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DEVELOPMENT IN CHILDREN BORN VERY PRETERM AFTER INTRAUTERINE GROWTH RESTRICTION WITH ABNORMAL FETAL BLOOD FLOW

Eva Morsing

LUNDS UNIVERSITET

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”When you are a bear of very little brain, and think of Things, you find sometimes that a Thing which seemed very Thingish inside you is quite different when it gets out into the open and has other people looking at it.” AA Milne

To David, Karin, Sara and Petter

Abstract

Delivery of fetuses with intrauterine growth restriction (IUGR) with abnormal umbilical artery blood flow in the second trimester represents a clinical dilemma. So far, no evidence based management protocols are available adressing when to deliver these fetuses. The high risk of hypoxia and fetal death has to be balanced against that of extreme preterm birth with associated morbidity. The clinical routine in Lund has been a proactive management, that is to deliver on fetal indication before occurence of more severe hemodynamic changes. The aim of this study was to evaluate short and long-term consequences in very preterm IUGR fetuses (PT-IUGR) with abnormal blood flow in the umbilical artery.

Study I: Mortality and neonatal morbidity did not differ between the PT-IUGR group and the very preterm background population, born before 30 gestational weeks (GW) in 1998-2004, with the exception of chronic lung disease ($p < 0.01$). Survival without major neurological handicap at two years was equal between the two groups.

Study II: At early school age, cognitive impairment was more prevalent in boys born very preterm with IUGR compared to a matched very preterm group with BW appropriate for gestational age (PT-AGA). Attention deficit disorders were more prevalent in children in both preterm groups compared to term children with birth weight AGA (T-AGA) ($p < 0.01$). There was a trend towards more behavioral problems in the preterm groups.

Study III: Lung function, assessed with spirometry in children at 6-10 years, was reduced in the PT-IUGR group compared to the T-AGA group. The PT-IUGR

group had worse lung function when born after 26 GW in comparison to the PT-AGA group ($p<0.05$).

Study IV: Cardiovascular measurements were assessed at 5-8 years. Systolic and mean blood pressure, adjusted for height z-score, was higher in both preterm groups compared to the T-AGA group ($p<0.05$). Findings in the vascular measurements were different between the preterm groups; the PT-IUGR group had lower aortic stiffness ($p<0.01$) and lower endothelial-dependent vasodilation compared to the PT-AGA group ($p<0.05$), and thinner intima media thickness in the carotid artery compared to the T-AGA group ($p<0.05$).

Conclusions: IUGR in very preterm birth did not have an impact on overall mortality or major handicaps. Impairment in lung function was only apparent in the preterm IUGR group after 26 GW, a period of gestation when delivery on fetal indication in general is considered acceptable. The adverse impact of IUGR on cognitive function was present at all gestational ages, but cognitive impairment was only significant in IUGR boys compared to preterm controls. The question whether the cognitive impairment in boys is transient or persistent remains unanswered. It is difficult to understand the implication of the outcomes of lower IMT and aortic stiffness seen in the preterm IUGR group on later cardiovascular health. Further studies have to be performed in older ages.

The observed outcome would not seem to be sufficiently severe to refrain from delivery in these very preterm IUGR fetuses and we suggest that it is justified to deliver fetuses with IUGR and abnormal blood flow in the umbilical artery at early gestational age in order to prevent intrauterine death.

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III. Morsing E, Gustafsson P, Brodszki J. Lung function in children born after fetal growth restriction and very preterm birth. *Acta Paediatrica 2011 Aug 8. Doi: 10.1111/j.1651-2227.2011.02435.x.* [Epub ahead of print]

IV. Morsing E, Liuba P, Fellman V, Maršál K, Brodszki J. Cardiovascular function in children born very preterm after intrauterine growth restriction with severely abnormal umbilical artery blood flow *In manuscript 2011*

Abbreviations

AA - Abdominal aorta

ACE - Angiotensin converting enzyme

ACH - Acetylcholine

ADD - Attention deficit disorder

AGA - Appropriate for gestational age

ARED - Absent or reversed end-diastolic blood flow

BDR - Bronchodilator response

BMI - Body mass index

BP - Blood pressure

BSA - Body surface area

BW - Birth weight

CCA - Common carotid artery

CHD - Coronary heart disease

CLD - Chronic lung disease

CNS - Central nervous system

CVD – Cardiovascular disease

DC - Distensibility coefficient

DT - Deceleration time

ECG - Electrocardiogram

FEV_1 - Forced expiratory volume in 1 sec

$FEF_{25-75\%}$ - Forced mid-expiratory flow rate

FHR - Fetal heart rate

FOAD - Fetal origin of adult disease

FSIQ - Full scale intelligence quotients

FVC - Forced vital capacity

GW - Gestational weeks

HPA - Hypothalamus-pituitary-adrenal axis

IMT - Intima-media-thickness

IVRT - Isovolumic relaxation time

IUGR - Intrauterine growth restriction

IVH - Intraventricular haemorrhage

IVST - Interventricular septum thickness

LBW - Low birth weight

LVM - Left ventricular mass

LVSF - Left ventricular shortening fraction

LVPWT - Left ventricle posterior wall thickness

LDF - Laser Doppler flowmetry

MCA - Middle cerebral artery

NEC - Necrotizing enterocolitis

NO - Nitric oxide

PA - Popliteal artery

PDA - Persistent ductus arteriousus

PI - Pulsatility index

PIQ - Performance intelligence quotient

PT - Preterm

PSV - Peak systolic velocity

PU - Perfusion unit

PVL - Periventricular leukomalacia

RAS - Renin-angiotensin system

ROP - Retinopathy of prematurity

SGA - Small for gestational age

SD - Standard deviation

SDQ - Strengths and difficulties questionnaires

SNP - Sodium nitroprusside

T - Term

UA - Umbilical artery

VIQ - Verbal intelligence quotients

WPPSI - Wechsler preschool and primary scale of intelligence

WISC - Wechsler intelligence scale for children

Introduction

Intrauterine growth restriction (IUGR) due to abnormal fetal blood flow in very early gestation constitutes a clinical dilemma. The hemodynamic changes presenting in the IUGR fetus, as measured by Doppler ultrasound velocimetry, are associated with hypoxia and ultimately fetal death. The clinician must weigh the risk of delivery in very early gestation with associated morbidity against the risk of fetal death if the fetus remains *in utero*. So far, no evidence-based management protocols are available. In the last 15 years, at the perinatal clinic in Lund, the clinical management of very preterm IUGR fetuses with severe changes in umbilical artery flow (*i.e.* absent or reversed end-diastolic blood flow-ARED), has been proactive, that is to deliver on fetal indication with emergency or elective cesarean section. The severity of changes in umbilical artery and ductus venosus blood flow indicates whether to deliver or not. The long-term combined effects of IUGR and very preterm birth on postnatal development are not fully understood. The events in utero, e.g. the changes in fetal blood flow, might aid the survival of the fetus, however, they may, alter the structure, physiology and metabolism in the fetus and in that way prejudice health later in life. The aims of this thesis are to investigate cognitive, pulmonary and cardiovascular outcomes in growth restricted fetuses born in very early gestation.

Definition of IUGR

The concept of growth restriction has been known since 1950. Historically, infants with low birthweight were considered to be born prematurely. After the introduction of ultrasound biometry as tool to date pregnancies based on size of

the fetus in early pregnancy, the term “small for gestational age” (SGA) in a fetus became more accurate. Dating of pregnancy by ultrasound is currently the most reliable method of estimating gestational age, with an accuracy ± 7 days, if the dating is done between the 16 and 20 weeks of pregnancy.[1] The diagnosis SGA is recommended if estimated fetal weight is more than 2SD below the mean of the Swedish reference population.[2]

A more sophisticated method of fetal surveillance in pregnancies with SGA fetuses became possible with Doppler ultrasound. Evaluation of fetal state with Doppler examination of the utero-placental and umbilical circulation forms the base to determine whether a fetus is growth-restricted or not. True IUGR due to placental dysfunction, a subgroup of small fetuses, is associated with abnormal blood flow in the umbilical vessels.[3]

Risk factors for IUGR

Several chronic maternal conditions are recognized to influence fetal growth. Hemoglobinopathies and heart diseases in the mother can result in decreased oxygen supply to the fetus and thereby cause IUGR. Hypertensive disorder in pregnancy with uteroplacental insufficiency is the most important factor causing IUGR.[4] Impaired placental nutrional exchange with resulting IUGR can be of maternal, placental, uterine or fetal origin.[5] Congenital infections, cytomegalovirus and rubella, are associated to IUGR.[6] Environmental factors like cigarette smoking during pregnancy is a major cause of IUGR.[7]

Fetal and placental circulation

The placenta supplies the fetus with oxygen and nutrients. During the pregnancy the placental blood flow adapts to meet the metabolic needs of the growing fetus. Maternal blood from the uterine artery travels via the spiral arteries into the intervillous space. The oxygenated fetal blood returns to the fetus via the

capillaries in chorionvilli to the umbilical vein and flows into the ductus venosus and the inferior vena cava into the fetal right atrium. Oxygenated blood passes the foramen ovale to the left atrium, left ventricle and to the aorta and by that provides the brain with oxygen supply. Fetal deoxygenated blood passes through the right atrium and ventricle to the pulmonary artery past the lungs via the ductus arteriosus and fetal descending aorta to the umbilical arteries and the placenca.

