.001
O ₂ therapy at 36 weeks' corrected gestation, n (%)	17 (76)	2 (11)	<0.001

Differences between groups were tested by Mann–Whitney test, *t*-test, Chi-square test or Fisher's exact test. SGA, small for gestational age; PDA, patent ductus arteriosus; O₂, oxygen.

TABLE 2— History of Asthma and Family Background in Very Low Birth Weight Infants With and Without Radiological BPD and Term Controls

	RadBPD (n = 21), n (%)	NonBPD (n = 19), n (%)	Term (n = 19), n (%)	P-value
Asthma diagnosed by a physician	5 (25)	2 (11)	0 (0)	0.058
Inhaled corticosteroid after hospital discharge	11 (55)	6 (32)	0 (0)	0.001
Inhaled corticosteroid in the past 12 months	2 (10)	0 (0)	0 (0)	0.323
Hospitalization due to respiratory symptoms after hospital discharge	8 (38)	4 (21)	1 (5)	0.030
Atopy or asthma in the family	14 (67)	10 (53)	9 (47)	0.442
Hairy pets ever	13 (68)	17 (90)	7 (37)	0.004
Exposure to tobacco smoke at home	13 (62)	9 (47)	4 (21)	0.032
High SES (employer/own account worker, upper- or lower-level white collar worker)	7 (33)	10 (56), n = 18	17 (90)	<0.001

Differences between groups were tested by Pearson Chi-square or Fisher's test. SES, socioeconomic status.

adiponectin and alveolar NO (Table 4). The statistically significant results in logistic regression analysis of low inflammatory marker levels (lowest quartile) among VLBW children are presented in Table 5. Chorioamnionitis was associated with low IL-8, and exposure to tobacco smoke with low 8-isoprostane. In addition, atopy in the family was associated with less risk of low IL-6, and postnatal systemic corticosteroids were associated with less risk of low adipsin (Table 5).

DISCUSSION

The main result of the study was that school-aged VLBW children with severe radiographic BPD did not present with increased inflammatory marker levels in

plasma or exhaled breath compared to VLBW children without BPD or non-asthmatic term-born controls. Inflammation plays a role in the pathogenesis of BPD but, according to our hypothesis, the inflammatory activity attenuated in BPD survivors by school age. Perinatal factors such as postnatal corticosteroid therapy and chorioamnionitis had some associations with inflammatory markers in plasma, the former with high adiponectin and the latter with low IL-8. In addition, some evidence was found for the role of excessive weight gain, since age- and sex-specific BMI was associated with high adipsin and leptin in plasma.

In this study, inflammatory markers in plasma or EBC or NO in exhaled breath were not associated with radBPD, although 25% of radBPD and 17.5% of all VLBW

TABLE 3—Inflammatory Markers in Plasma, Exhaled Breath Condensate and Exhaled Breath in Very Low Birth Weight Children With and Without Radiological BPD and Term Children

	RadBPD (n = 21), MD (range)	NonBPD (n = 19), MD (range)	Term (n = 19), MD (range)	P-value
Plasma				
ECP (pg/ml) ¹	2.4 (0.1–6.8)	2.2 (0.1–3.0)	2.3 (0.1–3.6)	0.432
IL-8 (pg/ml) ¹	2.4 (0.8–5.5)	3.4 (0.8–8.1)	3.1 (0.8–8.7)	0.812
IL-6 (pg/ml) ¹	1.1 (0.2–4.8)	1.0 (0.2–12.2)	1.4 (0.2–14.2)	0.563
Adiponectin (ng/ml)	3,300 (1,866–5,316)	3,280 (2,129–5,616)	3,018 (1,192–5,887)	0.578
Adipsin (ng/ml)	890 (556–1,409)	826 (483–1,140)	826 (598–1,363)	0.423
Leptin (pg/ml)	2,903 (532–49,992)	6,217 (765–37,691)	2,201 (503–24,013)	0.169
Resistin (pg/ml)	2,764 (1,458–4,120)	2,452 (1,529–4,736)	2,741 (1,460–7,217)	0.307
Breath condensate				
8-isoprostane (pg/ml) ¹	1.5 (0.2–11.5), n = 19	1.0 (0.2–28.6)	3.5 (0.2–10.4)	0.178
LTB4 (pg/ml) ¹	1.0 (1.0–30.5), n = 19	6.4 (1.0–35.3)	8.9 (1.0–35.7)	0.136
Exhaled breath				
FENO50 (ppb)	8.2 (4.40–21.30), n = 16	8.7 (4.70–32.1), n = 18	7.1 (4.0–35.1), n = 18	0.976
Alveolar NO (ppb)	2.2 (1.9–3.0), n = 14	2.5 (1.2–4.7), n = 16	2.3 (1.2–7.2), n = 18	0.319
Bronchial NO (ppb)	0.27 (0.05–0.87), n = 14	0.27 (0.09–1.59), n = 16	0.29 (0.01–1.45), n = 18	0.900

