Department of Clinical Science, Intervention and Technology

Karolinska Institutet, Stockholm, Sweden

CARDIOVASCULAR DEVELOPMENT OF THE PRETERM INFANT

Ulf Schubert

Karolinska Institutet

Stockholm 2016

Published by Karolinska Institutet.
Printed by Eprint AB 2016

ISBN 978-91-7676-412-1

Cardiovascular development of the preterm infant

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Ulf Schubert

Principal Supervisor:
Professor Mikael Norman
Division of Pediatrics,
Department of Clinical Science,
Intervention and Technology
Karolinska Institutet, Stockholm

Opponent:
Associate Professor Katarina Hanséus
Clinical Director of Pediatric Cardiology,
Children´s Heart Center
University Hospital Lund

Co-supervisor(s):
Associate Professor
Anna-Karin Edstedt Bonamy
Department of Women´s and
Children´s Health
Karolinska Institutet, Stockholm

Examination Board:
Professor Nina Nelson Follin
Department of Clinical and Experimental
Medicine/ Division of Pediatrics
University of Linköping
Department of Quality and Patient Safety,
Karolinska University Hospital, Stockholm

Professor Hashim Abdul-Khaliq
Clinical Director of Pediatric Cardiology,
Children´s Heart Center
Universität des Saarlandes,
Germany

Associate Professor Anders Jonzon
Department of Women´s and
Children´s Health
University of Uppsala

Associate Professor Baldvin Jonsson
Department of Women´s and
Children´s Health
Karolinska Institutet, Stockholm

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
Journal of Developmental Origins of Health and Disease 2011, 2(4): 218-225

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

CONTENTS

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

IVH	Intraventricular hemorrhage
IVS	Interventricular septum
Kcal	Kilocalorie
Khz	Kilohertz
LBW	Low birth weight
LV	Left ventricle
MI	Mechanical index
MMP	Matrix metalloproteinase
MPI	Myocardial performance index
MRI	Magnetic resonance imaging
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
NO	Nitric oxide
PDA	Patent ductus arteriosus
PDGF	Platelet-derived growth factor
PPROM	Prelabor premature rupture of membranes
PVL	Periventricular leukomalacia
RDS	Respiratory distress syndrome
ROI	Region of interest
ROP	Retinopathy of prematurity
RV	Right ventricle
SD	Standard deviation
SDS	Standard deviation score
SF	Shortening fraction
SGA	Small for gestational age
SMC	Smooth muscle cell
STE	Speckle-tracking echocardiography
STIC	Spatio-temporal image correlation
SV	Stroke volume
TAPSE	Tricuspid annular plane systolic excursion
TI	Thermal index
TTTS	Twin-to-twin transfusion syndrome
VEGF	Vascular endothelial growth factor
VLBW	Very low birth weight
Vs.	Versus
WHO	World Health Organization

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[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[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[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[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)[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[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[12].

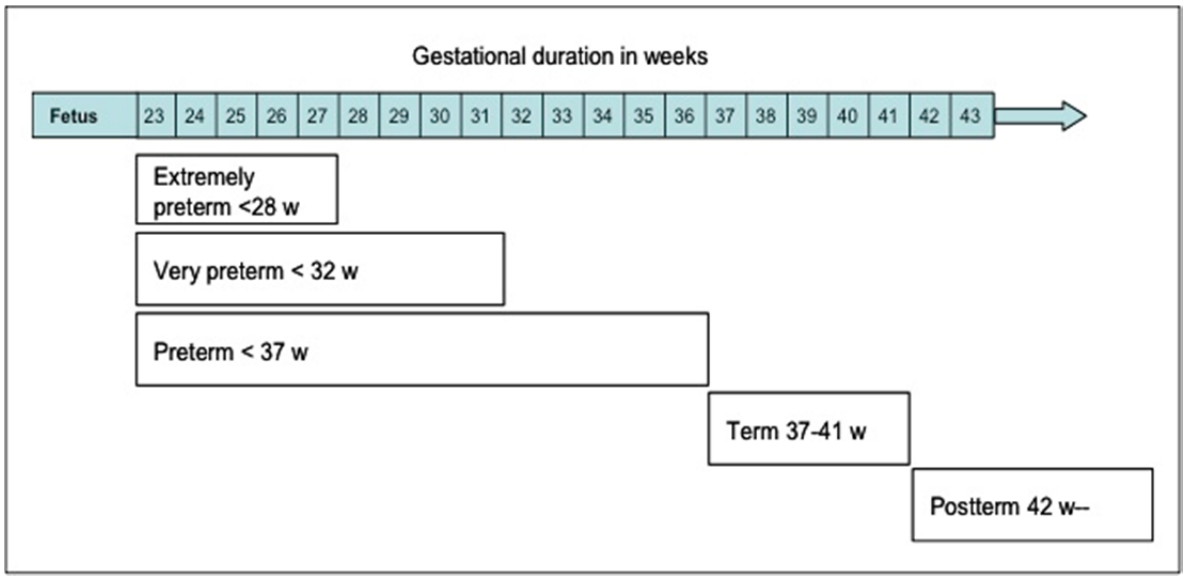


Figure 1: Definition of pregnancy length (adapted from Tucker et al.[10])

About 16 million infants are born prematurely around the world each year[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[14]. According to data from the WHO[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[16]. 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[17]. 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[16]. 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[18-22]. Finally, low or advanced maternal age and assisted reproductive techniques have resulted in an important increase of preterm deliveries worldwide[1-3,23].

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[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[25].

Persistent impairments include cognitive problems and behavioral disorders, hearing loss, visual problems/blindness and cerebral palsy[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[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[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)[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[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[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[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[34].

Another large meta-analysis confirmed the association between prematurity and hypertension[5], and a third population-based study indicated that the risk of hypertension among young men increased with the degree of prematurity[4].

Intima-media thickening is an established risk factor for atherosclerosis. Some studies have shown that individuals born preterm exhibit increased IMT[5], 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[35].

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[33].

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[36]. 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[37]. 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[38].

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[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[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[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[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).

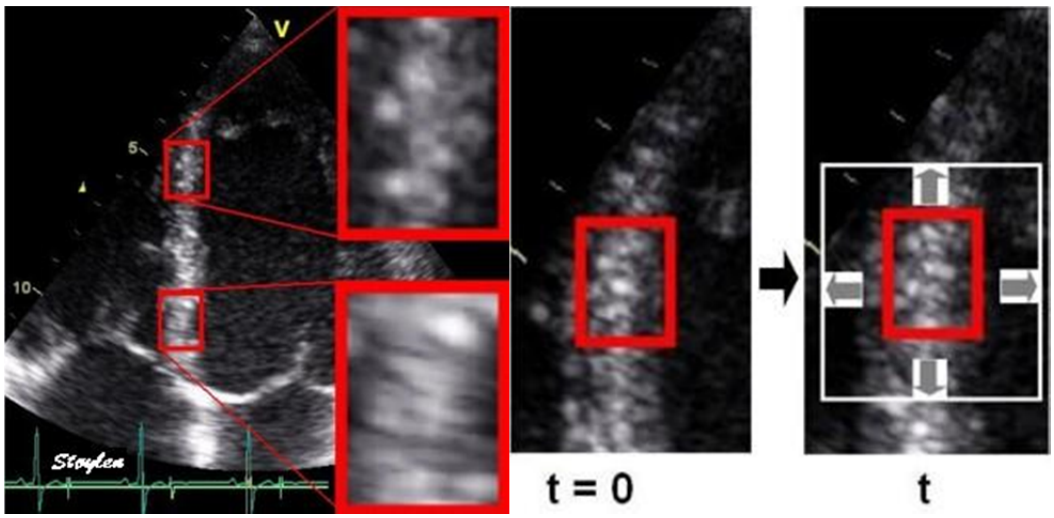


Figure 2: Speckle-tracking search algorithm in the interventricular septum. Magnification of the kernel (red square) in the original frame (t=0) and in the subsequent frame (t) and corresponding search area (white square) for the matching kernel. Reproduced with permission from: http://folk.ntnu.no/stoylen/strainrate

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 ($\varepsilon = L - L0 / L0$), 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[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)[44]. STE measures have been shown to be very sensitive to changes in load and cardiac function[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[48], rejection after a heart transplant[49] or cardiac resynchronization therapy[50]. The ability to detect subclinical myocardial dysfunction before the appearance of clinically apparent ventricular impairment has been recognized even in preterm infants[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[52], the technique has been used to evaluate the right ventricle and showed good feasibility and reproducibility[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/cm^2. 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 function[54]. 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, thrombomodullin, 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 velocity[55]. 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 pressure[56], the pathological pathway can lead to a circulus vitiosus and the evolution of atherosclerosis[57]. 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 formation[58].

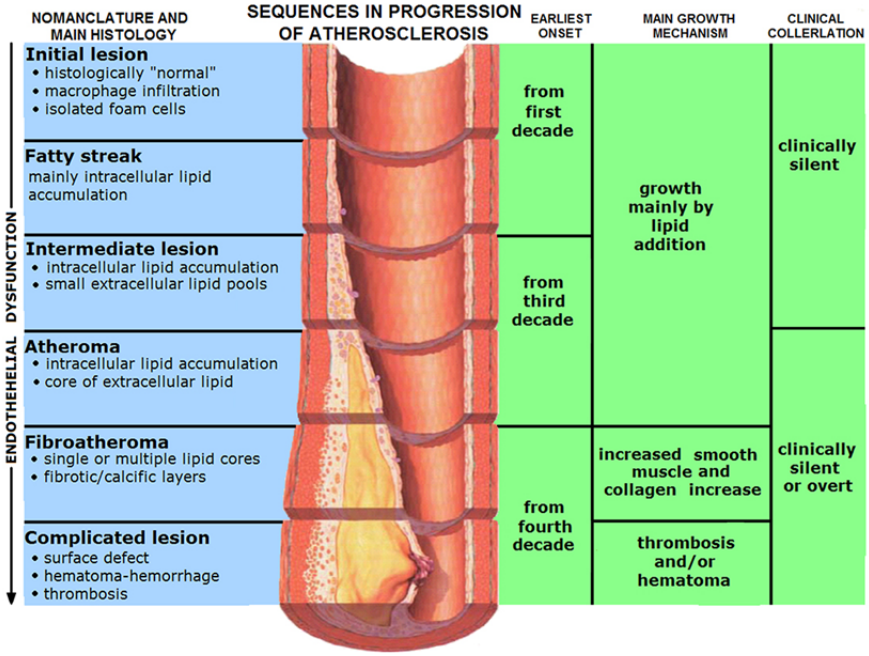


Figure 3: Different stages in the development of atherosclerosis from early life to symptomatic disease. Adapted from http://en.wikipedia.org/wiki/File:Endo_dysfunction_Athero.PNG, free documentation license

2.6.2 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[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[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[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[63]. Moreover, children born small for gestational age continue to exhibit low levels of IGF-1 and vascular dysfunction[64]. Taken together, these findings could explain why fetal growth restriction is a developmental risk factor for lasting arterial narrowing[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)[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[68].

