CARDIOVASCULAR DEVELOPMENT OF THE PRETERM INFANT

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Cardiovascular development of the preterm infant

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To Ivan and Ilse

“Man sieht nur mit dem Herzen gut, 
das Wesentliche ist für die Augen unsichtbar”

Der Kleine Prinz, Antoine de Saint-Exupéry
ABSTRACT

The numbers of preterm births and cardiovascular deaths are increasing in most countries. The causes of both developments are multiple and apparently not related to each other. However, preterm birth might provide an increasing contribution to the burden of cardiovascular morbidity and mortality, since epidemiological evidence is growing that cardiovascular disease risk factors such as hypertension, ischemic heart disease and cerebrovascular events are linked to preterm birth. Despite this, most of the underlying mechanisms remain unknown.

The overall concept of this thesis was to seek evidence for a perinatal origin of the changes seen in adults who were born preterm, regarding both vascular and cardiac function. For this purpose, we performed a longitudinal observational study, investigating changes in diameter and intima-media thickness of the aorta and carotid artery, and in cardiac function during the first six months after preterm birth. In addition to well-established ultrasound methods, we applied innovative technology such as speckle-tracking echocardiography, since advances in myocardial imaging modalities have facilitated the echocardiographic examination of preterm infants and even the detection of subclinical functional impairment.

In preterm born infants, we found significant alterations of the development of the cardiovascular system. The large arteries we examined became significantly narrower and the intima-media thickened in relation to vessel diameter when they were compared to healthy infants born at term. In addition, we found significant differences in the left ventricular systolic and diastolic function, suggesting that myocardial remodeling may occur as an adaptive process of premature exposure towards the extra-uterine circulation.

Early changes in the cardiovascular development of the preterm infant may persist and have long-term implications. In fact, adults born preterm exhibit similar alterations in cardiovascular structure and function to those found in our studies. As it is not currently possible to prevent preterm birth or influence the developmental changes described in this thesis, we will meet more children and adolescents with remodeled vessels and hearts in the future. Further research on the underlying mechanisms is warranted. In addition, early and continued follow-up will be required if we are to determine the long-term and clinical significance, and to improve cardiovascular health in the growing population of individuals born preterm.
LIST OF SCIENTIFIC PAPERS

I. Aortic growth arrest after preterm birth: a lasting structural change of the vascular tree
   Ulf Schubert, Matthias Müller, Anna-Karin Edstedt Bonamy, Hashim Abdul-Khaliq, Mikael Norman

II. Relative intima-media thickening after preterm birth
   Ulf Schubert, Matthias Müller, Hashim Abdul-Khaliq, Mikael Norman, Anna-Karin Edstedt Bonamy
   Acta Paediatrica 2013, 102: 965-969

III. Transition from fetal to neonatal life: Changes in cardiac function assessed by speckle-tracking echocardiography
   Ulf Schubert, Matthias Müller, Mikael Norman, Hashim Abdul-Khaliq
   Early Human Development 2013, 89: 803-808

IV. Preterm birth is associated with altered myocardial function in infancy
   Ulf Schubert, Matthias Müller, Hashim Abdul-Khaliq, Mikael Norman
   Journal of the American Society of Echocardiography 2016, 29(7): 670-8
LIST OF ABBREVIATIONS

aEDD  Aortic end-diastolic diameter
AGA   Appropriate for gestational age
a-IMT Aortic intima-media thickness
aSD   Aortic systolic diameter
BMI   Body mass index
BPD   Bronchopulmonary dysplasia
cEDD  Carotid end-diastolic diameter
c-IMT Carotid intima-media thickness
CO    Cardiac output
cSD   Carotid systolic diameter
CVD   Cardiovascular disease
DOHaD Developmental Origins of Health and Disease
ECG   Electrocardiogram
EDD   End-diastolic diameter
EF    Ejection fraction
FAC   Fractional area change
GV    Growth velocity
IGF   Insulin-like growth factor
IMT   Intima-media thickness
IUGR  Intra-uterine growth restriction
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>IVH</td>
<td>Intraventricular hemorrhage</td>
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<td>IVS</td>
<td>Interventricular septum</td>
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<tr>
<td>Kcal</td>
<td>Kilocalorie</td>
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<tr>
<td>Khz</td>
<td>Kilohertz</td>
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<td>LBW</td>
<td>Low birth weight</td>
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<td>LV</td>
<td>Left ventricle</td>
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<td>MI</td>
<td>Mechanical index</td>
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<td>MMP</td>
<td>Matrix metalloproteinase</td>
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<td>MPI</td>
<td>Myocardial performance index</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>NEC</td>
<td>Necrotizing enterocolitis</td>
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<td>NICU</td>
<td>Neonatal intensive care unit</td>
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<td>NO</td>
<td>Nitric oxide</td>
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<td>PDA</td>
<td>Patent ductus arteriosus</td>
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<tr>
<td>PDGF</td>
<td>Platelet-derived growth factor</td>
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<td>PPROM</td>
<td>Prelabor premature rupture of membranes</td>
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<td>PVL</td>
<td>Periventricular leukomalacia</td>
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<td>RDS</td>
<td>Respiratory distress syndrome</td>
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<td>ROI</td>
<td>Region of interest</td>
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<td>ROP</td>
<td>Retinopathy of prematurity</td>
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<td>RV</td>
<td>Right ventricle</td>
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<td>SD</td>
<td>Standard deviation</td>
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<td>SDS</td>
<td>Standard deviation score</td>
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<td>SF</td>
<td>Shortening fraction</td>
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<tr>
<td>SGA</td>
<td>Small for gestational age</td>
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<tr>
<td>SMC</td>
<td>Smooth muscle cell</td>
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<tr>
<td>STE</td>
<td>Speckle-tracking echocardiography</td>
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<tr>
<td>STIC</td>
<td>Spatio-temporal image correlation</td>
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<tr>
<td>SV</td>
<td>Stroke volume</td>
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<tr>
<td>TAPSE</td>
<td>Tricuspid annular plane systolic excursion</td>
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<td>TI</td>
<td>Thermal index</td>
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<td>TTTS</td>
<td>Twin-to-twin transfusion syndrome</td>
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<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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<td>VLBW</td>
<td>Very low birth weight</td>
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<td>Vs.</td>
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<td>WHO</td>
<td>World Health Organization</td>
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1 INTRODUCTION

Every year there are around 16 million infants born preterm across the world. That number is equivalent to the entire population of the Netherlands and is increasing worldwide. In high-income countries this rise is partly due to the more frequent use of assisted reproductive technology like in-vitro fertilization and artificial insemination, and increasing maternal age\textsuperscript{1-3}. In addition, advances in prenatal and postnatal care over the last decades have contributed to the fact that the number of infants who survive preterm birth is constantly increasing, leading to a growing population of adults born preterm.

There is some epidemiological evidence that preterm birth might be a risk factor for cardiovascular and metabolic disease in later life, but very little is known about the mechanisms. Preterm delivery induces adaptive processes in hemodynamics that stabilize the infants’ condition ex-utero, which might also be implicated in life-long anatomical and functional changes in the cardiovascular system. These changes seem to contribute to adverse cardiovascular development, such as arterial hypertension, stroke and impaired myocardial function\textsuperscript{4-7}.

Cardiovascular disease represents the most important cause of morbidity and mortality in developed countries. Important changes in lifestyle in lower income and developing countries are leading to a situation where cardiovascular disease will replace infections as the dominant cause of death even in these countries already during the next decade.

From a public health perspective, it is still unclear how preterm birth will contribute to the global burden of cardiovascular disease.

In this thesis, I will discuss three studies presenting early cardiovascular changes after preterm birth and one study investigating the transition from fetal to neonatal life using novel echocardiographic techniques.
2 BACKGROUND

2.1 PRETERM BIRTH

2.1.1 Definitions and epidemiology

The normal human pregnancy length is estimated to be 280 days after the first day of
the last menstrual period\textsuperscript{8}. In relation to this estimate, the World Health Organization
(WHO) has defined preterm birth as a pregnancy that ends before 37 gestational weeks
have been completed\textsuperscript{9}. A categorization into further subdivisions of moderately preterm
(32-36 weeks), very preterm (<32 weeks) and extremely preterm (<28 weeks) infants is
widely accepted (Figure 1)\textsuperscript{10}. In order to date gestational age, and define the expected
date of delivery, the so-called pregnancy wheel is used, counting 280 days from the last
menstrual period\textsuperscript{11}. In conditions where ultrasound is available, a more correct
estimation of pregnancy length is possible due to the fact that fetal size is proportional to
gestational length\textsuperscript{12}.

![Figure 1: Definition of pregnancy length (adapted from Tucker et al.)](image)

About 16 million infants are born prematurely around the world each year\textsuperscript{13}. Although
the majority of preterm births, about 85\%, occur after 31 weeks, prematurity is the single
biggest cause of newborn death and the second-leading cause of death after
pneumonia in children under the age of five\textsuperscript{14}. According to data from the WHO\textsuperscript{15}, the
number of preterm births is continuously increasing worldwide, and the present rate is
more than 11%. The range across 184 countries is 5-18%, with the highest numbers of
premature births in Southern Asia and Sub-Saharan Africa. In 2011, the first World
Prematurity Day was arranged, and public health policies recognized the fact that global
progress in child survival and health cannot be achieved without addressing the problem
of preterm birth. These strategies should address education and health for girls and
women, the prevention and management of sexually transmitted diseases, family
planning and the promotion of healthy nutrition and a healthy lifestyle.

2.1.2 Etiology of preterm birth

Approximately 60% of all preterm deliveries have a spontaneous onset and 40% are
medically induced. The main causes for spontaneous deliveries are premature rupture
of the membranes, placental abruption, premature labor and vaginal bleeding, often due
to infection, inflammation or stress. Medical indications for delivering preterm infants
include pregnancy complications such as preeclampsia, and fetal conditions such as
intrauterine growth restriction (IUGR), hydrops, infection or anemia.

Several risk factors for preterm birth have been identified. Genetic influences account for
about 30% of the development of preeclampsia, a common cause of preterm delivery,
but even a family history of preterm birth without preeclampsia raises the risk for a
subsequent spontaneous preterm delivery. Other factors, such as maternal obesity,
low socioeconomic status of the family, maternal smoking, multiple pregnancies and
bacterial colonization of the reproductive tract, are associated with higher rates of
prematurity. Finally, low or advanced maternal age and assisted reproductive
techniques have resulted in an important increase of preterm deliveries worldwide.

In summary, preterm infants have been exposed to very different biological conditions
before delivery and cannot be considered as a single homogenous group. This
heterogeneity is important to consider when studying short and long-term outcomes
after preterm birth.
2.1.3 Neonatal morbidity

Neonatal morbidity and mortality rates are inversely related to gestational age, meaning that the probability of suffering from medical problems related to preterm birth increases with the immaturity of the infant\textsuperscript{24}. Moderately preterm infants may have difficulties in maintaining their temperature and stable blood glucose levels and have feeding and respiratory problems. However, these conditions are relatively easy to treat and do usually not imply severe complications.

On the other hand, very preterm and extremely preterm infants may suffer from a variety of medical problems and complications due to the immaturity of their organ systems and inappropriate adaptation to the extra-uterine environment. A number of severe morbidities can exist during more acute periods of hospitalization. Respiratory distress syndrome (RDS), infections, patent ductus arteriosus (PDA), necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), periventricular leukomalacia (PVL) and retinopathy of prematurity (ROP) account for severe conditions that often coexist.

In the long-term, the development of chronic lung disease or bronchopulmonary dysplasia (BPD) is a common respiratory problem. The most severe cases are complicated by pulmonary hypertension and cor pulmonale\textsuperscript{25}.

Persistent impairments include cognitive problems and behavioral disorders, hearing loss, visual problems/blindness and cerebral palsy\textsuperscript{26}.

2.2 CARDIOVASCULAR DISEASE (CVD)

Cardiovascular disease is a class of morbidities that involve the heart and/or blood vessels. Common CVDs include: ischemic heart disease, congestive heart failure, rheumatic heart disease, cardiomyopathy, congenital heart disease, peripheral artery disease and stroke. Atherosclerosis is the most common cause of CVD and may be accelerated by arterial hypertension, smoking, diabetes, lack of exercise, obesity, high blood cholesterol, poor diet and drugs. According to a report published by the WHO in collaboration with the World Heart Federation\textsuperscript{27}, cardiovascular diseases are the leading cause of death worldwide, except in Africa, where infections are still responsible for most deaths. According to the report on the Global Burden of Disease Study\textsuperscript{28}, CVDs resulted in 17.3 million deaths (31.5% of all deaths) in 2013 compared to 12.3 million (25.8%) in 1990. Ischemic heart disease and stroke account for approximately 80% of
CVD deaths in men and 75% in women, at an average age of 80 in the developed world and 68 in the developing world. Thus, age is the most important risk factor for CVD. Another risk factor is a low socioeconomic and educational status. Because of this, the Commission on Social Determinants of Health (WHO 2008)\textsuperscript{29} has recommended that more equal distributions of power, wealth, education, housing, environmental factors, nutrition and health care are needed to address inequalities in cardiovascular disease and non-communicable diseases\textsuperscript{30}.