Differences between groups were tested by Kruskal–Wallis test.

ECP, eosinophilic cationic protein; IL, interleukin; LTB4, leukotriene B4; NO, nitric oxide; FENO50, fractional exhaled NO at 50 ml/s

¹Levels below the detection limit were coded as half of the detection limit.

TABLE 4—Significant Predictors for High Inflammatory Marker Levels (Highest Quartile) According to Logistic Regression Analysis in Very Low Birth Weight Children

	Univariate analysis			Multivariate analysis		
	P-value	OR	95% CI	P-value	OR	95% CI
ECP						
Chorioamnionitis no/yes	0.042	0.23	0.06–0.95			
Hairy pets ever no/yes	0.045	0.18	0.03–0.96	0.037	0.14	0.02–0.89 ¹
IL-8						
Hairy pets ever no/yes	0.100	0.25	0.05–1.30	0.033	0.11	0.01–0.83 ¹
Adiponectin						
Chorioamnionitis no/yes	0.010	0.13	0.03–0.61	0.019	0.09	0.01–0.67 ²
Systemic corticosteroid no/yes	0.030	5.00	1.17–21.4	0.034	32.0	1.29–793 ²
Adipsin						
Systemic corticosteroid no/yes	0.003	14.1	2.45–81.6			
Cystic BPD no/yes	0.033	6.38	1.16–34.9			
BMI-SDS	0.067	1.82	0.96–3.46	0.035	2.47	1.07–5.75 ¹
Leptin						
BMI-SDS	0.002	4.13	1.65–10.31	0.003	5.76	1.83–18.2 ¹
Bronchial NO						
BMI-SDS	0.053	2.50	1.65–10.3	0.080	2.60	0.89–7.58 ¹
Alveolar NO						
Chorioamnionitis no/yes	0.049	0.15	0.02–0.99	0.038	0.03	0.001–0.82 ²

ECP, eosinophilic cationic protein; IL, interleukin; BMI-SDS, body mass index standard deviation score; NO, nitric oxide.

¹Multivariate analysis adjusted for atopy or asthma in the family (no/yes), hairy pets ever (no/yes), exposure to tobacco smoke at home (no/yes), BMI-SDS.

²Multivariate analysis adjusted for birth weight (g), male gender (no/yes), chorioamnionitis (no/yes), postnatal sepsis (no/yes), ventilator therapy (no/yes), postnatal systemic corticosteroid therapy (no/yes), cystic BPD (no/yes).

children had asthma. In line, eNO concentrations have not been elevated in prematurely born schoolchildren with a history of BPD compared to those without.^{15,16} In another study, school-aged BPD survivors evinced lower eNO levels than preterm children without BPD and term-born children with and without asthma,¹⁷ regardless of the

similar degree of spirometric airflow limitation in the BPD survivors and asthmatic children. The airflow reduction in the BPD children was not reversible, suggesting that structural changes in the airways rather than ongoing inflammation may be the cause of the symptoms. Our finding of low EBC 8-isoprostane and

