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PRENATAL INFLUENCES ON HEALTH OUTCOMES OVER THE LIFESPAN

Effects of smoking, socio-economic status and foetal growth

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Stockholm 2012

Till minnet av Sonja Lundén

Min mormor som lärde mig att vara tacksam över att få studera

ABSTRACT

The most remarkable development during a person's life takes place in utero. Through about 280 days of proliferation and differentiation one single cell is developed into a human being. As this is the time when the whole body is formed, it is easy to imagine that exposures during this time can have health consequences over the lifespan. Indeed, a large number of studies have shown that early exposures, such as low birth weight and maternal smoking during pregnancy, influence health later in life, for example the risks of hypertension and cardiovascular disease. However, it has been questioned if the found associations really reflect causal relationships, or if they are due to genetic or environmental confounders. The aim of this thesis was to study how factors during prenatal/early life can influence health outcomes over the lifespan, after taking common genetic and shared environmental factors into account. The focus lies on intergenerational influences of smoking, socio-economic status and foetal growth.

In the first study it is investigated if a change in maternal smoking habits between two consecutive pregnancies influences the risk of stillbirth. It was found that women who quit smoking between pregnancies have the same risk of stillbirth in their second pregnancy as women who never smoked when pregnant. Women, who smoked in one of their two pregnancies, had an increased risk of stillbirth in the pregnancy where they smoked compared to the pregnancy when they did not smoke. These findings support the conclusion of a causal association between maternal smoking during pregnancy and stillbirth.

In the second study it is investigated if maternal smoking during pregnancy has a long term influence on offspring blood pressure. A small but statistically significant increase in systolic and diastolic blood pressure was found in sons whose mothers had smoked during pregnancy. This association remained, although not statistically significant, within brother pairs. The conclusion is that there might be a long term influence of maternal smoking during pregnancy on offspring blood pressure.

In the third study it is investigated if intergenerational social mobility influences the risk of hypertension. The results showed a decreased risk of hypertension among twins with upward social mobility and indicated an increased risk among twins with downward social mobility. The conclusion is that social inequity in hypertension is initiated early in life (indicated by parental social status), but modifiable through later factors (indicated by adult social status).

In the fourth study it is investigated if the intergenerational influence on birth weight/birth length is due to genes or environment. An association between mother's and offspring's size at birth was found within birth weight discordant dizygotic twin pairs, but not within monozygotic twin pairs. The conclusion is that the intergenerational association in size at birth is due to direct or indirect genetic factors.

LIST OF PUBLICATIONS

This thesis is based on the following studies which will be referred to by their Roman numbers.

I.   Högberg L, Cnattingius S. The influence of maternal smoking habits on the risk of subsequent stillbirth: is there a causal relation? *British Journal of Obstetrics and Gynaecology* 2007; 114: 699-704

II.   Högberg L, Cnattingius S, Lundholm C, D'Onofrio B M, Långström N, Iliadou A N. Effects of maternal smoking during pregnancy on offspring blood pressure in late adolescence. *Journal of Hypertension* 2012; 30: 693-699

III.   Högberg L, Cnattingius S, Lundholm C, Sparén P, Iliadou A N. Intergenerational social mobility and the risk of hypertension. *Journal of Epidemiology and Community Health* 2012; 66: e9

IV.   Högberg L, Lundholm C, Cnattingius S, Öberg S, Iliadou A N. Birth weight discordant female twins and their offspring: is the intergenerational influence on birth weight due to genes or environment? *Submitted manuscript*

CONTENTS

LIST OF ABBREVIATIONS

AGA	Appropriate for Gestational Age
BMI	Body Mass Index
CI	Confidence Interval
CMV	Cytomegalovirus
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DOHaD	Developmental Origins of Health and Disease
DZ	Dizygotic
ECG	Electrocardiogram
GEE	Generalized Estimation Equation
GH	Growth Hormone
HbA1c	Haemoglobin Adult 1c
HIV	Human Immunodeficiency Virus
HPA	Hypothalamic-Pituitary-Adrenal
IGF	Insulin-like Growth Factor
Igf	Insulin-like growth factor allele
IQ	Intelligence Quote
IUGR	Intrauterine growth restriction
LGA	Large for Gestational Age
LMP	Last Menstrual Period
MZ	Monozygotic
OR	Odds Ratio
PKU	Phenylketonuria
Q73	Questionnaire 1973
SALT	Screening Across the Lifespan Twin Study
SBP	Systolic blood pressure
SD	Standard Deviation
SES	Socio-economic status
SGA	Small for Gestational Age
SIDS	Sudden Infant Death Syndrome
STAGE	The Study of Twin Adults: Genes and Environment
TTTS	Twin-to-twin transfusion syndrome
WHO	World Health Organization

1 INTRODUCTION

The most remarkable development during a person's life takes place in utero. Through about 280 days of proliferation and differentiation one single cell is developed into a human being. As this is the time when the body is formed, it is easy to imagine that exposures during this time can have health consequences over the lifespan. A large number of studies have shown that low birth weight in new-borns is associated with increased risk of, for example, hypertension and cardiovascular disease later in life [1]. The risk is also increased among those who had a low birth weight without being born preterm, i.e. those who were growth restricted. These findings have given rise to the Developmental Origin of Health and Disease (DOHaD) hypothesis. It states that adverse foetal conditions can alter tissue structure and function, and may thereby influence the risk, or even program/predispose for later disease. This hypothesis have however been criticized. It has been argued that other factors than foetal conditions can explain the association between low birth weight and adult disease [2-4], for example genetic or social factors that influence the risk of both low birth weight and cardiovascular disease. One way to investigate if an association is due to shared genes or common environmental factors (familial factors) is to study if the association remains within related individuals, for example within pairs of siblings or twins.

This thesis builds on four studies (Study I-IV) that all are based on Swedish register data. The first two deals with adverse effects of maternal smoking during pregnancy. Smoking is a known risk factor for several adverse pregnancy outcomes such as miscarriage, preterm birth and foetal growth restriction [5]. In Study I, maternal smoking during pregnancy is investigated as a cause of stillbirth. Maternal smoking during pregnancy has also been suggested to have long term influences on offspring health, for example through effects on childhood blood pressure and overweight [6,7]. In Study II, it is investigated if maternal smoking during pregnancy has an influence on late adolescent offspring blood pressure. Blood pressure is known to track across the lifespan [8]; hence adolescents with higher blood pressure are more likely to become hypertensive as adults. Smoking in general and maternal smoking during pregnancy in particular have over the last decades become strongly socially patterned where women from lower socio-economic groups smoke more often than women from higher socio-economic groups [5]. Health is also often socially patterned. In Sweden, as in other countries, there is a social gradient in health where, for most conditions, those with lower social status have a higher risk of disease [9]. In the third study of this thesis, social mobility (between parent's and own socio-economic status group) is studied in relation to risk of hypertension. The interest in determinants of birth weight has been renewed in the light of the DOHaD hypothesis. Social status is related to birth weight and there is also a known association between parent's and offspring's size at birth. In the fourth study this intergenerational association in size at birth is investigated among female twins and their offspring, aiming to separate genetic and environmental influences.

2 BACKGROUND

2.1 FOETAL GROWTH

Appropriate foetal growth is important for health in both the short and the long perspective. Infants, who fail to reach their full growth potential, face increased risks of perinatal morbidity and mortality [10]. Adults, who were growth restricted at birth, face increased risks of hypertension, diabetes and cardiovascular disease later in life [11]. Growth restricted infants are often born preterm, which is a risk factor in itself. In this thesis, the focus lies on growth restriction; therefore risks of preterm birth will not be further discussed. Before we go into determinants and consequences of intrauterine growth restriction, some aspects of normal pregnancy and some definitions need to be clarified.

Pregnancy duration

Usually, it is not possible to know the exact date of conception. Therefore, information about the first day of the last menstrual period (date of LMP) is used to estimate pregnancy duration (that date, plus seven days, minus three months gives the expected date of delivery). This gives a pregnancy duration of 40 weeks or about 280 days [12]. Pregnancy duration can, more accurately, be estimated through early ultrasound measurements of foetal dimensions (usually the biparietal diameter or the crown-rump length). This has been done in almost all pregnancies in Sweden since the 1990's [13]. A term birth takes place between the 37th and 42nd week of gestation [12]. An infant born before the 37th week of gestation is said to be preterm and if born after 42 completed weeks the infant is said to be post-term [12]. To match the current viability limits, the cut off between late miscarriage and preterm birth has been set to 22 completed weeks of gestation.

Birth weight – a proxy for foetal growth

Birth weight is the result of both gestational age and foetal growth. Hence, a new-born can have low birth weight because of (1) preterm birth, (2) restricted foetal growth or (3) both. Normal birth weight (±2SD) for a term infant (40 weeks of gestation) in Sweden is between 2700 and 4400 g [14,15]. Low birth weight is defined as a weight below 2500 g. Low birth weight infants have increased risks of infant morbidity and mortality, and these risks increase with a reduction in birth weight [16]. In many epidemiological studies, birth weight is used as a proxy for foetal growth, as information on birth weight (but not gestational age) is often available. However, it only provides a measurement of one single time point and does not take differences in gestational age into account. To assess foetal growth and foetal growth restriction, repeated measurements of foetal size are required. Most often, this information is not available; instead small for gestational age (SGA) is used as a proxy for intrauterine growth restriction (IUGR). A foetus or infant is classified as SGA if the estimated foetal weight/birth weight is below a certain cut off, e.g. two standard deviations or the 10th percentile of the mean value for the specific gestational week [16]. Among infants diagnosed as SGA, there will be those who have suffered from foetal growth restriction, but there will also be some who are just constitutionally small [17]. Intrauterine growth restriction is defined as a reduction in growth rate that prevents the

Table 1. Definition of measures used to assess foetal growth.

Low birth weight	Birth weight below 2500 g.
Macrosomia	Birth weight above 4500 g.
Small for gestational age (SGA)*	Birth weight below -2SD of the expected birth weight given the gestational age.
Appropriate for gestational age (AGA)	Birth weight within ±2SD of the expected birth weight given the gestational age.
Large for gestational age (LGA)	Birth weight above +2SD of the expected birth weight given the gestational age.
Intrauterine growth restriction (IUGR)	A failure to reach the full genetic growth potential, seen as a deviation from the own growth curve.

*To determine SGA, AGA and LGA birth weights are compared towards a standard growth curve for the population. SGA can also be defined using other cut offs, such as a birth weight below the 10th percentile.

foetus to reach his or her full genetic growth potential [10]. Most IUGR infants will be classified as SGA, but some can also be born with appropriate birth weight for gestational age (AGA). These and other definitions are summarized in Table 1.