Fetal brain development

Neural development starts early in gestation and the CNS is generated from the ectoderm. By the end of the fourth week after conception the formation of the neural tube is completed. The nervous system contains a network of cells, neurons, that communicate with other cells in synapses by transmitting signals through the axons, and of glial cells, that provide structural and nutritional support. There are different glial cells in the central nervous system: oligodendrocytes generating myelin, a fatty substance, that insulates and wraps around the axons, microglia providing immune-defense[8] and astrocytes providing nutrients to the neurons and involvement in the scarring process and tissue repair.[9]

Dobbing described the period of human brain growth spurt, a transient period of rapid growth of the brain, occuring after midgestation and leveling out at 3-4 years of age, as especially vulnerable to adverse influences. This period is characterized by glial multiplication, myelinisation, and establishment of dendritic and synaptic connections. Neuronal multiplication is completed at midgestation.[10] However, emerging evidence has shown that neurogenesis can also take place in the adult brain, e.g in hippocampus and that the neurons have ability to integrate into the brain and are influenced by environmental factors.[11] More recently, Kostovic et al[12] have described, by using MRI and histochemistry techniques, the organization of cerebral connections in the fetus and preterm infant and they depicted different intensity and pattern of cerebral pathways during development. The axon fibers in

the transient subplate zone feature intensive growth, ”waiting” period and accumulation. Developmental lesions occuring during intense growing phases of axon fibers partly account for the selective vulnerability of the periventricular white matter, while occuring in ”waiting” periods the axons may be re-routed and explain structural plasticity of the developing cortex.

Fetal lung development

Lung development in human fetuses can be divided into four periods; the embryonic, pseudoglandular, canicular and saccular phases. In the early stage of organogenesis, formation of the lung appears as a ventral bud from the future esophagus. After separation from the primitive esophagus, the formation of lobes and segmental portion of the airway tree begins. In parallell with the formation of airways the development of the vascular connection occurs. The pulmonary arteries bud off from the sixth pair of the aortic branches. The pseudoglandular phase begins by the end of the 5th postconceptional week. This stage represents formation of all conducting airways, progenitors of type I and type II cells and the surrounding tissue which will be the future acini of the gas exchanging parenchyma. The canicular phase occurs between the 16th and 26th postconceptional weeks and denotes formation of the lung parenchyma. Lamellar bodies storing surface active material produced by differentiated type I cells occur in this stage. In the beginning of the saccular phase, 24 postconceptional weeks through birth, the conducting airways end in thin-walled sacculas, the last generation of airways. After further growth, the sacculas will eventually form the ducts of alveoli. During this phase further maturation of the surfactant system occurs and the interstitial tissue decreases in favour of the capillary network and thereby enables the gas-exchange over air-blood barriers[13] The number of airway generations is complete at birth, but the alveoli are formed after birth. Postnatal maturation of alveoli is ongoing continuously until the age of 36 months.[14] In the

alveolization phase the lung undergoes remodeling to a mature adult state and thereby the gas exchange surface area increases.

Hemodynamics in IUGR

The technology of Doppler velocimetry has enabled surveillance of high-risk fetuses by recording fetal and placental blood flow. Abnormal uteroplacental perfusion is characterized by increased placental resistance.[3] Postnatal studies of the placentas of IUGR infants with abnormal fetal blood flow have demonstrated reduced vascularization and maldevelopment in the terminal villi compartment.[15-17] These studies have been taken as evidence that abnormal fetal blood flow reflects abnormal placental function. Monitoring of the blood velocity waveform in the umbilical artery (UA) forms the base of assessing fetal well-being and has been demonstrated to reduce mortality and morbidity of the IUGR fetus.[18] Resistance to blood flow is measured as pulsatility index (PI), defined as the peak systolic minus the least diastolic flow velocity divided by the mean velocity over the heart cycle. As placental insufficiency worsens, the end-diastolic flow in the UA decreases and eventually becomes absent or reversed (ARED) (Fig. 1). The ”brain sparing effect” is characterized by increased flow in the fetal middle cerebral artery (MCA) at the expense of blood flow to other tissues, an early adaptive reaction of the fetus to placental insuffiency. The impact of increased flow in MCA on the development of central nervous system (CNS) has been studied to some extent. Some studies demonstrated no associations,[19,20] other either a lower risk[21] or a higher risk of adverse neurodevelopment[22] at follow up. A longitudinal study of MCA flow showed that a high peak systolic velocity (PSV) in MCA predicts mortality better than a low MCA-PI in IUGR fetuses delivered very preterm.[23] Pathological changes in the circulation of growth restricted fetuses develop in a sequential way.[24] Reduced flow in the ductus venosus (DV) during the atrial contraction occurs later and predicts more serious perinatal outcome.[25,26]

Serial Doppler examinations of the umbilical artery, MCA and DV together with assessment of fetal heart rate (FHR) variability, provide information facilitating the clinical decision on when to deliver the IUGR fetus in the second trimester. At present, there is an on-going multicenter randomised clinical trial called TRUFFLE, that investigates three management protocols based on the early or late ductus venosus blood flow changes and short term fetal heart rate variability for indication of intervention in preterm IUGR fetuses.[27]

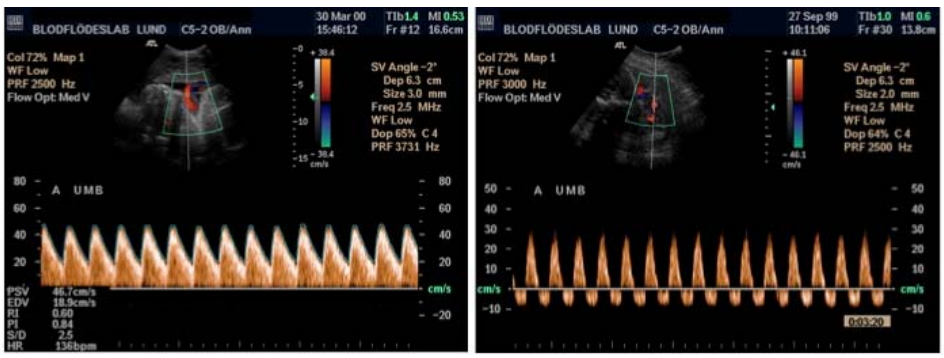

Figure 1. Doppler recording of blood flow velocities recorded from the umbilical artery. Reversed end-diastolic blood flow in the umbilical artery. 1 a) Normal blood flow velocity waveform with positive diastolic velocity and low pulsatility index (0.84) indicating low resistance to flow in the placenta. 1 b) Reversed end-diastolic blood flow velocity.

Neonatal mortality and morbidity in preterm IUGR

High perinatal mortality in IUGR fetuses delivered very preterm has been described by several authors. The survival rates vary from 64-89% in very preterm IUGR fetuses with abnormal fetal blood flow.[28-30] Mari et al found that gestational age at birth and the combination of abnormal MCA peak systolic velocity and reversed blood flow in ductus venosus were the most predictive factors for mortality in IUGR fetuses delivered before 32 weeks.[31]

Further, predictive parameters for adverse neonatal outcome in infants with IUGR and abnormal fetal blood flow were gestational age at birth, birthweight and

abnormal ductus venosus Doppler in a study by Baschat et al.[29] The association with increased neonatal morbidity in infants born preterm with IUGR and abnormal fetal blood flow has been described in many studies: necrotizing enterocolitis (NEC),[28,32] chronic lung disease (CLD)[33,34] and retinopathy of prematurity (ROP).[35]

Neurodevelopment and IUGR

Preterm birth is associated with neurodevelopmental disabilities in childhood. Several studies have shown that children born very preterm are at risk for impairment in cognitive and neuromotor skills, and have educational and behavioral problems[36-39] The neurodevelopmental deficits seem to be correlated to degree of immaturity at birth. Adverse neurodevelopmental outcome in children born moderately preterm or term with IUGR and abnormal fetal blood flow has been described by several authors.[40-42] Fattal-Valevski et al demonstrated a relationship between postnatal somatic growth and neurocognitive outcome in children born IUGR at near term.[43] Torrance et al found that low birthweight, acidosis and placental villitis in growth restricted fetuses with abnormal fetal blood flow in the umbilical artery were predictive for adverse neurodevelopment.[44] Neurodevelopmental follow-up studies are sparse in very preterm IUGR children at higher ages. Kaukola et al showed suboptimal neurodevelopment in one-year old children born very preterm and subjected to abnormal blood flow in *utero*.[45] Strong correlation between abnormal fetoplacental blood flow and poorer cognitive outcome in 3-6 years old children born after 28 GW was found by Kutschera.[46] Reversed but not absent end-diastolic flow in the UA was associated with lower IQ in a study by Schreuder et al.[47] MRI-studies in preterm IUGR neonates have documented specific brain patterns with reduced cerebral grey matter volumes[48] and decreased volume of the hippocampus[49] correlating to less mature behaviour (e.g attention-interaction ability). A recent study by Padilla et al

showed that grey matter volumes in preterm IUGR infants examined at 1 year of age were reduced in several brain regions compared to AGA preterm and term controls.[50]

Lung development and IUGR - experimental studies

Animal studies suggest several mechanisms that may be involved in the adverse effect of IUGR on lung development. Hypoxia in newborn mice interfered with alveolar and pulmonary artery development mediated by inhibition of transforming growth factor (TGF)-beta.[51] Umbilico-placental embolisation in sheep did not affect the size of the fetal lung but rather resulted in structural alterations like smaller number of alveoli, thicker interalveolar septa and by that thicker pulmonary blood-air barrier in IUGR offspring.[52] Furthermore, IUGR lambs exhibited smaller lung capacity, reduced lung compliance and increased chest wall compliance compared to controls at postnatal follow up.[53] In an IUGR rat model the authors found decreased gene-expression for formation of elastin, essential for normal alveolar development, decreased elastic fiber deposition and increased lung compliance.[54] Lung liquid, lung growth and surfactant protein expression was unaltered in IUGR fetal lambs compared to controls.[55] It appears that in animals, the structural alterations within the lung following IUGR have a greater functional impact than just the reduced lung size.

