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CARDIOVASCULAR FUNCTION IN 6-YEAR-OLD CHILDREN BORN EXTREMELY PRETERM

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Cardiovascular function in 6-year-old children born extremely preterm

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To life

ABSTRACT

In western countries, approximately 6 to 12 per cent of all pregnancies end preterm, i.e., before 37 weeks of gestation. After the introduction of antenatal corticosteroids, surfactant therapy and neonatal intensive care, survival rates after preterm birth have increased markedly worldwide. While this progress is very welcome, concerns have been raised that preterm birth may carry increased risk for adult morbidity and shortened life-span due to conditions associated with aging, such as stroke and cardiovascular disease. This may be particularly important in populations with increasing overweight and sedentary.

This thesis aims at providing new knowledge on blood pressure, growth and dynamics of the arterial tree as well as cardiac structure and function in children born extremely preterm. Addressing these issues may help our current understanding of cardiovascular development after preterm birth, if and when cardiovascular follow-up is needed in childhood, and provide important clues on how lasting cardiovascular health after preterm birth may be promoted.

We studied a population based cohort EXPRESS (Extremely Preterm Infants in Sweden Study) consisting of children born between 2004 and 2007 at 22-26 weeks of gestation, and age and sex-matched controls born at term. At the age of 6½ years, we measured blood pressure and performed a comprehensive evaluation of cardiovascular structure and function using echocardiography. In addition, we measured lung function using spirometry.

The conduit and coronary arteries were of similar or smaller size in children born extremely preterm than in controls born at term, also after adjusting for body size. Reassuringly, the arterial tree had no signs of accelerated intima media thickening or premature arterial stiffening – two early markers of a predisposition for atheroma formation. Casual blood pressures were normal but higher in children born extremely than in their peers born at term. This finding could not be explained by fetal growth restriction, antenatal corticosteroid exposure or neonatal morbidity. Children born extremely preterm also exhibited a unique cardiac phenotype characterized by smaller left ventricles with altered systolic and diastolic functions than same-aged children born at term. Finally, reduced lung function or airway obstruction in children born extremely preterm at age of 6 ½ years did not predict right ventricular hypertrophy or pulmonary hypertension.

In conclusion, children born extremely preterm exhibit a cardiovascular phenotype that differ from same-aged peers born a term. The long term significance for cardiovascular health of these findings remains to be established.

LIST OF SCIENTIFIC PAPERS

I. **Mohlkert LA**, Hallberg J, Broberg O, Hellström M, Pegelow Halvorsen C, Sjöberg G, Edstedt Bonamy AK, Liuba P, Fellman V, Domellöf M and Norman M. **Preterm Arteries in Childhood: Dimensions, Intima-Media Thickness and Elasticity of The Aorta, Coronaries, Carotids in 6-Year-Old Children Born Extremely Preterm**. *Pediatr Res*. 2017 Feb;81(2):299-306. doi: 10.1038/pr.2016.212.

II. Edstedt Bonamy AK, **Mohlkert LA**, Hallberg J, Liuba P, Fellman V, Domellöf M, Norman M. **Blood Pressure in 6-Year-Old Children Born Extremely Preterm.** *J Am Heart Assoc*. 2017 Aug 1;6(8). pii: e005858. doi: 10.1161/JAHA.117.005858.

III. **Mohlkert LA**, Hallberg J, Broberg O, Rydberg A, Pegelow Halvorsen C, Liuba P, Fellman V, Domellöf M, Sjöberg G, Norman M. **The Preterm Heart in Childhood: Left Ventricular Structure, Geometry and Function Assessed by Echocardiography in 6-Year-Old Survivors of Periviable Births**. *J Am Heart Assoc*. 2018 Jan 20;7(2). pii: e007742. doi: 10.1161/JAHA.117.007742.2.

IV. **Mohlkert LA**, Sjöberg G, Rydberg A, Pegelow Halvorsen C, Thuvfesson E, Hallberg J, Domellöf M, Norman M. **Lung Function and Pulmonary Circulation in 6-Year-Old Survivors of Extremely Preterm Birth**. Submitted 2018

INNEHÅLL

LIST OF ABBREVIATIONS

AA	Abdominal aorta
AoV	Aortic valve
BPD	Bronchopulmonary dysplasia.
BSA	Body surface area
CA	Coronary artery
CCA	Common carotid artery
cIMT	Carotid intima media thickness
CO	Cardiac output
CTRL	Control
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
ECG	Electrocardiogram
EXPT	Extremely preterm
FEV1	Forced expiratory volume in one second
FMD	Flow mediated dilatation
FRC	Forced respiratory capacity
FVC	Forced vital capacity
GA	Gestational age
IUGR	Intra uterine growth restriction
IVH	Intraventricular hemorrhage
IVS	Intraventricular septum
LV	Left ventricle
LVM	Left ventricular mass
LVOT	Left ventricular outflow tract
MAPSE	Mitral annular plane systolic excursion
MRI	Magnetic resonance imaging
NEC	Necrotizing enterocolitis
PDA	Persistence of ductus arteriosus
PFO	Persistence of foramen ovale
PVL	Periventricular leucomalacia

PVR	Pulmonary vascular resistance
PW	Posterior wall
RDS	Respiratory distress syndrome
ROP	Retinopathy of prematurity
RWT	Relative wall thickness
RVOT	Right ventricular outflow tract
SBP	Systolic blood pressure
SV	Stroke volume
TAPSE	Tricuspid annular systolic excursion
TDI	Tissue Doppler imaging
TRV	Tricuspid regurgitant velocity
2D	two-dimensional
2DST	two-dimensional speckle tracking
2DSTE	two-dimensional speckle tracking echocardiography
VTI	Velocity time itnegral

1 INTRODUCTION

Although preterm birth still is a major cause of death on a global level, survival after preterm birth has increased significantly in many countries.[1] As a result, there are approximately 20 million children (0-18 years) born preterm only in Europe and North America, and the population of young adults born preterm is growing. This development has recently been associated with previously unknown adult health issues: several studies link preterm birth to an increased risk for a number of adverse conditions and morbidities in adulthood, such as later hypertension[2, 3], diabetes[4, 5], stroke[6-8], accelerated cardiac aging[9] and even early death from cardiovascular disease (CVD)[10].

Studies of cardiovascular structure and function in children born preterm may help clarifying how birth-related events interact with and predict childhood and adult outcomes. Immediately after preterm birth, growth of retinal microvessels[11] and of large arteries such as the carotids and aorta[12] slow down compared with fetal development. Long-term follow-up data suggest that this birth-related decrease in vascular growth velocity after preterm birth may be a persisting and generalized phenomenon[13-17]. Some reports but not all[14, 18-21] also suggest that, besides arterial narrowing, accelerated intima-media thickening[17, 22] and early loss of arterial elasticity[23, 24] may be involved in the causal pathway from preterm birth to CVD. More recently and supported by animal data, studies of the preterm heart have revealed altered myocardial performance already in infancy, increased myocardial collagen deposition, ventricular remodelling and hypertrophy in young adult life.

Even among infants born extremely preterm, i.e. before 28 weeks of gestation, survival is nowadays the most probable outcome[1]. Given that a decrease in gestational age has been associated with increasing cardiovascular risk in adult life[2, 10], the overall hypotheses in this thesis were that children born extremely preterm exhibit early signs of adverse arterial growth and premature vascular aging, as well as blood pressure elevation linked to altered cardiac structure and function.

2 BACKGROUND

2.1 PRETERM BIRTH

2.1.1 Definitions and epidemiology

The duration of a normal (term) human pregnancy is defined as 37- 42 weeks of gestation. Postterm is defined as birth >42 weeks and preterm is defined as birth before 37 weeks of gestation. In addition, preterm birth can be categorized as very preterm if delivery occurs <32 weeks of gestation and extremely preterm if delivery occurs <28 weeks of gestation[25].

In Sweden, approximately 120 000 infants have been born in recent year. Since the start of the Medical Birth Registry in 1973, 5-6% of all pregnancies have ended preterm (<37 weeks), about 1% very preterm and 0.3-0.4% have ended extremely preterm according to statistics from the National Board of Health and Welfare (www.socialstyrelsen.se). In many other countries, rates of preterm birth are higher than in Sweden. In USA, 10-12% of all deliveries occur preterm and in Europe, most countries report increasing proportions[26].

2.1.2 Risk factors for preterm birth

Preterm birth has different underlying mechanisms and causes: maternal, uteroplacental or fetal risk factors are presented in Table I. Two thirds of the preterm deliveries are spontaneous. Spontaneous preterm delivery often starts with preterm prelabor rupture of membranes, pPROM and ends with preterm labor. One third of preterm births are induced or medically indicated. Medical indications to induce preterm birth include maternal complications such as severe preeclampsia or fetal complications such as severe intrauterine growth restriction (IUGR), or combined maternal-fetal indication such as chorioamnionitis with maternal and fetal infection.

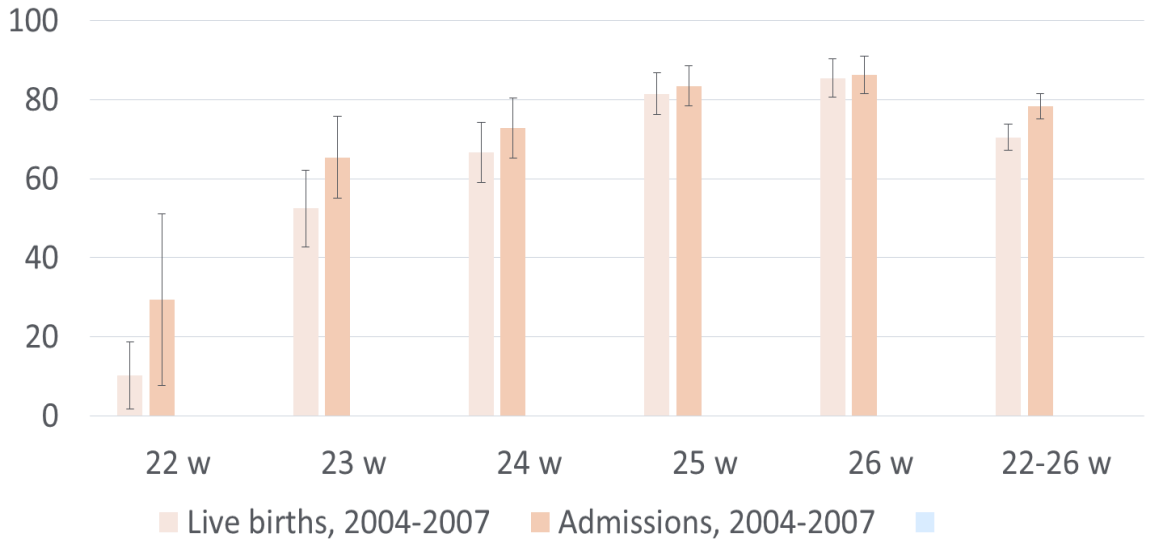
Table I. Risk factors for and causes of preterm birth

Maternal	Fetal	Obstetric
Low or advanced maternal age	Twins, triplets	Preeclampsia
Low education, low social status, single mother	Intrauterine growth retardation	Premature rupture of membranes
Smoking in pregnancy	Fetal distress	Preterm labor
Low or high body mass index	Chromosomal aberration	Chorioamnionitis
Obstetric history of previous miscarriage or stillbirth		Bleeding (placenta previa, ablatio)
Familial/common genetic factors		Assisted reproduction

2.1.3 Survival after very preterm birth

The focus of this thesis is on cardiovascular health in children born extremely preterm. Before 1980, almost all deliveries occurring before 28 weeks of gestation resulted in infant death. But after this time period, mortality after extremely preterm birth has gradually declined due to introduction of antenatal corticosteroids for lung maturation, surfactant therapy for respiratory distress syndrome (RDS) and development of modern neonatal intensive care. In Sweden, the mortality rate after very preterm birth is close to 5% and after extremely preterm birth it is nowadays below 20 %, Figure 1.