2.3 DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE (DOHAD) AND FETAL PROGRAMMING

The hypothesis that early life exposures predict later health outcomes has been discussed extensively during the last 30 years. The original hypothesis was a result of observations in different studies that found an association between low birth weight (LBW) and coronary heart disease later in life\textsuperscript{31,32}. The basic idea from an evolutionary perspective is that early life exposure has already induced an adaptive response in the fetus in order to provide it with an advantage when it comes to surviving in its current situation, and make a prediction of future environmental conditions.

Undernutrition was a common exposure during pregnancies in the last century and led to many LBW infants. This has also given researchers the opportunity to investigate associations between inadequate intrauterine growth patterns and later disease. Changes in organ growth and in fetal circulation can be interpreted as adaptive responses towards undernutrition and might be beneficial under the current precarious situation. Nevertheless, these, sometimes irreversible changes, may result in disadvantageous health developments after birth. Thus, other cardiovascular diseases such as hypertension and stroke, as well as diabetes and metabolic syndrome, have been linked to LBW. The underlying mechanisms of how environment is shaping developmental trajectories are nowadays investigated, including fetal programming by epigenetic modification of gene expression and telomere attrition\textsuperscript{33}.

The DOHaD hypothesis has been criticized because the associations between exposures very early in life and the risk of morbidity and mortality half a century later do not seem to be plausible. According to the critics, other environmental and genetic factors may confound the associations between birth weight and disease risk in a lifelong perspective.
Since most of the early studies in the DOHaD field originate from the beginning of the 20th century, LBW was a consequence of intrauterine growth restriction at term, and not prematurity. But now that the number of infants who survive preterm birth increases, the main cause of LBW is preterm birth, often with appropriate birth weights. Thus, the mechanisms leading to LBW are very different, and it is doubtful that the results from growth-restricted individuals can be extrapolated to preterm infants. However, there is increasing evidence that even prematurity might operate as an early exposure and signal for cardiovascular diseases at a later date. For example, in a large cohort of more than 600,000 young adults, the proportion of those who were born preterm and were given antihypertensive drugs as a proxy for hypertension was higher compared to the group of individuals born at term.

Another large meta-analysis confirmed the association between prematurity and hypertension, and a third population-based study indicated that the risk of hypertension among young men increased with the degree of prematurity.

Intima-media thickening is an established risk factor for atherosclerosis. Some studies have shown that individuals born preterm exhibit increased IMT, and that in young adults born preterm, thickening of the carotid intima-media is associated with an unfavorable lipid profile and a higher current body mass index.

Given that preterm birth contributes to cardiovascular disease, it may have important implications for public health. We are not just observing an increasing number of preterm deliveries, but also a substantial increase in the numbers of preterm survivors. In 2010, about 90% of preterm births occurred in low-income and middle-income countries. As many of the cardiovascular risk factors emerge with ageing, the burden on healthcare costs will rise in the future, especially in these countries.

### 2.4 PRINCIPLES AND SAFETY OF ULTRASOUND

The basic idea of ultrasound is that electrical signals are converted into mechanical pulses that are reflected in anatomical tissues according to differences in the acoustic impedance of these structures. The transducer of an ultrasound machine consists of piezoelectric material, which transforms electrical into mechanical signals by changing size when voltage is applied. But the transducer is not just responsible for the emission of the pulse, it also receives the echoes, transforming mechanical signals back to
electrical signals that are used to produce the image on the screen of the ultrasound machine. In medical applications, the frequencies of the waves are above 20 kHz and cannot be perceived by the human ear (beyond sound = ultrasound). As the propagation speed of the waves in human tissue only varies within a small range (1480-1580 m/s), the position of a specific target can be calculated by the time between the emission and reception of the signal. The intensity of the reflected signal is visualized as brightness and is mainly dependent on the attenuation coefficient of the respective tissue.

Attenuation is the combined effect of absorption and scattering and is most prominent in bone and soft tissues and lower in liquids and blood. The fact that different signal intensities produce different levels of brightness gave the B-mode scan its name. However, it is better to use the expression "cross-sectional scan", as echo intensity determines the brightness even in M-Mode, or motion, imaging. The main difference between the two modes is that only one scan line is interrogated versus time in M-Mode, while multiple scan lines are used in B-Mode imaging. The cross-sectional image is produced by sweeping a beam through different scan lines, and the repetition rate needs to be high enough to build up moving targets. As fetal and newborn hearts move at high velocities and rates, it is crucial to use high frame rates in order to achieve optimal temporal resolution.

In infants and fetus, hearts are not only beating faster than in adults. They are also much smaller. Therefore, optimal spatial resolution is necessary to discriminate different points. Lateral resolution depends on beam width and line density. An increase in line density can be achieved by reducing the sector width or frame rate. Axial resolution is determined by pulse length, and the shorter the pulse wave, the better the point-to-point differentiation in the axial direction. Obviously, higher frequencies may provide shorter pulses and better axial resolution, but again, attenuation is greater and tissue penetration poorer.

The Doppler technique is used in addition to M-Mode and B-Mode imaging in order to visualize moving objects such as blood cells or the myocardium. This imaging technology is based on the effect that the frequency of sound is shifted when it is reflected against a moving object. By analyzing the shift, it is possible to measure velocities that are recorded in relation to the position of the transducer and displayed on the ultrasound machine. Both pulsed wave Doppler and continuous Doppler techniques are applied, and velocity signals are usually coded in red and blue color maps, depending on whether the object is moving towards the transducer or away from it.
A loudspeaker is normally used to produce an audible signal, which is equal to the Doppler shift and represents the intensity of the scattered wave.\(^\text{39}\)

### 2.4.1 Mechanical and thermal indices

As mentioned before, electrical energy is transformed into mechanical energy by the transducer, thus emitting ultrasonic waves that are travelling through different kinds of tissue in the human body. Part of the energy is scattered back towards the transducer, but a considerable amount of the energy is absorbed by the tissue, a physiological effect that leads to heating. The absorption or attenuation of energy is very similar in soft tissues, but considerably higher in bone, for example. The amount of heating depends on the imaging modes, with B-mode being the lowest, and M-Mode, color Doppler and pulsed Doppler being higher. This means that the shortest time should be spend on Doppler imaging during a routine ultrasound examination.\(^\text{40}\)

Secondly, ultrasound waves lead to pressure variations in body tissue that may cause bubbles. Two different types of these so-called cavitations have been described:

a) stable cavities oscillate causing shear stress to cell membranes that might be damaged and b) inertial cavities may collapse and produce high temperature and chemical radicals that may induce tissue destruction.\(^\text{41}\)

Because of the potentially detrimental effects of heating and cavitation, manufacturers of ultrasound machines are advised to provide online updates of the so-called thermal (TI) and mechanical index (MI). This ensures that the investigator is aware of possible risks and is able to adapt acoustic output settings and limit the duration of examination.\(^\text{42}\)

### 2.5 SPECKLE-TRACKING ECHOCARDIOGRAPHY (STE)

All ultrasound images consist of a huge number of speckles that are the result of reflected echoes and backscatters. This can be easily observed when an operator uses the zoom facility and the image is becoming less smooth. Each region of the myocardium includes a characteristic pattern of speckles that are unique and reasonably stable even under conditions of movement, and can be re-identified or tracked after displacement and deformation (Figure 2).
This is essential in order to be able to use this technique in echocardiography, as the myocardium is contracting and relaxing during the heart cycle and the position of the speckle pattern is changing according to fiber movements. Thus, the displacement (cm), velocity of displacement (cm/s), deformation (=strain%) and rate of deformation (strain-rate 1/s) can be measured during systole and diastole. Lagrangian strain is defined as the deformation of an object in relation to its original length ($\varepsilon = \frac{L - L_0}{L_0}$), meaning that a negative strain of 20% compresses the object by one-fifth, whereas the corresponding positive strain is the result of stretching. Usually, the speckles of the myocardium reach the original position and configuration at the end of the heart cycle, and both the displacement and strain are zero for that time point and the maximal strain is equal in systole and diastole\textsuperscript{43}. Strain can be measured in all dimensions and longitudinal, radial and circumferential strain are used to characterize myocardial movements.

Myocardial velocity is defined as displacement per time unit and the strain rate as strain per time unit. Both variables are expressed in systole and diastole, with the diastolic part of the cardiac cycle being differentiated in early and late ventricular filling phases.

STE has been validated for the left ventricle in vivo and in vitro by sonomicrometry and tagged magnetic resonance imaging (MRI)\textsuperscript{44}. STE measures have been shown to be very sensitive to changes in load and cardiac function\textsuperscript{45-47}, and have, therefore, been considered a valuable tool in detecting myocardial impairment that was not recognized.
by conventional echocardiography, for example in children with septic shock\textsuperscript{48}, rejection after a heart transplant\textsuperscript{49} or cardiac resynchronization therapy\textsuperscript{50}. The ability to detect subclinical myocardial dysfunction before the appearance of clinically apparent ventricular impairment has been recognized even in preterm infants\textsuperscript{51}. Since the right ventricle is of particular interest in fetal and neonatal examinations, and a strong predictor of outcome in many pathological conditions in preterm infants\textsuperscript{52}, the technique has been used to evaluate the right ventricle and showed good feasibility and reproducibility\textsuperscript{53}. The lack of objective echocardiographic tools to describe right ventricular function has made STE even more valuable.

STE on infants with smaller hearts and higher heart rates depends on optimal spatial and temporal resolution. As the quality of tracking is a function of the frames that can be analyzed during each heart cycle, the frame rates have to be high enough to ensure excellent tracking conditions. However, if the frame rates are too high, the lateral resolution might be impaired due to the effects of smearing.

### 2.6 THE VASCULAR TREE

#### 2.6.1 Arterial structure, intima-media thickening and the development of atherosclerosis

A normal artery can be divided into three layers: the intima, media and adventitia. The tunica intima consists of a single layer of endothelium cells, which are in direct contact with the blood flow. The main function of this layer is to provide a barrier function and maintain vascular homeostasis via a mechano-biochemical response to differences in shear stress, thus modulating permeability, coagulation and vascular tone.

The tunica media is composed of concentrically arranged smooth muscle cells (SMC), the extracellular matrix, collagen and elastic tissues. In larger arteries, such as the aorta and carotid artery, the amount of elastic tissue is considerably higher than in the smaller arteries or arterioles. Arterioles constitute the main site for peripheral resistance due to their smaller diameters, and contribute to the translation of a pulsatile towards a more continuous flow. The interaction between the intima and media occurs via myoepithelial bridges, which are sensitive to shear stress and essential for the regulation of the vascular tone.
The tunica adventitia is the outermost connective tissue around the artery and is made of longitudinally arranged collagen fibers, which contain nerves and capillaries (vasa vasorum) that supply the larger arteries with oxygen and nourishment from the abluminal side.

The biomechanical force on the vessel wall – which is the perpendicular force of blood pressure and the cyclic stretch of pulsatile flow – is dependent on blood flow, arterial diameter, resistance and viscosity and is usually expressed as dynes/cm². Normal vascular homeostasis is maintained when all of these factors are in balance. However, changes in diameter, blood pressure or laminar flow lead to altered shear stress, activate intracellular biochemical pathways and result in modulation of cellular structure and function⁵⁴. The number of known translational factors has been constantly increasing over the last decades, and include: vasoactive substances such as nitric oxide (NO), endothelin-1, prostacyclin and natriuretic peptide; growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and insulin-like growth factor 1 (IGF-1); pro-inflammatory mediators and complement factors such as tPA, thrombomodulin, and also matrix molecules such as MMP 9 and Collagen XII.

At the critical point when atherogenic influences overweigh atheroprotective mechanisms, atherosclerosis might develop. One mechanism is translated via the activation and proliferation of SMC, resulting in intimal hyperplasia and a decrease in maximal shortening velocity⁵⁵. As a consequence, the artery is less adaptive towards changes in shear stress and becomes stiffer and less compliant. As the total arterial compliance has a direct impact on systolic blood pressure⁵⁶, the pathological pathway can lead to a circulus vitiosus and the evolution of atherosclerosis⁵⁷. Simultaneously, the synthesis of MMPs induces the retention of lipoproteins and fatty acids, which can be seen histologically as fatty streaks in the early stage of atheroma formation⁵⁸.
2.6.2 Vessel growth after preterm birth

During the last trimester of pregnancy, high levels of circulating IGF-1 induce vascular growth and elastin deposition in order to prepare sufficient elastic recoil in the great arteries in the feto-neonatal transition\textsuperscript{59}. Several studies have shown that human aortic smooth muscle cells are sensitive to IGF-1 at the receptor level and that a normal concentration of IGF-1 is necessary for normal vascular development\textsuperscript{60,61}. The accumulation of elastin in the arterial wall is a process that has been shown to be particularly active in the immediate perinatal period in an animal model\textsuperscript{62}. In humans, the down-regulation of IGF-1 caused by placental insufficiency has been associated with thinner and stiffer umbilical arteries in newborn infants who had suffered from intrauterine growth restriction\textsuperscript{63}. Moreover, children born small for gestational age continue to exhibit low levels of IGF-1 and vascular dysfunction\textsuperscript{64}. Taken together, these findings could explain why fetal growth restriction is a developmental risk factor for lasting arterial narrowing\textsuperscript{65,66}.

Low serum concentrations of IGF-I in very preterm infants have been associated with arrested growth of the microvasculature in the eye, preceding retinopathy of prematurity (ROP)\textsuperscript{67}. This disease affects the immature retina of preterm infants. Due to deficient vasculogenesis, hypoxia leads to excessive levels of vascular endothelial growth factor (VEGF), which causes pathological neovascularization\textsuperscript{68}.