2.6.3 Arterial dimensions and IMT after preterm birth

Prematurity and the programming of cardiovascular disease has become an important field of research[33], and it seems that vascular ageing is starting already in the fetal period and continues throughout life[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[71,72], arterial elasticity[73,74] and blood pressure[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 [65,66,76-78]but also in individuals born preterm[72,79,80]. The most common methodological approach is non-invasive ultrasound of the aorta, carotid and coronary arteries, which measures diameters and IMT[81]. Results concerning IMT can be somewhat contradictory as some studies have shown an association between low birth weight and intima-media thickening[82-86], while others, mostly in elderly people, have failed to demonstrate this association[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[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[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[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.

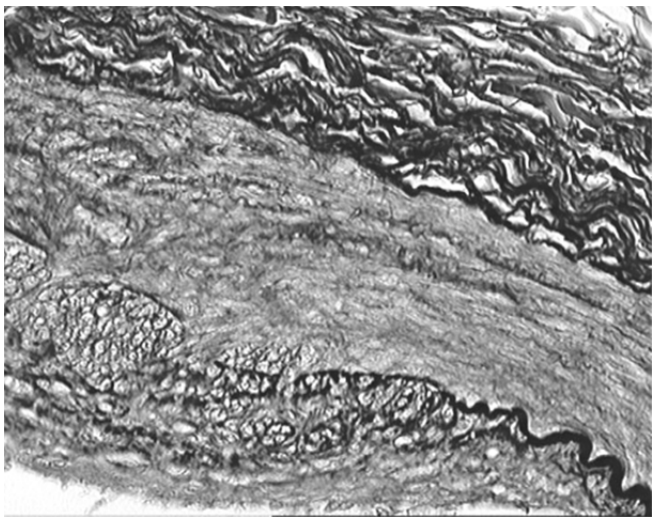


Figure 4: Histology after fetal death: intimal thickening in the left coronary artery with fragmentation of the internal elastic membrane (original magnification x 200). Reproduced with permission from Milei 2008

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[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[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[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[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 sympathic stimulation due to sparse sympathic nerve endings. Additionally, the myocytes are smaller than in a term newborn with a single nucleus and less mitochondria and a higher water content[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[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[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[100]. 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[101]. During the fetal period, the heart is growing and the myocardial mass is constantly increasing[102]. Systolic and diastolic pressures show a linear rise during gestation and the combined cardiac output decreases with higher afterload[103].

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[104]. 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[105]. 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[106]. 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[107].

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[108-115], myocardial imaging proved to be useful in pathological conditions such as congenital heart defects[116,117], growth restriction and preeclampsia[118], amniotic infection[119] and twin-to-twin transfusion syndrome[120]. 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[121].

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[122]. 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[123,124].

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[125,126]. 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[127], 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[36]. 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[143]. 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[144].

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[6]. 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[145].

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 data[146].

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.

	Preterm infants (n=25)	Term controls (n=30)	p-value
Maternal data			
Age, years	32.6 (4.2)	31.1 (4.3)	0.20
Parity, n	1.7 (1.0)	1.9 (1.0)	0.46
Family history of CVD	3/25 (12%)	5/30 (17%)	0.62
Smoking in pregnancy, n	5/25 (20%)	1/30 (3.3%)	0.048
Perinatal data			
Gestational age, weeks	27.7 (1.2)	39.0 (1.4)	-
Boys, n	13/25 (52%)	11/30 (33%)	0.16
Birth weight, g	1153 (258)	3456 (437)	-
Birth weight SDS	-0.82 (1.01)	0.09 (1.10)	0.003
Birth length, cm	37.2 (2.7)	50.3 (1.9)	-
Infant data at final assessment			
Postmenstrual age, days	380 (15)	371 (10)	0.13
Weight, kg	5.61 (0.48)	6.09 (0.62)	0.003
Length, cm	59.8 (2.5)	61.2 (2.7)	0.072
Heart rate, beats/min	144 (17)	143 (15)	0.90
Syst. blood pressure, mmHg	91 (13)	94 (9)	0.41
Diast. blood pressure, mmHg	59 (11)	63 (12)	0.32

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.

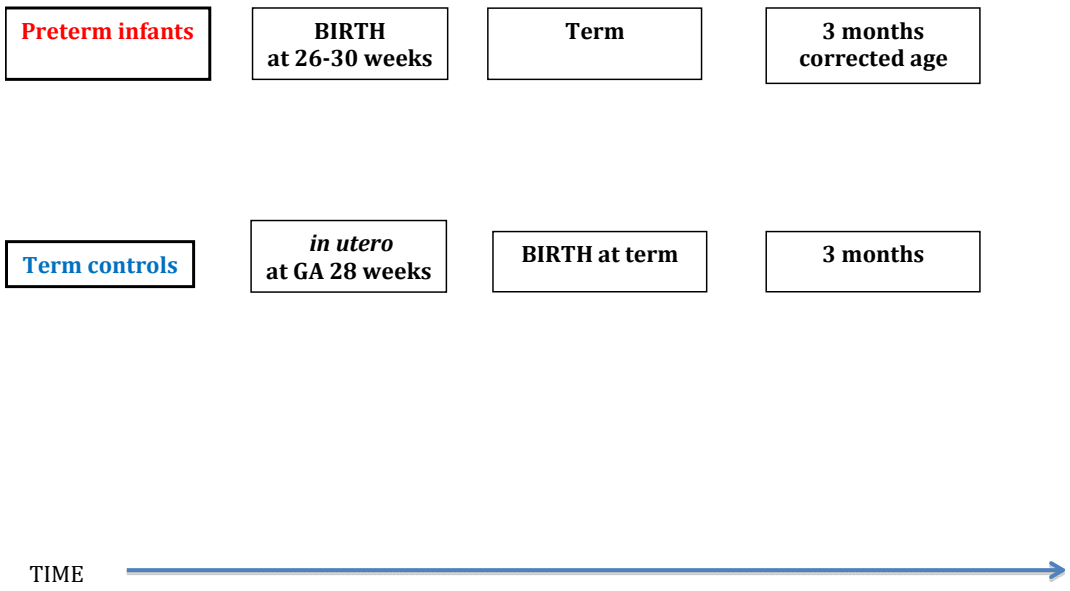


Figure 5: Time points when the preterm and term control groups were examined

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[148] (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[149].

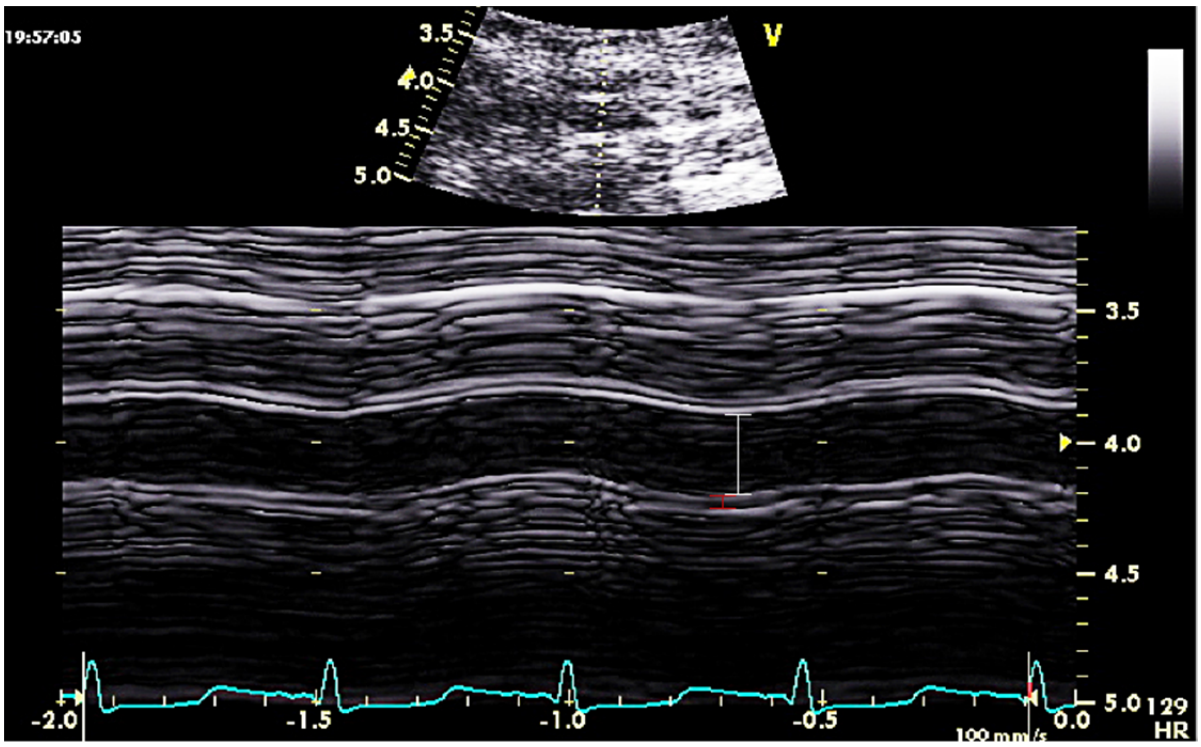


Figure 6: M-Mode image of the abdominal aorta of a preterm infant. Illustration of the measurements of the end-diastolic diameter (white) and intima-media thickness (red)

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 [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.

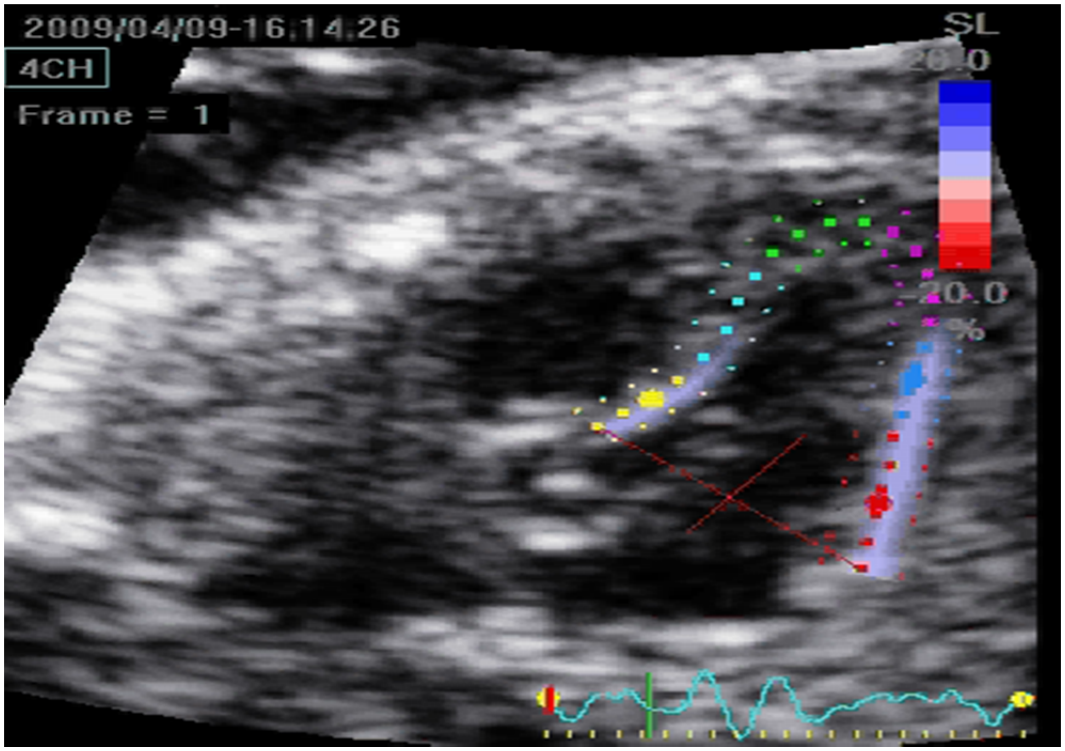


Figure 7: Identification of six regions of interest in the left ventricle of a fetus at a gestational age of 28+2 using STE: red, dark-blue and purple at the free left ventricular wall, and yellow, light-blue and green at the interventricular septum from basis to apex