Figure 1. Infant survival (proportions with 95% confidence intervals) after extremely preterm birth (22-26 weeks of gestation) in Sweden 2004-2007. Data from the EXPRESS study[1]



2.1.4 The EXPRESS study

Entering the new millennium and following improved survival for infants born before 28 weeks of gestation, a growing interest about the prognosis of these "new survivors" evolved. Reviewing the literature, rates of neonatal complications were found to vary significantly, most likely reflecting rapid improvement in perinatal care, making data historic already a few years after publication. But also selection bias and a lack of population-based data contributed to a large variation in reported complication rates. After the neonatal period, there was only one report – the EPICURE-study from UK and Ireland - on developmental outcomes at 30 months of age showing that half of the children had some disability and that one on four had a severe disability[27].

Given this scarcity of data, a decision was taken to launch a national, population-based cohort study named EXPRESS – EXtremely PREterm infants in Sweden Study with three overall objectives to:

- Provide estimates for perinatal mortality and morbidity by gestational age
- Identify perinatal risk factors for adverse outcome
- Evaluate long-term health

EXPRESS included all deliveries in Sweden before 27 weeks of gestation between April 1st 2004 and March 30st 2007, and long-term health evaluation of the cohort is still ongoing.

2.1.5 Neonatal morbidity

The most obvious neonatal morbidity in preterm infants is lung disease. Lack of surfactant in the immature lungs and alveoli constitutes a high risk of developing respiratory distress syndrome (RDS), an acute condition with inadequate gaseous exchange. Antenatal corticosteroids accelerates fetal lung maturation and surfactant production which has improved neonatal outcome substantially by preventing RDS[28]. RDS can also be effectively treated by administration of exogenous surfactant in the airways. Despite these improvements, most extremely preterm infants need mechanical ventilation at some point and in the Swedish EXPRESS-study, 85% of the infants had been treated with ventilator[29]. After the acute phase, many infants suffer from prolonged oxygen dependency as a result of chronic lung disease. If there is a need of supplemental oxygen at 36 weeks of postmenstrual age, the infant is diagnosed with bronchopulmonary dysplasia (BPD).

Delayed circulatory transition, i.e., persistence of ductus arteriosus (PDA) and foramen ovale (PFO), is also common after preterm birth. A hemodynamically significant PDA affected 61% of the infants in EXPRESS. Left-to-right shunting over a PDA may cause pulmonary congestion and in worst cases contribute to severe chronic lung disease[30]. In addition, systemic hypoperfusion could cause symptoms from the central nervous system (such as apneas), the gut (poor growth) and kidneys (renal failure). This is why a hemodynamically significant PDA is most often treated with drugs or surgery[31].

The premature brain is immature and sensitive to periventricular ischemic lesions such as periventricular leukomalacia (PVL)[32]. Intraventricular hemorrhage (IVH), bleeding into the brain ventricles is another cerebrovascular lesion which if small and limited, often has good prognosis. In EXPRESS, 14% of the infants suffered from cystic PVL or significant IVH grades 3-4[29]. The mechanisms underlying cerebrovascular insults in preterm infants include an instable autoregulation of cerebral blood flow over a wide range of perfusion pressures.

Another important complication following very preterm birth is retinopathy of prematurity (ROP). This disease affects the microcirculation of the eye and it has been suggested that ROP may reflect a general microvascular complication from preterm birth[33], Table II.

Table II. Severe neonatal morbidity in extremely preterm infants[29]

Neonatal morbidity	Rate
SGA, fetal growth restriction	16%
IVH grade 3-4/Cpvl	14%
Treated PDA	61%
NEC	5.8%
ROP stage 3 or more	34%
Any BPD	73%
Sepsis	41%

2.2 DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

2.2.1 Intrauterine programming

The embryonic phase starts from conception and last during the first 8 weeks of gestation. The fetal period begins from 9 weeks of gestation and lasts to term (37 – 42 weeks). Malformations occur from 3 to 7 weeks of gestation, which is the most vulnerable period. From 8 weeks to term, functional disturbances and minor abnormalities may result from different environmental threats to the fetus[34].

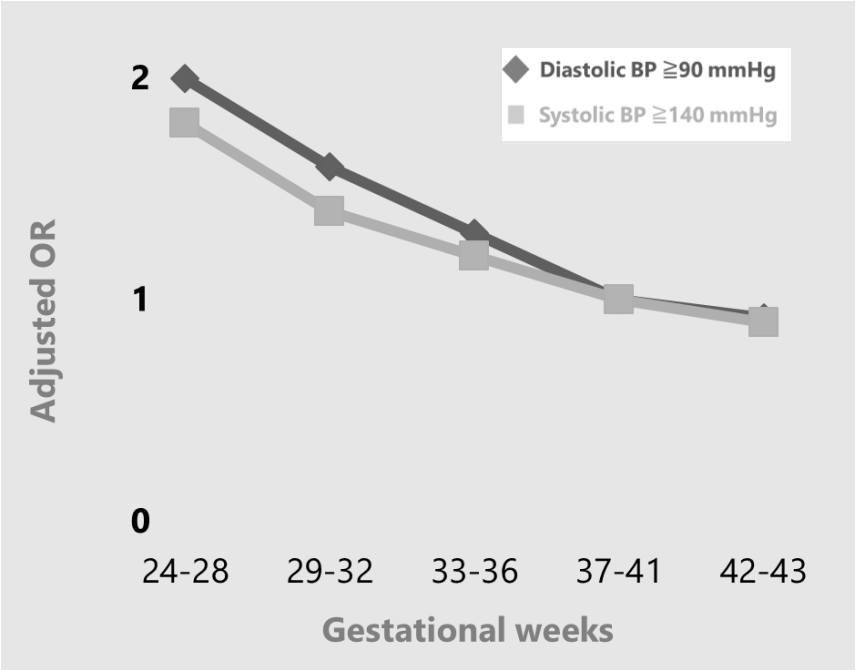
Preeclampsia, placental dysfunction, maternal smoking and undernutrition are perinatal risk factors that have been associated with poor fetal growth and low birth weight[35]. Exposure to a suboptimal in utero environment may affect the fetus at critical stages of development which is believed to cause long-term effects in tissue structure and function, processes known as intrauterine programming[36]. This is considered to be a major explanation to why there is an association between low birth weight, accelerated aging and death from ischemic heart disease before 65 years of age[37]. The underlying mechanisms are still obscure. In recent years, epigenetic changes have been suggested to play an important role for developmental programming[38, 39].

2.2.2 Preterm birth – a perinatal risk factor for cardio vascular disease?

While programming of the fetus seems to occur, less has been known about developmental adaptations after preterm birth. In a large Swedish birth cohort born 1925 to 1949, preterm birth was not associated with increased for hypertension or coronary heart disease[40, 41]. However, in this historic cohort, most infants born preterm died and survival was very selective and confined to the strongest individuals.

In a more contemporary cohort of young adult men, a gradually increasing risk for high blood pressure was associated with decreasing gestational age at birth[2], Figure 2. Children and teenagers born very preterm has also been reported to have elevated blood pressure[14], Bonamy et al showed that extremely preterm children already at 2.5 years-of-age had significant higher blood pressure than a control group born at term[42]. A recent meta-analysis concluded that preterm birth is a risk factor for hypertension[3]. This seems to be particularly true if early growth restriction is followed by accelerated postnatal weight gain[35].

Figure 2. Adjusted odds ratios for a blood pressure in the hypertensive range by gestational age in young, Swedish men (n= 329 495). After Johansson S et al, Circulation 2005.



Besides hypertension, the effects of other risk factors for adult cardiovascular disease – such as familial hypercholesterolemia, diabetes, obesity, cigarette smoking, and family history of cardiovascular disease – have been reported to be measureable already in childhood[43-45]. How these factors relate to and interact with preterm birth has only recently been addressed. Glucose intolerance and diabetes have been shown to occur more frequently in young people

born preterm[4, 46, 47]. And compared with controls born at term, Finnish adults who were born preterm had - besides higher blood pressure – also higher body fat percentages and waist circumferences, and they were more likely to have metabolic syndrome[48].

Preterm has not only been linked to increased risks for hypertension and metabolic syndrome. Several authors have reported an association between preterm birth and cerebrovascular disease and stroke[6]. And in a large Swedish registry study, gestational age was shown to be inversely correlated to the risk of cardiovascular death in young middle age[10].

2.2.3 The preterm heart

In a contemporary cohort of young adults, very preterm birth was associated with structural and functional changes of the heart, also after adjusting for other risk factors such as blood pressure[9, 49]. These changes corresponded to being ten years older or having a body mass index that was nine units higher[9], and exhibit a dose-response relationship to gestational age. In an animal study – in isolation from selection bias and confounding factors – moderately preterm lambs showed irreversible myocardial remodeling already nine weeks after term equivalent age[50]. Very preterm infants have also recently been shown to exhibit altered left ventricular systolic and diastolic myocardial functions already six months after birth[51], possibly preceding structural changes.

2.2.4 The vascular tree after preterm birth

Immediately after preterm birth, growth of retinal micro vessels[11] and of large arteries such as the carotids and aorta[12] slow down compared with fetal conditions. Follow-up data suggest that this birth-related arrest in vascular growth after preterm birth may be a lasting and generalized phenomenon[13-16]. Some reports but not all[14, 18, 21] also suggest that, besides arterial narrowing, accelerated intima-media thickening[17, 22] and early loss of arterial elasticity[23, 24] may be involved in the causal pathway from preterm birth to CVD.

2.3 ASSESSING CARDIOVASCULAR HEALTH IN CHILDREN

2.3.1 Physical examination

History and physical examination of the pre-school child can reveal signs of overt diseases such as hypertension, some but not all congenital cardiovascular conditions and heart failure. However, physical examination cannot detect normal variations or subclinical problems, and is an insufficient method to assess developmental physiology.

2.3.2 ECG and exercise test

Electrocardiogram, ECG, is helpful to evaluate pediatric arrhythmias, signs of myocardial dysfunction, and is sensitive to detect changes in the conducting system. Advanced ECG with vector analysis has recently been reported as a tool to detect cardiomyopathy in children[52].

Exercise test is an important method to evaluate cardiovascular health also in children. It includes simultaneous ECG, blood pressure registrations, and respiratory frequency. It rules out exercise induced arrhythmias, conducting disorders and myocardial events and also provides information about blood pressure in relation to work load. Usually, exercise test can be reliable and performed from the age of 10 years.

2.3.3 Imaging

X-ray is often used to answer questions about cardiac size in conjunction with pulmonary over-circulation associated with ventricular or atrial septum defects, or a patent ducts arteriosus. The value of X-ray for physiology research in small children is however limited.

Magnetic resonance imaging (MRI) is a good way to evaluate the heart and arterial tree without exposing the patient for irradiation. MRI can measure cardiac dimensions, myocardial performance in health and disease, and also coronary flow. It is however expensive and difficult to conduct in lower pediatric ages without sedation or anesthesia.

Computed cardiac tomography is superior to MRI to investigate and map cardiac and coronary anomalies. This technique is faster to perform compared to MRI but because of exposure to radiation, the latter is to be preferred in children.

Ultrasound can depict the heart and vessels in real time and measure dimensions and function. Ultrasound can be performed bedside and does not expose the patient to any risks. It is therefore the method-of-choice when investigating a pediatric population and can be used in all age-groups, from birth to adulthood. The image quality of ultrasound in a pediatric population is however challenged by high heart rates, which means that time resolution needs to be prioritized as well as reducing the sector width.

