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VERY PRETERM BIRTH - etiological aspects and short and long term outcomes

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Hans Arp

ABSTRACT

Very preterm birth, occurring before 32 completed weeks of gestation, is an often unexplained pregnancy complication affecting approximately 1 to 2 percent of all births. This thesis includes four studies regarding the etiology, and short and long term outcomes of very preterm birth, using Swedish population-based data. The aims were to investigate if viral infections during pregnancy increase the risk of very preterm delivery, to study the role of level of care for infant mortality in very preterm infants, and to explore long-term health in adults born very preterm, with regard to high blood pressure and type 2 diabetes.

Among pregnant women in Stockholm, we identified 269 cases of very preterm birth and 301 controls with term delivery, with archived blood sampled for the Rubella serology screening in early pregnancy. Serum was investigated for presence of viral genetic material. Any viremia was detected in 10 cases and in 5 controls, corresponding to an adjusted odds ratio (95 percent confidence interval) of 2.21 (0.71-6.84). Although risk estimates were consistently elevated for any viremia and for Parvovirus B19, none were significant on a 5 percent level. Whether viral infections during pregnancy increase the risk of very preterm birth needs to be investigated in larger studies.

During 1992-98, 2,253 liveborn singleton infants were born very preterm in Swedish general and university hospitals. Infant mortality rates increased by decreasing gestational age, from 5 percent at 31 weeks to 56 percent at 24 weeks. Very preterm birth at a general hospital was associated with an increased risk of infant mortality, but the risk increase was confined to extremely preterm infants born at 24 to 27 weeks, having an adjusted odds ratio for infant mortality of 2.00 (1.15-3.49).

Among 329,495 young men born in Sweden 1973-81 and conscripted for military service, gestational age at birth was inversely related to high systolic blood pressure at conscription (≥ 140 mm Hg). Adjusted odds ratios among men born very and extremely preterm (29-32 weeks and 24-28 weeks, respectively) were 1.45 (1.28-1.64) and 1.88 (1.33-2.68), respectively. The association was not confounded by familial (common genetic and shared environmental) factors. In addition, being born small for gestational age was not a risk factor of high systolic blood pressure among men born at 24 to 32 gestational weeks, but increased the risk among men born moderately preterm (33-36 weeks) and at term (≥ 37 weeks).

In a cohort of 18,230 Swedish twins, rates of type 2 diabetes increased with decreasing gestational age and with decreasing birth weight. In cohort analyses there was no association between preterm birth and type 2 diabetes, whereas risk of type 2 diabetes increased with decreasing birth weight. However, in co-twin case-control analyses, an increased risk of type 2 diabetes with lower birth weight was found within dizygotic but not within monozygotic twin pairs. Odds ratios per 500 grams decrease in birth weight were 1.38 (1.02-1.85) and 1.02 (0.63-1.64), respectively, indicating genetic confounding of the association between low birth weight and type 2 diabetes.

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LIST OF PUBLICATIONS

- I. Johansson S, Buchmayer S, Harlid S, Iliadou A, Sjöholm M, Grillner L, Norman M, Sparén P, Dillner J, and Cnattingius S.
Infection with Parvovirus B19 and Herpes viruses in early pregnancy and risk of second trimester miscarriage or very preterm birth.
Submitted to Paediatric Perinatal Epidemiology

- II. Johansson S, Montgomery S M, Ekbohm A, Otterblad Olausson P, Granath F, Norman M, and Cnattingius S.
Preterm delivery, level of care, and infant death in Sweden:
a population-based study
Pediatrics 2004;113:1230-1235
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- III. Johansson S, Iliadou A, Bergvall N, Tuvemo T, Norman M, and Cnattingius S.
Risk of high blood pressure among young men increases with the degree of immaturity at birth
Circulation 2005;112:3430-3436
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- IV. Johansson S, Iliadou A, Bergvall N, de Faire U, Kramer M S, Pawitan Y, Pedersen N L, Norman M, Lichtenstein P, and Cnattingius S.
Genetic factors contribute to the association between low birth weight and type 2 diabetes - a large cohort study of Swedish twins
In Press. Epidemiology 2008
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LIST OF ABBREVIATIONS

AGA	appropriate for gestational age
BMI	body mass index
BP	blood pressure
BPD	bronchopulmonary dysplasia
CI	confidence interval
CRH	corticotropin-releasing hormone
CRP	c-reactive protein
DNA	deoxyribonucleic acid
DOHaD	developmental origin of health and disease
EDD	expected date of delivery
ELBW	extremely low birth weight
HPA	hypothalamic-pituitary-adrenal
ICD	international classification of diseases
i.e.	that is (<i>id est</i>)
IL	interleukin
IQ	intelligence quotient
IVH	intraventricular haemorrhage
LGA	large for gestational age
LMP	last menstrual period
MDI	mental development index
MBP	mannose-binding protein
nCPAP	nasal continuous positive airway pressure
NEC	necrotising enterocolitis
NICU	neonatal intensive care unit
PCR	polymerase chain reaction
PDA	patent ductus arteriosus
PVL	periventricular leukomalacia
RDS	respiratory distress syndrome
RNA	ribonucleic acid
ROP	retinopathy of prematurity
SALT	screening across the lifespan twin
SCB	Statistiska Centralbyrån
SD	standard deviation
SGA	small for gestational age
TNF	tumour necrosis factor
VLBW	very low birth weight
vs.	versus

1. INTRODUCTION

Very preterm birth, occurring before 32 completed weeks of gestation, is a common and often unexplained pregnancy complication affecting approximately 1 to 2 percent of all pregnant women.

Genetic factors account for a substantial proportion of very preterm births, but several environmental risk factors have also been identified. For example, intrauterine infections, maternal smoking, and low socioeconomic status have all been associated with increased risk of very preterm delivery. However, this knowledge has not been able to translate into successful prevention strategies. Clearly, more knowledge is needed on the etiology of very preterm birth.

Infants born very preterm face substantial morbidity and mortality risks. The complex nature of intensive care for preterm infants demands highly qualified staffing as well as access to advanced technologies. Centralization of hospital care for very preterm infants may have an impact on short term prognosis, but it is not fully elucidated which infants benefit most from highly specialized neonatal intensive care units.

Nevertheless, the development of neonatal intensive care during the last decades has led to improved survival rates among very preterm infants. Today, survivors of very preterm birth constitute a new generation of young adults, but the life-long health effects are virtually unknown and late morbidity could be an increasing problem. Research on perinatal risk factors for the development of common diseases in adulthood suggests that low birth weight may increase the risk of cardiovascular disease and type 2 diabetes. It is not clarified whether poor foetal growth or preterm birth, the two principal explanations of low birth weight, are independently associated with those diseases. In addition, both low birth weight and type 2 diabetes may be explained by a common genetic predisposition.

In this thesis I will present four studies regarding etiology, and short term and long term prognosis of very preterm birth, using Swedish population-based data. The aims are to investigate whether viral infections during pregnancy increase the risk of very preterm delivery, to study the role of level of care for infant mortality in very preterm infants, and to explore long term health in adults born very preterm, with regard to high blood pressure and type 2 diabetes.

2. BACKGROUND

In this chapter I will present the epidemiology of preterm birth, as well as an over-view of short term and long term prognosis after very preterm birth.

EPIDEMIOLOGY OF PRETERM BIRTH

Definitions

Normal gestational length of human pregnancy has been estimated to 282-283 days^{1,2}. Gestational age of a newborn infant is categorized as preterm, term or postterm (Figure 1), as proposed by the World Health Organization in the 1970s³. Preterm birth occurs before 37 completed gestational weeks and could further be subdivided into moderately preterm, very preterm and extremely preterm. As in this thesis, “very preterm birth” usually refers to all births at ≤ 31 weeks, including also extremely preterm births at ≤ 27 weeks.

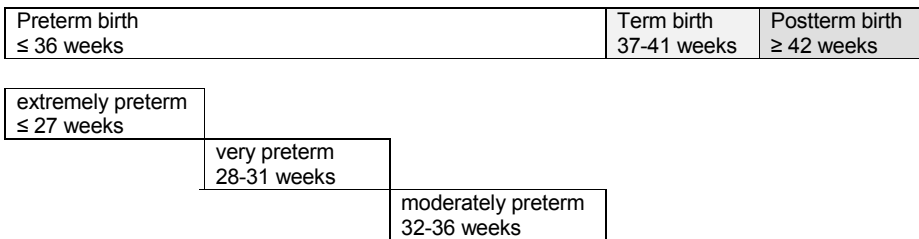


Figure 1. Categorization of gestational age by completed gestational weeks at birth.

To determine gestational age at birth, it is necessary to date the pregnancy and calculate the expected date of delivery (EDD) occurring at 40 completed gestational weeks. One commonly used method is to define EDD as 280 days from the last menstrual period (LMP), using the so-called pregnancy wheel (Figure 2)⁴.

The simplicity of this method makes it well suited for low-resource communities. Despite problems to recall correct date of LMP^{5,6} the estimations of gestational age are reasonably good^{4,7} and can be used in perinatal epidemiology research when other dating methods are unavailable.

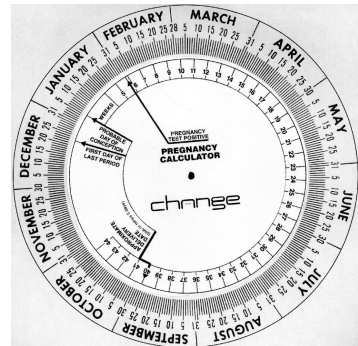


Figure 2. The pregnancy wheel.

A more accurate way to date the pregnancy is to measure foetal size in early pregnancy, using ultrasound⁸. Foetal growth velocity is constant during early pregnancy⁹, and measures such as femoral length and head circumference are proportional to gestational length. Hence, such measures can be used to predict EDD and calculate gestational age in clinical practice¹⁰. In addition to improved precision, ultrasound dating also leads to more valid gestational age estimates. Estimates derived from LMP typically overestimate gestational age about 2-3 days⁷. Importantly, changing pregnancy dating method from LMP to ultrasound could have an impact on gestational age distribution, leading to an increase in preterm birth rate and a concomitant decrease in postterm birth rate¹¹. Hence, rates of preterm birth may not be comparable if rates are based on different methods for gestational age determination.

Rates of preterm birth

Contrary to the general belief, preterm birth is a common pregnancy complication. Internationally, the variation of preterm birth rates is striking. About 6 percent of all pregnancies end preterm in Sweden (2003)¹², whereas the corresponding figure for the US is reported to be almost 13 percent (2005)¹³. In developing countries, rates may be even higher. In a study including ultrasound-dated pregnancies in Malawi, 20 percent of women delivered preterm¹⁴.

Very preterm births, occurring before 32 completed gestational weeks, account for about 15 percent of preterm births, which means that 1 to 2 percent of all pregnancies end very preterm^{12,13}.

Rates of preterm birth seem to be constant or even decreasing in the UK and Sweden^{15,16}, but several countries report increasing rates over recent decades^{13,17-20}. This observation has been attributed to a number of factors, such as the introduction of ultrasound pregnancy dating, more frequent medically induced preterm deliveries, assisted reproduction, and more frequent multiple births^{17,18,20,21}. Increasing preterm birth rates seem to be explained by a greater number of moderately preterm births, since rates of very preterm birth have been stable over time^{13,16,21}.

Risk factors of preterm birth

Preterm birth has been associated with a number of risk factors (Table 1).

Table 1. Risk factors of preterm birth.

- Ethnicity
 - Family history
 - Infections
 - Maternal characteristics
 - Socioeconomic status
 - Multiple pregnancies
 - Smoking and substance abuse
 - Air pollution
-

Ethnicity

Epidemiological studies have shown ethnic differences in rates of preterm birth²². In the US during 2005, 19 percent of pregnancies ended preterm among black non-Hispanic women, whereas only 12 percent of white non-Hispanic women delivered preterm¹³. Corresponding rates for very preterm birth was 2.3 percent and 1.1 percent, respectively. In addition, black women do not only face an increased risk of preterm birth. Compared to white women, they are also at an increased risk of repeated preterm birth²². Although such findings may be explained by environmental or socioeconomic factors, they could also indicate that some ethnic groups have a genetic predisposition for preterm birth^{23,24}.

Family history

One preterm delivery increases the risk of preterm delivery in subsequent pregnancies^{25,26} and the risk of re-occurrence is especially high for very preterm birth²⁵. The heritability of preeclampsia, a common cause of preterm delivery, has been estimated to 31 percent, and genetic factors may account for one third of all preterm deliveries^{27,28}. The mechanisms behind such genetic influences remain to be determined, but case-control studies support that inflammatory responses may be influenced by genetic factors²⁹⁻³².

Infections

Bacterial vaginosis and intrauterine bacterial infections are well-established risk factors of preterm delivery^{33,34}. Bacterial vaginosis may increase the risk of very preterm delivery more than two-fold³⁵, and intrauterine infection is reported to be associated with even higher risks, especially for extremely preterm birth³⁶. Infections localized to organ systems other than the reproductive tract may also be important. Periodontal infections have been reported to more than double the risk of very preterm birth³⁷.

The uterus and amnionitic membranes can become infected in several ways. Bacteria can migrate to the uterus from the vagina or the abdominal cavity, be introduced during invasive procedures such as chorionic villi sampling³⁸, or through haematogenous spread^{39,40}. If chorioamnionitis develops, the risk of very preterm delivery is increased⁴¹, especially if an inflammatory response is also elicited in the foetus, when the risk of extremely preterm birth may increase ten-fold³⁶.

A number of bacteria has been cultured from amniotic fluid and chorioamnionitic membranes in preterm deliveries; vaginal organisms with low virulence, such as *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Gardnerella vaginalis* and *Bacteriodes* species, and several other bacteria such as *Escherichia coli*, *Enterococcus faecalis*, *Streptococcus* species and *Chlamydia trachomatis*^{33,34}.

While much focus has been on bacteria, less is known about the role of viral infections. The only larger epidemiological study suggested that *Parvovirus B19* may be associated with an increased risk of late spontaneous abortion and stillbirth⁴². The prevalence of IgM seropositivity for *Parvovirus B19* among women with such pregnancy complications was 13 percent as compared to 1.5 percent in the remaining pregnant population⁴². Smaller clinical studies and case-series also report that viral infections may increase the risk of preterm delivery. Levels of antibodies against *Cytomegalovirus* was found to be higher in women with early onset preeclampsia and preterm delivery, compared to women with normal pregnancies ending at term⁴³. *Cytomegalovirus* was also more commonly detected in dried neonatal blood spots, sampled after birth, in infants born preterm than in term infants (prevalence 33 vs. 24 percent)⁴⁴.

Maternal characteristics

Several maternal characteristics have been associated with preterm birth. Firstly, as already described, preterm birth rates differ by ethnicity. Secondly, maternal age is reported to influence pregnancy outcome. Low and high maternal age increases the risk of preterm birth^{45,46}. Moreover, maternal age has been shown to interact with parity, i.e. the risk of preterm birth being highest in younger multiparae and older primiparae⁴⁷. Compared to 25- to 29-year old primiparae women, the risk of preterm birth was approximately doubled for multiparae women aged less than 18 years and for primiparae women aged more than 40 years. One may speculate that teenage mothers with several children are exposed to less favorable socio-economic conditions^{48,49}. Delayed child-bearing at an older age is related to more prevalent assisted reproduction, higher risk of preeclampsia and more frequent twin pregnancies^{50,51}.

Finally, reproductive history may be important. Previous induced abortions may increase the risk of very preterm births with spontaneous onset⁵². Women with a previous second trimester spontaneous abortion or a previous very preterm delivery are at increased risk of very preterm delivery in a subsequent pregnancy^{22,25,53}. A short interval between subsequent pregnancies (<6 months) has also been reported to be a risk factor, doubling the risk of extremely preterm delivery in subsequent pregnancies⁵⁴. However, the association between a short interpregnancy interval and

other adverse pregnancy outcomes including preterm birth may be confounded by socioeconomic factors⁵⁵ or adverse outcomes in previous pregnancies⁵⁶.

Socioeconomic status

There are marked socioeconomic inequalities in preterm birth rates. The differences in preterm birth rates between countries like Sweden, USA and Malawi are probably partly explained by different socioeconomic contexts¹²⁻¹⁴. Within developed countries, socioeconomic status is also related to risk of preterm birth. A recent British study demonstrated that very preterm birth was twice as common among women living in most deprived areas compared to women in least deprived areas⁵⁷. Similar conclusions were drawn in Norway, where maternal characteristics such as single motherhood and low education were associated with a 25 and 50 percent increase in risk of preterm birth, respectively¹⁹.

Multiple pregnancies

An American study reported that 54 percent of twins were born preterm⁵⁸. In Europe, preterm birth rates in twin pregnancies vary from 42 percent in Ireland to 68 percent in Austria, attributing to 20 percent of all preterm births⁵⁹. Twins resulting from subfertility treatment are more commonly born preterm compared to naturally conceived twins⁶⁰. The majority of preterm births in singletons are due to spontaneous onset of labour, but induced deliveries account for about half of preterm births among twins⁵⁹.

In absolute terms, neonatal outcome of multiple pregnancies is generally worse than in singleton pregnancies⁶¹. However, besides intrauterine growth retardation, prematurity is the principal factor driving increased mortality and morbidity rates in twins and triplets. When gestational age is taken into account, risks of neonatal mortality and morbidity are not increased in multiple pregnancies compared to single pregnancies⁶².

Smoking and substance abuse

Maternal smoking has a dose-dependent impact on risk of preterm birth⁶³. Heavy smoking (≥ 10 cigarettes per day) may increase the risk of very preterm delivery more than two-fold. Exposure to environmental tobacco smoke (passive smoking) has also been associated with an increased risk, yet lower than for active smoking^{64,65}. The association between snuff (smokeless tobacco) and preterm birth is less well studied but investigations from Sweden and India found that snuff increases the risk of preterm birth^{66,67}. A South African study concluded that snuff did not affect the rate of preterm birth although women using snuff had slightly shorter gestational length in term births compared to women not using snuff⁶⁸.