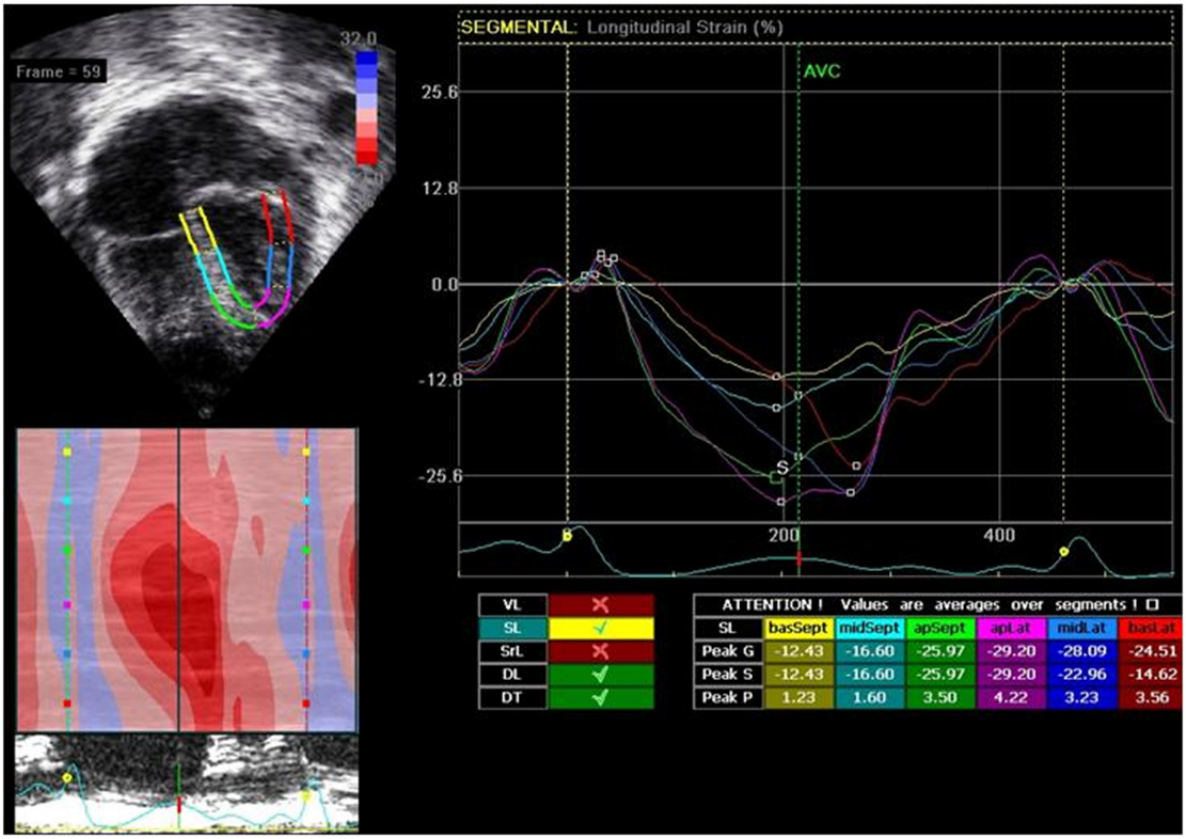


Figure 8: Graphical and numerical presentation of strain in six regions of interest (ROI) in the LV of a preterm infant 3 months after birth with the same color coding of the free left ventricular wall and interventricular septum as in figure 7

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[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 ($\beta= 0.42$ mm/kg, $r= 0.26$, $p= 0.07$) and between infant and maternal aEDD ($\beta=0.072$ 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 ($r^2= 0.54$, $p<0.001$). 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.

	Very preterm (n=21)	Term controls (n=29)	p-value
Aortic IMT*(mm)			
IMT, 28 w	0.41 (0.31-0.46)	0.58 (0.52-0.63) †	0.001
IMT, Term	0.45 (0.38-0.57)	0.47 (0.38-0.56)	0.78
IMT, 3 m	0.56 (0.51-0.63)	0.58 (0.52-0.70)	0.37
Aortic end-diastolic diameter*(mm)			
EDD, 28 w	3.6 (3.3-4.1)	3.4 (2.9-3.7) †	0.03
EDD, Term	4.9 (4.1-5.6)	5.8 (5.4-6.4)	< 0.001
EDD, 3 m	5.8 (5.6-6.3)	7.4 (6.7-8.0)	< 0.001
Relative aortic IMT**			
IMT/EDD, 28 w	0.109 (0.094-0.125)	0.182 (0.168-0.196) †	< 0.001
IMT/EDD, Term	0.105 (0.092-0.118)	0.085 (0.073-0.097)	0.01
IMT/EDD, 3 m	0.096 (0.087-0.105)	0.085 (0.077-0.093)	0.03

*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.

	Very preterm (n=21)	Term controls (n=29)	p-value
Carotid IMT*(mm)			
IMT, 28 w	0.33 (0.28-0.36)	-	-
IMT, Term	0.35 (0.32-0.38)	0.36 (0.31-0.39)	0.89
IMT, 3 m	0.37 (0.33-0.48)	0.37 (0.32-0.48)	0.64
Carotid end-diastolic diameter*(mm)			
EDD, 28 w	1.7 (1.5-1.9)	-	-
EDD, Term	2.0 (1.8-2.1)	2.7 (2.4-3.0)	< 0.001
EDD, 3 m	2.6 (2.4-2.9)	3.1 (2.7-3.3)	< 0.001
Relative carotid IMT**			
IMT/EDD, 28 w	0.198 (0.166-0.240)	-	-
IMT/EDD, Term	0.185 (0.165-0.205)	0.138 (0.121-0.156)	< 0.001
IMT/EDD, 3 m	0.157 (0.139-0.176)	0.132 (0.115-0.149)	0.03

*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.

		mean (SD), fetal	mean (SD), neo	p-value
RV	S	4.83 (1.86)	6.38 (2.34)	0.006
	E	-4.40 (1.93)	-6.44 (1.54)	<0.001
	A	-4.53 (2.30)	-5.97 (2.52)	0.053
LV	S	4.49 (1.56)	4.44 (1.45)	0.99
	E	-4.70 (1.60)	-5.51 (1.84)	0.07
	A	-4.71 (1.49)	-4.22 (1.86)	0.39
IVS	S	4.27 (1.44)	4.00 (1.13)	0.46
	E	-3.28 (0.71)	-5.02 (1.83)	0.002
	A	-4.11 (1.73)	-4.82 (1.84)	0.10

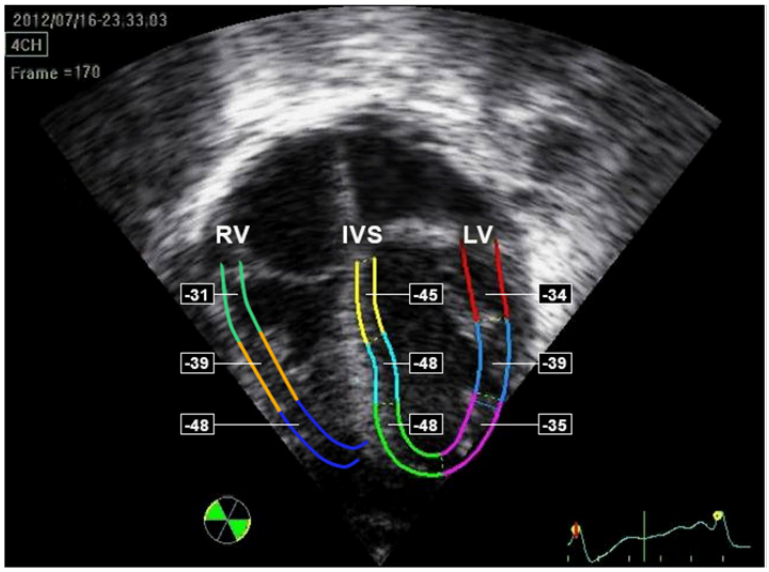
Table 4: Maximal basal velocities (cm/s) of the right ventricle (RV), left ventricle (LV) and interventricular septum (IVS) in systole (S), early diastole (E) and late diastole (A) in echocardiographic examinations at 28th week of gestation (fetal) and after birth (neo). Data are mean (SD), p-values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

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

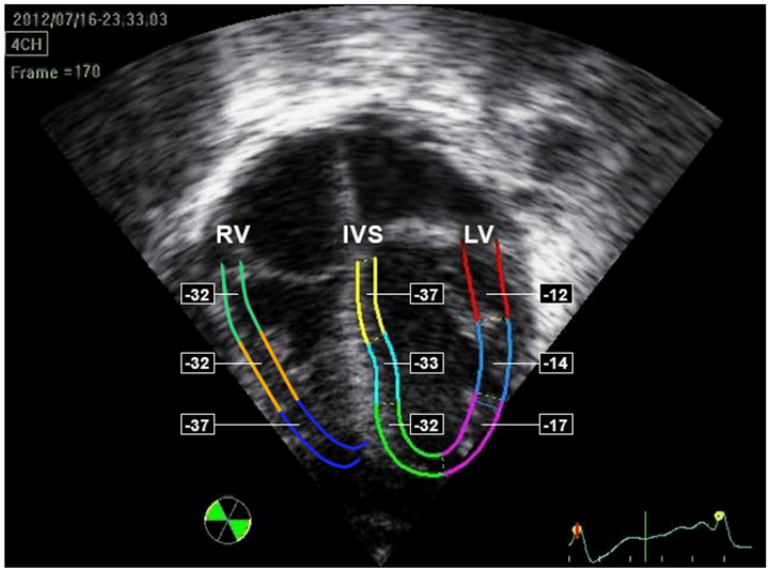
Table 5: Maximal and mean systolic longitudinal strain (%) of the right ventricle (RV), left ventricle (LV) and interventricular septum (IVS) at the base, mid-ventricular (medial) and apex in echocardiographic examinations at 28th week of gestation (fetal) and after birth (neo). Data are mean (SD), p-values according to paired t-test