Postnatal follow up of lung function

Barker described the association between low birth weight (LBW) and impaired long-term respiratory health.[56] Numerous studies have described impaired lung function and/or increased respiratory morbidity during infancy,[57] childhood[58-61] and adolescence[62] after preterm birth. Furthermore, follow-up studies have shown higher incidence of asthma in children born preterm.[63,64] In a large Swedish national cohort study, prescription of asthma medication was significantly more

frequent among young adults with a history of extreme preterm birth.[65] Other studies showed evidence of higher respiratory morbidity and reduced lung function in children and adolescents born preterm with a history of chronic lung disease (CLD) as compared to preterm subjects with no previous CLD.[66,67] The adverse effect of pre- and postnatal exposure to smoking on respiratory health has been demonstrated.[68,69] In the EPICure study, a population based study of survival and follow up of infants born before 26 weeks in England, 50 % of the extremely preterm children had impaired lung function at 11 years of age.[61]

The knowledge of the impact of IUGR and extreme prematurity on long term lung function and morbidity is limited. IUGR in infants born at term has been associated with reduced lung function in infancy[70] and in childhood.[58,71] In contrast, Matthes et al found no association between LBW at term and reduced lung function in adolescence.[72] IUGR infants born preterm had higher airway resistance than preterm AGA children.[73] However, the IUGR infants were born at higher gestational age than the control AGA group of infants. In contrast, reduced lung function was not associated with IUGR in a population based cohort study of very LBW preterm children at 8 years of age.[74]

IUGR and cardiovascular morbidity

An increasing body of literature has been published on the associations between low birth weight and cardiovascular dysfunction and morbidity later in life referring to the concept of “fetal origin of adult disease” (FOAD). The hypothesis postulates that adverse events occuring during early development in fetal life (*i.e* growth restriction) might permanently altering the patterns of growth and organ development in the adult offspring. This process has been called “fetal programming”. Fetal adaptation may cause alterations in arterial function that predispose for cardiovascular diseases in later life. Alterations in the

cardiovascular system involve the renal function, alteration in vascular structure and function as well as sympathetic regulation.

Many experimental studies, using models of nutritional deprivation or decreased utero-placental perfusion resulting in LBW, have investigated the developmental programming hypothesis. Nutritional deprivation in pregnancy resulting in increased BP in the offspring has been associated with an up-regulation of the renin-angiotensin system (RAS),[75] reduced nephron numbers[76] and increased circulating catecholamines suggesting elevated sympatho-adrenergic activity.[77] Increased sympathetic hyperinnervation have also been reported in animals exposed to hypoxia.[78] Alterations in the hypothalamus-pituitary-adrenal (HPA) axis, the neuro-endocrine system involved in mediating stress responses, have been described in an animal IUGR model.[79]

Experimental studies support the fetal origin hypothesis postulated by Barker, who described the link between LBW and hypertension and coronary heart disease (CHD) in epidemiological studies.[80,81] Precursors of atherosclerosis, increased IMT and endothelial dysfunction, constitute a risk for cardiovascular disease (CVD) and have been associated with LBW.[82,83] Carotid IMT was increased in children born to mothers with low energy intake during pregnancy.[84] There is a clear link between IUGR born at near-term or term and impairment of endothelial function.[83,85-87] However, this association has not been demonstrated in subjects born preterm[87] IUGR offspring of rats exposed to reduced uteroplacental perfusion had impaired endothelium impairment and higher arterial BP.[88]

In children born extremely preterm, brachial blood pressure was not elevated at 6 and 11 years old compared to matched controls.[89,90] In contrast, although studied in subjects at older ages, two large epidemiologic studies have reported on associations between elevated BP/hypertension and preterm birth; a negative correlation between gestational age and BP was demonstrated in a study by Johansson et al,[91] and the other study showed a strong correlation of prescription

of antihypertensive medication and preterm birth.[92] Other studies that support the link between preterm birth and alterations in BP are those by Bonamy et al, who reported a higher BP in adolescent girls born preterm[93] and Feldt et al who found associations between BP reactivity to stress and gestational age at birth[94] Growth in infancy has been reported to have an impact on later cardiovascular morbidity in LBW children. Rapid weight gain in individuals born with LBW near term has been linked to arterial hypertension.[95,96] Whether IUGR in individuals born very preterm adds to later cardiovascular health remains unclear.

Aims of the study

IUGR caused by utero-placental insufficiency in very preterm fetuses might have a harmful influence on subsequent outcome in a wide range of physiological functions. It is unclear whether growth restriction prior to very preterm birth modifies or accentuates the risks of prematurity.

The specific aims of the present studies were:

To evaluate the effect of IUGR with abnormal umbilical artery blood flow in very preterm fetuses delivered on fetal indication on:

- Mortality, neonatal morbidity and outcome up to two years of age.
- Cognitive and behavioral outcome at 5-8 years of age.
- Lung function at 6-11 years of age.
- Cardiovascular outcome at 5-8 years of age.

Subjects and study design

Index (ARED) group in study I

During the period 1998-2004, 46 IUGR fetuses (36 singletons and 10 twins) with ARED flow in the umbilical artery before 30 gestational weeks (ARED group) were managed and delivered at the Department of Obstetrics and Gynecology in Lund - a level 3 perinatal center. Paper I describes retrospectively the mortality, neonatal morbidity and neurological outcome at 2 years of age in this cohort compared to controls. Inclusion criteria were estimated fetal weight more than 2 SD below the mean of the Swedish reference population,[2] no malformation or abnormal karyotype known before birth, and no twin-to-twin transfusion syndrome. Gestational age was determined by routine ultrasound fetometry at 17-18 postmenstrual weeks. In seven fetuses the ultrasound estimated gestational age was found to be ≥14 (range 14-49) days shorter than gestational age according to the last menstrual period. Of the 46 fetuses with IUGR and ARED flow in the umbilical artery, four were stillborn. In three of the cases, parents declined active management and in the fourth case the mother was pregnant with twins and admitted at 22+4 weeks with severe preeclampsia and heart failure. The first twin had absent end-diastolic flow while the other twin had normal umbilical artery flow. On behalf of the twin with normal blood flow, decision was taken not to intervene.

All fetuses in the index group had absent or reversed diastolic blood flow in the UA at admission, assessed by Doppler ultrasound recordings of flow velocities. Fetal and placental circulation was also monitored by recording flow velocities in

the umbilical vein, the fetal MCA, the ductus venosus and the maternal uterine arteries. Flow in the umbilical vein was considered abnormal if pulsations occurred. Redistribution of fetal blood flow was defined as the MCA PI > 2 SD below the gestational age-related mean.[97] Abnormal ductus venosus flow was defined as absent or reversed flow during the atrial contraction (a-wave). To assess flow in the uterine arteries a scoring system according to Hernandez-Andrade et al was used.[98] All fetuses were delivered by a cesarean section and in all cases the mothers had received at least one dose of 12 mg Betamethason for acceleration of fetal lung maturation. Indications for delivery were deterioration of fetal circulation assessed by Doppler ultrasound and/or severely abnormal FHR tracing.

Control groups in study I

Two control groups were identified for study I. Control group A (n=399) consisted of all other infants born in Lund before 30 gestational weeks during the corresponding time period (1998-2004). Of these, 24 infants were stillborn and 4 died in the delivery room. 371 infants were admitted to the neonatal intensive ward. Control group B (n=42), a subgroup of group A, comprising liveborn infants admitted to the neonatal intensive ward, was selected to be the control group to the index group in the prospective follow-up studies (papers II-IV). Group B was matched to the index group for gender, gestational age and year of birth. All infants of group B were born appropriate for gestational age (AGA). Twins formed a matched pair if one twin was IUGR with ARED flow and the other twin was AGA of same gender with normal blood flow in the UA. In seven cases the co-twin served as a matched control.

Subjects in Studies II-IV

Study population

From the original study with 42 liveborn infants with IUGR and ARED, admitted to the neonatal intensive ward, four infants died during the hospital stay. Of the surviving 38 infants, 34 children (18 boys and 16 girls; 27 singletons and 7 twins) were available for follow-up examinations. The gestational age at birth was median (range) 26+6 (24-29) GWs.

Table 1. Number of children who participated in the follow-up studies and the mean age at assessment.

Study	Median age, years (range)	PT-IUGR n	PT-AGA n	PT-AGA n
WPPSI/WISC	7.1 (5-8)	14/20	14/20	14/20
Spirometry	8.4 (6-11)	31	31	31
Exhaled NO	8.8 (6-11)	19	21	24
Laser Doppler	7.2 (5-8)	32	32	32
Carotid artery diameter	7.2 (5-8)	25	27	27
Aortic diameter	7.2 (5-8)	22	23	19
Popliteal artery diameter	7.2 (5-8)	26	24	25
Intima media thickness	7.2 (5-8)	31	30	32
Echocardiography	7.2 (5-8)	27	21	21

WPPSI= Wechsler Preschool and Primary Scale of Intelligence. WISC=Wechsler Intellegence Scale for Children. NO=Nitric Oxide. PT-IUGR=preterm IUGR and ARED. PT-AGA=preterm with birth weight AGA. T-AGA=term with birth weight AGA.

Control infants

Two matched control groups were selected for the follow-up studies. One group of preterm infants with AGA birth weight (PT-AGA) and one group of AGA infants born at term (T-AGA). The children in the the PT-AGA group were the infants of the control group B in study I. They were matched for gender, gestational age at

birth and year of birth. The T-AGA group was matched for gender and year of birth. All infants in the T-AGA group were vaginally delivered after an uncomplicated pregnancy and they had Apgar scores >7. Table 1 shows the numbers of children who participated in the various studies.

Methods

Study I

Fetal monitoring

As previously mentioned, all fetuses in the ARED group had absent or reversed end-diastolic blood flow in the umbilical artery before delivery and an estimated fetal weight more than 2 SD below the mean of the Swedish reference population.[2] Fetal and placental circulation in the ARED group were assessed by recording flow velocity signals from the umbilical artery, the umbilical vein, the middle cerebral artery, the ductus venosus and the maternal uterine arteries. Doppler velocimetry was performed transabdominally using either an Aspen (Acuson, Mountain View, CA, USA) or a HDI 5000 (Philips Medical Systems, Bothell, WA, USA) ultrasound system. All recordings were performed during voluntary maternal apnoe and during periods of absent fetal breathing and movements. Redistribution of fetal blood was defined as MCA pulsatility index > 2 SD below the gestational age-related mean.[97] Abnormal ductus venosus flow was defined as absent of reverse flow during the a-wave. Flow in the umbilical vein was considered abnormal if there were pulsations present. Flow velocity waveforms in the uterine arteries were assessed using a scoring system by Hernandez-Andrade et al.[98] Indication for delivery was detoriation of fetal circulation. In three cases there was an additional maternal indication (preeclampsia). Maternal and obstetric characteristics of the three groups are shown in Table 2. For further details see paper I.