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**Figure 3:** Different stages in the development of atherosclerosis from early life to symptomatic disease. Adapted from http://en.wikipedia.org/wiki/File:Endo_dysfunction_Athero.PNG, free documentation license.
2.6.3 Arterial dimensions and IMT after preterm birth

Prematurity and the programming of cardiovascular disease has become an important field of research\textsuperscript{33}, and it seems that vascular ageing is starting already in the fetal period and continues throughout life\textsuperscript{58,69,70}. Therefore, many researchers have developed methods to predict cardiovascular disease risk. It is obvious that the earlier valid parameters are available, the sooner medical and lifestyle interventions can be initiated.

In addition to measurements of endothelial function\textsuperscript{71,72}, arterial elasticity\textsuperscript{73,74} and blood pressure\textsuperscript{75} in individuals born preterm, arterial dimensions and IMT have been determined in several studies and at different ages. There is actually some evidence that narrowing of the vascular tree is present not only in subjects born at term with low birth weight\textsuperscript{65,66,76-78} but also in individuals born preterm\textsuperscript{72,79,80}. The most common methodological approach is non-invasive ultrasound of the aorta, carotid and coronary arteries, which measures diameters and IMT\textsuperscript{81}. Results concerning IMT can be somewhat contradictory as some studies have shown an association between low birth weight and intima-media thickening\textsuperscript{82-86}, while others, mostly in elderly people, have failed to demonstrate this association\textsuperscript{35,87-91}. Even in autopsies performed after neonatal deaths, coronary IMT was not increased in infants born small for gestational age in one study\textsuperscript{87}. In contrast, another study investigating 22 unexplained intra-uterine deaths and 36 victims due to sudden-infant-death-syndrome, has shown early atherosclerotic coronary lesions in the prenatal and neonatal period, especially in infants whose mothers smoked\textsuperscript{69} (Figure 4).

Another study compared 92 young adults born with very low birth weight with 68 individuals born at term with adequate birth weight\textsuperscript{92}. The authors found that the carotid arteries in the low birth weight subjects were insignificantly narrower, but IMT was significantly thicker in relation to vessel diameter.

However, it is important to emphasize, that most of these studies included subjects who were born with low birth weight but not necessarily preterm. And although studies will be able to show that VLBW infants - both preterm and term - exhibit intima-media thickening, pathophysiological mechanisms might be different.
2.7 CARDIAC DEVELOPMENT

2.7.1 Circulatory transition at birth

A lot of processes occur over a very short period of time when the placental circulation is interrupted at delivery. Fluid from the lungs is removed by tissue absorption and expulsion through the mouth and regular breathing is established. The location of gas exchange is transferred from the placenta to the lungs and sufficient pulmonary blood flow has to be established in order to facilitate oxygen uptake. During late gestation, pulmonary perfusion accounts for only about 17% of the combined cardiac output\textsuperscript{93,94}. In the postnatal period, when blood is flowing serially through the right and left ventricles, practically all of the blood circulates through the lungs. The sudden increase in alveolar and arteriolar oxygen tension in the pulmonary artery, lung expansion and vasoactive products from the vascular endothelium, such as endogenous NO and prostacyclin lead to decreased pulmonary vascular resistance\textsuperscript{95}. The increase of pulmonary blood flow and venous return to the left atrium results in higher left atrial pressures, and in combination with the increase of left ventricular afterload, the foramen ovale closes. Left ventricular afterload rises immediately at birth, when the low resistant placental circulation is replaced by a high resistant systemic circulation. The underlying mechanism seems to be peripheral vasoconstriction, due to labor-induced endogenous catecholamines, renin-angiotensin, vasopressin and corticosteroids\textsuperscript{95}.

In healthy conditions, the closure of the ductus arteriosus occurs slightly later than the foramen ovale and the ductus venosus and this process can take place hours or even days after birth. The closure occurs due to higher partial oxygen pressures and decreased concentrations of the circulating prostaglandin E2.
In the fetus, oxygenated blood returns through the umbilical venous system, passes through the ductus venosus and mixes with poorly oxygenated blood from the fetal systemic circulation before entering the right atrium. With the clamping of the umbilical cord the ductus venosus usually closes and the right atrial preload decreases\textsuperscript{96}. Simultaneously, intrathoracic pressure levels fall from about 8-10 mmHg prenatally to subatmospheric and makes the ventricular function less restrictive, which could compensate for the drop in right ventricular preload.

Fetal ventricular function is restrictive due to an incompliant thorax that is surrounded by amniotic fluid. The myocardium is both less compliant and less contractile than during the postnatal period, and the main mechanism to cope with changes in preload and afterload is to change heart rate. The fetal myocardium consists of less contractile units, a developing sarcoplasmatic reticulum and T-tubular system, a deficiency in the ability of calcium uptake and reduced responsiveness towards sympathetic stimulation due to sparse sympathetic nerve endings. Additionally, the myocytes are smaller than in a term newborn with a single nucleus and less mitochondria and a higher water content\textsuperscript{97}.

In the event of a preterm delivery, the gradual development of contractile units and T-tubular systems, sympathetic innervation and increase of myocardial mass is suddenly interrupted\textsuperscript{98}. Further development then occurs under adverse – or at least very different conditions in the extra-uterine environment.

After preterm birth, the immature myocardium of the left ventricle is exposed to a significantly higher afterload and additionally, to persisting atrial and ductal shunts, leading to a volume overload. But even a delay in the decrease of pulmonary vascular resistance, due to respiratory distress syndrome, might put an extra load to the myocardium of the right ventricle.

To date, there have been very few human studies that focused on myocardial development and preterm birth, and there is little knowledge about how the adaptive mechanisms of the myocardium cope with the extra load. However, there is interesting data of myocardial remodelling in preterm lambs. According to their findings, the adaptive process towards higher afterload was characterized by the deposition of extracellular matrix and collagen in the myocardium in order to maintain the integrity and strength of the ventricular wall. In addition, T-lymphocytes were present in most of the preterm lamb hearts, suggesting an inflammatory response similar to the cardiac inflammatory changes found in patients with hypertension\textsuperscript{99}. 
2.7.2 Cardiac function in the fetus

The heart begins as a primitive tube and starts to contract already at four weeks of gestational age, although blood is not pumped into the circulation at this early stage. When the transition to a looped heart occurs, peristaltic movements are replaced by a suction pump model where the ejection fraction is about 100%, something that is never achieved later in life. Septation takes place between week 5-7, and differentiation of the myocardium and ventricle formation starts during this period and has usually finished before week 12. Animal studies suggest that the Frank-Starling mechanism already applies at the embryonic stadium. During the fetal period, the heart is growing and the myocardial mass is constantly increasing. Systolic and diastolic pressures show a linear rise during gestation and the combined cardiac output decreases with higher afterload.

Fetal cardiac function has been quantified by planimetric methods, flow velocity integral and calculation of stroke volume and cardiac output, shortening fraction by M-Mode, myocardial performance index (MPI) and early and late ventricular filling parameters. Additionally, three-dimensional ultrasound technologies – the so-called spatio-temporal image correlations (STIC) – have been used to calculate stroke volume and ejection fraction from systolic and diastolic ventricular volumes. MRI is still considered the gold standard, as measurements of ventricular mass and volume and the calculation of cardiac output are possible, and the image quality is not dependent on the examiner, maternal obesity, fetal position or gestational age. However, fetal movements might complicate interpretation of the images, and rapid heart rates require high frame rates that have a direct impact on image resolution.

Myocardial imaging modalities have contributed to a better understanding of fetal cardiac function. The main advantage of the speckle-tracking technique is that it is independent of the angle of insonation, which is particularly important for difficult fetal positions where Doppler techniques cannot be applied. After the initial phase of feasibility studies in 2004-2012, myocardial imaging proved to be useful in pathological conditions such as congenital heart defects, growth restriction and preeclampsia, amniotic infection and twin-to-twin transfusion syndrome. Recently, attempts have been made to use three-dimensional STE in a fetal cohort, and a mean temporal resolution of 31 volumes per second was achieved. However, the authors concluded that three-dimensional STE was only possible in a few individuals and that further improvement of this technique was necessary to use it in fetuses.
2.7.3 Cardiac function in the newborn

Historically, the basic concerns of neonatologists have been the lungs. This is understandable because of the immediate postnatal challenge in the delivery room to establish adequate ventilation and the assumption that heart rate and function will just follow ventilation. In other words: the immaturity of the respiratory system has been considered more acute in comparison with the cardiac, and the focus on the neonatal intensive care unit (NICU) has mainly been on respiratory issues. Additionally, a comprehensive approach towards cardiac function in newborn infants has always been complicated. Hemodynamic monitoring has usually been limited to the continuous measuring of heart rate and oxygen saturation, continuous or intermittent blood pressure, urine output and estimates of capillary refill and skin color. Practically all of these measurements depend on various factors other than cardiovascular, and do actually not help the clinician when it comes to making decisions or providing treatment. For example, the normal blood pressure range adjusted for gestational and postnatal age is still unknown, and the treatment of “hypotension” does not improve relevant clinical outcome in preterm infants.

Because of this dilemma, the importance of echocardiographic assessments in NICUs has been widely understood, and practical guidelines on so-called functional or point-of-care echocardiography have been formulated. The standard techniques for left ventricular function are usually based on left ventricular size and Doppler measurements of both left ventricular inflow and outflow, obtained using M-Mode in the parasternal short or long axis view and cross-sectional images in the three and four-chamber views. Many of these measurements are load-dependent, which is of particular importance in the transitional period after birth. Although it is well known that the assessment of shortening fraction (SF) is associated with considerable inter- and intraobserver variability, this technique is widely used in the evaluation of left ventricular systolic function. An alternative might be the biplane volumetric measurement and calculating the ejection fraction according to the modified Simpson’s formula, although the geometrical assumptions are not really met in newborn infants. Combining Doppler with cross-sectional imaging permits the calculation of stroke volume (SV) and cardiac output (CO). The Doppler signal can be obtained from the suprasternal or three-chamber view, while the aortic diameter is usually measured using M-Mode. It is recommended to use the mean of at least three measurements as an error in diameter leads to a squared miscalculation of CO. SV is equal to the aortic flow velocity time
integral (VTI) multiplied by the aortic valve area, and CO equals SV multiplied by heart rate. Normal values of VTI for term infants are available and can be used to estimate cardiac function\(^{128}\).

The myocardial performance index (MPI) combines systolic and diastolic function, since the sum of the isovolumetric contraction and relaxation time is divided by the ejection time. Although not specific for either systolic or diastolic performance and load-dependent, MPI is considered a useful parameter when it comes to differentiating between pathological and normal conditions in the neonatal period\(^{129}\).

The assessment of diastolic function by conventional echocardiography is mainly based on left ventricular inflow patterns. During the transitional period, these patterns progressively change, from a more restrictive inflow with lower E/A ratios, lower E-wave velocities and higher A-waves to a more mature sequence. At higher heart rates, the fusion of E and A-waves may complicate the analysis. Acceleration and deceleration times as well as velocity time integrals for both the E and A-wave may give additional information, and reference values are available for preterm and term infants\(^{130}\). Differences between preterm and term infants seem to be resolved by three months of age. In contrast to adult cardiology, the right ventricle represents an interesting target, but the limitations of conventional echocardiography usually reduce the evaluation to simple visual assessment. More advanced techniques like tricuspid annular plane systolic excursion (TAPSE) and fractional area change (FAC) have been used to quantify right ventricular function, and reference values are available for preterm and term infants\(^{51,131}\).

Recently, other techniques like tissue Doppler imaging\(^{51,132-135}\) and speckle-tracking echocardiography\(^{136-138}\) seem to have overcome some of the limitations of conventional echocardiography, showing good feasibility and reproducibility, even in preterm infants\(^{53,139-141}\). Some of these have found altered left ventricular diastolic function\(^{140}\) and others have revealed changes in systolic function in infants undergoing ligation of PDA\(^{141}\).

As the myocardium of the LV consists of two helically orientated muscle layers with an interposed circumferential midwall layer, rotational movements make an important contribution to the ejection of blood from the LV cavity and can be tracked by two-dimensional STE\(^{142}\). The torsion or wringing motion towards the LV outflow tract in
systole is the net twist between apical and basal twist, untwisting is considered a surrogate for diastolic function.

Another technique that has been introduced to the pediatric population is real-time three-dimensional STE, analyzing myocardial deformation in all three dimensions without losing speckles because of out-of-plane motion. During the acquisition of 3D-images, the number of achievable frames/volumes is crucial in order to obtain acceptable temporal resolution, and the authors concluded that the resolution was good enough to use it in the pediatric population. In contrast, the precision of real-time three-dimensional echocardiography has been questioned in another study that tested calibrated dynamic objects of the size of neonatal and pediatric hearts. The authors described relevant spatiotemporal inaccuracies and concluded that the precision of real-time three-dimensional echocardiography was not sufficient for a small, fast-moving structure such as the neonatal or pediatric heart.

In summary, it is important to establish robust nomograms for STE values adjusted for gestational and postnatal age, in order to use myocardial imaging in the clinical evaluation of newborn hearts.