		Fetal		Neo		p-value
RV	SV (ml)	3.25	(0.58)	-†		-
	CO (ml)	443	(58)	-†		-
	EF (%)	65.29	(6.69)	55.51	(11.6)	< 0.001
	MPI	0.24	(0.17)	0.13	(0.04)	0.01
	E/E´	11.3	(5.8)	8.6	(4.5)	0.07
	E/A	0.94	(0.39)	1.00	(0.56)	0.66
	E´/A´	1.12	(0.94)	1.26	(0.59)	0.48
LV	SV (ml)	2.26	(0.44)	6.27	(1.66)	-
	CO (ml)	321	(56)	797	(214)	-
	EF (%)	64.66	(8.06)	63.58	(6.32)	0.57
	MPI	0.26	(0.15)	0.26	(0.11)	0.67
	E/E´	11.3	(7.3)	11.9	(5.9)	0.67
	E/A	0.95	(0.45)	1.16	(0.20)	0.12
	E´/A´	1.06	(0.39)	1.48	(0.71)	0.005

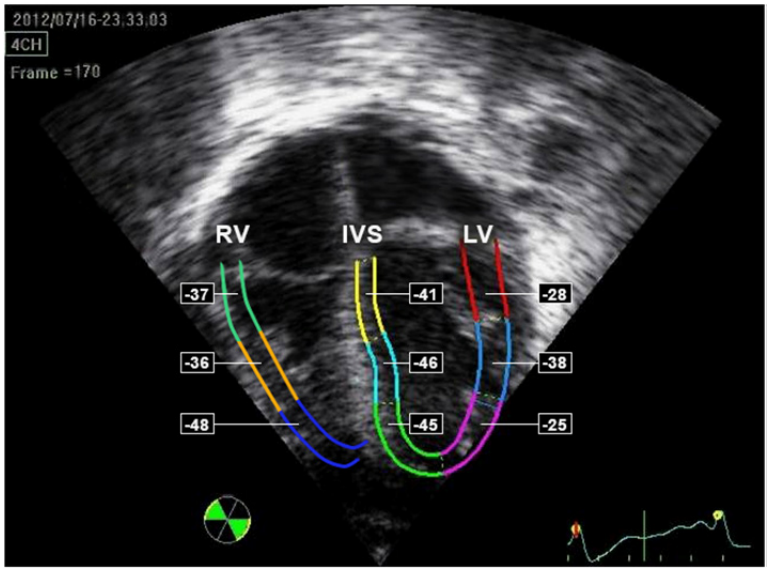
Table 6: Stroke volume (SV in ml), cardiac output (CO in ml/min), EF (%) by biplane volumetry and myocardial performance index (MPI), relation between max. velocities in early diastole in pulsed Doppler (E) and 2D-S (E´) and relation between early and late diastolic max. velocities in pulsed Doppler (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



a



b



c

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.

	28 weeks		40 weeks		53 weeks		
Region of interest	Preterm	Control	Preterm	Control	Preterm	Control	p-value for difference between groups at 53 wks
Left ventricle (LV)							
LV-basal	-18.6 (3.5)	-	-19.3 (4.6)	-20.0 (3.8)	-19.8 (4.2)	-22.1 (3.8)	0.03
LV-mid	-17.5 (3.2)	-	-18.1 (3.4)	-19.1 (2.6)	-19.8 (3.4)	-21.7 (3.8)	0.05
LV-apical	-17.5 (4.2)	-	-18.7 (3.3)	-19.4 (2.8)	-20.5 (3.4)	-22.2 (4.5)	0.12
LV-mean	-17.9 (2.5)	-	-18.7 (2.6)	-19.5 (2.1)	-20.0 (2.2)	-22.0* (3.2)	0.010
Interventricular septum (IVS)							
IVS-basal	-18.4 (3.6)	-	-20.4 (4.1)	-20.6 (3.7)	-21.1 (2.8)	-21.7 (3.3)	0.48
IVS-midseptal	-18.8 (3.4)	-	-20.2 (3.1)	-19.6 (3.5)	-22.1 (2.5)	-22.2 (3.4)	0.86
IVS-apical	-19.8 (3.8)	-	-20.3 (3.0)	-19.9 (3.7)	-22.5 (3.0)	-23.6 (4.7)	0.36
IVS-mean	-19.0 (3.4)	-	-20.3 (3.0)	-20.1 (3.4)	-21.9 (2.2)	-22.5* (3.3)	0.44
Right ventricle (RV)							
RV-basal	-20.7 (8.9)	-	-24.7 (7.6)	-25.3 (7.3)	-25.2 (6.7)	-28.0 (7.1)	0.17
RV-mid	-20.0 (6.2)	-	-24.0 (4.7)	-22.6 (4.7)	-24.2 (4.3)	-23.9 (6.0)	0.86
RV-apical	-21.0 (4.9)	-	-21.4 (4.7)	-21.2 (5.0)	-22.2 (7.2)	-21.4 (5.8)	0.69
RV-mean	-20.5 (5.5)	-	-23.3 (4.0)	-23.0 (4.2)	-23.9 (4.2)	-24.4 (5.2)	0.69

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.

	28 weeks		40 weeks		53 weeks		
Region of interest	Preterm	Control	Preterm	Control	Preterm	Control	p-value for difference between groups at 53 wks
Left ventricle (LV)							
Systole	3.17 (2.6-4.5)	-	4.99 (4.4-6.3)	4.41 (3.2-5.5)	5.49 (4.2-7.0)	6.47* (5.1-7.4)	0.27
Early diastole	-3.74 (-4.9;-2.7)	-	-5.79 (-6.9;-5.1)	-5.63 (-6.5;-4.0)	-7.37* (-11;-5.2)	-10.9* (-13.1;-7.9)	0.003
Late diastole	-3.61 (-5.6;-2.5)	-	-5.26 (-6.1;-3.6)	-4.16 (-5.3;-3.1)	-5.11 (-7.0;-3.1)	-6.95* (-8.9;-5.5)	0.009
Interventricular septum (IVS)							
Systole	2.85 (2.5-3.2)	-	4.38 (3.9-4.9)	3.81 (3.2-4.7)	5.88* (5.3-7.2)	5.80* (5.0-7.0)	0.83
Early diastole	-3.84 (-5.2;-2.6)	-	-5.68 (-7.7;-4.6)	-4.62 (-5.9;-3.5)	-7.27* (-8.5;-6.2)	-7.25* (-8.9;-6.1)	0.90
Late diastole	-4.14 (-4.8;-3.5)	-	-5.33 (-6.2;-4.2)	-4.22 (-5.6;-3.4)	-6.46* (-7.3;-5.3)	-7.06* (-9.3;-5.7)	0.13
Right ventricle (RV)							
Systole	4.31 (3.5-5.1)	-	6.72 (5.2-7.8)	5.62 (4.6-7.7)	7.98* (6.7-9.2)	7.08 (5.6-8.7)	0.11
Early diastole	-4.60 (-6.5;-4.2)	-	-7.32 (-8.9;-5.2)	-6.47 (-7.6; -5.6)	-7.55 (-11;-4.9)	-6.80 (-9.8;-4.3)	0.49
Late diastole	-4.82 (-7.0;-2.2)	-	-7.30 (-8.4;-5.4)	-5.90 (-8.1;-3.9)	-8.45 (-10;-5.7)	-5.90 (-7.9;-4.8)	0.05

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.

	28 weeks		40 weeks		53 weeks		
	Preterm	Control	Preterm	Control	Preterm	Control	p-value for difference between groups at 53 wks
Left ventricle (LV)							
Systole	-2.33 (-2.9;-2.1)	-	-2.60 (-3.5;-2.3)	-2.40 (-3.2;-2.0)	-3.37* (-3.8;-2.7)	-3.06* (-3.7;-2.6)	0.53
Early diastole	2.78 (2.4-3.5)	-	3.51 (3.1-3.7)	3.05 (2.7-4.0)	3.33 (3.1-5.1)	4.33* (3.5-5.3)	0.28
Late diastole	2.28 (1.8-2.9)	-	2.76 (2.0-3.3)	2.10 (1.6-3.2)	2.52 (2.1-3.6)	3.22* (2.2-3.9)	0.28
Interventricular septum (IVS)							
Systole	-2.12 (-2.6;-1.8)	-	-2.32 (-2.8;-1.9)	-2.08 (-2.4;-1.7)	-2.62 (-3.2;-2.0)	-2.60* (-3.1;-2.2)	0.75
Early diastole	2.72 (2.0-3.1)	-	2.62 (2.2-3.2)	2.32 (2.0-2.9)	3.22* (2.9-4.3)	3.44* (2.6-4.3)	0.93
Late diastole	2.45 (1.8-2.9)	-	2.47 (1.9-2.9)	1.88 (1.5-2.4)	2.56 (2.0-3.0)	2.57* (2.2-3.4)	0.23
Right ventricle (RV)							
Systole	-2.79 (-3.2;-2.6)	-	-3.81 (-4.1;-3.1)	-2.70 (-3.7;-2.3)	-4.17 (-4.7;-2.8)	-3.79* (-5.3;-3.3)	0.38
Early diastole	3.20 (2.3-3.5)	-	3.59 (2.8-4.5)	3.00 (2.3-4.3)	4.54* (3.9-5.5)	4.16* (2.9-6.1)	0.82
Late diastole	2.64 (2.1-3.1)	-	3.33 (2.5- 4.2)	2.30 (1.7-3.0)	2.90 (2.2-4.1)	3.53* (2.7-4.3)	0.38

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).

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

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[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[152]. This insufficient nutritional status is not just present in infants with fluid restriction[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[154,155]. In a prospective study from England, which included more than 100 infants with a gestational age ≤ 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 ≤ 31 weeks and ≥ 31 weeks, respectively[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[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[59,62], it is also essential for normal vessel growth in humans[60,61]. Down-regulation of IGF-1 by placental insufficiency has been associated with thinner and stiffer umbilical arteries[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[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 thickening[92]. 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 controls[158].

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 circulation[159]. 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 system[161]. 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 syndrome[69].

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 IMT[162]. 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[95]. Additionally, left ventricular preload rises after birth, because pulmonary resistance and right ventricular afterload decrease and pulmonary blood flow increases[96]. Finally, arterial blood pressure as a proxy for left ventricular afterload increases postnatally[163].

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[45-47,164-168]. However, right ventricular strain and strain rate values were expected to be higher after birth as pulmonary resistance decreases. This was actually not the fact. 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[169]. 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[45].

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[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 occured 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[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[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[172].

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[145].

In a comparable cohort of clinically stable preterm infants born before 30 weeks of gestation, Hirose et al[140] 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[99]. 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[173]. 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[174]. 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[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 Anzahl der Frühgeburten und der kardiovaskulären Todesfälle nimmt weltweit stetig zu. Die Ursachen für beide Entwicklungen sind vielfältig und offenbar nicht miteinander verknüpft. Gleichwohl scheinen Frühgeborene ein erhöhtes Risko für Herzkreislaufprobleme wie z.B. Bluthochdruck, koronare Herzerkrankung und Schlaganfälle zu haben, auch wenn die genauen Mechanismen weiterhin unbekannt sind.