Abuse of other drugs during pregnancy, including narcotics and alcohol, is associated with a number of poor perinatal outcomes, including preterm birth⁶⁹. Prenatal drug exposure to tobacco and cocaine has been estimated to account for 5.7 percent of preterm births in American settings⁷⁰. Excessive alcohol use is also reported to be more common among women with preterm births⁷¹. However, narcotics and alcohol may be part of a low socio-economic lifestyle, and it is difficult to disentangle the independent roles of substance abuse versus deprived socioeconomic circumstances.

Air pollutants

Exposure to ambient air pollution, such as particulate matters, ozone, carbon monoxide and nitric dioxide, has been reported in several studies to increase the risk of preterm birth in a dose-dependent manner⁷²⁻⁷⁴. However, there are also negative

results published⁷⁵. Despite attempts to adjust for socioeconomic status in studies reporting positive findings⁷²⁻⁷⁴, one cannot exclude that residual socioeconomic confounding explains the association between air pollution and preterm birth.

Etiologies and biological mechanisms

The variety of identified risk factors could be translated into different etiologies of preterm birth (Table 2).

Table 2. Main etiologies of preterm birth.

- Premature labour
 - Preterm premature rupture of the membranes
 - Placental abruption and vaginal bleeding
 - Preeclampsia and other maternal illnesses.
-

Firstly, one needs to consider two principally different etiological concepts; spontaneous preterm birth and medically induced preterm birth⁷⁶. The majority of preterm births have a spontaneous onset, initiated by premature labour, rupture of the membranes or vaginal bleeding⁵⁹. The remaining preterm births are medically induced on maternal or foetal indications, typically due to preeclampsia. This heterogeneity of preterm birth needs to be considered in research on etiological concepts and biological mechanisms⁷⁶. Neonatal outcome may depend more on gestational age at birth than etiology of preterm birth⁷⁷, but risk factors may have differential impact on spontaneous and induced preterm birth, respectively^{78,79}.

Secondly, the various etiologies of preterm birth are related to several biological pathways (Table 3).

Table 3. Biological pathways for preterm birth.

- Genetic mechanisms
 - Inflammation
 - Vascular mechanisms
 - Neuroendocrine stress responses
 - Mechanical stress
-

Genetic mechanisms

Ethnic differences²², risk of repeated preterm delivery^{25,26}, and familial aggregation of preeclampsia and preterm birth^{27,28}, indicate that genetic mechanisms are important for preterm birth.

One may speculate about genetic influences on several physiological processes leading to preterm delivery. Polymorphisms of genes involved in the immune system could be related to preterm delivery. One genotype of a promoter gene for interleukin-6 (IL-6), regulating responses to stressful stimuli, was found in 38 percent of mothers with very preterm deliveries, and in 29 percent of mothers with term deliveries²⁹. Tandem repeat polymorphism of the gene for the interleukin-1 receptor antagonist, involved in duration and severity of inflammation, was found in 27 percent of women with preterm deliveries, compared to 12 percent of women with term deliveries³¹. Polymorphisms of immunoregulatory genes for interleukin-10 (IL-10) and mannose-binding protein 2 (MBL2), have also been more commonly found in women with preterm births and may increase the risk of chorioamnionitis^{30,32}, a pregnancy complication often preceding spontaneous preterm birth.

A low intake of dietary vitamin C may increase the risk of preterm birth⁸⁰, and genetic variants of a membrane-bound vitamin C transporter may double the risk of spontaneous preterm delivery⁸¹. Polymorphisms in folate metabolizing genes, affecting

homocystein levels, may also play a role for spontaneous preterm delivery, especially in black women with low folate intake⁸².

Inflammation

The association between intrauterine infections and preterm birth involves biological pathways related to inflammation^{33,83}. Bacterial colonization and release of toxins activates the production of cytokines, such as tumour necrosis factor α (TNF α) and interleukin-6 (IL-6). Cytokines stimulate prostaglandin production in the chorioamniotic membranes and placenta and lead to infiltration of neutrophilic white blood cells. Activation of metalloproteases leads to weakening of chorioamniotic membranes and cervical ripening. Prostaglandins also stimulate myometrial contractions. The inflammatory response culminates in preterm labour and rupture of the membranes.

An inflammatory response in the foetus also contributes to preterm labour and rupture of the membranes. Chorioamnionitis could results in foetal stress involving the hypothalamic-pituitary-adrenal axis (HPA axis). Foetal release of cortisol contributes to increased levels of prostaglandins.

Figure 3 is a schematic view of inflammatory pathways leading to preterm delivery.

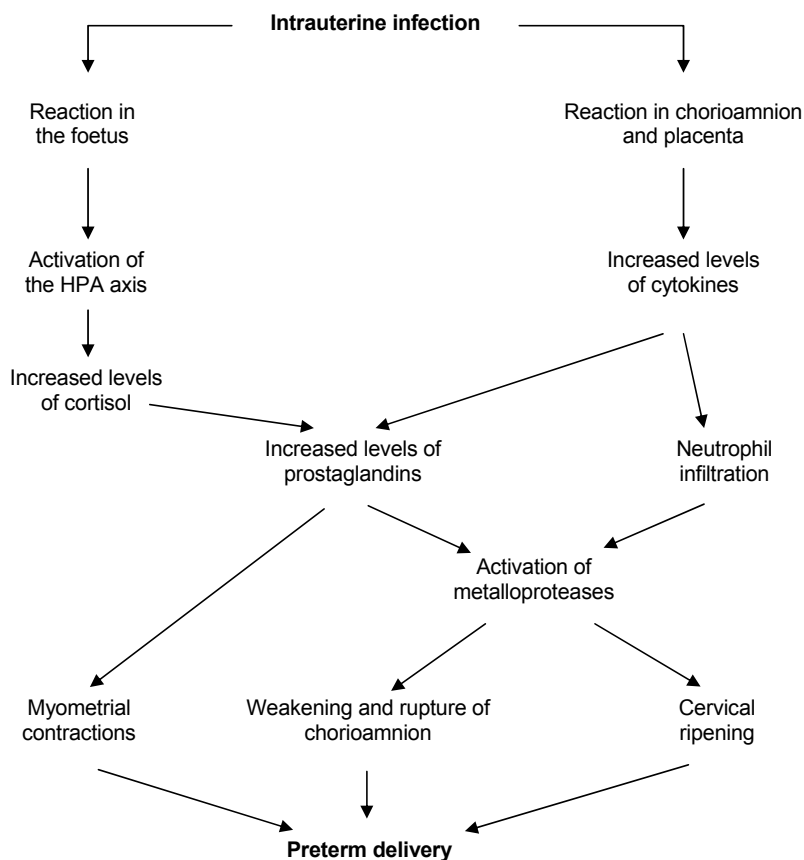


Figure 3. Potential pathways from intrauterine infection to preterm delivery. Adapted from Goldenberg³³.

Vascular mechanisms

Preeclampsia and placental abruption are pregnancy complications often resulting in medically induced preterm delivery. Although principally different, both complications can be attributed to impaired placental vascular function.

Preeclampsia, affecting 3 to 5 percent of pregnant women, is a complex disorder initiated already during the critical process of implantation and placentation shortly after conception⁸⁴⁻⁸⁶. Inadequate invasion of endovascular cells, placental production of anti-angiogenic factors and development of endothelial dysfunction leads to small-bore, high-resistant placental vessels that cannot respond to the increasing demand of blood supply and nutrition to the foetus. Clinical manifestations of preeclampsia, such as hypertension, renal dysfunction and neurological symptoms, may necessitate preterm delivery on maternal indication. More commonly, delivery is induced on foetal indication, due to signs of foetal stress including abnormal umbilical blood flow and growth restriction.

Placental abruption, complicating 0.5 to 1 percent of pregnancies, is a too early separation of the placenta from the uterine wall, diagnosed by a combination of ultrasound findings and clinical signs such as vaginal bleeding, abdominal pain and foetal distress^{87,88}. Consequences depend on degree of placental detachment, degree of foetal distress and gestational age at abruption, but the risk of perinatal mortality in very preterm deliveries following abruption is substantially increased⁸⁹.

Neuroendocrine stress responses

The association between low socioeconomic status and preterm delivery could be mediated by psychological distress during pregnancy⁹⁰⁻⁹². Maternal stress activates the HPA axis, illustrated by elevated cortisol levels in gestational week 15 in women who later delivered preterm⁹³. Increased secretion of cortisol stimulates placental secretion of corticotropin-releasing hormone (CRH), interacting with prostaglandins and oxytocin, which mediate uterine contractions. Secretion of CRH is reported to be elevated in pregnant women who later deliver preterm^{94,95} and it has been suggested that serum levels of CRH may be a useful marker in the clinical assessment of the risk of parturition in women presenting with preterm contractions⁹⁶.

Mechanical stress

Finally, mechanical stress of the uterus and cervix could be associated with preterm delivery. Caesarean section in a first pregnancy increases the risk of preterm birth in a second pregnancy⁹⁷. Uterine overdistension is assumed to increase the risk of preterm delivery, exemplified by shorter gestations in twin pregnancies, especially in those with excessive amniotic fluid (polyhydramnios)⁹⁸. Leiomyomata, benign neoplasms in the uterine wall, is associated with an increased risk of preterm delivery, supposedly due to increased mechanical strain of the uterus⁹⁹. One proposed mechanism is that stretching of foetal membranes increases interleukin-8 concentrations and collagenase activity, implicated in cervical ripening¹⁰⁰. Finally, incompetence of the cervix has been regarded to be causally related to preterm delivery, and cerclage has been widely used in attempts to prevent preterm birth. However, cervical cerclage is largely an unsuccessful strategy¹⁰¹, which suggests that the cervix plays more than just a mechanical role¹⁰².

Relations between risk and biology

There are probably complex relationships between risk factors of preterm birth and biological mechanisms. One risk factor may be important for several pathways and vice versa. In Figure 4 I have made an attempt to summarize these relationships, by indicating the likelihood of an association. A very likely association is denoted “+++”, while a possible association is represented by “+”.

Biological pathway Risk factor	Genes	Inflammation	Vascular	Neuroendocrine	Mechanical
	Ethnicity	+++	+		+
Family history	++	+		+	
Infections	+	+++		+	
Low maternal age		+		+	
High maternal age		+	+	+	
Reproductive history	+	+	+	+	+
Socioeconomic status	+	+		++	
Multiple pregnancy			+	+	++
Smoking		+	++	+	
Air pollution		+		+	

Figure 4. Relations between risk factors and biological mechanisms of preterm birth.

Prevention efforts

The ultimate goal of research on risk factors, etiologies and biological mechanisms of preterm birth is to develop preventive strategies. Especially very preterm infants face substantial risks of mortality or long term neurological sequelae^{103,104}. There are also economic implications. Neonatal intensive care is associated with significant costs, which increase exponentially with decreasing gestational age^{105,106}. Preventing preterm deliveries would not only save lives, but also yield large cost savings.

The majority of preterm births have a spontaneous onset⁵⁹. Given the association between preterm delivery and infections^{33,83}, antibiotic treatment seems like a potential strategy for prevention. However, large randomised controlled trials have drawn rather disappointing conclusions. Antibiotic treatment of women in preterm labour with intact membranes does not delay or prevent preterm delivery¹⁰⁷. Similarly, treatment does not prevent preterm birth in pregnant women with bacterial vaginosis¹⁰⁸. Antibiotic treatment of women with premature rupture of the membranes does not reduce the rate of preterm birth, but can to some extent delay the preterm delivery¹⁰⁹. Still, it is possible that antibiotic treatment could be beneficial if targeted to high-risk groups, as indicated by a large American study. In black urban women screened for reproductive tract infections, antibiotics reduced the risk of preterm delivery (relative risk 0.16, 95 percent CI 0.04-0.66)¹¹⁰.

Maternal periodontal disease is associated with an increased risk of preterm birth³⁷. If bacterial load in the oral cavity contributes to chorioamnionitis, through haematogenous spread or due to increased systemic inflammatory activity, treatment of periodontal disease could be beneficial for pregnancy outcome. One small pilot study showed that treatment of periodontal disease during pregnancy reduced

systemic inflammation, measured by levels of interleukin-6, and reduced the risk of preterm birth (odds ratio 0.26, 95 percent CI 0.08-0.85)¹¹¹. However, in a larger study treatment of periodontitis had no effect on risk of preterm birth, despite improved periodontal health in treated women¹¹².

Prevention of preeclampsia, as a mean to prevent preterm birth, has been extensively studied¹¹³. Many strategies have been tested, including lifestyle choices (rest or exercise), various nutritional measures and drugs. However, almost all strategies have been unsuccessful, with the exception of moderate benefits of low-dose aspirin and calcium supplementation^{114,115}. Antioxidants seem to decrease the risk of preeclampsia, but results should be interpreted cautiously, especially since antioxidants may increase the risk of preterm birth¹¹⁶. Treatment of preeclamptic women with antihypertensive drugs is widely used, but there are limited data supporting that such treatment may reduce the risk of preterm delivery¹¹⁷.

Smoking and substance abuse are potentially preventable factors associated with preterm birth. Women who stop smoking from first to second pregnancy reduce their risk of preterm birth to that of non-smoking women²⁵. A recent meta-analysis including randomised controlled trials concluded that smoking cessation during pregnancy was associated with a 16 percent reduction of the risk of preterm delivery¹¹⁸. Studies on treatment of substance abuse with regard to infant outcomes are scarce, but two small studies have found that gestational length increases somewhat in women undergoing such treatment^{119,120}.

Similarly, social disadvantages may be a target for intervention programs. However, a large randomised trial of psychosocial support and health education during high-risk pregnancies could not find that such interventions reduced the risk of preterm birth¹²¹. A recent meta-analysis of studies on social support during pregnancy came to the same conclusion¹²².

SHORT TERM PROGNOSIS OF VERY PRETERM BIRTH

Mortality during the neonatal period and during infancy

Infant mortality (death during the first year of life) has decreased during the last decades for all infants, as demonstrated by national birth statistics from Sweden (Figure 5, unpublished data from the Medical Birth Register). The reduction in infant mortality over time is mainly explained by decreased neonatal mortality (death during the first four weeks of life), although postneonatal mortality (death after the first four weeks of life but before 1 year of life) has declined somewhat.

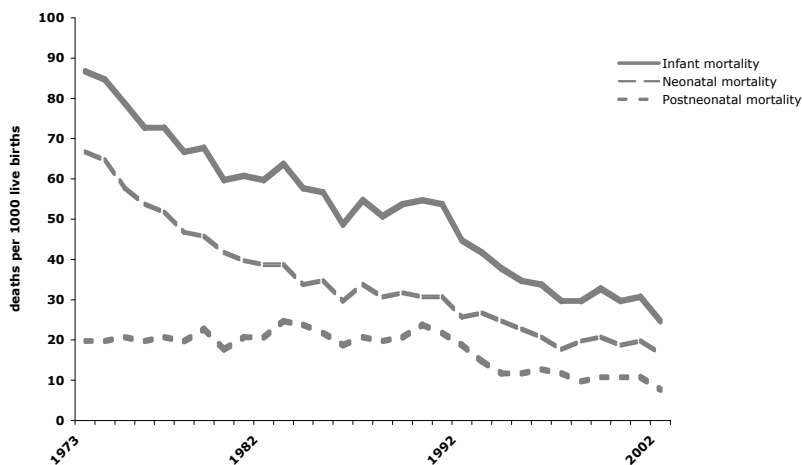


Figure 5. Infant, neonatal and postnatal mortality in infants born in Sweden 1973 to 2002.

The same pattern in improved survival during infancy is seen among term, moderately preterm and very preterm infants, but in absolute numbers, the improvement is most dramatic for very preterm infants (≤ 31 weeks) (Figure 6). From 1973 to 2002, infant mortality rates decreased from 401 to 90 per 1000 live-born very preterm infants, whereas the corresponding rates per 1000 live-born moderately preterm infants (32-36 weeks) and term infants (≥ 37 weeks) decreased from 34 to 8 and from 3 to 1, respectively. This improvement in survival among very preterm infants during the neonatal period has primarily been attributed to improvements of neonatal intensive care, including the introduction of antenatal steroids and surfactant for prevention and treatment of respiratory distress syndrome¹²³.

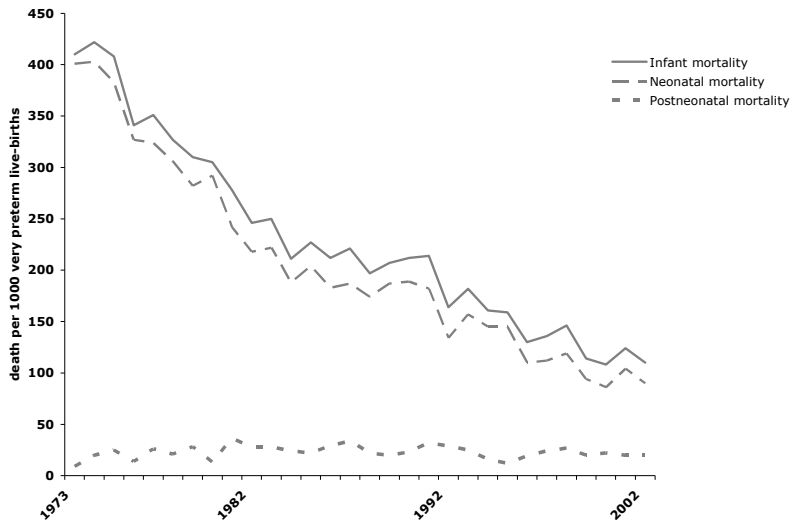


Figure 6. Infant, neonatal and postnatal mortality in very preterm infants born in Sweden 1973 to 2002.

Mortality in very preterm infants is inversely related to gestational age. Despite the overall reduction, the most immature infants still face a substantial risk of death, as illustrated by recently reported mortality rates from Sweden, and the Australia and New Zealand Neonatal Network (Figure 7)^{124,125}.