Echocardiography and vascular ultrasound includes two-dimensional imaging (2D) for dimensions, and color Doppler to detect shunts, obstructions or insufficiencies, pulsed and continuous spectral Doppler to evaluate blood velocities, and pulsed wave spectral Tissue Doppler (TDI) for myocardial velocities (cardiac only). Two-dimensional myocardial speckle tracking echocardiography (2DSTE) or for the intima media in artery walls (2DST) is the least angle dependent technic compared to spectral Doppler and tissue Doppler that allows

measuring myocardial or wall alterations in longitudinal, radial, and circumferential directions to obtain strain, strain rate and velocities.

2.3.4 Vascular function tests

Endothelial function can be estimated with brachial artery flow mediated dilatation (FMD) using ultrasonographic measurement of the arterial diameter before and after arterial occlusion. This method is known to indicate arterial endothelial dysfunction, an important predictor of cardiovascular events[53]. FMD cannot be used in smaller children. In addition, in previous studies using other techniques, preterm birth has not been associated with later endothelial dysfunction in children or adults[15, 17, 54, 16].

Pulse-wave velocity measure arterial stiffness, i.e., the biological aging of the artery wall. It has been used in both adult and pediatric populations, and is nowadays considered to be the gold standard for arterial assessment[55]. Adolescent girls born preterm did not exhibit increased pulse wave velocity, in fact their aorta appeared to be more elastic than same-aged controls born at term[14].

2.3.5 Blood pressure measurements

Hypertension is considered to be one of the most important risk factors for developing CVD in adulthood. Determination of casual or office blood pressure is a standard method in clinical settings. It is important to measure blood pressure with the recommended cuff-size, covering two thirds of the right upper arm. A too small cuff size results in falsely high blood pressure readings. Ambulatory 24-hour blood pressure measurements is advocated as the most reliable method to diagnose hypertension. Blood pressure should normally decrease when sleeping night time and loss of night blood pressure dipping indicates true hypertension. It is important to report what blood pressure method that was used, especially in small children in which oscillometric devices are usually used.

3 AIMS

The overall aim of this thesis was to explore and describe cardiovascular structure and function using ultrasonography, evaluate blood pressure and study the relation between lung function and pulmonary circulation in 6-year-old children born extremely preterm.

Study 1. The aim was to estimate diameter, intima-media thickness and stiffness of the large arteries (the coronaries, carotid arteries and aorta) in children born extremely preterm as compared to same-aged controls born at term, and to determine any associations to different perinatal risk factors.

Study 2. The aim was to measure blood pressure in a population-based cohort of children extremely preterm, to test for sex differences and any dose-response relationship between gestational age and blood pressure. Contributions from other perinatal risk factors was also investigated.

Study 3. The aim was to characterize the cardiac phenotype of children born extremely preterm, and explore whether or not fetal and neonatal growth restriction, as well as a patent ductus arteriosus in infancy could may be in the causual pathway for adverse later cardiac structure and function.

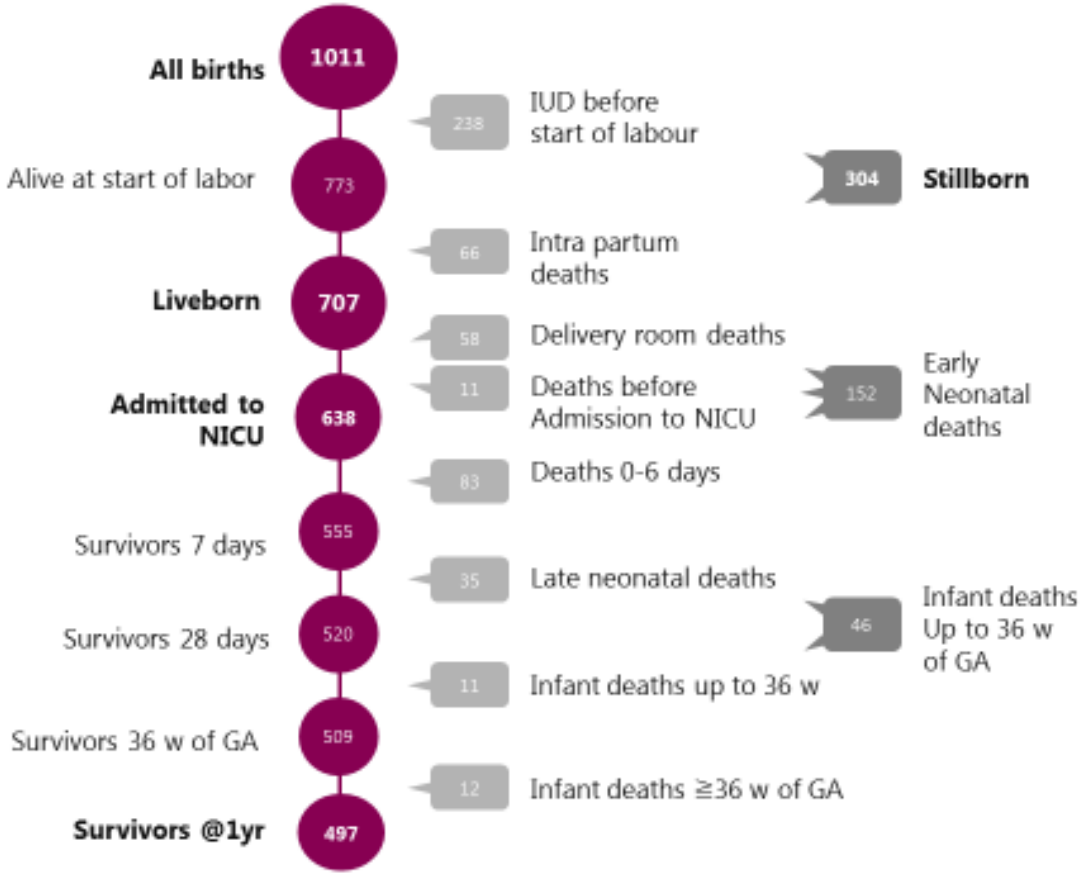
Study 4. The aim was to examine associations between lung function and pulmonary circulation, including right heart dimensions and function, in children born extremely preterm.

4 METHODS

4.1 THE EXPRESS CHILDREN

The EXPRESS (Extremely Preterm Infants in Sweden Study) is a prospective population based cohort including all children who were born before 27 weeks of gestation between April 1st 2004 and March 31st 2007 in Sweden, Figure 3.

Figure 3. Flow chart for the EXPRESS study



The cohort have prospectively collected perinatal data on mothers health during pregnancy including treatment with antenatal corticosteroids, smoking in pregnancy, education, age, delivery hospital and data of the infants with gestation age, birthweight, medical treatments, need of respiratory support as mechanical ventilation and diagnoses such as sepsis, ROP, NEC, BPD, and PDA.

4.2 THE CONTROL CHILDREN

A control group consisting of children born between April 1st 2004 and March 31st 2007 at a gestational age of 40 weeks ± 2 days were recruited. Inclusion criteria were uncomplicated pregnancies to infants born term without perinatal complications.

To each preterm child, a list of 10 eligible participants, matched on birthdate, sex, delivery hospital, residency and mothers' country of birth, were retrieved from the Swedish Medical Birth Register held at the National Board of Health and Welfare and if none of the potential controls on the list accepted the invitation to participate, it was registered as missing.

In total, 172 controls were included, and 6 were coded as missing.

4.3 THE FOLLOW-UP

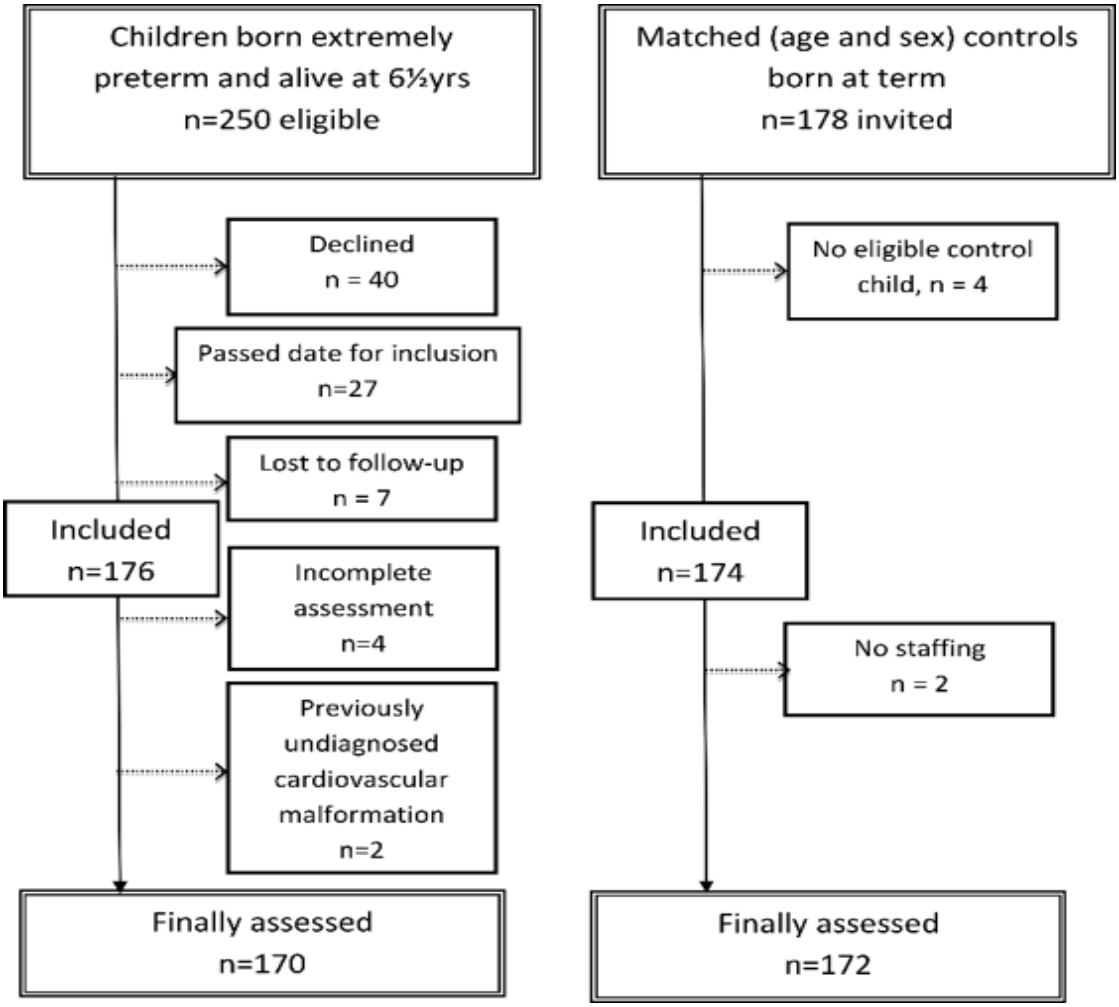
Between years 2010 and 2013 when the children reached age of 6 ½ -years-old ±3 months, a neuropsychological follow up study were conducted in the seven regions with the highest level of perinatal intensive care in Sweden. Simultaneously and in parallel three regions Umeå, Lund and Stockholm performed a cardiovascular and lung function follow-up named CHARM (Comprehensive Heart And Respiratory Measurements).

From these three regions the extremely preterm children and their families were invited to participate in the cardiovascular and lung function study. All families who agreed to attend were given written and oral information and either both parents or legal guardians had to sign the written consent.

