DEPARTMENT OF WOMAN AND CHILD HEALTH
Karolinska Institutet, Stockholm, Sweden

LONG-TERM CARDIOVASCULAR FOLLOW-UP AFTER PRETERM BIRTH

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To Max and Selma

"Toutes les grandes personnes ont d'abord été des enfants,
mais peu d'entre elles s'en souviennent."
Antoine de Saint-Exupéry (Le Petit Prince)

ABSTRACT

Cardiovascular disease is a leading cause of morbidity and mortality worldwide. A large number of studies show that the risk of cardiovascular disease is increased in people born with low birth weight. The aim of this thesis is to study the contribution of preterm birth, the most common cause of low birth weight, to later cardiovascular function and disease risk.

Clinical follow-up studies of children and adolescents born very preterm (total n=118) in the 1980's and 1990's were performed. Vascular endothelial function was assessed using Laser-Doppler measurements of skin perfusion responses to acetylcholine, an endothelium-dependent vasodilator (paper I-II). Dermal capillary density was studied using intra-vital video microscopy (paper II). Arterial stiffness was measured using pulse wave analysis, pulse wave velocity and ultrasound techniques (paper I and III). Arterial dimensions were studied using ultrasound and magnetic resonance imaging (paper I, III and IV).

Paper I shows that adolescent girls, born at a mean gestational age of 29 w, had narrower abdominal aorta and lower skin perfusion, as compared to controls born at term. No signs of arterial stiffening were found and the endothelial function was unaffected after preterm birth. Paper II demonstrates that in 9-year old children born very preterm, the skin capillary density was reduced, but not the endothelial function, as compared to controls. Paper III shows that the 9-year old children born very preterm had the same carotid dimensions and stiffness as controls. Paper IV reports results from magnetic resonance imaging of the aorta in 86 healthy adolescents, of whom half were born very preterm. This study confirms the findings from paper I, showing lasting aortic narrowing after preterm birth. In addition, the aortic size was also strongly and independently associated with maternal smoking in pregnancy. Papers I, II and IV also show that children and adolescents born preterm have increased blood pressure. In paper II-III, the heart rate was higher in preterm children, but the heart rate was not related to their blood pressure.

Paper V investigates the association between preterm birth and fetal growth restriction and later risk of hypertension in a cohort of 6,425 men and women born 1925-1949 in Sweden, of whom 2,931 were born preterm. At follow-up in 1987 through 2006, the risk of hypertension was increased by 53% in those born small for gestational age. Preterm birth was not associated with risk of subsequent hypertension.

In conclusion, young subjects born very preterm exhibit altered vascular development, as illustrated by a lower capillary density and aortic narrowing. They also have higher blood pressure and heart rate. No signs of premature arterial stiffening or endothelial dysfunction– early markers of atheromatous disease – were found. The significance of these findings for future cardiovascular disease risk is not yet known.

LIST OF PUBLICATIONS

This thesis is based on the following papers. The papers will be referred to by their Roman numerals (I-V).

I. Anna-Karin Edstedt Bonamy, Ana Bendito, Helena Martin, Ellika Andolf, Gunnar Sedin, Mikael Norman.
Preterm birth contributes to increased vascular resistance and higher blood pressure in adolescent girls.
Pediatric Research 2005; 58:845-9.

II. Anna-Karin Edstedt Bonamy, Helena Martin, Gun Jörneskog, Mikael Norman.
Lower skin capillary density, normal endothelial function and higher blood pressure in children born preterm.
Journal of Internal Medicine 2007; 262:635-42.

III. Anna-Karin Edstedt Bonamy, Ellika Andolf, Helena Martin, Mikael Norman.
Preterm birth and carotid stiffness and diameter in childhood.
Acta Paediatrica 2008; 97:434-437.

IV. Anna-Karin Edstedt Bonamy, Johan Bengtsson, Zoltan Nagy, Hans De Keyzer, Mikael Norman.
Preterm birth and maternal smoking in pregnancy are strong risk factors for aortic narrowing in adolescence.
Submitted to Acta Paediatrica.

V. Anna-Karin Edstedt Bonamy, Mikael Norman, Magnus Kaijser.
Being born too small, too early or both- does it matter for risk of hypertension in the elderly?
Submitted.

CONTENTS

LIST OF ABBREVIATIONS

ACh	acetylcholine
AGA	appropriate for gestational age
AI	augmentation index
BP	blood pressure
BPD	bronchopulmonary dysplasia
bpm	beats per minute
BW	birth weight
CI	confidence interval
CPAP	continuous positive airway pressure
CV	coefficient of variation
CVD	cardiovascular disease
DBP	diastolic blood pressure
ELBW	extremely low birth weight
FMD	flow-mediated dilatation
GA	gestational age
HR	hazard ratio
IMT	intima-media thickness
IUGR	intrauterine growth retardation
IVH	intraventricular hemorrhage
LBW	low birth weight
LD	Laser Doppler
MAP	mean arterial pressure
MRI	magnetic resonance imaging
NEC	necrotizing enterocolitis
PDA	patent ductus arteriosus
PP	pulse pressure
PPROM	preterm premature rupture of membranes
PU	perfusion units, arbitrary
PVL	periventricular leucomalacia
PWMI	periventricular white-matter injury
PWV	pulse wave velocity
RDS	respiratory distress syndrome
ROP	retinopathy of prematurity
SBP	systolic blood pressure
SDS	standard deviation score
SEM	standard error of the mean
SGA	small for gestational age
SI	stiffness index
UAC	umbilical artery catheter
VLBW	very low birth weight

1 INTRODUCTION

Hypertension affects 28 – 45% of middle-aged men and women[1] and it is an important risk factor for coronary heart disease and stroke, the leading causes of death worldwide[2]. Given that even small increments in blood pressure at the population level will have large impact on cardiovascular disease risk[3], social, environmental and biological factors that contribute to set blood pressure on a higher level are of great significance for public health.

Preterm birth is one of the most common pregnancy complications worldwide. Due to recent advances in ante- and neonatal care the number of infants surviving preterm birth is steadily increasing. Only in Sweden about 100,000 persons below 20 years of age have been born preterm[4]. A continuously growing number of adults are thus born preterm, but the long-term effects of preterm birth are still largely unknown.

In the 1980's, the first reports associating low birth weight to later hypertension and cardiovascular disease appeared. This link has since then been extensively studied in a large number of epidemiological and clinical studies. However, the main focus of these studies has been on low birth weight in term, or near term, births. Preterm birth is the major cause of low birth weight in industrialized countries today, where 5 to 12% of infants are born before 37 completed weeks of gestation. Poor fetal growth, resulting in low birth weight at term, and preterm birth do not share the same underlying mechanisms and are not comparable biological exposures for the developing organism. Results from studies in low birth weight children born late in pregnancy can thus not easily be generalized to subjects born preterm without further studies.

Long-term follow-up studies of cardiovascular health in adult survivors of preterm birth are lacking. New techniques to measure vascular function have been shown to enable prediction of cardiovascular disease risk already at young age. In the long run, forecasts of affected vascular function may allow preventive interventions already in pediatric populations exhibiting cardiovascular risk markers.

The main aim of this thesis is to study blood pressure, vascular function and structure in children and adolescents born very preterm in order to investigate whether vascular changes predictive of cardiovascular disease risk are present already in childhood and adolescence. In addition to vascular measurements in young people born preterm, the long-term risk of hypertension in those who actually survived preterm birth in the first half of the 20th century is studied.

2 BACKGROUND

2.1 EPIDEMIOLOGY OF PRETERM BIRTH

2.1.1 Definitions

Pregnancy length

A normal pregnancy is given an estimated date of delivery 40 weeks, or 280 days, from the first day of the last menstrual period. The currently accepted definitions of pregnancy lengths are found in figure 1[5]. In Sweden 5 to 6 % of all pregnancies end before 37 completed weeks of gestation and around 1% of babies are born very preterm, i.e. before 32 completed weeks of gestation[4]. Corresponding data for the United States show that in 2005 almost 13% of babies were born preterm[6].

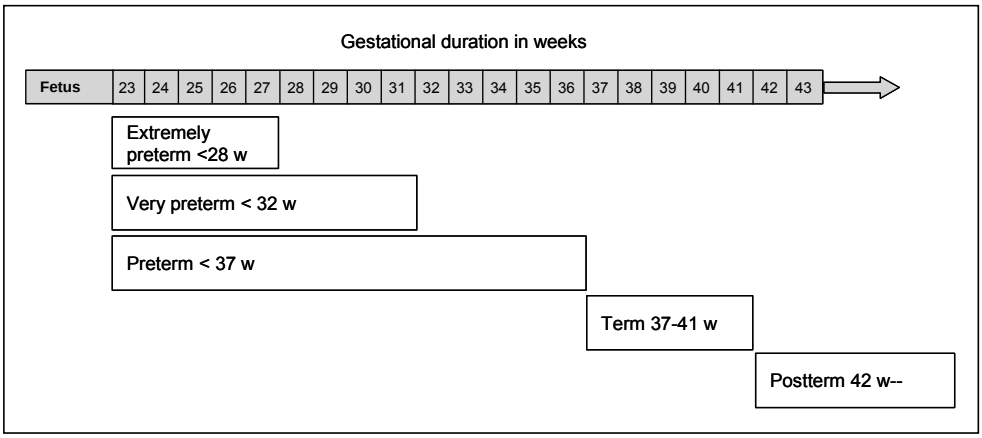


Figure 1. Categorization of pregnancy lengths (adapted from Tucker et al.[5])

Measures of size at birth

Most, but not all, children born preterm have low birth weight. Correspondingly, an infant born at term can also have low birth weight (Figure 2). WHO has defined low birth weight as a birth weight below 2,500g[7]. The current birth weight classification is found in Table 1.

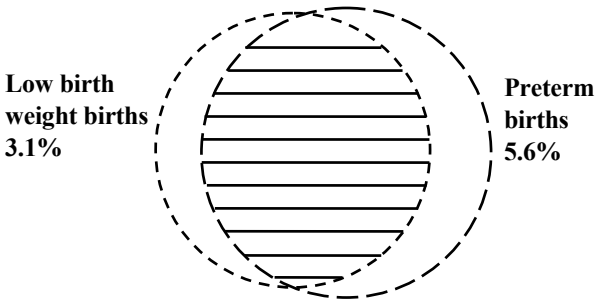


Figure 2. Relation between preterm birth and low birth weight in Sweden in 2005. Data from The National Board of Health and Welfare, www.socialstyrelsen.se.

Extremely low birth weight (ELBW)	< 1000 grams
Very low birth weight (VLBW)	< 1500 grams
Low birth weight (LBW)	< 2500 grams

Table 1. Classification of birth weights

Regardless if a pregnancy ends at term or preterm, the birth weight for a certain gestational age can be too low. The term used for this condition is *small for gestational age* (SGA) and refers to infants whose birth weight and/or length is at least 2 standard deviations (SD) below the mean for the gestational age. SGA has also been defined in some publications as birth weight or length below the 10th, 5th, or 3rd percentile[8]. In this thesis, the term SGA will be used to define children born with a birth weight less than 2 SD below the mean birth weight for the gestational age, according to fetal growth curves based on ultrasonically estimated fetal weights at different gestational ages[9]. SGA refers only to the size of the child at birth and is not synonymous with *intrauterine growth retardation* (IUGR), which is a pathologic condition defined as slowed growth velocity between two time-points in pregnancy, usually assessed using ultrasound. Being SGA does not always imply that an infant has suffered IUGR, since smallness at birth may be caused by other factors, e.g genetic[8]. Children with normal birth weights have birth weights between -2 and +2 SD and are referred to as *appropriate for gestational age* (AGA). *Large for gestational age* (LGA) infants have birth weights >2SD above the mean birth weight for the gestational age.

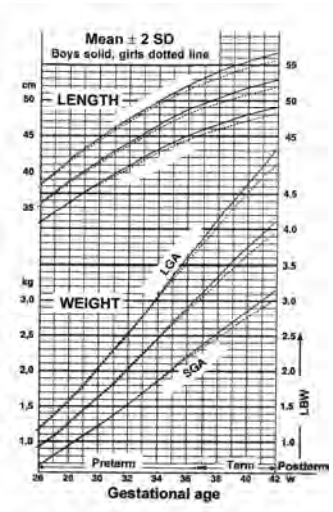


Figure 3. Birth length and birth weight curves according to ultrasonically estimated fetal weights at different gestational ages[9].

2.1.2 Causes of preterm birth

A preterm delivery can be either medically indicated, about 1/3, or spontaneous, about 2/3 of all preterm deliveries. The medically indicated preterm deliveries are undertaken because of fetal or maternal illness. One common cause is maternal pre-eclampsia (see 2.6.3), leading to fetal distress, poor fetal growth or severe maternal illness. Other causes of medically indicated preterm deliveries include IUGR, antepartum hemorrhage, fetal anemia or infection. Spontaneous preterm deliveries start with either preterm premature rupture of membranes (PPROM) or preterm labor. The underlying mechanisms include infection or inflammation, activation of the hypothalamic-pituitary-adrenal (HPA) axis because of maternal or fetal stress, antepartum hemorrhage, and uterine or cervical abnormalities[10].

Babies born preterm are thus not a homogenous group of infants. They have all been exposed to different biological conditions ultimately leading to preterm birth and to

different medical interventions. In addition, the gestational age (GA) at birth is often the most important factor for their short- and long-term outcome. The risks associated with preterm birth rapidly decrease with increasing GA[11, 12].

2.1.3 Mortality and morbidity after preterm birth

Neonatal mortality

Before the development of modern neonatal intensive care, the mortality among children born very preterm was elevated, but it has declined rapidly over the last decades. The infant mortality after very preterm birth is approximately 15% in Sweden, ranging from 5% in those with a gestational duration of 31 weeks to about 60% in those born after 23 weeks of pregnancy[13, 14].

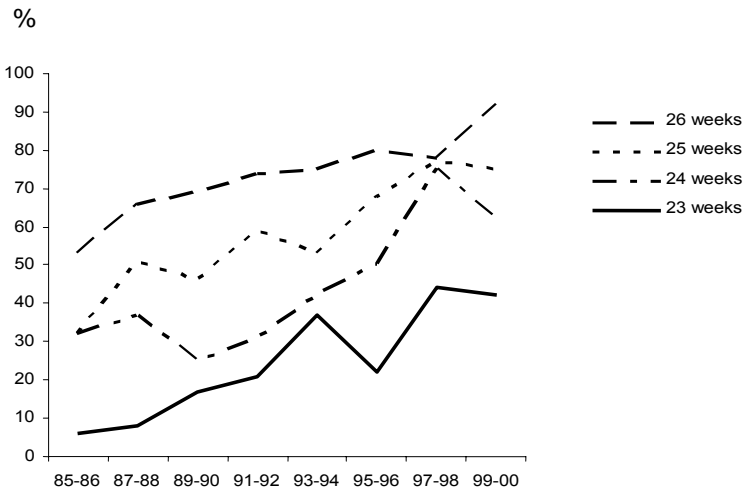


Figure 4. Survival rates after extremely preterm birth from 1985 to 2000 in Sweden. Data from The National Board of Health and Welfare, www.socialstyrelsen.se.