Data collection

In the first study, all data from pregnancies and from neonatal intensive care were collected retrospectively from the obstetric and pediatric patient records, from the database of perinatal data in the Southern Swedish Health Care Region (Perinatal Revision South Database) and from the Regional Registry of Cerebral Palsy.

Table 2. Maternal and obstetric characteristics of cases with liveborn infants

Characteristic	ARED Group (n=42)	Group A (n=371)	Group B (n=42)	*p-value*
Maternal age (years)	31 (20-42)	31 (16-43)	31 (17-43)	*ns*
Primiparas	19 [45]	216 [58]	22 [52]	*ns*
Cesarean section	42 [100]	249 [67]	26 [86]	*<.001*, <.001§*
Antenatal steroids	40 [95]	*na*	36 [86]	*ns*
Pre-eclampsia	16 [38]	44 [12]	3 [7]	*<.001*, <.001§*
Chorioamnionitis	0 [0]	*na*	8 [19]	*<.01*[§]
PPROM	0 [0]	133 [36]	13 [31]	*<.001*, <.001§*

Data expressed as median (range) or *n* [%]. ARED Group, index group with absent or reversed end-diastolic umbilical artery blood flow; Group A, all other infants born < 30 gestational weeks during 1998-2004; Group B, appropriate-for-gestational age infants matched for gestational age, gender and year of birth. *ARED Group vs Group A; [§]ARED Group vs Group B. na= data not available; ns=not significant. PPROM=preterm premature rupture of membranes.

Outcome variables

The perinatal outcome was assessed by perinatal mortality, birth weight, birth weight deviation from the expected weight according to the Swedish standard (as a percentage of the expected mean),[2] Apgar score at 5 minutes, umbilical cord arterial pH and base excess. Parameters of the respiratory and circulatory status during the first 12 hours of postnatal life were recorded. Data on the following neonatal morbidities were recorded; prevalence of persistent ductus arteriosus, (when medical or surgical treatment was needed), necrotizing enterocolitis, (when

an infant presented with signs of abdominal distension, elevated c-reactive protein and either signs of intramural gas on X-ray or need for surgical intervention), intraventricular hemorrhage grade III-IV according to Papile et al.[99] and cystic periventricular leukomalacia detected by cranial ultrasound imaging, chronic lung disease, (defined as need for extra oxygen at 36 postmenstrual weeks) and retinopathy of prematurity (ROP) stage 3-5. Intraventricular hemorrhage grade III-IV and cystic periventricular leukomalacia were both considered as severe brain damage. Survival and prevalence of cerebral palsy at 2 years of age were registred.

Methodological considerations

There are some limitations to this study. The fetuses in the control groups, with few exceptions, had not been monitored with fetal Doppler velocimetry, thus, no data were available on the fetal and placental circulation during intrauterine life. There was some infants in the background population that had SGA birth weight. However, all infants in the control group B had AGA birth weight and thus, abnormal umbilical artery blood flow was less probable. A mixture of singletons and twins in the groups may have influenced the results. However twins were matched carefully as described previously.

Study II. Cognitive tests

WPPSI and WISC

For 42 children (14 matched triplets) aged 60-83 months, the Wechsler Preschool and Primary Scale of Intelligence-III was used, whereas the Wechsler Intelligence Scale for Children-III, 1991 revision, British version was applied for 60 children (20 matched triplets), aged 84-105 months. Both tests consist of 2 IQ subscales (verbal IQ (VIQ) and performance IQ (PIQ)) and of full-scale IQ (FSIQ); all scales have a mean of 100 points and SD of 15. Cognitive impairment was considered when FSIQ was <70 (> 2 SD below the normative mean).

Behavior questionnaire

Two scoring questionnaires regarding prevalence of attention-deficit disorder (Brown´s ADD scales)[100] and of behavioral problems (Strengths and Difficulties Questionnaire, SDQ)[101] were filled out by the parents. Brown´s ADD consists of 44 items and examines the ability to sustain attention, activate and organize work tasks, sustain energy and effort to complete tasks, to regulate moods, use short-term working memory, and to recall learned material. A point summary of > 55 indicates attention and deficit disorders. The SDQ comprises 25 items divided into 5 subscales (prosocial, hyperactivity, emotional symptoms, conduct problems and peer problems). The total behavioral deviance score is calculated from the subscales with the exception of prosocial subscale. A total score of > 13 is considered to be borderline or high.

Methodological considerations

In the study protocol we had originally decided to perform cognitive evaluation with WISC-III at 7 years of age. Under the study period it became apparent that some of the children had already been assessed with WPPSI-III in the regional clinical follow up at 5 years of age. For that reason we had to assess the matched counterparts with the same test and at the same age. Correlation coefficents (*r*) between the two tests were previously determined to be 0.85, 0.73 and 0.85 in VIQ, PIQ and FSIQ, respectively.[102] The matched children were always assessed within 2 months and by the same psychologist as the index children.

Study III. Lung function tests

Exhaled nitric oxide

To assess airway inflammation, fractional exhaled nitric oxide (NO) was measured using the NIOX Mino (Aerocrine, Stockholm, Sweden). The NO measurements took place before the spirometric measurements.

Spirometry

Pulmonary function was measured using a spirometer (Vitalograph 2170; Spirotrac IV, Ennis, Ireland). Spirometry was performed in 31 children of PT-IUGR group at 8.3 (6.4–10.6) years. The matched controls were examined within ± 2 months of the examination of the index children. None of the children showed clinical signs of infection at the time of examination. The children were tested in a standing position breathing through a mouthpiece. No nose clips were used. One experienced nurse with special training, blinded to the children's background, performed the measurements according to ATS⁄ERS standard.[103] The maneuvers were repeated for at least three but maximal eight times to achieve the best measurements. Respiratory function was assessed from the measurements of forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV_1), FEV_1⁄FVC and forced mid-expiratory flow rate ($FEF_{25-75\%}$). The measurements were expressed as z-scores and were adjusted for age corrected for any prematurity, height and gender according to Stanojevic.[104] Eighty-five children were evaluated before and after bronchodilatation by two inhalations of 0.2 mg of salbutamol from a dry powder inhalator (Ventoline DiskusGlaxoSmithKline, Solna, Sweden). Reversible airway obstruction was considered present when FEV_1 increased > 10% after bronchodilatation.

Methodological considerations

In clinical practice it is common to consider values below the predicted mean -1.96 SDS as abnormal in order to prevent over-diagnosis of disease. We wanted to have sensitive tools to detect differences. It is common scientific practice to define values as abnormal if they are expected to be found in 5% of the population or less, accordingly, we defined the lower limit of normal as the predicted mean -1.64 SDS. A 10% increase in FEV_1 was regarded as a significant bronchodilator response (BDR) after inhaled salbutamol (based on the criteria of the two best FEV_1 values within 5%). Different cut-off levels for a significant BDR have been

suggested.[105] The greater the response, the more likely the patient is to have reversible airway disease. Cut-off levels will always be a trade-off between specificity and sensitivity. Spirometry was performed during a wide range of age. The younger children may have been disadvantaged in following instructions of the examiner and thus age at examination could be a confounding factor, although Eigen et al found that spirometry was reproducible in healthy preschool children.[106]

Questionnaire

A short questionnaire on respiratory illness was completed by the parents after the pulmonary function test. Patient medical records were also reviewed. Information on family history of allergy, transient or current wheezing disorder, the use of inhaled corticosteroids and exposure to tobacco smoke was collected. Children who had more than three episodes of airway obstruction and⁄or required bronchodilators or inhaled corticosteroids were considered to have wheezing disorder. Wheezing disorder was considered current if it had occurred during the last 12 months. History of previous positive skin prick test to pollen, mites or house pets was considered as presence of atopy.

Study IV. Cardiovascular examinations

Laser Doppler Flowmetry (LDF)

Microvascular endothelial function in the forearm was examined by using LDF and transdermal iontophoresis (Periflux 5000, Perimed AB, Järfälla, Sweden), a non-invasive method to introduce vasoactive drugs over the skin barrier by using a low-intensity electric current. Acetylcholine (ACH) (Sigma-Aldrich AB, Stockholm, Sweden) – an endothelial-dependent vasodilator, and sodium nitroprusside (SNP) (Sigma-Aldrich AB, Stockholm, Sweden) – an endothelial-independent vasodilator, were used. All investigations were performed by one of

the authors (EM) in a quiet room with temperature 23°C. Exercise was avoided for at least 4 hours prior to the examination. None of the children were on medication at the time of examination. The subject was examined sitting with the left arm fixed on a pillow and had rested for 15 minutes before the start of measurement. The LDF method has been described in detail by Morris et al.[107] Briefly, a drug-delivery probe (PF 383, Perimed AB) was placed on the volar side of the distal forearm. An indifferent electrode was attached to the upper arm. The temperature of the probe was 32°C. Iontophoresis was performed with 0.18 mL 1% ACH and 0.1% SNP, respectively, diluted in physiological saline. An electric field was created by a constant current of 0.1 mA. Skin perfusion was first measured for two minutes to assess the baseline value. To obtain a dose response blood flow, current was delivered for 20 seconds and repeated 6 times at 60- seconds interval (anodal, ACH) or at 180-second interval (catodal, SNP). Blood flow data (perfusion units, PU) were recorded continuously. Area under the curve (AUC), and the mean and maximum responses were computed digitally.