Eligible for inclusion were 250 children, 34 families were lost to follow up and 40 declined participation. Finally 176 children were included in the study. In drop-out analysis there were no disclose any significant differences in gestational age, birth weight or sex distribution between participants and those lost to follow-up

Exclusion criteria for cardiovascular and lung function follow up were diagnoses of cardiac (n=12) and lung malformations (n=0).

Figure 4. Flow chart for follow-up study.



4.4 STUDY PROTOCOL

A common protocol was used to collect the data for all included papers. Detailed information about the equipment used is described in the method sections of the respective scientific report.

The clinical examination was performed by trained medical staff and included a questionnaire focusing on the child's family history, cardiac and respiratory health, anthropometric measurements, assessment of blood pressure, cardiac and lung function.

4.5 DEFINITIONS OF RISK FACTORS AND COVARIATES

Data on risk factors and covariates were obtained from the questionnaire filled out by the parent/s on the examination day, data collected from the EXPRESS database, Table III.

Table III. Definition of risk factors/covariates

Variable	Source of data	Definition
Sex	EXPRESS	Biological sex of participant (female or male)
Family history of cardiovascular disease	Q6	A parent or grandparent with diabetes, dyslipidemia, cerebrovascular stroke, myocardial infarction or coronary bypass surgery
Maternal education	Q6	More or less than 12 years of formal education
Maternal smoking	Q6	The mother smoked during pregnancy
Pre-eclampsia	EXPRESS	BP ≥ 140/90 mmHg + proteinuria
Parity	EXPRESS	first child +
Placental abruption	EXPRESS	yes/no
Antenatal steroids	EXPRESS	yes/no
Multiple fetuses	EXPRESS	twins, triplets
Small for gestational age	EXPRESS	Birth weight < 2 SD below the mean birth weight for gestational age and sex according to intrauterine growth charts.[56]
Bronchopulmonary dysplasia	EXPRESS	Need for oxygen with a fraction of inspired oxygen of >30% or respiratory support (mechanical ventilation or nasal continuous positive airway pressure) at 36 weeks of postmenstrual age.
Patent ductus arteriosus, PDA	EXPRESS	Treated either medical or surgical or both.
ROP	EXPRESS	Stage ≥3
Gestational age	EXPRESS	In weeks week post menstrual
Birth weight	EXPRESS	in gram
Height	Clinical examination	in centimeters
Weight	Clinical examination	in kilogram
BSA, body surface area	Clinical examination	according to Haycock[57]
BMI, body mass index	Clinical examination	body weight / body height ^{2}
Asthma	Q6	or asthma like disease expressed as asthma ever.

EXPRESS: EXPRESS database, Q6: questionnaire at 6.5 year follow up

4.6 HEALTH OUTCOMES

4.6.1 Blood pressure

After a period of calm adaptation to the settings, a validated oscillometric device (Omron HEM 907, Omron Healthcare, Kyoto, Japan) was used to measure systolic (SBP) and diastolic (DBP) blood pressures, and heart rate, in the right arm with an appropriate cuff size covering two thirds of the upper arm. The blood pressure measurements was repeated three times, at least 2 minutes apart while the child was sitting in the parents´ lap or in a chair. Mean systolic and diastolic blood pressures were calculated and used in the analyses.

SBP and DBP z-scores were calculated using age-, sex- and height adjusted BP nomograms for children[58]. Age and sex adjusted z-scores for current height were calculated using the World Health Organization growth reference data from 2007 for 5-19 years (www.who.int/growthref/en/).

4.6.2 Cardiovascular assessments

4.6.2.1 *Examination protocol*

Ultrasonography was used to investigate arterial and cardiac structure and function, following guidelines and standards of the American Society of Echocardiography[59-61].

A complete echocardiography to exclude structural malformations and pulmonary hypertension was performed on all participants. All recordings were made with a simultaneous ECG. Resolution was set at 70-90 frames per second. In each region, one experienced cardiac sonographer performed all examinations. Off-line calculations were conducted by two operators; LAM for registrations from Umeå and Stockholm, and OB for registrations from Lund.

4.6.2.2 Measurements of carotid and coronary arteries, and aorta

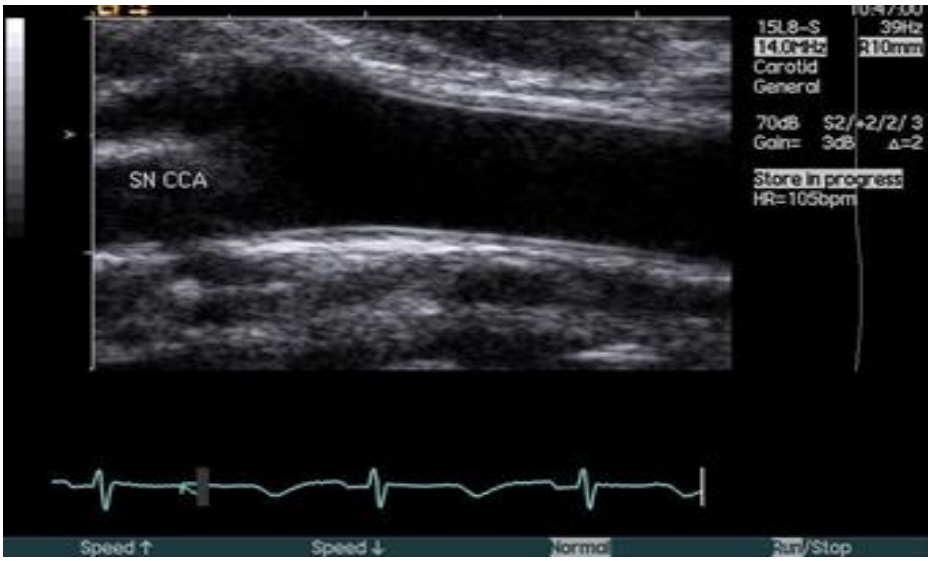
Both carotids were examined with the child comfortable in supine position. The right and left common carotid was documented in its longitudinal and short axis views 10 mm before the branching (carotid bulb). Recordings with three R-R intervals were intended and saved for later off-line analyses[61]. Coronary arteries were measured in diastole for maximal inner diameter or when at largest. Abdominal aorta and common carotid inner diameter were measured in peak systole and end diastole.

Strain was calculated as peak systolic diameter - end-diastolic diameter (ΔD)/end-diastolic diameter x 100.

The stiffness index (β) for CCA and AA was calculated using the formula:
ln (SBP/DBP)/strain[17, 62].

To avoid magnification errors, both common carotids were scanned perpendicular to near field and far field of intima media borders for calculation of true diameter and intima media thickness. The common carotid intima media thickness (cIMT) was measured from the far wall of the common carotid with a semi automatically software AHP (Arterial Health Package, Siemens Medical Solutions, USA Inc). The region of interest of the far wall was set to 10 mm and mean cIMT values were reported[61].

Figure 5. 2D-imaging of the common carotid artery.



The left main coronary artery, left anterior descending coronary artery, left circumflex coronary artery and right coronary artery were recorded from a parasternal high short axis view. In order to avoid respiratory disturbance, recumbent left sided position was used during this assessment.

4.6.2.3 Echocardiography

Echocardiography was performed with the child resting in a supine position, in order to acquire subcostal views. Abdominal aortic diameter was recorded in longitudinal axis with the near and far fields perpendicular to each other. The diameter and ability to collapse with breathing of the vena cava inferior were judged visually to estimate normal venous pressure, and a diameter change of ≥ 50% was considered normal. The four-chamber view was obtained with the child positioned on his or her left side.

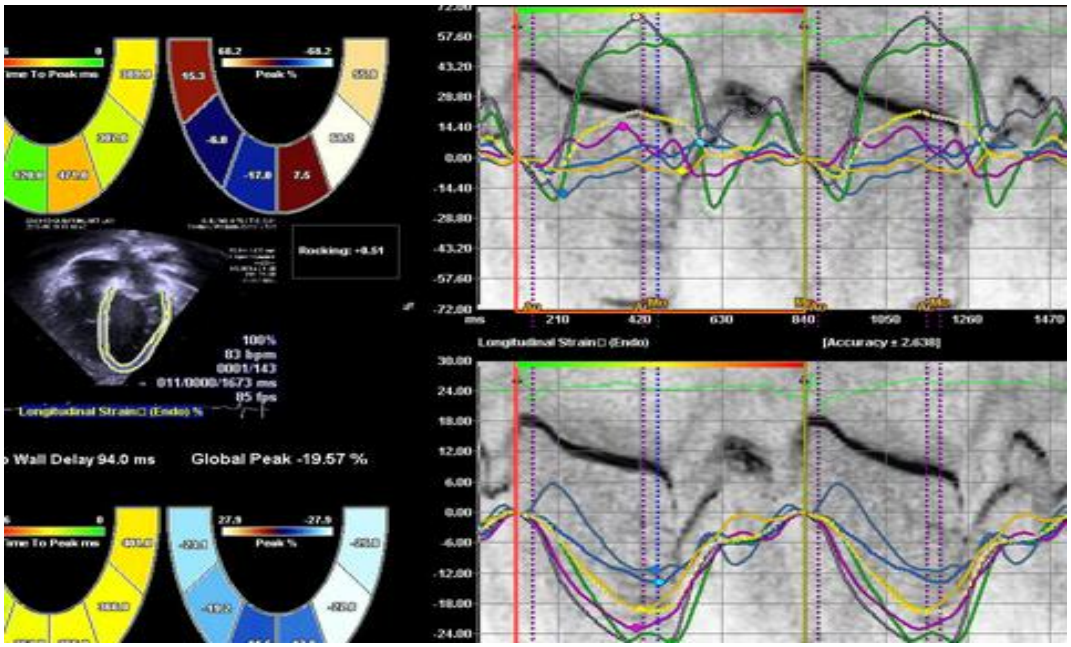
4.6.2.4 Cardiac dimensions and function

Left heart dimensions were extracted from M-mode and 2D digital stored clips. M-mode parasternal long axis view was used for measures of septal thickness (IVS), posterior wall (PW) and LV diameter in end-diastole. Calculation of relative wall thickness and left ventricular mass are presented in paper III. Aortic valve annulus systolic diameter, left atrium and left ventricle length and width were assessed from 2D images, and sphericity indices were calculated as length/width for each chamber.

Systolic function was estimated by M-mode tracings of mitral annular plane systolic excursion (MAPSE) towards the apex using parasternal four chamber view lateral wall. Stroke volume (SV) was calculated using left ventricular outflow tract velocity time integral and AoV annulus diameter: SV = pi x (AoV annulus^2)/4 x $LVOT_{VTI}$. Cardiac output (CO) was calculated as SV x HR/min. Diastolic filling pressure E/e', was estimated by the ratio of transmitral early diastolic velocity, E-wave and mitral lateral annular early diastolic myocardial velocity, e'. Left ventricular time intervals were extracted from tissue velocity recordings from basal lateral and interventricular septum. Myocardial performance index was calculated as follows by interventricular relaxation time + interventricular contraction time / interventricular ejection time.

Two-dimensional speckle tracking (2DSTE) of left ventricular global longitudinal myocardial strain, strain rate, and velocities were performed by a semi automatically software VVI (Vector Velocity Imaging, Siemens Medical Solutions, USA Inc). Manually traces of the ventricular endocardial border from mitral annulus hinge point in lateral free wall to mitral annulus interventricular septal hinge point were outlined and calculated by the software.

Figure 6. 2-dimensional speckle tracking echocardiography of the left ventricle.