Neonatal morbidity

Infants born before 35 weeks of gestation are not mature enough at birth to maintain homeostasis in the extrauterine environment without support. Their basic needs include heat, nutrition and respiratory and circulatory monitoring and support.

The lungs, their alveoli and the production of the surface tension reducing protein surfactant are not fully mature at very preterm birth. The lack of surfactant makes the lung non-compliant and the infant is at risk of developing respiratory distress syndrome (RDS), a condition which requires continuous positive airway pressure (CPAP) or mechanical ventilation to ensure adequate gaseous exchange. RDS can be treated by instillation of exogenous surfactant in the airways[15]. When a very preterm birth can be anticipated, corticosteroids are administered to the mother to accelerate fetal lung maturation. This fetal therapy has improved neonatal outcome substantially[16]. The respiratory drive is also immature and preterm infants often have apneas, treated with

methyl xanthines, e.g. theophylline or caffeine. Caffeine has recently been shown to improve both pulmonary and neurological outcome[17, 18].

The postnatal transition from fetal circulation is often delayed in the preterm infant. The ductus arteriosus, right- to- left shunting blood from the pulmonary artery to the aorta in fetal life, frequently remains open. With the drop in pulmonary vascular resistance that occurs after birth, the patent duct will eventually left-to-right shunt systemic oxygenated blood from the aorta back to the pulmonary circulation[19]. This puts the infant at risk of inadequate systemic circulatory output[20] and is also associated with adverse neonatal outcome[21]. Treatment by indomethacin or ibuprofen, two prostaglandin antagonists, promotes closure in the majority of cases. If that fails, the patent duct will be closed by surgery[19].

Infants born very preterm are at risk of cerebral lesions in the early neonatal period. Periventricular white-matter injury (PWMI) is common and includes both focal periventricular lesions (PVL- periventricular leucomalacia) and diffuse myelination disturbances. Immature vascularization, ischemia, inflammation and a maturation-dependent vulnerability in the white matter are involved in the pathogenesis[22]. Infants with PWMI are at high risk of developing cerebral palsy. Another cerebro-vascular lesion in the early neonatal period is intraventricular hemorrhage (IVH), which is a bleeding from the germinal matrix into the brain ventricules. It is usually associated with good prognosis, if the surrounding brain tissue remains unaffected.

Insensible water losses through the skin and respiratory tract, and the immature renal and autonomic nervous system function in infants born preterm make them especially susceptible to hypotension and rapid changes in blood pressure. Since the cerebral auto-regulation is instable in preterm infants, these blood pressure changes may affect cerebral blood flow and put them at risk of IVH and PVL[23, 24].

Infections manifesting as septicemia, pneumonia or, more rarely, meningitis are common. They may be contracted in utero, at birth, or iatrogenically in the neonatal ward. The immune system is immature, and so is the skin, one of the most important barriers against infection. The frequent use of indwelling devices, such as venous and arterial catheters, tracheal tubes etc. further increase the risk of bacterial and fungal colonization and infection. Infections are treated by antibiotics and/or anti-fungal agents[25].

Another rare, but potentially very serious, complication of preterm birth is necrotizing enterocolitis (NEC). It is an inflammatory condition of the bowel occurring in infants at the lowest gestational ages and more often in those who are SGA at birth. The pathogenesis is not fully understood yet, but NEC is associated with decreased intestinal blood flow and invasion of bacteria into the intestinal wall. It may progress to intestinal gangrene and rupture, peritonitis and the mortality in patients with NEC is high. Mild cases are treated by antibiotics and fasting, more severe forms with intestinal surgery[26].

Long-term morbidity

Neurological

The brain develops rapidly between 20 and 32 weeks of postmenstrual age. Consequences of PWMI and IVH, infections and undernutrition in this period may result in neurological disabilities, such as cerebral palsy, visual- and hearing impairments, cognitive difficulties and behavioral problems. The incidence of neurological impairment after preterm birth has declined in recent years, except for those who survive extremely preterm birth[27]. Outcomes vary between countries, but about 25% of all infants born before 28 weeks of gestation are affected by some kind of neurological impairment. Outcomes are worse in the lower gestational age strata[28].

Respiratory

The most severe respiratory complication in preterm infants is development of chronic lung disease of prematurity (CLD), now called bronchopulmonary dysplasia (BPD). A common definition is need of supplemental oxygen after 36 weeks postmenstrual age. The major risk factors for development of BPD are short gestation, being SGA, duration of mechanical ventilation, patent ductus arteriosus (PDA), chorioamnionitis and neonatal sepsis. The lungs in BPD are non-compliant and show inflammatory changes. The condition usually resolves with time, but for some infants home treatment with diuretics, corticosteroid- and bronchodilator inhalations and sometimes home oxygen therapy is needed after discharge[15]. Long-term follow-up studies of the new generation of survivors of extremely preterm birth with BPD are lacking.

The acute lung injury and the inflammatory processes involved also affect the developing pulmonary circulation, as does regional hypoxia causing pulmonary vaso-constriction. These processes may lead to pulmonary hypertension and subsequent increase in right ventricular cardiac afterload[29].

ROP

Retinopathy of prematurity (ROP) is a vascular disease that affects the immature retinal vessels. Hyperoxia is involved in the complex pathogenesis, which has not been fully clarified. The transition from intra- to extrauterine life involves a substantial increase in oxygen tension from the intrauterine levels of about 4kPa to extrauterine levels of 8-12 kPa. This increase in oxygen tension will supply the retina via passive diffusion and retinal vessel development will temporarily regress. However, this avascular retina subsequently develops hypoxia, when the metabolic needs outgrow the passive diffusion potential. Hypoxia stimulates release of vascoactive substances, such as vascular endothelial growth factor (VEGF), which will promote a pathological retinal neovascularization, similar to that seen in diabetic retinopathy[30]. Another factor affected by preterm birth and involved in ROP development is insulin-like growth factor I (IGF-I). Low IGF-I levels after preterm birth inhibit normal vessel growth, which may enhance hypoxia[31, 32].

The most important risk factor for ROP is low gestational age at birth. ROP has also been associated with almost all known pregnancy- and neonatal illnesses. ROP rarely has its onset before 31 weeks of postmenstrual age. There are different stages of

ROP (I-V), where the most severe form involves complete retinal detachment and subsequent blindness. To prevent this, very preterm infants are screened for ROP at regular intervals. Treatment by laser-coagulation or cryotherapy is available for infants who show progressive disease[28].

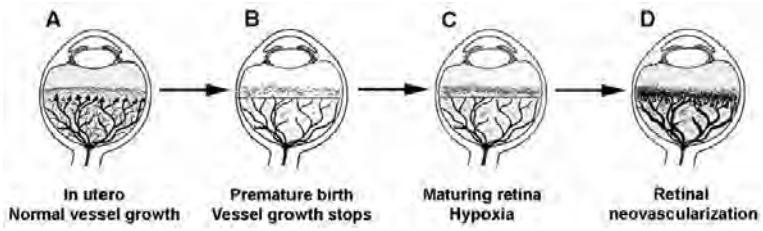


Figure 5. Development of ROP, a micro-vascular disease, following preterm birth. Picture adapted from Hellström et al[31]. © PNAS

2.2 EPIDEMIOLOGY OF CARDIOVASCULAR DISEASE

The term cardiovascular disease (CVD) covers a wide range of disorders, including diseases of the cardiac muscle and of the vascular system supplying the heart, brain, and other vital organs. The most common manifestations of CVD are ischemic heart disease, congestive heart failure, and stroke, which together account for approximately 80% of the burden of CVD. The major risk factors for CVD include tobacco use, high blood pressure, high blood glucose, lipid abnormalities, obesity, and physical inactivity. 50 percent of deaths in high-income countries and about 30 percent of deaths in low- and middle- income countries are the result of CVD. Even in areas where infections, nutritional deficiencies and HIV/AIDS are still the predominant causes of death, CVD is increasing and is predicted to be the leading cause of morbidity and mortality worldwide by 2020[33].

2.3 THE ARTERIES AND CAPILLARIES

Histology

An artery is composed of three layers; the tunica intima, the tunica media and the tunica adventitia. These three layers are separated by the internal elastic lamina between the intima and media, and the external elastic lamina separating the media and the adventitia.

The intima consists of vascular endothelium anchored to the basal lamina surrounded by a thin layer of supportive fibro-collagenous tissue. The endothelium is a single layer of specialized epithelial cells present in all blood vessels. It acts as interface between the flowing blood and the vascular wall and functions to maintain vascular homeostasis by modulating vascular tone, permeability, coagulation and fibrinolysis. The endothelium responds to acute and chronic changes in shear stress and transmural pressure by converting physical forces into a cellular response[34].

The media determines the elastic properties of an artery. In the elastic arteries (see below) the media is predominantly composed of elastic fibers; concentrically organized elastin bands and collagen fibers with a thin layer of smooth muscle cells surrounding

the elastic fibers. In muscular arteries and arterioles, the smooth muscle component dominates.

The tunica adventitia is a layer of collagen and some elastin, which merges with the connective tissue surrounding the blood vessel, and it contains nerves, fibroblasts and small blood vessels supplying the large arteries (vasa vasorum).

The arterial vascular tree

The arterial vascular tree can be divided into three functional compartments.

- **Large elastic arteries** - aorta and its large branches, e.g. the brachio-cephalic, carotid, renal and iliac arteries. These arteries store blood during systole and use their elastic properties to expel blood towards the periphery during diastole.

- **Muscular arteries** - conduits that distribute blood to the periphery. By changing the arterial muscle tone, they can modify wave propagation towards the periphery.

- **Arterioles** – small calibre vessels in the periphery. They are the major site of resistance to blood flow in the vascular tree and help converting the pulsatile blood flow into a continuous flow through the capillaries. Moreover, their high resistance protects the capillaries from the high systemic blood pressure.

Capillaries

The capillaries are the smallest blood vessels in the body. They have thin walls consisting of the endothelium and the basal lamina, surrounded by a few pericytes. The thin capillary walls allow exchange of gas, fluids and nutrients with the surrounding cells. The steady blood flow in the capillaries is modulated by pre-capillary sphincters consisting of a few smooth muscles cells located at the end of the terminal arterioles. Both local factors and the sympathetic nervous system are involved in relaxation and constriction of these sphincters, determining the number of capillaries perfused. There are also arteriovenous shunts present in the skin microcirculation, allowing blood to bypass the capillary network, e.g. to enhance heat loss when exercising.

2.4 PREDICTION OF CARDIOVASCULAR DISEASE RISK

Vascular ageing starts before birth and continues throughout life[35-37]. A number of methods have been developed to measure vascular structure and function to enable prediction of cardiovascular disease risk. Testing for presymptomatic atherosclerosis preferentially involve methods that are safe, non-invasive, reproducible and which correlate with the extent of the atherosclerotic process[38].

Blood pressure

Both systolic and diastolic blood pressures (BP) show an independent and graded relationship with cardiovascular disease and stroke risk[39, 40]. Systolic BP continuously rises throughout life, while diastolic BP peaks at around 60 years of age and then falls, making the pulse pressure (PP), i.e. the difference between systolic and diastolic BP,

increase. These processes are part of the vascular ageing[41], and explain why pulse pressure has been shown to be a good predictor of adverse cardiovascular events in older populations[42]. In children and young adults, systolic BP correlates to the presence of fatty streaks and fibrous plaques in the coronary arteries and aorta[43].

Although mean BP is similar in different large arteries, the correlation between aortic and brachial systolic BP has been shown to be poor, especially in young and non-hypertensive persons[44]. Aortic BP is a better determinant of cardiac workload and aortic PP has been shown to correlate better to atherosclerosis and cardiovascular events than brachial PP[45]. Aortic BP can be estimated non-invasively using a pulse wave analysis system, see below.

Endothelial function

Endothelial function is often referred to as the endothelium's capability to modulate the vasomotor tone. Dysfunction in the endothelium-dependent vasodilatation has been associated with all known cardiovascular risk factors, e.g. ageing, hypertension, smoking, diabetes mellitus and obesity[46].

There are a number of methods to assess vascular endothelial function[47]. The most commonly used non-invasive method is flow-mediated dilatation (FMD) of the brachial artery[48, 49]. This test uses ultrasound to measure changes in brachial artery diameter in response to increased blood flow stimulated by the release of an applied arterial occlusion. The shear stress induced by this increase in blood flow stimulates endothelial production of nitric oxide[50, 51], as shown in Figure 6, which acts as a vasodilator. FMD in the brachial artery correlates well with endothelial function in the coronary arteries[52].

Other methods for non-invasive testing of endothelial function include Laser Doppler (LD) measurements of blood flow responses to local application of endothelium-dependent vasodilators, e.g. iontophoresis of ACh (Figure 6) (described in detail in section 4.2.2). LD- measurements in the peripheral circulation is the only method that can be used in infants and small children and it correlates with FMD in the brachial artery[53].

Endothelial dysfunction usually precedes the development of atherosclerotic lesions and thrombotic events. In both children and adults, endothelial dysfunction is related to atherosclerotic risk factors[48, 54, 55].

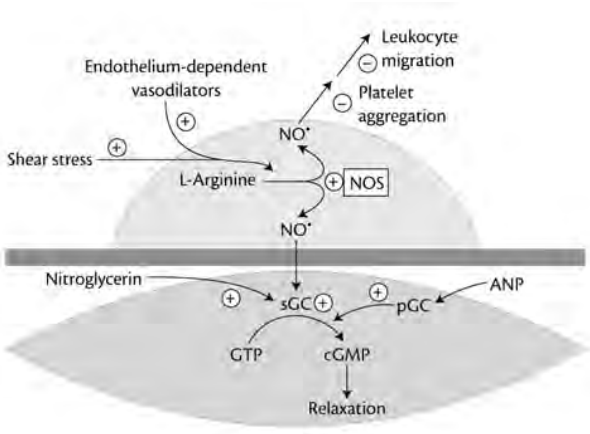


Figure 6. Shear stress and endothelium-dependent vasodilators, such as acetylcholine, act by stimulating eNOS activity thereby increasing endothelium-derived nitric oxide production. NO induces vasodilation by stimulating the production of cyclic guanosine monophosphate (cGMP) in vascular smooth muscle cells. In contrast, other vasodilators, such as nitroglycerin, act independently of the endothelium. GTP=guanosine triphosphate; eNOS=endothelial nitric oxide synthase; pGC=particulate guanylyl cyclase; sGC=soluble guanylyl cyclase. Image taken from: Goligorsky M, Lieberthal W. Atlas of Diseases of the Kidney: Acute Renal Failure (1999). Reproduced with permission from ©Current Medicine Group LLC, Philadelphia.