Determinants of intrauterine growth restriction (IUGR)

Birth weight is influenced by both genetic and environmental factors. Women and men who themselves were heavy at birth are more likely to be tall and heavy as adults and to have larger offspring than smaller parents [16]. Generally, these associations are stronger on the matrilineal side [16]. The risk of having an SGA infant is increased if the mother was born SGA herself, if her sister had an SGA infant, or to a lesser extent if her brother had an SGA infant [18]. There are many different causes to foetal growth restriction. In western countries, placental insufficiency and maternal smoking have been pointed out as the most important factors [10,16]. In developing countries, insufficient maternal nutrition and malaria play a larger role [10]. Determinants of IUGR can be divided into maternal, placental and foetal factors (Table 2 [10,16,17,19-21]).

Maternal factors

Short stature, low pre-pregnancy weight and poor gestational weight gain have all been associated with increased risk of IUGR [19]. In Sweden, there has been a trend of increasing mean birth weight during the last decades, which has been suggested to partly be due to a concurrent increase in BMI among pregnant women [22]. At the same time, there has been a slight decrease in infants with birth weight between 1500 and 2499 g [22]. Probably, the increase in mean birth weight is also due to a large decrease in maternal smoking during pregnancy. Cigarette smoking is causally related to foetal growth and decrease birth weight by around 135-300 g [5,21]. Alcohol consumption and illicit drugs also affect foetal growth negatively, as well as some prescribed drugs (antimetabolites, anticoagulants, anticonvulsants) [20]. High altitude influence foetal growth through hypoxia and the same influence can be seen in women with severe lung disease, cardiac disease or severe anaemia [10].

Table 2. Maternal, placental and foetal factors associated with foetal growth and IUGR.

Maternal

Demographic	*Nutritional*	*Drugs*
Maternal height, weight	Severe caloric restriction	Cigarette smoking
Maternal birth weight	*Medical conditions*	Alcohol consumption
Gestational weight gain	Preeclampsia	Illicit drugs
Inter-pregnancy interval	Hypertension	Some prescribed drugs
Parity	Renal disease	*Other*
Ethnic group	Diabetes	Reproductive technology
Maternal age	Autoimmune diseases	Previous SGA infant
Environmental	Hypoxemia	Socio-economic position
High altitude		

Placental | **Foetal** | |

Structural	*Demographic*	*Congenital malformations*
Abnormal placentation	Sex	Cardiovascular defects
Placenta praevia	Multiple gestations	Skeletal dysplasia e.g.
Chorioangioma	*Genetic*	*Intrauterine infections*
Velamentous insertion	Parent's size	Viral (CMV, Rubella)
Hormonal	Chromosomal abnormality	Bacterial (syphilis)
Insulin, steroids, GH etc.	GH, GHr mutation	Protozoal (malaria)

CMV=Cytomegalovirus. GH=Growth Hormone r=receptor.

Other medical conditions associated with IUGR are hypertension which increases the incidence 2-3 fold [20]. Hypertension can be further superimposed by preeclampsia, which is thought to be caused by inappropriate placentation and lead to insufficient nutritional supply for the foetus. Renal disease is associated with both hypertension and preeclampsia. Diabetes is often associated with macrosomia. Paradoxically there is also an association with IUGR, which might be due to damages in the microcirculation or common genetic causes [4,20]. Autoimmune diseases, such as systemic lupus erythematosus (SLE), increase the risk of foetal growth restriction eight-fold [20]. There is a large overlap between several of the factors influencing foetal growth restriction [19]. Socio-economic status has been suggested to influence foetal growth indirectly [19]. Further, ethnicity might at least partly have indirect influences. There are large differences in mean birth weight between different ethnic groups. For example, in India, mean birth weight is 700 g lower than in many western countries [23]. However, this difference can largely be explained by small maternal size and maternal under nutrition. A study of first and second generation Indian immigrants to the UK showed that women born in the UK gave birth to significantly larger infants than women born in India [24]. Ethnicity is often strongly linked to socio-economic status; it has been shown that African born black women in USA do not have the same increase in risk of IUGR as American born black women, suggesting an effect of social status rather than ethnicity [17]. Assisted reproductive techniques (ART) have also been associated with increased risk of IUGR [25], suggesting that very early disturbances of the embryo's environment can influence foetal growth.

Placental factors

Naturally, foetal growth is strongly dependent on placental function. Structural placental abnormalities can influence foetal growth negatively, e.g. if the placenta is small or affected by infarctions or lesions [17,20,21]. Placenta praevia, larger chorioangiomas (benign blood vessel tumours in the placenta) and a velamentous umbilical cord insertion (umbilical cord inserted in the foetal membranes instead of the placental mass) can also impair foetal growth [17,20]. The most important factor is the abnormal placentation often seen in pregnancies with preeclampsia, foetal growth restriction, placental abruption and stillbirth. In such pregnancies, there is insufficient cytotrophoblast invasion and incomplete reformation of the spiral arteries, leading to inadequate perfusion of the placenta, local placental hypoxia and impaired foetal growth [10,26]. The placenta also has endocrine functions. Alterations in hormone levels (insulin, steroids, growth hormone, placental lactogen) has been suggested to affect foetal growth [17].

Foetal factors

Male foetuses grow faster than female foetuses. In developed countries the sex difference in mean birth weight has been estimated to be 126 g [16]. Multiple gestation is a clear risk factor for IUGR, twins and triplets are significantly smaller than singletons at birth. Chromosomal abnormalities, congenital malformations and genetic disorders can cause foetal growth restriction [17]. Further, viral, bacterial and protozoal intrauterine infections can pose a threat to normal foetal growth, e.g. rubella, cytomegalovirus, herpes simplex, HIV, syphilis and malaria and toxoplasma [10,17].

Regulation of foetal growth

Some of the described factors that influence foetal growth are due to pathology, while some are due to physiology. Non-genetic and non-pathological maternal factors that restrict foetal growth can be referred to as maternal constraint [27]. This operates to some extent in all pregnancies through e.g. maternal size, parity, age and nutrition [27]. One of the first, and often cited, publications demonstrating maternal constraint was Walton and Hammond's cross-breeding experiments with Shire horses (at least 170 cm tall) and Shetland ponies (around 100 cm tall) from 1938 [28]. They found that the reciprocal crossbred fouls had the same size at birth as pure bred foals of the mare's breed [28]. Clearly, there was a large influence of maternal size. From an evolutionary perspective, mammals have to balance between the foetus and the mother's nutritional needs [27]. Further, the foetus cannot be larger than that it permits vaginal delivery [27]. Before the 16th week of gestation there is very little variation in foetal size and the growth is mainly determined by the foetal genes [29], but thereafter environmental and hormonal factors become increasingly important.

The most important hormone system for foetal and placental growth throughout gestation is the insulin-like-growth factor (IGF) system. This system includes IGF-1 and IGF-2 that, through the IGF-1 receptor has mitogenic and antiapoptotic effects [30]. The IGF-2 receptor has a regulatory function that leads to decreased IGF-2 levels. Both IGF-1 and IGF-2 are expressed throughout pregnancy. In embryonic development IGF-2 is most important, while the importance of IGF-1 increase later in gestation [30]. It has been found that foetal and cord serum concentrations of IGF-1 correlates with birth weight. Levels are decreased in IUGR infants and increased in LGA infants [30]. Furthermore, infants with IUGR have increased levels of IGF-binding proteins (there

are six such proteins). Knock-out studies in mice have showed that a disruption of Igf1, Igf2 or Igf1-receptor alleles results in foetal growth restriction [30]. Further, an overexpression of IGF-2, due to disruption of Igf2-receptor causes foetal overgrowth [30]. The Igf2 and the Igf2-receptor genes are imprinted, i.e. parent of origin determines which alleles that will be expressed [30]. Imprinting of genes is thought to have evolved as a conflict between the paternal genome, that promotes growth for his offspring, and the maternal genome, that wants to regulate growth [31]. Genes are imprinted through epigenetic mechanisms [32]. The Igf2 gene, that promotes growth, is paternally imprinted, and the Igf2-receptor gene that regulates growth is maternally imprinted [30]. Changes in normal imprinting have been linked to both IUGR and overgrowth in humans. One of the clinical presentations in Beckwith-Wiedemann syndrome is overgrowth. This syndrome can be caused by different genetic or epigenetic changes in a specific chromosomal region, which leads to upregulation of paternally imprinted genes and downregulation of maternally expressed genes [30,33]. In Silver-Russel syndrome, which presents with severe intrauterine growth restriction, two regions containing imprinted genes have been shown to have duplicated maternal copies [30,33].

Why foetal growth matters

Low birth weight influences health outcomes over the lifespan (Table 3). On short term, growth restriction is an important risk factor for stillbirth, neonatal mortality and morbidity [10]. Although a majority of growth restricted children have a catch up growth, around 10% have continued poor growth and might be shorter than expected as adults [10]. This can be a larger problem in low income countries with higher demands on physical work abilities. Intrauterine growth restriction has also been associated with increased risks of sudden infant death syndrome (SIDS) and cerebral palsy, small decreases in IQ, learning disabilities, and impaired airway function [10]. Decreases in IQ and learning problems can influence the child's educational attainment and possible life chances [34]. Low birth weight and IUGR is also associated with several adverse health outcomes in adulthood, this has given rise to the Developmental Origins of Health and Disease hypothesis which will be further discussed in the next section.

Table 3. Short and long term risks associated with low birth weight and IUGR.

Perinatal outcomes	*Childhood outcomes*	*Adult outcomes*
Mortality	*Mortality*	*Mortality*
Stillbirth	SIDS	Death from CVD
Neonatal death		
Morbidity	*Morbidity*	*Morbidity*
Hypoglycaemia	Continued poor growth	CVD
Hypothermia	Small decrease in IQ	Hypertension
Polycythaemia	Cerebral palsy	Diabetes type 2
Severe depression at birth	Learning disabilities	Preeclampsia
Infection	Impaired airway function	Osteoporosis, etc.

SIDS=Sudden Infant Death Syndrome CVD=Cardiovascular disease

2.2 DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

The hypothesis

The first to propose that early life deprivation could predispose to coronary heart disease was David Barker and Clive Osmond in 1986 [35]. Although, this was not a completely new idea [36,37], there was a new focus on the influence of nutrition. Clive and Osmond found an association between low birth weight and mortality from coronary heart disease. With time, these findings have been replicated numerous times and in many different populations [1,38]. Associations have also been shown between low birth weight and other outcomes, including stroke, hypertension and type 2 diabetes [38]. Further, the importance of postnatal growth has been demonstrated, as the highest risks are seen among those who were small at birth but large in childhood and adulthood [38]. The findings relating low birth weight/poor foetal growth to later disease has evolved into the Developmental Origins of Health and Disease (DOHaD) hypothesis, described by Professor Barker as follows: "The foetal origins hypothesis proposes that coronary heart disease, type 2 diabetes, stroke and hypertension originate in developmental plasticity, in response to under nutrition during foetal life and infancy" [38].