Die Grundidee dieser Doktorarbeit war es, den frühen Ursprung der Veränderungen sowohl des Gefäßsystem als auch der Herzfunktion nachzuweisen, unter denen Erwachsene leiden, die ehemals zu früh geboren wurden. Zu diesem Zweck führten wir eine Verlaufsstudie durch, welche einerseits den Gefässdurchmesser und die Intima-Media-Struktur der Aorta und der Halsschlagader, andererseits die Herzfunktion während der ersten sechs Monate nach Geburt untersuchte. Neben etablierten Ultraschallverfahren verwendeten wir innovative Technologien wie Speckle-Tracking-Echokardiographie, die durch die direkte Beurteilung des Herzmuskels die Diagnose subtiler funktioneller Beeinträchtigungen möglich gemacht haben.

Im Vergleich mit gesunden Neugeborenen zeigten sich signifikante strukturelle und funktionelle Veränderungen der Gefäße und des Herzens. Die untersuchten Arterien waren deutlich schmaler und die Intima-Media bezogen auf den Gefäßdurchmesser verdickt. Darüberhinaus fanden wir deutliche Unterschiede in der linksventrikulären systolischen und diastolischen Funktion des Herzens. Offenbar führt die vorzeitige Umstellung zum nachgeburtlichen Blutkreislauf zu einem Umbau des Herzmuskels und zu einer Beeinträchtigung der Funktion.

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 kardiellen 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.

9 SVENSK SAMMANFATTNING

Antalet barn som föds för tidigt och överlever till vuxen ålder ökar. Samtidigt ökar hjärtkärlsjukdomar på många håll i världen. Även om en koppling mellan för tidig födelse och hjärtkärlsjukdom förefaller långsökt, så finns idag forskning som visar på en ökad risk för underburna barn att drabbas av högt blodtryck, hjärtinfarkt och stroke. Orsakerna bakom dessa samband är okända.

Den övergripande 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, artärdiametrar och intima-media tjocklek under de första sex månaderna efter tidig födsel. Utöver väletablerade ultraljudsmetoder, 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 extrauterint 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.

10 ACKNOWLEDGMENTS

Firstly, I would like to thank all the infants and their parents for participating in the studies. I particularly appreciated that there were not any dropouts during the six-month study period. Without their enthusiasm, my work would not have been possible.

In addition, I would like to express my gratitude to:

Mikael Norman, my main supervisor, for being an excellent clinician and researcher, with a broad knowledge of the vascular effects in preterm children and a highly accurate approach to study planning and interpreting the results. He also taught me that ice-skating on lakes is a completely normal activity during lunch breaks in Swedish hospitals.

Hashim Abdul-Khaliq, my co-supervisor and co-worker at the Deutsche Herzzentrum in Berlin, for teaching me pediatric cardiology and the wisdom, that real echocardiography has little to do with standard projections.

Anna-Karin Edstedt, my co-supervisor, who helped me to write the paper on IMT, for her efficacy and diligence in formulating difficult issues in a simple way and for her great epidemiological and statistical knowledge.

Matthias Müller, my co-worker and cardiologist in Heidelberg, for performing the lengthy and detailed analysis of the echo data. The time he spent on this task is very much appreciated.

Mats Blennow, professor and co-worker, who recruited me to the neonatal department at Karolinska many years ago, although he actually had no clue what he was doing taking me on at that time. Over the years we have found out that having different opinions can be fruitful and enrich friendship.

Martino Corrias, a fabulous clinician and co-worker, who showed me how resistant preterm infants are – including to our treatments. They really have a great will to survive.

Kajsa Bohlin, my co-worker and friend, who convinced me in times of indignation that the term “major revision” actually means that the paper has almost been accepted and not almost been rejected.

Dirk Wackernagel, my friend and co-worker, who appreciated my extended presentations on “fraggle-tracking” and always understood the beauty of my hand-made figures and tables.

Lars Naver, assistant professor and co-worker, for helping me in critical situations in the NICU when I was excessively tired because of my many night duties, and who learned that Boogaloo is an excellent party accelerator.

Boubou Hallberg, my co-worker and chief at the NICU, for his medical and oenological advice. I will always remember his great energy in general, and especially in critical situations such as round pingis, kubb and boule competitions.

Ewa Henckel, my co-worker at Karolinska, for offering a helping hand in any kind of situation and for a careful proof-reading.

Queralt Ferrer, pediatric cardiologist in Barcelona and good friend, and **Wolfgang Henrich**, professor in the obstetric department at the Charité in Berlin, for teaching me fetal echocardiography.

Tessma Mesfin, statistician and co-worker, for quoting a famous person who said that statistics are always wrong, but sometimes useful.

All the **midwives** for recruiting the children to the control group, so that they were study participants even before they were born.

All the nurses working on the NICUs who helped me to perform the echocardiographic examinations, and especially the night sisters who participated in my crash course: from beginner to almost expert in echo in 5 minutes.

The **staff of AVA 1+2** in Solna, who allowed me to borrow the ultrasound machine day and night, in order to perform study examinations and teach my son´s school class “what is inside the body” with the help of ultrasound.

Elke Scheurich, a good friend from Hamburg, helping me with tables and figures to fit in smoothly.

All my **friends** around the world. I promise that I will spend more time with you again soon.

The papers included in this thesis were supported by grants from the Swedish Heart and Lung Foundation, the Childhood Foundation of the Swedish Order of Freemasons and by a regional agreement on medical training and research (ALF) between Stockholm County Council and the Karolinska Institutet.

11 REFERENCES

1. Dunietz GL, Holzman C, McKane P, et al. Assisted reproductive technology and the risk of preterm birth among primiparas. *Fertil Steril.* Apr 2015;103(4):974-979 e971.
2. Declercq E, Luke B, Belanoff C, et al. Perinatal outcomes associated with assisted reproductive technology: the Massachusetts Outcomes Study of Assisted Reproductive Technologies (MOSART). *Fertil Steril.* Apr 2015;103(4):888-895.
3. Hoffman MC, Jeffers S, Carter J, Duthely L, Cotter A, Gonzalez-Quintero VH. Pregnancy at or beyond age 40 years is associated with an increased risk of fetal death and other adverse outcomes. *Am J Obstet Gynecol.* May 2007;196(5):e11-13.
4. Johansson S, Iliadou A, Bergvall N, Tuvemo T, Norman M, Cnattingius S. Risk of high blood pressure among young men increases with the degree of immaturity at birth. *Circulation.* Nov 29 2005;112(22):3430-3436.
5. Parkinson JR, Hyde MJ, Gale C, Santhakumaran S, Modi N. Preterm birth and the metabolic syndrome in adult life: a systematic review and meta-analysis. *Pediatrics.* Apr 2013;131(4):e1240-1263.
6. Lewandowski AJ, Augustine D, Lamata P, et al. Preterm heart in adult life: cardiovascular magnetic resonance reveals distinct differences in left ventricular mass, geometry, and function. *Circulation.* Jan 15 2013;127(2):197-206.
7. Ueda P, Cnattingius S, Stephansson O, Ingelsson E, Ludvigsson JF, Bonamy AK. Cerebrovascular and ischemic heart disease in young adults born preterm: a population-based Swedish cohort study. *European journal of epidemiology.* Apr 2014;29(4):253-260.
8. Bergsjo P, Denman DW, 3rd, Hoffman HJ, Meirik O. Duration of human singleton pregnancy. A population-based study. *Acta obstetricia et gynecologica Scandinavica.* 1990;69(3):197-207.
9. WHO: recommended definitions, terminology and format for statistical tables related to the perinatal period and use of a new certificate for cause of perinatal deaths. Modifications recommended by FIGO as amended October 14, 1976. *Acta obstetricia et gynecologica Scandinavica.* 1977;56(3):247-253.
10. Tucker J, McGuire W. Epidemiology of preterm birth. *BMJ.* Sep 18 2004;329(7467):675-678.
11. Ross MG. Circle of time: errors in the use of the pregnancy wheel. *J Matern Fetal Neonatal Med.* Dec 2003;14(6):370-372.
12. Persson PH, Weldner BM. Normal range growth curves for fetal biparietal diameter, occipito frontal diameter, mean abdominal diameters and femur length. *Acta obstetricia et gynecologica Scandinavica.* 1986;65(7):759-761.
13. Torchin H, Ancel PY, Jarreau PH, Goffinet F. [Epidemiology of preterm birth: Prevalence, recent trends, short- and long-term outcomes]. *Journal de gynecologie, obstetrique et biologie de la reproduction.* Jul 1 2015.
14. Platt MJ. Outcomes in preterm infants. *Public health.* May 2014;128(5):399-403.
15. Blencowe H, Cousens S, Chou D, et al. Born too soon: the global epidemiology of 15 million preterm births. *Reproductive health.* 2013;10 Suppl 1:S2.

16. Ananth CV, Getahun D, Peltier MR, Salihu HM, Vintzileos AM. Recurrence of spontaneous versus medically indicated preterm birth. *Am J Obstet Gynecol.* Sep 2006;195(3):643-650.

17. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet.* Jan 5 2008;371(9606):75-84.

18. Pararas MV, Skevaki CL, Kafetzis DA. Preterm birth due to maternal infection: Causative pathogens and modes of prevention. *Eur J Clin Microbiol Infect Dis.* Sep 2006;25(9):562-569.

19. Kyrklund-Blomberg NB, Cnattingius S. Preterm birth and maternal smoking: risks related to gestational age and onset of delivery. *Am J Obstet Gynecol.* Oct 1998;179(4):1051-1055.

20. Gardner MO, Goldenberg RL, Cliver SP, Tucker JM, Nelson KG, Copper RL. The origin and outcome of preterm twin pregnancies. *Obstet Gynecol.* Apr 1995;85(4):553-557.

21. Smith LK, Draper ES, Manktelow BN, Dorling JS, Field DJ. Socioeconomic inequalities in very preterm birth rates. *Arch Dis Child Fetal Neonatal Ed.* Jan 2007;92(1):F11-14.

22. Marchi J, Berg M, Dencker A, Olander EK, Begley C. Risks associated with obesity in pregnancy, for the mother and baby: a systematic review of reviews. *Obesity reviews : an official journal of the International Association for the Study of Obesity.* Aug 2015;16(8):621-638.

23. Olausson PO, Cnattingius S, Haglund B. Teenage pregnancies and risk of late fetal death and infant mortality. *British journal of obstetrics and gynaecology.* Feb 1999;106(2):116-121.

24. Costeloe K, Hennessy E, Gibson AT, Marlow N, Wilkinson AR. The EPICure study: outcomes to discharge from hospital for infants born at the threshold of viability. *Pediatrics.* Oct 2000;106(4):659-671.

25. Kinsella JP, Greenough A, Abman SH. Bronchopulmonary dysplasia. *Lancet.* Apr 29 2006;367(9520):1421-1431.

26. Colvin M, McGuire W, Fowlie PW. Neurodevelopmental outcomes after preterm birth. *BMJ.* Dec 11 2004;329(7479):1390-1393.

27. Mendis S, Puska P, Norrving B, World Health Organization., World Heart Federation., World Stroke Organization. *Global atlas on cardiovascular disease prevention and control.* Geneva: World Health Organization in collaboration with the World Heart Federation and the World Stroke Organization; 2011.