4.6.2.5 Pulmonary circulation and right heart dimension and function

To assess the pulmonary circulation, we measured pulmonary artery diameter, pulmonary vascular resistance, right ventricular relative wall thickness, right ventricular sphericity index, TAPSE (linear tricuspid annular plane excursion towards apex) and free wall E/e'. Imaging, measurements and calculations correspond to those methods described above for the left side of the heart.

We defined pulmonary hypertension as $TRV/RVOT_{VTI}$ >0.275 (estimated according to Abbas formula[63, 64]).

4.6.3 Lung function assessments

Participants were informed to avoid the use of β2-agonists 24 hours before the study day. Dynamic spirometry was performed 20 minutes after inhalation of 200 micrograms of salbutamol (Airomir®, Teva) through a valved spacer. ATS/ERS guidelines[65] were followed using a Jaeger Masterscreen (Carefusion Technologies, San Diego, CA), with the child wearing nose clip sitting in an upright position. After a full inhalation, the child was encouraged to exhale as fast and long as possible. At least three acceptable attempts were obtained. Providing that the effort was judged as maximal by the test leader, the curve passed visual quality inspection and reproducibility criteria were fulfilled, the highest values of Forced Vital Capacity (FVC) and Forced Expiratory Volume in 1 second (FEV_1) was extracted for analysis. Using the Global Lung Initiative reference values, FVC, FEV1 and the

ratio FEV1/FVC were then converted to z-scores[66]. The spirometry system was calibrated daily using a 3 liter precision syringe.

4.7 STATISTICAL METHODS

4.7.1 Main statistical methods

The Pearson Chi-2 test or Fishers exact test were used to test for group differences in proportions. For continuous variables, normality was assessed using visual assessment of histograms and the Shapiro–Wilk test. The Students T-test or the Mann-Whitney U-test were used to assess group differences, depending on distribution. For multivariate assessment of associations, linear regression analysis was used. Results are expressed as beta-coefficients and 95% confidence intervals.

4.7.2 Covariate selection

Potential determining or confounding factors were identified from a priori knowledge or literature. Covariates were assessed individually or by stepwise regression with backwards selection, using a P-value cutoff of 0.15 for inclusion in the model. Possible clustering of data was evaluated using a mixed-effects linear model with examination site as the random-effect variable.

All analysis were made using the STATA software package (StataCorp LP, College Station, Texas, USA.

4.8 ETHICAL CONSIDERATIONS

Before the families accepted the invitation, they received oral and written information. All families, both custodians, signed a written consent. Children and their parents were reminded of the child's voluntary participation and the opportunity to cancel at any time during the study day.

5 RESULTS

5.1 COHORT CARACTERISTICS

5.1.1 Family, maternal and pregnancy characteristics

The proportions with a family history of cardiovascular disease did not differ between CTRL (74%) and EXPT children (73%, p=0.80). University education was more common among CTRL mothers (61%) than in EXPT mothers (47%, p=0.009). Maternal ages (mean 31.8 vs 31.4 years, p=0.47) were similar in the two groups whereas the proportion mothers with

Table IV. Neonatal characteristics, morbidity and treatments.

	EXPT (n=176)	CTRL (n=172)
Neonatal data		
Gestational age, weeks (range, SD)	24.9 (22-26)	39.4 (37-41)
Birth weight, g (range, SD)	788 (348-1161)	3591 (2430-4315)
Boys, n (%)	97 (55%)	99 (58%)
SGA at birth, n (%)	27 (16%)	
SGA at 36 weeks PMA, n (%)	76/162[b] (47%)	
BPD moderate, n (%)	69(45%)	
BPD severe, n (%)	29(19%)	
PDA, n (%)	103(61%)	
Neonatal treatments		
Antenatal corticosteroids	152/162 (94%)	
Surfactant[a]	42(60%)	
Postnatal corticosteroids	21(28%)	
Mechanical ventilation[b], days(SD) range	14.9(18.3) 0-121	
Blood transfusion[c]	12.6(8.3)	
Plasma transfusion[d]	7.3(9.1)	
Antibiotics[e], days	30.8(18.3)	
PDA pharmacological closure	100(58%)	
PDA surgical treatment	39(23%)	
O2 at discharge	14(19%)	

Data are mean (SD) or numbers (%) if not indicated otherwise. [a]Some missing data; [b] 13.6% had no ventilator days; [c]All children had at least one blood transfusion; [d] 14.8% received no plasma transfusion; [e] 4 infants had no treatment with antibiotics. Moderate BPD: need of <30 % oxygen, Severe BPD: need of > 30% oxygen at 36 weeks of postmenstrual age. BSA (body surface area, SGA (small for gestational age).

smoking during pregnancy was lower in CTRL (1%) than in EXPT (5%, p=0.03). Ten percent of the mothers to EXPT-children suffered from preeclampsia during pregnancy and

17% of the EXPT-children were twins, whereas by definition, the CTRL-group did not contain any cases of preeclampsia or multiple pregnancies.

Neonatal characteristics are presented in Table IV. At 6 ½ years-of-age, the EXPT children were still smaller – both in height (-4%) and weight (-15%) – than CTRL children and their BMI was 8% and BSA 10% lower than in CTRL, Table V.

Table V. Anthropometry of 6½-year-old children born extremely preterm (EXPT) and controls born at term (CTRL).

	EXPT n=176	CTRL n=172	p-value
Age, months	80 (2.1)	80 (2.3)	0.22
Weight, kg	20.6 (3.6)	24.2 (4.1)	<0.001
Height, cm	118 (5.6)	123 (4.8)	<0.001
BMI, kg/m^2	15.1 (1.6)	16.4 (2.1)	<0.001
BSA, m^2	0.82 (0.09)	0.91 (0.09)	<0.001

Data are mean (SD).

5.2 THE PRETERM ARTERIES IN CHILDHOOD

Arterial dimensions

The coronary arteries, carotid arteries, aortic valve annulus, and abdominal aorta inner diameters were significantly narrower in EXPT children than in CTRL children before adjustment for BSA and site. After adjustment, left main coronary artery, right common carotid, aortic valve annulus, and abdominal aorta remained significantly narrower in the EXPT group than in CTRL, Table VI.

Table VI. Arterial inner diameters (mm) in 6½-year-old children born extremely preterm (EXPT) and in controls born at term (CTRL).

	EXPT	CTRL	p-value	Adjusted differences[a] in means (95% CI)	p-value
Coronary arteries[b]	(n=149)	(n=163)			
Left main	3.0 (0.7)	3.2 (0.6)	0.002	-0.14 (-0.25;-0.02)	0.02
Right	2.4 (0.4)	2.6 (0.4)	<0.001	-0.08 (-0.17;0.02)	0.12
Left anterior descending	1.7 (0.3)	1.9 (0.3)	0.004	-0.05 (-0.15; 0.04)	0.28
Common carotid artery[c]	(n=135)	(n=150)			
Left	5.4 (0.4)	5.6 (0.5)	0.002	-0.008 (-0.02;0.004)	0.200
Right	5.5 (0.4)	5.8 (0.4)	<0.001	-0.02(-0.03;-0.006)	0.004
Aorta[c]	(n=151)	(n=159)			
Aortic valve annulus	13.9 (1.1)	15.2 (1.2)	<0.001	-0.87 (-1.1;-0.64)	<0.001
Proximal abdominal	9.4 (0.9)	10.3(1.0)	<0.001	-0.6 (-0.8;-0.3)	<0.001

Values in millimetres presented as mean (SD) and as adjusted differences in means (95% confidence intervals). [a] Differences in means adjusted for body surface area (m^2) and site. [b] Proximal innerdiameter. [c] Systolic proximal diameter.

Gestational age and appropriate for gestational age

In subgroup analyses restricted to EXPT children, we found that adjusted arterial dimensions were similar or slightly smaller (right and left anterior descending CA) in children born at 22–24 weeks of gestation than in 25–26 weeks of gestation. In contrast, arterial dimensions in EXPT-children did not differ in relation to being born SGA or AGA, or in relation to being SGA or AGA 10 to 14 weeks after birth, i.e., at 36 weeks of postmenstrual age, please see Tables 3-5 in paper I.

Intima media thickness

Absolute carotid artery intima media thickness (cIMT) was similar in CTRL and EXPT children. However, the relative cIMT of the right CCA was 1% thicker in EXPT han in CTRL, Table VII.

In EXPT-children, carotid intima media thickness did not vary in relation to gestational age, small for gestational age at birth or at hospital discharge.

Table VII. Intima media thickness (cIMT) of the common carotid arteries (CCA) in 6½-year-old children born extremely preterm (EXPT) and controls born at term (CTRL).

Absolute cIMT, mm	EXPT (n=114)	CTRL (n=121)	p-value	Adjusted* differences in means (95% CI)	p-value
Left CCA	0.38 (0.04)	0.38 (0.04)	0.30	0.001 (-0.01;0.01)	0.43
Right CCA	0.38 (0.04)	0.37 (0.04)	0.12	0.008 (-0.003;0.02)	0.16
Relative cIMT, %	(n=110)	(n=118)			
Left CCA	16.7 (2.3)	16.1 (2.1)	0.042	0.5 (-0.1;1.2)	0.10
Right CCA	16.9 (2.3)	15.6 (1.9)	<0.001	1.0 (0.5;1.6)	<0.001

Relative cIMT= [(IMT CCAx2)/CCA inner diameter] x 100. Absolute IMT-values presented in millimetres and relative IMT as percentages. Values represent mean (SD). *Adjusted for BSA (Haycock) and site.

Arterial elasticity

The strain in both left and right common carotids was higher in EXPT than in CTRL children, meaning that EXPT had larger ability to distend their CCAs in systole and release arterial strain in diastole as compared to CTRL children. The results were consistent with a lower CCA stiffness index in EXPT than in CTRL children, Table VIII.

Table VIII. Dynamic properties of the common carotid arteries and the proximal abdominal aorta in 6½-year-old children born extremely preterm (EXPT) and controls born at term (CTRL).

Common carotid artery	EXPT	CTRL	p-value
Left	(n=135)	(n=149)	
Strain, %	20 (4.6)	19 (4.3)	0.045
Stiffness index	2.8 (0.7)	3.1 (0.8)	0.002
Right	n=118	n=124	
Strain, %	21 (3.8)	20 (3.8)	0.024
Stiffness index	2.8 (0.8)	3.0 (0.8)	0.007
Abdominal aorta	(n=112)	(n=121)	
Strain, %	29 (7.9)	28 (7.5)	0.80
Stiffness index	2.1 (0.8)	2.1 (0.7)	0.67

Values represent mean (SD). Strain= (ΔD/EDD) x 100. Stiffness index = ln (SBP/DBP)/strain. ΔD=systo-diastolic diameter change; EDD=end-diastolic diameter; DBP=diastolic blood pressure; SBP = systolic blood pressure.

5.3 BLOOD PRESSURE

All children born extremely preterm and children born at term had unadjusted blood pressures within the normal range, and there were no differences in median systolic (SBP) or diastolic blood pressures (DBP) in absolute values between the two groups. According to the pediatric blood pressure nomograms by age, sex and height[58], a SBP >90th percentile was found in 6.5% EXPT children and 8.1% in CTRL children, and a DBP >90th percentile was seen in 3.6 % EXPT 3.6% and 0.6% CTRL. No child had BP >95th percentile.

After converting blood pressures into z-scores[58], both SBP and DBP were significantly higher in children born extremely preterm than in children born at term, Table IX.

Table IX. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) in 6½-year-old children born extremely preterm (EXPT) or at term (CTRL).