Arterial stiffness

Loss of arterial elasticity is a normal age-related process. Even physiological age-related arterial stiffening leads to increased systemic blood pressures and left ventricular afterload and to decreased coronary artery perfusion[56].

Arterial stiffness, or inversely arterial distensibility, can be determined using a range of non-invasive techniques. B- or M-mode ultrasound imaging can be used to measure changes in lumen diameter from diastole to systole. A stiffness index (SI) can be calculated by entering these diameter changes and the simultaneously measured brachial BP into an equation (see 4.2.5).

Another indirect measure of arterial stiffness is the pulse wave velocity (PWV). It can be estimated non-invasively, both in elastic and muscular arteries, by calculating the pulse wave transit time from one location to another, using either a photoplethysmographic method (see 4.2.4) or applanation tonometry. The transit time is divided by the distance between the measuring points to obtain the velocity. The stiffer the artery is, the faster the pulse wave travels.

A third method to obtain a measure of arterial stiffness is to analyze the pulse wave form to quantify the late systolic component of the pulse wave[57]. When the pulse wave arrives in the periphery, it will be reflected back along the arteries to the central circulation. The main wave reflection site is the arterioles. A stiffer artery will reflect the pulse wave back to the central arteries faster. If the reflected wave arrives early in systole, during ventricular contraction, it will increase the cardiac afterload. Early wave

reflection will also decrease diastolic pressure. In diastole, the arterial system normally benefits from the pressure augmentation by the reflected waves and too early wave reflection may thus indirectly impair myocardial perfusion[44].

The potential of arterial stiffness measurements to predict cardiovascular events is less well studied in healthy populations than endothelial function. Nevertheless, arterial stiffness has been shown to be predictive of primary coronary events and stroke in patients with essential hypertension[58, 59], and an increase in central arterial stiffness has been observed in patients with coronary artery disease[60, 61], hypertension and additional cardiovascular risk factors[61, 62], stroke[63], and also in healthy elderly individuals[64]. Recent data also show that aortic PWV is predictive of cardiovascular disease risk in apparently healthy individuals[65, 66]. However, in adolescents, arterial stiffness measured as carotid-femoral PWV does not correlate with brachial BP-levels[67].

Methodological aspects of arterial stiffness measurements and their clinical applications have been published in a comprehensive European expert consensus document in 2006[68].

Arterial dimensions and intima-media thickness

Arterial dimensions can be assessed non-invasively using ultrasound or other imaging techniques, such as computed tomography or magnetic resonance imaging (MRI). Dimensions can be measured either as luminal diameter or area, or as inter-adventitial diameter, thus including the intima and media in the measurements. The intima-media thickness (IMT) can also be assessed using high-resolution B-mode ultrasound, measuring the distance between the intima-lumen interface and the media-adventitia interface. The most common locations for IMT-measurements are the carotid arteries, aorta, femoral and brachial arteries. The IMT is a proxy measure of atherosclerotic burden[69] and is predictive of cardio-vascular disease risk[70, 71]. Cardiovascular risk factor profile in adolescence is associated with IMT in adulthood[72, 73]. In overweight and obese children IMT regressed when diet and exercise interventions were applied, although no change in BMI occurred over time[74], showing that early modification of lifestyle factors affects cardiovascular risk markers in young people.

The importance of arterial diameter with respect to cardiovascular disease risk is less clear. One study indicates that coronary artery diameter is an independent predictor of atherosclerosis in the coronary arteries and another recent study shows a close inverse relationship between aortic root diameter and PP[75, 76], which is an important predictor of cardiovascular events. Similar findings from the same group showing that a smaller aortic diameter is associated with increased PP have, however, been questioned previously[77, 78]. They are contradictory to the currently accepted hypothesis of mechanical fatigue in the arteries with ageing, leading to elastic fiber rupture, arterial dilatation, collagen replacement and subsequent arterial stiffening. A stiffer artery will make the reflected wave from the periphery travel faster and this premature wave reflection increases the pulse pressure[78, 79].

Capillaries

Although the increased peripheral resistance that characterizes essential hypertension resides mainly in the arterioles[80], capillary abnormalities are also present[81]. Capillary rarefaction, i.e. a decrease in capillary density, can either be structural or functional. Structural rarefaction means anatomic absence of capillaries, while functional rarefaction refers to non-perfusion of existing capillaries. The capillary density is readily assessed in the skin, usually in the fingers, using intra-vital video microscopy (see 4.2.3). To test the capillary recruitment, a cuff can be applied and inflated to either supra-venous pressure, to obtain venous congestion of capillaries that were not perfused at baseline, or to supra-arterial pressure, to obtain capillary recruitment by reactive hyperemia at release of the cuff[82].

Structural dermal capillary rarefaction is present in patients with established essential hypertension[83]. Both functional and structural capillary rarefaction also occur in border-line hypertension and even in the normotensive off-spring to hypertensive individuals[84, 85]. Capillary recruitment has also been shown to be impaired in patients who exhibit established risk factors for coronary heart disease[55].

2.5 LOW BIRTH WEIGHT AND CARDIOVASCULAR STUDIES

2.5.1 Developmental origins of health and disease (DOHaD)

The idea that early life exposures are associated with later morbidity and mortality first came from Kermack in the early 1930's[86]. Anders Forsdahl, Norway and David Barker, UK revived the early life hypothesis in the late 1970's and early 1980's by examining the relationship between infant mortality, birth weight and other indicators of fetal nourishment and later chronic disease patterns[87, 88]. Barker and his colleagues then formulated hypotheses to shed light on how undernutrition during different trimesters of pregnancy programs an individual's adult risk of coronary heart disease, stroke, high blood pressure and non-insulin-dependent diabetes, commonly referred to as the "fetal origins hypothesis" or "Barker hypothesis". One of the first studies lending support to this hypothesis was published by Gennser et al. in 1988, demonstrating a three-fold increase in risk of high diastolic blood pressure at military conscription in men born small for gestational age[89].

Barker and colleagues continued by investigating the hypotheses, originally generated by ecological studies, in historical cohorts of men and women born in the first half of the 20th century. They could show that mortality from ischemic heart disease declined with increasing birth weight[90]. His group later demonstrated that the same associations were valid also for high blood pressure, diabetes mellitus and stroke[91-95]. These results have been confirmed by a vast number of studies since then[96-108].

Results from these studies do not, however, imply that there is a causal relationship between smallness at birth and later disease. Low birth weight is a proxy measure of a number of intrauterine exposures. Some of these exposures, e.g. decreased fetal nutrient supply, may induce fetal adaptive responses, such as alterations in blood flow and organ growth. These adaptive responses provide immediate advantages for survival in utero and are also intended to improve survival after birth by prediction of the postnatal

environment based on intrauterine experiences. However, these predictive- adaptive responses, which cause irreversible changes to the organism, may show to be maladaptive after birth when for example the nutrient supply is no longer restricted[109].

There has been criticism of the DOHaD-hypothesis arguing that the association between size at birth and later disease risk may be confounded by genetic and socio-economic factors[110]. Moreover, most of the studies in the DOHaD-field do not contain a sufficient number of persons born preterm to be able to conclude whether the results associating low birth weight to later disease risk can also be extrapolated to subjects born preterm.

2.5.2 Fetal growth restriction at term and vascular studies

A large number of studies of vascular function and structure in infants, children and adults have been conducted in the search of mechanisms behind the association between low birth weight and cardiovascular disease risk. This section aims at summarizing the results from studies focusing mainly on vascular function and structure in subjects with low birth weight born at, or near, term.

Endothelial function

Endothelium-dependent vasodilatation is associated with birth weight when born at term. Most studies report that low birth weight subjects have impaired endothelial function in infancy, childhood, adolescence and adulthood[111-117]. Moreover, animal studies show that impaired intrauterine growth caused by maternal protein restriction during gestation gives off-spring endothelial dysfunction[118]. However, a few smaller studies could not find any association between birth weight and endothelial function[119-121]. In one study of middle-aged subjects, the authors speculate that adult lifestyle factors might become more important for endothelial function with ageing, and overwhelm any residual effects of developmental programming[122].

Arterial stiffness

Results from studies relating birth weight to later arterial stiffness are not conclusive. Martin et al. demonstrated an inverse relationship between birth weight and carotid stiffness, but the group differences in aortic and carotid stiffness between small for gestational age (SGA) children and appropriate for gestational age (AGA) children were not significant[123]. In middle-aged men and women, the arterial PWV was negatively related to birth weight, i.e. signs of arterial stiffening were found in those with low birth weight[124]. These findings could, however, not be replicated in later studies of young and middle-aged adults[125, 126]. In 2003, Oren et al. found a positive relationship between birth weight and PWV, but a negative relationship with gestational age and suggested that preterm birth and birth weight act through separate mechanisms in the development of arterial stiffness in healthy young adults[127].

Arterial dimensions

In 1998, carotid stenosis was reported to be related to low birth weight in subjects born in the 1920's[128], but these findings could not be reproduced by the same group in 2002, and they then concluded that impaired fetal growth was not linked to increased

atherogenesis[129]. However, babies born after intrauterine growth retardation (IUGR) have been shown to have increased aortic intima-media thickness (IMT)[130, 131]. Moreover, in young healthy adults, carotid IMT was found to be inversely related to birth weight in the lowest tertile of birth weights and among those who had accelerated postnatal weight gain. When restricting this analysis to those born at term, the inverse relationship between birth weight and carotid IMT was strengthened, indicating that preterm birth was not a risk factor for increased carotid IMT in this study[132]. Other studies in adults have failed to demonstrate carotid intima-media thickening in low birth weight subjects[133-135].

Vascular growth, measured as arterial dimensions, has also been shown to be permanently affected in low birth weight subjects. In 9-year old children, coronary artery diameter, aortic root diameter, and left ventricular outflow tract diameter correlate to birth weight standard deviation score[136]. Other elastic artery dimensions, such as the carotid, aorta and popliteal arteries have also been shown to be negatively affected by IUGR[119, 137, 138].

Blood pressure and hypertension

A vast number of studies, predominantly conducted in subjects born at term, show an inverse relationship between birth weight and blood pressure (in review[100]), but there has been criticism stating that this association is confounded by genetic, socio-economic and environmental factors[139]. Recent large studies do, however, show that the relationship between poor fetal growth and increased BP is independent of these confounding factors[140, 141]. A couple of studies also demonstrate that low birth weight is associated not only with higher blood pressure levels, but also with a diagnosis of hypertension later in life[99, 141].

Cardiovascular morbidity and mortality

Ever since Barker postulated his hypothesis in the 1980's, most studies have found that the risk of ischemic heart disease and stroke show an inverse relationship with birth weight. The risk reduction for ischemic heart disease was estimated to 16% for every kilogram increase in birth weight in a meta-analysis[142]. Moreover, in a large cohort of persons born preterm and/or with low birth weight 1925 through 1949 in Sweden, it was recently demonstrated that the inverse relationship between birth weight and ischemic heart disease is associated with poor fetal growth and not preterm birth[143].

2.5.3 Preterm birth and vascular studies

Low birth weight can be explained by either being small for gestational age or born preterm, or a combination of both. Once the association between low birth weight and poor cardiovascular outcome was established, both epidemiological and vascular studies started to investigate whether part of this association could be explained by preterm birth. In the review of the literature below, only studies also containing subjects born before 35 weeks of gestation are included.

Endothelial function

Although endothelial function is affected in all age groups of persons with low birth weight born at term, there are so far no studies showing endothelial compromise after preterm birth. Singhal et al. first investigated this in 2001 in a large group of adolescents born preterm. There were no signs of affected endothelial function as measured by FMD in subjects born very preterm[144, 145]. In 2003, Norman et al. published data on 54 infants, born at term or preterm, being either SGA or AGA at birth. Endothelial function was tested at 3 months of age, using Laser Doppler measurements of blood flow responses to iontophoresis of ACh. Results show similar perfusion increases in children born preterm (regardless of SGA or AGA at birth) and term AGA-infants. Term SGA-infants had impaired endothelial function[113]. Recently, endothelial function was studied in 5 year old children born very preterm, both SGA and AGA, and controls. No differences in endothelial function, as assessed by Laser Doppler measurements of blood flow responses to both arterial occlusion and iontophoresis of ACh, were found[146].

Arterial stiffness

Studied immediately after birth, the stiffness index (SI) in the abdominal aorta was positively related to gestational age in a mixed group of term and preterm infants, i.e. the arterial distensibility was better in infants born preterm. Stiffer arteries were found in those born to mothers with placental insufficiency and this difference was more pronounced if infants were born preterm[147, 148]. In contrast to these findings, arterial compliance was found to be lower in a group of infants born very preterm, as compared to near-term infants, when measured both at 5 days and 7 weeks of age[149]. Later in childhood, arterial stiffness was compromised only in subjects born preterm and SGA, while preterm AGA-subjects had the same arterial stiffness as term AGA-controls, when measured by brachio-radial pulse wave velocity[150]. At 28 years of age, lower gestational age was related to stiffer central arteries, although the group difference between subjects born term and preterm did not reach statistical significance[127].

Arterial dimensions and intima-media thickness

Data on arterial dimensions after preterm birth are scarce. Abdominal aortic and common carotid artery diameters are positively associated with GA when measured immediately after birth[147, 148]. Later on, brachial artery diameter is reduced in subjects born very preterm studied at 15 years of age[144]. Finken et al. performed 184 carotid IMT measurements in 19 year old subjects born very preterm and could not see that carotid IMT was related to gestational age within the preterm group, but rather to current cardiovascular disease (CVD) risk factor profile. Unfortunately, no term controls were included in this follow-up study[135].

Capillaries

One of the first proposals that vascular structure might be altered after preterm birth in a general way came from Hellström et al. in 1998, who had observed abnormal retinal vascularization in 4 to 9 year old subjects born preterm, independent of their degree of ROP[151, 152]. Similar findings are also present in adult women born preterm[153]. Data on capillary density and recruitment after preterm birth are otherwise scarce. One small

follow-up study in adult subjects born moderately preterm did not find any differences in dermal capillary density or recruitment between subjects born preterm AGA, SGA or term controls. Neither was the capillary density related to the higher BP found in the preterm and low-birth weight subjects[154].

Blood pressure and hypertension

One of the first studies investigating BP in relation to preterm birth was reported by Siewert-Delle and Ljungman in 1998. They showed that middle-aged men born preterm had higher systolic BP (SBP) than those born at term[155]. Many studies of blood pressure in different age groups of individuals born preterm have followed since then.