### Developmental plasticity or foetal programming

According to the DOHaD hypothesis these diseases originate in developmental plasticity or foetal programming. That is, a process in which a stimulus or insult during a critical or sensitive period of development has lasting effects on the structure or function of the body [11]. This process could be of advantage for the individual if the stimulus correctly predicts the extra uterine environment and leads to adaptions suitable for this milieu, a so called predictive adaptive response [39]. It has been suggested that poor intrauterine nutrition due to maternal starvation could lead to such as response; maternal under nutrition is then a signal that predicts a harsh environment, the foetus adapts by a smaller body size and insulin resistance which could be an advantage in a food deprived environment [38,40]. However, if the poor intrauterine nutrition is due to, for example maternal disease or eating disorders, there will be a mismatch between the predicted environment and the actual environment the infant is born into. The adaptions suitable for a harsh environment will then be a disadvantage in a milieu where food is abundant, and this leads to a predisposition for later disease [38,40].

The finding that risks of cardiovascular and metabolic diseases are modified by postnatal growth supports this theory, i.e. those who are born small and become overweight later in life have the highest risk of disease [38]. Another example comes from "natural experiments" of starvation. People who were born during or after the Dutch Hunger Winter had decreased glucose tolerance and evidence of insulin resistance at age 50 [41], while no such effects were seen in people born during or after the Leningrad siege [42]. In Holland the food shortage was immediately reversed after liberation from the German occupation, while in Russia the food shortage remained even after the war.

Possible mechanisms

Results from studies in several species have supported the DOHaD hypothesis. The inverse association between birth weight and both blood pressure and insulin resistance has, for example, also been found in guinea pigs [43]. Further, manipulations of maternal diet and glucocorticoid administration during sensitive periods of gestation in mouse, rat, guinea pig, sheep and pig have been shown to cause permanent changes in the offspring's physiology [43]. These changes include insulin resistance, altered appetite regulation, altered hypothalamic-pituitary-adrenal axis (HPA) function and endothelial dysfunction, which can be seen as changes in programmed set points of physiological systems [1,43]. There are also structural changes in organ development and growth, for example reduced nephron number in the kidneys, reduced β-cell mass in the pancreas, increased fat mass vs. lean body mass and reduction in skeletal muscle fibres [43,44]. Several of the proposed mechanisms are thought to be due to epigenetic changes, i.e. an altered gene expression without a change in the gene sequence [44]. Effects of maternal undernutrition and exposure to glucocorticoids have been suggested to have similar mechanisms, as undernutrition reduces the activity of the enzyme 11β-hydroxysteroid dehydrogenase, which deactivates cortisol [43]. In humans, low birth weight infants have increased cortisol concentrations in umbilical cord blood [45] and increased cortisol secretion also later in life [46]. Reduced number of glomeruli in the kidneys is thought to accelerate the decline in renal function that comes with ageing and predispose to hypertension [44]. Figure 1 shows a simplified overview of the mechanisms underlying the DOHaD hypothesis.

Figure 1. Simplified overview of mechanisms underlying the DOHaD hypothesis.
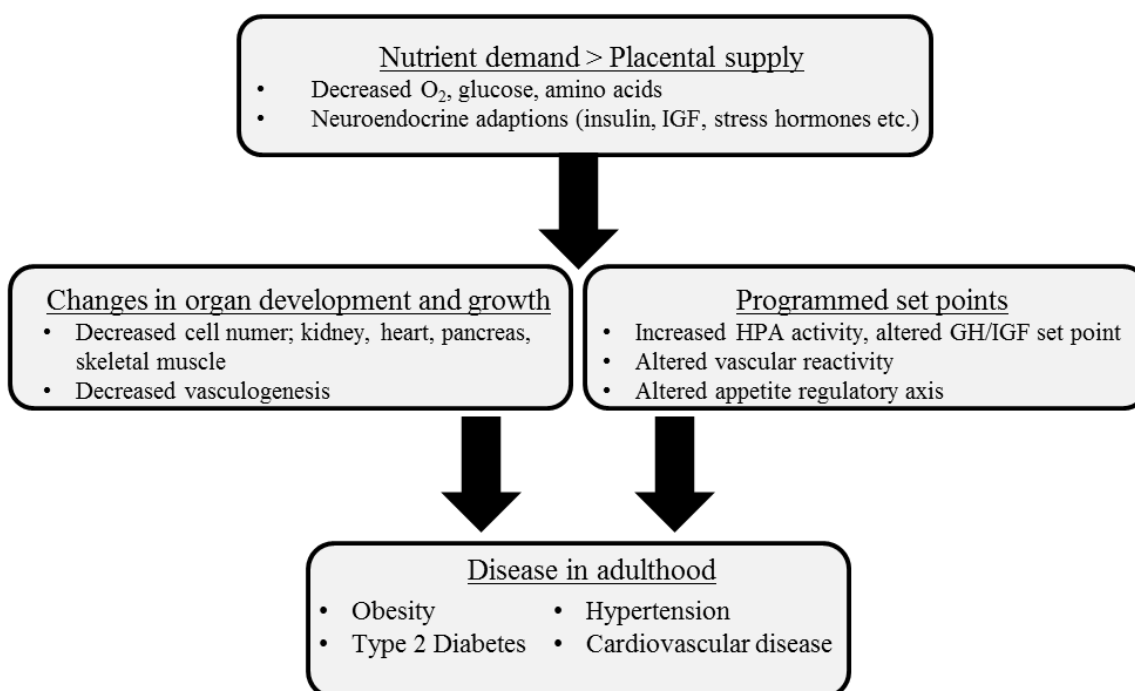
Adapted from McMillen and Robinson 2005 [1]

Critique or alternative explanations

The DOHaD hypothesis has been criticised on a number of points. It has been questioned if the association between foetal growth and adult disease is important today, as many studies used historical data. Further, concerns about socio-economic and genetic confounding have been expressed. Both low birth weight and cardiovascular disease are more common in low socio-economic groups [47,48] and genetic factors could also influence both foetal growth and disease development, for example genes predisposing to type 2 diabetes [4]. It has also been questioned how important these associations are, even if they are causal [2]. For example, in a large meta-analysis a one kilo decrease in birth weight was only associated with very small (less than 1 mm Hg) increase in blood pressure [49].
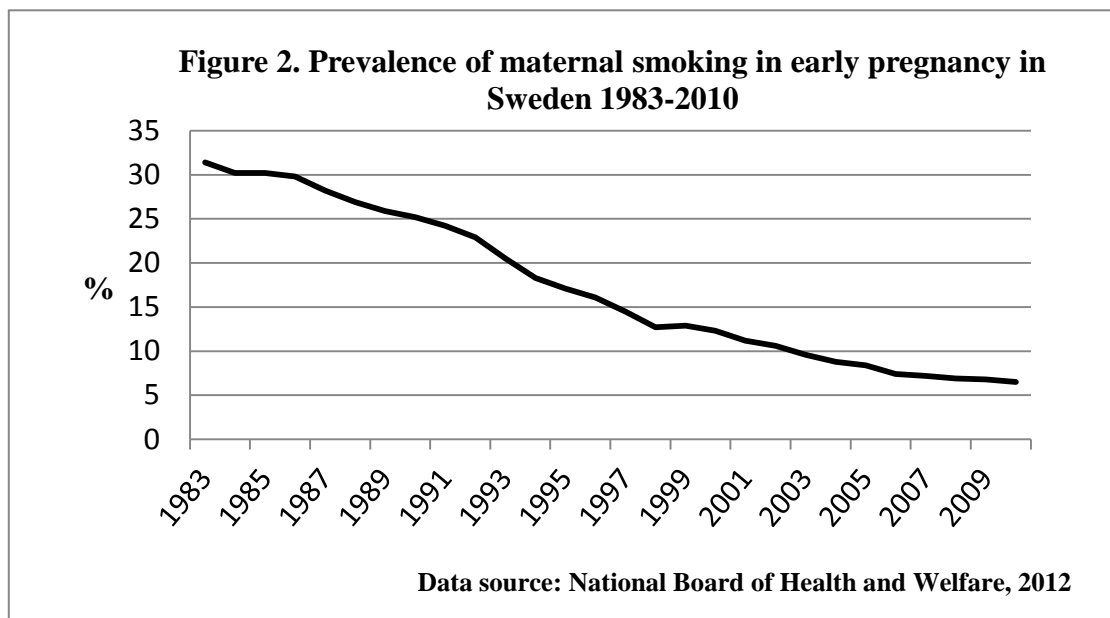
In response to this critique, it has been argued that many countries in the world still have similar conditions as industrialized countries had one hundred years ago. Considering the rapid changes in these societies in terms of diet and food availability, even larger effects of low birth weight could be expected [50]. Further, associations between low birth weight and adult disease remain within all socioeconomic strata and through the whole range of birth weights [51]. Studies within birth weight discordant twin pairs were hoped to provide some insight in underlying mechanisms, however they have yielded mixed results. The association between low birth weight and hypertension remains within monozygotic twins, suggesting an influence of foetal programming [52]. While the association between low birth weight and stroke and coronary heart disease does not remain within monozygotic twin pairs, suggesting that the association is due to genetic confounding [53]. With respect to type 2 diabetes, one study found evidence of foetal programming [54], while results from another study suggested that the association was confounded by genetic factors [55]. Although the influence on blood pressure may be very small, larger effects on hypertension risk have been reported. For example, in an American study of more than 22 000 men, the effect on blood pressure was less than 1 mm Hg per kilogram increase in birth weight, but the association between birth weight and treated hypertension was stronger with more than 30% difference in prevalence across the birth weight range [56].

It can also be questioned if the changes seen in growth restricted individuals really are due to a mismatched predictive adaptive response. Developmental response to the environment can be without any evolutionary significance [40]. The growth restricted foetus can relocate resources to prioritise some organs e.g. the brain on the expense of others, such as the kidneys [40]. This might be advantageous for short term survival, but increase the risk of disease on long term. Similarly, increased growth in childhood (catch up growth) might be beneficial on short term, but have costs in terms of altered hormone levels and increased demands on suboptimal developed organs, which in the long run increase the risk of disease [38].

2.3 MATERNAL SMOKING DURING PREGNANCY

How common is it to smoke during pregnancy?