	EXPT (n=171)	CTRL (n=172)	p-value
SBP, mm Hg	97.7 (92.7 to 103.0)	97.2 (93.5 to 103.3)	0.81
DBP, mm Hg	56.0 (52.3 to 60.3)	55.7 (51.3 to 60.3)	0.30
SBP, Z-score*	0.08 (-0.28 to 0.66)	-0.06 (-0.50 to 0.39)	0.02
DBP, Z-score*	-0.08 (-0.37 to 0.33)	-0.18 (-0.63 to 0.25)	0.01
SBP>90th percentile, n (%)	11 (6.5)	14 (8.1)	0.56
DBP>90th percentile, n (%)	6 (3.6)	1 (0.6)	0.06
Heart rate, beats/min	88.2 (80 to 95.7)	85.0 (78.3 to 90.3)	0.005

Data are median, interquartile range (IQR) or n (%). *according to pediatric BP nomograms by age, gender and height[58]

After stratification on sex, group differences in SBP and DBP z-scores, and in heart rate, were confined to boys, whereas girls born EXPT did not exhibit any statistically significant differences in blood pressure or heart rate compared with girls born at term.

In EXPT, shorter gestation, higher BMI and higher heart rate at follow-up were independently associated with blood pressure whereas perinatal exposures such as maternal smoking, neonatal morbidity or antenatal steroid treatment were not. For each week longer gestation, SBP decreased by 0.10 and DBP by -0.09 z-score.

5.4 THE PRETERM LEFT HEART IN CHILDHOOD

Cardiac dimensions

The proportion of EXPT children with LV length <10th percentile in the CTRL group was 51%. After adjustment for BSA, the left ventricle was 3-5% smaller in EXPT than in CTRL. However, stroke volume and cardiac output did not differ significantly between groups. And sex, fetal growth restriction or a patent ductus arteriosus in the neonatal period did not contribute to cardiac dimensions.

Before adjusting for body size, interventricular septum and LV posterior wall were significantly thinner in EXPT than CTRL, whereas the relative wall thickness – taking size of the ventricular cavity into account – did not differ between groups. After adjusting for BSA and site, there were no remaining group differences in LV wall thickness. In fact, LVM was lower in the EXPT group than in the CTRL group, Table X.

Table X. Left heart dimensions and stroke volume (SV) and LV mass (LVM) in 6½-year-old children born extremely preterm (EXPT) and in controls born at term (CTRL).

	EXPT (n=157)	CTRL (n=133)	p-value	Adjusted[†] differences in means (95% CI)	p-value
LV dimensions					
LV length	54.6(4.3)	58.6(3.9)	<0.001	-1.9(-2.8;-0.9)	<0.001
LV width	35.6(3.2)	37.3(3.2)	<0.001	-1.0(-1.8;-0.3)	0.009
AoV annulus	13.9(1.1)	15.5(1.0)	<0.001	-0.8(-1.0;-0.6)	<0.001
IVS	5.6(0.9)	6.1(0.8)	<0.001	-0.1(-0.3;0.1)	0.36
PW	5.4(0.8)	5.6(0.7)	0.004	0.1(-0.1;0.3)	0.50
RWT	0.31(0.04)	0.31(0.04)	0.98	0.01(-0.002;0.02)	0.09
Volume/mass					
SV, ml	15.8(2.7)	17.6(2.9)	<0.001	-0.8(-1.5;-0.02)	0.05
LVM[‡], g	48.5(11.4)	59.3(11.1)	<0.001	-3.0(-5.4;-0.4)	0.01

Data are mean (SD) and expressed in millimetres if not indicated otherwise. [†]Adjusted for body surface area (m^2) and site. [‡] LVM calculated according to Devereux.[67] AoV annulus=Aorta valve annulus diameter, LV=left ventricle, LVM=left ventricular mass, SV=stroke volume.

Cardiac function

Left ventricular longitudinal shortening (MAPSE) and systolic tissue velocity (lateral s') were 7-11% lower, and transversal shortening fraction (SF) was 6% higher in EXPT than in CTRL. EXPT also exhibited lower atrial emptying tissue velocities (lateral e') than CTRL, which is in line with the finding of a lower 2DSTE lateral peak velocity in EXPT than in CTRL.

EXPT children also had higher lateral wall E/e' ratio compared with CTRL children, indicating altered diastolic function with elevated (but still in the normal range) filling pressure in EXPT than in CTRL. In addition, tissue Doppler imaging at the LV lateral wall showed lower diastolic myocardial velocities and higher ratios of transmitral early velocity to tissue Doppler early diastolic mitral annular velocity, indicating a stiffer left ventricle in EXPT than in CTRL, Table XI.

Finally and in a subgroup of the sample (children investigated in Stockholm), endocardial deformation determined by STE did not reveal any statistically significant group differences in global or basal longitudinal strain, strain rate, or peak velocity.

Table XI. Left heart function in 6½-year-old children born extremely preterm (EXPT) and in controls born at term (CTRL).

	EXPT	CTRL	p-value	Adjusted[†]differences in means (95% CI)	p-value
Systolic function	n=157	n=130			
MAPSE, mm	12.4(1.9)	13.3(1.7)	<0.001	-0.9(-1.4;-0.4)	<0.001
Shortening fraction	0.36(0.04)	0.34(0.06)	<0.001	0.02(0.009;0.03)	0.001
Septal					
TDI s', cm/s	6.7(1.0)	6.6(0.7)	0.50	-0.1(-0.4;0.1)	0.30
MPI'	0.44(0.07)	0.45(0.07)	0.16	-0.01(-0.03;0.009)	0.26
Lateral					
TDI s', cm/s	8.3(1.4)	9.1(1.4)	<0.001	-1.0(-1.4;-0.6)	<0.001
MPI'	0.43(0.06)	0.42(0.07)	0.18	0.009(-0.009;0.03)	0.34
Diastolic function					
Lateral					
Mitral e´, cm/s	16.5(2.4)	17.8(2.5)	<0.001	-1.6(-2.3;-0.9)	<0.001
Mitral a', cm/s	5.2(1.4)	5.2(1.1)	0.94	-0.5(-0.8;-0.2)	0.003
E/e'	5.7(1.2)	5.0(1.0)	<0.001	0.6(0.3;0.9)	<0.001

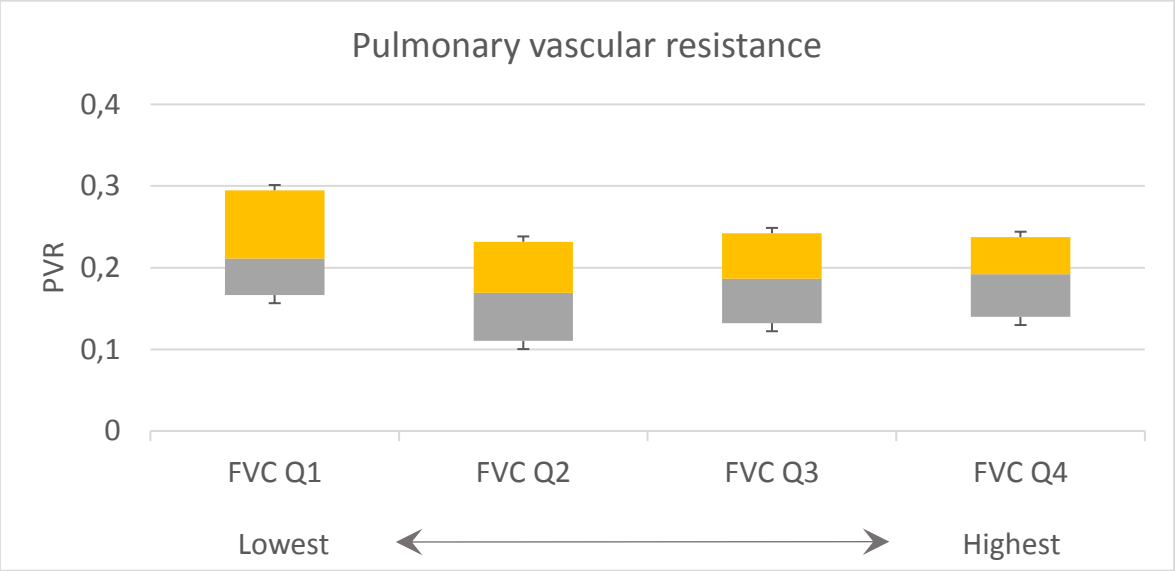
Data are mean (SD). [†]Adjusted for site. E/e'= transmitral early diastolic velocity indexed to mitral annular early diastolic velocity, MAPSE= Mitral annular plane systolic excursion, Mitral e'= mitral annular early diastolic velocity, Mitral a'= mitral annular late diastolic velocity, TDI=Tissue Doppler Imaging, TDI s'=mitral annular systolic ejection velocity in, MPI'= TDI derived myocardial performance index.

5.5 LUNG FUNCTION AND PULMONARY CIRCULATION

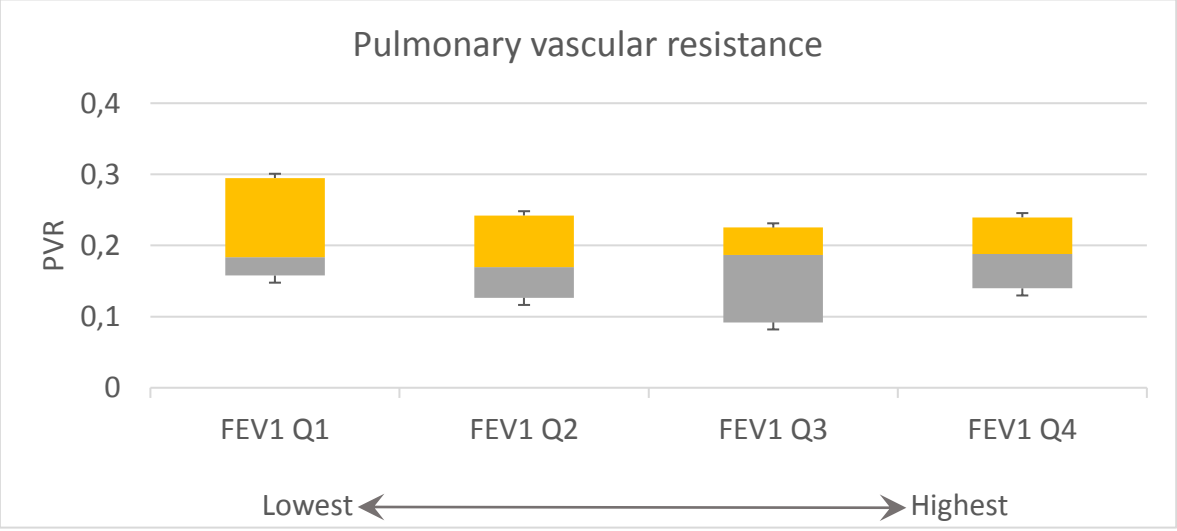
Over a wide range (z-scores for FVC ranging from -4.6 to +2.8); for FEV1 ranging from -4.0 to +2.8; and for FEV1/FVC ranging from -2.8 to +1.8), there were no associations between lung function and PVR, E/e', RWT or sphericity index of the right heart in extremely preterm children aged 6 ½ years.

Figure 7. Relation between 7A) lung volume (forced vital capacity; FVC) and 7B) airway flow (forced expiratory volume in 1 second, FEV1) and pulmonary vascular resistance (PVR) in 6½-year-old children born extremely preterm. FVC and FEV1 presented in quartiles and PVR box plots represent medians and 25th to 75th centiles.