In fact, myocardial imaging has become routine in adult cardiology and is widely used as a valid diagnostic tool. However, follow-up studies on preterm individuals are scarce. In one cross-sectional study of 20 to 39-year-old adults who were born preterm, functional cardiovascular magnetic resonance revealed significant reductions in left ventricular longitudinal strain and strain rate, as well as in left ventricular systolic and diastolic myocardial velocities. In addition to functional alterations, the left ventricular shape, size and mass were also found to differ from the heart structure in adults born at term. The authors of that study concluded that adults born very preterm exhibited structural changes of the left side of the heart that they would expect to see in an adult who was ten years older or who had a body mass index (BMI) that was nine units higher. This was also the case after adjusting for other risk factors such as blood pressure. In the same cohort, quantification of right ventricular function and anatomy showed that ejection fraction is significantly reduced, and that ventricles are smaller and exhibit greater mass compared to adults born at term.
3 AIMS

The overall objective of this thesis was to investigate cardiovascular physiology after preterm birth.

The specific aims were:

- To compare aortic and carotid growth in preterm infants with those of fetuses and term newborn infants

- To determine intima-media thickness of the aorta and carotid artery after preterm birth

- To characterize the circulatory transition from fetal to neonatal life by using novel echocardiographic techniques such as speckle-tracking

- To assess the development of cardiac function during the first six months after preterm birth by conventional and speckle-tracking echocardiography
4 METHODS

4.1 STUDY COHORT

We studied cardiovascular physiology in two groups during two developmental periods corresponding to the last trimester of pregnancy and the first three months after term-equivalent age.

The study group comprised 25 very preterm infants (13 boys) born at Karolinska University Hospital between August 2008 and September 2009 at the start of the third trimester. They all had a gestational age of between 26 and 30 weeks and a mean birth weight and standard deviation (SD) of 1153 (258) grams. All the very preterm infants were singletons without any malformations. 21 very preterm infants were appropriate for gestational age (AGA), defined as a birth weight within 2 SD from the mean for normal fetal weight, and four infants were small for gestational age (SGA), defined as a birth weight of more than 2 SD below the mean according to Swedish sex and gestational age-specific reference data146.

There were no cases of maternal diabetes and one mother delivering very preterm had a diagnosis of preeclampsia. Umbilical artery blood flow velocity measurements had been performed on clinical indication in 10 of 25 pregnancies ending very preterm and the pulsatility index had been found to be normal in all cases (within ± 2SD for gestational length)147. Besides one case of preeclampsia (maternal indication for Cesarean section), primary causes of preterm delivery were preterm labor (n=7), pre-labor premature rupture of membranes (n=9), vaginal bleeding (n=7) and one delivery was medically indicated because of intercurrent maternal disease (liver tumor).

In the very preterm group, 22 out of 25 (88%) had received antenatal steroids to induce lung maturation and ten (40%) needed ventilator support during their initial hospitalization. Twenty (80%) had an umbilical artery catheter inserted after birth, with the catheter tip located in the lower thoracic aorta (Th 6-10). All umbilical catheters were removed before the first ultrasonographic assessment. Five infants (20%) were pharmacologically treated with ibuprofen for a hemodynamically significant patent ductus arteriosus, but none of the infants needed surgical ligation for this condition. Ten infants (40%) suffered from neonatal septicemia, three (12%) had a diagnosis of mild intraventricular hemorrhage (grades 1–2), four (16%) had bronchopulmonary dysplasia, defined as the need for supplementary oxygen at 36 weeks of postmenstrual age, and
one infant (4%) had retinopathy of prematurity stage 1. There were no cases of
necrotizing enterocolitis.

As control group, we selected 30 (11 boys) healthy fetuses, who were subsequently
born at term with normal birth weights: mean birth weight (SD): 3456 (437) grams. 29 of
the term infants were AGA and one was proportionately large for gestational age without
any history of maternal diabetes. The recruitment and enrollment of the controls was
performed during routine antenatal visits to three primary healthcare maternity clinics in
the second trimester of pregnancy. Gestational age had been prospectively determined
in all of the pregnancies by a fetal ultrasound examination at 17–18 postmenstrual
weeks, according to Swedish recommendations for antenatal care.

All of the parents were interviewed about their family history of cardiovascular disease
and a positive history was defined as a report of myocardial infarction, stroke,
pharmacologically treated hypertension or hyperlipidemia among their first-degree
relatives. The infant and maternal characteristics are presented in table 1. Once the
infants were included in the study, there were no dropouts because of pregnancy or
postnatal complications, fetal or infant deaths, or withdrawal of parental consent.
<table>
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<tr>
<th></th>
<th>Preterm infants (n=25)</th>
<th>Term controls (n=30)</th>
<th>p-value</th>
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<td>Smoking in pregnancy, n</td>
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<td>1/30 (3.3%)</td>
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<td><strong>Perinatal data</strong></td>
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<td>39.0 (1.4)</td>
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<td>Boys, n</td>
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<td>11/30 (33%)</td>
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<td>Birth weight, g</td>
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<td>3456 (437)</td>
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<td>Birth weight SDS</td>
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<td>Birth length, cm</td>
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<td>50.3 (1.9)</td>
<td>-</td>
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<td><strong>Infant data at final assessment</strong></td>
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<td>Postmenstrual age, days</td>
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<td>371 (10)</td>
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<td>Length, cm</td>
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<td>Heart rate, beats/min</td>
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<td>Syst. blood pressure, mmHg</td>
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<td>94 (9)</td>
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<td>Diast. blood pressure, mmHg</td>
<td>59 (11)</td>
<td>63 (12)</td>
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</tr>
</tbody>
</table>

**Table 1:** Maternal and infant characteristics, mean (SD) values or proportions (%). CVD=cardiovascular disease, SDS= standard deviation score
4.2 STUDY PROTOCOL

The protocol in study I, II and IV consisted of three consecutive assessments. Ultrasonography of the major arteries and the heart was performed at 3 months before term, at term and three months after term-equivalent age (Figure 5). The first ultrasound was performed at a mean (SD) of 206 (10) days of postmenstrual age in very preterm infants and at 198 (6) days of postmenstrual age in the fetal reference group. At the third and last ultrasonographic investigation, the very preterm infants were 380 (16) days and the reference infants born at term 371 (10) days of postmenstrual age. At the final investigation, weight, length and blood pressure (mean of two measurements using an oscillometric device; Omron HEM-907, Omron Healthcare Inc., IL, USA) were measured in all infants. At the same time and using the same protocol (anthropometry, blood pressure and ultrasonography of the large arteries and the heart), we also examined the mothers once. In studies I and II, we excluded all infants that were not AGA, in study IV we did not include fetal data.

In study III, we compared cardiac function in the healthy control group before and after birth. The first intra-uterine echocardiographic examination was performed at a mean gestational age of 28+3 weeks and the second extra-uterine assessment was carried out at term, on average 170 hours after birth.

![Figure 5: Time points when the preterm and term control groups were examined](image-url)
4.2.1 Ultrasound assessments

All recordings were performed by one experienced examiner using the same ultrasound machine (GE Vingmed Vivid 7, General Electric, Horten, Norway, http://www.ge.com/no/). We used a phased array matrix sector probe (GE M3S 1.5–4.0 MHz) for fetal, and another phased array sector probe (GE 10-S 4.0–10.5 MHz) for infant examinations. A complete diagnostic echocardiographic assessment was performed on all subjects to rule out malformations and significant patent ductus arteriosus in the newborn infants. All recordings in infants included an electrocardiogram (ECG), in fetal investigations we used a dummy ECG based on mitral and aortic valve motion in order to define systole and diastole.

All recordings were analyzed off-line by an independent examiner, blinded for group belonging in studies I, II and IV, using commercially available software (Echo-PAC, GE Healthcare, USA).

4.2.2 Aortic and carotid diameters and IMT

At each investigation, we performed three consecutive angle-corrected M-mode recordings, each including at least six heart cycles, to determine the end-diastolic and systolic diameters of the upper abdominal aorta at the level of the diaphragm, and the diameter of the common carotid artery just before the bifurcation. Aortic and carotid IMT were measured in the distal arterial wall using M-Mode according to international guidelines (Figure 6). The reason for the measurement of the distal wall was that a proper differentiation between tunica adventitia and media is not possible in the proximal wall.
4.2.3 Conventional echocardiography

All standard echocardiographic views were performed: parasternal long and short axis, four and two chamber, subcostal and suprasternal views, M-Mode and Doppler investigation of the four valves and the great arteries. We determined systolic parameters such as: a) shortening fraction of the LV in the M-Mode recording of the parasternal long axis view, b) ejection fraction by biplane volumetric estimation according to the modified Simpson’s formula and c) cardiac output as the product of stroke volume and heart rate. Diastolic function was assessed by measuring the relationship between early and late ventricular filling (E/A-ratio). Finally, we calculated the myocardial performance index (MPI) as a parameter for both systolic and diastolic function. MPI is the ratio of isovolumetric contraction and relaxation time divided by ejection time. It has been shown to be useful in both ventricles, even in infants.\textsuperscript{129}
4.2.4 Speckle-tracking echocardiography

For each examination, digital loops containing at least five heart cycles in a B-Mode apical four-chamber projection were acquired in high 2D-quality. In order to obtain optimal frame rates, apical two-chamber views including either the right or left ventricle and the corresponding atrium were recorded when frame rates were too low in the four-chamber-projection. No harmonic imaging was used.

A semi-automatic system traced the myocardium/endocardium border of each ventricle in separate analyses, marking 6 regions of interest (Figure 7). When necessary, the tracking process was visually optimized by the examiner, as the midpoint of the endocardial tracking line was not necessarily the anatomical apex of the respective ventricle. We measured at the basal (atrio-ventricular annulus), mid-septal and apical level in the free walls of the left (LV) and right ventricle (RV) and the interventricular septum (IVS) in systole and diastole. Values for the interventricular septum were recorded and analyzed together with those of the left ventricle (Figure 8).

Peak systolic longitudinal strain, strain rate and myocardial velocities were determined. For estimations of each of these variables, three measurements were performed and the mean value calculated.

Figure 7: Identification of six regions of interest in the left ventricle of a fetus at a gestational age of 28+2 using STE: red, dark-blue and purple at the free left ventricular wall, and yellow, light-blue and green at the interventricular septum from basis to apex
4.2.5 Calculated indices

We measured the early diastolic filling velocity in the conventional PW-Doppler (E) and by STE (E’) and calculated the relation between them (E/E’) in both ventricles. This parameter has become central in the evaluation of diastolic function and an estimate of diastolic filling pressure\textsuperscript{150}. Additionally, we assessed the relationship of early and late ventricular filling velocities in the conventional PW-Doppler, and myocardial velocities during early and late diastolic filling by STE in both ventricles.

4.3 STATISTICAL METHODS

The results are presented as means and standard deviations (SD), medians and interquartile ranges or proportions. Shapiro Wilk test was used to test the normality of distributions. If p>0.05, the data distributions were considered to be normal. Student’s t-test and Wilcoxon rank-sum test were used to test for group differences, the paired
t-test was applied in the analysis of within group changes. A p-value of < 0.05 was considered significant in studies I-III. In order to avoid type 1 statistical errors due to multiple comparisons, a significance level of 0.01 was chosen for study IV. Associations between covariates were first tested using univariate analyses, and associations with a p<0.25 were then entered into a stepwise multivariate regression model. Accordingly, perinatal characteristics and neonatal morbidities were analyzed for associations with the respective outcome variables. All data were analyzed using JMP 11.0.0 (SAS Institute Inc., Cary, NC, USA).

4.4 ETHICAL CONSIDERATIONS AND INFORMED CONSENT

The study protocols were in accordance with the ethical principles for medical research involving human subjects laid out in the Declaration of Helsinki. Parental oral and written informed consent was obtained before the infants were included in the studies. All the studies had been approved by the Regional Ethics Review Board in Stockholm (2008/294-31/3).
5 RESULTS

5.1 STUDY I

Aortic diameters and growth

At the first assessment, which corresponded to an average post-conceptional age of 28 weeks, the aortic end-diastolic diameter (aEDD) was somewhat larger in very preterm infants than in the age-matched controls assessed as fetuses (median 3.6 vs. 3.4 mm, p=0.03), whereas there was no significant group difference in systolic diameters (aSD; median 4.0 vs. 4.3 mm, p=0.30). At the next investigation three months later, both aEDD (4.9 vs. 5.8 mm) and aSD (5.3 vs. 6.4 mm) were significantly smaller in infants born very preterm as compared with infants born at term (p<0.001 for both comparisons). At the final assessment six months after the first comparison, these group differences remained highly significant: on average, the aEDD was 1.6 mm or 22% smaller in infants born very preterm when they were compared with those born at term (5.8 vs. 7.4 mm, table 2). In the fetuses, the aEDD increased by +2.6 mm during the third trimester. In contrast, the very preterm infants only exhibited +0.9 mm increase in aEDD during the same developmental period (p<0.001). During the following three-month period, aortic growth velocity (aGV) continued unchanged (+0.9 mm) in very preterm infants, whereas the aGV in term controls slowed down to +1.3 mm (p<0.001 vs. fetal aortic growth), and the group difference in aGV was no longer statistically significant. At the end point of our study, aEDD did not vary in relation to the infants’ sex, postmenstrual age, maternal smoking in pregnancy or a family history of cardiovascular disease.