Since 1983 there has been a steady decrease in maternal smoking in early pregnancy (self-reported at first visit to antenatal care) from 31.4% in 1983 to 6.5% in 2010 (Figure 2).

Figure 2. Prevalence of maternal smoking in early pregnancy in Sweden 1983-2010

Data source: National Board of Health and Welfare, 2012

Although this is a very positive development, there are still subgroups where the prevalence of maternal smoking is high. Among teenage mothers smoking is more common (27%) than in other age groups [57]. Furthermore, the prevalence of smoking during pregnancy shows large variations between different areas. In 2006, there were still some municipalities with a prevalence around 20-30%, while only 0-2% of women in other areas smoked during pregnancy [57]. Smoking in general and maternal smoking during pregnancy in particular are strongly related to socio-economic status and these differences have increased over time [58]. Table 4 shows the three municipalities in Stockholm County with the highest and the lowest prevalence of parental smoking, respectively. The three areas with the lowest smoking prevalence are all among the ten wealthiest municipalities in Sweden [59]. There is also a strong association with education. In Sweden, 3% of high educated women (≥15 years) smoked during pregnancy in 1997, compared to 34% among low educated women (≤9 years) [5]. In the 1960's, when the adverse effects of smoking were relatively unknown in society, 97% of smoking women continued to smoke during pregnancy [58]. Today, most pregnant women are aware of risks for their foetus and 20-40% quit smoking in early pregnancy [5]. Most quit already before the first visit to antenatal care, some quit later in pregnancy, and among those who continue to smoke about 50% report that they have cut down the amount smoked [5,60]. Risk factors for continued smoking during pregnancy are severity of addiction (amount smoked and age at onset of smoking), low maternal education, multiparity, exposure to passive smoking at home and not cohabiting with the infant's father [61]. Partner smoking is also a major risk factor for taking up smoking after delivery [60].

Table 4. Smoking prevalence among parents in the three municipalities in Stockholm County where the prevalence was highest and lowest, respectively in 2006.

| | Parental smoking (%) | | | | |
| | In pregnancy | When the child is 0-4 weeks | | When the child is 8 month | |
Municipality	Week 8-12	Mother	Father	Mother	Father
Norrtälje	12.5	9.8	14.1	12.2	13.9
Nynäshamn	11.0	7.2	11.8	7.7	11.0
Södertälje	9.4	7.1	19.7	9.8	18.7
Danderyd	1.0	0.8	4.8	0.0*	4.8*
Vaxholm	1.8	1.2	3.7	2.6	5.2
Lidingö	1.9*	0.2	5.1	0.6	5.3

*Missing data in more than 20%.
Adapted from "Tobaksvanor bland gravida och spädbarnsföräldrar 2006" [57].

Adverse effects of smoking during pregnancy

Smoking during pregnancy has been associated with several adverse pregnancy outcomes and some studies also suggest long term influences on offspring's health [5,62]. Smoking during pregnancy is causally related to foetal growth restriction; smoking of 10 cigarettes per day has been associated with a 200 g reduction in birth weight [5]. Further, there is a dose-response relationship and smoking cessation in early pregnancy has been shown to increase birth weight [63]. Potential mechanisms for this are chronic foetal hypoxemia due to increased levels of carbon monoxide haemoglobin and reduced uterine and placental blood flow due to increased vascular resistance [5]. Smoking is also associated with increased risks of placenta complications such as placental abruption (premature separation of the placenta from the uterine wall) and placenta praevia (when the placenta partly or completely covers the internal os), which both are potential causes of foetal and maternal death [5]. Underlying mechanisms for placental abruption has been suggested to be degenerative and inflammatory alterations in the placenta, decreased levels of ascorbic acid, which is important for collagen synthesis, and increased risk of premature rupture of the membranes in smoking mothers [5]. Foetal growth restriction and placental complications have been shown to explain the increased risk of stillbirth seen in mothers who smoke during pregnancy [5]. Further, maternal smoking during pregnancy has been associated with increased risks of infertility (defined as inability to conceive after twelve months of unprotected intercourse), ectopic pregnancy, spontaneous abortion, preterm delivery, neonatal mortality (first week of life), oro facial clefts and sudden infant death syndrome (SIDS) [5]. Paradoxically, smoking during pregnancy is inversely associated with preeclampsia risk [5].

Women exposed to passive smoking during pregnancy also have increased risks of adverse pregnancy outcomes, although risks are generally lower than in active smokers [64]. Further, smokeless tobacco such as the Swedish moist snuff ("snus") is associated with both preterm birth and stillbirth [65,66]. In 2006, the prevalence of oral moist snuff ("snus") use among pregnant women ranged between 0.5 and 6.9% in different Swedish counties, with an average of 1.4% [57].

Are there long term effects of maternal smoking during pregnancy?

While it is very clear that smoking threatens the health of the foetus and the new-born, it is much less clear whether exposure to smoking in utero also has long term effects on health. In the light of the DOHaD hypothesis, several studies have investigated if smoking during pregnancy has long term influences on, for example offspring blood pressure, obesity, type 2 diabetes and neurobehavioral outcomes [62]. The proposed mechanisms are largely the same as outlined for the DOHaD hypothesis, with the addition that nicotine itself and other components in cigarettes (carbon monoxide, cadmium, and additives) can have detrimental long term effects on the individual [62]. Several experimental studies on animals exposed to nicotine during pregnancy have also shown effects in offspring, such as elevated blood pressure, loss of β-cell mass in the pancreas and impaired insulin sensitivity in peripheral tissues [67]. Although several studies on humans have shown associations with in utero exposure to smoking and later disease [6,68], it is difficult to determine whether these associations are causal. Power et al. found associations with in utero exposure to smoking and adult BMI, waist circumference, blood pressure, HbA1c (a measure of long time blood sugar levels) and triglycerides (blood lipids), but after adjustment for postnatal influences only BMI and waist circumference remained significant [69]. It has also been shown that the association with smoking during pregnancy and both offspring BMI and IQ can be attributed to familial confounding [70,71]. Studies investigating maternal smoking and offspring blood pressure have yielded very mixed results. About half of the studies have reported positive associations, while the other half have reported null findings or attenuations towards the null after adjustments. In their review, Bakker and Jaddoe conclude that further studies that account for potential confounders are needed [62].

2.4 STILLBIRTH

Stillbirth causes great distress and grief among afflicted parents. Around 3 million stillbirths have been estimated to occur each year and 98% of them occur in low and middle income countries [72]. There are different definitions of stillbirth ranging from foetal death from the 20th to the 28th gestational week or from a foetal weight of 350-1000 g [73]. In Sweden, stillbirth is now defined as foetal death after at least 22 completed weeks of gestation (before 2008, the definition was 28 completed weeks of gestation) [74]. The incidence of stillbirth in Sweden is 3-4 per 1000 births and has been rather constant during the last thirty years (Figure 3).

In a recent meta-analysis, maternal overweight/obesity, maternal smoking, high maternal age, primiparity, a small for gestational age foetus, placental abruption and pre-existing maternal diabetes/hypertension were pointed out as the most important risk factors of stillbirth in high income countries [75]. Overweight/obesity combined has been estimated to increase the odds of stillbirth by 28-58%. Maternal smoking has been associated with at least 40% increase in odds and high maternal age ($\geq$35 years) also increase risk of stillbirth, especially in primiparous women [75]. Other risk factors of stillbirth are; low socio-economic status [76], low maternal education [77], stillbirth or IUGR in a previous pregnancy [78], infections, congenital malformations/genetic abnormalities, multiple births, placental and umbilical cord complications, maternal injury and other maternal medical conditions, such as lupus and cholestasis in pregnancy [73,79,80]. Despite this, the cause remains unknown in at least 10-15% of stillbirths [74]. Bendon has described how complex chains of risk factors can lead to stillbirth and suggests three common death mechanisms; hydrops, asphyxia and shock [81]. To reduce stillbirth rates, it has been suggested that risk factors such as smoking, overweight and high maternal age should be reduced in the pregnant population and supervision of pregnancies complicated by other risk factors should be increased [75]. Primary prevention of smoking has been pointed out as the most efficient way to reduce smoking during pregnancy, while effective tools to reduce other risk factors, such as overweight and high maternal age are lacking [82].

Figure 3. Stillbirth in Sweden 1973-2010

Data source: National Board of Health and Welfare, 2012
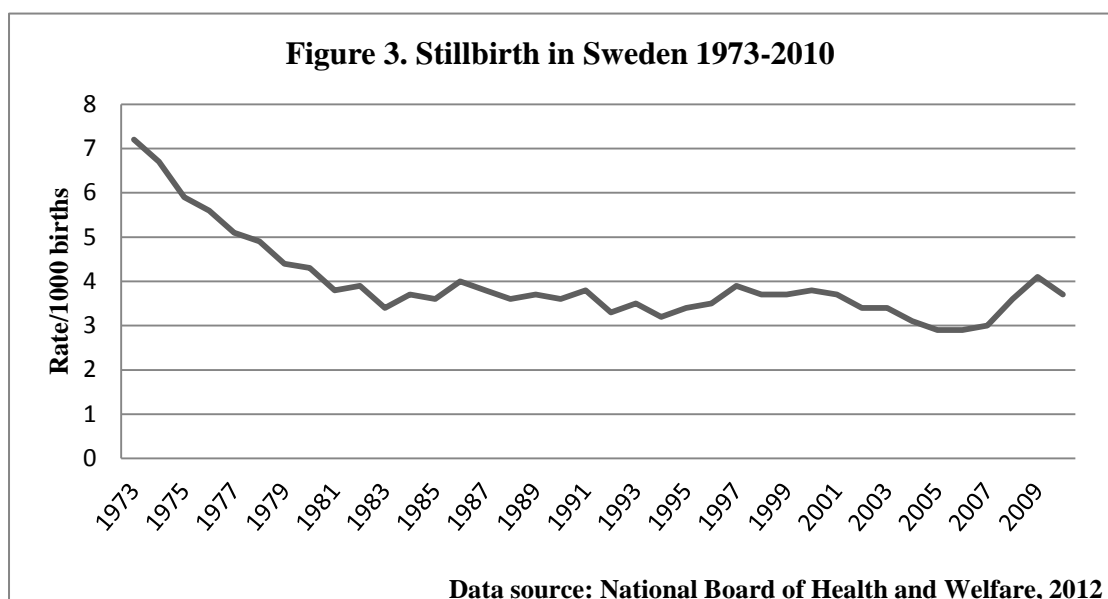
2.5 HYPERTENSION

An adequate blood pressure is necessary to sustain the circulation and perfusion of organs and tissues in the body. As the name says, blood pressure is the pressure exerted by circulating blood on the inner walls of the arteries. (There is a pressure in the veins too, but it is the arterial blood pressure that is measured). Both blood pressure and blood flow is generated by the heart and is hence pulsatile. Maximum blood pressure is reached during systole – when the heart contracts and pumps out blood in the circulation, this is the systolic blood pressure. The lowest blood pressure is reached during diastole – when the heart relaxes and is filled with blood, this is the diastolic blood pressure. The difference between systolic and diastolic blood pressure is called pulse pressure [83]. The body regulates the blood pressure through both central and peripheral mechanisms, e.g. baroreceptors, sympathetic innervation and through hormones (epinephrine, norepinephrine, renin-angiotensin-aldosterone axis) [83]. Blood pressure can be increase through increased total peripheral resistance, or through increased cardiac output [83]. With age, blood vessels often become stiffer due to atherosclerosis. This leads to a decreased blood vessel compliance and increased peripheral resistance which can result in hypertension [83].