Intima media thickness

A high-resolution ultrasound system (Acuson Sequoia C512, Siemens AG, Erlangen, Germany) equipped with a 15 MHz transducer was used. Longitudinal two-dimensional scans of the 1 cm long distal end of the left common carotid artery were imaged so that the lumen-intima and media-adventitia interfaces were distinguishable. All images corresponded to the R-wave on electrocardiogram (ECG). In short, four scans obtained from each individual were recorded on videotape for off-line analysis of intima-media thickness (IMT). The mean carotid IMT of four measurements along a 1-cm segment was calculated from each scan. Mean IMT values obtained from all scans from the same subject were averaged.

Arterial diameter and distensibility measurements

Dynamic properties of large arteries were assessed by echo-tracking ultrasonography. This technique provides, in addition to vessel diameters, indirect

measures of arterial elastic properties derived from diameter changes of arteries in response to the change in distending pressure. We used an echo-tracking system (Diamove, Teltec AB, Lund. Sweden) capable of detecting vessel wall movements of <10 μm and with a time resolution of 1.15 ms.[108] The echo-tracking instrument measures the instant distance between vessel walls perpendicular to the longitudinal axis of the vessel.[109] Details of the technique and measurement procedure have been described previously.[110] Three consecutive recordings of pulsatile diameter changes were obtained from the abdominal aorta (AA), the common carotid artery (CCA), and the popliteal artery (PA). The vessel diameters were adjusted for body surface area (BSA).[111] The AA was insonated from the epigastrium, and the measurements were performed below the xiphoid process. The CCA was insonated from behind the sternocleidomastoid muscle, and measurements were performed 1 cm proximal to the bifurcation. Measurements of the PA were performed in the central part of the popliteal fossa. Arterial blood pressure was measured by a digital blood pressure monitor (OMRON, Boso medicus, Jungingen, Germany) on the right arm at baseline and after each recording of pulsatile diameter changes. Pulse pressure (ΔP) is the difference between the systolic and diastolic blood pressure. Stiffness (β)[112] was defined as:

$$\beta = \ln(Psyst/Pdiast)/(Dsyst-Ddiast)/Ddiast,$$

where Psyst and Pdiast are the maximum systolic and diastolic pressures in mm of mercury, respectively, and Dsyst and Ddiast are the corresponding vessel diameters in mm. Stiffness (β) was calculated off-line from the diameter curves; a high value of β denotes a stiffened arterial wall. The coefficients of variation for repeated vessel diameter measurements were 5.7 %, 4.1 % and 5.5 % for the AA, CCA and PA, respectively. The corresponding coefficients of variation for stiffness of the AA, CCA and PA were 9.8%, 11.5% and 12.3%, respectively. The variabilities in the blood pressure measurement were 3.3% and 3.5% for systolic and diastolic blood pressure, respectively. Distensibility coefficient (DC), the

relative increase of arterial cross-section area for a given increase in pressure,[113] was calculated off-line:

$DC= (2\Delta D*D + \Delta D2) / (\Delta P*D2)$

ΔP is pulse pressure in kPa, D is the minimum diastolic diameter in mm, ΔD is pulsative diameter change in mm.The unit for DC is 10-3/kPa.

Echocardiography

Echocardiography was performed by two cardiologists using Acuson Sequoia C 512 (Siemens AG, Erlangen, Germany). They were blinded to the children's background. The end-diastolic thickness of the interventricular septum (IVST) and left ventricular posterior wall (LVPWT), the end-diastolic diameter and area of left atrium, and the diameter of proximal ascending aorta (valve annulus and sinotubular junction) were all measured in parasternal long-axis view, and thereafter adjusted for BSA.[111] Left ventricular shortening fraction (LVSF) was derived from the end-systolic and end-diastolic diameters of the left ventricle. The left ventricular mass (LVM) was estimated according to the American Society of Echochardiography recommendations, which assumes LV as a prolate ellipse of revolution:[114]

$LVM =0.8*\{1.04[(LVD+LVPWT+IVST)^3-(LVD)^3]\}+0.6$ g

LVM index was calculated as LVM (g) divided by height (cm). Doppler studies were performed to assess LV inflow and outflow; a 4-chamber view of the heart was acquired to obtain signals of flow parallel to ultrasound beam (within 15 degrees). Doppler spectra of mitral inflow were recorded with the sample volume placed at the level of the mitral valve leaflets at which the velocity was maximal. The peak velocity of early (E) and late (A) diastolic filling, E/A, deceleration time of the E wave (DT), and isovolumic relaxation time (IVRT) were derived from the mitral inflow recordings.

Methodological considerations

The cardiovascular assessments were difficult to perform in some of the children. The investigations are time-consuming and children found it difficult to lie or sit still resulting in poor technically satisfactory recordings and analysis had to be discarded. Other children refused to be examined. The success rate was 72 % in the echocardiography assessment. In the arterial measurements of the CCA, AA and PA the success rates were 82%, 67% and 78 % respectively

Data collection and analysis

All data on pregnancies and neonatal intensive care were collected retrospectively from the perinatal registry of the Southern Swedish Health Care Region (Perinatal Revision South) and from the obstetric and pediatric patient records. Information on socioeconomic factors was obtained from questionnaires given to the parents when children attended the tests. Anthropometric measurements (height and weight) were performed by a pediatrician (EM) at two different occasions. Height, weight and body mass index (BMI) were expressed as z-scores estimated from the growth curve of the Swedish reference population.[115]

The study was approved by the Regional Research Ethics Committee of the University of Lund, and the examinations of children were performed after their parents gave written informed consent.

Statistical analysis were performed using SPSS statistical software, version 15.0, 16.0 and 18.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were compared between the groups by using the Chi-square test or Fishers exact test, as appropriate. Group differences in continuous variables were assessed with Mann-Whitney *U* or ANOVA with *post hoc* Bonferroni. P-values < 0.05 were considered statistically significant. Confounders were explored by using general linear model, binary logistic regression or linear regression analysis as appropriate.

Results and comments

Neonatal mortality and morbidity (study I)

In the first study we described the outcome of IUGR fetuses with absent or reversed end-diastolic flow (ARED) delivered on fetal indications before 30 gestational weeks. The ARED group was compared to the background population and a matched control group born with AGA birth weight during the same time period and at corresponding gestational weeks as described in the method section. Characteristics at birth and during the first 12 hours of postnatal life are presented in Table 3. The IUGR fetuses had lower birth weight, increased birth weight deviation and lower placental weight than the control groups. Apgar score at 5 min was higher and base excess lower at birth in the ARED group compared to the background population. Postnatal requirement of oxygen during the first 12 hours, was lower in the ARED group compared to the infants in the control groups. Mortality and neonatal morbidity did not differ between groups with the exception of CLD that was more prevalent in the ARED group compared to the control groups (p=0.001 and 0.03, respectively). Survival rate and prevalence of cerebral palsy at 24 months of age was similar in the three groups.

The findings that the ARED group had higher Apgar scores and lower base excess in the umbilical artery might reflect the active management to deliver the IUGR fetuses before deterioration by cesarean section. We did not have data on the antenatal state of the fetuses in the control groups; some fetuses of the Control group A were delivered vaginally and some by cesarean section. As data on antenatal steroids were missing in the background population, no conclusion can be drawn on how well prepared the fetuses were regarding respiratory function

after birth and this may possibly explain the higher oxygen requirement during the first 12 hours of postnatal life in the background population. The higher rate of CLD in the ARED group is remarkable considering the lower early postnatal requirement of supplemental oxygen.

Table 3 Infant characteristics at birth and morbidity during first 12 hours of postnatal life.

Characteristics	ARED group	Control group A	Control group B	*p*
Number of subjects	42	371	42	
Gestational age (weeks + days)	27+1 (24 to 29)	27+1 (23 to 29)	26+6 (24 to 29)	*ns*
Male gender	21 [50]	202 [54]	21 [50]	*ns*
Birth weight (g)	642 (395-1165)	945 (466-2590)	1015 (660-1790)	*<.001*,<.001*[§]
Birth weight deviation (%)#	-37 (-64 to -21)	-10 (-57 to 72)	-4 (-18 to 14)	*<.001*,<.001*[§]
Placental weight (g)	240 (130-830)	380 (150-1045)	390 (200-830)	*<.001*,<.001*[§]
Umbilical artery pH	7.29 (7.02-7.43)	7.30 (6.76-7.51)	7.34 (6.79-7.51)	*ns*
Umbilical artery BE (mmol/L)	-2.9 (-16.0 to 8.7)	-4.2 (-26.8 to 4.0)	-3.7 (-25.4 to 0.0)	*<.05*, ns*[§]
Apgar score at 5 min < 7	7 [17]	124 [33]	9 [21]	*<.05*, ns*[§]
Surfactant treatment	24 [57]	228/371 [61]	26 [62]	*ns*
Lowest FiO_2	0.21 (0.21-0.36)	0.21 (0.21-1.0)	0.23 (0.23-1.0)	*<.05*, <.01*[§]
Highest FiO_2	0.4 (0.21-1.0)	0.43 (0.21-1.0)	0.52 (0.21-1.0)	*<.05, <.05*[§]
Lowest BE (mmol/L)	-5.5 (-10.4 to 7.2)	-5.1 (-29.5 to 8.3)	-6.0 (21+6 to 7.2)	*ns*
Lowest mean arterial pressure (mmHg)	27.0 (18-39)	26.5 (10-41)	26.5 (18-38)	*ns*

Data expressed as median (range) or n [%]. #Calculated as percentage of the expected birth weight according to Swedish standard. * ARED group vs Control group A, [§] ARED group vs Control group B. ns=non significant. BE=base excess

The study by Laughon showed a strong association between IUGR and development of CLD even with little exposure to oxygen in the first two postnatal weeks in very preterm infants.[116] In contrast to our study, Voβeck et al reported on higher mortality, higher rate of ROP and NEC and increased risk of severe motor impairment at follow-up in infants born with ARED before 30 gestational weeks

compared to gestational age matched controls.[117] The same study showed higher incidence of CLD, in accordance with our study which has also been described by others.[28,33] A population-based case-control study of infants born 1983-90 in western Sweden showed an association between BW SGA and cerebral palsy in infants born at term but failed to show such an association in subjects born preterm.[118]

Other published studies, comparable to our have reported on lower survival rates in infants born very preterm with ARED flow.[28,29,117] The high survival rate in the ARED group was similar to that of the background population which suggests that early intervention was beneficial. Perinatal mortality and survival rate at 2 years of age are described in Table 4.