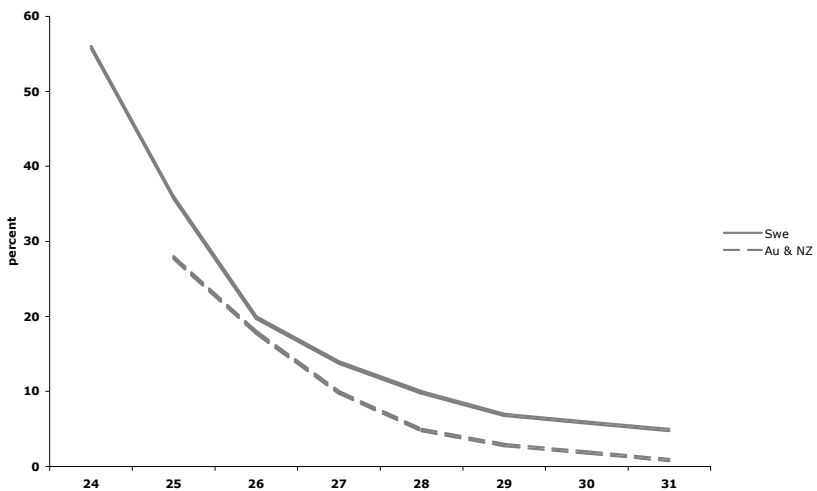


Figure 7. Infant mortality rates in Sweden 1992-1998, and mortality rates before discharge from neonatal intensive care in Australia and New Zealand 1998-2001, by gestational week.

Another way to express the relation over time between mortality and gestational age, is that the so-called “border-of-viability” has shifted to the left. Today, extremely small and immature infants could be considered as candidates for resuscitation and admission to neonatal intensive care units. However, as demonstrated by data from the Vermont Oxford Network*, mortality rates are exceptionally high among the tiniest infants¹²⁶. Of infants born with a birth weight of 401-500 grams (mean gestational age 23 weeks), overall survival was only 17 percent.

A large proportion of infants born at the “border-of-viability” die because of decisions taken shortly after delivery to limit intensive care and provide only palliative treatment¹²⁷. Therefore, management policies could be important for survival in the most immature infants. Studies from Sweden and Germany support that proactive management promotes survival in infants born at 22 to 25 gestational weeks^{128,129}.

To improve survival, regional and/or national organisation of neonatal intensive care also needs to be considered. The complex nature of neonatal intensive care demands highly qualified staffing as well as access to advanced technologies. In several studies from different countries, level-III neonatal intensive care units, i.e. university hospitals, have had lower mortality rates when compared with smaller level-II units. However, the older studies have limitations. They categorised infants according to birth weight instead of gestational age¹³⁰⁻¹³², were performed before recent improvements of neonatal practice^{133,134}, or did not adjust for potential confounders such as obstetric complications^{130,134}. Still, more recent studies also support that centralisation of neonatal intensive care is associated with reduced mortality. A large American study, including 48,237 very-low-birth-weight infants (<1500 grams, 75 percent born at ≤ 31 weeks), found that both volume of care (number of admissions) and level of care were associated with risk of neonatal mortality¹³⁵. According to a report from Finland, 69 of 170 annual deaths could be prevented if all very preterm infants (≤ 31 weeks) were born in university hospitals¹³⁶. A British study investigating variations in standards of neonatal care showed that poor quality of ventilatory support, cardiovascular support and thermal care increased the risk of mortality two- to threefold for infants born at 27 and 28 weeks¹³⁷. In addition, poor quality of care was especially associated with deaths among infants in good condition at birth.

* Vermont Oxford Network is a worldwide network of neonatal intensive care units, which report their outcome data to a central database. Any neonatal intensive care unit can join the Vermont Oxford Network.

Neonatal morbidity

Preterm infants face high morbidity risks during the neonatal period, although advances in neonatal care during recent decades have led to reduced rates of some conditions. For instance, the introduction of antenatal steroids has almost halved risks of respiratory distress syndrome and brain haemorrhage¹³⁸. However, improvements in neonatal care and nursing is not only related to new treatments but also due to implementation of refined strategies, such as improved nutrition, better infection control and more gentle ventilatory support. Thus, neonatology has learnt to better deal with the medical problems related to preterm birth.

Similar to mortality, morbidity risks are inversely related to gestational age^{128,139,140}. Compared to term infants, infants born close to term (34-36 weeks) is a population at risk for problems related to immaturity, such as feeding difficulties, temperature instability, infections and respiratory distress, occasionally even necessitating mechanical ventilation^{141,142}. Nevertheless, moderately preterm infants are generally spared from complicated morbidity. In contrast, very and extremely preterm infants commonly suffer from multiple and interacting morbidities. Medical problems are especially prevalent among 23-25 week infants^{126,128,129,139}, since their extremely immature organ systems at birth complicate the transition from intrauterine to extrauterine life.

Typical medical conditions affecting preterm infants are listed in Table 4¹⁴³. Although those conditions are separate clinical entities, they are also strongly correlated. For example, the acute lung problem shortly after birth (RDS) is correlated with circulatory problems (PDA), brain haemorrhage (IVH) and later lung disease (BPD).

Table 4. Medical problems in preterm infants.

CLINICAL ENTITY	SYNOPSIS
Respiratory Distress Syndrome (RDS)	Lung problem developing shortly after birth due to lack of endogenous surfactant in the lungs. Surface tension increases in the smallest airways and lungs get non-compliant (stiff). Treated with instillation of exogenous surfactant in the airway. Common reason for mechanical ventilation.
Patent Ductus Arteriosus (PDA)	The duct is a blood vessel between the pulmonary artery and the aorta, essential for foetal blood circulation. The duct should close after birth but can stay open in preterm infants, shunting too much blood to the lungs and leaving too little blood for other organs. Can be closed with drugs or surgery.
Necrotising Enterocolitis (NEC)	Inflammation and necrosis of the bowel, leading to various abdominal symptoms. Treated with bowel rest and antibiotics, but surgical bowel resection is commonly performed in cases of bowel necrosis and/or perforation.
Broncho-Pulmonary Dysplasia (BPD)	A more chronic lung problem, related to short gestational age, RDS, PDA and mechanical ventilation. Months of ventilatory support and supplementary oxygen may be needed in severe cases. Some, but not all children can later be prone to asthma-like problems and have reduced lung function.
Retinopathy Of Prematurity (ROP)	Over-growth of blood vessels in the immature retina of the eye, related to factors such as short gestational age and oxygen administration. Low-grade retinopathy usually resolves without specific therapy but laser treatment may be needed in severe forms. Worst-case scenario includes retinal detachment and blindness.
IntraVentricular Haemorrhage (IVH)	Bleedings originating in the germinal matrix, a vascularised and cellularly active tissue beside the brain ventricles. Localised bleedings may not be associated with poor outcomes, but those extending into the brain tissue may have a poor prognosis, and could contribute to decisions to withdraw care (end-of-life-decisions).
PeriVentricular Leukomalacia (PVL)	Damage of brain white matter, related to hypoxia and inflammation. The initial insults may occur before, shortly after birth or during a sudden clinical deterioration, and PVL then develops over 2-3 weeks. Some forms of PVL are strongly associated with cerebral palsy. Can be diagnosed with ultrasound of the brain.
Infections	Very common, due to an immature immune system and much exposure to bacteria from the environment (including staff). Bacteria of low virulence and fungi are common pathogens. Can usually be treated successfully with antibiotics, but infection-related mortality is significant.

While some studies report relatively low or decreasing morbidity rates among survivors after extremely preterm birth (≤ 27 weeks)¹⁴⁴⁻¹⁴⁶, other studies conclude that the improved survival over recent decades have led to increased morbidity rates^{140,147}. Table 5 provides rates of some neonatal morbidities among extremely preterm infants, reported in studies from Europe and the US.

Table 5. Neonatal morbidity in extremely preterm infants surviving till discharge. Data presented as numbers (%).

	Serenius ¹⁴⁴	Markestad ¹⁴⁵	Vanhaesebrouck ¹⁴⁷	Wilson-Costello ¹⁴⁶
<i>Study characteristics</i>				
Setting	Sweden	Norway	Belgium	Cleveland, USA
Time period	1992-1998	1999-2000	1999-2000	2000-2002
Gestational age	23-25 weeks	22-27 weeks	22-26 weeks	500-999 grams
Total no. of births	224	502	525	not reported
Admissions	213	366	303	233
Survivors till discharge	140 (66%)	290 (79%)	175 (58%)	165 (71%)
<i>Morbidity in survivors</i>				
BPD (oxygen at 36w)	50 (36%)	106 (36%)	78 (44%)	84 (51%)
ROP (treated)	21 (15%)	14 (5%)	35 (20%)	not reported
Any IVH or PVL	33 (24%)	124 (43%)	84 (48%)	43 (26%)

As already mentioned, neonatal morbidity rates are inversely related to gestational age. Compared to the most immature infants (≤ 27 weeks), very preterm infants born at 28-31 weeks have less medical problems during the neonatal period. For example, a Dutch study reported 87 percent survival until discharge in very preterm infants and that the rate of bronchopulmonary dysplasi (BPD) was only 10 percent among survivors¹⁴⁸.

LONG TERM PROGNOSIS OF VERY PRETERM BIRTH

Outcomes in children born very preterm

Very preterm birth coincides with a period of intense growth and development of the brain¹⁴⁹. Given the high rates of medical problems during neonatal intensive care, it is not surprising that maturation processes in the central nervous system could be affected, leading to reduced regional brain volumes and risk of long term sequelae¹⁵⁰.

Similar to the inverse relation between gestational age and short term morbidity, rates of disabling impairments in childhood, such as severe hearing loss, blindness and low intelligence quotient (IQ), increase with decreasing gestational age at birth^{151,152}.

Several recent follow-up studies are reporting neurodevelopmental outcomes of children surviving extremely preterm birth in the current era of neonatal intensive care. Although some reports conclude that neurosensory outcome has improved over time^{104,146,153}, those children face significant risks. A hospital-based American study including two-year-old children with birth weights below 1000 grams, reported that cerebral palsy was present in 5 percent and abnormal mental development was present in 21 percent (Bayley Mental Development Index (MDI) <70)¹⁴⁶. A regional study from Belgium including three-year-old children born before 27 gestational weeks reported that cerebral palsy was diagnosed in 25 percent, and that mental development was classified as abnormal in 31 percent (MDI <70)¹⁵⁴.

High rates of neurodevelopmental impairments are also reported in older children born extremely preterm. A national study from Finland including five-year-olds with birth weights below 1000 grams found that 14 percent had cerebral palsy, 9 percent were diagnosed with cognitive impairment, and 4 percent needed a hearing aid¹⁵⁵. In all, only 61 percent of these Finnish children were considered to be free from neurosensory abnormalities. A study from the United Kingdom and Ireland including six-year-old children born before 26 gestational weeks also found similarly high long term morbidity rates¹⁵⁶.

Consequently, functional limitations and special care needs are common, as demonstrated by an American study including eight-year-old children with birth weights below 1000 grams¹⁵⁷. According to responses in a parental questionnaire, common limitations (not mutually exclusive) were trouble understanding simple instructions (22 percent), trouble speaking or communicating (22 percent), reduced time and effort in activity (19 percent), difficulty dressing (13 percent) and difficulty using the toilet (10 percent). The need of health care was also common: 22 percent visited a physician on a regular basis and 31 percent had physical or occupational therapy. Finally, 39 percent had an individualised education plan at school¹⁵⁷, although socioeconomic factors such as parental education level and family structure influence school performance for children born very preterm¹⁵⁸. Given the prevalence of functional limitations and special care needs, it is not surprising that extremely preterm birth may have a negative impact on families, in terms of increased financial and caretaker burden¹⁵⁹.

There are limited data from Sweden on outcomes in children surviving extremely preterm birth during the last two decades. Two recent reports including a national cohort of 86 eleven-year-old children born before 26 gestational weeks, indicate that neurosensory morbidities are not uncommon also among Swedish children born extremely preterm. Cerebral palsy was present in 6 percent, severe visual impairment in 12 percent, and deafness or impaired hearing in 6 percent¹⁶⁰. Although 85 percent were attending mainstream schools, children born extremely preterm had worse mental health, less social competence and more learning problems compared to their term born peers¹⁶¹. For example, internalising and attention problems reported by

parents were overrepresented (33 percent vs. 10 percent, and 30 percent vs. 9 percent), and repetition of grade in school or special educational resources was also more common (59 percent vs. 12 percent).

Outcome in adults born very preterm

With regard to preterm birth and outcomes in adulthood, we need to consider that modern neonatal intensive care has a short history. Antenatal steroids and surfactant for prevention and treatment of respiratory distress syndrome (RDS) were broadly implemented less than 20 years ago. Consequently, very little is known about health in adult life for the growing number of children who have survived very and extremely preterm birth since the 1990s. The possibility to gain knowledge on outcomes in adulthood is further complicated by the continuous short term evaluation and development of technologies and treatment strategies of morbidities during the neonatal period.

In long term follow-up studies, methodological issues may introduce bias, confounding and random errors:

- study design: hospital-based versus population-based cohorts
- study population: size, short term mortality, non-participating survivors
- inclusion criteria: birth weight versus gestational age
- statistics: power, confounding
- outcomes: age at assessment, and outcome definitions
- external validity: adult survivors versus current population of surviving infants

In our attempts to predict outcomes for today's population of very preterm infants, we need to specifically reflect on external validity of the findings from current studies on outcomes in adulthood. Given the increased survival of preterm infants, it is unlikely that preterm survivors during the 1970s are representative for the population of preterm infants surviving in the modern era of neonatal intensive care. Outcomes may be better than reported till date due to refined care, or outcomes may be worse due to an increased proportion of non-healthy survivors with sequelae after complicated clinical courses. Therefore, it is essential to perform follow-up studies including the growing generation of children born very preterm. Still, the studies discussed below provide some insights into outcomes in adults born very preterm.

Follow-up studies of adults born very preterm predominantly include individuals born during the late 1970s and early 1980s. In Table 6 I have summarised design and main outcomes recently reported by five different research groups¹⁶²⁻¹⁶⁹.

One American hospital-based cohort^{162,163} and one Canadian population-based cohort¹⁶⁴⁻¹⁶⁶ are the most well-known and thoroughly described cohorts of adults born preterm. Both cohorts share several characteristics; individuals were born around 1980, the inclusion criterion was based on birth weight, mortality rates were high, study populations were relatively small, and outcomes were assessed before 25 years of age.

The American cohort included individuals born between 1977-1979, with birth weights <1500 grams (very low birth weight, VLBW). With today's perspective, gestational age was relatively high (mean 29.7 weeks) but only 64 percent survived till adulthood. Compared to the control group of adults with normal birth weights, adults with VLBW had more neurosensory impairments (10 percent vs. 1 percent), significantly lower IQ (87 vs. 92) and a lower rate of longer university education (30 percent vs. 53 percent)¹⁶². The differences in educational achievements remained when adults with neurosensory impairments or subnormal IQ (<70) were excluded from the analyses. Despite those differences, both groups had similarly good self-reported health, with the

exceptions that the VLBW group reported less physical activity and greater risk avoidance¹⁶³.

The Canadian cohort included individuals born in 1977-1982. The inclusion criterion was birth weight between 501-1000 grams (extremely low birth weight, ELBW). Gestational age of included individuals was relatively low (mean 27.1 weeks) and only 42 percent survived till adulthood. Neurosensory impairments, mainly cerebral palsy, blindness and cognitive impairments, were present in 27 percent of survivors. Surprisingly, few differences were found when the ELBW group was compared to a group of individuals with normal birth weight. For example, high school graduation was high in both groups, 82 percent vs. 87 percent of ELBW survivors and controls, respectively. Similar proportions in the two groups lived independently or had become parents¹⁶⁶. Although adults with ELBW had more chronic morbidities, no differences in health care consumption were found except that prescription of glasses, medications for depression and home-care services were more common in the ELBW group¹⁶⁴. Generally, self-perceived quality of life was similar in the two groups¹⁶⁵.

When scrutinising the publications on the two cohorts described above, results do not appear to be very different given that both cohorts are relatively small and hampered by low power. For example, ex-preterm adults in both cohorts are less frequently enrolled to university and men seem to have a worse outcome than women. I personally think that the biggest difference is how the research groups interpret their findings, and that the diverging interpretations are not mutually exclusive. While the American researchers discuss “educational disadvantages” after preterm birth, the Canadian researchers conclude that most of ELBW survivors have “overcome their earlier difficulties”.

Other recent publications support that very preterm birth has lasting effects. A hospital-based cohort study from Canada, including adolescents with birth weights ≤ 800 grams, found reduced reading and arithmetic skills, and reduced scholastics, athletic, and romantic confidence among ex-preterm adolescents¹⁶⁷. Parents also reported more behavioural problems, both internalising and externalising, especially among males. A national study from the Netherlands reported that 13 percent of young adults born very preterm had moderate to severe cognitive or neurosensory problems¹⁶⁸. Compared to age-peers in the general Dutch population, twice as many young adults born very preterm were poorly educated (24 percent vs. 13 percent) and 3 times as many were neither employed or in school (7.6 percent vs. 2.6 percent). Another national study, including all births in Sweden during 1973-1979, found a stepwise increase in disability with decreasing gestational age¹⁶⁹. In total, 13 percent of adults born at 24-28 weeks and 6 percent of adults born at 29-32 weeks received economic societal assistance due to handicap or persistent illnesses, compared to 1.5 percent of adults born at term. Gestational age was also inversely associated with university education and net salary, i.e. adults born preterm were less frequently attending university and had lower salaries compared to adults born at term.

Table 6. Summary of studies of outcomes in adults born very preterm.

Author Setting	Hack ¹⁶² USA	Saigal ¹⁶⁶ Canada	Grunau ¹⁶⁷ Canada	Hille ¹⁶⁸ Netherlands	Lindström ¹⁶⁹ Sweden
Study design	Hospital-based cohort	Population-based cohort	Hospital-based cohort	Population-based cohort	Population-based cohort
Birth years	1977 – 1979	1977 – 1982	1981 – 1986	1983	1973 – 1979
Inclusion criteria	birth weight <1500 grams	birth weight 501 – 1000 grams	birth weight ≤800 grams	birth weight <1500 grams or gestational age <32 weeks	Individuals born at 24 – 41 weeks, and alive in 2002
Study base and study population	490 infants admitted to NICU 312 adult survivors 242 individuals included	397 infants admitted to NICU 166 adult survivors 149 individuals included	250 infants admitted to NICU 98 adult survivors 79 individuals included ²	1338 infants admitted 959 adult survivors 705 individuals included	In all 522 310 adults: 317 born at 24-28 weeks 2630 born at 29-32 weeks
Mean birth weight and gestational age	1179 grams 29.7 weeks	841 grams 27.1 weeks	719 grams 25.8 weeks	only distributions reported ³	only distributions reported ⁴
Main results (vs. control group)	more neurological impairments lower academic achievements less alcohol and drug abuse	more neurological impairments similar health care use, quality of life, and academic and social achievements.	lower cognitive skills less confidence and more neuropsychological problems	poor education and unemployment more common ⁵	lower education lower salary public financial assistance more common

² Adults (n=19) with major sensorimotor handicaps and/or IQ <70 impairments were excluded.