Normal blood pressure has been defined as below 120 mm Hg systolic and 80 mm Hg diastolic [84]. Hypertension is defined as a systolic blood pressure (SBP) above 140 mm Hg and/or a diastolic blood pressure (DBP) above 90 mm Hg [84]. Repeated measurements are usually required for the diagnosis. Often, hypertension gives no symptoms in itself; although very high levels can cause headache, dizziness, irritability and palpitations [85]. The interest in finding people with high blood pressure lies instead in prevention of cardiovascular disease. Hypertension is a risk factor for myocardial infarction, stroke, angina pectoris, claudicatio intermittens and nephrosclerosis [85]. As previously mentioned, hypertension during pregnancy is also related to adverse outcomes for the foetus, such as IUGR and stillbirth, especially when the increased blood pressure is superimposed by preeclampsia [86].

In 95% of cases, there is no known cause to the increased blood pressure, this is then referred to as primary or essential hypertension. Risk factors for essential hypertension are high age, overweight/obesity (especially abdominal), high alcohol consumption, stress, high salt consumption, low socio-economic status and hereditary factors [85]. As mentioned earlier, developmental factors are also associated with increased blood pressure, such as IUGR, preterm birth and in some studies maternal smoking during pregnancy [87,88].

Hypertension is a very common condition; the prevalence in the adult (>20 years) Swedish population has been estimated to 27% [89]. In the population over 70 years more than 50% have hypertension [85]. Blood pressure has been shown to "track" over the lifespan, i.e. there is a correlation between childhood and adult blood pressure [90,91]. Therefore, childhood blood pressure can be a predictor of adult blood pressure and risk of hypertension.

2.6 LIFE COURSE PERSPECTIVE

It is known that adult lifestyle factors (e.g. smoking, alcohol, physical activity, diet) and other adult risk factors (e.g. high blood pressure, high blood lipids and glucose) are important for disease development. We have also seen that early development is important for later disease (DOHaD). Life course epidemiology aims to bridge over these different perspectives; developmental and adult risk factors, as well as integrating biological and social risk processes within and over generations [92]. The life course perspective is often applied in research regarding socio-economic influences on health. There are different theoretical models used in life course epidemiology to explain how factors across the lifespan can influence health, e.g. the critical period model and the accumulation model. These are not competing hypothesis, but rather related and able to operate at the same time [93].

Critical/Sensitive period model

The critical period model assumes that irreversible changes in a body, that were induced during a specific period of development, is important for future health, hence timing of exposure is important [94]. This model has also been called biological programming or latency model and underlies the DOHaD hypothesis [94]. A critical period has been defined as a time window during which an exposure can have protective or adverse effects on development and subsequent health; outside this time window the exposure is not associated with disease risk [94]. Usually critical periods take place during foetal development or other times of rapid growth and differentiation. An example of a critical period is limb development in pregnancy week 3-8, when exposure to thalidomide causes limb malformations; exposure outside of this critical period does not cause malformations [94]. A sensitive period is a time window where an exposure has a stronger influence on development and subsequent health than it has at other time points [92].

Social mobility model

The social mobility model can also be seen as a critical period model with later effect modification [95]. For example, the influence of social factors during early life (critical/sensitive period) might be modified by later social factors that depend on social mobility. The focus lies on change in socio-economic status (upward or downward mobile groups) and later disease. Social mobility can be assessed either during a person's own working life (intragenerational) or between generations (intergenerational).

Accumulation model

In contrast to the critical/sensitive period and the social mobility models, the accumulation model assumes that cumulative exposures over the life course influence the risk of later disease irrespective of their timing [96]. According to the accumulation model, the effect of, for example, low socio-economic status in childhood and in adulthood would be the same. There are different types of accumulation models [96]. First, exposures can be separate and independent of each other. Second, they can cluster together, often in socially patterned ways. Third, exposures can be linked to one another in such a way that one exposure leads to the next; a chain of risk model. Fourth, if only the last exposure in the chain of risk causes the disease, this last exposure can be said to have a trigger effect.

2.7 SOCIAL STATUS AND SOCIO-ECONOMIC INEQUITIES

Socio-economic status (SES) can be of interest in research for descriptive purposes, when investigating how health effects of social differences is mediated, or as a confounder. Low socio-economic status is associated with increased mortality and morbidity from a large number of diseases, including hypertension, cardiovascular disease and adverse pregnancy outcomes such as IUGR and stillbirth [97]. In Sweden, there is for example a five year difference in life expectancy between those with the lowest and those with the highest education [9]. The social gradient in health is apparent over the whole range of social groups; it has even been shown that men with a doctoral degree live longer than men with "just" a high university degree [98]. Health differences that are perceived as unjust, unnecessary and avoidable are referred to as social inequity. Differences that are not perceived as unjust, for example a higher mortality in old people compared to young people, are referred to as social inequality [99].

What causes social inequities in health?

To a large part, social differences in health are caused by social differences in determinants of health [9]. Such determinants are life-style factors, access to health care, area of living, social network, work conditions, stress etc. Around 30-50% of socio-economic differences in mortality have been explained by health related life-style factors [9]. In one study, this proportion was even higher; 72%, out of which smoking alone explained 35% [100]. Other important life-style factors are physical activity, diet and alcohol consumption [9]. Psychosocial factors that influence these life-style factors are also of great importance [101]. Further, there can also be some degree of reverse causation, where healthy people are more likely to experience upward social mobility and vice versa.

Operationalization of social status

Social status is often operationalized through education, occupation or income [102]. These measurements are strongly correlated but not completely interchangeable [102]. Education captures more of knowledge related assets [102]. It is usually completed in young adulthood and contains the transition from the parents status to the own adult status. Thereby, education reflects both childhood and young adult social status. Further, it is a strong predictor of occupation and income and might also capture cognitive abilities. Advantages with education as a measure of SES are that it is easy to measure and valid over different ages and working circumstances in a person's life. A disadvantage is that there can be cohort effects, where a certain length of education means different things in different age groups, in women or among people who have studied abroad. Income is a more direct measure of material resources. Money in itself is not likely to be related to health, but rather what you can buy for money in terms of housing, food and services. It might also influence self-esteem and social participation. Money is considered the best indicator of material living standards; however it might be sensitive information with lower response rates in questionnaires and it can fluctuate over time [102]. Occupation is related to both prestige and income and reflects a person's place in society [102]. Occupation is strongly related to both education and income. It also reflects social privileges such as access to health care or education, social networks, work stress, psychosocial milieu, toxic environments and physical

demands [102]. Information about occupation is often easily available, but it can change over the life course and people who are outside the work force, such as unemployed, students, house-wives and retired citizens are often excluded. There is not one measure of social status that always is preferable [102]. As said, they are strongly related but at the same time they capture somewhat different aspects of status or position in society.

There are also correlations across generations, for example in educational attainment [103]. In Sweden, about 45% of the age groups born in the mid 1980's had started a university education at age 25. However, the probability to start university was strongly related to the parent's level of education; among those with parent's who only had primary/lower secondary (in Swedish: förgymnasial) education, 21% went on to university [103]. Among those who had a parent with a research education, the corresponding figure was 83%. Further, these differences were even higher for university educations that required high grades.

An example from six municipalities

Socio-economic status, whether it is measured as education, occupation or income, is related to behaviours (smoking, diet, physical activity) and to health both among children and adults [97]. As an illustration we can go back to the municipalities in Stockholm County that had the highest and lowest prevalence of maternal smoking during pregnancy in 2006. Where maternal smoking was highest (Norrtälje, Nynäshamn and Södertälje), the part of the population with the lowest group of educational attainment (primary/lower secondary school) ranged between 17-21% and the highest group of education (≥3 year's post-secondary education) ranged between 14-18%. The corresponding percentages for the municipalities with lowest maternal smoking (Danderyd, Vaxholm and Lidingö) were 7-9% in the lowest educational group and 32-54% in the highest group [104]. The relative share of employment (% gainfully employed) was between 72-78% in Norrtälje, Nynäshamn and Södertälje vs. 82-86% in Danderyd, Vaxholm and Lidingö [104]. As expected, there were also differences in income between the municipalities [105]. Life expectancy was on average three years longer for a girl born 2007-2011 in Danderyd, Vaxholm or Lidingö compared to a girl born in Norrtälje, Nynäshamn or Södertälje (Figure 4) [106].