However, we found borderline significant correlations between the infants’ aEDD and current weight (β= 0.42 mm/kg, r= 0.26, p= 0.07) and between infant and maternal aEDD (β=0.072 mm/1 mm increase in maternal aEDD, r= 0.27, P= 0.06).

In the final multiple regression model including all three covariates (group, infant weight at last examination and maternal aEDD), only group belonging (very preterm-term) contributed significantly to the variation in infant aEDD (r²= 0.54, p<0.001). The result of this regression analysis was the same after log-transforming infant aEDD.
Carotid artery diameters and growth

At the first assessment, the cEDD was 1.7 mm (interquartile range 1.5–1.9 mm) in very preterm infants (no fetal data available). At term, both cEDD (2.0 vs. 2.7 mm, p<0.001) and the systolic diameters (cSD; 2.4 vs. 2.9 mm, p<0.001) were significantly smaller in very preterm infants than term infants. Three months later and at the end point of our study, the average group difference in cEDD was 0.43 mm, corresponding to a 14% smaller carotid diameter in infants born very preterm (p=0.002), table 3.

In very preterm infants, the cEDD increased by an average of 0.3 mm during the first three months of postnatal life. During the next three-month period, carotid growth velocity (cGV) increased in very preterm infants to 0.6 mm (p=0.01 for within group comparison) and it was higher in preterm infants than in controls born at term (+0.31 mm, p=0.02 for group difference). At the last examination, infant cEDD did not vary in relation to infants’ sex, infants’ current weight, maternal smoking, maternal cEDD or a family history of cardiovascular disease.

We found no significant associations between gestational age, SD scores of birth weight and birth weight and the corresponding aEDD and cEDD at the final follow-up examination. In addition, there were no statistically significant associations between neonatal exposures (antenatal steroids, ventilator treatment or umbilical artery catheterization) or neonatal morbidity (septicemia and patent ductus arteriosus) and later aEDD and cEDD.

5.2 STUDY II

Aortic intima-media thickness

At the first assessment, corresponding to a mean post-conceptional age of 28 weeks, a-IMT was thinner in the very preterm infants compared with the age-matched controls assessed in fetal life. During the following investigations, three and six months later, a-IMT was not significantly different between infants born very preterm and infants born at term. When related to vessel diameter, a-IMT represented 10.5% of the vessel lumen diameter in the preterm group at term and 9.6% three months later, compared with 8.5% in the control group at term and three months after term (p < 0.05 for both comparisons, table 2). When related to body weight three months after term, a-IMT/kg was similar in both groups: 104 mikrom/kg in the preterm group vs. 101 mikrom/kg in the control group.
(p = 0.73). There was no significant association between birth weight SDS and a-IMT at any age. Nor was there any relationship between increased weight nor length from birth to final follow-up and increased a-IMT. At the end point of our study, a-IMT did not vary in relation to infants’ sex, maternal smoking, postmenstrual age, maternal a-IMT or a family history of CVD.
<table>
<thead>
<tr>
<th></th>
<th>Very preterm (n=21)</th>
<th>Term controls (n=29)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aortic IMT</strong> (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMT, 28 w</td>
<td>0.41 (0.31-0.46)</td>
<td>0.58 (0.52-0.63) †</td>
<td>0.001</td>
</tr>
<tr>
<td>IMT, Term</td>
<td>0.45 (0.38-0.57)</td>
<td>0.47 (0.38-0.56)</td>
<td>0.78</td>
</tr>
<tr>
<td>IMT, 3 m</td>
<td>0.56 (0.51-0.63)</td>
<td>0.58 (0.52-0.70)</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Aortic end-diastolic diameter</strong> (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDD, 28 w</td>
<td>3.6 (3.3-4.1)</td>
<td>3.4 (2.9-3.7) †</td>
<td>0.03</td>
</tr>
<tr>
<td>EDD, Term</td>
<td>4.9 (4.1-5.6)</td>
<td>5.8 (5.4-6.4)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>EDD, 3 m</td>
<td>5.8 (5.6-6.3)</td>
<td>7.4 (6.7-8.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Relative aortic IMT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMT/EDD, 28 w</td>
<td>0.109 (0.094-0.125)</td>
<td>0.182 (0.168-0.196) †</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IMT/EDD, Term</td>
<td>0.105 (0.092-0.118)</td>
<td>0.085 (0.073-0.097)</td>
<td>0.01</td>
</tr>
<tr>
<td>IMT/EDD, 3 m</td>
<td>0.096 (0.087-0.105)</td>
<td>0.085 (0.077-0.093)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Data are median (25-75th percentile), p-values according to Wilcoxon rank-sum test.
**Data are mean (SD), p-values according to Student’s t-test
†Measurements performed in utero

*Table 2: Aortic measurements in millimeters (mm) of intima-media thickness (IMT), end-diastolic diameter (EDD) and calculation of relative intima-media thickness for the preterm and term control group at 28 weeks of gestation, at term and at three months after term.*
Carotid intima-media thickness

At the first assessment, the c-IMT was 0.33 (0.28–0.36) mm in very preterm infants. There are no data available for the fetal controls, because of the variation in the insonation angle when measuring the fetal carotid artery. At term and three months after term-equivalent age, there were no differences in c-IMT between the two groups. When related to lumen diameter, c-IMT represented 18.5% at term and 15.7% three months after term in the very preterm group compared with 13.8% and 13.2% in the control group for the respective time points during the investigation (p < 0.05 for both comparisons, table 3). When c-IMT was related to body weight three months after term, there were no significant differences between the groups: 76 mikrom/kg in the very preterm group vs. 68 mikrom/kg in the control group (p = 0.27). There was no significant association between birthweight SDS and c-IMT at any age. Nor was there any relationship between increased weight nor length from birth to final follow-up and increased c-IMT. At the last examination, infant c-IMT did not vary in relation to infants’ sex, infants’ current weight, maternal smoking, maternal c-IMT or a positive family history of CVD.
<table>
<thead>
<tr>
<th></th>
<th>Very preterm (n=21)</th>
<th>Term controls (n=29)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carotid IMT</strong>(mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMT, 28 w</td>
<td>0.33 (0.28-0.36)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IMT, Term</td>
<td>0.35 (0.32-0.38)</td>
<td>0.36 (0.31-0.39)</td>
<td>0.89</td>
</tr>
<tr>
<td>IMT, 3 m</td>
<td>0.37 (0.33-0.48)</td>
<td>0.37 (0.32-0.48)</td>
<td>0.64</td>
</tr>
<tr>
<td><strong>Carotid end-diastolic diameter</strong>(mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDD, 28 w</td>
<td>1.7 (1.5-1.9)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EDD, Term</td>
<td>2.0 (1.8-2.1)</td>
<td>2.7 (2.4-3.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>EDD, 3 m</td>
<td>2.6 (2.4-2.9)</td>
<td>3.1 (2.7-3.3)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Relative carotid IMT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMT/EDD, 28 w</td>
<td>0.198 (0.166-0.240)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IMT/EDD, Term</td>
<td>0.185 (0.165-0.205)</td>
<td>0.138 (0.121-0.156)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IMT/EDD, 3 m</td>
<td>0.157 (0.139-0.176)</td>
<td>0.132 (0.115-0.149)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Data are median (25-75th percentile), p-values according to Wilcoxon rank-sum test.

**Data are mean (SD), p-values according to Student’s t-test

**Table 3**: Carotid measurements in millimeters (mm) of intima-media thickness (IMT), end-diastolic diameter (EDD) and calculation of relative intima-media thickness for the preterm and term control group at 28 weeks of gestation, at term and at three months after term
5.3 STUDY III

The ultrasound performances and analyses were technically feasible and reproducible in all 30 fetuses and in the neonatal period (intra-observer variability: 5.8% for STE and 9.8% for conventional echocardiography, inter-observer variability 6.5% vs. 10.7% respectively).

Longitudinal systolic strain
Compared to fetal values, systolic strain values were significantly lower after birth in all regions of interest and in both ventricles. RV strain was still higher in comparison to LV and IVS, and strain was still higher at the basal parts of the heart compared to medial and apical regions of interest (Table 5).

Strain rate
Strain rate values decreased significantly in all regions of interest after birth, with the exception of early diastolic strain rate in the LV (Figure 9 a-c). The trend of higher strain rates at the base of the heart remained unchanged. Strain rate values continued to be higher in the RV compared to LV and IVS, although the differences became less pronounced (for absolute values: see tables in the published article in part two of this thesis).

Myocardial velocities
Systolic, early and late diastolic myocardial velocities increased after birth. In particular, velocities increased in the RV, which exhibited significantly higher velocities than the LV and IVS. Only systolic and late diastolic velocities in the LV and IVS remained unchanged (Table 4).

Cardiac indices, ejection fraction and cardiac output
Regarding the LV, MPI and E/E´ were almost identical pre- and postnatally. The ratio of early and late diastolic filling (E/A) and E´/A´ increased after birth, which was also true for the RV. In contrast, MPI and E/E´ decreased in the RV after birth. The ejection fraction according to Simpson was similar in the left ventricle even after birth. Cardiac output (CO) in the LV was comparable to the combined CO in utero (Table 6). When CO was related to birth weight, it was 232 ml/kg/min. No fetal weight estimations were performed.
<table>
<thead>
<tr>
<th></th>
<th>mean (SD), fetal</th>
<th>mean (SD), neo</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>4.83 (1.86)</td>
<td>6.38 (2.34)</td>
<td>0.006</td>
</tr>
<tr>
<td>E</td>
<td>-4.40 (1.93)</td>
<td>-6.44 (1.54)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A</td>
<td>-4.53 (2.30)</td>
<td>-5.97 (2.52)</td>
<td>0.053</td>
</tr>
<tr>
<td><strong>LV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>4.49 (1.56)</td>
<td>4.44 (1.45)</td>
<td>0.99</td>
</tr>
<tr>
<td>E</td>
<td>-4.70 (1.60)</td>
<td>-5.51 (1.84)</td>
<td>0.07</td>
</tr>
<tr>
<td>A</td>
<td>-4.71 (1.49)</td>
<td>-4.22 (1.86)</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>IVS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>4.27 (1.44)</td>
<td>4.00 (1.13)</td>
<td>0.46</td>
</tr>
<tr>
<td>E</td>
<td>-3.28 (0.71)</td>
<td>-5.02 (1.83)</td>
<td>0.002</td>
</tr>
<tr>
<td>A</td>
<td>-4.11 (1.73)</td>
<td>-4.82 (1.84)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

**Table 4:** Maximal basal velocities (cm/s) of the right ventricle (RV), left ventricle (LV) and interventricular septum (IVS) in systole (S), early diastole (E) and late diastole (A) in echocardiographic examinations at 28th week of gestation (fetal) and after birth (neo). Data are mean (SD), p-values according to paired t-test.

<table>
<thead>
<tr>
<th></th>
<th>mean (SD), fetal</th>
<th>mean (SD), neo</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>basal</td>
<td>-29.3 (6.4)</td>
<td>-25.3 (7.3)</td>
<td>0.06</td>
</tr>
<tr>
<td>medial</td>
<td>-26.5 (4.4)</td>
<td>-22.6 (4.7)</td>
<td>0.003</td>
</tr>
<tr>
<td>apical</td>
<td>-26.0 (4.9)</td>
<td>-21.2 (5.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>mean</td>
<td>-27.3 (4.4)</td>
<td>-23.0 (4.3)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>LV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>basal</td>
<td>-23.9 (4.3)</td>
<td>-20.0 (3.8)</td>
<td>0.002</td>
</tr>
<tr>
<td>medial</td>
<td>-21.8 (3.3)</td>
<td>-19.1 (2.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>apical</td>
<td>-22.1 (3.6)</td>
<td>-19.3 (2.7)</td>
<td>0.02</td>
</tr>
<tr>
<td>mean</td>
<td>-22.2 (2.9)</td>
<td>-19.5 (2.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>IVS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>basal</td>
<td>-24.3 (5.4)</td>
<td>-20.6 (3.6)</td>
<td>0.01</td>
</tr>
<tr>
<td>medial</td>
<td>-22.9 (4.6)</td>
<td>-19.6 (3.5)</td>
<td>0.009</td>
</tr>
<tr>
<td>apical</td>
<td>-22.1 (3.6)</td>
<td>-19.9 (3.7)</td>
<td>0.11</td>
</tr>
<tr>
<td>mean</td>
<td>-22.2 (2.9)</td>
<td>-20.1 (3.4)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Table 5:** Maximal and mean systolic longitudinal strain (%) of the right ventricle (RV), left ventricle (LV) and interventricular septum (IVS) at the base, mid-ventricular (medial) and apex in echocardiographic examinations at 28th week of gestation (fetal) and after birth (neo). Data are mean (SD), p-values according to paired t-test.
<table>
<thead>
<tr>
<th></th>
<th>Fetal</th>
<th>Neo</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SV (ml)</td>
<td>3.25 (0.58)</td>
<td>-†</td>
<td>-</td>
</tr>
<tr>
<td>CO (ml)</td>
<td>443 (58)</td>
<td>-†</td>
<td>-</td>
</tr>
<tr>
<td>EF (%)</td>
<td>65.29 (6.69)</td>
<td>55.51 (11.6)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MPI</td>
<td>0.24 (0.17)</td>
<td>0.13 (0.04)</td>
<td>0.01</td>
</tr>
<tr>
<td>E/E´</td>
<td>11.3 (5.8)</td>
<td>8.6 (4.5)</td>
<td>0.07</td>
</tr>
<tr>
<td>E/A</td>
<td>0.94 (0.39)</td>
<td>1.00 (0.56)</td>
<td>0.66</td>
</tr>
<tr>
<td>E´/A´</td>
<td>1.12 (0.94)</td>
<td>1.26 (0.59)</td>
<td>0.48</td>
</tr>
<tr>
<td><strong>LV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SV (ml)</td>
<td>2.26 (0.44)</td>
<td>6.27 (1.66)</td>
<td>-</td>
</tr>
<tr>
<td>CO (ml)</td>
<td>321 (56)</td>
<td>797 (214)</td>
<td>-</td>
</tr>
<tr>
<td>EF (%)</td>
<td>64.66 (8.06)</td>
<td>63.58 (6.32)</td>
<td>0.57</td>
</tr>
<tr>
<td>MPI</td>
<td>0.26 (0.15)</td>
<td>0.26 (0.11)</td>
<td>0.67</td>
</tr>
<tr>
<td>E/E´</td>
<td>11.3 (7.3)</td>
<td>11.9 (5.9)</td>
<td>0.67</td>
</tr>
<tr>
<td>E/A</td>
<td>0.95 (0.45)</td>
<td>1.16 (0.20)</td>
<td>0.12</td>
</tr>
<tr>
<td>E´/A´</td>
<td>1.06 (0.39)</td>
<td>1.48 (0.71)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