Blood pressure in the neonatal period increases with increasing gestational age. Measured within 2 hours after birth in healthy infants born with a GA ranging from 29 to 40 weeks, the SBP increases by 1.5 mm Hg for every week increase in GA[147]. Blood pressure has been shown to be normal in infancy in children born preterm. At 3 months of age, BP correlates with body weight, but is not related to preterm birth or being SGA at birth[113].

In small clinical follow-up studies of school-age children born preterm, blood pressure differences are small and increases in SBP or mean arterial pressure (MAP) confined to those born preterm and SGA[150]. However, when comparing school-age children born SGA and preterm to children born SGA at term, those born preterm hade significantly higher SBP[156]. Using 24-hour ambulatory blood pressure measurements, daytime BP-readings did not differ between subjects born preterm or at term, but the night-time SBP was higher in those born preterm and correlated to a higher heart rate, indicating sympathetic nervous system activation[157]. In women in their mid-twenties born preterm, casual BP-measurements showed increased BP-levels, while 24-hour ambulatory BP-measurements could not confirm that mean BP was increased. However, the number of readings above 130 mm Hg was significantly higher in women born preterm[153, 158].

In larger clinical follow-up studies after preterm birth, SBP is higher at young adult age. No additional risk has been observed in those born preterm and SGA[159-163].

In large register studies, males born preterm have been shown to have higher BP at military conscription[97, 164, 165]. In one of these studies, the risk of having a SBP above 140 mm Hg was increased by 93% in those born between 24 and 28 weeks of gestation, as compared to those born at term[164].

Cardiovascular morbidity and mortality

In a cohort of men and women born 1915 to 1929, the risk of cerebrovascular disease, and especially occlusive stroke, has been found to be particularly high among those born preterm[166]. The risk of ischemic heart disease in that cohort was not increased after preterm birth, a finding recently confirmed in another historical cohort by Kaijser et al[143].

With the exception of data from these two historical Swedish cohorts, long-term follow-up studies of cardiovascular health after preterm birth are lacking, mainly due to the limited survival of subjects born very preterm before the development of modern neonatal intensive care.

2.6 OTHER PERINATAL EXPOSURES AND THE VASCULAR TREE

2.6.1 Maternal smoking in pregnancy

Maternal smoking is one of the most common noxious fetal exposures. It increases the risk of pregnancy complications, such as spontaneous abortions, PPROM and placental abruption. The risk of preterm birth and fetal growth restriction is also increased in smokers[167].

Inhaled smoke contains both nicotine and a large number of other potentially harmful substances. Nicotine has in animal models been shown to increase fetal arterial blood pressure and placental vascular resistance and to decrease fetal heart rate and umbilical blood flow[168]. In humans, there are studies showing acute central blood flow changes in the fetus in response to maternal cigarette smoking[169, 170]. Maternal smoking has also been associated with chronically increased resistances in uterine, umbilical and fetal middle cerebral arteries[171]. After birth, infants to smoking mothers have higher SBP in infancy[172]. In human fetuses, pre-atherosclerotic intimal thickening of the coronary arteries has also been associated with maternal smoking[173].

In atherosclerotic arteries of adult smokers, the elastin content is reduced and collagen content increased[174, 175] Similar findings are present in the pulmonary arteries in off-spring to sheep treated with nicotine during gestation[176]. Recent data show that maternal smoking in pregnancy is related to increased aortic IMT in neonates[177]. Human data indicating structural changes in the vascular tree beyond the neonatal period in the off-spring to smoking mothers are scarce. Källén investigated the effect of maternal smoking on incidence of congenital heart defects and found a 30% increase in risk of PDA, even when intrauterine growth and gestational age were controlled for, and an increased risk of truncus anomalies and atrial septal defects[178].

2.6.2 Neonatal estrogens

Fetal exposure to placental estrogens and progesterone normally increases markedly toward the end of pregnancy and birth weight can be used as a proxy for antenatal estrogen exposure[179]. When born preterm, exposure to placental steroids abruptly ends. Preterm girls can, to various degrees, compensate for this loss by increasing endogenous estrogen production during the first months after birth[180]. Whereas endogenous estrogens are known to have beneficial effects on arterial stiffness and endothelial function, offering cardioprotection in women of reproductive age, their role in early vascular development and influence on later BP is far less clear[181, 182]. Experimental data show that estrogen can modulate the process of abnormal vascularization in ROP-development[183]. Moreover, variations in neonatal gonad hormones correlate with later BP in animal models of hypertension[184]. In addition, a preterm drop in estrogen during angiogenesis can silence gene expression of estrogen receptors in the vascular tree, a phenomenon which has been proposed to be linked to

accelerated atherosclerosis[185]. However, there are so far no studies relating neonatal hormonal changes to later cardio-vascular function in subjects born preterm.

2.6.3 Hypertensive disorders of pregnancy and pre-eclampsia

Hypertensive disorders of pregnancy affect approximately 10-20% of all pregnancies worldwide. Pre-eclampsia occurs in 15-25% of these hypertensive pregnancies and is usually defined as the combination of hypertension and proteinuria, occurring in late second and third trimester of pregnancy[186]. Symptoms include abdominal pain, headache and general malaise. The severity ranges from mild, without systemic involvement, to severe forms with multi-organ failure and/or convulsions–eclampsia – which carries a high risk of maternal and fetal mortality.

Pre-eclampsia is linked to systemic vascular endothelial dysfunction and occurs more often in women who have a predisposition for cardiovascular disease[187]. The pathogenesis involves failure of the normal invasion of trophoblast cells from the embryo into the endometrium, leading to maladaption of the maternal arterioles which provide fetal blood supply. This leads to poor villous development and can result in placental insufficiency. There is also a strong maternal immune response involved in the pathogenesis, which could be one of the reasons behind the endothelial dysfunction associated with pre-eclampsia. Even among women with cardiovascular risk factors, pre-eclampsia occurs more often in first pregnancies and in multiparae who have changed partner between pregnancies, suggesting that immunologic factors are also of importance[186]. Pre-eclampsia is treated with anti-hypertensive drugs to reduce the risk of complications while prolonging the pregnancy to diminish the risks with preterm birth. Delivery of the baby is the ultimate cure for pre-eclampsia.

Infants to pre-eclamptic mothers are more often SGA and also more frequently in need of neonatal care, even if born near or at term. The umbilical artery wall is thicker and the elastin content reduced[188]. It is not known if these findings have any lasting consequences for blood vessel development in the off-spring, but subjects born to mothers with pre-eclampsia are at higher risk of hypertension later in life. Women born to mothers with pre-eclampsia are more likely to develop pre-eclampsia themselves, and men are more likely to trigger pre-eclampsia in their partners[189]. It has been assumed that the effects of developmental programming in subjects exposed to pre-eclampsia follow the same pattern as other causes of intrauterine deprivation and undernutrition[190].

3 AIMS

The overall objective of this thesis is to investigate long-term cardiovascular function, vascular structure and blood pressure after preterm birth.

The specific aims of the included studies are:

- To study arterial stiffness, endothelial function and blood pressure in adolescent women born preterm in the 1980's and if these variables are associated with neonatal estradiol levels. (Paper I).

- To study if capillary rarefaction or endothelial dysfunction are related to blood pressure in school-age children born very preterm. (Paper II).

- To study carotid artery dimensions and elasticity in school-age children born very preterm. (Paper III).

- To measure aortic size using magnetic resonance imaging in a prospectively collected cohort of very low birth weight children born preterm 1989 to 1992. (Paper IV).

- To study the risk of hypertension in a cohort of persons born preterm and/or with low birth weight 1925 through 1949. (Paper V).

4 METHODS

4.1 STUDY POPULATIONS

Paper I-IV

Three groups of children and adolescents born very preterm (total n=118, including 8 subjects born with a GA of 32-34 weeks in paper I) and their controls born at term (total n=94) were studied in paper I to IV. The exclusion criteria in these clinical follow-up studies were: multiple pregnancy, major malformation or known chromosomal aberration, congenital infection (CMV, rubella, toxoplasmosis or HSV), diabetes mellitus and present cardiovascular disease.

Data on family history of cardiovascular disease among first- and second-degree relatives, maternal smoking during the index pregnancy, and when applicable, current smoking, use of oral contraceptives and age at menarche were recorded. Weight, height and waist circumference were measured according to standard clinical practice. For children born preterm, perinatal data were obtained from medical records (paper I-IV) and in a prospectively collected database (paper IV). Birth weight and gestational age for the control subjects were reported by the parents (paper I); found in the maternity ward records (paper II-III); or in the study database, where data on maternal and paternal education level were also available (paper IV).

Paper I

Neonatal estradiol levels were collected prospectively in all girls born preterm (≤34 weeks of gestation) 1982 through 1989 in Uppsala. All survivors who did not meet any of the exclusion criteria (n=60) were invited to participate in the study. Thirty-four of them agreed to take part. Age-matched controls (n=32) were found among healthy volunteers born AGA at term.

Paper II and III

The preterm children (n=39) were identified by searching hospital records from 1992 through 1998 at the Karolinska and Danderyd Hospitals, Stockholm, Sweden. All SGA-children were identified and an equal number of AGA-children were frequency-matched to those according to GA, birth year and gender. The control children (n=21 in paper II and n=17 in paper III) born at term were identified through the maternity ward records.

Paper IV

The *Stockholm Neonatal Project* [191], a prospective population based study, was initiated in September 1988 and continued until March 1993. All children with a birth weight of 1500 g or less were included if they were born at, or transferred to, the neonatal intensive care unit at the Karolinska Hospital or to an annex unit at St. Görans Hospital in Stockholm.

Of the 291 infants originally included, 182 were available for follow-up at 5 ½ years of age. At that age, a control group of 125 term-born children was assembled from a population-based register according to birth date, birth hospital and gender. In 2005, we invited all 114 study subjects who had a GA of <32 weeks and met none of the exclusion criteria and 75 of the original controls to participate. The response rate was close to 90% in both groups. Because of metallic implants of unknown composition (after surgical closure of a patent ductus arteriosus in the neonatal period), 16 children from the study group were excluded from magnetic resonance imaging (MRI). Another 35 adolescents in the study group and 26 in the control group declined participation. In four examinations (one of them a control), the data quality was suboptimal. In two subjects, one from each group, the exams could not be finished due to claustrophobia, leaving 45 datasets for the study group and 41 datasets for the control group. The mean gestational age, birth weight and maternal age did not differ between participants and non-participants in either group.

Paper V

The source population for this cohort study was all births from 1925 through 1949 at four major delivery units in Sweden (Allmänna BB and Södra BB in Stockholm, Uppsala University Hospital and Sundsvall County Hospital). Information about maternal age, date of last menstrual period (LMP), maternal/paternal occupation, proteinuria or pre-eclampsia during pregnancy and proteinuria at time of admission was collected at time of admission. Immediately after delivery, birth weight, birth length, sex and twin status were noted. At discharge, information about proteinuria post-partum and breast-feeding at hospital discharge were collected. By manually examining the ~ 250,000 birth records during this period, a cohort of infants born preterm, SGA, or both, was identified. All newborn infants with a gestational duration of <35 weeks or a birth weight of < 2000 g for girls and < 2100 g for boys were included. Different cut-off points for girls and boys were used to obtain groups of equal size, since boys on average weigh more than girls at birth. Subjects for whom no information was available on gestational duration or for whom only the month for the LMP was given were not included in the cohort. As a reference cohort, we selected infants born after 35 weeks of gestation with a birth weight above 2,000 grams (girls) or 2,100 grams (boys). For convenience, we selected the first subject of same sex and hospital of birth born after each study subject. Subjects who emigrated or deceased prior to 1987 were excluded.

4.2 VASCULAR MEASUREMENTS

4.2.1 Blood pressure

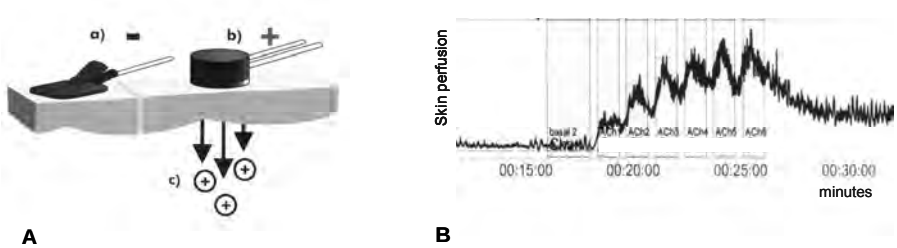
Paper I-IV

The heart rate and brachial blood pressure were recorded with an automated oscillometric method (Boso Medicus™, Bosch+Sohn, Germany), using an appropriately sized arm cuff, after 10 minutes' rest in the supine position (study I-III, or upright sitting position in paper IV). A mean value of 3 (9 in paper I) consecutive determinations taken at intervals of at least 3 minutes was regarded as the subject's blood pressure. The intra-subject coefficient of variation (CV) for repeated measurements was 5% for systolic and 4-6 % for diastolic BP in the different studies.

4.2.2 Endothelial function

Paper I-II

A Laser Doppler (LD) instrument (Periflux 4001, laser wavelength 780 nm) and a micropharmacology system were used to measure blood flow in the dorsal hand skin (Perimed AB, Kista, Sweden)[123, 192]. The LD signal is proportional to the number and velocity of moving blood cells in the skin micro-vessels and expressed in perfusion units of output voltage (1 perfusion unit (PU)=10 mV). The temperature of the LD probe was standardized to 32ºC. To study endothelium-dependent vasodilation, basal perfusion was recorded for ≥2 minutes, after which 2% acetylcholine (ACh, acetylcholine chloride, Sigma-Aldrich) was transferred across the skin by iontophoresis with an anodal current of 0.1 mA for 20 seconds repeated 5 times at 60-second intervals. Basal perfusion and changes in response to ACh were measured as the area under the PU/time curve. The CV for repeated measurements of maximum perfusion change induced by ACh has previously been found to be 18% in our laboratory[192].



A B

Figure 7. A Laser Doppler instrument with an integrated micropharmacology system (A) for trandermal delivery of acetylcholine, an endothelium dependent vasodilator. B shows skin perfusion at baseline and successive perfusion responses to each dose of ACh.

4.2.3 Capillary density

Paper II

Dermal capillary density was studied using intravital video-microscopy[82]. The dorsum of the middle phalanx of the index, middle and ring finger in the left hand was examined twice. One microscopic field (1 mm^2) in each finger was studied before and during venous occlusion obtained by inflating a finger cuff at 40 mm Hg for two minutes before the recording started. The skin was prepared using paraffin oil. A system comprising a video microscope (Micro-Watcher, VS-10, Mitsubishi Kasei Corp, Tokyo, Japan) with a 200x magnification lens and a VCR-TV was used to record the films.