28. Mortality GBD, Causes of Death C. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet.* Jan 10 2015;385(9963):117-171.

29. WHO Commission on Social Determinants of Health., World Health Organization. *Closing the gap in a generation : health equity through action on the social determinants of health : Commission on Social Determinants of Health final report.* Geneva, Switzerland: World Health Organization, Commission on Social Determinants of Health; 2008.

30. Berkman LF, Sivaramakrishnan K. WHO Commission on the social determinants of health: a bold new venture. *European journal of public health.* Dec 2008;18(6):547.

31. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet.* Sep 9 1989;2(8663):577-580.

32. Barker DJ, Osmond C, Simmonds SJ, Wield GA. The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. *BMJ.* Feb 13 1993;306(6875):422-426.

33. Bayman E, Drake AJ, Piyasena C. Prematurity and programming of cardiovascular disease risk: a future challenge for public health? *Arch Dis Child Fetal Neonatal Ed.* Nov 2014;99(6):F510-514.

34. Crump C, Winkleby MA, Sundquist K, Sundquist J. Risk of hypertension among young adults who were born preterm: a Swedish national study of 636,000 births. *Am J Epidemiol.* Apr 1 2011;173(7):797-803.

35. Finken MJ, Inderson A, Van Montfoort N, et al. Lipid profile and carotid intima-media thickness in a prospective cohort of very preterm subjects at age 19 years: effects of early growth and current body composition. *Pediatr Res.* Apr 2006;59(4 Pt 1):604-609.

36. Skinner J, Alverson D, Hunter S. *Echocardiography for the neonatologist.* London ; New York: Churchill Livingstone; 2000.

37. Yagel S, Silverman NH, Gembruch U. *Fetal cardiology : embryology, genetics, physiology, echocardiographic evaluation, diagnosis, and perinatal management of cardiac diseases.* 2nd ed. New York, NY: Informa Healthcare; 2009.

38. Stoylen A. Basic ultrasound, echocardiography and Doppler for clinicians. 2010; http://folk.ntnu.no/stoylen.

39. Park MK. *Pediatric cardiology for practitioners.* 3rd ed. St. Louis: Mosby; 1996.

40. Miller MW, Nyborg WL, Dewey WC, Edwards MJ, Abramowicz JS, Brayman AA. Hyperthermic teratogenicity, thermal dose and diagnostic ultrasound during pregnancy: implications of new standards on tissue heating. *International journal of hyperthermia : the official journal of European Society for Hyperthermic Oncology, North American Hyperthermia Group.* Sep-Oct 2002;18(5):361-384.

41. Holland CK, Deng CX, Apfel RE, Alderman JL, Fernandez LA, Taylor KJ. Direct evidence of cavitation in vivo from diagnostic ultrasound. *Ultrasound Med Biol.* 1996;22(7):917-925.

42. Abbott JG. Rationale and derivation of MI and TI--a review. *Ultrasound Med Biol.* Mar 1999;25(3):431-441.

43. D'Hooge J, Heimdal A, Jamal F, et al. Regional strain and strain rate measurements by cardiac ultrasound: principles, implementation and limitations. *Eur J Echocardiogr.* Sep 2000;1(3):154-170.

44. Amundsen BH, Helle-Valle T, Edvardsen T, et al. Noninvasive myocardial strain measurement by speckle tracking echocardiography: validation against sonomicrometry and tagged magnetic resonance imaging. *J Am Coll Cardiol.* Feb 21 2006;47(4):789-793.

45. Abraham TP, Laskowski C, Zhan WZ, et al. Myocardial contractility by strain echocardiography: comparison with physiological measurements in an in vitro model. *Am J Physiol Heart Circ Physiol.* Dec 2003;285(6):H2599-2604.

46. Culwell NM, Bonagura JD, Schober KE. Comparison of echocardiographic indices of myocardial strain with invasive measurements of left ventricular systolic function in anesthetized healthy dogs. *Am J Vet Res.* May 2011;72(5):650-660.

47. Carasso S, Cohen O, Mutlak D, et al. Differential effects of afterload on left ventricular long- and short-axis function: insights from a clinical model of patients with aortic valve stenosis undergoing aortic valve replacement. *Am Heart J.* Oct 2009;158(4):540-545.

48. Basu S, Frank LH, Fenton KE, Sable CA, Levy RJ, Berger JT. Two-dimensional speckle tracking imaging detects impaired myocardial performance in children with septic shock, not recognized by conventional echocardiography. *Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies.* May 2012;13(3):259-264.

49. Sera F, Kato TS, Farr M, et al. Left ventricular longitudinal strain by speckle-tracking echocardiography is associated with treatment-requiring cardiac allograft rejection. *Journal of cardiac failure.* May 2014;20(5):359-364.

50. Onishi T, Saha SK, Ludwig DR, et al. Feature tracking measurement of dyssynchrony from cardiovascular magnetic resonance cine acquisitions: comparison with echocardiographic speckle tracking. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance.* 2013;15:95.

51. James AT, Corcoran JD, Jain A, et al. Assessment of myocardial performance in preterm infants less than 29 weeks gestation during the transitional period. *Early Hum Dev.* Dec 2014;90(12):829-835.

52. Czernik C, Rhode S, Metze B, Schmalisch G, Buhrer C. Persistently elevated right ventricular index of myocardial performance in preterm infants with incipient bronchopulmonary dysplasia. *PLoS One.* 2012;7(6):e38352.

53. Levy PT, Holland MR, Sekarski TJ, Hamvas A, Singh GK. Feasibility and reproducibility of systolic right ventricular strain measurement by speckle-tracking echocardiography in premature infants. *J Am Soc Echocardiogr.* Oct 2013;26(10):1201-1213.

54. Cunningham KS, Gotlieb AI. The role of shear stress in the pathogenesis of atherosclerosis. *Lab Invest.* Jan 2005;85(1):9-23.

55. Dzau VJ, Braun-Dullaeus RC, Sedding DG. Vascular proliferation and atherosclerosis: new perspectives and therapeutic strategies. *Nat Med.* Nov 2002;8(11):1249-1256.

56. Berry KL, Cameron JD, Dart AM, et al. Large-artery stiffness contributes to the greater prevalence of systolic hypertension in elderly women. *J Am Geriatr Soc.* Mar 2004;52(3):368-373.

57. Dart AM, Kingwell BA. Pulse pressure--a review of mechanisms and clinical relevance. *J Am Coll Cardiol.* Mar 15 2001;37(4):975-984.

58. Napoli C, D'Armiento FP, Mancini FP, et al. Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal

accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J Clin Invest.* Dec 1 1997;100(11):2680-2690.

59. Wagenseil JE, Ciliberto CH, Knutsen RH, Levy MA, Kovacs A, Mecham RP. Reduced vessel elasticity alters cardiovascular structure and function in newborn mice. *Circ Res.* May 22 2009;104(10):1217-1224.

60. Chisalita SI, Johansson GS, Liefvendahl E, Back K, Arnqvist HJ. Human aortic smooth muscle cells are insulin resistant at the receptor level but sensitive to IGF1 and IGF2. *J Mol Endocrinol.* Dec 2009;43(6):231-239.

61. Colao A. The GH-IGF-I axis and the cardiovascular system: clinical implications. *Clin Endocrinol (Oxf).* Sep 2008;69(3):347-358.

62. Bendeck MP, Langille BL. Rapid accumulation of elastin and collagen in the aortas of sheep in the immediate perinatal period. *Circ Res.* Oct 1991;69(4):1165-1169.

63. Burkhardt T, Matter CM, Lohmann C, et al. Decreased umbilical artery compliance and igf-I plasma levels in infants with intrauterine growth restriction - implications for fetal programming of hypertension. *Placenta.* Feb 2009;30(2):136-141.

64. Sesso R, Franco MC. Abnormalities in metalloproteinase pathways and IGF-I axis: a link between birth weight, hypertension, and vascular damage in childhood. *Am J Hypertens.* Jan 2010;23(1):6-11.

65. Brodszki J, Lanne T, Marsal K, Ley D. Impaired vascular growth in late adolescence after intrauterine growth restriction. *Circulation.* May 24 2005;111(20):2623-2628.

66. Ley D, Stale H, Marsal K. Aortic vessel wall characteristics and blood pressure in children with intrauterine growth retardation and abnormal foetal aortic blood flow. *Acta Paediatr.* Mar 1997;86(3):299-305.

67. Hellstrom A, Engstrom E, Hard AL, et al. Postnatal serum insulin-like growth factor I deficiency is associated with retinopathy of prematurity and other complications of premature birth. *Pediatrics.* Nov 2003;112(5):1016-1020.

68. Chen J, Smith LE. Retinopathy of prematurity. *Angiogenesis.* 2007;10(2):133-140.

69. Milei J, Ottaviani G, Lavezzi AM, Grana DR, Stella I, Matturri L. Perinatal and infant early atherosclerotic coronary lesions. *The Canadian journal of cardiology.* Feb 2008;24(2):137-141.

70. Stary HC. Evolution and progression of atherosclerotic lesions in coronary arteries of children and young adults. *Arteriosclerosis.* Jan-Feb 1989;9(1 Suppl):I19-32.

71. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. *Circulation.* Mar 13 2007;115(10):1285-1295.

72. Singhal A, Kattenhorn M, Cole TJ, Deanfield J, Lucas A. Preterm birth, vascular function, and risk factors for atherosclerosis. *Lancet.* Oct 6 2001;358(9288):1159-1160.

73. Mori A, Uchida N, Inomo A, Izumi S. Stiffness of systemic arteries in appropriate- and small-for-gestational-age newborn infants. *Pediatrics.* Sep 2006;118(3):1035-1041.

74. Akira M, Yoshiyuki S. Placental circulation, fetal growth, and stiffness of the abdominal aorta in newborn infants. *J Pediatr.* Jan 2006;148(1):49-53.

75. Bonamy AK, Martin H, Jorneskog G, Norman M. Lower skin capillary density, normal endothelial function and higher blood pressure in children born preterm. *J Intern Med.* Dec 2007;262(6):635-642.

76. Jiang B, Godfrey KM, Martyn CN, Gale CR. Birth weight and cardiac structure in children. *Pediatrics.* Feb 2006;117(2):e257-261.

77. Painter RC, de Rooij SR, Bossuyt PM, et al. Maternal nutrition during gestation and carotid arterial compliance in the adult offspring: the Dutch famine birth cohort. *J Hypertens.* Mar 2007;25(3):533-540.

78. te Velde SJ, Ferreira I, Twisk JW, et al. Birthweight and arterial stiffness and blood pressure in adulthood--results from the Amsterdam Growth and Health Longitudinal Study. *Int J Epidemiol.* Feb 2004;33(1):154-161.

79. Edstedt Bonamy AK, Bengtsson J, Nagy Z, De Keyzer H, Norman M. Preterm birth and maternal smoking in pregnancy are strong risk factors for aortic narrowing in adolescence. *Acta Paediatr.* Aug 2008;97(8):1080-1085.