7A



7B

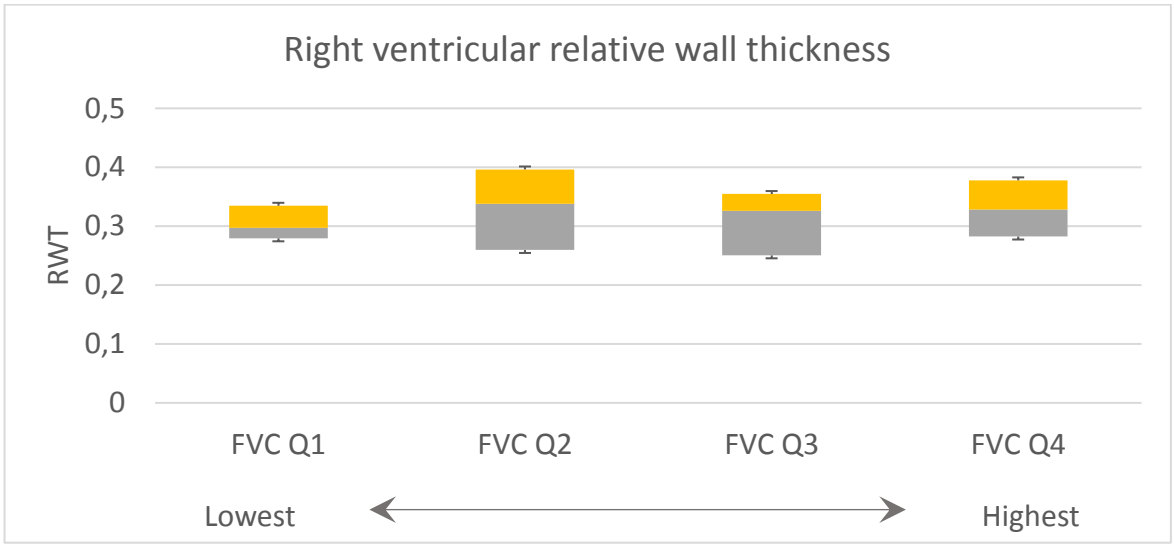


The only significant associations disclosed were positive correlations between FVC z-score and pulmonary artery diameter (r=0.52, β=0.55 mm, p=0.015) and FEV1 z-score and

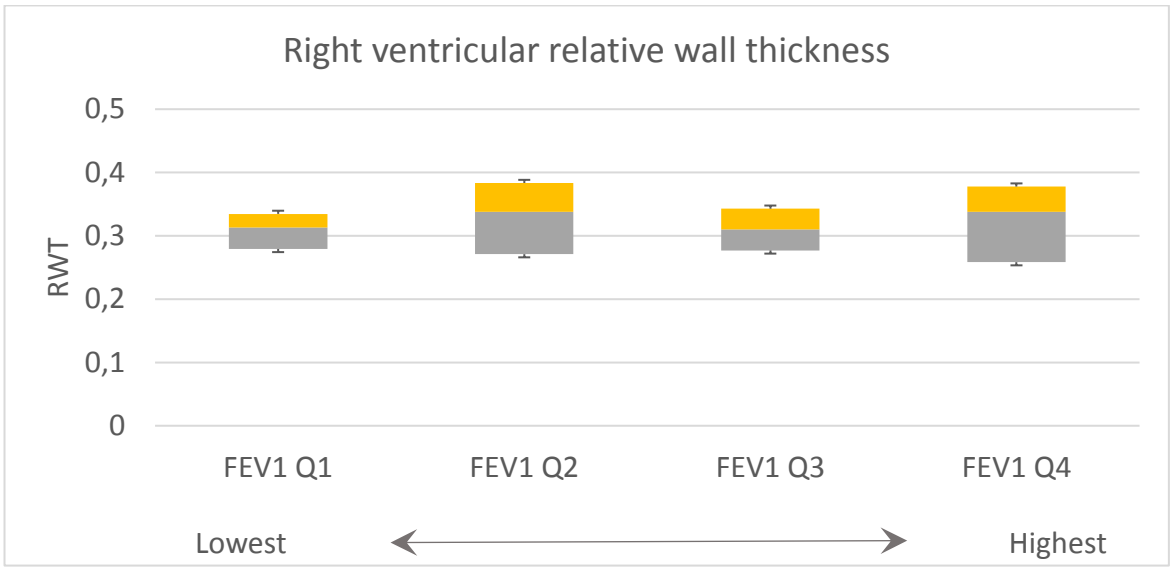
pulmonary artery diameter (r=0.58, β=0.73 mm, p=0.001), as well as between FEV1 / FVC z-score and TAPSE (r = 0.22, β= 0.76 mm, p = 0.026). The relation between lung function and pulmonary vascular resistance, as well as between lung function and right ventricular mass are presented in Figures 7-8.

Figure 8. Relation between 8 A) lung volume (forced vital capacity; FVC) and 8 B) airway flow (forced expiratory volume in 1 second, FEV1) and pulmonary right ventricular relative wall thickness (RWT) in 6½-year-old children born extremely preterm. FVC and FEV1 presented in quartiles and RWT box plots represent medians and 25th to 75th centiles.

8 A



8 B



6 DISCUSSION

The overall purpose of this thesis was to describe the development and growth of the cardiovascular system after extremely preterm birth. To do so, we explored cardiovascular structure and function in 6-year-old children born before 27 weeks of gestation and compared our findings with those of same-aged control children born at term. Several interesting group differences were discovered. The main structural differences were that childhood survivors of extremely preterm birth had narrower large arteries - especially the abdominal aorta, but also the right common carotid artery and left main coronary artery - and smaller left cardiac chambers than sex and age-matched controls born at term. Functional differences included higher systemic blood pressure and higher resting heart rate, as well as altered left ventricular systolic and diastolic function comparing children born extremely preterm and at term. Finally, and reassuringly, although children born extremely preterm often suffer from asthma-like symptoms and reduced lung function, this was not associated with increased pulmonary vascular resistance or right ventricular hypertrophy of the heart.

Arterial narrowing has previously been reported in very preterm infants and adolescens[12, 13], also in those born more moderately preterm[68]. Animal studies of the early development of large vessels have shown that these processes – normally occuring in fetal life - are sensitive to unexpected pathophysiological stimulus. The extracellular matrix consists of vascular smooth muscle cells which are sensitive to hypoxia and altered pressure. In the intima, smooth muscle cells exposed to hypertension or hypoxia either increase in size or proliferate. A thicker intima, media and adeventia layers and narrower vessel lumen may result[69].

In fetal life, half of the combined cardiac output is distributed via the aorta to the placenta. Accordingly, placental needs and blood flow drive aortic growth, especially in the third trimester. If the birth-related termination of the placental circulation occur well before term, aortic size will be superfluous and its growth will decelerate[12]. If this adaptation to preterm circulatory conditions could play a role in the postnatal development of the preterm heart remains to be clarified. We note that the aortic annulus diameter was approximately 1 standard deviation narrower in EXPT than in CTRL, also after adjusting for current body size.

Growth and elasticity of the great arteries involve early production of elastin and collagen in the arterial wall. Elastin production seems to be particularly active in the third trimester [70] i.e., a process that could be disturbed by extremely preterm birth. However, our data do not indicate any signs of accelerated arterial stiffening in small children born extremely preterm.

The coronary arteries belong to muscle arteries with greater ability to actively dilate or constrict. The significance of a narrower left coronary artery which was seen in EXPT children for the development of later cardiovascular events in adults, such as disturbed coronary flow and myoardial ischemia, is still unclear. In adult patients referred for coronary angiography, a small coronary diameter was an independent predictor of atheroma formation in both the right and left coronary arteries[71].

Interestingly, we and others report thicker cIMT of the right carotid artery, despite different methodology and study populations with different degrees of prematurity, in ages from pre-school age to young adults and from marginally to significantly elevated blood pressure with preserved elasticity[17, 20, 72]. Remodeling of the carotid arteries with slower artery growth, a narrower lumen diameter and a thicker intima media may result in increased blood velocity and shear stress. Together with increased carotid pulsatility found in children born preterm, a compromised carotid function may develop later in life. However, carotid intima media thickening in preterm children was only seen after taking arterial diameter into account. Our cIMT-findings are comparable to recent studies in adolescents[19] and young adults born preterm[17]. In view of these findings, and of normal or even enhanced carotid elasticity as well as normal endothelial function[14-17], there are presently no obvious vascular clues that could explain the association between low gestational age at birth and increased adult mortality from stroke[6-8].

The EXPT children had slighly higher systolic and diastolic blood pressures than controls, most pronounced in boys. The association between preterm birth and later increase in blood pressure was strengthened by the finding of approximately 0.1 SD lower blood pressure for every week increase in gestational age whereas there were no associations to maternal risk factors or perinatal morbidity.

Since tracking of blood pressure from childhood to adulthood has been reported to be strong[73, 74], a small difference in early childhood may become larger later in life. In a previous population-based cohort study, blood pressure at 6- and 11 years of age did not differ between those born extremely preterm or at term[75, 76]. In adolescence and at young adult age, however, several studies including a systematic review have reported up to 2-4 mmHg higher

systolic and diastolic blood pressures in people born preterm[2, 3, 14, 77-80]. Although this increase in blood pressure may seem small on an individual level, increasing the mean blood pressure in a population by 2-5 mmHg may have significant effects on the numbers who will suffer from hypertension[10], and from later stroke or ischemic heart disease[81-83]. Given a dose-response relationship with gestational age[2, 10, 79] and that the association between preterm birth and elevated blood pressure has been found to be independent of shared familial factors[2] as well as of intrauterine growth restriction[77, 78, 84], there may be a causal relationship to preterm birth.

In our cohort, follow-up at 2½[42] and at 6 years of age showed larger blood pressure elevation in boys born extremely preterm than in girls. If this reflects increased neonatal morbidity in boys born extremely preterm[85] or relates to other early sex-differences remain to be clarified. In fact, CTRL girls had higher SBP and DBP z-scores than CTRL boys, and EXPT girls had higher DBP z-scores than EXPT boys. Follow-up in adolescence and adult life after preterm birth have shown elevated blood pressure in those born extremely preterm without any sex differences[78, 80].

The mechanisms by which blood pressure levels or control appear to be compromised in children and young adults born preterm are not yet fully understood. Possible contributing factors include impaired morphological development of glomeruli and fewer nephrons on the basis of abrupted kidney development resulting in smaller kidneys[86, 87], microvascular growth arrest and rarefaction building up peripheral vascular resistance[14, 16, 88], and sympathoadrenal overactivity[89, 90]. As long as the underlying mechanisms remain ambiguous, early interventions are difficult to design. Interestingly, preterm infants randomized to breastmilk instead of formula had significantly lower blood pressure at follow-up 16 years after the intervention[15] indicating that early nutritional factors may play a role.

Our data could not confirm that being small for gestational age at birth is a perinatal risk factor for high blood pressure in children born extremely preterm. Over a life-course perspective, being born small for gestational age has been considered to be more harmful in terms of risk for hypertension than preterm birth[40, 91]. However, life-course studies of historic perinatal cohorts may be biased by selective survival among those born preterm and do not contain today´s survivors born at extremely short gestations. Our present and previous[18] results indicate that small for gestational age is not a perinatal risk factor for high blood pressure in follow-up of subjects born preterm.

Perinatal risk factors such as antenatal corticosteroid therapy and severe neonatal morbidity were not associated with blood pressure at 6 years of age. The long-term safety of antenatal corticosteroid therapy regarding blood pressure has previously been documented[92]. The lack of an association between severe neonatal morbidity and later blood pressure also indicates that increased blood pressure after preterm birth may reflect a developmental origin rather than the end result of strikes from perinatal morbidity.