All recordings lasted at least one minute and the films were analyzed both off-line and by live playback of the videotapes until all identifiable capillaries within the microscopic field of 1 mm^2 had been marked on an acetate sheet. Calibration of the 1 mm^2 area was verified with a glass micrometer. The intra-subject coefficient of variation (CV) between fingers was 8.1% before and 6.3% during venous occlusion for observer 1, and 7.9 and 8.3% respectively for observer 2, who blindly analyzed a random sample of the recordings (120/360) to assess inter-observer variability (CV=10.4%).

4.2.4 Muscular artery measurements

Paper I

Pulse wave velocity

The pulse wave velocity (PWV) was measured to estimate arterial stiffness in a muscular artery. It is related to arterial vessel wall compliance- i.e.in a stiffer artery, the pulse wave travels faster. A photoplethysmographic method was used to determine the transit time of the arterial pulse wave from the brachial artery (antecubital fossa) to the radial artery just proximal to the wrist[193]. The mean transit time for at least 10 heart cycles was calculated and divided by the distance between the probes. The PWV measurements were repeated 3 times in each subject and the CV for repeated measurements was 19%.

Radial pulse wave analysis

A pulse wave analysis system (SCOR-Px, AtCor Medical, Australia) was used together with applanation tonometry (Millar tranducer SPT-301, Millar Instruments, USA) to analyze the radial artery pressure waveform. The central aortic systolic pressures were deduced from the radial artery pressure waveforms by a data transfer algorithm, validated in adults[194].

The sum of the mechanical properties in elastic and muscular arteries determines the velocity of the outgoing systolic pressure wave and the extent to which this wave is reflected back to the proximal aorta. The reflected waves augment the forward traveling pressure wave generated by left ventricular ejection. Identification of early and late systolic peaks in the aortic pressure curve allows quantification of an augmentation

index (AI, presented at a standardized heart rate of 75 bpm, Figure 8[195]). The AI is related to the speed of the central and peripheral pressure wave reflections, and can therefore be used to estimate total arterial stiffness. The mean value of 3 recordings, each comprising 10 consecutive pressure waves, was taken as the subject's reading. The CV for AI measurements was 9.7%.

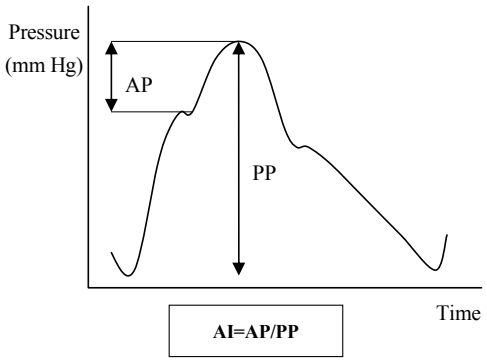


Figure 8. Calculation of augmentation index (AI, %) from the arterial pressure wave form. AP=augmented pressure. PP=pulse pressure. Adapted from O`Rourke et al[195].

4.2.5 Elastic artery measurements

Paper I, III

The mechanical properties and dimensions of the left common carotid artery (paper I and III) and the aorta (paper I) were studied by measuring their pulsatile diameter changes[192]. A real-time B mode ultrasonic scanner (Hitachi EUB 240) and a computer-generated pair of electronic echo-trackers (Diamove™, Teltec AB, Sweden) were used to measure the pulsatile movements of the vessel wall, Figure 9. Ultrasonic signals were sampled at a frequency of 100 MHz, implying that the system could detect vessel wall movements of less than 10 micrometers. The end-diastolic diameter (Dd, mm) and the pulse amplitude of the diameter (ΔD, mm) were determined. These data and those of simultaneously measured blood pressures in the brachial artery were computed to yield the stiffness index (β) of the arterial wall, see Equation 1.

$$\beta = \frac{\ln (SBP/DBP)}{\Delta D} \times Dd$$

Equation 1. Equation for calculation of stiffness index β. SBP= systolic blood pressure, DBP= diastolic blood pressure, ΔD= pulse amplitude of the diameter (mm), Dd=end-diastolic diameter (mm).

The mean value of 3 recordings, each comprising 6 to 10 consecutive heart cycles, was regarded as the subject's reading. The CV for repeated measurements of arterial stiffness index has been found to be 10–13% at our laboratory[192].

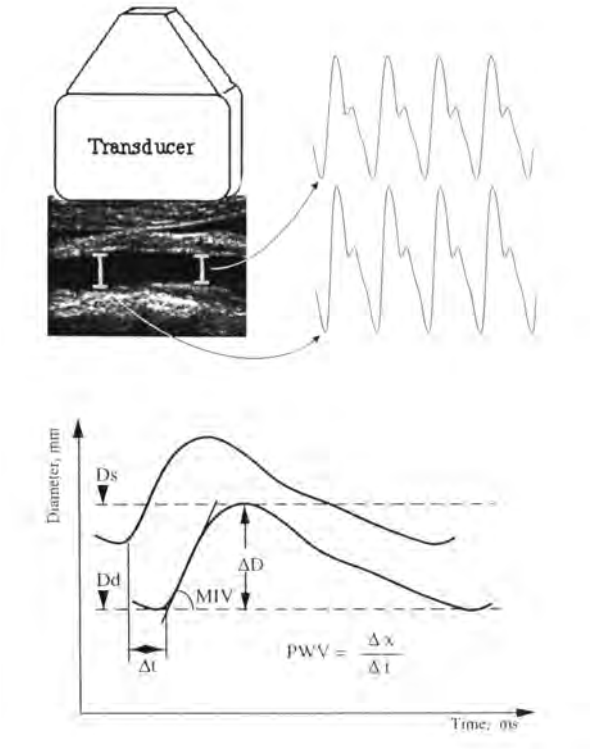


Figure 9. Ultrasonic echo-tracking system for measurements of vessel wall pulsations. The systolic and diastolic vessel diameters (Ds and Dd), the pulse amplitude (ΔD) and the maximum incremental velocity (MIV) are obtained from the wave forms. The time shift between the two curves can be used to calculate the pulse wave velocity. (The PWV-function was not used in this thesis). Image reproduced with permission from dr Jie Hu (thesis, Karolinska Institutet, Stockholm, 1998).

Paper IV

Each participant underwent a single scanning session on a 1.5 T MR scanner (GE, Milwaukee, USA). The protocol included localizer scans along all 3 axes of the body (Figure 10). Based on these images, and depending on the height of the subject, five to seven 5 mm thick slices were positioned along and approximately perpendicular to the descending aorta. The slices were positioned 4.5 cm apart. For reference, the first and most inferior slice was positioned just below the iliac bifurcation. The images which were used in further analysis were acquired during breath-hold with a retrospectively pulse-gated Fiesta sequence[196], providing 25 images within the subject's heart-cycle for each slice. The image acquisition parameters were as follows: field of view = 240 mm, repetition time = 4.2 ms, echo time = 1.8 ms, flip angle = 45^{o}, acquisition matrix = 224x224 and then padded with zeros to 256x256 before image reconstruction. The field of view was reduced to 80% in the phase encoding directions (anterior-posterior).

The images of the aorta were segmented by an observer blinded to subject group, using a subpixel-precision Active Contour Model[197, 198] implemented in Matlab, permitting semi-automatic calculations of aortic end-diastolic cross-sectional areas, defined as the smallest aortic area within the heart cycle. Corresponding methods have previously been used for aortic segmentation from MRI data[199, 200]. For statistical analyses the second, fourth and fifth to seventh slices were used, representing the distal abdominal aorta, the proximal abdominal aorta and the thoracic aorta, respectively (Figure 10). For calculation of the coefficient of variation (CV) for the aortic segmentation, we acquired 5 consecutive image series of an adult volunteer using the same MR image acquisition parameters as for the participants. The CV varied between 0.6% and 2.3% at the different aortic levels. In a random subset of end-diastolic images (n=90) we also compared aortic measurements by the Active Contour Model to those manually traced by an experienced blinded MR-observer. The overall CV for the different aortic levels was 4.2%.

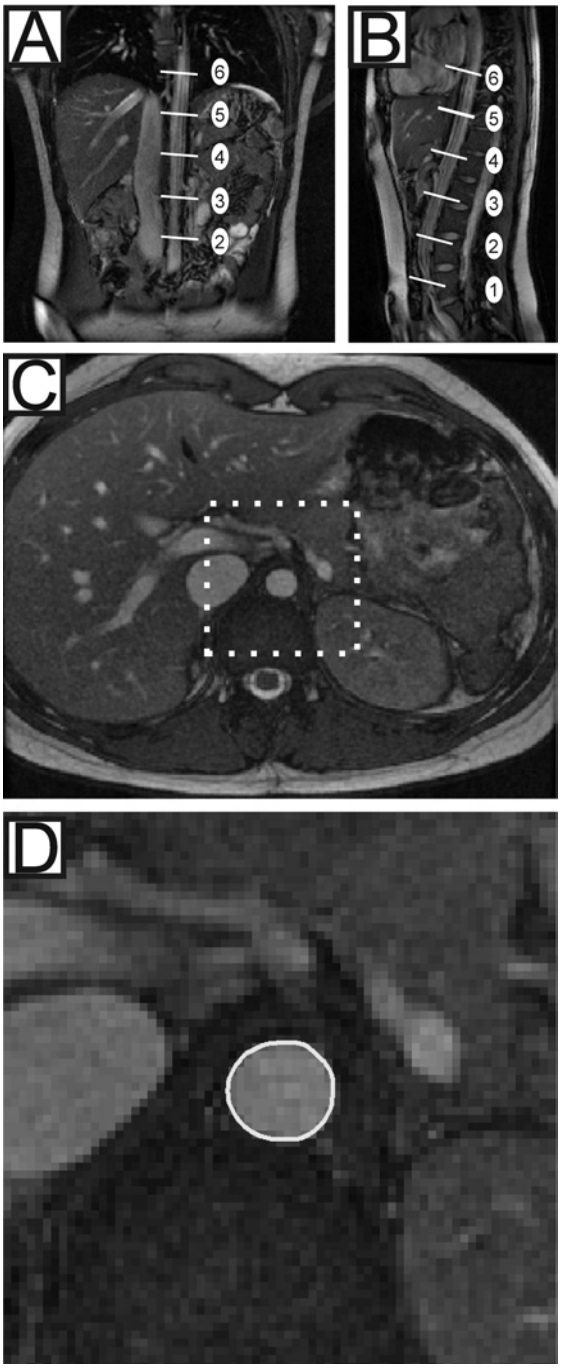


Figure 10. MRI slice positioning and segmentation in the descending aorta. Part A and B depict the positioning of the MRI slices. Part C displays slice 4 in A and B. The square indicates the area magnified in Part D. Part D illustrates the result of the aortic segmentation (white outline).

4.3 COHORT STUDY OF PRETERM BIRTH AND LATER RISK OF HYPERTENSION

Paper V

Categorization of perinatal data

We used the last menstrual period to estimate gestational duration, which was categorized into 4 groups: 32 completed weeks or less (very preterm), 33 to 36 (preterm), 37 to 42 (term), and 43 weeks or more (postterm). Birth weight was categorised in 7 groups of 500 gram intervals, from less than 1,500 grams up to 4,000 grams or more.

As a measure of fetal growth, we used birth weight for gestational age according to Swedish reference curves for estimated intrauterine growth[9]. Birth weight for gestational age was categorized in five groups according to their distance from average in numbers of standard deviations (≤-2 standard deviations (SD), >-2 to -1 SD, >-1 to 0 SD, >0 to +1 SD, and >+1 SD).

Follow-up and analysis

Follow-up started January 1, 1987 and continued to December 31, 2006. We used the Register of Population and Population Changes to ascertain emigration or death during follow-up. Hypertension was defined from the Hospital Discharge Register using the diagnostic codes for essential hypertension, 401 and I10, as principal or contributory diagnosis, according to the 9th and 10th revisions of the *International Classification of Diseases*, respectively.

Data were modelled through Cox proportional hazards regression, using the TPHREG procedure in SAS Statistical Software, version 9.1 (SAS Institute Inc., Cary, NC, USA). Hypertension was the failure event, and subjects with no hypertension were censored at death, emigration or December 31, 2006, whichever occurred first. Time was modelled from January 1, 1987, to date of event or to date of censoring. We controlled for age at entry in 5 year categories and sex in all analyses by adding these variables in the strata statement. When controlling for birth weight, gestational duration, or fetal growth, the method was the same. Testing for trend was done by scoring categories with equidistant points and using these scores as a continuous variable.

To assess whether our results were influenced by case-ascertainment bias or by an underlying association between fetal growth and ischemic heart disease or diabetes– other diseases of the metabolic syndrome– we did a restricted analysis. In this analysis, time was modelled with first admission to hospital as starting date and only hypertension with no previous or simultaneous diagnosis of ischemic heart disease or diabetes was counted as failure event. In addition, censoring occurred also at time of first diagnosis of ischemic heart disease or diabetes.

4.4 STATISTICAL METHODS

The statistical methods used in this thesis are described in detail in each of the five papers.

Paper I-IV

The results are presented as mean (SEM) or proportions. Student's t-test, chi-squared test, analysis of variance and rank-sum test were used to test for group differences. A p-value of <0.05 was considered significant. The association between covariates or potential confounders and outcome was tested using simple, linear regression or ANOVA. Associations with $p<0.10$ (paper II and II), $p<0.20$ (paper I) or $p<0.25$ (paper IV), were investigated in multivariate regression models. Separate models were constructed in the same way within the preterm group in each paper to investigate the association between neonatal characteristics and outcome. All data in paper I-IV were analyzed using JMP 5.0.1, (SAS Institute Inc, Cary, NC) or Intercooled Stata 9.1 (StataCorp LP, College Station, TX).

4.5 INFORMED CONSENT AND ETHICS

The study protocols comply with the declaration of Helsinki. The parents and children gave informed consent before inclusion (paper I-IV). All studies had been approved by the local ethics committees (Ups 02-464, KI 02-395, 02-210, 04-527/3, 01-366).

5 RESULTS

5.1 SUBJECT CHARACTERISTICS

In paper I to IV, subjects born preterm more often had a family history of cardiovascular disease in first- and second degree relatives, but the difference was statistically significant only in Paper IV. Children and adolescents born preterm had also been exposed to maternal smoking during pregnancy more often than controls in paper I-III, although the group difference did not reach statistical significance. Table 3 displays background, perinatal and anthropometric data for subjects in Paper I-IV.

In Table 2, the perinatal characteristics for children and adolescents born preterm are given. Some perinatal data, such as cause of preterm birth and the proportion of infants being SGA, vary between studies depending on inclusion criteria (see 4.1).