80. Bonamy AK, Andolf E, Martin H, Norman M. Preterm birth and carotid diameter and stiffness in childhood. *Acta Paediatr.* Apr 2008;97(4):434-437.

81. McCloskey K, Vuillermin P, Ponsonby AL, Cheung M, Skilton MR, Burgner D. Aortic intima-media thickness measured by trans-abdominal ultrasound as an early life marker of subclinical atherosclerosis. *Acta Paediatr.* Feb 2014;103(2):124-130.

82. Skilton MR, Evans N, Griffiths KA, Harmer JA, Celermajer DS. Aortic wall thickness in newborns with intrauterine growth restriction. *Lancet.* Apr 23-29 2005;365(9469):1484-1486.

83. Gunes T, Koklu E, Yikilmaz A, et al. Influence of maternal smoking on neonatal aortic intima-media thickness, serum IGF-I and IGFBP-3 levels. *Eur J Pediatr.* Oct 2007;166(10):1039-1044.

84. Koklu E, Kurtoglu S, Akcakus M, et al. Increased aortic intima-media thickness is related to lipid profile in newborns with intrauterine growth restriction. *Horm Res.* 2006;65(6):269-275.

85. Gale CR, Jiang B, Robinson SM, Godfrey KM, Law CM, Martyn CN. Maternal diet during pregnancy and carotid intima-media thickness in children. *Arterioscler Thromb Vasc Biol.* Aug 2006;26(8):1877-1882.

86. Lamont D, Parker L, White M, et al. Risk of cardiovascular disease measured by carotid intima-media thickness at age 49-51: lifecourse study. *BMJ.* Jan 29 2000;320(7230):273-278.

87. Pesonen E, Johnsson J, Berg A. Intimal thickness of the coronary arteries in low-birthweight infants. *Acta Paediatr.* Oct 2006;95(10):1234-1238.

88. Oren A, Vos LE, Uiterwaal CS, Gorissen WH, Grobbee DE, Bots ML. Birth weight and carotid intima-media thickness: new perspectives from the atherosclerosis risk in young adults (ARYA) study. *Ann Epidemiol.* Jan 2004;14(1):8-16.

89. Painter RC, de Rooij SR, Hutten BA, et al. Reduced intima media thickness in adults after prenatal exposure to the Dutch famine. *Atherosclerosis.* Aug 2007;193(2):421-427.

90. Tilling K, Smith GD, Chambless L, et al. The relation between birth weight and intima-media thickness in middle-aged adults. *Epidemiology.* Sep 2004;15(5):557-564.

91. Gale CR, Ashurst HE, Hall NF, MacCallum PK, Martyn CN. Size at birth and carotid atherosclerosis in later life. *Atherosclerosis.* Jul 2002;163(1):141-147.

92. Hovi P, Turanlahti M, Strang-Karlsson S, et al. Intima-media thickness and flow-mediated dilatation in the Helsinki study of very low birth weight adults. *Pediatrics.* Feb 2011;127(2):e304-311.

93. Sutton MG, Plappert T, Doubilet P. Relationship between placental blood flow and combined ventricular output with gestational age in normal human fetus. *Cardiovasc Res.* Jul 1991;25(7):603-608.

94. Rudolph AM, Heymann MA. The circulation of the fetus in utero. Methods for studying distribution of blood flow, cardiac output and organ blood flow. *Circ Res.* Aug 1967;21(2):163-184.

95. Blackburn S. Placental, fetal, and transitional circulation revisited. *The Journal of perinatal & neonatal nursing.* Oct-Dec 2006;20(4):290-294.

96. Rudolph AM. Congenital cardiovascular malformations and the fetal circulation. *Arch Dis Child Fetal Neonatal Ed.* Mar 2010;95(2):F132-136.

97. Rudolph AM. Myocardial growth before and after birth: clinical implications. *Acta Paediatr.* Feb 2000;89(2):129-133.

98. Younoszai AK, Saudek DE, Emery SP, Thomas JD. Evaluation of myocardial mechanics in the fetus by velocity vector imaging. *J Am Soc Echocardiogr.* May 2008;21(5):470-474.

99. Bensley JG, Stacy VK, De Matteo R, Harding R, Black MJ. Cardiac remodelling as a result of pre-term birth: implications for future cardiovascular disease. *Eur Heart J.* Aug 2010;31(16):2058-2066.

100. Sedmera D. Function and form in the developing cardiovascular system. *Cardiovasc Res.* Jul 15 2011;91(2):252-259.

101. Dunnigan A, Hu N, Benson DW, Jr., Clark EB. Effect of heart rate increase on dorsal aortic flow in the stage 24 chick embryo. *Pediatr Res.* Oct 1987;22(4):442-444.

102. Jouk PS, Usson Y, Michalowicz G, Grossi L. Three-dimensional cartography of the pattern of the myofibres in the second trimester fetal human heart. *Anatomy and embryology.* Aug 2000;202(2):103-118.

103. Tobita K, Garrison JB, Liu LJ, Tinney JP, Keller BB. Three-dimensional myofiber architecture of the embryonic left ventricle during normal development and altered mechanical loads. *The anatomical record. Part A, Discoveries in molecular, cellular, and evolutionary biology.* Mar 2005;283(1):193-201.

104. Godfrey ME, Messing B, Cohen SM, Valsky DV, Yagel S. Functional Assessment of the Fetal Heart. *Ultrasound Obstet Gynecol.* May 24 2011.

105. Ahmed BI. The new 3D/4D based spatio-temporal imaging correlation (STIC) in fetal echocardiography: a promising tool for the future. *J Matern Fetal Neonatal Med.* Jul 2014;27(11):1163-1168.

106. Ertl-Wagner B, Lienemann A, Strauss A, Reiser MF. Fetal magnetic resonance imaging: indications, technique, anatomical considerations and a review of fetal abnormalities. *Eur Radiol.* Aug 2002;12(8):1931-1940.

107. Fogel MA, Wilson RD, Flake A, et al. Preliminary investigations into a new method of functional assessment of the fetal heart using a novel application of 'real-time' cardiac magnetic resonance imaging. *Fetal Diagn Ther.* Sep-Oct 2005;20(5):475-480.

108. Di Salvo G, Russo MG, Paladini D, et al. Quantification of regional left and right ventricular longitudinal function in 75 normal fetuses using ultrasound-based strain rate and strain imaging. *Ultrasound Med Biol.* Sep 2005;31(9):1159-1162.

109. Pu DR, Zhou QC, Zhang M, Peng QH, Zeng S, Xu GQ. Assessment of regional right ventricular longitudinal functions in fetus using velocity vector imaging technology. *Prenat Diagn.* Nov 2010;30(11):1057-1063.

110. Matsui H, Germanakis I, Kulinskaya E, Gardiner HM. Temporal and spatial performance of vector velocity imaging in the human fetal heart. *Ultrasound Obstet Gynecol.* Feb 2011;37(2):150-157.

111. Ta-Shma A, Perles Z, Gavri S, et al. Analysis of segmental and global function of the fetal heart using novel automatic functional imaging. *J Am Soc Echocardiogr.* Feb 2008;21(2):146-150.

112. Willruth AM, Geipel AK, Fimmers R, Gembruch UG. Assessment of right ventricular global and regional longitudinal peak systolic strain, strain rate and velocity in healthy fetuses and impact of gestational age using a novel speckle/feature-tracking based algorithm. *Ultrasound Obstet Gynecol.* Feb 2011;37(2):143-149.

113. Onugoren O, Gottschalk E, Dudenhausen JW, Henrich W. Assessment of long-axis ventricular function in the fetal heart with a tissue-tracking algorithm. *J Perinat Med.* Apr 2012;40(3):297-305.

114. Crispi F, Sepulveda-Swatson E, Cruz-Lemini M, et al. Feasibility and reproducibility of a standard protocol for 2D speckle tracking and tissue Doppler-based strain and strain rate analysis of the fetal heart. *Fetal Diagn Ther.* 2012;32(1-2):96-108.

115. Ishii T, McElhinney DB, Harrild DM, et al. Circumferential and longitudinal ventricular strain in the normal human fetus. *J Am Soc Echocardiogr.* Jan 2012;25(1):105-111.

116. Germanakis I, Matsui H, Gardiner HM. Myocardial strain abnormalities in fetal congenital heart disease assessed by speckle tracking echocardiography. *Fetal Diagn Ther.* 2012;32(1-2):123-130.

117. Barker PC, Houle H, Li JS, Miller S, Herlong JR, Camitta MG. Global longitudinal cardiac strain and strain rate for assessment of fetal cardiac function: novel experience with velocity vector imaging. *Echocardiography.* Jan 2009;26(1):28-36.

118. Zhou Q, Ren Y, Yan Y, Chu C, Gui Y, Li X. Fetal tissue Doppler imaging in pregnancies complicated with preeclampsia with or without intrauterine growth restriction. *Prenat Diagn.* Nov 2012;32(11):1021-1028.

119. Di Naro E, Cromi A, Ghezzi F, Giocolano A, Caringella A, Loverro G. Myocardial dysfunction in fetuses exposed to intraamniotic infection: new insights from tissue Doppler and strain imaging. *Am J Obstet Gynecol.* Nov 2010;203(5):459 e451-457.

120. Van Mieghem T, Giusca S, DeKoninck P, et al. Prospective assessment of fetal cardiac function with speckle tracking in healthy fetuses and recipient fetuses of twin-to-twin transfusion syndrome. *J Am Soc Echocardiogr.* Mar 2010;23(3):301-308.

121. Enzensberger C, Degenhardt J, Tenzer A, Doelle A, Axt-Fliedner R. First experience with three-dimensional speckle tracking (3D wall motion tracking) in fetal echocardiography. *Ultraschall Med.* Dec 2014;35(6):566-572.

122. Azhibekov T, Noori S, Soleymani S, Seri I. Transitional cardiovascular physiology and comprehensive hemodynamic monitoring in the neonate: relevance to research and clinical care. *Seminars in fetal & neonatal medicine.* Feb 2014;19(1):45-53.

123. Batton B, Li L, Newman NS, et al. Use of antihypotensive therapies in extremely preterm infants. *Pediatrics.* Jun 2013;131(6):e1865-1873.

124. Dempsey EM, Barrington KJ. Treating hypotension in the preterm infant: when and with what: a critical and systematic review. *Journal of perinatology : official journal of the California Perinatal Association.* Aug 2007;27(8):469-478.

125. Mertens L, Seri I, Marek J, et al. Targeted Neonatal Echocardiography in the Neonatal Intensive Care Unit: practice guidelines and recommendations for training. Writing Group of the American Society of Echocardiography (ASE) in collaboration with the European Association of Echocardiography (EAE) and the Association for European Pediatric Cardiologists (AEPC). *J Am Soc Echocardiogr.* Oct 2011;24(10):1057-1078.

126. Singh Y, Gupta S, Groves AM, et al. Expert consensus statement 'Neonatologist-performed Echocardiography (NoPE)'-training and accreditation in UK. *Eur J Pediatr.* Sep 12 2015.

127. Lipshultz SE, Easley KA, Orav EJ, et al. Reliability of multicenter pediatric echocardiographic measurements of left ventricular structure and function: the prospective P(2)C(2) HIV study. *Circulation.* Jul 17 2001;104(3):310-316.