Left ventricular dimensions including the outlet were significant smaller. Also the contraction patterns were different, favoring concentric instead of longitudinal contractions. The diastolic filling were decreased indicating a stiffer left ventricle in EXPT children than in children born term. The absence of associations to poor fetal or neonatal growth, as well as the finding of the smallest cardiac volumes and most deviating LV contraction patterns in those with the shortest gestations, provide support for the assumption of a causal relationship between extremely preterm birth and adverse cardiovascular development. Patency of ductus arteriosus (PDA) is a common complication after extremely preterm birth. Studies in infancy have not shown an association between PDA and lasting effects on cardiac structure and function[93], observations that seem to be confirmed by our follow-up study.

Children[94], adolescents[72] and young adults born very preterm[9] have previously been reported to have a similar reduction in cardiac dimensions as in our study, i.e., short left ventricles with smaller internal diameters. Also adults born preterm were reported to have higher LVM than those born at term[9]. The greater LVM in adults could be ascribed to higher blood pressure in preterm adults than in those born at term[9], while the blood pressure differences in our cohort were small or non-existent. Infants[51], children[94] and adolescents[72] born preterm have, similar to our cohort, comparable or even lower LVM than peers born at term, also in the occurrence of elevated blood pressure[95]. Overall, these findings indicate that LV hypertrophy of the preterm heart may be a late phenomenon which occurs after childhood and adolescence. In accordance with a lower LVM, we have previously found a smaller left main coronary artery in EXPT than in CTRL[96].

Global longitudinal systolic strain or strain rate of the LV showed no differences between the groups. Compared, previous STE-studies in infants[51] and adults[9] have demonstrated reduced strain, strain rate and myocardial velocity in the preterm offspring. We assessed strain with echocardiography which differ from results acquired with magnetic resonance used in adult studies[9]. In addition, global strain may conceal regional reductions in myocardial deformation such as those found at the free wall of the left ventricular in 6 month-old infants born very preterm[51].

The underlying mechanisms for adverse cardiac development following preterm birth are still largely unknown. In an animal study of preterm birth, cardiomyocyte hypertrophy and collagen deposition were observed in the offspring already nine weeks after term-equivalent age[50]. In human studies, changed myocardial function seems to occur before structural changes and has been reported weeks and months after preterm birth[51, 97, 98]. These observations suggest that the triggering events are birth-related.

Children born SGA exhibit similar left heart dimensions and function from those born AGA. Our findings are comparable to those reported in a smaller cohort of 5-year-old-children[99]. Most of the participants had postnatal growth restriction in infancy, which has been interpreted as suboptimal neonatal nutrition. Although, giving the preterm infant formula[100] or intralipid[101] have been linked to impaired adult cardiovascular physiology, we found no association between worse neonatal growth and LV structure and function at 6-years-of-age.

Kwon et al, conducted a cardiac follow-up of children born very preterm and with BPD at mean age of 7.7 years, and found normal cardiac function except for decreased right ventricular longitudinal strain measured with speckle tracking echocardiography (STE)[102]. In The EPICURE-study, lung function was performed in children born at 25 weeks of gestation or less and at the age of 11 years, the authors reported significant reduced lung function, mainly in those with a history of bronchopulmonary dysplasia, but there were no data on pulmonary circulation or right heart performance[103]. Moreover, lasting reductions in lung function following after preterm birth have been found, without signs of catch-up in adult age[104]. Against this background, long-term changes in lung function have been suggested to influence right ventricular structure and contribute to smaller right ventricles with greater mass in adults born preterm[49]. However, our data do not provide support for such assumption because over a wide range of lung function, from significantly reduced to above normal – we were unable to demonstrate any significant association between lung volume or airway flow, respectively, and pulmonary vascular resistance. Likewise, FVC, FEV1 or FEV1/FVC z-scores did not contribute to right ventricular relative wall thickness and no child suffered from right ventricular hypertrophy. In children with the lowest lung volumes, somewhat narrower pulmonary arteries and slightly lower right ventricular systolic displacement (TAPSE) towards the apex were seen.

Most of our participants had moderate to severe bronchopulmonary dysplasia in the neonatal period, some were born small for gestational age and a substantial proportion suffered from postnatal growth restriction. In the whole EXPRESS-cohort, asthma like disease was reported

in 33% at follow-up and among EXPRESS-children born at 22-24 weeks of gestation, 24% had FVC and 44% had FEV1 below the lower limit of normal[105].

However, all with exception for one EXPT-child examined herein had $TRV/RVOT_{VTI}$ <0.275, indicating normal PVR. In children with the lowest FVC and FEV1, there were small but statistically insignificant trends towards increases in PVR which could indicate an effect on pulmonary vascular resistance, albeit still in the normal range. Even if, echo-Doppler estimated PVR has the potential to detect pulmonary hypertension, there are indications that it may not define precise PVR, especially not in the upper range, and the participants in the reference studies have had other referral causes than prematurity[106]. For this reason, we cannot be sure that there may have been EXPT children in our study with slightly increased and concealed pulmonary vascular resistance. Such an interpretation is supported by a slightly reduced TAPSE – a good indicator of right ventricular systolic function[107] – in children with low FEV1/FVC.

Our findings of a narrower main pulmonary artery in children with the smaller lung volumes may point towards silent and subclinical evidence of underdevelopment of the pulmonary vascular tree seen in animal studies as a result of an interruption in lung growth and lung remodeling after preterm birth[50]. Interestingly and as described above, the systemic arteries were also narrower in the EXPRESS-cohort than in same-aged controls born at term[96] maybe indicating a systemic effect on vascular growth after extremely preterm birth.

From a clinical perspective, our study seems reassuring. While lung function follow-up seems compulsory for all survivors of extremely preterm birth, based on our results they do not support echocardiographic evaluation for pulmonary hypertension and right ventricular hypertrophy on a routine basis before school-start. To identify early signs of right ventricular dysfunction, only those EXPT-children with a history of increased PVR already from infancy, or those with declining lung function, need echocardiographic assessment at early age.

6.1 STRENGTHS AND LIMITATIONS

Strengths of this study include the national population-based and prospective design, a large cohort of survivors born at 22-26 weeks of gestation having a detailed cardiovascular, including lung function with spirometry, follow-up at 6-years-of-age. The control group was carefully selected to avoid selection bias. Blinding of those who conducted the measurements and analyses minimized observer bias and drop-out analysis did not indicate any response bias. Almost all pregnancies were dated by obstetric ultrasound. The number of study participants were large enough to allow for subgroup and risk factor analysis.

Limitations include that causality cannot be proven for any association between preterm birth and later cardiovascular outcome, and genetic or other sources of confounding cannot be excluded. We studied some but not whole systemic and pulmonary circulation. All cardiovascular assessment were performed at rest, and we didn't investigated 24-h ambulatory blood pressure. Blood sampling for lipid profiling, inflammatory status or other biomarkers of vascular aging was not performed. Arterial stiffness was studied in segments of the common carotid artery and the abdominal aorta, but we did not evaluate pulse wave velocity over the entire aorta. Finally, arterial blood flow and vascular resistance were not assessed.

7 CONCLUSIONS AND CLINICAL IMPLICATIONS

In summary, healthy 6-year-old children surviving extremely preterm birth have

- smaller or similar sized arterial tree, similar carotid intima media thickness, and similar or even decreased arterial stiffness
- smaller left hearts featuring signs of deviating systolic and diastolic functions (also after adjusting for body surface area and also in the absence of arterial hypertension)
- higher systolic and diastolic blood pressures, as well as resting heart rate

than age- and sex-matched control children born at term. In addition, we found

- no association between reduced lung function and increased pulmonary vascular resistance or right ventricular wall thickening in 6-year-old children born extremely preterm

The preterm arteries, heart and blood pressure in childhood are likely reflections of early developmental deviations in cardiovascular physiology. Their significance for cardiovascular morbidity and mortality in adult life remain to be established. If our findings could be linked to disadvantageous cardiovascular health in future life, we think that the roots and opportunities for intervention should primarily be sought in the neonatal period[12].

The most important clinical implication of our findings is that before 6 years of age, there seems to be no need for routine investigation of the cardiovascular system in children born extremely preterm. In this relatively large cohort of pre-school children we found no signs of accelerated arterial aging as assessed by carotid IMT and arterial stiffness and there was no child with overt signs of myocardial hypertrophy or heart failure, or with systemic or pulmonary hypertension in need of further investigation and treatment.

That said, we suggest that blood pressure is monitored in older children and young adults born preterm. Early detection of hypertension is important as hypertension at the age of 30 years increases the life-time risk for cardiovascular disease by 40%[83]. We also think that at some point before adulthood, children born extremely preterm should be assessed with echocardiography and those detected with significant reductions in LV size or function should be included in a follow-up program. As a first step, such an assessment could be included in a coming longitudinal research agenda for the EXPRESS cohort.

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9 SVENSK SAMMANFATTNING

Globalt sett slutar 6 till 12 procent av alla graviditeter med förtidig födelse, dvs födelse före graviditetsvecka 37. Efter introduktion av antenatala kortikosteroider, surfaktantbehandling och neonatal intensiv vård har överlevnaden efter tidig födelse ökat markant över hela världen. Trots att dessa framsteg är mycket välkomna så har på senare tid uppkommit en oro för att tidig födelse kan medföra ökad risk för sjuklighet i vuxen ålder och kanske en förkortad livslängd. Bakgrunden är rapporter om samband mellan tidig födelse och ökad risk för kardiovaskulära sjukdomar. Sambanden kan vara särskilt viktiga i populationer med ökad förekomst av andra riskfaktorer för hjärtkärlsjukdom såsom övervikt och minskad rörlighet.

Denna avhandling syftar till att ge ny kunskap om blodtryck, tillväxt och funktion av hjärtkärlsystemet hos barn som var födda extremt förtidigt, d.v.s. 14-18 veckor före beräknad tid. Att lyfta dessa områden kan hjälpa vår nuvarande förståelse av kardiovaskulär utveckling efter tidig födelse, om och när kardiovaskulär uppföljning behövs i barndomen, och ge viktiga ledtrådar om hur varaktig kardiovaskulär hälsa efter tidig födelse kan främjas.

Vi har studerat en populationsbaserad kohort EXPRESS (Extremely Preterm Infants in Sweden Study) med barn födda mellan åren 2004-2007 i graviditetsvecka 22-26 och kontroller matchade för ålder och kön födda i fullgången tid. Vid 6 ½ års ålder mätte vi blodtryck och utförde en fullständig ekokardiografisk utvärdering av kardiovaskulära strukturer och funktioner. Vi utförde också lungfunktionsmätning med spirometri.

De stora artärerna och kranskärlen var lika stora eller mindre hos de förtidigt födda barnen jämfört med kontrollerna vilket kvarstod efter justering för kroppsstorlek. Vi hittade inga tecken till ökad intima media tjocklek eller artärstelhet, två tidiga markörer för en predisposition för arterioskleros.

Viloblodtryck var normala men högre hos de tidigt födda barnen jämfört med jämnåriga barnen födda i normal tid. Blodtrycksförhöjningen kunde inte förklaras av andra faktorer såsom fetal tillväxthämning, exponering för antenatala kortikosteroider eller neonatal sjuklighet. Barn som fötts extremt tidigt uppvisade också en unik hjärtfenotyp som karaktäriserades av en mindre vänsterkammare med annorlunda systolisk och diastolisk funktion jämfört med barn i samma ålder som fötts i fullgången tid. Slutligen fann vi att reducerad lungfunktion eller luftvägsobstruktion hos extremt tidigt födda 6-åringar inte påverkade höger hjärtkammare eller trycken i lungcirkulationen.

Sammanfattningsvis, barn som fötts extremt tidigt uppvisar en hjärtkärl fenotyp som skiljer sig från jämnåriga barn födda i fullgången tid. Den långsiktiga betydelsen för kardiovaskulär hälsa av dessa fynd återstår att fastställa.

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