Figure 4. Life expectancy for a girl born 2007-11
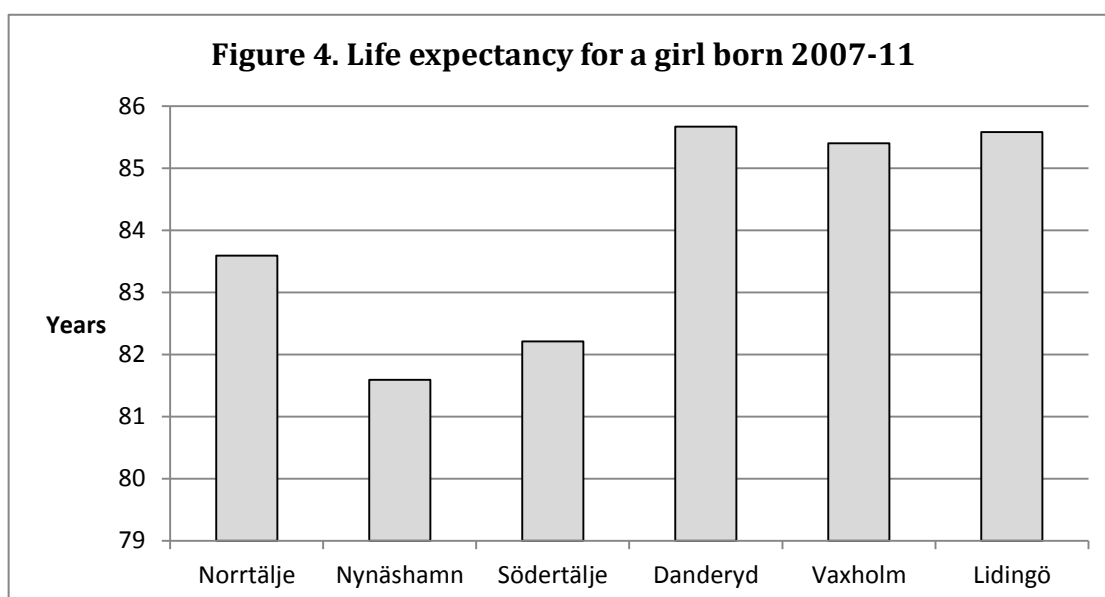
2.8 TWINS

In Sweden, twin pregnancies constitute 1.5-2.0% of all pregnancies [12]. Twins can be dizygotic (DZ) or monozygotic (MZ). Dizygotic or fraternal twins originates from two different fertilized eggs, they can have either the same or the opposite sex and are as similar as siblings in general. Monozygotic or identical twins originate from one single fertilized ovum and are hence genetic clones. Roughly, one third of the twins are like-sexed DZ, one third is opposite-sexed DZ and one third is MZ. Dizygotic twin pregnancy is associated with higher maternal age, fertility treatment and inheritance [12]. The differences in twinning frequency between ethnic groups are due to differences in dizygotic twinning [12]. Monozygotic twin pregnancy is considered as a random event [12], although rare familial cases have been reported e.g. due to abnormalities in the zona pellucida [107].

Twins are on average smaller at birth than singletons and are generally born after shorter gestations. Average gestational length in twin pregnancies is 36-37 weeks compared to 39-40 weeks in singleton pregnancies [12]. Mean birth weight is up to one kilo lower among twins than among singletons [12]. Discordance in birth weight in twin pairs can be due to unequal placenta sharing or peripheral/velamentous umbilical cord insertion [108,109]. Birth weight differences above 25% of the larger twin's birth weight have been suggested as a cut off for what should be regarded as clinically relevant [12]. While differences in birth weight within a twin pair is due to differences in nutritional supply, differences in birth weights between unrelated twins are likely to be due to the same factors determining birth weight in singletons.

Twin studies

The aim of twin studies is often to separate effects of environment and genes. In the classical twin study dizygotic and monozygotic twins are compared. Briefly, the idea is that all twins share intrauterine and childhood environment, while DZ twins also share on average 50% of their genes and MZ twins share 100% of their genes. Hence, MZ twins constitute a way to, at least theoretically, completely control for genetic factors. If the studied association is due to familial factors (genes and environment) the association is expected to be weakened or diminished within twins. A further decrease among MZ twins compared to DZ twins suggest importance of genetic factors [110].

Chorionicity

If monozygotic and dizygotic twins differ in other ways than genetic similarity, this threatens to complicate the interpretation of twin studies. One such suggested possibility is chorionicity [111]. Chorion is the outer membrane enclosing the foetus (the inner membrane is called amnion). All dizygotic twins are dichorionic, i.e. the twin siblings have separate chorion, amnion and placentas (although the placentas can be fused) [107]. Monozygotic twins that were separated day 0-3 after fertilization also become dichorionic (about 25-30%), while those separated after this time becomes monochorionic and share one single placenta [107]. Monozygotic twins that are separated late (after day 7) also become monoamniotic, i.e. both twins lie in the same amniotic sac. This occurs in about 1-2% of monozygotic twin pregnancies [107]. Twins separated after day 14 do not separate completely and become conjoined twins.

3 AIMS

The overall aim of this thesis was to study how factors during prenatal and early life can influence health outcomes over the lifespan, taking familial confounding into account.

The specific aims were:

- To study if maternal smoking cessation from one pregnancy to another will influence the risk of stillbirth, in order to seek support for the hypothesis of a causal relationship. (Study I)

- To study if maternal smoking during pregnancy influences blood pressure in adolescent offspring, and to investigate if a possible association could be explained by shared familial factors. (Study II)

- To study if intergenerational social mobility (between parental and adult socio-economic status group) influences risk of hypertension and if a possible association is explained by shared familial factors. (Study III)

- To study if the tendency to repeat birth weights across generations is explained by genetic or environmental factors. (Study IV)

4 MATERIALS AND METHODS

4.1 SETTINGS

The studies that this thesis is based upon are all set in Sweden and utilize information from population based registers. Sweden has a long history of population based statistics; the first national census was for example conducted in 1749 [112]. Since 1947, all Swedish residents have a unique personal registration number that is given at birth or immigration. This is a ten digit number consisting of year, month, day of birth and four additional digits (YYMMDD-XXXX). The personal registration number enables register linkage at the individual level which provides great opportunities for epidemiological research [113].

4.2 DATA SOURCES

The Medical Birth Register

The Medical Birth Register started in 1973 and serves the purpose of surveillance of antenatal, obstetric and neonatal care. It covers 97-99% of all pregnancies resulting in a delivery in Sweden [114], and reporting to the register is required by law. Copies of medical records from prenatal, delivery and neonatal care are sent to the National Board of Health and Welfare (Socialstyrelsen) who keeps the register. The amount of collected information has changed and expanded several times. Smoking in early pregnancy has been registered since 1983. This information is obtained by midwifes at the women's first visit to antenatal care which, in 95% of pregnancies, takes place before the 15th week of gestation [115]. The information is collected in a standardized way, using check boxes, where women are categorized as: non-smokers (i.e. non-daily smokers), moderate smokers (1-9 cigarettes per day) and heavy smokers (10 or more cigarettes per day). These categories have been validated by cotinine measurements and shown to have acceptable validity [116]. Since 1990, information about smoking three months prior to pregnancy has been collected; however the coverage was low during the first years. In 1999, smoking in pregnancy week 30-32 and information about use of oral moist snuff ("snus") was added. In Studies I and II, only information about maternal smoking in early pregnancy is used as the other variables were introduced later and have insufficient coverage. Birth weights and birth lengths of the new-borns, which have been reported since the start of the register are measured directly after birth to at least the nearest 10 g and cm, respectively. Stillbirth has also been registered since 1973. Up to July 2008, stillbirth was defined as foetal death after at least 28 completed weeks of gestation. Thereafter the definition was changed to foetal death after at least 22 completed weeks of gestation, in accordance with international practise.

The Conscript Register

In 1901, Sweden introduced compulsory national service for all young men. During conscription, which most often took place at age 18, tests were performed to grade and place the young men for military service. Basic examinations included tests of IQ, hearing, vision and colour vision, measurements of height and weight, a general examination by a physician and an estimate of physical fitness. The young men who were considered physically and mentally suited for military service proceeded to additional tests, including electrocardiogram (ECG), pulse and blood pressure

measurements and maximal fitness tests (cycle and muscle capacity). The measurement of blood pressure was taken once in supine position, in the right arm, after five minutes of rest. Both manual and automatic cuffs were used, and in case of an uncertain reading an extra manual measurement was performed. Weight was measured in kg (in light indoor clothes) and height in cm (without shoes). In the beginning of the 1990s, the number of men drafted to complete military service decreased rapidly, however they still went through conscription. In 2007, a self-rating test of suitability for military service was introduced and calls for conscription were based on this rating. This resulted in a substantial decline in number of conscripts, which limited the possibilities for epidemiological research based on the Conscript Register from 2007. In 2010, the compulsory national service was abolished in peace time and conscription ceased. The data that has been collected over the years is still a rich source for research, but it is no longer renewed. The register is managed by The National Service Administration (Rekryteringsmyndigheten) former Swedish Defence Recruitment Agency (Pliktverket).

The Swedish Twin Register

The Swedish Twin Register started in the late 1950's with the primary purpose to study effects of smoking and alcohol on health. Over the years different birth cohorts of twins have been included. Information has been collected through questionnaires and interviews and additional information have been obtained through linkage to other registers. Today the Swedish Twin Register holds information about more than 170 000 individual twins in 85 000 twin pairs [117]. The register is managed by Karolinska Institutet and constitutes a fantastic source for research aiming at separating effects of genetic and environmental influences. The second and the third birth cohort in the twin register were used in this thesis (Study III and IV). The second cohort comprised twins born 1926-1958. All like-sexed twins were in 1973 asked to respond to a postal questionnaire (Q73), with questions about health and lifestyle factors. In 1998-2002, all twins from this cohort alive and living in Sweden were contacted again and asked to participate in a telephone interview called SALT (Screening Across the Lifespan Twin Study) [118]. The purpose was to screen for common complex diseases. Questions involved symptoms, diagnoses and lifestyle factors. In 2005, the third cohort in the Twin Register, comprising twins born 1959-1985, was contacted and asked to answer a similar web-based questionnaire called STAGE (The Study of Twin Adults: Genes and Environment) [117]. Response rates were generally high: 83% responded to Q73, 74% responded to SALT and 66% responded to STAGE [117,118]. The twins' zygosity was determined from questions of childhood similarity. In Q73, twins were asked if they as children were as like as "two peas in a pod" (in Swedish "lika som bär"). In SALT and STAGE, the twins were also asked how often strangers had difficulties distinguishing them from their co-twin during childhood. In SALT, this method of zygosity determination was validated with DNA analysis in a subsample of 199 twins and proved correct in 99% of the cases [118]. Birth characteristics (birth weight, birth length, gestational age etc.) for the twins born 1926-1972 have been collected manually from original birth records, found in archives throughout Sweden. Birth characteristics for twins born 1973 or later have been retrieved from the Medical Birth Register.

The Multi-Generation Register

The first version of the Swedish Multi-Generation Register was created in year 2000 and a new version is created annually. It contains all people born 1932 or later who have been registered by the Swedish authorities at some time since 1961. These people are called index persons. The Multi-Generation Register then links all index persons to their biological and, if applicable, adoptive parents. At present there are about nine million index persons in the register. This provides a great opportunity for research as it enables family linkages.

Education Register

The Education Register is available from 1985 and is updated annually. Information about the population comes from the Register of the Total Population and information about education comes from universities, schools and education providers. For immigrants information about education is collected through questionnaires.