TABLE 5—Significant Predictors for Low Inflammatory Marker Levels (Lowest Quartile) According to Logistic Regression Analysis in Very Low Birth Weight Children

	Univariate analysis			Multivariate analysis		
	<i>P</i> -value	OR	95% CI	<i>P</i> -value	OR	95% CI
IL-6						
Atopy in the family no/yes	0.046	0.21	0.05–0.97	0.017	0.07	0.01–0.62 ¹
IL-8						
Chorioamnionitis no/yes	0.003	13.0	2.34–72.1	0.003	29.0	3.27–258 ²
Adipsin						
Systemic corticosteroid no/yes	0.049	0.11	0.01–0.99	0.016	0.01	0.00–0.45 ²
Resistin						
Birth weight (g)	0.018	1.01	1.00–1.01			
8-isoprostane						
Male gender no/yes	0.045	4.28	1.04–17.7			
Exposure to tobacco smoke no/yes	0.023	5.25	1.26–21.8	0.028	7.11	1.23–41.0 ¹

IL, interleukin.

¹Multivariate analysis adjusted for atopy or asthma in the family (no/yes), hairy pets ever (no/yes), exposure to tobacco smoke at home (no/yes), body mass index standard deviation score.

²Multivariate analysis adjusted for birth weight (g), male gender (no/yes), preeclampsia (no/yes), postnatal sepsis (no/yes), ventilator therapy (no/yes), postnatal systemic corticosteroid therapy (no/yes) and cystic BPD (no/yes).

normal exhaled NO in former VLBW children with asthma supports this conception.

Chorioamnionitis was associated with low plasma IL-8, a Th-1-type proinflammatory cytokine. Indirect evidence was found that chorioamnionitis may even protect from high ECP and alveolar NO responses. Low expression of IL-8-promoting genes at school age may be the result of immunologic hypo-responsiveness induced by chorioamnionitis during pregnancy. Antenatal inflammation has led to immunologic hypo-responsiveness increasing susceptibility to postnatal infections in preterm infants⁹ and to respiratory infections during the first years of life.⁶ On the other hand, down-regulation of the genes controlling inflammation may protect from permanent tissue injury.⁹

There was an association between neonatal systemic corticosteroid therapy and high anti-inflammatory adiponectin concentrations. Postnatal corticosteroids were administered only to severely lung-injured ventilator-dependent patients. Thus, our finding may be associated with a direct anti-inflammatory influence of corticosteroids, or with early severe lung damage itself.

Interestingly, higher relative weight as assessed by BMI-SDS was associated with high pro-inflammatory markers leptin and adipsin in plasma, and tended to be associated with high bronchial NO. Overweight was not common in the VLBW cases, which means that the associations revealed must be fairly strong. In other studies, overweight was not associated with increased eNO in 10–16-year-old²⁵ or 6–18-year-old²⁶ school-children. Overweight has been found to represent systemic inflammation, attributable to overproduction of adipokines and induction of free fatty acid release in

response to TNF- α in both macrophages and adipocytes.¹² Thus, inflammation-induced airway injury, while having its origin in the neonatal period, may progress in obese children.

In our study exposure to tobacco smoke at home was associated with low 8-isoprostane levels. In contrast with a previous study,²⁷ no effect on eNO was found. In vitro, tobacco smoke has reduced the capacity of alveolar macrophages to synthesize and release prostaglandins and LTB₄.²⁸ However, passive smoking has increased the risk of respiratory symptoms in preterm children at 6 years of age,⁵ and is a well-known risk factor for asthma.²⁹