Table 4 Perinatal mortality and outcome at 2 years of age

	ARED group	Control group A	*p*
All infants	46	399	
Stillborn	4 [9]	24 [6]	*ns*
Neonatal deaths in delivery room	0 [0]	4 [1]	
Neonatal deaths of infants admitted to NICU (0-6 days)	0 [0]	27 [7]	
Perinatal mortality	4[(9]	55 [14]	*ns*
Liveborn infants	42	375	
Survival to home discharge	38 [90]	326 [87]	*ns*
Survival at 2 years of age	38 [14]	34/321 [11]	*ns*

Data expressed as n [%]. NICU, neonatal intensive care unit. *ns*, not significant

Cognitive and behavioral outcome (study II)

Cognitive outcome

The main findings in this study were that cognitive outcome in terms of full-scale IQ and verbal IQ in the PT-IUGR group was lower in comparison to the PT-AGA

group (p=0.007 and 0.003, respectively) and to that of the T-AGA group (p<0.001 and 0.001, respectively). The PT-AGA group had lower full-scale IQ and performance IQ than the T-AGA group (p=0.002 and 0.001, respectively). There was a clear gender-related difference in the results - the group differences were only apparent between boys in the preterm groups. Cognitive impairment, defined as full-scale IQ < 70 was more prevalent in the PT-IUGR group than in the PT-AGA group (p=0.011) Effects of risk factors, chronic lung disease, septicemia, postnatal steroid treatment, level of parental education were assessed by using logistic regression analysis. PT-IUGR remained a significant risk factor after taking these confounding variables in account. Gestational age per se did not have an impact on full-scale IQ in either preterm group. The impact of IUGR on cognitive outcome did not differ according to gestational age at birth (Fig. 2).

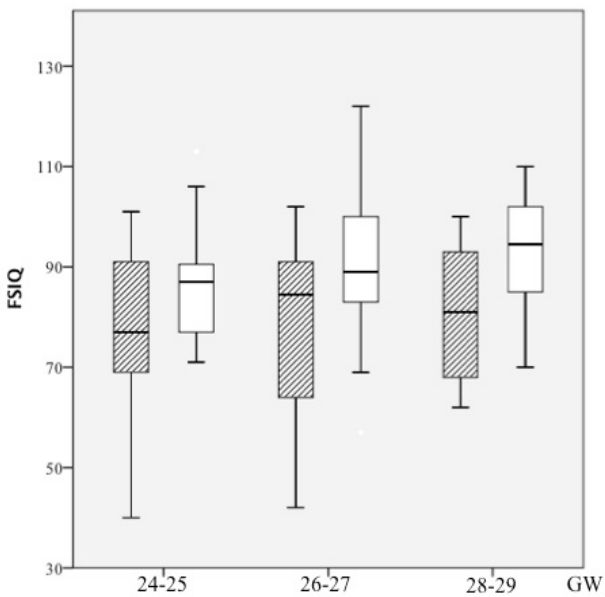

Figure 2. Distribution of Full-scale IQ (FSIQ) according to gestational age in the respective preterm group. Filled boxes represent the Preterm-IUGR group. Open boxes represent the Preterm-AGA group. GW; gestational weeks. Boxes represent medians, quartiles and the 2.5 and 97.5 percentiles.

The finding that gestational age was not associated with cognitive outcome in the

present study is in contrast to others.[20,119] In other studies, neonatal morbidity including CLD and postnatal steroid treatment, and head circumference at the time of assessment have been related to cognitive outcome in preterm children.[120-122] These associations were not found in our study. To our knowledge this is the first study to describe a gender-difference in cognitive outcome in children born preterm with IUGR and ARED. Unfavourable neurological outcome in males born preterm has been described previously.[123,124]

Less tissue damage after hypoxia-ischemia in female animals[125] and lower volumes of white matter, as detected by MRI, in boys born preterm compared to boys born preterm have been described.[126] These studies implicate an increased susceptibility in male gender.

Behavioral outcome

Scores from Brown´s ADD scales and behavior scales according to SDQ did not differ between the preterm groups. However, the PT-IUGR group scored higher in Brown´s ADD and had a higher total behavior deviance score than children in the T-AGA group. There was a negative correlation between FSIQ and Brown´s ADD and SDQ ($p<0.001$ and <0.001, respectively).

A higher risk of inattention-hyperactivity in children born SGA after 29 GW was demonstrated by Guellec et al[127] who also reported on the association between SGA and lower cognitive function. In children born SGA before 29 GW the behavioral and inattentive difficulties were equal to those born AGA. The Helsinki study of very-low-birth weight adults reported on associations between SGA and behavioral problems and emotional instability but not with inattention deficits in adults born preterm SGA.[128] Findings of reduced volumes of hippocampus[49] and cortical grey matter[48] and less mature infant behavior in preterm IUGR subjects have been described earlier. Hippocampal volumes have been linked to deficits in every day memory in adolescents born preterm.[129]

Outcome of lung function (study III)

Nitrix oxide measurements

Incidence of allergy and exhaled NO values were similar in the groups. The results are in accordance with the study by Lum et al[130] which show that allergic asthma is not essential in the lower lung function observed in subjects with preterm birth.

Spirometry

There was a trend for the PT-IUGR group having the lowest and the T-AGA group the highest spirometric values respectively. However, there was no significant difference between the two preterm groups. We could not find any gender difference in spirometric values. Gestational age and weight deviation at birth within the preterm groups were associated with lower values of $FEF_{25-75\%}$. The subgroup of PT-IUGR children with a gestational age at birth $\geq$ 26 weeks had significantly lower values of $FEF_{25-75\%}$ than PT-AGA children of corresponding gestational age at birth (p=0.008). Respiratory impairment (z-score of $FEF_{25-75\%} \leq$ -1.64) occured independently of gestational age at birth in the PT-IUGR group, whereas lung impairment was only present in PT-AGA subjects born at lower gestational ages. Within the preterm groups only one child with a birth weight below 1000 g had a z-score for $FEF_{25-75\%} \geq$ -1.64.

Our results showed that IUGR had a more adverse impact on lung function than prematurity at higher gestations, however, the BW *per se* was the predominant risk factor for impaired lung function at school age. Our study is consistent with Greenough et al, who suggested that low BW regardless of degree of IUGR or prematurity is associated with later respiratory morbidity.[73] Structural alterations in the lung following IUGR may be significant in the pathogenesis of impaired lung function. Experimental studies have demonstrated increased thickness of the

air-blood barrier,[131] reduced number of alveoli and pulmonary vessel density and impaired endothelial function in the pulmonary artery following IUGR in sheep.[132]

Cardiovascular outcome (study IV)

Laser Doppler Flowmetry

In a repeated measure analysis including all time-points the PT-IUGR group had decreased endothelium-dependent vasodilatation compared to the PT-AGA group (p=0.019). When analyzing all three study groups simultaneously with ANOVA differences were only significant between the two preterm groups after the first and the second dose of ACH, respectively. The baseline skin perfusion values were similar between the three groups (p=0.22*).* No significant difference in the endothelial-independent response was noted between the groups. Age at testing, family history of CVD in first grade relatives and z-scores for current weight and height did not confound the differences in the endothelial-dependent response between the respective groups.

Our results are in contrast to other studies where neonates[87] and 5-year old children[133] born SGA preterm did not demonstrate lower vasodilation in response to acetylcholine as compared to preterm AGA subjects. The groups were smaller and there was no information on fetal hemodynamics of the SGA individuals in those studies. In the study by Norman,[87] SGA at term was associated with impaired endothelium-dependent function. In accordance with our study, endothelial vasodilatation was reduced when measured in large arteries in a subgroup of preterm SGA children.[134] In addition, preterm offspring born after hypertensive pregnancy had reduced endothelial-dependent vasodilatation compared to preterm offspring born after normotensive pregnancy.[135] In our study a high proportion of children in the PT-IUGR group was born after hypertensive pregnancies. The application of SNP, an endothelial-independent vasodilator, was

used as a control and we could not find differences in response as measured by laser Doppler between groups. In an experimental study on rats exposure to chronic intermittent hypoxia resulted in attenuated endothelium-dependent vasodilation in skeletal muscle resistance arteries.[136] All individuals in the PT-IUGR group in our study, had abnormal fetal blood flow and hence an increased risk of fetal hypoxia.

Intima media thickness

Intima media thickness is a well-established marker for CVD. IMT in the carotid artery was significantly decreased in the PT-IUGR group compared to the T-AGA group ($p=0.047$) and when adjusted for diastolic diameter the difference became even more apparent ($p=0.037$). There was a trend towards thicker intima media in the PT-AGA group, however the difference did not reach significance. IMT was not associated to family history of cardiovascular disease, gestational age or weight deviation at birth.

Our results are consistent with those of Painter et al,[137] who demonstrated reduced IMT in the carotid artery of adults prenatally exposed to the Dutch famine, especially during early gestation, however, without any associated reduction of CVD risk. Further support is given by an animal study showing that offspring of rats exposed to maternal protein restriction had thinner aortic walls.[138] In contrast, term IUGR neonates had increased aortic wall thickness in comparison with AGA controls.[82] Increased IMT in the carotid artery seems to be a later event than that in the aortic wall.[139] Unfortunately, we did not measure aortic wall IMT in our study population.

Blood pressure

Blood pressure and heart rate were measured. There was no significant difference between the groups in either systolic/diastolic BP or heart rate. When adjusted for z-score for height, we found significantly higher systolic and mean BP in the

preterm groups compared to the term group of children. The results suggest that IUGR did not seem to have an additional impact on BP at 7 years of age.