	Paper I	**Paper II & III**	**Paper IV**
Number of subjects born preterm	34	39	45
Calendar period of birth	1982-1989	1992-1998	1988-1993
Age at study, y	16.5 (1.6)	9.1 (1.7)	15.0 (1.2)
Gestational duration, weeks	29.1 (2.9)	28.9 (1.6)	27.6 (2.0)
- range, weeks	23- 34	25-30	24-31
Birth weight	1343 (469)	1106 (305)	1011 (211)
SGA, n (%)	9 (26%)	20 (51%)	11 (24%)
Cesarean section, n (%)	25 (74%)	25 (64%)	26 (58%)
Cause of preterm delivery			
- PPROM or preterm labour, n (%)	11 (32%)	5 (13%)	20 (44%)
- pre-eclampsia, n (%)	12 (35%)	17 (44%)	10 (22%)
- bleeding, n (%)	8 (24%)	8 (21%)	5 (11%)
- miscellaneous*, n (%)	3 (9%)	3 (8%)	4 (9%)
- missing, n (%)	0	6	6
Gender, female	34 (100%)	20 (51%)	23 (51%)
Mechanical ventilation, n (%)	15 (44%)	11 (28%)	22 (49%)
CPAP, n (%)	31 (91%)	25 (64%)	39 (87%)
Umbilical artery catheter, n (%)	26 (76%)	13 (33%)	22 (49%)
PDA , n (%)	missing	7 (18%)	19 (42%)

Table 2. Perinatal characteristics for children and adolescents born preterm in paper I to IV. Data are given as mean or numbers. Standard deviations (SD) or percent in brackets. *Miscellaneous causes of preterm delivery: infection, IUGR, diabetes, Rh-immunization. CVD= cardiovascular disease

	Paper I			*Paper II& III**			*Paper IV*		
	Preterm n=34	**Control n=32**	**p**	**Preterm n=39**	**Control n=21**	**p**	**Preterm n=45**	**Control n=41**	**p**
Family history of cardiovascular disease, n	26 (76%)	20 (62%)	0.15	35 (92%)	16 (76%)	0.09	40 (89%)	28 (68%)	0.03
Perinatal data									
Maternal age, yrs	29.9 (4.8)	28.5 (3.9)	0.20	31.4 (5.1)	29.6 (6.0)	0.24	30.1 (0.8)	30.7 (0.8)	0.64
Smoking in pregnancy, n	10 (29%)	5 (16%)	0.10	12 (31%)	3 (14%)	0.16	13 (29%)	11 (27%)	0.83
Gestational length, w	29.1 (2.9)	39.6 (1.0)	<0.001	28.9 (1.6)	40.3 (1.0)	<0.001	27.6 (2.0)	39.8 (1.2)	<0.001
Birth weight, g	1343 (469)	3602 (316)	<0.001	1106(305)	3704 (404)	<0.001	1011 (211)	3510 (371)	<0.001
Current data									
Age, yrs	16.5 (1.6)	16.5 (1.6)	0.91	9.1(1.7)	9.7 (1.5)	0.19	15.1 (1.2)	14.6 (1.2)	0.06
Weight, kg	57.9 (12.6)	57.1 (7.5)	0.76	30.8 (8.1)	31.7 (5.7)	0.69	54.3 (10.8)	56.1(8.2)	0.39
Height, m	1.63 (0.06)	1.66 (0.06)	0.03	1.35 (0.10)	1.40 (0.08)	0.07	1.65 (0.11)	1.67 (0.10)	0.45
BMI, kg/m^2	21.7(4.3)	20.7 (2.6)	0.23	16.8 (2.8)	16.2 (2.0)	0.4	19.8 (2.6)	20.2 (2.6)	0.49
Waist circumference, cm	72.1 (9.5)	68.5 (4.8)	0.06	59.3 (7.4)	58.9 (5.3)	0.85	70.8 (6.6)	69.9 (7.5)	0.57

Table 3. Background, perinatal and anthropometric data in healthy children and adolescents participating in studies in Paper I-IV. Data are given as mean (SD) or number of subjects (%). p-values according to Student's t-test or chi-squared test. * Four controls included in this table data did not undergo ultrasound examination in paper III and are thus not included in results of paper III.

5.2 BLOOD PRESSURE AND HEART RATE

Paper I

Systolic and diastolic blood pressures were higher in women born preterm, as well as pulse pressure (Table 4). In addition to preterm birth, systolic BP was associated with family history of cardiovascular disease, height, and smoking, but not with age, waist circumference, BMI, age at menarche, use of oral contraceptives or heart rate (Table 5). A corresponding analysis with diastolic BP as the dependent variable showed significant relations with preterm delivery, smoking, and heart rate. The pulse pressure was associated with preterm birth and height. Heart rate did not differ between groups. Both systolic and diastolic aortic BP, estimated using the pulse wave analysis system, were also significantly higher in girls born preterm (Table 7).

Paper II &III

Blood pressure did not differ between subjects born preterm and term in univariate analysis (Table 4). In multivariate analysis, adjusting for gender, height (in accordance with recommendations made by the American Academy of Pediatrics) and family history of cardio-vascular disease, SBP was 2.6 mm Hg higher in children born very preterm than in controls (p=0.010). Adjusting for BMI instead of height diminished the contribution of preterm birth to systolic BP and the association was no longer significant. Female sex was associated with 2.3 mm Hg lower systolic BP when including height in the model, and 3.5 mm Hg lower SBP when including BMI (p=0.01 and 0.0007, respectively). Pulse pressure was lower in children born preterm, median 36 vs. 40 mm Hg (Wilcoxon rank-sum test, p=0.047). Heart rate was higher in preterm children in both univariate and multivariate analyses (Table 4). Heart rate was also negatively associated with BMI in the multivariate analysis, but BP and heart rate were not related. Within the preterm group, no differences in heart rate or BP were found between subjects born SGA or AGA.

Paper IV

Both systolic and diastolic BP were higher in the preterm group, but there was no difference in heart rate between groups (Table 4). The blood pressures in adolescents exposed to maternal smoking in pregnancy did not differ from those without such exposure. Height, preterm birth and heart rate, but not sex, were associated with systolic BP in the final multiple regression model (R^2=0.33). Finally, neither systolic, nor diastolic blood pressure correlated with aortic cross-sectional area.

	Paper I		*Paper II& III*		*Paper IV*	
	preterm	**control**	**preterm**	**control**	**preterm**	**control**
Systolic BP	115 (1.5)*	104 (1.6)	99 (1.3)	97 (1.8)	122 (1.9) †	115 (2.0)
Diastolic BP	68 (1.0)*	63 (1.0)	58 (0.9)	59 (1.2)	72 (1.3)‡	68 (1.3)
Pulse pressure	46 (1.0)*	41 (1.0)	41 (1.1)	38 (1.5)	50 (1.3)	47 (1.4)
Heart rate	67 (2.0)	65 (2.0)	76† (1.6)	68 (2.2)	80 (1.7)	78 (1.8)

Table 4. Brachial blood pressures (mm Hg) and heart rate (bpm) in children and adolescents born preterm or at term (paper I-IV). Data are mean (SEM). p-values according to Student's t-test. * p≤ 0.001, † p≤0.01, ‡ p< 0.05

Dependent variable	Regression factor	Regression coefficient	p-value
SBP	Preterm delivery	5.7	<0.001
	Current smoking	4.2	0.02
	Family history of CVD	2.0	0.05
	Height (cm)	0.5	0.008
DBP	Preterm delivery	2.7	<0.001
	Current smoking	3.2	0.01
	Heart rate	0.18	0.007

Table 5. Factors independently related to blood pressure in two groups of adolescent girls born preterm (n=34) or at term (n=32) (paper I). Regression coefficients expressed in mm Hg. CVD=cardiovascular disease.

5.3 ENDOTHELIAL FUNCTION

Paper I

Resting skin perfusion was lower in preterm girls than in control girls (mean 5.9 vs. 9.5 PU, p=0.01). The maximum perfusion response induced by the endothelium dependent vasodilator ACh, however, was similar in the two groups (Figure 11). The ACh response was higher in girls using oral contraceptives (mean 137 PU, n =15) than in those who did not (107 PU, p=0.01). We found no other associations between endothelium-dependent vasodilatation and background variables, including smoking. BP correlated inversely with basal skin perfusion (r= -0.33 for systolic BP, r= -0.30 for diastolic BP, p≤0.01), but not with the maximum perfusion response.

Paper I **Paper II**

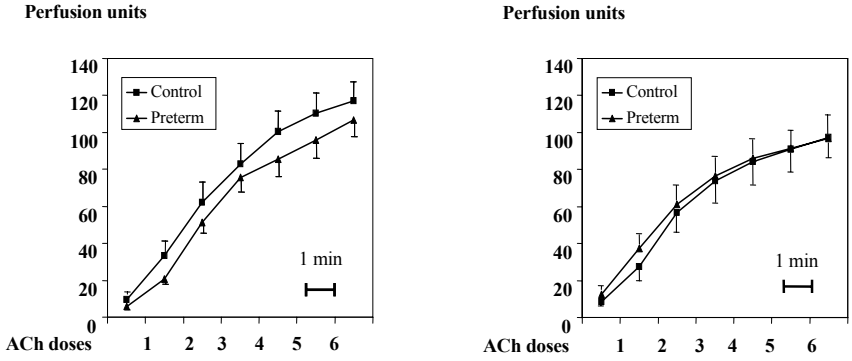


Figure 11. Skin perfusion in response to ACh, an endothelium-dependent vasodilator

Paper II

In LD measurements, the total skin perfusion at baseline was higher in preterm children, 13 (1.0) PU vs. 9 (1.4) PU in controls (p=0.026). The endothelial function did not differ between groups; the maximum response to ACh was the same in both groups, 96.9 (8) PU for children born preterm and 97.3 (11) for term controls (p=0.98, Figure 11). Resting skin perfusion and baseline capillary density were inversely correlated (β= -0.17, r=0.31, p=0.017). No other correlations between capillary density and endothelial function were found. Endothelial function did not correlate to any of the other background variables, nor to BP.

5.4 CAPILLARY DENSITY

Paper II

Children born preterm had fewer dermal capillaries at baseline. During venous occlusion, this difference was no longer significant, but the trend towards fewer capillaries in preterm subjects remained (Table 6). When performing simple, linear regression, capillary density was not related to any of the other potential confounders or risk factors. Within the preterm group, the off-spring to pre-eclamptic mothers had 87.3 (2.7) capillaries/mm^2 at baseline compared to 92.0 (3.1) capillaries/mm^2 in those born without having been exposed to pre-eclampsia, but this finding did not reach statistical significance (p=0.25). Children born preterm who had had retinopathy of prematurity (ROP) in infancy tended to have fewer capillaries, 85.3 (3.2) capillaries/mm^2 vs. 92.8 (2.5) capillaries mm^2 (p=0.07). BP or heart rate did not correlate with capillary density.

	Preterm n=39	Term n=21	p-value
Capillary density/mm^2			
Functional capillary density (baseline)	89.9 (1.9)	96.6 (2.6)	0.04
Structural capillary density (venous occlusion)	91.4 (2.0)	96.8 (2.7)	0.12

Table 6. Capillary density before and during venous occlusion in 9 year old children born preterm or at term. Data are mean (SEM). p-values according to Student's t-test.

5.5 ARTERIAL DIMENSIONS AND STIFFNESS

Paper I

Preterm girls had narrower abdominal aortas than the control girls: the end-diastolic diameter was 10.0 mm (0.2) compared to 11.0 mm (0.3) in the control group (p=0.01). In both groups, the average carotid diameter was 6.5 mm (0.1).

Preterm girls also had lower aortic stiffness index, but no group differences in carotid stiffness, brachio-radial PWV or augmentation index were found (Table 7). The relative pulsatile diameter change– i.e. the vessel wall strain– was 15.9% (0.7) in the aorta of preterm girls and 13.4% (0.8) in the control group (p=0.03). Multivariate analysis, including height and weight in the regression model, showed that in addition to group, the aortic diameter also correlated positively with age (r=0.42, p=0.01). Aortic and carotid strain correlated to pulse pressure (r=0.26 and 0.39, respectively, p<0.05). The PWV correlated to BP (r=0.42 for systolic BP, r=0.57 for diastolic BP, p<0.001). After adjustment for BP, the group difference in PWV remained insignificant. The AI did not correlate to any of the background variables.

	Preterm	Control	p-value
Aortic SBP, mm Hg	100 (1.4)	93 (1.5)	0.001
Aortic DBP, mm Hg	70 (1.2)	66 (1.3)	0.03
Brachial pulse wave velocity, m/s	7.8 (0.4)	7.5 (0.4)	0.54
Augmentation index, %	- 2.4 (1.7)	-4.2 (1.8)	0.46
Stiffness index, abdominal aorta	3.3 (0.2)	3.9 (0.2)	0.02
Stiffness index, carotid artery	5.2 (0.2)	5.0 (0.2)	0.33

Table 7. Aortic BP and different measures of arterial stiffness in healthy adolescent girls born preterm or at term. Data are mean (SEM).

Paper III

The mean carotid end-diastolic diameter was 6.4 mm in all three groups (p=0.99) and there was no significant difference in carotid stiffness index (Table 8). Carotid diameter correlated to body surface area (r=0.38, p=0.004). There were no other correlations between carotid diameter or carotid stiffness and tested covariates, including heart rate.

	Preterm SGA n = 20	Preterm AGA n = 19	Term controls n = 17	p
Carotid diameter, mm	6.4 (0.13)	6.4 (0.14)	6.4 (0.15)	0.99
Carotid pulse amplitude, mm	0.73 (0.02)	0.70 (0.02)	0.70 (0.03)	0.53
Carotid stiffness index	4.7 (0.25)	5.3 (0.25)	4.7 (0.27)	0.18

Table 8. Carotid end-diastolic diameter, pulse amplitude and stiffness index in healthy children born preterm AGA or SGA or at term. Data are mean (SEM), p-values according to Student's t-test.

Paper IV

Subjects born very preterm had significantly smaller aortic end-diastolic area at all levels. Table 9 shows unadjusted group comparisons for four levels: the thoracic, proximal, mid- and distal abdominal aorta. In multivariate analysis, preterm birth and maternal smoking in pregnancy were found to be strong and highly significant contributors to a narrower aorta, even after adjustment for body surface area (Table 10). Male sex was associated with a larger aortic cross-sectional area. Maternal education level, a proxy variable for socio-economic status, was not associated with aortic narrowing in uni- or multivariate analyses. No interaction between maternal smoking in pregnancy and maternal education level could be found with respect to aortic narrowing.