Our results should be interpreted with caution. The study was limited by the small sample size from a single center. On the other hand, only 22 subjects in each group would have been needed to demonstrate a between-group difference equal to 1 standard deviation (effect size of 1.0) at the 5% alpha error and 90% statistical power. It is possible that due to under-powering, some associations though in fact present, were not detected. Many confidence intervals were wide, especially in adjusted analyses. On the other hand, all participants were initially treated in the same hospital with uniform intensive care protocols. Due to small number of eligible patients and the enrichment of BPD cases in the most premature newborns, matching for birth weight was not optimal between the BPD and nonBPD cases. Some of the data were collected retrospectively. At the start of the follow-up, BPD was diagnosed based on both the need for O₂ supplementation and radiological criteria. Currently, the consensus criteria include duration of O₂ therapy, and the O₂ reduction test is used to assess the severity of BPD.³⁰ Chorioamnionitis was diagnosed without placental

histology. Information on physician-diagnosed asthma was obtained from the questionnaire and patient records.

On the other hand, the inflammatory markers measured covered several inflammatory mechanisms and both systemic and pulmonary inflammatory responses. Further, the eNO analyzing methods applied account for both central and peripheral NO output. The large number of markers measured may be of advantage, since the inflammatory response in BPD is multi-factorial. Multiple testing may result in incidental findings, which we controlled by using multivariate analyses. Thus the findings, although to our knowledge unique, should be regarded as preliminary.

In conclusion, school-aged former VLBW children with severe radiographic BPD do not evince higher levels of inflammatory markers in plasma or exhaled breath than nonBPD VLBW or non-asthmatic term controls. Other perinatal factors, including chorioamnionitis and postnatal corticosteroid treatment may induce changes reflected as altered expression of inflammatory genes even up to school age. Higher BMI-SDS was associated with higher levels of pro-inflammatory markers, suggesting that prevention of excessive weight gain might improve the respiratory outcome in VLBW infants.

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REGULAR ARTICLE

Very low birthweight bronchopulmonary dysplasia survivors show no substantial association between lung function and current inflammatory markers

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Adipokines, Bronchopulmonary dysplasia, Impulse oscillometry, Inflammation, Lung function, Very low birthweight

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ABSTRACT

Aim: The role of inflammation in the bronchopulmonary dysplasia (BPD) survivors is indistinct. We evaluated lung function in relation to inflammatory markers in plasma, exhaled breath condensate and exhaled air in school-aged very low birthweight (VLBW) survivors with and without radiographic BPD.

Methods: Pre- and postbronchodilator impulse oscillometry were performed by 21 six to 14-year-old VLBW children with radiographic BPD, 19 VLBW children without radiographic BPD and 19 age-matched nonasthmatic term controls. Eosinophilic cationic protein, interleukins 6 and 8, adiponectin, adipisin, leptin and resistin in plasma, leukotriene B4 and 8-isoprostane in exhaled breath condensate, and bronchial and alveolar nitric oxide output were measured.

Results: Abnormal lung function was found in 12.5% of the former VLBW children. Airway resistance at 5 Hz was highest in the radiographic BPD, but bronchodilator responses were most prominent in the non-BPD group. Plasma adiponectin had a modest positive correlation with obstruction and with bronchodilator responses, and alveolar nitric oxide and plasma interleukin 6 with bronchodilator responses.

Conclusion: Very low birthweight children with radiographic BPD had poorest lung function. The most pronounced bronchodilator responses were found in VLBW children without radiographic BPD. Current detected inflammatory markers had only a minor association with lung function in school-aged BPD survivors.

INTRODUCTION

Inflammation has an active role in the emergence of bronchopulmonary dysplasia (BPD) (1). Premature and very low birthweight (VLBW) infants (birthweight <1500 g) have presented with lower lung function at school age and in adolescence than term infants, and BPD survivors have presented with lower lung function than other preterms (2–7).

Impulse oscillometry (IOS) is a validated technique which allows evaluation of lung function during spontaneous tidal breathing (8). Impedance can be divided into resistance and reactance. Resistance is composed of both

central and peripheral airway resistance and increases in airway obstruction. Reactance decreases in restriction, hyperinflation and obstruction. The resonance frequency

Abbreviations

BMI-SDS, Body mass index standard deviation score; BPD, Bronchopulmonary dysplasia; Hz, Hertz; IL, Interleukin; IOS, Impulse oscillometry; SD, Standard deviation; VLBW, Very low birthweight.