Several studies have shown the inverse correlation between LBW and BP.[81,140] However, in those studies, the gestational age in the subjects was not known and it is uncertain whether the LBW was due to preterm birth or growth restriction. More recent studies reported on the larger impact of preterm birth rather than BW SGA on subsequent BP.[91,133] However, in the EPICure study, BP measured in the brachial artery at 6 and 11 years was not different between extremely preterm children compared to controls. Possible underlying mechanisms for hypertension following growth restriction have been extensively studied in animals as previously mentioned. The nutrition-deprivation model mimics the slow postnatal growth until 36 postmenstrual weeks observed in both preterm groups, suggesting a common cause for increased BP in the PT-IUGR as well as the PT-AGA group.

Arterial stiffness and diameters

The diameters in the abdominal aorta, CCA and PA were not significantly different between the groups after adjustment for BSA. Stiffness was lower and DC was higher in the abdominal aorta in the PT-IUGR group compared to the PT-AGA group ($p=0.001$ and $p<0.001$). Stiffness and DC in the CCA and PA did not differ between the groups.

Previously, our group found reduced diameters in the abdominal aorta in children and young adults born at term after IUGR and ARED flow,[141,142] which is in contrast to the present findings. One explanation might be that the difference in BSA in childhood in relation to fetal growth in very preterm subjects is more pronounced than at later age which could influence the results. Difference in vessel diameters might be more apparent in adolescence when the children have achieved catch-up growth.

Increased arterial stiffness was demonstrated in children born SGA after moderately preterm birth and at term[143,144] while the stiffness in the thoracic aorta was lower in growth-restricted fetuses compared to normally grown fetuses, suggesting a fetal adaptive mechanism.[145] To our knowledge arterial stiffness has not been assessed in children born very preterm with IUGR and abnormal fetal blood flow, however, our findings of lower stiffness in aorta in the PT-IUGR group are in line with those of Lazdam et al[135] who reported on young adults born preterm after hypertensive pregnancy having lower arterial stiffening than offspring of normotensive pregnancy. As mentioned earlier, a higher proportion of the PT-IUGR group was born after hypertensive pregnancy. Impaired synthesis of elastin in the vessel wall of aorta has been proposed to cause alteration in mechanical properties.[146] Experimental studies have suggested a correlation between the mechanical properties of arteries and the ratio of elastin to collagen content in the vessel wall.[147] Synthesis of elastin and collagen peaks during prenatal life, and collagen amount peaks earlier than elastin during gestation.[148] We speculate that the PT-IUGR group may experience adverse events earlier than their PT-AGA peers, with a resulting predominant impairment of collagen synthesis as compared to that of elastin synthesis. This may explain the decreased stiffness in arteries of the PT-IUGR group as compared to the PT-AGA group. Alternatively, the reduced IMT seen in the PT-IUGR group might be a result of reduced matrix in the vessel wall with a fewer smooth muscle cells and reduced synthesis of collagen accompanied by a reduction of stiffness.

Echocardiography

After adjustment for BSA, left ventricle systolic and diastolic function, dimensions and mass were within normal ranges and did not differ between the groups. Others have demonstrated altered cardiac shape and a subclinical diastolic dysfunction in IUGR fetuses[149,150] as well as in younger children born PT and IUGR.[151] Cardiac morphology and function were altered in children born preterm regardless of SGA

BW in a study by Mikkola et al.[133] It is unclear whether these morphological and functional alterations within the heart are transient or persistent. The children in the present study were older at assessment than the children in the studies by Mikkola and Crispi et al and we do not know if the findings had been the same if the children had been assessed at a younger age. The groups in the study by Crispi were larger and the present study might be under-powered to detect differences between groups.

Anthropometric outcome

Postnatal growth expressed as z-scores for weight up to 7 years of age is shown in Figure 3. The PT-IUGR group had significantly lower z-scores than the PT-AGA and T-AGA groups at all time-points. The PT-AGA group did not differ significantly in z-scores for weight from the T-AGA group after 24 months of age. To note, the PT-AGA group had a lower relative weight gain than the PT-IUGR group during the period between birth and 36 GW (postnatal age corresponding to gestational age of 36 weeks), reflecting poor postnatal growth during the immediate postnatal period. A large proportion of the PT-IUGR group had a z-score for weight < -2 at discharge (71 %) compared to 3 % in the PT-AGA group. Growth retardation persisted in childhood in the PT-IUGR group. At 5 years of age, z-score for weight was < -2 in 45 % *vs* 9 % of the children in the PT-IUGR and PT-AGA groups, respectively.

Poor postnatal growth in preterm infants persisted into childhood in previous longitudinal studies of growth[89,152] while a high proportion of healthy SGA-infants born at term had reached catch-up at 24 months of age.[153] To our knowledge, no studies of longitudinal postnatal growth in very preterm infants exposed to IUGR and ARED have been published. Relative weight-gain between birth and 40 GW was decreased in the PT-AGA group compared to that of the PT-IUGR group.

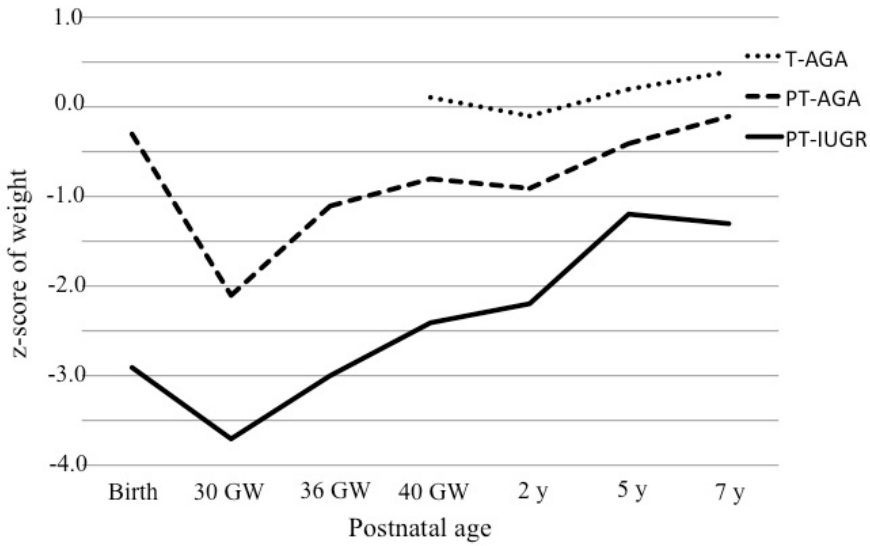

Figure 3. Postnatal growth expressed as z-score of weight. PT-IUGR; very preterm after intrauterine growth restriction (IUGR) with absent or reversed end-diastolic blood flow (ARED) PT-AGA; preterm with BW appropriate for gestational age (AGA), T-AGA; born at term with BW AGA. BW: birth weight; GW: gestational weeks, y: years.

After very preterm birth, low IGF-1 concentrations are associated with decreased catch-up growth.[154] The lowest concentrations of IGF-1 were found in very preterm infants born SGA. Although crossing the weight-curves upwards, the PT-IUGR children remained significantly lighter and shorter than the PT-AGA children at 7 years of age. The mechanism behind failure of catch-up growth in children born IUGR is often unclear but low concentrations of circulating IGF-1[155] and mutations in the IGF-1 receptor have been demonstrated to cause persisting short stature.[156]

Weight gain and nutrient-enriched diets during early postnatal development have been shown to be associated with less favourable cardiovascular outcome.[95] Another study demonstrated that infants who were short and thin at birth and with catch-up after 2 years of age had higher BP as adults.[157] In contrast, early catch-up

growth in very preterm SGA-children is associated with better cognitive outcome.[158] The different growth patterns in our PT-IUGR and PT-AGA groups may be of importance for differences in several outcome parameters in our study. The slow postnatal weight gain in the PT-IUGR group may be beneficial for future cardiovascular health but unfavourable for cognitive outcome.[158]

General discussion

In this thesis we have examined various outcome entities in infants and children born very preterm after IUGR and ARED blood flow, and compared the results to those of matched preterm and term controls with normal BW. Adaptation to abnormal fetal blood flow *in utero* might result in long-term consequences in agreement with the fetal origin of adult disease hypothesis.[81] Physiological and structural alterations of the cardiovascular,[75,136,138] respiratory[51-53] and nervous system[159,160] have been extensively examined in animal studies which support the fetal origin hypothesis. Epidemiological studies have linked low birth weight to adverse outcome in several organ systems,[56,81,161,162] but it is unclear, at least in some of them, whether they explore the adverse outcome of individuals born moderately preterm with normal birthweight or of those born at term with growth restriction. A major question remains whether we in part examine the same pathophysiology in subjects born extremely preterm where postnatal growth restriction may have a similar effect to that of adverse fetal growth during the corresponding time period in the infant born at term? The timing of the adverse events during gestation is critical to the fetal adaptive response.[163] Insults that coincide with the development of organs will alter size as well as function.[164] A re-setting of endocrine functions has also been suggested to influence outcome after prenatal adverse events.[75,165] Epigenetic changes such as DNA methylation and histone acetylation induced by prenatal influences can permanently alter gene expression and have been described in epidemiological as well as in experimental studies.[166,167]

Essential to discuss and examine in future studies is how IUGR *in early vs later gestation* affects outcome. So far, we have no means of estimating for how long a fetus has experienced growth restriction with abnormal placental blood flow at the time of admission. Only in cases when ultrasound biometry has been performed both in early gestation and at 18 GW and where a discrepancy was found between the two examinations, may one retrospectively understand that the fetus has been exposed to an early growth restriction. In addition, it is essential to evaluate the possibly differentiated effects, in PT-IUGR and PT-AGA subjects respectively, of postnatal exposures known to have an impact on both cardiovascular health and brain development. These postnatal exposures include steroid treatment,[168] infection,[169] and diverse nutritional strategies.[95]

Two main IUGR models have been used in animal studies, namely maternal nutritional deprivation[75] and models using interventions causing reduction of utero-placental perfusion.[88] The latter model resembles more closely the nutritional and hypoxic insult occurring *in utero* in the preterm IUGR infant. However, results emanating from models using maternal nutritional deprivation may be relevant for preterm birth *per se* as this is strongly associated with postnatal growth restriction and nutritional deficit.