³ Gestational age distribution: 9% ≤27 weeks; 62% 28-31 weeks; 29% ≥32 weeks. Birth weight distribution: 14% <1000 g; 65% 1000-1500 g; 20% ≥1500 g.

⁴ Birth weight distribution at 24-28 weeks: 34% <1000 g; 66% 1000-1999 g. At 29-32 weeks: 2% <1000 g; 76% 1000-1999 g; 21% ≥2000 g.

⁵ No formal control group. Comparisons were made with age-peers in the general population.

The developmental origin of health and disease in adulthood

The hypothesis that exposures in early life may influence health outcomes in adulthood has been an extensively growing research field during the last 20 years. In a public health perspective, such associations may have important implications in the context of primary prevention. To reduce public health impact of common diseases like cardiovascular disease and type 2 diabetes, prevention efforts may be needed much earlier than generally anticipated, during childhood or even during foetal life.

The Norwegian general practitioner, professor Anders Forsdahl, investigated the considerable variations in cardiovascular mortality rates in Norwegian counties during the 1970s, and found that high infant mortality rates preceded high cardiovascular mortality rates¹⁷⁰. Given that high infant mortality was a proxy for poverty, Forsdahl suggested that poverty during childhood could be risk factor of later cardiovascular disease¹⁷⁰. This interpretation was supported by a later study in which poverty during childhood was associated with increased serum cholesterol levels in adulthood¹⁷¹. In 1988, Swedish researchers published a study based on 104 young men, suggesting that intrauterine growth restriction was associated with high diastolic blood pressure¹⁷².

Professor David Barker and co-workers in the UK further developed the idea that early exposures may lead to poor health later in life. After replicating Forsdahl's findings, that infant mortality correlated with cardiovascular mortality in a time-shifted fashion¹⁷³, Barker and co-workers published a larger cohort study in 1989 that showed that birth weight was inversely related to risk of cardiovascular mortality¹⁷⁴. The British research group proposed a hypothesis that poor foetal nutrition was associated with cardiovascular disease in adulthood, by "programming" the foetus for cardiovascular disease decades later^{175,176}.

Today, the theoretical framework is extended and the hypothesis is usually referred to as the Developmental Origin of Health and Disease (DOHaD)¹⁷⁷. The hypothesis states that environmental exposures act via the general phenomenon (across species) of developmental plasticity, i.e. structure and function in organs change in response to environmental exposures. If exposures operate during critical time windows during development, induced changes may become irreversible.

A central concept in the DOHaD hypothesis is "predictive adaptive response"¹⁷⁷. Basically, this means that responses in the foetus/child serves to adapt to a "predicted" future environment. From an evolutionary point of view, predictive adaptive responses are designed to optimise the phenotype and promote survival. However, if the prediction is wrong, there will be an inappropriate "mis-match" between expected and actual environment that may increase the risk of disease. For example, if the foetus is exposed to poor nutrition prenatally, its physiology will be adapted to a postnatal environment that is sparse. If the foetus is born into a nutritionally plentiful environment, a predictive adaptive response including energy conservation may not be advantageous, but increase the risk of obesity.

Until recently, the DOHaD research field has mainly focused on low birth weight in individuals born at term, assumingly related to intrauterine growth restriction. This research direction was not surprising since most of earlier studies were based on cohorts born in the early 20th century when few preterm born infants survived. Today, the most common cause of low birth weight is preterm birth in most countries¹⁷⁸. As opposed to low birth weight at term, the majority of preterm infants have a normal birth weight for gestational age¹⁷⁹.

Preterm birth is also suggested to be a perinatal contribution to disease in adulthood^{180,181}, although inappropriate predictive adaptive responses related to exposures during the foetal life is probably not operating. Preterm birth may be considered as

very “un-natural” from an evolutionary perspective. It seems unlikely that a foetus would predict the environment of neonatal intensive care, especially since most pregnancies have proceeded normally till the time of preterm delivery. One may speculate that postnatal factors experienced during neonatal intensive care predispose preterm infants to health problems later in life. Exposures such as inflammation and pain could “prime” inappropriate response patterns and disrupt organ development leading to structural abnormalities.

Criticism of the DOHaD hypothesis

The DOHaD hypothesis has been criticised since associations between perinatal exposures and diseases many decades later may seem far-fetched¹⁸²⁻¹⁸⁵. It has been argued that alternative pathways explain observed associations, i.e. genetic or environmental factors confound the associations between perinatal exposures and outcomes later in life.

Genetic confounding occurs when there is a common genetic background for the exposure and the outcome (Figure 8). For example, it has been suggested that genetic factors influencing the glucose/insulin homeostasis may lead to both reduced foetal growth and increased risk of cardiovascular disease and type 2 diabetes¹⁸⁶.

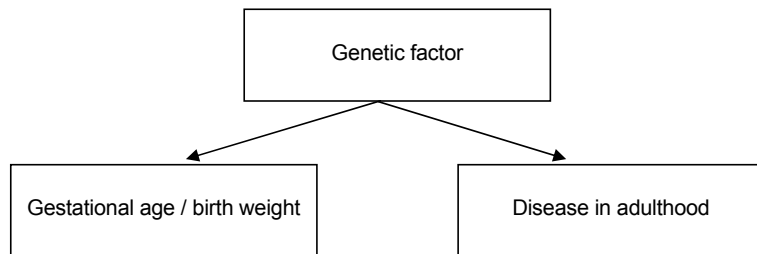


Figure 8. Genetic confounding of the association between perinatal characteristics and disease in adulthood.

Environmental confounding (Figure 9) is another plausible explanation of the DOHaD hypothesis, since it has been shown that socioeconomic factors could be associated with birth weight¹⁸⁷, gestational age²⁵ and disease in adulthood¹⁸⁸.

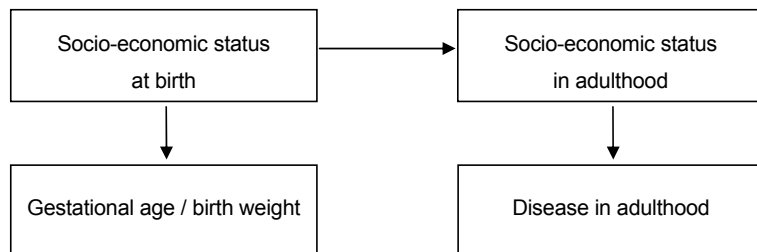


Figure 9. Environmental confounding of the association between perinatal characteristics and disease in adulthood.

Whether perinatal exposures are causally related to disease in adulthood is important to consider, since environmental exposures and genetic factors could have independent effects on pregnancy outcomes and adult health. Family-based studies can be used to study confounding due to familial (i.e. shared genetic and early environmental) factors (Figure 10). If an association is only found among independent individuals but not within families, the association is probably explained by familial factors. On the other hand, if an association is evident within families, the association could be explained by exposures unique for the individual, such as individual specific factors resulting in low birth weight.

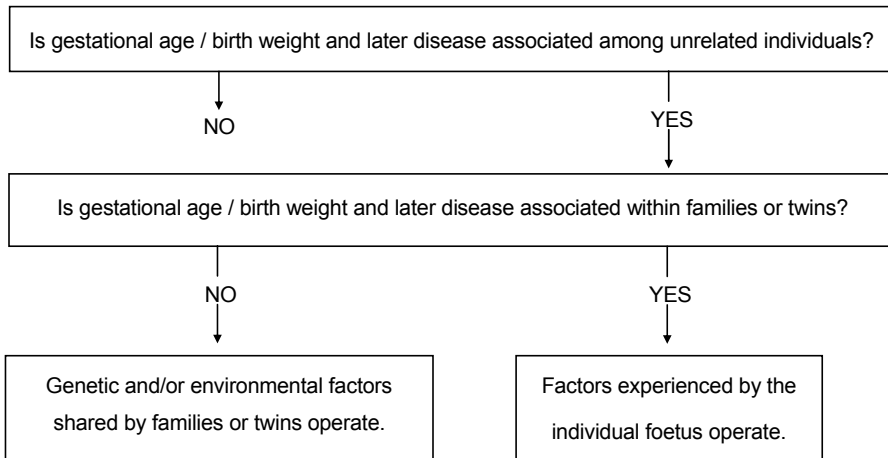


Figure 10. Importance of unique and shared factors with respect to suggested associations between perinatal characteristics and disease in adulthood.

Developmental origin of cardiovascular disease

The DOHaD hypothesis originated from studies on associations between low birth weight and coronary heart disease^{174,176}. Today, several studies have replicated the original findings and a recent review article, based on 18 studies, found that 1 kg lower birth weight increased the risk of coronary heart disease by 16 percent¹⁸⁹. Whether low birth weight is associated with increased blood pressure has also been addressed, and studies report an inverse relation between birth weight and blood pressure^{190,191}. This association was questioned in a meta-analysis that found weaker associations in larger studies, selective emphasis on results supporting the DOHaD hypothesis, and inappropriate or inadequate adjustments for potential confounders¹⁸⁴. However, results from a large twin-study has confirmed that decreasing birth weight is associated with increasing risk of hypertension, independently of genetic factors and shared familial environmental factors in childhood and adolescence, and socioeconomic factors in adulthood¹⁹².

Birth weight is a function of foetal growth and gestational length, and a low birth weight could be explained by intrauterine growth restriction or preterm birth. It has been suggested that the association between low birth weight and risk of cardiovascular disease is mediated by foetal growth restriction in term birth rather than preterm birth¹⁹³. However, that study was based on a birth cohort from the early twentieth century, when mortality rates among preterm infants were very high. Today, the most common cause of low birth weight in most countries is preterm birth¹⁷⁸.

Although a developmental origin of cardiovascular disease is a well-supported hypothesis, the independent effects of low birth weight due to preterm birth or foetal growth restriction has not been fully elucidated. Preterm birth may attenuate the association between being born small for gestational age (SGA) and risk of high blood pressure^{194,195}, and gestational age may modify the association between body mass index (BMI) in adulthood and blood pressure¹⁹⁶.

In small clinical studies, adolescents and young adults born very preterm were found to have structural changes in the vascular tree^{197,198} and higher systolic blood pressure^{194,195,198} than individuals born at term. Results from Swedish population-based cohort studies suggested that gestational age may be inversely associated with blood pressure^{180,181,199}. However, the strength of the association has not been studied among survivors of very short gestations and one of the previous studies did not include individuals with gestational ages less than 35 weeks¹⁹⁹. Moreover, these studies did not account for genetic and socioeconomic factors, which may influence both gestational age and risks of cardiovascular diseases^{25,27,188,200}. Finally, intrauterine growth restriction is overrepresented among preterm births¹⁷⁹, emphasising the importance of using reference curves based on foetal growth rather than recorded birth weights, when assessing the relation between birth weight for gestational age and later blood pressure.

Developmental origin of type 2 diabetes

The increasing prevalence of type 2 diabetes is a global public health challenge²⁰¹, largely due to the strong association between type 2 diabetes and cardiovascular disease²⁰². Lifestyle education²⁰³ and pharmacological treatment of the metabolic syndrome^{203,204} might mitigate this expected pandemic, but the ideal situation would be to intervene before the occurrence of reduced glucose tolerance.

An association between low birth weight and risk of type 2 diabetes in adulthood has been reported in several epidemiological studies^{191,205}. A recent meta-analysis concluded that individuals with a birth weight of less than 2500 grams had a 32 percent increased risk of type 2 diabetes, compared to individuals with birth weights above 2500 grams²⁰⁶.

It has been suggested that poor nutrition during foetal life induces permanent adaptive changes in the foetus that predispose to type 2 diabetes, a mechanism referred to as the thrifty phenotype hypothesis²⁰⁷. The finding that prenatal exposure to famine is linked to reduced glucose tolerance in adults lends support to this hypothesis²⁰⁸. However, genetic factors account for close to 50 percent of the variation in foetal growth²⁰⁹, and type 2 diabetes risk is also influenced by genetic factors²¹⁰ or gene-environment interactions²¹¹. Thus, a common genetic background related to the glucose/insulin homeostasis may result in both low birth weight and development of type 2 diabetes, and explanation referred to as the foetal insulin hypothesis^{186,212}.

Twin studies may serve as a model to resolve issues regarding the importance of environmental and genetic factors for the association between foetal growth and type 2 diabetes. Since twins have the same gestational age, birth weight differences within twin pairs reflect individual differences in foetal growth. Twins are generally brought up together, and analyses within twin pairs also provide control for socioeconomic and other shared environmental factors during childhood and adolescence. Finally, dizygotic and monozygotic twins share 50 percent and 100 percent of their genes, respectively. Thus, analyses stratified by zygosity allows for varying degrees of control for genetic factors.

To study the presumably subtle effects of genetic factors related to both foetal growth and insulin-glucose homeostasis, large twin studies are needed²¹². The small size of previous twin studies may well explain previous conflicting results²¹³⁻²¹⁶. While birth weight was associated with type 2 diabetes in within-pair comparisons including 14 monozygotic and 14 dizygotic twin pairs discordant for type 2 diabetes, the same study found no association between birth weight and reduced glucose tolerance among 13 monozygotic and 26 dizygotic twin pairs discordant for reduced glucose tolerance²¹³. A study including 58 monozygotic and 140 dizygotic twin pairs found no association between birth weight and glucose tolerance²¹⁴. A large Swedish twin study estimating between- and within-pair effects of birth weight differences on diabetes risk found that mean birth weight between pairs was associated with type 2 diabetes²¹⁵. However, within-effects were consistently lower compared to between-effects, suggesting that the association between birth weight and type 2 diabetes was influenced by genetic factors shared by twins²¹⁵. Also, a recent publication found no association between birth weight and insulin resistance neither within nor between twin pairs, suggesting that both shared genetic and environmental factors may be important for the association²¹⁶.

Adding to the complexity are reports showing that preterm birth could predispose to type 2 diabetes. Compared to term born peers, young children born very preterm had a reduced glucose tolerance, irrespective of their birth weight being appropriate or low for gestational age²¹⁷. Similarly, young adults born very preterm had higher indices of insulin resistance and glucose intolerance, compared to subjects born at term²¹⁸.

3. AIMS

The overall objective of this thesis was to increase the knowledge about the etiology and prognosis of very preterm birth.

The specific aims were:

- To study the association between common viral infections during pregnancy and the risk of very preterm birth (Paper I).
- To elucidate the role of level of care for infant mortality in very preterm infants (Paper II).
- To investigate whether low gestational age at birth increases the risk of high systolic blood pressure in young men (Paper III).
- To study the importance of gestational age and birth weight for the risk of developing type 2 diabetes later in life, focusing on the possibility of genetic confounding (Paper IV).

4. MATERIAL AND METHODS

SETTING

The studies included in this thesis were conducted in Sweden. The well-defined organization of the health care system, the systematic use of civic registration numbers, and the existence of national population-based registers, enable well-designed epidemiological studies in Sweden²¹⁹.

The health care system in Sweden is uniformly designed. It is almost entirely financed by taxes. Private care solely funded by private insurances or the patients themselves, is very rare. Hospital care is organized in three levels (Figure 11). Sweden is divided into 20 geographically defined counties, each operating local hospitals with basic facilities and one larger general hospital with more advanced care. Specialized care is centralized region-wise to seven university hospitals.

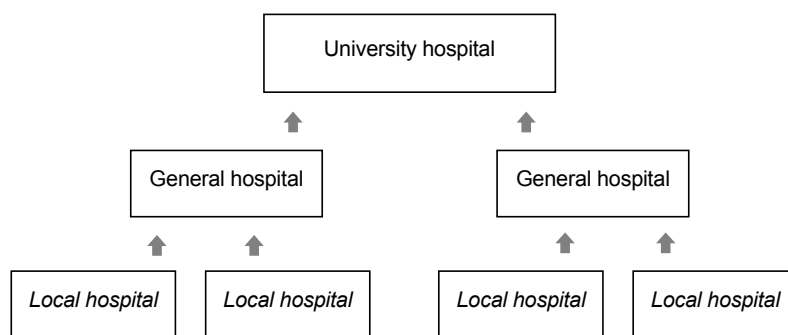


Figure 11. Schematic illustration of the organization of hospital care in Sweden.

The civic registration number is a unique identification number assigned to each Swedish resident at birth or at immigration²²⁰. Ten digits build up the civic registration number. To the six first digits that indicate date of birth, four digits are added to make the number unique for each individual. The civic registration number is used in health care settings to label medical records, referrals and analyses. It is also used for record identification in national registers. Consequently, it is possible to link individual information from various data sources.

DATA SOURCES

The studies included in this thesis used information from the following national population-based data registers: the Medical Birth Register, the Cause of Death Register, the Conscript Register, the Multigeneration Register, the Population and Housing Census 1990, and the Swedish Twin Registry. For validation purposes in Paper I, information on gestational age and birth weight was also collected from original antenatal, obstetric and neonatal records.

Medical Birth Register

The Medical Birth Register contains data on essentially all births in Sweden since 1973²²¹. Starting at the first antenatal visit, information is prospectively collected on standardized records, and forwarded to the registry. Mortality data is added through

linkage to the Cause of Death Register. The Medical Birth Register has been validated, and the quality of the variables used in studies included in this thesis is considered high²²¹. The National Swedish Board of Health and Welfare is formally responsible for the Medical Birth Register.

Cause of Death Register

The Swedish statistics on causes of deaths are among the oldest in the world, going back to 1749 when a nationwide report system was introduced²²². Statistics on causes of death have been published annually since 1911. Today, the National Swedish Board of Health and Welfare has the formal responsibility for the Swedish mortality statistics. The underlying cause of death is selected from the conditions reported on the death certificate, according to instructions issued by the World Health Organisation in the International Classification of Diseases (ICD). There are no recent figures available on the accuracy of Swedish death certificates, but a validation study is currently ongoing.