Key notes

- Inflammation plays an important role in the development of bronchopulmonary dysplasia (BPD), but the association of inflammation with lung function is not known in BPD survivors.
- Abnormal lung function was found in 12.5% of the very low birthweight children at school age, but among 12 inflammatory markers measured, only plasma adiponectin had modest correlation with reduced lung function.
- Current inflammation had no substantial association with lung function in school-aged BPD survivors.

increases in obstruction and restriction (9). The most frequent lung function abnormality in school-aged prematurely born children has been airway obstruction (2,10,11).

How long and how much airway inflammation influences lung function in BPD survivors after infancy? Recently, we published our results on inflammatory markers in school-aged VLBW BPD survivors compared to term and VLBW controls without BPD in infancy (12) and concluded that airway inflammation, although present in infancy, had attenuated before school age.

The aim of this study, conducted in the same study population, was to evaluate lung function and respiratory symptoms in relation to inflammatory markers in plasma, exhaled breath condensate and exhaled air in school-aged VLBW survivors with and without cystic radiographic BPD. The hypothesis was that VLBW children with severe BPD have more respiratory symptoms and poorer lung function than VLBW children without BPD, but respiratory symptoms and lung function are not associated with the current rate of inflammation.

PATIENTS AND METHODS

The BPD group consisted of 21 VLBW children with severe cystic radiographic BPD of Northway grade III–IV (13). The age-matched control groups included 19 VLBW children without cystic radiographic BPD (non-BPD group) and 19 term-born nonasthmatic children (term group). The children were studied at six to 14 years of age.

Background data collected from hospital records and parental questionnaires have been published recently (12). Birthweight [mean (SD) 919 g (252) vs. 1198 g (222)] and use of prenatal antibiotics did not differ between the two VLBW groups. The children in the radiographic BPD group had shorter gestational age [mean (SD) 26.6 weeks (1.6) vs. 28.9 weeks (1.8)], more septic infections during primary hospitalisation and longer duration of oxygen therapy [median (range) 73 days (50–730) vs. 11 days (1–74)] and needed postnatal corticosteroids more often than VLBW controls. In the VLBW control group, two children needed supplemental oxygen until gestational age of 36+2 and 36+5 weeks representing moderate BPD and four children with severe radiological changes did not need oxygen supplementation at a gestational age of 36 weeks representing mild BPD according to the current classification (14). High socio-economic status was seen in 17 of 40 (42.5%) VLBW and 17 of 19 (89.5%) term children, and exposure to tobacco smoke at home in 22 of 40 (55.0%) and four of 19 (21.1%) children, respectively. Doctor-diagnosed asthma was found in five (24%) children in the radiographic BPD group and two (11%) in the non-BPD group. Respiratory symptoms, including wheezing, cough over 4 weeks, respiratory infections, hospitalisations due to respiratory symptoms and use of inhaled corticosteroids or β_2 -agonists were registered.

Clinical examination was conducted by a paediatrician (TK). Laboratory examinations consisted of total serum immunoglobulin E, eosinophil cationic protein, interleukin

(IL) 6 and 8, adiponectin, adipisin, leptin and resistin concentrations in plasma, leukotriene B4 and 8-isoprostane concentrations in exhaled breath condensate and exhaled nitric oxide concentration, bronchial nitric oxide flux and alveolar nitric oxide concentration, as recently published (12). Fully informed consent was obtained from the parents. The study was approved by the Ethics Committee of the hospital district.