It is of great importance to follow very preterm children regardless of growth restriction into adulthood. In the last decade at our clinic we have routinely offered and performed neurological follow-up with psychological tests and assessments of motor functions in infants and children born very preterm. Since very preterm birth carries increased risk for cognitive and behavioral deficits in the child it is essential to provide early assistance and support in school. Unfortunately, information on the cognitive and behavioral assessments is seldom transferred to the school system. In the future, efforts should be made to increase awareness of the special learning and behavioral difficulties occuring after very preterm birth among staff members at school and to secure implementation of transferred

information. More research is warranted about the self-esteem and psychological health in both school-age children and in adults born very preterm.

We should also provide follow-up of lung function and cardiovascular function as an integrated part of clinical follow-up schemes. Is there a critical window to intervene and thereby prevent or reduce later adverse outcome? Children with impaired lung function, without underlying allergy, might benefit from bronchodilatory treatment before physical activities. Interventions such as reducing body weight, increasing exercise, or avoiding smoking might alleviate adverse outcomes.

Summary and conclusions

The main findings of our study were that mortality, neonatal morbidity or major neurological handicap did not differ between the preterm IUGR infants and the background population except for chronic lung disease. However, when lung function was assessed in childhood the adverse effect of IUGR was only apparent in children born after 26 gestational weeks, a period of gestation when delivery on fetal indication is more generally accepted. Before 27 GW, the impact of gestational age on lung function was of more importance.

Regarding cognitive function, the adverse impact of IUGR was present throughout the range of gestational ages. There was a more pronounced impairment in boys born very preterm with IUGR and ARED flow, suggesting a particular susceptibility in males. It is unclear whether the findings of lower cognitive results in preterm-IUGR boys are due to transient neurological immaturity, or if the impairment will persist into adulthood.

Findings differed in cardiovascular function and structure between the preterm groups. The preterm IUGR had lower microvascular endothelial-dependent vasodilatation and the preterm-AGA group had increased aortic stiffness. IMT was reduced in the carotid artery in the preterm-IUGR group compared to the term group. These different vascular ”phenotypes” are difficult to explain and the impact on later cardiovascular health remains unclear. These findings suggest that IUGR might have a different impact on pathophysiological mechanisms acting on the vessel wall in early gestation. Blood pressure was higher in both preterm groups compared to the term group, which might be explained by postnatal growth restriction. Previous studies in adults born preterm, suggest that this elevation of

BP will persist into adulthood. Further studies are warranted on the mechanisms underlying the different vascular behavior observed in preterm AGA vs preterm IUGR subjects and longitudinal follow-up studies will find out whether our findings track into adulthood.

The observed outcomes would not seem to be sufficiently severe to refrain from delivery in these very preterm IUGR fetuses. To note, it may be wrong to generalize these results to the very preterm IUGR population *per se*, as reported mortality, morbidity and outcome might mainly reflect the standard of fetal monitoring and neonatal care at Lund University Hospital.

In summary:

- Very preterm elective delivery of IUGR fetuses with severely abnormal blood flow in the umbilical artery, but before the occurence of severe changes in the ductus venosus blood flow, did not cause an increase in perinatal mortality or neonatal morbidity, with the exception of chronic lung disease.

- Survival without major neurological handicap at two years of age was equal to that of the very preterm background population.

- Cognitive impairment at early school age was more prevalent in boys born very preterm with IUGR and ARED flow compared to boys born preterm with BW AGA. The effect of IUGR on cognitive outcome at 5-8 years of age was not related to degree of prematurity.

- Attention-deficits disorder, as assessed by questionnaires, was more prevalent in both preterm groups than in the term group of children.

- Lung function, assessed at 6-11 years of age with spirometry, was reduced in children born preterm after IUGR and ARED flow compared to the term group. In comparison to the preterm AGA group, the adverse effect of IUGR on lung function was only apparent in children born after 26 GW.

- Children born very preterm with IUGR and ARED flow had thinner IMT than children born at term. Aortic wall stiffness was decreased and endothelial vasodilation lower in the IUGR group compared to the preterm AGA group. Blood pressure was increased in both preterm groups.

In conclusion, the results of our study suggest that it is justified to deliver fetuses with IUGR and abnormal fetal blood flow in the umbilical artery even at early gestations, in order to prevent intrauterine death.

Swedish summary

Tillväxthämning hos foster, med avvikande blodflöde i navelartären under tidig graviditet är ett tillstånd som är komplicerat att handlägga och det finns ingen evidens för när tidpunkt för förlossning bör ske. Förlossning i mycket tidig graviditet är associerad med ökad sjuklighet och död efter födelsen och måste vägas mot att fostret exponeras för syrebrist och risk för fosterdöd. Med ultraljud Doppler kan man övervaka foster-cirkulationen och blodflödet mellan moderkakan och fostret. Man har i studier kunnat visa att försämringen sker i en viss ordning i fostrets cirkulation. Allvarliga blodflödesförändringar i ductus venosus, pulsationer i navelvenen liksom avvikelser i fostrets hjärtfrekvens kommer sent och antyder att fostret är allvarligt sjukt och risken för intrauterin fosterdöd är stor. Tidigare studier har visat ökad perinatal dödlighet och sjuklighet i nyföddhetsperioden hos barn födda mycket för tidigt med tillväxthämning och avvikande blodflöde. Det är svårt att dra några slutsatser av de studierna eftersom fostren föddes både spontant och efter kejsarsnitt och i många fall hade hunnit utveckla allvarliga blodflödesförändringar innan födelsen. Det är oklart om tillväxthämning och patologiskt blodflöde innebär ökad risk för avvikelser hos det för tidigt födda barnet om man har en så kallad *”aktiv”* handläggning.

Kvinnokliniken i Lund har sedan 15 år tillbaka handlagt foster med tillväxthämning och avvikande blodflöde i tidig graviditet *aktivt*, så till vida att fostret förlöses med planerat kejsarsnitt, innan fostret har utvecklat allvarliga blodflödesförändringar. Syftet med den här avhandlingen har varit att utvärdera vilka konsekvenser en sådan handläggning i tidig graviditet medför för barnets sjuklighet på kort såväl som på lång sikt.

Delarbete I visade att foster med tillväxthämning och avvikande blodflöde i navelsträngen som förlöstes aktivt med kejsarsnitt i mycket tidig graviditet inte hade ökad perinatal dödlighet eller allvarlig sjuklighet i nyföddhetsperioden jämfört med foster som föddes för tidigt av andra anledningar under samma tidsperiod (1998-2004). Ett undantag var att de tillväxthämmade barnen hade ökad förekomst av kronisk lungsjukdom, det vill säga, att de krävde extra syrgastillförsel för att uppnå normal syrgasmättnad i blodet vid en ålder motsvarande 36 veckors graviditet. Vi följde upp barnen till 2 års ålder och fann ingen skillnad mellan grupperna beträffande överlevnad och förekomst av cerebral pares.

I delarbete II testades barnen vid ca 7 års ålder med psykologiska test (WPPSI eller WISC). Barnens föräldrar intervjuades och fyllde i frågeformulär avseende beteende (Strength and Difficulties, SDQ) och uppmärksamhetsstörningar (Brown´s attention deficit disorder, ADD). Resultaten visade att pojkarna som föddes tidigt efter tillväxthämning hade sämre resultat i begåvningstest jämfört med pojkar som föddes tidigt med normal födelsevikt för graviditetstiden. Vi kan inte svara på frågan om våra resultat beror på en ökad känslighet eller en försenad mognad hos pojkar jämfört med flickor. Uppmärksamhetsstörningar var vanligare hos de för tidigt födda barnen jämfört med barnen som föddes i normal tid.

I delarbete III undersökte vi lungfunktionen med spirometri. Resultaten visade att de för tidigt födda barnen hade sämre lungfunktion än barnen som föddes i fullgången tid, sämst lungfunktion hade barnen med födelsevikt under 1000 g. Hälften av de för tidigt födda barnen hade behandlats med astma-läkemedel under de första levnadsåren jämfört med 10 % av barnen som föddes i normal tid. Förekomst av allergi var lika mellan grupperna.

I delarbete IV undersökte vi kärl- och hjärtfunktionen vid 7 års ålder. Vi fann ingen skillnad i hjärtfunktionen mellan grupperna men de för tidigt födda barnen hade högre systoliskt blodtryck, om man tog hänsyn till längd och ålder vid

undersökningen, än barnen som föddes i normal tid. Styvheten i aortas kärlvägg var högre hos de för tidigt födda barnen med normal vikt vid födelsen och kärlväggen i halskärlet var tunnare hos de för tidigt födda barnen med tillväxthämning vid födelsen.

Sammanfattningsvis, pekar resultaten på att det finns en ökad risk för ett långvarigt behov av extra syrgas efter födelsen vid tillväxthämning i tidig graviditet. För tidigt födda barn hade mer astma under uppväxten och när man undersökte barnen i skolåldern hade de lägre lungfunktion än de barn som föddes i fullgången tid, särskilt de barn som vägde under 1000 g vid födelsen. Pojkar som är tillväxthämmade och för tidigt födda hade en ökad risk för lägre begåvning än flickor. Barn som föds mycket för tidigt hade högre blodtryck än de som föddes i normal tid.

Sammanfattningsvis talat våra uppföljningsresultat för att det är försvarbart att förlösa foster som är tillväxthämmade med allvarliga flödesförändringar i tidig graviditet för att undvika risk för syrebrist och fosterdöd. Det är viktigt att betona att betydelsen av våra undersökningsresultat är för det enskilda barnet svårbedömda och det är oklart om avvikelserna i skolåldern är bestående in i vuxen ålder. Det är av största vikt att vi erbjuder uppföljning av alla barn som föds mycket för tidigt upp till skolåldern och kanske ännu längre och, att i de fall där det behövs, sätta in stödinsatser på ett tidigt stadium.

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