4.3 FAMILY BASED METHODS

An association between an exposure and an outcome does not necessarily imply that the association is causal, that is, that the exposure causes the outcome. Other factors can influence the found association and one can only adjust for such factors if they are known and if they can be measured. As it is impossible to have knowledge about and measure all such factors, observational studies contain both unknown and unmeasured confounding to some degree. Related individuals, such as siblings and twins, may share many of these factors as they to different extent share genes and family environment. This is what family based methods take advantage of. They are often used to investigate if a found association is due to shared familial factors, i.e. shared environment and/or common genes. Full siblings share similar childhood environment and on average 50% of their segregating genes. They also share some, but not all maternal factors (the prenatal environment). Twins are a special kind of siblings, who share both intrauterine and childhood environment. Further, monozygotic (MZ) twins are genetic clones and share 100% of their genes, while dizygotic (DZ) twins are as similar as ordinary siblings and share on average 50% of their segregating genes. By stratifying an analysis on twins' zygosity it is possible to gain insights about the influence of genetic vs. environmental factors. If familial factors are important for a found association, it is expected that the association is weaker within family members (assuming no confounding from unshared environmental exposures or measurement error [119]). If the association is non-present within siblings or twins, the association could be due to familial confounding. If the association remains within DZ twins but not within MZ twins, this points towards an influence of genetic factors. Children of twins can also be informative as children of MZ twins are genetically half siblings and children of DZ twins are common cousins. In this thesis, siblings differentially exposed to maternal smoking during pregnancy were investigated in Studies I and II. In Studies III and IV twin data was used to assess familial confounding. These within twin/sibling pair analysis were all nested within cohort studies. The two following designs were used.

Co-twin control design

In the co-twin control design, twins discordant for the *exposure* are followed over time and the risk of the outcome is evaluated. In the similar co-twin case-control design, twins discordant for the *outcome* are used. The latter is equivalent to a case-control study with a 1:1 matching within twin pairs [110]. The twin with the outcome is the case and his or her healthy co-twin is the control, the matching refers to all shared familial factors. Only discordant (for exposure or outcome, respectively) pairs contribute with information in these analyses. In Study III, the co-twin case-control design was used with the hypertensive twin as case and the healthy co-twin as control. If the twin with higher socio-economic status in adulthood has lower odds of hypertension than his/her co-twin, this would indicate that the association between low social status and hypertension is not attributed to familial factors.

In Study I, a "co-sibling control design" or "consecutive birth analysis" was used. This is very similar to the co-twin control design, but utilizes consecutive siblings instead of twins. Siblings (foetuses) differentially exposed to maternal smoking during pregnancy were followed and their stillbirth risk was estimated. If maternal smoking is causally

related to stillbirth, it is expected that the exposed foetus will have a higher risk compared to its non-exposed sibling.

Between and within design

In the "between and within" design, the regression coefficient of the exposure is decomposed into a between-cluster or cluster-level component and a within-cluster or individual-level component [120]. The between component is estimated by the twin/sibling pair mean. The within component is estimated by the individual deviance from the twin/sibling pair mean. In this thesis the between component is not reported since it was not the focus of the analyses. The within component can be thought of as an estimate of the exposure on the outcome that is independent of all factors shared by twin/sibling pairs. A simplistic explanation of the conceptual idea is that the more family members resemble each other the less the deviate from each other. In Study II, the within family effect estimates the expected change in offspring blood pressure by a one unit change in maternal smoking behaviour between pregnancies (that is, going from smoker to non-smoker or the other way around). In Study IV, the within twin pair component estimates the expected change in offspring size at birth by a one unit change in mother's size at birth (one unit is defined as 500 g or 1 cm).

Table 5. Overview of study design and study population in Study I-IV.

	Study I	Study II	Study III	Study IV
Study design	Cohort (with "co-sibling control" analysis, not published in the article)	Cohort with nested within sibling analysis	Cohort with nested co-twin case-control analysis	Cohort with nested within twin analysis
Data sources	Medical Birth Register, Education Register, Immigration Register	Medical Birth Register, Conscript Register, Multi-Generation register	Swedish Twin Register	Swedish Twin Register, Medical Birth Register
Study population	All Swedish women who had their first and second births between 1983 and 2001	All young men born in Sweden 1983-1988 who conscripted for military service in 2001-2006	All like-sexed twins born in Sweden between 1926 and 1958	All like-sexed female twins born in Sweden 1926-1985 who had their first child between 1973 and 2009
Numbers (%)*	467 878 (89%)	87 223 (43%) 9448 full brothers 780 discordant brother pairs	20 560 (55%) [1] 12 324 (33%) [2] 12 030 (32%) [3]	8685 (81%) 1479 DZ pairs 1526 MZ pairs
Exposure	Maternal smoking in early pregnancy		Parental SES[1] Adult SES[2] Social mobility[3]	Mother's birth weight and length
Outcome	Stillbirth	Blood pressure	Hypertension	Offspring's birth weight and length
Confounders	Mother's age, education, birth country, cohabitation with the infant's father, inter-pregnancy interval, previous stillbirth and year of second delivery	Age, height, BMI, parent's education and cohabitation, mother's parity and blood pressure disease during pregnancy.	Birth year, sex, birth weight, gestational age, mother's age at delivery, parity, adult height, BMI, alcohol consumption and smoking.	Mother's education and gestational age, offspring's birth year and gestational age.

*Numbers in the cohorts with full information about both the exposure and the outcome. In parenthesis, how large this fraction is compared to the whole study population (after exclusions not listed in this table).

4.4 STUDY DESIGN, POPULATION AND STATISTICAL METHODS

Study I: Maternal smoking during pregnancy and stillbirth risk

This is a population based cohort study where maternal smoking habits during two successive pregnancies are investigated in relation to stillbirth risk in the second pregnancy. Data mainly comes from the Medical Birth Register but linkage was also done to the Education and the Immigration Registers.

The study population was defined as all women in Sweden who delivered their first and second consecutive single births between 1983 and 2001. In total, there were 526 691 such women, of whom 467 878 (89 %) had information about first trimester maternal smoking in both pregnancies. The women were categorized as non-smokers in both pregnancies (reference group), smokers in first pregnancy/non-smokers in second pregnancy (quitters), non-smokers if first pregnancy/smokers in second pregnancy (starters) and smokers in both pregnancies. The outcome of interest was stillbirth in the second pregnancy (defined as foetal death after at least 28 completed weeks of gestation). Gestational age was determined by early second-trimester ultrasound screening when available, otherwise the last menstrual period was used. Routine ultrasound screening was introduced in Sweden in the 1980's. Since 1990 all pregnant women in Sweden have been offered this examination and 95% accept the procedure [13]. As potential confounders mother's country of birth, education, age, cohabiting with the infant's father, inter-pregnancy interval and previous stillbirth was considered. Year of second delivery was also included to capture a possible time effect.

Logistic regression was used in the analyses. Crude and adjusted estimates are presented as odds ratios (OR) with 95% confidence intervals (CI). Adjustments were made for potential confounding factors as mentioned above. An additional conditional logistic regression analysis was performed after the study was published. This additional analysis utilizes information from differentially exposed siblings, by comparing the odds of stillbirth for the exposed sibling compared to the unexposed "co-sibling" (equivalent to the co-twin control design). The outcome was then stillbirth in either pregnancy, while in the cohort analyses only risk of stillbirth in the second pregnancy was studied. This analysis was adjusted for mother's age, cohabitation with the infant's father and birth order of the children. The analyses were performed using SAS version 9.1 and 9.3. (SAS Institute Inc. Cary, NC, USA).

Study II: Maternal smoking during pregnancy and adolescent offspring blood pressure

This is a population based cohort study with a nested within sibling analysis. Maternal smoking during pregnancy was studied in relation to late adolescent offspring blood pressure. Full brother pairs where one brother had been exposed to maternal smoking during pregnancy and the other had not were identified in the cohort. Data from the Conscript Register, the Medical Birth Register, the Multi-Generation Register and the Education Register (in 1990) was used.

The study population consisted of all men born in Sweden between 1983 and 1988 who conscripted for mandatory military service between 2001 and 2006. Those who died or emigrated before age 18 (the age of conscription) were naturally excluded. To achieve higher homogeneity in the cohort, those with mothers' born outside the Nordic countries, those who had a congenital malformation or were multiples were excluded. After these exclusions, there were 259 515 men registered in the Medical Birth Register, of whom 201 701 went through the conscription procedure. Information about blood pressure was available for 92 730 of these young men. Full information about both maternal smoking during pregnancy and blood pressure was available for 87 223 men. Within this cohort 9 448 full brothers were identified, and among them 780 brother pairs were discordant for maternal smoking. Maternal smoking was categorized as non-smoker, moderate smoker (1-9 cigarettes per day) and heavy smoker (10 or more cigarettes per day) in the cohort analysis. In the within brother analysis only non-smoker and smoker was used. The outcomes were systolic (SBP) and diastolic (DBP) blood pressure measured at conscription. We chose to look at blood pressure as a continuous variable, rather than using a cut of for prehypertension or hypertension. This was because one single measurement of blood pressure is not enough to give a diagnosis of hypertension and hypertension is not very common in young healthy teenagers. From the registers, we also obtained information on potential confounders.

Generalized estimation equation (GEE) models were used to estimate regression coefficients with 95% confidence intervals for late adolescent SBP and DBP as a function of maternal smoking during pregnancy. The reason to use GEE instead of a plain linear regression model was the correlated structure of the data (siblings). Univariate and multivariate analyses are presented. Adjustments were made for conscript characteristics (age, height and BMI), parental characteristics (education and cohabitation during pregnancy, which can be seen as a way to adjust for the family's socio-economic status), and pregnancy characteristics (parity and maternal blood pressure disease during pregnancy). In the within brother analysis, the effect on blood pressure within brothers differentially exposed to maternal smoking in utero was estimated. These analyses were adjusted for age, height, BMI at conscription, parental cohabitation during pregnancy and mother's blood pressure disease during pregnancy. All analyses were performed using SAS version 9.2. (SAS Institute Inc. Cary, NC, USA).

Study III: Intergenerational social mobility and the risk of hypertension

This is a cohort study with a nested co-twin case-control analysis. The influence of parental social status, own social status in adulthood and social mobility were studied in relation to risk of hypertension. In the co-twin case-control analysis, twins who were discordant for hypertension were compared in relation to their adult socio-economic status. Data from the Swedish Twin Register was used.

The study population consisted of all like-sexed twins born in Sweden from 1926 to 1958. In total there were 37 392 such twins in the Swedish Twin Register. Among them, 88% had responded to both the questionnaire in 1973 (Q73) and the telephone interview between 1998 and 2002 (SALT) [118]. Among them 20 560 (97%) had information about socio-economic status (SES) in adulthood and hypertension, 12 324 (58%) had information about both parental SES and hypertension, and 12 030 (57%) had information about both parental and adult SES as well as hypertension.