	Preterm n=45	Term controls n=41	p-value
Thoracic aorta, mm^2	114 (99- 135)	139 (129- 153)	<0.001
Proximal abdominal aorta, mm^2	103 (84- 117)	126 (104- 147)	<0.001
Mid-abdominal aorta, mm^2	69 (61- 86)	91 (75- 107)	<0.001
Distal abdominal aorta, mm^2	73 (65- 89)	96 (83- 110)	<0.001

Table 9. Aortic end-diastolic cross-sectional area (mm^2) measured by magnetic resonance imaging in healthy adolescents born preterm or at term (total n=86). Data are presented as median (inter-quartile range). p-values according to Wilcoxon rank-sum test.

Thoracic aorta	% change (95% CI)	p-value
Preterm birth	-16.2 (-10.2 to -21.8)	<0.001
Male	9.8 (2.0 to 18.2)	0.013
Maternal smoking in pregnancy	-12.4 (-5.4 to -18.8)	0.001
Proximal abdominal aorta		
Preterm birth	-17.0 (-10.9 to -22.6)	<0.001
Male	12.5 (4.5 to 21.2)	0.002
Maternal smoking in pregnancy	-13.2 (-6.2 to -19.6)	0.001
Distal abdominal aorta		
Preterm birth	-19.0 (-12.9 to -24.8)	<0.001
Male	16.8 (8.0 to 26.3)	<0.001
Maternal smoking in pregnancy	-9.6 (-2.0 to -16.7)	0.015

Table 10. Change in end-diastolic aortic area in adolescent subjects (n=86) in relation to preterm birth, gender and exposure to maternal smoking in pregnancy. Estimates are based on results of multivariate linear regression analysis of log aortic area adjusted for body surface area.

Within the preterm group, the aortic area did not correlate to perinatal covariates, such as pre-eclampsia, SDS for birth weight, use of umbilical artery catheter or PDA, at any of the levels of measurement. Multivariate analyses, adjusted for body surface area and gender, demonstrated that lower gestational age was independent contributor to a narrower thoracic and distal abdominal aorta and maternal smoking to aortic narrowing at all levels (Table 11).

Thoracic aorta	% change (95% CI)	p-value
Gestational age, per week	3.0 (0.6 to 5.5)	0.015
Maternal smoking in pregnancy	-14.7 (-5.3 to -23.1)	0.004
Proximal abdominal aorta		
Gestational age, per week	1.6 (-1.4 to 3.6)	0.38
Maternal smoking in pregnancy	-15.2 (-5.6 to -23.8)	0.003
Distal abdominal aorta		
Gestational age, per week	3.0 (0.3 to 5.7)	0.029
Maternal smoking in pregnancy	-16.1 (-5.8 to -25.2)	0.004

Table 11. Change in end-diastolic aortic area in 15 year old subjects born preterm (n=45) in relation to gestational age and exposure to maternal smoking in pregnancy. Estimates are based on results of multivariate linear regression analysis of log aortic area adjusted for body surface area and gender.

5.6 COHORT STUDY OF PRETERM BIRTH AND LATER RISK OF HYPERTENSION

Paper V

At start of follow-up, there were 6,425 subjects in the cohort; 2,931 were born preterm, 2,176 subjects had low birth weight and 576 were born small for gestational age. There were 851 cases of hypertension in the cohort, of whom 69 had hypertension as principal diagnosis, whereas 105 had a principal diagnosis of ischemic heart disease.

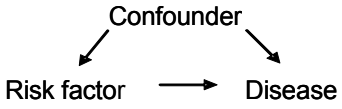
Low birth weight was associated with an increased risk of hypertension (p for trend=0.0003, Table 12). There was a stepwise increase in risk of hypertension with decreasing category of fetal growth. Compared to subjects with a birth weight of 0-1 SD above the mean for gestational age, those born small for gestational age had a risk increase of 53%. Gestational duration was not associated with hypertension (Table 12) and we found no interaction between fetal growth, preterm birth and risk of hypertension. Calendar period of birth did not affect the results.

In the analysis of other covariates, we found a negative association between socioeconomic status and risk of hypertension. Compared to the lowest category, those in the highest category had a decreased risk of hypertension (HR 0.65; 95% CI 0.44 to 0.97). Stratifying this analysis on fetal growth had no effect on the association. When stratified on fetal growth, neither of the covariates maternal age, pregnancy hypertensive disorder and breastfeeding at hospital discharge was associated with later risk of hypertension. In the restricted analysis, results were unchanged, and we found no indication of our results being due to case ascertainment bias or an underlying association between fetal growth and ischemic heart disease or diabetes.

All	N	# cases	HR	C.I.
Total:	***6425***	**851**		
Birth weight				
<1500	*151*	19	1.08	(0.67-1.74)
1500–1999	*835*	123	1.21	(0.96-1.53)
2000–2499	*1190*	183	1.20	(0.98-1.47)
2500–2999	*1000*	132	1.02	(0.81-1.27)
3000–3499	*1367*	180	1	ref
3500–3999	*1309*	149	0.84	(0.68-1.05)
>=4000	*573*	65	0.85	(0.64-1.14)
P for trend:				*0.0003*
Gestational duration				
<=32	*986*	128	1.01	(0.83-1.24)
33–36	*1945*	275	1.08	(0.93-1.26)
37–42	*3221*	422	1	ref
>=43	*273*	26	0.79	(0.53-1.18)
P for trend:				*0.34*
Fetal growth				
≤-2 SD	*576*	98	1.53	(1.20-1.95)
> -2 to -1 SD	*942*	141	1.32	(1.06-1.65)
> -1 to 0 SD	*1836*	251	1.17	(0.97-1.42)
> 0 to +1 SD	*1560*	185	1	ref
> +1 SD	*1511*	176	0.95	(0.78-1.17)
P for trend:				*<0.0001*

Table 12. Hazard ratios for hypertension in relation to birth weight, preterm birth and fetal growth in a cohort of men and women born in Sweden 1925 through 1949.

6 EPIDEMIOLOGICAL TERMINOLOGY

Confounding	Confounding is the distortion of the effect of one risk factor by the presence of another. Confounding occurs when another risk factor for a disease is also associated with the risk factor being studied, but acts separately. Age, gender and socio-economic status are often confounding risk factors, because subjects with different values of these are often at different risk of disease. 
Selection bias	Systematic error that occurs when, because of design and execution errors in sampling, selection, or allocation methods, the study comparisons are between groups that differ with respect to the outcome of interest for reasons other than those under study.
Information bias	A flaw in measuring outcome or exposure. Many different biases (recall, reporting, measurement, withdrawal etc.) are collectively grouped in this class.
Internal validity	Truth within a study. A study is internally valid if the study conclusions represent the truth for the individuals studied, because the results were not likely due to the effects of chance, bias, or confounding because the study design, execution, and analysis were correct.
External validity or generalizability	Truth beyond a study. A study is externally valid if the study conclusions represent the truth for the population to which the results will be applied. The important characteristics are those that would be expected to have an impact on a study's results if they were different (e.g., age, disease severity, co-morbidity etc). External validity can occur only if the study is first internally valid.

7 DISCUSSION

The main findings of this thesis are that children and adolescents born very preterm have higher blood pressure, reduced functional dermal capillary density and narrower aorta. They do not exhibit classical markers of cardiovascular disease risk, such as early arterial stiffening or endothelial dysfunction, at this age. Moreover, we could not show that preterm birth in the first half of the 20th century is associated with an increased risk of hypertension later in life.

The rationale for the included studies was to investigate if the association between low birth weight and signs of increased cardiovascular disease risk is also valid for children born preterm. It has been suggested that the fetus might be particularly susceptible to programming effects of undernutrition and other adverse exposures in utero during the third trimester of pregnancy[201]. Infants born very preterm spend this period ex utero, where exposures differ from those in the intrauterine environment. Although both similarities and differences regarding cardiovascular risk markers were found in the studies included, answers about the underlying mechanisms cannot be given by this thesis. In the following section the results from the different studies will be discussed, and potential confounding and methodological limitations considered.

Blood pressure, hypertension and heart rate

The finding of a higher BP after preterm birth confirms results from a number of both clinical and epidemiological studies[97, 159-162, 164, 165]. The increase in systolic BP in subjects born preterm in study II and III were not significant in univariate analyses, but when including height in the model, BP was significantly higher in those born preterm. In pediatric clinical practice, BP-levels are always age- and height standardized[202]. Including BMI in the multivariate model in paper II diminished the contribution from preterm birth. In a study of 400 adolescents born preterm, BMI and current weight were the best predictors of BP at 19 years of age. However, the prevalence of hypertension was 11 % in that study and 45 % were pre-hypertensive[163], indicating that a higher BMI after preterm birth might well be part of the causal pathway, rather than a confounder of the association between preterm birth and later BP-elevation.

BP-differences in paper I and IV, studying subjects who were post-pubertal, were more pronounced. A person with a SBP ≥120 mm Hg is considered to be pre-hypertensive[202]. Using this definition, 24% of preterm girls in paper I and 50% of adolescents born preterm in paper IV were pre-hypertensive, compared to 3 and 34%, respectively, in controls (p=0.01 and 0.11 for paper I and IV). In paper IV, the BP was measured in the upright sitting position, non-fasting, while in paper I the subjects were fasting, BP measured in the supine position and the study performed in the morning, which could explain differences in results between studies and the high number of pre-hypertensives even among controls in paper IV. In addition, in paper IV the subjects also underwent an MR-examination, a potentially stressful situation. Simultaneous salivary cortisol measurements were strongly related to SBP in boys born preterm, indicating that stress might have influenced the results (Elsmén E et al., unpublished data).

We only performed casual BP-measurements at one single research visit to the clinic. 24-hour ambulatory BP-measurements may more correctly reflect blood pressure control and cardiac workload. Results from studies using ambulatory BP-measurements in subjects born preterm show subtle abnormalities, such as absence of drop in systolic BP at night or increased number of readings above 130 mm Hg[157, 158], which may reflect differences in autonomic control (see below).

Aortic SBP and PP, as estimated by pulse wave analysis in paper I, were elevated in adolescent girls born preterm to the same magnitude as brachial SBP and PP. This indicates that the BP-elevation seen after preterm birth reflects an increase in cardiac workload and that the brachial BP-elevation is not due only to peripheral vascular properties.

In contrast to the finding of a higher BP in children and adolescents born preterm, we could not see that preterm birth in the first half of the 20th century was related to an increased risk of inpatient care for hypertension 37 to 82 years of age (paper V). Strengths with paper V include the large number of persons born preterm, the prospectively collected birth characteristics and the non-differential follow-up through nationwide registers. However, infant mortality among those with low birth weight used to be high and selective survival of the fittest preterm infants may have affected the results[106]. Caution must thus be taken when generalizing results from a cohort of persons born before the development of neonatal intensive care to today's populations of children born very and extremely preterm. Moreover, we studied diagnosis of hypertension in the hospital discharge register. It can therefore not be excluded that risk estimates would be different if we studied hypertension in the population receiving outpatient care.

Heart rate was higher in children born preterm in paper II and III, but heart rate did not correlate to systolic BP. In a recent study of 9 year old children born extremely preterm, the heart rate correlated to excretion of urinary catecholamines, suggesting that sympathoadrenal tone is increased after preterm birth[203]. In adults, sympathoadrenal overactivity is associated with development of hypertension and an increased mortality from cardiovascular disease in hypertensive patients[204, 205].

Endothelial function and capillary density

We found no signs of endothelial dysfunction after preterm birth, as assessed by iontophoresis of ACh, an endothelium-dependent vasodilator (paper I-II). These findings confirm previous reports of unaffected endothelial function after preterm birth[113, 144, 146]. The coefficient of variation for Laser Doppler measurements of endothelial function was relatively high (18%). However, using the same technique and sample sizes previously, large differences in endothelial function have been demonstrated between children born SGA and AGA at term[113, 115, 123]. The sample sizes in both study I and II allowed 80% power for detection of a difference in maximum perfusion response of 25 PU (~25%). It can thus not be excluded that smaller differences in endothelial function may be present already at young age and that these differences may amplify with age.

Use of oral contraceptives was associated with higher maximum perfusion responses in paper I. Estrogen replacement therapy after oophorectomy has been shown to restore endothelial function, as have oral contraceptives given to amenorrheic athletes[182, 206]. Estrogens are known to increase the bioavailability of nitric oxide, which may explain these findings[207]. In paper I, we hypothesized that vascular function might be permanently affected by estrogen deprivation after preterm birth and that the ability to compensate for this loss by endogenous estrogen production in the neonatal period would correlate to later vascular function. We could, however, not find any association between neonatal estradiol levels and later vascular function in this group of adolescent girls born preterm.

Skin perfusion at baseline was significantly lower in adolescent girls born preterm (paper I) and significantly higher in school-age children born preterm (paper II). These contradictory results may indicate differences in autonomic function in different age groups of children. Children in paper II had higher heart rate, indicative of increased sympathetic tone, and lower functional capillary density. The sympathetic nervous system controls pre-capillary sphincters in the arterioles and blood can be shunted through arterio-venous shunts by-passing the dermal capillary network. Such shunting would be reflected as higher baseline perfusion in the arterioles (from where the Laser Doppler signal mainly arises) and is also consistent with the inverse relationship between functional skin capillary density and baseline skin perfusion found in paper II.

We did not test endothelium-independent vasodilatation using nitroglycerin or sodium nitroprusside, NO-donors, in these studies. Previous reports show that impaired vasodilatation in low birth weight (LBW) children is endothelium-dependent and that endothelium-independent NO-donors induce the same vascular response in both LBW and normal BW children[123].

In paper II, we found that children born very preterm had lower functional dermal capillary density. However, the capillary density was not related to childhood BP and it remains to be studied longitudinally whether capillary rarefaction in children born very preterm will affect BP later in life, as shown in adult subjects[83]. If capillary rarefaction is present also in other organ systems, various body functions might be affected. Muscular capillary density is, for example, an important determinant of muscle metabolism and thus insulin sensitivity. Accordingly, capillary rarefaction could be one of the mechanisms linking preterm birth to insulin resistance[120, 162, 208].

Speculatively, there could be several mechanisms behind the observed capillary rarefaction. First, the second trimester of pregnancy is a period of very rapid fetal growth and organ development, which demands an expanding capillary network. Most of the infants born very preterm suffer from infections, hypoxia and stress during this sensitive period of development, which could affect capillary formation. Moreover, a major cause of preterm birth is maternal pre-eclampsia. In paper II, 17 of 39 preterm children (44%) had been exposed to pre-eclampsia, which is characterized by impaired microvascular function and capillary changes in the placenta[209, 210]. Thus, some of the children born very preterm could have had affected capillary growth already before birth.