**Table 6:** Stroke volume (SV in ml), cardiac output (CO in ml/min), EF (%) by biplane volumetry and myocardial performance index (MPI), relation between max. velocities in early diastole in pulsed Doppler (E) and 2D-S (E´) and relation between early and late diastolic max. velocities in pulsed Doppler (E/A) and 2D-S (E´/A´) for the right (RV) and left ventricle (LV) in echocardiographic examinations at 28th week of gestation (fetal) and after birth (neo). Data are mean (SD), p-values according to paired t-test. † = measurements not performed.
Figure 9: Changes in strain rate in the transitional period from fetal to neonatal life (in %) in the left (LV) and right (RV) ventricle and interventricular septum (IVS) in systole (a), early diastole (b) and late diastole (c)
5.4 STUDY IV

At term-equivalent age, we found no significant differences in cardiac function between infants born very preterm and at term. When we repeated the measurements three months later, there were still no group differences detectable by conventional echocardiography. However, using speckle-tracking echocardiography, we found significantly lower left ventricular longitudinal strain and significantly lower left ventricular systolic and diastolic myocardial velocities in infants born very preterm than in infants born at term. In addition, the postnatal increase in myocardial strain, strain rate and myocardial velocities seen in the left ventricle was slower in preterm infants compared to controls.

Longitudinal Myocardial strain

At term-equivalent age, there were no significant differences in LV or RV mean free wall strain, or IVS strain between the preterm and term infants.

In term infants but not in preterm infants, there was a significant increase in LV free wall strain from birth to three months of age. There were no other statistically significant age-related intra-group changes in strain.

At three months of corrected age, mean free wall LV strain was significantly lower in the preterm infants (p=0.010), whereas IVS and RV mean free wall strain did not differ significantly from the values found in infants born at term (Table 7).

Strain rate

There were no inter-group differences in the strain rate at term-equivalent age, with the exception of a higher right ventricular systolic strain rate in preterm infants than in term infants.

In term infants, there was a significant increase in all LV, IVS and RV strain rates from birth to three months of age. In preterm infants, there was a significant increase in LV systolic, IVS and RV early diastolic strain rates from term-equivalent age to three months of corrected age.

At three months of corrected age, there were no inter-group differences in LV, IVS or RV strain rates (Table 9).
Myocardial velocity

At term-equivalent age, there were no significant differences in maximal myocardial velocities between preterm and term infants.

In term infants, all (systolic, early and late diastolic) LV and IVS myocardial velocities increased significantly from term to three months of age, whereas RV myocardial velocities remained unchanged. In preterm infants, the systolic myocardial velocity of the RV, the early diastolic velocity of the LV, and all IVS myocardial velocities increased from term-equivalent age to three months of corrected age.

At three months of corrected age, LV diastolic myocardial velocities were significantly lower, both in early (p=0.003) and late diastole (p=0.009), in preterm infants than in term controls. There were no other between group differences in myocardial velocity at any region of interest (Table 8).

Conventional echocardiography and indices

At three months of corrected age, there were no statistically significant differences between preterm infants and term-born controls when it came to the measurements of diastolic indices such as E/A, E’/A´, E/E´ and the myocardial performance index (MPI) of both the right and left ventricles. There were also no group differences in the ejection fraction (EF), shortening fraction (SF), stroke volume (SV) and cardiac output (CO) of the left ventricle (Table 10)

Covariate analyses

Mean LV strain in all preterm infants at 3 months of corrected age was -20.0 and without the 4 SGA-infants it was -19.9. Corresponding means for RV strain were -23.9 (all preterm infants) and -23.3 (without SGA-infants), and for IVS strain: -21.9 (all preterm infants) and -22.2 (without SGA-infants), respectively

Moreover, at three months of corrected age, mean myocardial strain values (all infants included in the analysis; n=55) did not correlate – neither in the LV or RV, nor in the IVS – to maternal smoking in pregnancy, BW-SDS or the infants’ body weight (p-values for an association ranging from 0.22 to 0.79). Similar insignificant correlations were made for strain rate and maximal myocardial velocities (p-values not shown).
Finally, there were no significant associations between the speckle tracking outcome variables at three months of corrected age and exposure to antenatal steroids, gestational age, a diagnosis of patent ductus arteriosus or bronchopulmonary dysplasia.
Table 7: Myocardial longitudinal strain (%) in the free wall of the left (LV) and right (RV) ventricle and the interventricular septum (IVS) as assessed by speckle tracking echocardiography. Preterm infants (n=25) are compared with controls born at term (n=30) during a 6 month period, starting at 28 weeks of postmenstrual age and ending at 3 months of term corrected age (53 weeks of postmenstrual age). Mean (SD) values. *denotes p<0.05 for within-group difference from 40 to 53 weeks of postmenstrual age.

<table>
<thead>
<tr>
<th>Region of interest</th>
<th>28 weeks</th>
<th></th>
<th>40 weeks</th>
<th></th>
<th>53 weeks</th>
<th></th>
<th>p-value for difference between groups at 53 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preterm</td>
<td>Control</td>
<td>Preterm</td>
<td>Control</td>
<td>Preterm</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Left ventricle (LV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV-basal</td>
<td>-18.6</td>
<td>-19.3</td>
<td>-20.0</td>
<td>-19.8</td>
<td>-22.1</td>
<td>-22.0</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>(3.5)</td>
<td>(4.6)</td>
<td>(3.8)</td>
<td>(4.2)</td>
<td>(3.8)</td>
<td>(4.2)</td>
<td></td>
</tr>
<tr>
<td>LV-mid</td>
<td>-17.5</td>
<td>-18.1</td>
<td>-19.1</td>
<td>-19.8</td>
<td>-21.7</td>
<td>-22.2</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>(3.2)</td>
<td>(3.4)</td>
<td>(2.6)</td>
<td>(3.4)</td>
<td>(3.8)</td>
<td>(4.5)</td>
<td></td>
</tr>
<tr>
<td>LV-apical</td>
<td>-17.5</td>
<td>-18.7</td>
<td>-19.4</td>
<td>-20.5</td>
<td>-22.2</td>
<td>-22.2</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>(4.2)</td>
<td>(3.3)</td>
<td>(2.8)</td>
<td>(3.4)</td>
<td>(4.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV-mean</td>
<td>-17.9</td>
<td>-18.7</td>
<td>-19.5</td>
<td>-20.0</td>
<td>-22.0*</td>
<td>-22.5*</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>(2.5)</td>
<td>(2.6)</td>
<td>(2.1)</td>
<td>(2.2)</td>
<td>(3.2)</td>
<td>(3.3)</td>
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</tr>
<tr>
<td>Interventricular septum (IVS)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>IVS-basal</td>
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<td>-20.4</td>
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<td>-21.7</td>
<td>-21.7</td>
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</tr>
<tr>
<td></td>
<td>(3.6)</td>
<td>(4.1)</td>
<td>(3.7)</td>
<td>(2.8)</td>
<td>(3.3)</td>
<td></td>
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</tr>
<tr>
<td>IVS-midseptal</td>
<td>-18.8</td>
<td>-20.2</td>
<td>-19.6</td>
<td>-22.1</td>
<td>-22.2</td>
<td>-22.2</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>(3.4)</td>
<td>(3.1)</td>
<td>(3.5)</td>
<td>(2.5)</td>
<td>(3.4)</td>
<td></td>
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</tr>
<tr>
<td>IVS-apical</td>
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<td>-20.3</td>
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<td>-23.6</td>
<td>-23.6</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>(3.8)</td>
<td>(3.0)</td>
<td>(3.7)</td>
<td>(3.0)</td>
<td>(4.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVS-mean</td>
<td>-19.0</td>
<td>-20.3</td>
<td>-20.1</td>
<td>-21.9</td>
<td>-22.5*</td>
<td>-22.5*</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>(3.4)</td>
<td>(3.0)</td>
<td>(3.4)</td>
<td>(2.2)</td>
<td>(3.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right ventricle (RV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>RV-basal</td>
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<td>-24.7</td>
<td>-25.3</td>
<td>-25.2</td>
<td>-28.0</td>
<td>-28.0</td>
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</tr>
<tr>
<td></td>
<td>(8.9)</td>
<td>(7.8)</td>
<td>(7.3)</td>
<td>(6.7)</td>
<td>(7.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV-mid</td>
<td>-20.0</td>
<td>-24.0</td>
<td>-22.6</td>
<td>-24.2</td>
<td>-23.9</td>
<td>-23.9</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>(6.2)</td>
<td>(4.7)</td>
<td>(4.7)</td>
<td>(4.3)</td>
<td>(6.0)</td>
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<td></td>
</tr>
<tr>
<td>RV-apical</td>
<td>-21.0</td>
<td>-21.4</td>
<td>-21.2</td>
<td>-22.2</td>
<td>-21.4</td>
<td>-21.4</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>(4.9)</td>
<td>(4.7)</td>
<td>(5.0)</td>
<td>(7.2)</td>
<td>(5.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV-mean</td>
<td>-20.5</td>
<td>-23.3</td>
<td>-23.0</td>
<td>-23.9</td>
<td>-24.4</td>
<td>-24.4</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>(5.5)</td>
<td>(4.0)</td>
<td>(4.2)</td>
<td>(4.2)</td>
<td>(5.2)</td>
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</tr>
</tbody>
</table>
Table 8: Maximum myocardial velocity (cm/s) in systole, early and late diastole in the left ventricle (LV), the interventricular septum (IVS) and the right ventricle (RV) in preterm infants (n=25) and in controls born at term (n=30) as assessed by speckle tracking echocardiography. Longitudinal assessments during a 6 month period, starting at 28 weeks of postmenstrual age) and ending 3 months of term corrected age (53 weeks of postmenstrual age).
Median (interquartile range) values. * denotes p<0.05 for within-group difference from 40 to 53 weeks of postmenstrual age.

<table>
<thead>
<tr>
<th>Region of interest</th>
<th>28 weeks</th>
<th>40 weeks</th>
<th>53 weeks</th>
<th>p-value for difference between groups at 53 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preterm</td>
<td>Control</td>
<td>Preterm</td>
<td>Control</td>
</tr>
<tr>
<td>Left ventricle (LV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systole</td>
<td>3.17 (2.6-4.5)</td>
<td>-</td>
<td>4.99 (4.4-6.3)</td>
<td>4.41 (3.2-5.5)</td>
</tr>
<tr>
<td>Early diastole</td>
<td>-3.74 (-4.9;-2.7)</td>
<td>-</td>
<td>-5.79 (-6.9;-5.1)</td>
<td>-5.63 (-6.5;-4.0)</td>
</tr>
<tr>
<td>Late diastole</td>
<td>-3.61 (-5.6;-2.5)</td>
<td>-</td>
<td>-5.26 (-6.1;-3.6)</td>
<td>-4.16 (-5.3;-3.1)</td>
</tr>
<tr>
<td>Interventricular septum (IVS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systole</td>
<td>2.85 (2.5-3.2)</td>
<td>-</td>
<td>4.38 (3.9-4.9)</td>
<td>3.81 (3.2-4.7)</td>
</tr>
<tr>
<td>Early diastole</td>
<td>-3.84 (-5.2;-2.6)</td>
<td>-</td>
<td>-5.68 (-7.7;-4.6)</td>
<td>-4.62 (-5.9;-3.5)</td>
</tr>
<tr>
<td>Late diastole</td>
<td>-4.14 (-4.8;-3.5)</td>
<td>-</td>
<td>-5.33 (-6.2;-4.2)</td>
<td>-4.22 (-5.6;-3.4)</td>
</tr>
<tr>
<td>Right ventricle (RV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systole</td>
<td>4.31 (3.5-5.1)</td>
<td>-</td>
<td>6.72 (5.2-7.8)</td>
<td>5.62 (4.6-7.7)</td>
</tr>
<tr>
<td>Early diastole</td>
<td>-4.60 (-6.5;-4.2)</td>
<td>-</td>
<td>-7.32 (-8.9;-5.2)</td>
<td>-6.47 (-7.8;-5.6)</td>
</tr>
<tr>
<td>Late diastole</td>
<td>-4.82 (-7.0;-2.2)</td>
<td>-</td>
<td>-7.30 (-8.4;-5.4)</td>
<td>-5.90 (-8.1;-3.9)</td>
</tr>
</tbody>
</table>
Table 9: Myocardial strain rate (1/s) in systole, early and late diastole in the left (LV) and right ventricle (RV) and in the interventricular septum (IVS) in preterm infants (n=25) and in controls born at term (n=30) as assessed by speckle tracking echocardiography. Longitudinal assessments during a 6 month period, starting 3 months before (28 weeks of postmenstrual age) and ending 3 months of term corrected age (53 weeks of postmenstrual age). Median (interquartile range) values. *denotes p<0.05 for within-group difference from 40 to 53 weeks of postmenstrual age.