Conscript Register

The Conscript Register, computerized since 1983, contains information about young men assessed for military service. Conscription is mandatory for men and enforced by law, but men with handicaps or congenital malformations generally receive an exemption. Information collected during conscription, including results from cognitive and physical testing, is recorded in the Conscript Register. The National Service Administration, Ministry of Defence, is formally responsible for the Conscript Register (Ingvar Ahlstrand, National Service Administration, personal communication, 2007).

Multigeneration Register

The Multigeneration Register was compiled in 2000 by Statistics Sweden (SCB) using available register data on the total Swedish population²²³. The Multigeneration Register includes information about Swedish residents born 1932 or later (index-persons) and their first-degree relatives. Parents, siblings and children to an index-person can be identified through this register.

Population and Housing Census 1990

In the Population and Housing Census 1990, data on individuals and families was collected by postal enquiries in 1990 by Statistics Sweden²²⁴. The census included information on socioeconomic characteristics such as education, profession and housing. Participation was mandatory for all residents in Sweden with an age of 16 years and older. The response rate was 97.5 percent.

Swedish Twin Registry

The Swedish Twin Registry, started in the early 1960s and administered by the Department of Medical Epidemiology and Biostatistics at Karolinska Institutet, is a national health-related database of twins²²⁵. It is updated annually with regards to the Address Registry, the Cancer Registry, and the Cause of Death Registry. Additional information is collected depending on specific needs for ongoing projects. Information from original medical records, such as maternal socio-demographic characteristics, gestational age, birth weight, birth length and head circumference, have recently been added to the Swedish Twin Registry.

Antenatal, obstetric and neonatal records

Starting at the first antenatal visit and ending at discharge after delivery, information is recorded on standardized records and forwarded to the Medical Birth Register, using the mother's civic registration number. The original antenatal, obstetric and neonatal records are stored in hospital archives. It is mandatory to store hospital records indefinitely, and records may be used for research purposes after approval²¹⁹. Thus, information in the Medical Birth Register can be validated and supplemented.

PAPER I

This case-control study about viral infections and risk of second trimester miscarriage and very preterm birth was based on blood sampled from pregnant women residing in Stockholm County, and stored at the Virology Laboratory of Karolinska University Hospital in Stockholm, Sweden. The Swedish maternity care program includes serological screening for Rubella immunity in early pregnancy. Sera from the Rubella screening are stored according to requirements issued by the National Board of Health and Welfare²²⁶. In this section of the thesis, only methods and results related to the risk of very preterm birth is presented. For information about methods and results on second trimester miscarriage, please read the complete manuscript of Paper 1, in the next section.

During the years 1990 to 2002, 75,037 blood samples from the Rubella screening were stored at the Virology Laboratory. On average, blood was sampled in gestational week 11, and 90 percent of samples had been collected between 7 and 18 completed weeks of gestation. Individual blood samples were coupled with individual data on maternal characteristics and pregnancy outcomes, by linkage of the register for stored serum samples with the Medical Birth Register.

After linkage of the registers, cases and controls were selected. Very preterm deliveries (≤ 31 weeks) of live born singleton infants were defined as cases ($n=419$). Controls were defined as singleton term deliveries (≥ 37 weeks), and we randomly selected 500 controls among the remaining records.

Although the quality of recorded gestational age and birth weight in the Medical Birth Register has been shown to be good²²¹, recorded gestational age and birth weight were validated for all cases, by abstracting this information from original medical records. Information in original medical records was also used to hierarchically categorize the etiology of very preterm delivery as follows: preeclampsia, vaginal bleeding including placental abruption, preterm premature rupture of the membranes, premature labour, or unclassified etiology^{227,228}.

A complete set of serum samples for virus analyses from all cases and controls could not be retrieved. Serum samples older than ten years had, after the study was initiated, been cleared from the Virology laboratory. The reason for this was that the National Board of Health and Welfare mandates storage of samples only for 10 years²²⁶. Consequently, serum could only be analyzed for 269 cases and 301 controls, born 1993 to 2002. Excluded cases and controls, with missing information on viremia, had birth weight and gestational age distributions compared to included cases and controls.

Serum was investigated for presence of viral RNA (PCR technique) from Human Parvovirus B19 and the following Herpesviridae: Varicella Zoster, Cytomegalovirus, Epstein Barr virus, Human herpes virus 6, Human herpes virus 7, and Human herpes virus 8. Presence of viral RNA was defined as viremia/viral infection.

Statistical methods

The effect of viremia on very preterm delivery was estimated by logistic regression. Maternal age, parity and smoking were a priori considered as potential confounders and were included in adjusted models. Differences in gestational age, birth weight and etiology of very preterm delivery, between virus positive and virus negative cases were assessed by Wilcoxon-Mann-Whitney test or Chi2-test.

PAPER II

This cohort study about very preterm birth, level of care and infant mortality, was based on the Swedish Medical Birth Register.

Among primiparous women giving birth between 1992 and 1998 (n=291,059), 2,374 liveborn singleton infants were delivered before 32 gestational weeks. Since there was no consensus on resuscitation of infants born at 22-23 weeks, 16 births at 22 weeks and 41 births at 23 weeks were excluded from further analysis (infant mortality rates 81 percent and 66 percent, respectively). In addition, 32 accidental preterm births in 22 local maternity units (designed for term deliveries and without access to neonatal service) were excluded, as were 32 infants with apparently misclassified gestational age. Thus, the analyses were performed on the remaining 2,253 live singleton infants, born at 24-31 weeks.

Maternal diagnoses were coded according to the International Classification of Diseases (ICD-9 or ICD-10). Pregnancy complications were grouped into four main groups; placental complications, hypertensive illnesses, diabetes and other maternal diseases. Placental complications were defined as placenta previa, placental abruption, or unspecified antepartum haemorrhage. Hypertensive illnesses were classified as preeclampsia and other hypertensive illness. Diabetes was classified as pregestational and gestational diabetes.

During the study period, gestational age was mainly determined by ultrasound²²⁹. Birth weight for gestational age was classified as small-, appropriate-, and large for gestational age (SGA, AGA, and LGA, respectively), according to the Swedish reference curve for foetal growth¹⁷⁹. Delivery hospitals were categorized into general hospitals (in Swedish: *länsjukhus*) and university hospitals (in Swedish: *regionssjukhus*), according to a classification by the Swedish National Board of Health and Welfare²³⁰.

Statistical analysis

Odds ratios for risk of infant mortality associated with level of care were calculated, using logistic regression. Independent variables in the multivariate logistic regression models were mode of delivery, hospital type (level of care), gestational age, birth weight for gestational age, infant sex, foetal presentation, placental complications, and maternal hypertensive illness.

PAPER III

This cohort study about gestational age at birth and high blood pressure in young adulthood, was based on the Medical Birth Register, the Conscript Register, the Multigeneration Register and the Population and Housing Census 1990. The civic registration number was used for individual record linkage.

From 1973 to 1981, there were 458,371 male live births recorded in the Medical Birth Register. In order to increase the homogeneity of the study population, we excluded multiple births, congenital malformations, infants to non-Nordic mothers and those who died before 18 years of age (n=54,649). Thus, the study population included 404,306 men of whom 379,963 (94 percent) were conscripted between 1991 and 2001. Information on systolic blood pressure was available for 329,495 men.

Information about gestational age and birth weight was obtained from the Medical Birth Register. During the study period, gestational age was estimated from the date of the last menstrual period. Gestational age was classified as extremely preterm (24-28 weeks), very preterm (29-32 weeks), moderately preterm (33-36 weeks), term (37-41 weeks), and postterm (42-43 weeks). This classification of extremely preterm birth was

introduced in order to include a sufficient number of individuals for statistical analyses. Birth weight for gestational age was classified as small-, appropriate-, and large for gestational age (SGA, AGA, and LGA, respectively), according to the Swedish reference curve for foetal growth¹⁷⁹.

At the military conscription, blood pressure was measured under standardized conditions according to written instructions [Dr Wågermark, National Service Administration, Sweden, personal communication 2005]. After 5 to 10 minutes rest in supine position, nurses assigned for fitness testing measured the blood pressure in the right arm. High systolic blood pressure was defined as ≥ 140 mm Hg and high diastolic blood pressure was defined as ≥ 90 mm Hg²³¹.

Information about parental socioeconomic and educational status and family structure was obtained from the Population and Housing Census 1990, and classified according to recommendations by Statistics Sweden²³².

Statistical analysis

Odds ratios for high systolic blood pressure were calculated using logistic regression. Independent variables in the analyses included gestational age, birth weight for gestational age, maternal age, parity, height at conscription, conscription year, family structure of the conscriptor, parents' educational and socio-economic status. Conscription year was added to the model to control for possible cohort effects.

Analyses between and within families were performed, to explore whether an association between gestational age and high blood pressure was confounded by familial factors²³³. These analyses were restricted to males with at least one full brother in the cohort ($n=106,576$). In analyses of familial effects, the exposure is decomposed into between- and within-family components. The between-family component is measured by the family mean of the exposure measurement, and the within-family component is measured by the individual deviation from the family mean. If familial factors are important they tend to make family members more alike. Hence, the within-family component would be non-significant, whilst the between-family component would be significant. If the within-family component remains significant, this indicates that the association between exposure and outcome depends on other than familial factors.

PAPER IV

Eligible participants in this study about perinatal characteristics and offspring risk of type 2 diabetes were like-sexed twins born in Sweden from 1926 to 1958 ($n=37,392$), included in the Swedish Twin Registry. In 1998, twins who were alive and living in Sweden, were invited to a telephone interview, the "Screening Across the Lifespan Twin (SALT) Study"²²⁵. SALT was initiated for screening of diseases, and was used to diagnose type 2 diabetes in this study. The response rate to SALT was 74 percent ($n=24,295$). In this study, the cohort was restricted to twins with known zygosity ($n=23,543$).

Information about parental and perinatal characteristics was abstracted from original birth records by visiting delivery archives, located all over Sweden. Birth records were obtained from 18,442 (78 percent) individuals (7,410 complete twin pairs), including information on birth weight and gestational age, based on date of last menstrual period. Socio-economic status was classified according to recommendations by Statistics Sweden²³².

During the SALT interview, twins were asked questions regarding their medical history and use of prescribed medications²²⁵. Diabetes status was determined using the algorithm presented in Figure 12. Among the 592 twins with type 2 diabetes, 390 were treated with oral antidiabetic drugs. Among twin pairs, 303 were discordant for type 2

diabetes (twin with type 2 diabetes had a non-diabetic co-twin), and 58 were concordant for type 2 diabetes (both twins had type 2 diabetes). Diabetes status was unknown for non-responding co-twins of 173 twins with type 2 diabetes.

Statistical methods

Cohort analyses of the association between birth weight and type 2 diabetes were done by logistic regression. Besides the main exposures (birth weight and gestational age), the adjusted regression models included covariates contributing significantly to the models ($p < 0.05$); sex, birth year, maternal age and parity, and socioeconomic status in adulthood.

Additional analyses of the effect of birth weight on risk of type 2 diabetes were estimated in co-twin case-control analyses stratified by zygosity. The co-twin case-control analyses were restricted to twin pairs discordant for type 2 diabetes. In co-twin case-control analyses, healthy co-twins are used as matched controls for the cases. Since twins within a pair share intrauterine exposures, maternal factors, 50 or 100 percent of their genes, and childhood environment, the matched nature of the co-twin case-control design minimizes confounding by these factors. Since dizygotic twins share 50 percent of their genes, the estimated paired effect of birth weight on risk of type 2 diabetes in dizygotic twins only partly controls for genetic factors. In contrast, analyses within monozygotic twin pairs fully control for genetic factors. Thus, if the effect of birth weight on risk of type 2 diabetes is smaller within monozygotic than dizygotic twins, this indicates that the association is confounded by genetic factors.

ETHICAL CONSIDERATIONS

The studies were approved by the research ethics committee of the Karolinska Institutet (Paper I-IV, Dnr 02-284, 01-368, 03-033 and 00-410).

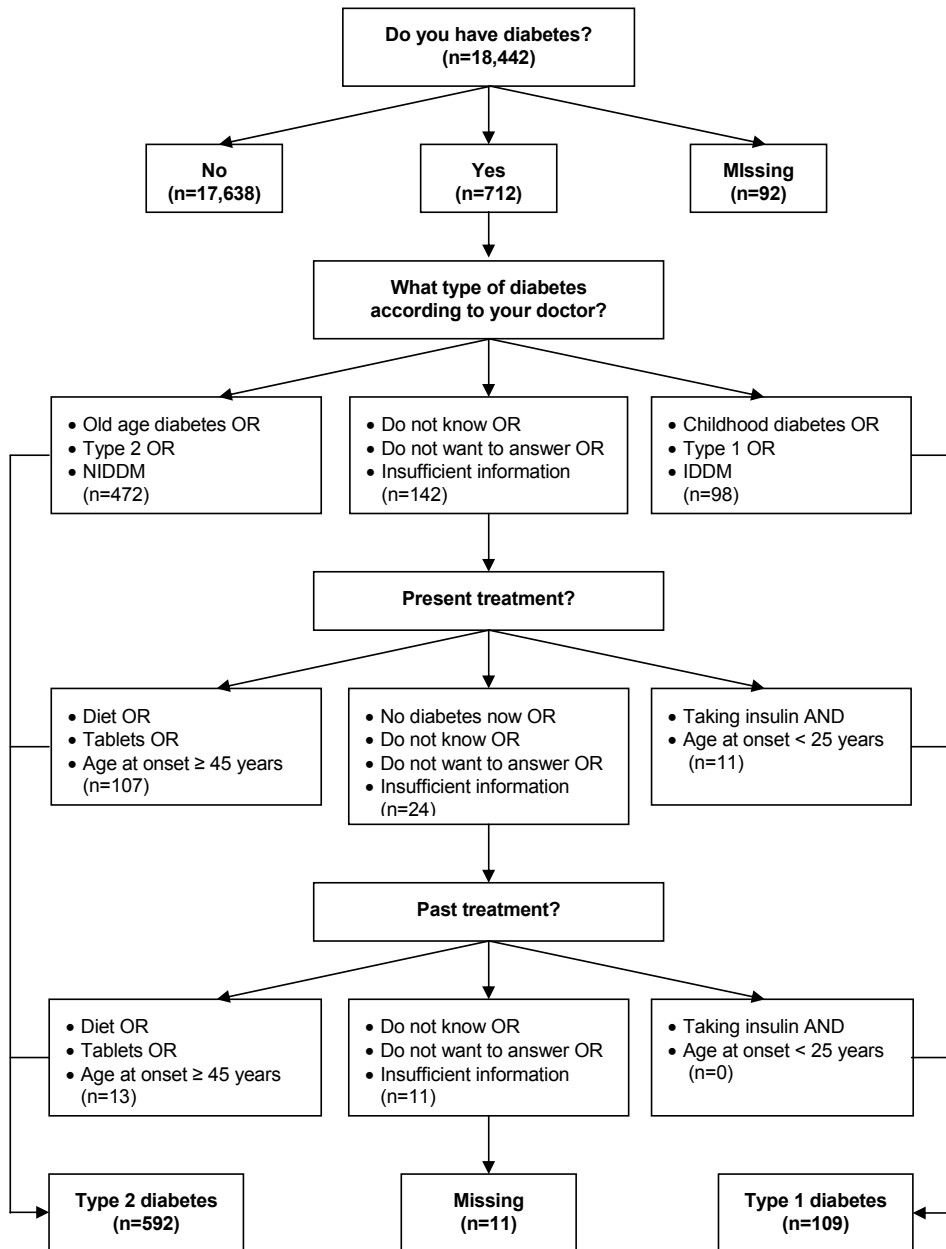


Figure 12. Flow chart on the categorization of diabetes status, according to responses in the SALT interview, in like-sexed twins born 1926-1958.

5. RESULTS

VIRAL INFECTIONS AND VERY PRETERM BIRTH (I)

Viremia was more often detected in first trimester blood samples from pregnant women who delivered very preterm, compared to women who delivered at term (Table 7). Any viremia was found in 10 women with very preterm deliveries and in 5 women with term deliveries. Of the positive samples, Parvovirus B19 was detected in 7 preterm cases and in 3 term controls. Although non-significant, risk estimates indicated that any viremia and Parvovirus B19 was associated with a twofold increase in risk of very preterm delivery.

Table 7. Prevalences of viral infections and odds ratios of very preterm delivery. Cytomegalovirus, found in one preterm case, is not included in the table below.

	Virus +		Virus -		Odds ratios (95% CI)			
	No.	(%)	No.	(%)	crude		adjusted [†]	
Any viral infection								
Very preterm delivery	10	(3.7)	259	(96.3)	2.28	(0.77-6.77)	2.21	(0.71-6.84)
Term delivery*	5	(1.7)	296	(98.3)	1.00		1.00	
Parvovirus B19[‡]								
Very preterm delivery	7	(2.6)	258	(97.4)	2.66	(0.68-10.4)	2.66	(0.64-11.1)
Term delivery*	3	(1.0)	294	(99.0)	1.00		1.00	
Human herpes virus 6								
Very preterm delivery	2	(0.7)	267	(99.3)	1.12	(0.16-8.00)	1.08	(0.14-8.08)
Term delivery*	2	(0.7)	299	(99.3)	1.00		1.00	

* reference

[†] adjusted for maternal age and parity

[‡] not analyzed in 4 cases and 4 controls

One may speculate that early pregnancy viremia would increase the risk especially for spontaneous preterm labour and extremely preterm birth (delivery before 28 weeks). However, the group of very preterm infants to virus positive mothers were not different from the group of very preterm infants to virus negative mothers. Etiologies of very preterm delivery were similarly distributed in the two groups, as were gestational ages and birth weights.

PRETERM DELIVERY, LEVEL OF CARE AND INFANT MORTALITY (II)

During the years 1992 to 1998, 2,253 infants were born before 32 completed weeks of gestation, of whom 267 (12.6 percent) died during the first year of life. The majority of deaths occurred during the neonatal period (n=232).

In the univariate analysis, infants born in general hospitals had a reduced risk of infant mortality compared with infants born in university hospitals (Table 8). In the multivariate analysis, infants born in general hospitals faced a 33 percent increased risk of infant mortality. This shift was primarily due to different gestational age distributions in university and general hospitals. Besides level of care, gestational age, birth weight for gestational age and infant sex were also associated with risk of infant mortality.