In contrast to our data, Irving et al. could not demonstrate any reduction in capillary density after moderately preterm birth[154]. Being born at 30 to 35 weeks of gestation might not imply the same changes to the organism as being born between 25 and 30 weeks of gestation. In agreement with this assumption, ROP is very seldom found in moderately preterm infants, whereas the disease is common in infants born at lower gestational ages. Kistner et al. have previously found that low gestational age is associated with both abnormal retinal vascularization and increased BP in young adults[153].

In paper II, we could only observe a significant difference in capillary density at baseline, although there was a trend towards a lower capillary density also after venous occlusion. Therefore, we cannot with certainty answer the question if the capillary rarefaction in children born very preterm is structural. There is evidence that both functional and structural entities might be affected in hypertension. When Serné et al. compared capillary density in hypertensive and normotensive adults, 62% of the difference in capillary numbers could be explained by structural and 38% by functional defects[211].

Arterial stiffness and dimensions

Arterial stiffness in the aorta, brachial and carotid arteries was studied in paper I and in the carotid artery in paper III. In paper I, adolescent girls born preterm had more elastic abdominal aorta. Muscular artery stiffness, measured as PWV, and combined muscular and elastic artery stiffness, measured as augmentation index, did not differ between groups. Carotid artery stiffness was also similar in children born preterm, irrespective of being SGA or AGA at birth, and controls in both paper I and III.

The finding of a more elastic aorta and higher strain in adolescent girls born preterm is interesting considering the aortic narrowing observed in paper I and IV. According to Laplace's law, tensile stress (σ) is directly proportional to arterial transmural pressure (P) and radius (r), and inversely proportional to arterial wall thickness (h) according to the formula: $\sigma = Pr/h$. Increased pulsatility despite a smaller radius could indicate that the aortic wall is thinner after preterm birth. Several factors influence arterial growth and vessel wall composition. Flow induced shear stress promotes arterial growth. In animal models, uncoupling of the placental circulation at birth and the subsequent drop in aortic blood flow reduce aortic dimensions[212]. Elastin synthesis rate is normally very high in the perinatal period[213]. In experimental models, the aortic blood flow reduction at birth disrupts elastin deposition, as do periods of protein malnutrition in utero[214, 215]. Infants born preterm are exposed to several of the factors that impede with elastin deposition and aortic growth in animal models: premature cessation of placental circulation, blood flow disturbances caused by PDA, the use of umbilical artery catheters and periods of hypotension. Moreover, it is often difficult to maintain a sufficient protein intake in the first weeks of extrauterine life. Since mature cross-linked elastin is not synthesized in the adult aorta, I suggest that perinatal changes in elastin deposition might have life-long consequences[216]. Previous studies on arterial dimensions after the neonatal period in subjects born preterm are lacking, with the exception of one study showing reduced brachial artery diameter.[144]

The association between preterm birth and later elastic artery narrowing could hypothetically be confounded by maternal vascular factors leading both to preterm birth and altered vascular structure in the off-spring. The finding of unaltered carotid dimensions does, however, speak against a general impairment in elastic artery development. "Brain-sparing" mechanisms, redistributing blood flow to meet the high cerebral metabolic needs, are operating in the fetus and preterm infant and maintain carotid artery blood flow. Nevertheless, preterm birth has recently been shown to be associated with a 70 % increase in stroke risk in a historical cohort[166]. Whether this risk reflects cerebral vascular changes, or is merely due to hypertension in persons born preterm, remains to be established.

In addition to preterm birth, we also found that maternal smoking in pregnancy is strongly and independently associated with aortic narrowing in adolescence (paper IV). This is one of the first reports associating fetal tobacco exposure to lasting structural changes in the arterial tree. Nicotine has been shown to increase placental, umbilical and fetal vascular resistance and to increase fetal BP[168, 171]. Experimental data also indicate that nicotine directly modifies collagen and elastin expression[176]. Substances in smoked tobacco may in addition induce elastin degrading enzymes, such as elastase; a mechanism proposed to operate in aortic aneurysm development in smokers[174]. There may be socio-economic confounding of the association between maternal smoking and later vascular structure, but inclusion of maternal education level in the multivariate model did not affect our results.

Effects of gestational age and fetal growth

Adolescent aortic size in paper IV was correlated to GA within the preterm group. We could not find any other graded relationship between GA and later vascular function or structure. This lack of associations must be interpreted cautiously, considering that the studies were not powered to investigate the effect of GA as a continuous variable. Fetal growth within the preterm group, measured as being SGA or AGA at birth, did not influence any of the measured parameters, which is in line with results from most of the previous studies in children born very preterm. In contrast, paper V reports results from a historical cohort showing that risk of inpatient care for hypertension is related to fetal growth even among those born preterm. Limitations concerning that cohort are discussed under the heading "Blood pressure, hypertension and heart rate".

Internal and external validity

Paper I to IV report results from children born very preterm. They have spent most of, or the whole, third trimester of pregnancy ex utero. Our inclusion- and exclusion criteria were chosen to obtain groups representative of the majority of very preterm infants, exposed to common causes of prematurity and having as few simultaneous medical conditions unrelated to prematurity as possible.

The results in paper I to IV are based on comparisons between subjects born very preterm and controls born at term. The internal validity thus depends on selection of the controls. In paper I, controls were recruited mainly at schools in Stockholm and

Uppsala among volunteers born at term. In paper II and III, controls were invited from the maternity ward records at Danderyd Hospital. No information about confounders, other than maternal smoking in pregnancy and maternal age, was available for these groups. We can therefore not exclude confounding of the association between preterm birth and impaired cardiovascular structure and function. In paper IV, controls were invited from a population-based register according to birth date, gender and living area. Moreover, we also had information on maternal education level to be used as a proxy measure for socio-economic status. We found no evidence that maternal education level affected the results. However, a family history of cardiovascular disease was systematically more common in children and adolescents born preterm than in controls and may have confounded the relationship between preterm birth and cardiovascular outcome.

The findings of paper I to IV should be generalizable to similar populations of children born very preterm, taking a few limitations into account. In paper II and III the study group was sampled to obtain an equal number of preterm children born SGA and AGA, to be able to study the effects of being SGA when born preterm. This made the proportion of SGA-children twice as high in the study group as in the source population. In paper IV, the inclusion criterion in the original preterm cohort was very low birth weight and not gestational age. This means that the proportion of children born SGA was higher in the highest GA strata.

Clinical implications

A new and growing population of adults with a history of very or even extremely preterm birth is emerging in society. Their long-term health risks are not yet known. Results from this thesis and other studies suggest that this new population may be at increased risk of cardiovascular disease. There is strong evidence that persons born preterm have higher BP already at young age. BP tends to track throughout life, why it seems plausible that they will still be a high risk population later in life[217]. There are also studies showing that people born preterm more often have insulin resistance, which is part of the metabolic syndrome and also strongly linked to cardiovascular disease risk[162, 208].

Most cardiovascular measures used in this work are not available for clinical use. Blood pressure is, however, readily measured in all clinical in- and outpatient settings. Based on our results, standard clinical BP and heart rate measurements may be the most appropriate tools for initial screening for cardiovascular disease risk after preterm birth. It is only recently that BP measurements have been recommended at every office visit in pediatric populations and these recommendations have not yet been implemented in Sweden[202]. Awaiting this, BP should at least be measured at regular intervals in persons born preterm.

8 CONCLUSIONS

- Blood pressure is increased already at young age after very preterm birth. 25% to 50% of subjects born very preterm have pre-hypertensive systolic BP-levels at 15 to 19 years of age. Screening for hypertension at early adult age could be of importance in the emerging population of persons born very preterm.

- Adolescents born very preterm have narrower descending aorta. Maternal smoking in pregnancy is also an independent risk factor for aortic narrowing. The underlying mechanisms are unknown, but could involve impaired synthesis of elastin. Blood pressure was not related to aortic size.

- No signs of arterial stiffening are found after very preterm birth. In adolescent girls, the aorta is more elastic. This increase in elasticity could indicate differences in arterial composition or wall properties after preterm birth.

- Carotid dimensions and stiffness are unaffected after preterm birth.

- The functional dermal capillary density is lower in children born very preterm, but the capillary density is not related to their blood pressure.

- Vascular endothelial function is not affected after preterm birth.

- Heart rate is higher in subjects born very preterm when studied at 9 years of age, but the heart rate is not related to BP. The higher heart rate and BP could indicate altered autonomic control after very preterm birth.

- The association between low birth weight and an increased risk of hypertension in subjects born in the first half of the 20^{th} century is related to poor fetal growth and not to preterm birth. Caution must be taken when generalizing these findings to the new generation of survivors after very and extremely preterm birth today.

9 SVENSK SAMMANFATTNING

Hjärt-kärlsjukdom är en mycket vanlig orsak till sjuklighet och död i hela världen. Att vara född med låg födelsevikt ökar risken att insjukna i hjärt-kärlsjukdom senare i livet. Syftet med studierna i denna avhandling är att undersöka kopplingen mellan för tidig födsel, den vanligaste orsaken till låg födelsevikt, och senare blodkärlsfunktion och risk för hjärt-kärlsjukdom.

I avhandlingen ingår fyra studier av barn och ungdomar som fötts för tidigt på 1980- och 1990-talet (totalt 118 stycken). Deras endotelfunktion studerades med Laser Dopplermätningar av blodflödesförändringar som svar på jontofores av acetylkolin, en endotelberoende blodkärlsvidgare (delarbete I och II). Hudens kapillärtäthet undersöktes med hjälp av videomikroskopi (delarbete II). Artärstelheten mättes med pulsvågsanalys, pulsvågshastighetsmätningar och med ultraljud (delarbete I och III). Halspulsåderns och aortas dimensioner mättes med ultraljud och magnetkamera (delarbete I, III, IV).

Delarbete I visar att de för tidigt födda tonårsflickorna hade smalare bukaorta och lägre basalt hudblodflöde jämfört med flickorna i kontrollgruppen som fötts fullgångna. Inga tecken på ökad blodkärlsstelhet sågs och endotelfunktionen var opåverkad hos de för tidigt födda.

9-åriga barn som fötts mycket för tidigt hade lägre kapillärtäthet, men inte heller här kunde några skillnader i endotelfunktion ses mellan för tidigt födda och deras kontroller (delarbete II). Inga skillnader i halspulsådrornas kärlstelhet och dimensioner kunde hittas (delarbete III).

Resultaten från magnetkameraundersökningen i delarbete IV bekräftade fynden från delarbete I och visade att aorta descendens i hela sin sträckning hade mindre tvärsnittsyta hos mycket för tidigt födda ungdomar än hos kontroller. Aortastorleken var också mindre hos de som exponerats för maternell rökning under graviditeten.

Delarbete I, II och IV visar också att för tidigt födda barn och ungdomar hade högre blodtryck. I delarbete II och III noterades också en högre hjärtfrekvens hos de för tidigt födda, men hjärtfrekvensen korrelerade inte till blodtrycket.

Delarbete V undersökte risken att slutenvårdas för hypertoni i en kohort bestående av 6425 svenska män och kvinnor födda 1925 till 1949, av vilka 2931 stycken var för tidigt födda. Risken för hypertoni 53% högre hos dem som fötts tillväxthämmade. För tidig födsel i sig var inte kopplad till senare hypertonirisk.

Sammanfattningsvis så är för tidig födsel associerat med bestående blodkärlsförändringar, såsom lägre kapillärtäthet och smalare aorta. För tidigt födda barn och ungdomar har också högre puls och blodtryck. Inga tecken på ökad kärlstelhet eller endoteldysfunktion kunde hittas. Vilken betydelse dessa fynd har för risken att insjukna i hjärt-kärlsjukdom efter mycket för tidig födsel är ännu ej känt.

10 RÉSUMÉ EN FRANÇAIS

Les maladies cardio-vasculaires représentent une des causes principales de morbidité et de mortalité à travers le monde. De nombreuses études mettent en évidence un risque accru de ces maladies chez les personnes de faible poids de naissance. Les études présentées dans cette thèse décrivent la relation entre prématurité (principale cause de faible poids de naissance) et risques futurs de pathologies cardio-vasculaires.

Quatre études ont été réalisées sur 118 enfants et adolescents nés prématurément entre 1982 et 1998. La fonction endothéliale a été étudiée en mesurant les variations de flux sanguin par vélocimétrie Doppler laser en réponse à une iontophorèse d'acétylcholine (vasodilatateur dépendant de l'endothélium) (études I et II). La densité capillaire cutanée a été étudiée par vidéo-microscopie (étude II). La rigidité des artères a été mesurée par analyse de l'onde de pouls, la vitesse de l'onde de pouls (VOP), et par échographie (études I et III). Les diamètres carotidiens et aortiques ont été mesurés par échographie et imagerie par résonance magnétique (IRM) (études I, III, IV).

Par rapport à un groupe témoin né à terme, des filles adolescentes nées prématurément présentent une aorte abdominale de diamètre plus étroit et un flux sanguin cutané réduit. En revanche, ni l'élasticité des artères, ni leur fonction endothéliale ne sont réduites (étude I).

Un groupe d'enfants de 9 ans nés grands prématurés présente une densité capillaire cutanée inférieure à celle d'un groupe témoin, mais leur fonction endothéliale n'est pas réduite non plus (étude II). D'autre part, leurs carotides ont une élasticité et un diamètre normaux (étude III).

Les résultats des examens IRM de l'aorte d'un groupe d'adolescents nés grands prématurés confirment les résultats de l'étude I en montrant que leurs aortes sont plus étroites que celles d'un groupe témoin (étude IV). D'autre part, les dimensions de l'aorte s'avèrent être clairement liées à l'usage du tabac par la mère pendant la grossesse, indépendamment de la prématurité des sujets.

Les études I, II et IV montrent que les sujets nés prématurément ont une tension artérielle plus élevée que les autres. Ils présentent aussi une fréquence cardiaque plus élevée (études II et III), mais sans qu'elle soit corrélée à leur tension artérielle.

Afin d'analyser les effets à long terme de la prématurité et de l'insuffisance de poids à la naissance, l'étude V compare 6425 hommes et femmes nés entre 1925 et 1949, dont 2931 sont nés prématurément. Entre les âges de 37 et 82 ans, le risque d'hypertension artérielle est augmenté de 53% pour ceux nés avec une insuffisance de poids. La prématurité n'est pas liée en tant que telle au risque d'hypertension dans cette tranche d'âge.

En conclusion, la grande prématurité prédispose à une modification du développement vasculaire, ce qui est illustré par une densité capillaire cutanée réduite et une aorte plus étroite. Les enfants nés prématurément présentent une fréquence cardiaque ainsi qu'une

tension artérielle plus élevées. En revanche, aucun signe précoce de maladies athéromateuses n'a été mis en évidence : ni élasticité artérielle réduite, ni dysfonctionnement endothélial. Il reste à déterminer un rapport entre ces résultats et le risque futur de maladies cardio-vasculaires.

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