<table>
<thead>
<tr>
<th></th>
<th>28 weeks</th>
<th></th>
<th>40 weeks</th>
<th></th>
<th>53 weeks</th>
<th></th>
<th>p-value for difference between groups at 53 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preterm</td>
<td>Control</td>
<td>Preterm</td>
<td>Control</td>
<td>Preterm</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td><strong>Left ventricle (LV)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systole</td>
<td>-2.33 (-2.9;-2.1)</td>
<td>-</td>
<td>-2.60 (-3.5;-2.3)</td>
<td>-</td>
<td>-2.40 (-3.2;-2.0)</td>
<td>-</td>
<td>-3.37* (-3.8;-2.7)</td>
</tr>
<tr>
<td>Early diastole</td>
<td>2.78 (2.4-3.5)</td>
<td>-</td>
<td>3.51 (3.1-3.7)</td>
<td>3.05 (2.7-4.0)</td>
<td>3.33 (3.1-5.1)</td>
<td>4.33* (3.5-5.3)</td>
<td></td>
</tr>
<tr>
<td>Late diastole</td>
<td>2.28 (1.8-2.9)</td>
<td>-</td>
<td>2.76 (2.0-3.3)</td>
<td>2.10 (1.6-3.2)</td>
<td>2.52 (2.1-3.6)</td>
<td>3.22* (2.2-3.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Interventricular septum (IVS)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systole</td>
<td>-2.12 (-2.6;-1.8)</td>
<td>-</td>
<td>-2.32 (-2.8;-1.9)</td>
<td>-</td>
<td>-2.08 (-2.4;-1.7)</td>
<td>-</td>
<td>-2.62 (-3.2;-2.0)</td>
</tr>
<tr>
<td>Early diastole</td>
<td>2.72 (2.0-3.1)</td>
<td>-</td>
<td>2.62 (2.2-3.2)</td>
<td>2.32 (2.0-2.9)</td>
<td>3.22* (2.9-4.3)</td>
<td>3.44* (2.6-4.3)</td>
<td></td>
</tr>
<tr>
<td>Late diastole</td>
<td>2.45 (1.8-2.9)</td>
<td>-</td>
<td>2.47 (1.9-2.9)</td>
<td>1.88 (1.5-2.4)</td>
<td>2.56 (2.0-3.0)</td>
<td>2.57* (2.2-3.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Right ventricle (RV)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systole</td>
<td>-2.79 (-3.2;-2.6)</td>
<td>-</td>
<td>-3.81 (-4.1;-3.1)</td>
<td>-</td>
<td>-2.70 (-3.7;-2.3)</td>
<td>-</td>
<td>-4.17 (-4.7;-2.8)</td>
</tr>
<tr>
<td>Early diastole</td>
<td>3.20 (2.3-3.5)</td>
<td>-</td>
<td>3.59 (2.8-4.5)</td>
<td>3.00 (2.3-4.3)</td>
<td>4.54* (3.9-5.5)</td>
<td>4.16* (2.9-6.1)</td>
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</tr>
<tr>
<td>Late diastole</td>
<td>2.64 (2.1-3.1)</td>
<td>-</td>
<td>3.33 (2.5-4.2)</td>
<td>2.30 (1.7-3.0)</td>
<td>2.90 (2.2-4.1)</td>
<td>3.53* (2.7-4.3)</td>
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</table>
Table 10: Cardiac function in preterm infants (n=25) and in controls born at term (n=30) as assessed by conventional echocardiography and calculated indices. Longitudinal assessments during a 6 month period, starting 3 months before (28 weeks of postmenstrual age) and ending 3 months of term corrected age (53 weeks of postmenstrual age).

<table>
<thead>
<tr>
<th></th>
<th>28 weeks</th>
<th></th>
<th>40 weeks</th>
<th></th>
<th>53 weeks</th>
<th></th>
<th>p-value for between group difference at 53 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preterm</td>
<td>Control</td>
<td>Preterm</td>
<td>Control</td>
<td>Preterm</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td><strong>Right ventricle (RV)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E/A</td>
<td>0.96 (0.39)</td>
<td>-</td>
<td>1.04 (0.27)</td>
<td>1.01 (0.59)</td>
<td>1.23 (0.61)</td>
<td>1.00 (0.37)</td>
<td>0.10</td>
</tr>
<tr>
<td>E'/A'</td>
<td>1.29 (0.61)</td>
<td>-</td>
<td>1.09 (0.40)</td>
<td>1.27 (0.58)</td>
<td>1.17 (0.69)</td>
<td>1.33 (0.72)</td>
<td>0.46</td>
</tr>
<tr>
<td>E/E'</td>
<td>9.31 (3.16)</td>
<td>-</td>
<td>11.28 (4.90)</td>
<td>8.59 (4.60)</td>
<td>11.01 (5.70)</td>
<td>10.63 (4.26)</td>
<td>0.81</td>
</tr>
<tr>
<td>MPI</td>
<td>0.20 (0.13)</td>
<td>-</td>
<td>0.06 (0.01)</td>
<td>0.13 (0.04)</td>
<td>0.09 (0.05)</td>
<td>0.12 (0.07)</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Left ventricle (LV)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF, %</td>
<td>62.8 (7.1)</td>
<td>-</td>
<td>60.7 (6.8)</td>
<td>63.6 (6.3)</td>
<td>60.2 (8.9)</td>
<td>61.3 (6.4)</td>
<td>0.59</td>
</tr>
<tr>
<td>E/A</td>
<td>0.98 (0.33)</td>
<td>-</td>
<td>1.28 (0.50)</td>
<td>1.13 (0.24)</td>
<td>1.16 (0.55)</td>
<td>1.20 (0.25)</td>
<td>0.71</td>
</tr>
<tr>
<td>E'/A'</td>
<td>1.14 (0.58)</td>
<td>-</td>
<td>1.41 (0.74)</td>
<td>1.49 (0.71)</td>
<td>1.70 (0.50)</td>
<td>1.52 (0.59)</td>
<td>0.45</td>
</tr>
<tr>
<td>E/E'</td>
<td>15.93 (7.4)</td>
<td>-</td>
<td>14.84 (4.53)</td>
<td>12.00 (6.02)</td>
<td>11.75 (4.29)</td>
<td>9.60 (4.21)</td>
<td>0.06</td>
</tr>
<tr>
<td>MPI</td>
<td>0.24 (0.13)</td>
<td>-</td>
<td>0.25 (0.14)</td>
<td>0.26 (0.11)</td>
<td>0.30 (0.11)</td>
<td>0.22 (0.09)</td>
<td>0.07</td>
</tr>
<tr>
<td>SF, %</td>
<td>32.1 (6.8)</td>
<td>-</td>
<td>31.2 (6.6)</td>
<td>30.0 (5.3)</td>
<td>33.0 (6.2)</td>
<td>32.2 (3.8)</td>
<td>0.55</td>
</tr>
<tr>
<td>SV, ml</td>
<td>2.3 (0.9)</td>
<td>-</td>
<td>5.3 (1.7)</td>
<td>4.8 (1.6)</td>
<td>7.4 (2.3)</td>
<td>7.7 (2.1)</td>
<td>0.60</td>
</tr>
<tr>
<td>CO, ml</td>
<td>347 (157)</td>
<td>-</td>
<td>795 (280)</td>
<td>797 (214)</td>
<td>1054 (319)</td>
<td>1112 (356)</td>
<td>0.52</td>
</tr>
</tbody>
</table>
6 DISCUSSION

The fundamental aim of this thesis was to investigate cardiovascular physiology during the first six months of postnatal life after preterm birth. Studies I and II illustrate changes in the vascular tree, study III shows the feasibility and reproducibility of conventional and speckle-tracking echocardiography in fetuses and neonates, and study IV compares cardiac function in preterm and term infants using both conventional and speckle-tracking techniques.

Study I demonstrates three major and novel findings: first, the data confirms our hypothesis that the aortic narrowing previously found in children and adolescents born very preterm has a perinatal origin, occurring during the developmental period that corresponds to the third trimester of pregnancy. Secondly, infants born very preterm exhibit smaller carotid arteries, suggesting that impaired growth of the arterial vascular tree may be a generalized phenomenon induced by very preterm birth. Thirdly, fetal aortic growth velocity is found to be high and the aortic diameter increases by 60% during the third trimester of pregnancy. This rapid and physiological aortic growth decelerates dramatically after birth.

The effect of preterm delivery on arterial growth is not only abrupt – as it occurs immediately after birth – but it is also strong: already six months later, the aorta is 22% and the carotid artery 14% narrower compared to infants born at term. An important factor for this patient group is the fact that there is apparently no compensation for the initial growth retardation. In previous studies of healthy adolescents born preterm, the aortic cross-sectional area was still 16-19% smaller than in healthy controls born at term. The mechanical properties, flow characteristics and compliance of the great arteries in preterm individuals will be an important topic for future research.

As previously mentioned, the extra-uterine condition has an immediate and important influence on vessel growth in preterm infants and future studies will have to clarify the exact mechanisms. To date, three different hypotheses have been discussed in the scientific literature. Firstly, the reduction of flow in the great arteries after the premature uncoupling of the placental circulation results in lower shear stress. As normal shear stress activates transcriptional factors of the expression of vascular endothelial derived growth factor receptors, and is therefore essential for adequate vessel growth\textsuperscript{151}, a reduced mechanical stimulus might lead to impaired vessel development.
Secondly, many preterm infants suffer from severe undernutrition during the first weeks of life\textsuperscript{152}. This insufficient nutritional status is not just present in infants with fluid restriction\textsuperscript{153}. The current recommendations for preterm infants are to provide nutrients that are approximately the same as the requirements for the rate of growth and composition of weight gain for a normal fetus of the same conceptional age. However, these aims are rarely achieved or maintained in preterm infants during their hospital stay\textsuperscript{154,155}. In a prospective study from England, which included more than 100 infants with a gestational age $\leq$ 34 weeks, the cumulative energy and protein deficit at the end of the fifth week were 813 and 382 kcal/kg and 23 and 13 g/kg in infants $\leq$ 31 weeks and $\geq$ 31 weeks, respectively\textsuperscript{156}. As the recommended dietary intakes are based on maintenance of normal growth and not catch up growth, it is not surprising that catch-up usually occurs after hospital discharge and is not completed before the age of two to three years. Interestingly, slow weight gain during the first year of life has been associated with an increased risk for CVD\textsuperscript{157}. In general, postnatal malnutrition and growth retardation are considered as inevitable in preterm infants, and there is no evidence that more aggressive enteral or parenteral feeding can be achieved without adverse effects. However, it is unclear why growth retardation should have a particular effect on the vascular tree, as all values for diameters at the end-point of our studies were corrected for current body weight. Without knowing the exact mechanisms, we can assume that vascular growth might be particularly sensitive to undernutrition during the first months of postnatal life.

Thirdly, IGF-1 levels reach a peak at late gestation that will be missed by preterm born infants. IGF-1 not only regulates elastin incorporation in the arterial wall\textsuperscript{60,62}, it is also essential for normal vessel growth in humans\textsuperscript{60,61}. Down-regulation of IGF-1 by placental insufficiency has been associated with thinner and stiffer umbilical arteries\textsuperscript{63}. Accordingly, the arteries of preterm infants might grow at a slower rate since they are also deficient in IGF-1. In addition, they suffer from insufficient vascularization of the retina, a process that precedes ROP\textsuperscript{67}. In our study population, only one infant developed ROP, but almost all of them displayed significant narrowing of the large arteries. This observation and the physiology of elastin deposition into the wall of the great arteries might indicate that the elastic arteries are even more sensitive to insufficient IGF-1 concentration than the capillaries.
In Study II we illustrate a relative intima-media thickening of the great arteries in infants born preterm compared to the healthy control group. This is due to smaller vessel diameters found in study I and cannot be attributed to fetal growth restriction as these children were excluded from this study. Three months after term, a-IMT represented 9.6% of the lumen diameter compared to 8.5% in term infants, c-IMT 15.7% versus 13.2%, respectively. Data are available from a large cohort of young adults born with very low birth weight, which demonstrated relative but not absolute intima-media thickening⁹². Thus, it seems possible that changes in the arterial vessel wall persist until adulthood. A small study of preschool children in Japan agreed with our findings, as it showed that the mean aortic IMT of the preterm group was significantly thicker compared to controls¹⁵⁸.