Table 8. Risk of infant mortality associated with level of care.

	Infant mortality (%)	Odds ratios (95% CI)	
		unadjusted	adjusted [†]
Hospital type			
- university hospitals*	14.2	1.00	1.00
- general hospitals	10.3	0.70 (0.54 – 0.90)	1.33 (0.88 – 2.02)
Gestational week			
- 24	55.6	23.96 (13.48 – 42.62)	41.48 (18.23 – 94.38)
- 25	35.5	10.78 (6.54 – 17.79)	16.67 (8.78 – 31.62)
- 26	19.9	4.75 (2.76 – 8.18)	6.12 (2.87 – 13.03)
- 27	13.5	3.01 (1.76 – 5.15)	3.46 (1.89 – 6.35)
- 28	9.7	2.08 (1.20 – 3.62)	2.46 (1.37 – 4.41)
- 29	6.8	1.40 (0.81 – 2.44)	1.42 (0.75 – 2.67)
- 30	6.4	1.32 (0.77 – 2.26)	1.41 (0.73 – 2.74)
- 31*	5.0	1.00	1.00
Birth weight for gestational age			
- large (LGA)	44.4	7.54 (3.46 – 16.43)	10.90 (3.18 – 37.38)
- appropriate (AGA)*	9.6	1.00	1.00
- small (SGA)	14.7	1.63 (1.24 – 2.16)	3.08 (1.94 – 4.89)
Infant sex			
- male	13.2	1.33 (1.02 – 1.73)	1.64 (1.13 – 2.38)
- female*	10.3	1.00	1.00

* reference

[†] adjusted for mode of delivery, hospital type, gestational age, birth weight for gestational age, infant sex, foetal presentation, placental complications, and maternal hypertensive illness

There was a significant interaction between gestational age and hospital type with regard to infant mortality (p=0.049). Stratified analyses showed that the increased mortality risk in general hospitals was confined to extremely preterm infants (Table 9).

Table 9. Infant mortality by hospital type, stratified by gestational age.

	Infant mortality (%)	Odds ratios (95% CI)	
		unadjusted	adjusted [†]
24–27 weeks			
- university hospital*	22.8	1.00	1.00
- general hospital	32.2	1.60 (1.02 – 2.50)	2.00 (1.15 – 3.49)
28–31 weeks			
- university hospital*	8.1	1.00	1.00
- general hospital	5.8	0.69 (0.45 – 1.07)	0.83 (0.51 – 1.33)

* reference

[†] adjusted for the covariates as in Table 8.

PRETERM BIRTH AND BLOOD PRESSURE IN ADULTHOOD (III)

Characteristics of the conscripted men (n=329,495) are presented in Table 10.

Table 10. Characteristics of men conscripted for military service.

	Gestational age (weeks)				
	24-28	29-32	33-36	37-41	42-43
At birth					
Birth weight, grams	1,192 (270)	1,825 (426)	2,745 (507)	3,590 (484)	3,815 (479)
At conscription					
Age, years	18.2 (0.4)	18.2 (0.4)	18.2 (0.4)	18.2 (0.4)	18.2 (0.4)
BMI, kg/m ²	22.1 (3.2)	22.1 (3.1)	22.3 (3.3)	22.3 (3.2)	22.4 (3.3)
Systolic BP, mm Hg	132 (13)	131 (12)	130 (11)	129 (11)	128 (11)

Values are mean (SD).

The proportion of men with high systolic blood pressure varied from 32 percent among men born extremely preterm (≤ 28 weeks) to 19 percent among men born postterm (≥ 42 weeks) (Table 11). Linear regression analyses showed that systolic blood pressure increased with decreasing gestational week (0.31 mm Hg/week, $p < 0.001$). Compared to men born at term (37-41 weeks), men born extremely preterm faced a two-fold increased risk of high systolic blood pressure (Table 11). Being born SGA was associated with a 10 percent increased risk of high systolic blood pressure, compared to being born AGA.

Table 11. Perinatal characteristics and risk of high systolic blood pressure at conscription.

Gestational age (weeks)	High systolic BP (%)	Odds ratios (95% CI)			
		crude	adjusted [†]		
24 – 28	31.5	1.81	(1.30-2.52)	1.88	(1.33-2.68)
29 – 32	26.6	1.44	(1.27-1.62)	1.45	(1.28-1.64)
33 – 36	23.5	1.21	(1.16-1.26)	1.24	(1.19-1.30)
37 – 41*	20.2	1.00		1.00	
42 – 43	18.7	0.91	(0.89-0.94)	0.90	(0.88-0.93)
Birth weight for gestational age					
SGA	21.5	1.09	(1.04-1.14)	1.10	(1.05-1.15)
AGA*	20.2	1.00		1.00	
LGA	19.2	0.94	(0.89-0.99)	0.90	(0.85-0.95)

* reference

[†] adjusted for gestational age, birth weight for gestational age, age and parity of the mother, height at conscription, conscription year, family situation of the conscriptor, and parents' educational level and socio-economic status.

The risk of high systolic blood pressure (≥ 140 mm Hg) per one-week decrease in gestational age increased both within and between families (adjusted odds ratios were 1.03 [1.01-1.05] and 1.06 [1.04-1.08], respectively), indicating that an association between gestational age and systolic blood pressure exists after controlling for common genetic and shared environmental factors after birth.

Finally, there was a significant interaction between gestational age and birth weight for gestational age ($p=0.03$) with regard to high systolic blood pressure (≥ 140 mm Hg). To enable stratified analyses by gestational age, extremely and very preterm births were collapsed into one category (≤ 32 weeks). SGA was not a risk factor of high systolic blood pressure among men born at 24 to 32 gestational weeks, but increased the risk of high systolic blood pressure among men with longer gestations.

PRETERM BIRTH, BIRTH WEIGHT, AND TYPE 2 DIABETES (IV)

In the cohort of 18,230 like-sexed twins, rates of type 2 diabetes consistently increased with decreasing birth weight (Table 12). Compared to twins born at term (37 to 41 gestational weeks), type 2 diabetes was slightly more common among preterm twins.

Table 12. Rates of type 2 diabetes by perinatal characteristics.

	Rate of type 2 diabetes (%)
Birth weight (grams)	
-1,999	5.3
2,000 to 2,499	3.9
2,500 to 2,999	2.7
3,000 to 3,499	2.6
3,500 -	2.4
Gestational age (weeks)	
31 – 34	3.7
35 – 36	3.3
37 – 41	3.0
42 – 45	3.3

In cohort analyses, there was no association between gestational age and type 2 diabetes, while the risk of type 2 diabetes increased with decreasing birth weight. Compared to twins with birth weight from 2,500 to 2,999 grams (reference), twins with birth weight less than 2,000 grams had a twofold increase in risk of type 2 diabetes, after adjustment for perinatal and adult covariates. When birth weight was used as a continuous measure, a 500-gram decrease in birth weight was associated with a 44 percent increase in risk.

To elucidate whether the association between low birth weight and type 2 diabetes was confounded by genetic factors, risks were calculated in twin pairs discordant for type 2 diabetes (Table 13). An increased risk of type 2 diabetes with lower birth weight was found within dizygotic but not within monozygotic twin pairs.

Table 13. Risks for type 2 diabetes among twin pairs, discordant for type 2 diabetes, per 500 grams difference in birth weight.

	Co-twin case-control	
	odds ratio	(95% CI) †
Dizygotic twin pairs (N=206)		
twin with type 2 diabetes	1.38	(1.02-1.85)
non-diabetic twin*	1.00	
Monozygotic twin pairs (N=97)		
type 2 diabetes	1.02	(0.63-1.64)
non-diabetic twin*	1.00	

* Reference

† Twin pairs are by definition matched for shared environmental and common genetic factors.

Finally, the association between birth weight and type 2 diabetes was explored in additional co-twin case-control analyses, using subsets of discordant twin pairs. Genetic confounding was found also within twin pairs in which the diabetic twin was treated with an antidiabetic drugs, within twin pairs born at term, and within twin pairs with moderate as well as large differences in birth weight.

6. GENERAL DISCUSSION

METHODOLOGICAL CONSIDERATIONS

Study design

To achieve the aims in this thesis, two different types of epidemiological study designs were used, the cohort study (paper II, III and IV) and the case-control study (paper I and IV).

As demonstrated by Figure 13 and further discussed below, the temporal relation between exposure and outcome is a principal difference between cohort and case-control studies. While a cohort study examines how exposures in a healthy population affect the distribution of future outcomes, a case-control study compares the distribution of past exposures among individuals with and without the outcome.

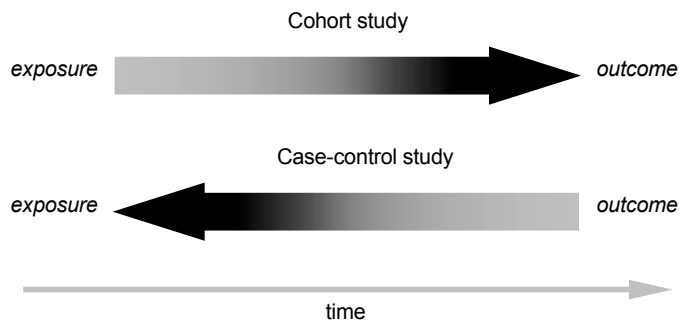


Figure 13. The temporal relation between exposure and outcome in cohort and case-control studies, respectively.

Cohort studies

A cohort study can be used to study how a certain exposure relates to an outcome, i.e. exposed individuals are compared with non-exposed individuals with regard to an outcome occurring after the exposure. The cohort study design has several advantages, especially if the study population is large and population-based, and information on exposures is prospectively collected before the outcome:

- estimates of relative risks can be accurate
- rare exposures can be studied
- a temporal relationship between the exposure and outcome can be inferred
- time-to-event analysis is possible
- can be used when randomization is not possible
- selection and information biases are decreased
- multiple outcomes can be studied

Cohort studies can also have limitations:

- lengthy and expensive
- may require very large samples
- not suitable for rare diseases
- less suitable for diseases with long latency
- can suffer from non-response, migration and loss-to-follow-up biases

In the present thesis, information in national registers was used in paper II, III and IV. The study populations were large, and the main exposures, gestational age and birth weight, were prospectively recorded at the time of delivery. The main limitation of register-based cohort studies is that they are restricted to information on exposures and confounders included in the registers. In this thesis, this limitation was partly overcome by individual record linkage of data from several data sources, using the civic registration number. For example, in paper III, the dataset included individually linked information from the Medical Birth Register, the Conscript Register, the Multigeneration Register and the Population and Housing Census 1990.

Case-control studies

In case-control studies, two groups are selected in the population under study: individuals with the outcome of interest (cases), and individuals without the outcome (controls). For good external validity, it is important that the controls are representative of the population from which the cases are obtained. There are several advantages of the case-control design:

- cheap, relatively easy and quick studies
- multiple exposures can be examined
- rare diseases and diseases with long latency can be studied
- suitable when randomization is unethical

Case-control studies also have disadvantages:

- case and control selection can be troublesome
- subject to bias
- it can be difficult to show a difference between cases and controls, if the exposure is common
- direct incidence estimation is not possible
- temporal relationship is not clear
- reverse causation is a problem in interpretation
- multiple outcomes cannot be studied

Nested case-control studies imply that cases and controls are selected within a defined cohort. Such case-control studies can often be performed at a fraction of the cost of a cohort study, simplify data analysis, and yet achieve nearly the same level of precision. In paper I, the case-control study was nested within a cohort of pregnant women in Stockholm, and cases (very preterm deliveries) were compared with un-matched, randomly selected controls (term deliveries). In paper IV, methods included a co-twin case-control study of twin pairs discordant for type 2 diabetes, nested within a cohort of Swedish twins. Generally, over-matching can limit the number of exposures in case-control studies. By definition, twins within a pair share several exposures, such as genetic factors, familial socio-economic factors, maternal exposures during pregnancy and gestational age. Therefore, the association between gestational age and type 2 diabetes could not be assessed in the co-twin case control analyses. However, the idea of the co-twin case-control analyses in paper IV was to study the independent effect of birth weight with regard to risk of type 2 diabetes, controlling for shared familial (genetic and environmental) factors.

Internal validity

A study has high internal validity if it is unlikely that findings are explained by systematic or random errors. A systematic error is generally referred to as bias: incorrect selection of study subjects can introduce selection bias, erroneous measurement of exposures and outcomes can result in information bias, lack of control for factors important for the association under study can lead to confounding, and including a factor with differential impact on the association between exposure and

outcome can result in effect modification. Systematic errors are independent of study size but can be reduced by careful study planning and design.

In the absence of systematic errors, random errors may still explain the observed association, but in contrast to systematic errors, a larger study size can reduce the risk of random errors.

This section will discuss the various aspects of internal validity in relation to findings presented in this thesis.

Selection bias

Selection bias occurs if sampling of study subjects is disproportional with regard to their probability of being exposed. For example, in a case-control study about preterm birth and environmental pollution, sampling of cases in a city and controls from the countryside would lead to selection bias. Selection bias is more common in case-control studies, but may also occur in cohort studies. Although information about exposures in cohort studies are generally collected or recorded before the outcome occurs, selection bias can result from differential loss to follow-up. Selection bias can also be the result of incomplete or poorly defined sampling of the cohort, for example if persons who have died are excluded from the cohort.

In the case-control study in paper I, cases and controls were selected from a cohort of pregnant women residing in the Stockholm County. Selection of cases and controls was done by linkage of the Medical Birth Register and the register for the archive of stored blood samples. Unfortunately, all selected cases and controls could not be included, since serum samples older than ten years had been discarded from the archive, after the study was initiated. The reason for this was that the National Board of Health and Welfare mandates storage only for ten years. This setback reduced the study size with about one third, but it should not have introduced selection bias, since samples were cleared depending on sample year, and similar proportions of cases and controls were lost.

Paper II included all singleton very preterm infants reported to the Medical Birth Register from 1992 to 1998, and we cannot exclude the possibility that some preterm born infants born in Sweden were not reported to the Medical Birth Register. However, selection bias would only be introduced if rates of not included infants differed by type of hospital, the main exposure of interest. In the cohort study in paper III, males were followed from birth to conscription. Conscription rates varied between 63 percent and 83 percent across the gestational age range, with the lowest rate among men born extremely preterm. In addition, among men born extremely preterm (24-28 weeks), those who participated in but did not complete conscription had a lower mean gestational age compared with those who completed conscription (26.9 vs. 27.4 weeks, $p < 0.05$). However, since conscripted men are probably healthier than non-conscripting men, such selection bias would probably underestimate the effect of preterm birth on later risk of high blood pressure.

Similar to paper III, some selection bias cannot be ruled out in paper IV, based on a cohort from the Swedish Twin Register. One may speculate that twins responding to the SALT questionnaire (response rate 74 percent) are healthier than non-responding twins. Even though such selection bias would drive risk estimates towards the null, it is unlikely that such bias would explain our findings.

Information bias

Inaccurate recording and classification of exposures and outcomes can be referred to as information bias or misclassification. If misclassification is the same across study groups, it is referred to as non-differential. If misclassification is unevenly distributed between study groups, it is referred to as differential.

In this thesis, the main exposures were gestational age and birth weight. In paper I, II and III, information about gestational age and birth weight was obtained from the Medical Birth Register, including data on virtually all deliveries in Sweden since 1973. The Medical Birth Register has recently been validated and the quality of recorded gestational age and birth weight is considered high²²¹. Since birth weight is a function of gestational age, analyses in paper II and III included a measurement of birth weight for gestational age, classified as small, appropriate and large for gestational age (SGA, AGA and LGA, respectively), using the Swedish reference curve for normal foetal growth¹⁷⁹. Foetal growth restriction is over-represented among preterm infants, emphasizing the importance of using a reference curve based on foetal growth rather than recorded birth weight.

In paper IV including twins born 1926 to 1958, information on gestational age and birth weight was abstracted from original medical records, stored in delivery archives throughout Sweden. Gestational age was based on date of last menstrual period. One can assume that data on last menstrual period was more uncertain in preterm deliveries during 1926 to 1958. In the cohort, mean birth weight decreased down to week 31 (Table 14), while gestational ages less than 31 weeks had increasing mean birth weights. Therefore, the cohort was restricted to include births between 31 and 45 weeks, since gestational ages of less than 31 weeks were probably more commonly misclassified.

Table 14. Mean birth weight and standard deviation by gestational week 28 to 45, among Swedish twins born 1926 to 1958.

Gestational week (weeks)	mean birth weight (grams)	standard deviation (grams)
28	2224	593
29	1938	528
30	1859	500
31	1844	347
32	1971	337
33	2096	336
34	2247	349
35	2388	362
36	2534	379
37	2666	397
38	2792	422
39	2892	432
40	2963	461
41	2996	471
42	2898	488
43	2930	458
44	2873	465
45	2817	469

We also compared mean gestational age and birth weight in the twin cohort with information on multiple births in 2003, as recorded in the Medical Birth Register, and found that the agreements were good. Mean gestational age was 264 days and 256 days in our cohort and in 2003, respectively. Corresponding values for mean birth weight was 2544 grams and 2588 grams.

Of the outcomes in this thesis, the definition of type 2 diabetes in paper IV could be subjected to misclassification (Figure 11, page 40), even though the agreement between questionnaire data and medical records has been shown to be good for chronic

diseases²³⁴. In order to improve specificity of the diagnosis, analyses restricted to twin pairs in which the diabetic twin used antidiabetic drugs were performed, without any change in risk estimates. Still, the algorithm could misclassify diseased twins as “healthy”, since undiagnosed glucose intolerance is common in elderly people²³⁵. Given the genetic liability to develop type 2 diabetes, one could question whether it is possible to identify truly disease-discordant monozygotic twin pairs²³⁶. In the cohort, there were 206 discordant and 17 concordant dizygotic twin pairs, compared to 97 discordant and 41 concordant monozygotic twin pairs, and only discordant pairs were included in the co-twin case-control analyses. Since the heritability of type 2 diabetes has never been estimated to 100 percent²³⁷, it is unlikely that all healthy co-twins in the monozygotic discordant twin pairs are misclassified. Further, a conceptually important methodological feature of selecting controls is their eligibility to become cases²³⁸. Finally, major misclassification seems unlikely since the prevalence of type 2 diabetes in our cohort is similar to the prevalence in the general Swedish population²³⁹.