128. Pees C, Glagau E, Hauser J, Michel-Behnke I. Reference values of aortic flow velocity integral in 1193 healthy infants, children, and adolescents to quickly estimate cardiac stroke volume. *Pediatr Cardiol.* Jun 2013;34(5):1194-1200.

129. Fouzas S, Karatza AA, Davlouros PA, et al. Neonatal cardiac dysfunction in intrauterine growth restriction. *Pediatr Res.* May 2014;75(5):651-657.

130. Schmitz L, Stiller B, Pees C, Koch H, Xanthopoulos A, Lange P. Doppler-derived parameters of diastolic left ventricular function in preterm infants with a birth weight <1500 g: reference values and differences to term infants. *Early Hum Dev.* Feb 2004;76(2):101-114.

131. Koestenberger M, Nagel B, Ravekes W, et al. Systolic right ventricular function in preterm and term neonates: reference values of the tricuspid annular plane systolic excursion (TAPSE) in 258 patients and calculation of Z-score values. *Neonatology.* 2011;100(1):85-92.

132. Lee A, Nestaas E, Liestol K, Brunvand L, Lindemann R, Fugelseth D. Tissue Doppler imaging in very preterm infants during the first 24 h of life: an observational study. *Arch Dis Child Fetal Neonatal Ed.* Jan 2014;99(1):F64-69.

133. Murase M, Morisawa T, Ishida A. Serial assessment of left-ventricular function using tissue Doppler imaging in premature infants within 7 days of life. *Pediatr Cardiol.* Aug 2013;34(6):1491-1498.

134. Poon CY, Edwards JM, Joshi S, Kotecha S, Fraser AG. Optimization of myocardial deformation imaging in term and preterm infants. *Eur J Echocardiogr.* Mar 2011;12(3):247-254.

135. Joshi S, Edwards JM, Wilson DG, Wong JK, Kotecha S, Fraser AG. Reproducibility of myocardial velocity and deformation imaging in term and preterm infants. *Eur J Echocardiogr.* Jan 2010;11(1):44-50.

136. Klitsie LM, Roest AA, Haak MC, Blom NA, Ten Harkel AD. Longitudinal follow-up of ventricular performance in healthy neonates. *Early Hum Dev.* Dec 2013;89(12):993-997.

137. Marcus KA, Mavinkurve-Groothuis AM, Barends M, et al. Reference values for myocardial two-dimensional strain echocardiography in a healthy pediatric and young adult cohort. *J Am Soc Echocardiogr.* Jun 2011;24(6):625-636.

138. Lorch SM, Ludomirsky A, Singh GK. Maturational and growth-related changes in left ventricular longitudinal strain and strain rate measured by two-dimensional speckle tracking echocardiography in healthy pediatric population. *J Am Soc Echocardiogr.* Nov 2008;21(11):1207-1215.

139. de Waal K, Lakkundi A, Othman F. Speckle tracking echocardiography in very preterm infants: feasibility and reference values. *Early Hum Dev.* Jun 2014;90(6):275-279.

140. Hirose A, Khoo NS, Aziz K, et al. Evolution of left ventricular function in the preterm infant. *J Am Soc Echocardiogr.* Mar 2015;28(3):302-308.

141. El-Khuffash AF, Jain A, Dragulescu A, McNamara PJ, Mertens L. Acute changes in myocardial systolic function in preterm infants undergoing patent ductus arteriosus ligation: a tissue Doppler and myocardial deformation study. *J Am Soc Echocardiogr.* Oct 2012;25(10):1058-1067.

142. James A, Corcoran JD, Mertens L, Franklin O, El-Khuffash A. Left Ventricular Rotational Mechanics in Preterm Infants Less Than 29 Weeks' Gestation over the First Week after Birth. *J Am Soc Echocardiogr.* Mar 26 2015.

143. Zhang L, Gao J, Xie M, et al. Left ventricular three-dimensional global systolic strain by real-time three-dimensional speckle-tracking in children: feasibility, reproducibility, maturational changes, and normal ranges. *J Am Soc Echocardiogr.* Aug 2013;26(8):853-859.

144. Herberg U, Klebach C, Faller J, Trier HG, Breuer J. Spatiotemporal accuracy of real-time 3D echocardiography in the neonatal and pediatric setting--validation studies using small dynamic test objects. *Ultraschall Med.* Dec 2013;34(6):580-589.

145. Lewandowski AJ, Bradlow WM, Augustine D, et al. Right ventricular systolic dysfunction in young adults born preterm. *Circulation.* Aug 13 2013;128(7):713-720.

146. Marsal K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. *Acta Paediatr.* Jul 1996;85(7):843-848.

147. Gudmundsson S, Marsal K. Umbilical artery and uteroplacental blood flow velocity waveforms in normal pregnancy--a cross-sectional study. *Acta obstetricia et gynecologica Scandinavica.* 1988;67(4):347-354.

148. Stein JH, Korcarz CE, Hurst RT, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. *J Am Soc Echocardiogr.* Feb 2008;21(2):93-111; quiz 189-190.

149. Schmidt C, Wendelhag I. How can the variability in ultrasound measurement of intima-media thickness be reduced? Studies of interobserver variability in carotid and femoral arteries. *Clin Physiol.* Jan 1999;19(1):45-55.

150. Ommen SR, Nishimura RA, Appleton CP, et al. Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures: A comparative simultaneous Doppler-catheterization study. *Circulation.* Oct 10 2000;102(15):1788-1794.

151. Urbich C, Stein M, Reisinger K, Kaufmann R, Dimmeler S, Gille J. Fluid shear stress-induced transcriptional activation of the vascular endothelial growth factor receptor-2 gene requires Sp1-dependent DNA binding. *FEBS Lett.* Jan 30 2003;535(1-3):87-93.

152. Clark RH, Thomas P, Peabody J. Extrauterine growth restriction remains a serious problem in prematurely born neonates. *Pediatrics.* May 2003;111(5 Pt 1):986-990.

153. Westin V, Stoltz Sjostrom E, Ahlsson F, Domellof M, Norman M. Perioperative nutrition in extremely preterm infants undergoing surgical treatment for patent ductus arteriosus is suboptimal. *Acta Paediatr.* Mar 2014;103(3):282-288.

154. Wilson DC, Cairns P, Halliday HL, Reid M, McClure G, Dodge JA. Randomised controlled trial of an aggressive nutritional regimen in sick very low birthweight infants. *Arch Dis Child Fetal Neonatal Ed.* Jul 1997;77(1):F4-11.

155. Carlson SJ, Ziegler EE. Nutrient intakes and growth of very low birth weight infants. *Journal of perinatology : official journal of the California Perinatal Association.* Jul-Aug 1998;18(4):252-258.

156. Embleton NE, Pang N, Cooke RJ. Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? *Pediatrics.* Feb 2001;107(2):270-273.

157. Eriksson JG. Early growth and coronary heart disease and type 2 diabetes: findings from the Helsinki Birth Cohort Study (HBCS). *Am J Clin Nutr.* Dec 2011;94(6 Suppl):1799S-1802S.

158. Shimizu T, Fujii T, Iwasaki J, et al. Abdominal aortic intima-media thickness in preschool children born preterm. *Pediatr Cardiol.* Jan 2014;35(1):121-125.

159. Norman M. Low birth weight and the developing vascular tree: a systematic review. *Acta Paediatr.* Sep 2008;97(9):1165-1172.

160. Stary HC, Blankenhorn DH, Chandler AB, et al. A definition of the intima of human arteries and of its atherosclerosis-prone regions. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Arteriosclerosis and thrombosis : a journal of vascular biology / American Heart Association.* Jan 1992;12(1):120-134.

161. Angelini A, Thiene G, Frescura C, Baroldi G. Coronary arterial wall and atherosclerosis in youth (1-20 years): a histologic study in a northern Italian population. *Int J Cardiol.* Sep 1990;28(3):361-370.

162. Koklu E, Ozturk MA, Kurtoglu S, Akcakus M, Yikilmaz A, Gunes T. Aortic intima-media thickness, serum IGF-I, IGFBP-3, and leptin levels in intrauterine growth-restricted newborns of healthy mothers. *Pediatr Res.* Dec 2007;62(6):704-709.

163. Johnson P, Maxwell DJ, Tynan MJ, Allan LD. Intracardiac pressures in the human fetus. *Heart.* Jul 2000;84(1):59-63.

164. Burns AT, La Gerche A, D'Hooge J, MacIsaac AI, Prior DL. Left ventricular strain and strain rate: characterization of the effect of load in human subjects. *Eur J Echocardiogr.* Apr 2010;11(3):283-289.

165. Govind SC, Brodin LA, Nowak J, Ramesh SS, Saha SK. Acute administration of a single dose of valsartan improves left ventricular functions: a pilot study to assess the role of tissue velocity echocardiography in patients with systemic arterial hypertension in the TVE-valsartan study I. *Clin Physiol Funct Imaging.* Nov 2006;26(6):351-356.

166. Eyskens B, Weidemann F, Kowalski M, et al. Regional right and left ventricular function after the Senning operation: an ultrasonic study of strain rate and strain. *Cardiol Young.* Jun 2004;14(3):255-264.

167. Eyskens B, Ganame J, Claus P, Boshoff D, Gewillig M, Mertens L. Ultrasonic strain rate and strain imaging of the right ventricle in children before and after percutaneous closure of an atrial septal defect. *J Am Soc Echocardiogr.* Aug 2006;19(8):994-1000.

168. Caiani EG, Weinert L, Takeuchi M, et al. Evaluation of alterations on mitral annulus velocities, strain, and strain rates due to abrupt changes in preload elicited by parabolic flight. *J Appl Physiol.* Jul 2007;103(1):80-87.

169. Jain A, Mohamed A, El-Khuffash A, et al. A comprehensive echocardiographic protocol for assessing neonatal right ventricular dimensions and function in the transitional period: normative data and z scores. *J Am Soc Echocardiogr.* Dec 2014;27(12):1293-1304.

170. Di Salvo G, Russo MG, Paladini D, et al. Two-dimensional strain to assess regional left and right ventricular longitudinal function in 100 normal foetuses. *Eur J Echocardiogr.* Nov 2008;9(6):754-756.

171. Voigt JU, Pedrizzetti G, Lysyansky P, et al. Definitions for a common standard for 2D speckle tracking echocardiography: consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging. *J Am Soc Echocardiogr.* Feb 2015;28(2):183-193.

172. Crump C, Sundquist K, Sundquist J, Winkleby MA. Gestational age at birth and mortality in young adulthood. *JAMA.* Sep 21 2011;306(11):1233-1240.

173. Nichols WW, Edwards DG. Arterial elastance and wave reflection augmentation of systolic blood pressure: deleterious effects and implications for therapy. *Journal of cardiovascular pharmacology and therapeutics.* Jan 2001;6(1):5-21.

174. Mattace-Raso FU, van der Cammen TJ, Hofman A, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. *Circulation.* Feb 7 2006;113(5):657-663.

175. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet.* May 10 1986;1(8489):1077-1081.