However, the question remains about whether relative intima-media thickening signals an atherosclerotic disease process or whether it is a physiological and transient adaptation towards reduced aortic blood flow in the newborn infant, as a result of the cessation of the umbilical and placental circulation¹⁵⁹. The physiology of reduced shear stress and the development of “adaptive intima-media thickening”¹⁶⁰ seems to be reasonable in the perinatal period. In fact, intimal proliferation is considered to be a normal process in early childhood by some authors, observing coronary intimal proliferation in almost 100% of children between one and five years old, who died from causes unrelated to the cardiovascular system¹⁶¹. Other studies indicate that “real” atherosclerotic lesions develop through a number of morphological changes, and that foam cells and fatty streaks are present both in aortas and coronary arteries of infants who died in fetal life or as a consequence of sudden infant death syndrome⁶⁹.

However, there is no explanation currently available why preterm infants exhibit equal IMT compared to infants born at term, even though their vessels are much smaller. Again, IGF-1 seems to play an important role, as lower concentrations have been associated with thicker aortic IMT¹⁶². This association has been shown in growth restricted infants who suffer from insufficient IGF-1 levels during periods of gestation, but might also be valid in the preterm population, as they are IGF-1 deficient after birth. The lack of knowledge about the exact biochemical mechanisms of atherosclerosis later in life might explain, why the results of studies that have investigated IMT in adults who were born with low birth weight are contradictory. There is still considerable concern, that other influences during various decades of life might play a more important role than early developmental programming in terms of disease evolution.
In study III we observe changes in myocardial function in the transitional period from fetal to neonatal life in a healthy and homogenous group of infants. To date, there have been quite a few published studies on myocardial performance both in fetus and neonates, but to our knowledge, this is the only study that has investigated the same individuals before and after birth. Assessing cardiac function by using the speckle-tracking technique in both fetuses and newborns is feasible and reproducible. We describe a significant decrease in longitudinal strain and strain rate and increased myocardial velocities after birth compared with those of the fetus. This might be because of changes in loading conditions and the closure of fetal shunts. After an initial drop in right ventricular preload, due to the cessation of the umbilical flow, right ventricular preload is expected to rise, as the negative pleural pressure after lung expansion results in increased venous return and a less restrictive ventricular function. This is illustrated by the E/A-conversion after birth. Additionally, left ventricular preload rises after birth, because pulmonary resistance and right ventricular afterload decrease and pulmonary blood flow increases. Finally, arterial blood pressure as a proxy for left ventricular afterload increases postnatally.

When we compare our measurements from speckle-tracking echocardiography during the feto-neonatal transition with conditions of important changes in load that were produced by medical or pharmacological interventions, we find that changes in STE measurements illustrate changes in loading conditions accordingly. However, right ventricular strain and strain rate values were expected to be higher after birth as pulmonary resistance decreases. This was actually not the case. These observations were confirmed in a later study by Jain et al, who did not find an appreciable effect on right ventricular echocardiographic measures during early postnatal life. The authors of that study argued that physiological changes after the decrease in right ventricular afterload might not be large enough and the contractile properties of the myocardium too immature to be able to affect STE-derived values. In our opinion, this argument is not completely convincing, because the strain values have been shown to be very sensitive to changes in loading conditions.

One limitation of study III is the use of so-called dummy ECG, which is defining systole and diastole by the opening and closure of valves. Obviously, electrical and mechanical systole and diastole are different in terms of timing, but peak values in strain, strain rate and velocity should not be affected by temporal delays. In any case, there is really no alternative to the use of dummy ECGs in fetal cohorts. In all postnatal
examinations, we used ECGs and not valve movements because the software defined heart cycles automatically and we did not want to introduce a possible bias by manually defining heart cycles.

Our findings, in both the fetal and postnatal states, agree well with other studies that used the same ultrasound equipment and algorithm for STE\textsuperscript{108,111,136,170}. However, the sample size in our as well as in many of these studies has been limited. In order to develop nomograms with sufficient statistical power, it will be necessary to perform much larger studies with comparable protocols at different gestational and postnatal ages, correcting for frame and heart rates and extrapolating for gender and race. One important step to reduce inter-vendor variability occurred recently, when the American Society of Echocardiography and the European Association of Cardiovascular Imaging published a consensus document to standardize nomenclature and technical approach in STE\textsuperscript{171}.

The main finding in study IV is that speckle-tracking echocardiography detects significant changes in left ventricular function six months after preterm birth. However, no differences between preterm and term infants were seen at term-equivalent age, meaning that alterations in function evolve over time and can be interpreted as an early adaptive process. We measured significantly lower left ventricular longitudinal strain and systolic and diastolic myocardial velocities three months after term-equivalent age in preterm infants compared to controls. The differences cannot be explained by differences in heart rate or blood pressure or other factors such as growth restriction or maternal smoking. Our findings may be one of the earliest indications reported so far of a potentially long-lasting change in cardiac development after preterm birth. This interpretation is supported by adult data. In a cross-sectional study of 20 to 39-year-old adults who were born preterm\textsuperscript{6}, functional cardiovascular magnetic resonance imaging revealed significant reductions in left ventricular longitudinal strain and strain rate, as well as in left ventricular systolic and diastolic myocardial velocities. In addition to functional alterations, left ventricular shape, size and mass were also found to differ from the heart structure in adults born at term. The authors of that study concluded that adults born very preterm exhibited structural changes of the left side of the heart corresponding to ten years older age or nine units higher BMI, even after adjusting for other risk factors such as blood pressure.
Accelerated cardiovascular ageing may be an important contributory factor to the increased cardiovascular morbidity and mortality reported in adults born preterm. With regard to the right ventricle, there were no significant differences between the groups. This might be due to a better adaptation of the originally dominant right ventricle that is exposed to a comparable postnatal situation with increased pulmonary resistance, due to open fetal shunts after preterm birth. Developmental changes in right ventricular function, seen in adults who were born preterm, might therefore occur after the investigational period of our study.

In a comparable cohort of clinically stable preterm infants born before 30 weeks of gestation, Hirose et al. detected altered diastolic function already one month after preterm birth. The examiners used conventional and speckle-tracking techniques and reported lower ratios of early to late diastolic filling, lower basal circumferential strain rate in early diastole and higher strain rates in late diastole. As this hemodynamic situation resembled the fetal condition, the authors concluded that there might be a delay in myocardial maturation after preterm birth. These findings are particularly interesting, as they confirm our observation of altered diastolic function after very preterm birth at an even earlier stage. In contrast, systolic function was conserved in that study, which is consistent with our measurements at term-equivalent age. It would have been valuable to extend the follow-up of that study in order to investigate possible changes even in systolic function.

The observed alterations in strain and myocardial velocities in our study are subtle and do not involve clinical symptoms or indications for treatment or intervention. This is also true for an animal study with lambs that were delivered preterm. Although there was no evidence of cardiac dysfunction nine weeks after term equivalent age, irreversible myocardial changes were present in both ventricles on autopsy. These changes implied excessive deposition of extracellular matrix in the myocardium in order to maintain ventricular wall integrity. They also displayed an inflammatory reaction that is found in patients with arterial hypertension. This observation confirms our hypothesis that the immature myocardium of the left ventricle is probably not able to cope with the sudden rise of systemic arterial pressure at birth.

The vascular changes that are described in studies I and II might provide an additional factor for an increase in the left ventricular work load after preterm birth. According to Laplace’s law, the compliance of the aorta is reduced due to narrower diameters and
thicker walls, with elastin deficiency leading to increased stiffness and pulse wave velocities. The reflected pulse wave arrives earlier in late systole in the central aorta and increases left ventricular afterload and myocardial oxygen consumption. This phenomenon is interesting, because the increase of aortic pulse wave velocity has been shown to be an independent risk factor of coronary heart disease and stroke. In summary, vascular remodeling promotes myocardial remodeling.

The strengths of all of our studies include the prospective inclusion of participants, the longitudinal design and the comparison with a healthy control group in studies I, II and IV. The same experienced investigator performed all the recordings, data analyses were performed by an independent examiner who was blinded for group belonging in studies I, II and IV. We controlled for possible confounders, with all measurements being adjusted for current body weight at the final control. Maternal data was acquired at the final examination in order to be able to control for genetic influences on outcomes. Established methods were used when available, and protocols for novel techniques (e.g. frame rate intervals for image acquisition) were guided by the recommendations used in comparable studies. The echocardiographic tool provided by the manufacturer was adjusted to fetal and infant conditions when necessary.

The limitations of the studies include the small number of infants, the relatively long intervals between examinations and the total follow-up time being restricted to six months after birth in the preterm group. Additionally, the cohort represented a convenience sample and did not include all consecutive births during the study period. In addition, the fairly narrow range of gestational ages in the preterm group, from 26 to 30 weeks, makes it difficult to draw conclusions concerning other preterm infants with different gestational ages.

Apparently, the question if and how the detected changes in the vascular structure and cardiac function will signal for cardiovascular disease later in life, cannot be answered in our studies. However, the analogy of our results and those obtained in studies with adults who were born preterm is striking and supports the hypothesis of a perinatal origin of cardiovascular alterations in this patient group.
7 CONCLUSION AND FUTURE PERSPECTIVES

The main result of this thesis indicates that significant changes in the cardiovascular development occur already during the first six months of postnatal life following preterm birth. These changes involve both the vascular tree and the cardiac function, two entities that are tightly connected, since alterations in the vascular anatomy and function will influence cardiac function and vice versa. Advances in echocardiographic technique and the use of novel myocardial imaging facilitate the analysis of cardiac function in preterm infants. This particular patient group has not been investigated extensively by functional echocardiography, although indications of an early developmental contribution to later adverse cardiovascular events have been presented in the last 30 years\textsuperscript{175}. The fact that a larger number of preterm individuals are now entering the age when cardiovascular disease is becoming more apparent, will enable researchers to study disease development and underlying mechanisms to a greater extent and in larger cohorts.

As it has not been possible to prevent preterm births so far, we will have to manage patients with remodeled hearts and vessels in the future. Transitional hemodynamics include the preterm rise in left ventricular pre- and afterload, a process that is probably causing long-lasting alterations in the premature myocardium and leading to a growth arrest of the large arteries.

The knowledge about early changes in cardiac and vascular function will hopefully enable us in the future to predict disease risk in early life and to design strategies of lifestyle intervention and medical treatment, thus reducing the risk burden of cardiovascular disease in the growing population of individuals who are born preterm.
8 DEUTSCHE ZUSAMMENFASSUNG


Die Tatsache, dass ähnliche Veränderungen sowohl des Gefässsytems als auch der Herzfunktion bei zu frühgeborenen Erwachsenen nachweisbar sind, legt die Vermutung nahe, dass die hier beschriebenen Befunde bis ins Erwachsenenalter fortbestehen – mit möglicherweise negativen Auswirkungen auf die Gesundheit. Da auch weiterhin Frühgeburten in gewissen Situationen unvermeidbar und physiologische Gesetzmäßigkeiten wie die abrupte Zunahme der kardialen Nachlast nicht zubeeinflussen sind, werden wir in Zukunft deutlich mehr Kinder und Erwachsene mit einer grundsätzlich anderen Physiologie der Gefäße und des Herzens zu behandeln haben. Frühzeitige und regelmässige Nachuntersuchungen
von Frühgeborenen werden notwendig sein, um die exakten Mechanismen besser zu verstehen und die stetig steigende Anzahl Frühgeborener adequat zu behandeln.

Den övertygande frågeställningen i denna avhandling handlar om att söka belägg för ett perinatalt ursprung som skulle kunna predisponera för tidigt födda till senare hjärtkärlsjukdom. För detta ändamål genomfördes en longitudinell observationsstudie av hjärtfunktion, artäärdiametrar och intima-media tjocklek under de första sex månaderna efter tidig födsel. Utöver väletablerade ultraljudsometoder, tillämpades ny teknik, s.k. speckle-tracking ekokardiografi. Den nya tekniken har det gjort möjligt att upptäcka subtila förändringar i hjärtfunktion även hos små individer.

För tidigt födda barn uppvisade en avvikande utveckling av hjärtkärlsystemet jämfört med friska barn födda i fullgången tid. De undersökta artärerna (aorta och a. carotis communis) var betydligt smalare och kärlens intima-media var tjockare hos för tidigt födda barn jämfört med fullgångna barn. Dessutom fann vi signifikanta skillnader i systolisk och diastolisk vänsterkammarfunktion, som sannolikt återspeglar den för tidigt födda hjärtmuskelns anpassning till extrauterin liv.

Resultaten visar att den tidiga kardiovaskulära utvecklingen förändras av för tidig födelse. Det kan inte uteslutas att denna tidiga påverkan kvarstår – barn och vuxna som föddes för tidigt har i andra studier uppvisat liknande förändringar i både hjärt- och kärlsystemet.

Än så länge har för tidig födelse inte gått att förebygga. Eftersom cirkulationsomställningen vid födelsen är livsnödvändig, så kommer vi i framtiden att möta fler barn och vuxna med en modifierad fysiologi av hjärt- kärlsystemet till följd av för tidig födelse. Fortsatt uppföljning av dessa individer kommer att behövas, dels för att bättre förstå de underliggande mekanismerna och den långsiktiga betydelsen, dels för att genom tidig upptäckt och prevention av kliniska komplikationer förbättra den kardiovaskulära hälsan i den växande gruppen av för tidigt födda individer.
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