Confounding bias

Confounding, a central issue in epidemiological research, refers to a situation when the association between exposure and outcome is affected by a third factor. The confounding factor is related to both exposure and outcome, and accounts for some/all of the observed relationship between the two. Importantly, a confounding factor should not be in the causal pathway between the exposure and the outcome. In that case it is referred to as an intermediate factor.

Confounding can ideally be controlled for by randomization (assures equal distribution of confounders between study and control groups). In observational studies, confounding can be reduced by restrictions (subjects are restricted by the levels of a known confounder), matching (potential confounding factors are kept equal between the study groups), stratification (for various levels of potential confounders) and multivariable analysis (in which confounding factors are taken into account in the analysis).

In the case-control study on viral infections and risk of preterm birth (paper I), confounding was poorly controlled for. Sample size was unexpectedly reduced when the archive was cleared for samples older than ten years. Cases and controls were not matched when the study was initiated. Smoking, a potential confounder, could not be added as covariate due to a large proportion of missing data among cases and controls. In the final multivariate analyses, only maternal age and parity were included.

In the cohort study on very preterm birth, level of care and risk of infant mortality (paper II), data on several potential confounders and risk factors were controlled for in multivariate analyses: mode of delivery, hospital type, birth weight for gestational age, gestational age, infant sex, foetal presentation, placental complications, and maternal hypertensive illness. Similar to paper I, smoking status was not included in the analyses, due to a large proportion of missing data. However, it seems unlikely that maternal smoking would confound the association between delivery at general hospitals and risk of infant death.

It has been argued that associations between infant characteristics and disease in adulthood are confounded by socioeconomic factors¹⁸²⁻¹⁸⁵. In the study on preterm birth and elevated blood pressure at conscription (paper III), socio-economic confounding was addressed in several ways. Firstly, the multivariate analyses included information on parental socio-economic status, parental educational level, and family structure. Secondly, the association was investigated in within-family analyses restricted to men with at least one full brother in the cohort.

Twin-studies are well suited to address confounding, since twins within a pair share foetal life, and are usually brought up together during childhood and adolescence. In

addition, dizygotic and monozygotic twins share 50percent and 100 percent of their segregating genes, respectively. If an association is evident both among unpaired twins and within twin pairs regardless of zygosity, an association is unlikely to be confounded by either genetic or shared familial factors, but instead explained by unique factors experienced by the individual twin. Confounding by shared environmental factors, such as socio-economic status, is likely if an association found in a cohort of (unpaired) twins is attenuated within both dizygotic and monozygotic twin pairs discordant for disease. As concluded in paper IV, genetic confounding is suggested if an association is found within disease-discordant dizygotic twin pairs, but not within disease-discordant monozygotic twin pairs. In addition, differences in birth weight within twin pairs reflect differences in foetal growth. Thus, the internal validity of twin studies in addressing the foetal programming hypothesis should be regarded as very high.

Effect modification

If the strength of an association varies over a third factor, this is called effect modification or interaction. The third factor is changing the effect of the exposure. For example, gender is an effect-modifying factor if an exposure is associated with an outcome among males, but not among females. Effect modification can be difficult to detect in smaller studies, but should be looked for if there is a plausible biological hypothesis. If detected, stratified analyses should be performed to obtain stratum-specific odds ratios.

In paper II, we found that gestational age modified the association between delivery hospital type and risk of infant mortality. In general hospitals, infant mortality was only elevated among the most immature infants, born before 28 weeks. In paper III, an interaction was found between gestational age and birth weight for gestational age, with regard to risk of high blood pressure. Among infants born very preterm, being born small for gestational age was not a risk factor for high blood pressure, whereas smallness at birth was a risk factor among men born after longer gestations.

In the co-twin case-control analyses in paper IV, low birth weight was associated with type 2 diabetes within dizygotic twin pairs, but not within monozygotic twin pairs, implying genetic confounding of the association between low birth weight and type 2 diabetes. Another way to demonstrate genetic confounding is to investigate whether zygosity modifies the effect of birth weight on risk of type 2 diabetes. However, formal testing showed no significant interaction between birth weight and zygosity ($p=0.29$). The main findings in paper IV should therefore be interpreted with caution.

Random error

Even in the absence of bias (systematic errors), chance could explain observed findings. In epidemiological research, one common method to assess the likelihood of random findings is to calculate a confidence interval for a risk estimate. The confidence level is usually set to 95 percent, i.e. there is a 95 percent probability that the association is not explained by chance when the confidence interval for a risk estimate does not include 1.00.

Random errors depend on study size. The cohort analyses in paper II, III and IV were all based on large study populations, minimizing the risk of chance findings. The case-control analyses in paper I and IV were based on smaller samples of study subjects, and the possibility of random errors cannot be ruled out.

External validity

While internal validity is related to the probability that observed observations are true for the study population itself, external validity refers to whether results could be generalized to other (non-studied) populations. Firstly, external validity depends on

high internal validity. For example, if study results are flawed, external validity has no meaning. Secondly, external validity is a matter of discussion and judgement. Whether obtained results can be extrapolated to other settings depend on the degree of similarity between the study population and the unstudied population.

Due to methodological limitations and inconclusive results in paper I, it is difficult to assess external validity. Observed findings need to be confirmed in larger epidemiological studies. Paper II was based on a large national cohort of very preterm infants of both sexes born during the 1990s, implying that results are applicable to today's population of very preterm infants born in Sweden. Similarly, paper III was based on the majority of all Swedish male adolescents born during 1973 to 1981, and the findings should be applicable to a male adolescent population born after 1981. Given that conscripting men are probably healthier than non-conscripting men, the observed association may be underestimated.

Paper IV was based on the Swedish Twin Registry and included twins born between 1926 and 1958. Our conclusion that preterm birth is not associated with type 2 diabetes, may not apply to today's population of preterm infants. The impact of very preterm birth could not be studied since recorded gestational ages less than 31 weeks were excluded due to probable misclassification.

One concern is whether findings in twins could be generalized to singleton born individuals. According to the "foetal programming hypothesis", nutritional insults to the foetus during late gestation lead to disturbances of glucose-insulin metabolism²⁰⁷. Compared with singletons, twins have shorter gestations and slower foetal growth²⁴⁰. Weight gain in twins is less pronounced during the third trimester²⁴¹, but inter-twin disparity in foetal size increases with gestation²⁴². However, results from subanalyses indicated genetic confounding within twin pairs born at term, and within twin pairs with moderate as well as large differences in birth weight.

Since inverse association was found between low birth weight and type 2 diabetes in dizygotic but not in monozygotic twin pairs, a crucial question is whether results related to placentation and subsequent foetal environment of dizygotic and monozygotic twins can be compared. All dizygotic twins and one third of monozygotic twins are dichorionic and have two (separate or fused) placentas, whereas two thirds of monozygotic twins are monochorionic and share a single placenta²⁴³. The effect of zygosity on birth weight is unclear, but monochorionicity is associated with reduced birth weight^{244,245}. Unequal sharing of placental blood flow appears to be the primary contributor to birth weight discordancy in monozygotic monochorionic twin pairs²⁴⁶. Similarly, dizygotic dichorionic twins are at increased risk of reduced birth weight if the umbilical cord inserts peripherally in the placenta²⁴³. There should be no principal differences between inadequate placental blood supply and nutrition between singletons, monozygotic twins and dizygotic twins.

FINDINGS AND IMPLICATIONS

Viral infections and risk of very preterm birth

Paper I study suggests that viral infection, specifically with Parvovirus B19, may be associated with an increased risk of very preterm birth (<32 weeks). Although no significant results were obtained on a 5 percent level, risk estimates were consistently elevated for viral infections in early pregnancy, indicating a two-fold increased risk of subsequent very preterm delivery. Larger epidemiological studies are warranted since an association between viral infections and very preterm birth is plausible from a biological perspective.

One may speculate that viral infections initiate inflammatory processes in pregnant women that may increase the risk of preterm birth. It has been shown that women with preterm deliveries have higher concentrations of IgM antibodies compared to women with term deliveries²⁴⁷. Moderately elevated levels of C-reactive protein (CRP) in early pregnancy have been associated with preterm delivery²⁴⁸. Histopathological inflammatory changes have been found in placental tissue after both spontaneous and induced preterm delivery²⁴⁹.

An association between viral infections and preterm birth are also supported by a recent case-control study demonstrating that preterm infants are more commonly exposed to Cytomegalovirus compared to infants born at term⁴⁴. It is unclear whether *congenital* Cytomegalovirus infection contributes to an increased risk of preterm birth^{250,251} but there may be a link between *maternal* Cytomegalovirus infection and preeclampsia, a pregnancy complication often leading to preterm delivery. Individuals seropositive for Cytomegalovirus have impaired endothelial function²⁵², and endothelial dysfunction is important for the development of preeclampsia²⁵³. It has also been shown that levels of antibodies against Cytomegalovirus are higher among women with early onset preeclampsia and preterm delivery, compared to women with normal pregnancies ending at term⁴³.

Increased expression of pro-inflammatory cytokines in the reproductive tract and placenta has been associated with spontaneous preterm birth^{254,255}. In paper I, viral infections were not specifically associated with very preterm deliveries with spontaneous onsets, but the study was hampered by limited statistical power. It is also possible that inflammatory processes elicited by viral infections are mainly localized to other organ systems than the urogenital tract. In addition, viremia induced inflammation may act through other biological pathways, as exemplified by the association between Cytomegalovirus and preeclampsia.

If confirmed in larger epidemiological studies, the findings in paper I may contribute to further knowledge on the etiology of very preterm birth. In the long run, such knowledge may be important for developing strategies to prevent preterm birth, since viral infections are principally preventable diseases through vaccination.

Preterm delivery, level of care and infant mortality

Paper II demonstrated that extremely preterm infants (24-27 weeks) born at general hospitals suffered an excess mortality risk compared to those born at university hospitals. Infant mortality was 32 percent in general hospitals, and 23 percent in university hospitals, corresponding to a doubled mortality risk for extremely preterm infants born in general hospitals.

The results generally support previous findings that level of care is associated with neonatal short term outcome¹³⁰⁻¹³⁷. However, for infants born at 28-31 weeks, there was no difference in infant mortality by level of care. If anything, risk of infant mortality tended to be lower in general compared with university hospitals. Thus, these results differ from a recent American study¹³², in which mortality among infants with birth weights from 1,250 to 2,000 grams was found to be higher in community versus regional hospitals. However, the point estimates in both studies were non-significant and confidence intervals were over-lapping, suggesting that the apparent difference in mortality risks may be a random effect. Another explanation is that Swedish policies regarding treatment of neonatal lung disease, including non-invasive ventilatory support with nasal continuous positive airway pressure (nCPAP), reduce the need for mechanical ventilation of very preterm infants^{256,257}. Consequently, the need for level-3 intensive care and iatrogenic risks associated with mechanical ventilation would be lower in Sweden compared with the US.

Among extremely preterm infants, the results raise the question whether the increased risk of infant death in general hospitals was a matter of quality of care, and whether further centralization could improve survival. Immediate postnatal management is critical in extreme prematurity^{258,259}, and neonatal intensive care was possible to initiate in all hospitals. The higher number of mortality-associated placental complications in general hospitals may reflect unexpected obstetric emergencies in which antenatal transfer was contraindicated. Moreover, the survival of the extremely preterm infant delivered after placental abruption has in itself been found to be poor^{89,260}. However, a poorer outcome in general hospitals was not confined to situations of unforeseen obstetric complications. Less experience due to fewer cases of extreme preterm births may also have contributed to worse outcomes in general hospitals. The finding that the mortality risk among extremely preterm infants to preeclamptic mothers was higher in general hospitals compared with university hospitals, supports the assumption that quality-of-care differences exist. Possibly, a larger proportion of preeclamptic pregnancies could have been antenatally referred to a university setting.

Only seven of the delivery units in Sweden are affiliated to university hospitals. Although antenatal referrals are possible in the vast majority of cases, extremely preterm infants will be born unexpectedly in general hospitals. It is therefore important that well functioning networks are created, to decentralize experience in resuscitation and stabilization. Postnatal transports should be considered in collaboration with the accepting unit. If “mobile transport teams” were developed nation-wide, these could travel to general hospitals to support local staff, ideally before delivery. Since timing also matters, such organization of transport may enable postnatal transfers on the first day of life.

In the debate about organization of neonatal intensive care our study supports the idea that extreme prematurity should be managed in university hospitals. Case-control studies and clinical audits within regional networks are two possibilities to further study clinical practices including referral routines, aiming at finding new and innovative ways to improve the prognosis for this vulnerable group of infants.

Long term health after preterm birth

The development of neonatal intensive care during the last decades has led to improved survival rates among preterm infants. However, the life-long health effects after very or extremely preterm birth are virtually unknown and late morbidity could be an increasing problem. The hypothesis of a developmental origin of disease in adulthood (DOHaD) has also resulted in a paradigm shift how diseases develop over time. Previously, genetic and lifestyle factors in adulthood were seen at the main predisposing factors for disease. The DOHaD hypothesis suggests that common diseases may originate already during foetal life, through changes in organ structure and function induced by harmful exposures¹⁷⁷.

Preterm birth and risk of high blood pressure

Paper III showed that preterm birth is a risk factor for high systolic blood pressure in today's generation of young Swedish men, and the risk increased with decreasing gestational age. Systolic blood pressure increased with decreasing gestational age (0.31 mm Hg/week), and men born extremely preterm faced a two-fold increase in risk of high systolic blood pressure (≥ 140 mm Hg). SGA was also associated with increased risk of high systolic blood pressure, but only among men born at 33 weeks or later. In addition, the associations between gestational age and risks of high systolic blood pressure were similar among males from different families as within families of full brothers. Thus, it is unlikely that the risk of high systolic blood pressure related to

short gestational age is confounded by common genetic or shared environmental factors in childhood.

Analyses on diastolic blood pressure showed less consistency. In linear regression analysis, no association was found between gestational age and diastolic blood pressure. In contrast, the risk of high diastolic blood pressure increased consistently with decreasing gestational age in logistic regression analyses, although only men born moderately preterm (33-36 weeks) faced a significantly increased risk. This discrepancy may be due to a non-linear relation between gestational age and diastolic blood pressure. Decreasing birth weight for gestational age was associated with increasing diastolic blood pressure in linear regression analyses, whereas logistic regression analyses showed no association between SGA and risk of high diastolic blood pressure. The latter finding could be due to the low prevalence of high diastolic blood pressure in the study cohort. SGA may also be less expressive for the risk of high diastolic blood pressure compared with the risk of high systolic blood pressure.

Individuals born preterm may be prone to blood pressure elevation through several mechanisms. Abnormal vascular growth and development induced by preterm birth may contribute to increased vascular resistance^{197,198}. Autonomic blood pressure regulation is immature in very preterm infants and the postnatal maturation of this homeostatic control mechanism differs from that seen in infants born at term²⁶¹. Very preterm infants commonly suffer from metabolic problems such as transient hyperglycaemia, in spite of relatively high levels of insulin²⁶². Increased postnatal levels of insulin may cause persisting changes in glucose and lipid metabolism, predisposing to increased blood pressure later in life²⁶³. Activation of the HPA axis has been suggested to link low birth weight at term to high systolic blood pressure in adulthood²⁶⁴. The immature HPA axis in preterm infants²⁶⁵, and the possibility of early programming of the numbers of glucocorticoid receptors²⁶⁶, may be important for the association between preterm birth and high blood pressure later in life. It has recently been shown that children born preterm have increased heart rate, due to sympathoadrenal overactivity²⁶⁷. Finally, a reduction in nephron number cannot be ruled out as another etiologic factor, although women born preterm exhibit high blood pressure in spite of normal kidney function²⁶⁸.

Whereas preterm birth per se may be conditional for the risk of high blood pressure later in life, postnatal environmental exposures are likely to have an impact as well. In a randomized controlled trial, preterm infants fed with breast milk had lower blood pressure at follow-up in adolescence, compared to those receiving formula²⁶⁹. In addition, postnatal nutrient intake and growth patterns have been associated with insulin resistance²⁷⁰ and with vascular endothelial function in infancy²⁷¹ and later in life²⁷². The full nature of these associations needs to be clarified, but they indicate that the risk of high blood pressure in adults born preterm could be modulated already in the neonatal nursery.

SGA was not a risk factor for blood pressure elevation among men born very preterm, and similar conclusions were drawn in smaller clinical studies^{194,195}. One may argue that the strong effect of very preterm birth could mask a smaller effect of being SGA. However, there may also be principally different pathways of inducing later blood pressure elevation in individuals born preterm and term. For example, dysfunction of the vascular endothelium, causing impaired vascular relaxation, has been found in infants and children born SGA at term, whereas very preterm infants had normal endothelial function regardless of birth weight for gestational age^{271,272}.

There was no interaction between gestational age and BMI with respect to systolic blood pressure in early adulthood, which is in contrast to results from a recent study¹⁹⁶. Differences in study populations may contribute to this discrepancy, including different distributions of gestational age and age when blood pressure was measured.

Accelerated weight gain in childhood has been reported to influence the risk of high blood pressure²⁷³. However, we had no longitudinal information on weight and height during childhood and adolescence and negative findings in these interaction analyses should be interpreted cautiously. Timing of the accelerated growth pattern may be of more importance than achieved BMI in adulthood.

The strong effect of preterm birth on later blood pressure may, provided causal, have important public health implications, since blood pressure elevation could lead to hypertension with time²³¹. In Sweden, approximately 6 percent of pregnancies end preterm and the corresponding figure in the US is reported to be as high as 12 percent¹⁷⁸. Hence, a similarly large proportion of the offspring could be at risk of increased blood pressure later in life. A proposed strategy to diagnose blood pressure elevation in early life includes measurement of blood pressure any time a preterm born child presents in health care²⁷⁴. If these results can be confirmed, it would be appropriate to suggest routine follow-up of preterm born infants with regard to blood pressure. A more active approach to lifestyle counselling and interventions in childhood may also be needed to avoid end-organ damage in adulthood²⁷⁴.