Lung function

Impulse oscillometry (Master Screen IOS; Jaeger, Höchberg, Germany) was performed before and 15 min after inhalation of 0.3 mg salbutamol (Ventoline[®], Glaxo-SmithKline, UK). Pre- and postbronchodilator impedance, resistance, reactance and resonance frequency were determined and are presented as percentages of height-related population-based reference values (% of predicted) (15–17). Baseline values of resistance and reactance at 5 Hz over ± 1.96 SD and a decrease of $\geq 36\%$ in impedance, $\geq 45\%$ in resonance frequency or $\geq 37\%$ in resistance at 5 Hz after salbutamol were considered clinically significant (16,17). The participants had been free of any respiratory infection for at least 6 weeks before IOS.

Statistics

Statistical analyses were carried out with SPSS (SPSS Inc. Released 2009. SPSS Statistics for Windows, Version 18.0., Chicago, USA: SPSS Inc.). *p* Values < 0.05 were considered statistically significant.

Differences between the groups were tested by independent-samples *t*-test or one-way analysis of variance for normally distributed and with Mann–Whitney *U*-test or Kruskal–Wallis test for non-normally distributed continuous variables, and by chi-square or Fisher's exact tests for categorised variables.

In VLBW children, the correlations between lung function parameters and inflammatory markers were studied using Pearson's correlation test for normally distributed and Spearman's correlation test for non-normally distributed variables.

Logistic regression analysis was used to find predictors for the worst quartiles of the lung function among VLBW children. After univariate analysis, the variables were entered simultaneously to the multivariate model in three categories (i) male gender, gestational age, prenatal antibiotics, postnatal corticosteroids, neonatal sepsis, ventilator therapy and cystic radiographic BPD, (ii) body mass index standard deviation score (BMI-SDS), hairy pets at home, exposure to tobacco smoke at home and atopy in the family and (iii) all the studied inflammatory markers. Positive total serum immunoglobulin E as a marker of atopy was tested separately.

RESULTS

There were no significant differences between the groups in respiratory symptoms, respiratory infections, needed treatments or atopic manifestations (Table 1).

Resistance at 5 Hz was highest in the radiographic BPD, next in the non-BPD and lowest in the control group (Table 2). Resistance and impedance responses in the bronchodilator test were most marked in the non-BPD, next in the radiographic BPD and lowest in the term control group (Table 2). Importantly, the results remained similar when children with doctor-diagnosed asthma were excluded from the analyses and when the current definition of BPD was used.

Asthmatics presented with higher resistance at 5 Hz than nonasthmatics in both the radiographic BPD group [mean (SD) 112% (25) vs. 87% (11), $p = 0.008$] and all VLBW children [median (range) 99% (63–144) vs. 81% (57–132), $p = 0.047$]. In contrast, there were no significant differences in bronchodilator responses between asthmatics and nonasthmatics. Lung function of atopic and nonatopic VLBW children did not differ significantly (data not shown).

Abnormal lung function was found in five (12.5%) VLBW children. Baseline bronchial obstruction was presented by one survivor of radiographic BPD and two non-BPD children. A significant bronchodilator response was seen

in one child with radiographic BPD and three non-BPD children, including the two non-BPD survivors with baseline obstruction. Thus, there was only one child with irreversible obstruction, suggesting permanent airway changes.

A significant correlation was found between adiponectin and higher resistance at 5 Hz (Table 3). In the bronchodilation test, a significant correlation was seen between adiponectin and the resistance, impedance and resonance frequency responses, between IL-6 and resonance frequency responses, and between alveolar nitric oxide concentration and resistance or impedance responses (Table 3). When the correlation coefficients were calculated including the radiographic BPD group only, no correlations were found.