Preterm birth, low birth weight and type 2 diabetes

In paper IV, the results showed that that preterm birth did not increase the risk of type 2 diabetes in adulthood, in the cohort of Swedish twins born 1926 to 1958. As already discussed, the lack of an association between preterm birth and type 2 diabetes should be interpreted with caution. The study population included no individuals born at less than 31 weeks, i.e. the effect of very preterm birth on future risk of type 2 diabetes was not investigated. Recent studies have shown that very preterm birth may be associated with reduced glucose tolerance in childhood²¹⁷ and in young adulthood²¹⁸.

The contribution to the research field of a developmental origin of disease in adulthood, are the findings regarding the association between low birth weight and type 2 diabetes. Decreasing birth weight was associated with increasing risk of type 2 diabetes in cohort analyses, within dizygotic twin pairs, but not within monozygotic twin pairs. These results suggest that genetic factors contribute to the association between low birth weight and risk of type 2 diabetes later in life. This twin study is the first large enough to address and support a previously proposed hypothesis, that low birth weight and type 2 diabetes may have a common genetic background²¹².

In contrast to the results in the present study, no evidence of genetic confounding was found in a recent study, based on the same twin cohort and study design, on the association between low birth weight and risk of hypertension¹⁹². The most evident explanation for these discrepant results is that genetic factors are important for the association between low birth weight and type 2 diabetes, but not for the association between low birth weight and hypertension.

Genetic factors could be important for the association between low birth weight and type 2 diabetes in several ways. Insulin is a key determinant of foetal growth and has a central role in glucose metabolism. Reduced insulin secretion or insulin resistance may explain both low birth weight and glucose intolerance in adulthood¹⁸⁶. A common genotype of the insulin gene (class I allele) has been associated with lower birth weight²⁷⁵, insulin resistance, dyslipidemia, and development of obesity^{276,277}. Likewise, a common variant of mitochondrial DNA is related to insulin resistance, and has been associated with both thinness at birth and type 2 diabetes later in life²⁷⁸. It has also been shown that a haplotype of the glucocorticoid receptor gene could modify the association between size at birth and glucose tolerance²⁷⁹.

Genetic confounding of the association between low birth weight and type 2 diabetes seems plausible, but the conflicting results from other epidemiological studies based on unrelated individuals as well as twins^{206,213-215}, suggests that the true nature of perinatal contributions to type 2 diabetes remains to be elucidated. However, genetic factors should be taken into account in future attempts to disentangle risk factors and biological mechanisms underlying the developmental origin of type 2 diabetes.

7. CONCLUSIONS

- Women with viral infections, especially with Parvovirus B19, may face an increased risk of very preterm birth. Our findings should be interpreted cautiously, but warrant larger epidemiological studies.
- Taking obstetric complications into account, there is an excess mortality risk among extremely preterm infants born in general hospitals. When possible, obstetric and neonatal intensive care for extremely preterm infants should be regionalized to university hospitals. Strong regional networks may also be important to optimize postnatal management, when prenatal transfer is contraindicated.
- Preterm birth is a risk factor for high systolic blood pressure in young men. The risk of high systolic blood pressure associated with birth weight for gestational age is modified by gestational age, suggesting that perinatal contributions to blood pressure elevation later in life may be induced by different biological pathways. Future research should focus on biological mechanisms. In clinical practice, blood pressure should be surveilled at follow-up visits for children and teenagers born preterm.
- In older cohorts, preterm birth does not increase the risk of type 2 diabetes in adulthood, whereas low birth weight does. However, the attenuation of the risk between dizygotic and monozygotic twin pairs suggests that genetic mechanisms play an important role for the association between low birth weight and type 2 diabetes. Future studies should investigate whether today's population of preterm infants face an increased risk of type 2 diabetes, taking genetic factors into account.

8. FUTURE CHALLENGES

Very preterm birth is a burdensome outcome of pregnancy. Despite advances in neonatal intensive care, development of strategies to prevent very preterm birth should have high priority in future research. Our findings that viral infections might increase the risk of very preterm birth need to be confirmed in larger epidemiological studies. An infrastructure of “biobanks” is currently being set up in Sweden to archive biological samples from health care. Access to stored blood samples, collected from women before and during pregnancy, may enable large-scale case-control studies on viral infections and risk of very preterm delivery. A national study should be able to include enough cases to enable stratification by viral type. If an association between viral infections and very preterm delivery is confirmed, clinical research should aim to reveal biological mechanisms. In the long run, such research may also be important from a public health perspective, since viral infections are principally preventable diseases through vaccination.

Prevention of very preterm birth has so far had little success. Despite medical and technological progress, our study on level of care and infant mortality implies that facilities for neonatal intensive care should be concentrated to reduce mortality risks for the most immature infants. However, it is a great challenge to achieve organizational changes in hospital care, since it involves not only the medical profession, but also regional and national political decision-making. Interests related to health care politics may not necessarily be the same as the interests of very preterm infants and their parents. Consequently, professionals in neonatal intensive care should not only find ways to facilitate and improve regional and national referral routines and practices, but also get involved in strategic discussions with public health representatives.

As demonstrated by several studies, there are concerns about long term morbidity in survivors after very preterm birth. Our findings that young men born preterm have an increased risk of elevated blood pressure add to the literature on perinatal risk factors of cardiovascular disease. Since preterm birth is a common pregnancy complication, typically affecting 10 percent of pregnant women in developed countries, our findings are important in a public health perspective. However, several questions remain to be addressed. Do women born preterm face similar risks as men? Will preterm born individuals face increased risks of cardiovascular morbidity and mortality at older ages? Which are the underlying factors and through which biological pathways do they operate? Can neonatal care promote normal cardiovascular development, for example by reducing stress, and thereby lower risks of future cardiovascular outcomes?

It remains to be determined whether preterm born individuals have an increased risk of type 2 diabetes in adulthood. Recent clinical studies have found that preterm birth may be associated with impaired glucose tolerance in childhood and adolescence. However, our large study on Swedish twins suggests that preterm birth has little, if any, impact on the risk of type 2 diabetes. The conflicting results could be explained by survivorship bias in our study, since the study population born 1926 to 1958, is not representative for today’s population of surviving preterm infants. From clinical experience, we know that very preterm infants commonly have an instable glucose/insulin homeostasis and often experience hypo- and hyperglycaemia during neonatal intensive care. Even though an association between preterm birth and later glucose intolerance seems possible, much work remains to elucidate the role of very preterm birth on the pathogenesis of impaired glucose tolerance. Studies of the postnatal adaptation of endocrine and metabolic systems in very preterm infants may provide some insights.

With regard to a developmental origin of type 2 diabetes, the major conclusion of our twin study is that the association between low birth weight and risk of type 2 diabetes could be explained (confounded) by genetic factors. Our findings should stimulate the research field of “foetal programming” to consider both environmental exposures and genetic factors in studies of perinatal contributions to the development of type 2 diabetes.

9. SVENSK SAMMANFATTNING

Bakgrund

En normal graviditet är i genomsnitt 40 veckor lång och förlossning mellan 37 och 41 veckor definieras som en förlossning i normal "fullgången" tid. Förlossning före 37:e veckan är "för tidig", och kan klassas som måttligt för tidig (32-36 veckor), mycket för tidig (28-31 veckor) eller extremt för tidig (≤ 27 veckor). Begreppet "mycket för tidig förlossning" brukar ofta, som i denna avhandling, omfatta både mycket och extremt för tidiga förlossningar.

Mycket för tidig födsel är en komplikation som drabbar 1-2 procent av alla gravida kvinnor. Det finns flera omständigheter som kan öka risken. Förutom ärftlighet finns ett samband med faktorer som underlivsinfektioner, rökning, låg eller hög ålder hos modern, tidigare för tidig förlossning, låg socialgrupp, och tvillinggraviditeter.

Nämnda riskfaktorer verkar genom olika biologiska mekanismer som orsakar vattenavgång och värkar före fullgången tid. Inflammation och aktiverat immunförsvar är sannolikt den vanligaste orsaken till för tidig förlossning, särskilt vid underlivsinfektioner. Andra förklaringar kan vara onormal funktion i moderkakens blodkärl, ökad stress, och mekanisk påfrestning på livmoder och fosterhinnor. Det kan också vara så att man på grund av hälsorisker för fostret eller modern väljer att avsluta graviditeten för tidigt.

Tack vare förbättrad mödravård och framsteg inom nyföddhetsvården, har dödligheten minskat för barn som föds mycket för tidigt. Jämfört med fullgångna barn är dock dödligheten relativt hög och risken ökar kraftigt med sjunkande graviditetslängd. Medan barn födda i vecka 31 har en dödlighet under 5 procent, överlever ungefär hälften av de barn som föds i vecka 23-24.

Under de första veckorna efter födelsen är olika medicinska problem vanliga, på grund av att organen är omogna. I likhet med dödligheten, är risken för sjuklighet högre ju mer för tidigt barnet är fött. Särskilt vanliga är lung- och andningsproblem, omogen blodcirkulation och infektioner, men hjärnblödningar och näthinneskador förekommer också.

Eftersom mycket för tidig födsel sammanfaller med en period då hjärnan genomgår kraftig tillväxt och utveckling, riskerar för tidigt födda barn att drabbas av neurologiska funktionshinder. En svensk undersökning omfattande 11-åriga skolbarn födda före vecka 26 visade att CP-skada fanns hos 6 procent av barnen, svår synskada hos 12 procent och grav hörselnedsättning hos 6 procent. Även om 85 procent av barnen gick i vanlig skola, hade drygt hälften fått gå om en klass.

Kunskapen om långtidseffekter av mycket för tidig födsel är liten, helt enkelt på grund av att de medicinska framsteg som drastiskt ökat överlevnaden introducerades för mindre än 20 år sedan. Vissa undersökningar visar på små skillnader mellan vuxna som föddes för tidigt och de som föddes i normal tid, medan andra studier talar för att lägre utbildningsnivå, arbetslöshet och behov av ekonomiskt stöd från samhället är vanligare hos vuxna som föddes för tidigt. Det är emellertid osäkert om dessa långtidsresultat gäller idag, eftersom studierna omfattat personer som inte är representativa för de barn som nu överlever med modern intensivvård. Långtidsprognosen för dagens överlevare kan vara bättre då vården har utvecklats, men den kan också vara sämre eftersom fler barn överlever trots att de varit svårt sjuka under nyföddhetsperioden. Att öka kunskapen om långsiktiga hälsoeffekter av mycket för tidig födsel hos den nya och växande generationen av barn som överlever tack vare modern intensivvård, är en av de viktigaste uppgifterna för dagens forskning.

Ett nytt, snabbt växande forskningsfält handlar om att fostret eller det lilla barnet kan "präglas" till ökad benägenhet för folksjukdomar som högt blodtryck och åldersdiabetes. Efter pionjärarbeten från Norge och Sverige, visade en engelsk forskargrupp i slutet av 1980-talet att äldre personer som haft låg födelsevikt löpte högre risk att dö i hjärtkärlsjukdom, jämfört med personer med normal födelsevikt. Teorin var att "undernäring" av fostret, avspeglad i låg födelsevikt, resulterar i bestående förändringar i till exempel ämnesomsättningen, förändringar som predisponerar för sjukdom i vuxen ålder.

De äldre studierna tog inte hänsyn till att låg födelsevikt kan ha olika förklaringar. Låg födelsevikt kan visserligen bero på dålig fostertillväxt, men idag är den vanligaste orsaken för tidig födsel. Flera färskare undersökningar talar också för att för tidig födsel i sig kan vara en riskfaktor för hjärtkärlsjukdom och åldersdiabetes senare i livet.

Syfte

Det övergripande syftet med denna avhandling var att öka kunskapen om orsak till och prognos vid för tidig födsel. De specifika målsättningarna var:

- att studera samband mellan virusinfektioner i tidig graviditet och risk för mycket för tidig förlossning (delarbete I)
- att studera om sjukhusstyp (läns- kontra universitetssjukhus) har samband med dödligheten hos mycket för tidigt födda barn (delarbete II)
- att undersöka om graviditetslängd vid födelsen påverkar risken för högt blodtryck hos unga vuxna män (delarbete III)
- att studera om graviditetslängd och födelsevikt ökar risken för åldersdiabetes senare i livet (delarbete IV)

Material och metoder

Delarbetena i denna avhandling är baserade på den svenska befolkningen. Välorganiserad sjukvård, systemet med personnummer, och nationella hälsoregister, ger särskilt goda förutsättningar för epidemiologisk forskning i Sverige.

Nedanstående datakällor har utnyttjats i denna avhandling:

- Medicinska födelseregistret, med information om nästan alla förlossningar sedan 1973
- Dödsorsaksregistret, med uppgifter om dödsorsaker kodade enligt ett system utarbetat av Världshälsoorganisationen (WHO)
- Mönstringsregistret, med resultat från mönstring till militärtjänstgöring
- Flergenerationsregistret, med information om släktskap mellan individer - med hjälp av detta register kan till exempel syskon och föräldrar identifieras
- Folk- och bostadsräkningen 1990, med data om socioekonomisk status
- Svenska tvillingregistret, med uppgifter om tvillingars hälsa - detta register har nyligen uppdaterats med information ur förlossningsjournaler
- ett register över arkiverade blodprover vid Viruslaboratoriet, Karolinska universitetssjukhuset
- uppgifter hämtade ur medicinska journaler

I samtliga delarbeten gjordes statistiska beräkningar i syfte att uppskatta så kallade relativa risker. En relativ risk innebär att risken för en grupp är skattad i förhållande till en jämförelsegrupp med "standardrisk". Till exempel, om den relativa risken för högt blodtryck hos en grupp är 1,5 betyder det att den gruppen har en 50 procentig riskökning jämfört med standardrisken.

Delarbete I

I det första delarbetet undersöktes om virusinfektioner under tidig graviditet ökade risken för mycket för tidig förlossning (≤ 31 veckor). Blodprover från 269 kvinnor med mycket för tidig förlossning och 301 från kvinnor med fullgångna graviditeter analyserades för viruspartiklar. Om viruspartiklar kunde påträffas, tolkades detta som att kvinnan haft en aktiv virusinfektion i tidig graviditet.

Virusinfektion i tidig graviditet förekom hos tio kvinnor med mycket för tidig förlossning (3.7 procent), och hos fem kvinnor med förlossning i fullgången tid (1.7 procent). Hos sju respektive tre av kvinnorna var det *Parvovirus B19* som påträffades. Virusinfektion fördubblade risken för mycket för tidig förlossning, men på grund av att antalet virusinfektioner totalt sett var litet var riskökningen inte statistiskt säker.

Delarbete II

I delarbete II studerades om dödligheten bland mycket för tidigt födda barn påverkades av om förlossningen skedde på länsjukhus eller universitetssjukhus. Under åren 1992 till 1998 föddes 2 253 barn efter en graviditetstid mellan 24 och 31 veckor.

För hela gruppen barn ökade dödligheten med sjunkande graviditetstid, från 5 procent vid 31 veckor till 56 procent vid 24 veckor. Jämfört med universitetssjukhus var dödligheten högre på länsjukhus, men den riskökningen gällde bara barn födda extremt för tidigt, i vecka 24-27. För dessa allra minsta barn var dödsrisken fördubblad vid förlossning på länsjukhus, medan dödligheten var lika på läns- och universitetssjukhus för barn födda i vecka 28-31.

Delarbete III

Frågeställningen för detta delarbete var om för tidig födsel ökade risken för högt blodtryck senare i livet. Genom att länka personuppgifter från bland annat Medicinska födelseregistret och Mönstringsregistret skapades en databas med 329 495 unga män.

Ju mer för tidig förlossning, desto högre var risken för förhöjt systoliskt blodtryck ("övertrycket" ≥ 140). Jämfört med män som fötts i fullgången tid, hade de som fötts efter 24-28, 29-32 respektive 33-36 veckor en riskökning på 88 procent, 45 procent respektive 24 procent. Sambandet förklarades inte av familjära faktorer.

Delarbete IV

Det sista delarbetet omfattade 18 230 tvillingar födda mellan 1926 och 1958. Syftet var att studera om för tidig födsel eller låg födelsevikt ökade risken för åldersdiabetes. Information om graviditetstid och födelsevikt samlades in från arkiverade förlossningsjournaler, och information om åldersdiabetes erhöles från en enkät som tvillingarna besvarade i vuxen ålder.

För tidig födsel ökade inte risken för åldersdiabetes senare i livet men däremot fanns ett samband mellan låg födelsevikt och åldersdiabetes. Bland tvillingar med en födelsevikt under 2000 gram förekom åldersdiabetes hos 5.3 procent, medan motsvarande siffra var 2.4 procent bland de med en födelsevikt över 3500 gram. Genom att studera tvillingpar där ena tvillingen var frisk och den andra hade diabetes, fanns emellertid ett samband mellan låg födelsevikt och åldersdiabetes bara hos tvåäggstvillingar men inte hos enäggstvillingar. Detta talar för att det är samma ärftliga faktorer hos en individ som ger upphov till *både* låg födelsevikt och åldersdiabetes.

Slutsatser

- Kvinnor som drabbas av virusinfektioner i tidig graviditet, särskilt med *Parvovirus B19*, kan löpa ökad risk för mycket för tidig förlossning. Mer forskning behövs på området.
- Dödligheten hos extremt för tidigt barn (≤ 27 veckor) är högre på länsjukhus jämfört med universitetssjukhus. När det är möjligt bör sådana förlossningar centraliseras, men starka regionala nätverk behövs också för att optimera barnets chanser när transport till universitetsklinik inte är möjlig före födelsen.
- För tidig födsel är en riskfaktor för högt blodtryck. Ju kortare graviditeten varit, desto högre är risken. Blodtrycket bör mätas vid läkarkontroller av barn och ungdomar som varit mycket för tidigt födda för att upptäcka högt blodtryck innan det ger tydliga besvär. Mer kunskap behövs om hälsosam livsstil och kanske bör man också studera om läkemedelsbehandling minskar risken för etablerad hjärtkärlsjukdom senare i livet.
- För tidig födsel ökar inte risken för åldersdiabetes. Däremot finns ett samband mellan låg födelsevikt och åldersdiabetes. Våra resultat talar emellertid för att detta inte är ett orsakssamband, utan att vissa individer har en genetisk predisposition för både låg födelsevikt och åldersdiabetes.

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