In the logistic regression, hairy pets at home predicted a significant risk of high resistance at 5 Hz in the univariate (OR 6.29, 95% CI 1.24–31.96, $p = 0.027$) and multivariate (adjusted OR 7.15, 95% CI 1.04–49.00, $p = 0.045$) analyses. Logistic regression detected no significant associations between lung function and BPD defined radiologically or

Table 1 Respiratory morbidity and medical history of very low birthweight infants with and without cystic radiological bronchopulmonary dysplasia and term controls

	radBPD (n = 21)	non-BPD (n = 19)	Term (n = 19)	p
Respiratory symptoms in the past 12 months*, n (%)	8 (38)	5 (26)	3 (16)	0.308
Respiratory infection in the past 12 months, n (%)	21 (100)	17 (89)	14 (74)	0.884
Inhaled corticosteroid in the past 12 months, n (%)	2 (10)	0 (0)	0 (0)	0.323
Inhaled β_2 -agonist in the past 12 months, n (%)	1 (5)	0 (0)	0 (0)	1.000
Regular physical exercise in the past 12 months, n (%)	15 (71)	14 (74)	16 (84)	0.678
Symptoms during physical exercise in the past 12 months [†] , n (%)	3 (14)	6 (32)	1 (5)	0.055
Atopic status				
Positive IgE, n (%)	6 (29)	3 (16)	3 (16)	0.556
Allergic symptoms, n (%)	11 (52)	11 (58)	7 (37)	0.420
Atopic eczema, n (%)	3 (14)	5 (26)	4 (21)	0.643

BPD, Bronchopulmonary dysplasia.

Differences between groups were tested by Fisher's test.

*Respiratory symptoms, at least one of the following: wheezing, cough >4 weeks while having an infection, cough at night, shortness of breath when waking up, paroxysmal shortness of breath, visiting doctor because of breathing difficulties.

[†]Most common symptoms fatigue and palpitation.

Table 2 Impulse oscillometry results of very low birthweight children with or without radiological bronchopulmonary dysplasia and term children

	radBPD (n = 19)	non-BPD (n = 19)	Term (n = 19)	p
Rrs5Hz of predicted (%), median (range)	92 (65–144)	79 (63–132)	70 (57–120)	0.011*
Xrs5Hz of predicted (%), median (range)	92 (50–197)	80 (41–229)	69 (40–150)	0.137
Rrs5Hz br response (%), mean (SD)	–17 (9)	–21 (10)	–11 (11)	0.010**
Zrs5Hz br response (%), mean (SD)	–18 (9)	–22 (11)	–12 (11)	0.013**
Fr br response (%), median (range)	–22 (–55 to –3)	–24 (–39 to 3)	–14 (–47 to 50)	0.267

Rrs, Resistance; br, Bronchodilator; Xrs, Reactance; Zrs, Impedance; Fr, Resonance frequency; BPD, Bronchopulmonary dysplasia.

The mean values and standard deviations (SD) are given for parameters with normal distribution and median and range for non-Gaussian parameters.

Differences between the groups were tested by one-way ANOVA or Kruskal–Wallis test. When the difference was statistically significant ($p < 0.05$), the post hoc LSD test or Mann–Whitney *U*-test was performed. Bonferroni correlation was used. * $p < 0.05$ (radBPD vs term), ** $p < 0.05$ (non-BPD vs. term).

Table 3 Statistically significant correlation coefficients between the inflammatory markers and lung function parameters in very low birthweight children

	Adiponectin (n = 38)			IL-6 (n = 38)		Alveolar NO (n = 30)	
	r	ρ	p	ρ	p	r	p
Rrs5Hz % of predicted		0.45	0.005				
Br response of Rrs5Hz	-0.38		0.020			-0.41	0.023
Br response of Zrs5Hz	-0.41		0.010			-0.44	0.012
Br response of Fr		-0.47	0.003	-0.42	0.009		

IL-6, Interleukin 6; NO, Nitric oxide; Br, Bronchodilator; Rrs5Hz, Resistance at 5 Hz; Zrs5Hz, Impedance at 5 Hz; Fr, Resonance frequency.

Correlation coefficients determined by Pearson (r) or Spearman (ρ) correlation. Correlation coefficients more than 0.60 or <-0.60 mean a strong correlation, between 0.3–0.6 and -0.3 to -0.6 a modest correlation, and between 0–0.3 and -0.3 to 0 a low correlation.

according to the current criteria, or other neonatal factors, exposure to tobacco smoke at home, atopy in the family, high BMI-SDS, positive immunoglobulin E or inflammatory markers in children.

DISCUSSION

There were two main results in this study. First, BPD survivors had more impaired baseline lung function with less bronchial reversibility than VLBW children without a history of BPD. Second, no correlations between the inflammatory markers and lung function were seen in the radiographic BPD group, supporting our hypothesis that reduced lung function in BPD survivors at 6–12 years of age is not anymore associated with inflammation.

Evidence of abnormal lung function, in terms of baseline obstruction or increased bronchial reactivity documented with the bronchodilation test, was found in 12.5% of VLBW children. In a recent study, spirometry, plethysmography and exercise challenge test were performed by 151 school-aged children born at a gestational age of ≤ 32 weeks and 47% of them presented with bronchial obstruction, 11% with pulmonary restriction and 41% with exercise-induced bronchoconstriction (10). In another study with spirometry and plethysmography, bronchial obstruction was found in 45% and pulmonary restriction in 3% of the 11-year-old children born as extremely premature (11). IOS is not sufficiently sensitive to study pulmonary restriction (9), and therefore we probably missed some cases. Reactance at 5 Hz was lowest in term children and highest in children with radiographic BPD, suggesting that the elastic properties of the lungs in our BPD survivors with severely damaged lungs in infancy had in fact recovered well by school age.

Bronchodilator responses were most marked in the non-BPD group. This is in line with the conception that severe BPD is associated with a risk of permanent airway damage and lung function reduction and that prematurity and respiratory distress syndrome without BPD are associated with bronchial hyper-responsiveness. Preterm birth is an important risk factor for wheezing disorders throughout childhood (18). Previously, both increased (7) and inconsistent (2,19) bronchodilator responses have been found in school-aged BPD survivors compared to other preterms.

In our VLBW children, greater bronchodilator response assessed by resistance and impedance correlated with alveolar nitric oxide flux, but not with exhaled or bronchial nitric oxide concentrations. In previous Finnish studies, nonatopic prematurely born children with and without BPD had exhaled nitric oxide levels similar to term controls at school age (6,20), even in the presence of impaired lung function (6).

Although the VLBW children evinced impaired lung function compared to nonasthmatic term-born controls, they did not report more respiratory symptoms. The absence of an association between respiratory symptoms and lung function has been observed also previously (2,3,19). Thus, a scheduled long-term follow-up of lung function seems to be indicated in all VLBW survivors.

Adiponectin levels correlated with airway obstruction and with greater bronchodilator responses in VLBW children, but the correlations were only modest. The link between adiponectin and airway inflammation is not clear, and, although mainly anti-inflammatory, adiponectin also has pro-inflammatory effects under certain conditions (21,22). In a recent study, adults with chronic obstructive pulmonary disease had plasma adiponectin levels similar to healthy controls, but in patients with chronic obstructive pulmonary disease the adiponectin levels were associated 24–26 with more peripheral obstruction and more effect of inhaled steroid treatment (23). Interestingly, human airway smooth muscle cells have been found to express adiponectin receptors (24) and adiponectin has been reported to increase contractility in smooth muscle cells (25), and therefore adiponectin may contribute to airway obstruction.

Elevated circulating IL-6 levels have previously been associated with asthma. In the present study, plasma IL-6 had a negative correlation with the bronchodilator response assessed by resonance frequency, but no correlations were seen for responses assessed by resistance, reactance or impedance.

The results of the present study must be interpreted with caution, as the number of cases was small and the design of the study, in terms of neonatal factors, was retrospective. The degree of inflammatory activity was low. Thus, the negative results may be due to insufficient power of the study. On the other hand, multitesting might have been a problem in terms of positive results because as many as 12

inflammatory markers were studied. The role of inflammation during exacerbation was not studied. All VLBW neonates were mainly treated in the same hospital with uniform intensive care protocols, which is a clear benefit, although compared with multicentre studies, it reduces the number of eligible patients with a rare problem such as cystic radiographic BPD.

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