The exposures in this study were parental SES, SES in adulthood and social mobility. The latter was defined as a combination of parental and adult SES. Parental SES was defined as the highest SES of either parent, based on their occupation. Information about parents' occupation originally came from birth and delivery records collected from archives all over Sweden. This information was hence recorded prospectively. Information about the twins SES in adulthood was defined by their own occupation as reported in the interview in 1998-2002. Those currently employed were asked about their occupation during the last 12 months, those unemployed were asked about their last occupation, and those retired were asked about their primary occupation in adulthood. Both parental and adult SES was classified in line with the guidelines of Statistics Sweden [121]. The groups were then further merged into three categories: low SES (unskilled and skilled blue-collar workers and low-level white-collar workers), high SES (intermediate and high level white collar workers) and self-employed (entrepreneurs and farmers). The classification of occupations into SES groups relies to a large extent on education and type of union organization. Blue-collar workers and low-level white-collar workers often have the same amount of education [121]. Low level white collar workers have also been suggested to share other characteristics with blue collar workers rather than with higher levels of white collar workers [9]. This was hence the rational for our categorization of SES. Social mobility was defined as the change between parental SES and SES in adulthood, which is the intergenerational mobility between the parental and offspring generation. It was categorized as stable low, upward mobile, downward mobile and stable high SES. Because of the diversity in background among self-employed workers we were not able to determine whether their mobility was upward or downward. This group was therefore excluded from analysis of social mobility.

Information about the outcome measure, i.e. hypertension, came from the interview in 1998-2002. Hypertension was defined as answering 'yes' to the questions: 'Do you have or have you had high blood pressure?' and 'Do you take any medication daily?' and then naming an antihypertensive drug. Hence, this is self-reported and treated hypertension. A subject was defined as non-hypertensive if answering 'no' to both

these questions, or naming a drug not listed as an antihypertensive medication. Subjects not answering the two questions, answering yes to the question about having hypertension but not naming an antihypertensive drug or answering no to the question but naming an antihypertensive drug were defined as unclassifiable (n=2 934; 12.1% of the defined study population).

Generalized estimating equations (GEE) were used to obtain OR with 95% CI's for the association between SES and hypertension in the cohort of twins. GEE was used to take the dependency in the data into account (twin data). Because hypertension risk is so strongly associated with age no crude analysis is presented, instead the first model was adjusted for birth year and sex. The second model was further adjusted for pregnancy and birth characteristics (birth weight, gestational age, mother's age at delivery and parity) and adult characteristics (height, BMI, smoking and alcohol consumption). To investigate whether shared familial factors confounded the associations, we conducted a co-twin case-control analyses using conditional logistic regression. All analyses were performed using SAS version 9.2. (SAS Institute Inc. Cary, NC, USA).

Study IV: Intergenerational influence on birth size – genes or environment?

This is a cohort study with a nested within twin pair analysis. The intergenerational influence on birth weight and birth length was studied in female twins and their offspring. In the within twin pair analysis, we wanted to see if female twin sisters discordant for birth weight reproduced their differences in birth weight in their own offspring. Data came from the Medical Birth Register which was linked to the Swedish Twin Register and Education Register.

The study population consisted of all like-sexed twins with known zygosity who were born in Sweden between 1926 and 1985. These twins should further have given birth to their first offspring between 1973 and 2009. Only first born offspring were selected as parity is known to influence offspring birth weight [122]. Further exclusions were made for stillborn, multiples and infants with congenital malformations. Within a like-sexed twin pair, there is a risk that birth data of one twin has been mixed up with the co-twin's information. To minimize this potential misclassification, the study population was restricted to twins with known birth order. For twins born 1926-1958, birth order was considered certain if they had been given names at birth or agreed on birth order in the questionnaires (Q73, SALT). For twins born 1959-1972, an algorithm was used that combined information from the medical records (birth weight, name if this was given at birth and time of birth) and the STAGE questionnaire (birth weight and birth order). For twins born 1973-1985, a different algorithm adapted from Johansson and Rasmussen was used [123]. This algorithm was based on information on birth order, birth weight and who had been the heavier infant at birth, using information from both medical records and the STAGE questionnaire. After these restrictions, our cohort consisted of 9 418 twin mothers with first born offspring, of whom 8 685 had full information about mothers' and offspring's birth weight and birth length. Within this cohort, we had 3 005 complete female twin pairs, of whom 1 479 pairs were dizygotic and 1 526 were monozygotic. The exposure in this study was mother's birth weight in grams and birth length in cm. This information was obtained from original birth records for twins born 1926-1973 and from the Medical Birth Register for twins born 1973-1985 (information in the Medical Birth Register also originates from birth records). The outcome was the first born offspring's birth weight and birth length, which was obtained from the Medical Birth Register.

We used generalized estimation equations (GEE) to account for the correlated structure of the data, i.e. twin pairs and present regression coefficients with 95% confidence intervals for the influence of a 500 g or 1 cm increase in mother's birth weight/birth length on offspring birth weight/birth length, respectively. We present both crude and adjusted estimates. Adjustments were made for mother's education, mother's gestational age, offspring's birth year, and offspring's gestational age. To investigate whether the association between mother's and offspring's birth weight is independent of common genetic or shared environmental factors, we performed within twin pair analyses [120]. We stratified these analyses on mother's zygosity to gain some insights about the potential contribution of genetic factors. All analyses were performed using SAS version 9.3 (SAS Institute Inc. Cary, NC, USA).

4.5 ETHICAL CONSIDERATIONS

Medical research requires the public's trust. Without trust in that researchers have good intentions and are competent and honest, nobody would offer their time or money to support scientific work, nor follow recommendations based on the findings. This trust must not be violated; therefore research ethics are extremely important. To consider a study for publication, medical journals demand that it has been approved by an ethics committee. Since 2004, this has also been regulated by law in Sweden (Lag om etikprövning 2003:460). All studies based on sensitive personal information, such as health information, must be approved by an ethics committee before start up. The ethics committees are composed by a chairman and members representing both scientific (10 members) and public (5 members) interests. Fundamental is that the ethics committee only approve research that "can be conducted with respect for human dignity and if human rights and fundamental freedoms are constantly heeded" [124]. This has its history in the Second World War, where many torture-like and deadly experiments were carried out by physicians in concentration camps. At the end of the war, the physicians were held responsible during the Doctor's Trial. From this trial the *Nuremberg code* stems. It was the first written ethical code concerning medical research and has become the most influential [125]. While the Nuremberg code focus on research subject's human rights, another influential code, the *Helsinki declaration*, focus on the physician's/ investigator's obligations to the research subjects [125]. Good ethics goes hand in hand with good research [126]. The codes point out that research should be well designed and carried out by qualified persons. If not, it would be a waste of both resources and study participants time, or worse, lead to incorrect conclusions. There is also researcher ethics that applies to the individual researcher. This is about being honest, thorough and just to colleagues, to not cheat or modify the results; again, it is a matter of trustworthiness [126].

The studies in this thesis are register based and includes many thousand participants. It would not be practical or economically defensible to get an informed consent from every single registered person. Instead, the ethics committee judge if the potential benefit from the studies outweighs the potential harm. The used register data was anonymous, i.e., no names or personal numbers are given out to researchers from the responsible authorities (National Board of Health and Welfare and Statistics Sweden). I believe that possible harm from violation of integrity by these studies is minimal. It is further important to think about how findings are presented and discussed. Increased stigmatization of vulnerable groups or victim blaming should be avoided, at the same time as the results must be presented truthfully. Sensitive topics in this thesis might be characteristics of women who smoke during pregnancy and differences in behavioural factors between socio-economic groups. In a respectful way, I believe most topics can be addressed.

All studies in this thesis were approved by the research ethics committee at Karolinska Institutet (Study I-IV dnr numbers 4863/2005; 2007-546-31; 00-410; 2006-874-32; 2006-1297-32; 2010-1585-32).

5   RESULTS

Study I: Maternal smoking during pregnancy and stillbirth risk

In this study population, 21.4 % of the pregnant women smoked in their first pregnancy (14.3% were moderate smokers and 7.1 % were heavy smokers), and 17.8 % smoked in their second pregnancy (11.5 % were moderate smokers and 6.3 % were heavy smokers). The majority of women who smoked during their first pregnancy also smoked during their second pregnancy (64.6% of moderate smokers and 81.4% of heavy smokers). Stillbirth rate was 4.5/1000 in first pregnancy (N=2 363) and 2.7/1000 in the second pregnancy (N=1 420). Maternal smoking of ten or more cigarettes per day was significantly associated with increased odds of stillbirth.

Odds of stillbirth in the second pregnancy were also associated with several maternal characteristics. A previous stillbirth in the first pregnancy was associated with substantially increased odds of a second stillbirth (adjusted OR 2.42 95% CI 1.32-4.41). The risk of stillbirth was further increased among women with high maternal age ($\geq$35 years), a non-Nordic country of birth, and a long inter-pregnancy interval ($\geq$3 years). Among teenage mothers ($\leq$19 years) the rate of stillbirth was relatively high (4.7 per 1000); however after adjustment for smoking and other variables, low maternal age was no longer significantly associated with stillbirth risk. High maternal education was associated with a decreased risk of stillbirth.

For women who were former (moderate or heavy) smokers, odds of stillbirth in the second pregnancy were similar to the odds among women who were non-smokers in both pregnancies (Table 6). Compared with non-smokers in both pregnancies, women who were smokers in both pregnancies generally experienced increased odds of stillbirth in the second pregnancy. No effects on odds of stillbirth were seen for women who stated that they were non-smokers during the first pregnancy but smokers during the second pregnancy.

Table 6. Cohort analysis of stillbirth rates in second pregnancy in relation to smoking habits in first and second pregnancy.

| Maternal smoking | | Stillbirth in 2nd pregnancy | | |
| | | Rate / | Odds ratio (95% CI) | |
1st pregnancy / 2nd pregnancy	No	1000	Crude	Adjusted*
Non-smoker/Non-smoker†	863	2.4	1.00	1.00
Non-smoker/Smoker	26	2.2	0.89 (0.60-1.32)	0.84 (0.56-1.26)
Smoker/Non-smoker	79	2.7	1.11 (0.88-1.40)	1.01 (0.79-1.30)
Smoker/Smoker	236	3.4	1.41 (1.22-1.63)	1.35 (1.15-1.58)

*Adjusted for the effects of maternal age, education, cohabiting with infant's father, mother's country of birth, inter-pregnancy interval, stillbirth in the first pregnancy and year of (second) delivery.
†Reference group.

In the additional sibling analysis (not available in Study 1), increased odds of stillbirth was seen for the sibling who had been exposed to maternal smoking during pregnancy compared to the unexposed sibling (Table 7). The point estimates were even higher in the sibling analysis compared to those seen in the cohort, for maternal smoking of ten or more cigarettes per day the risk of stillbirth was more than doubled.

Table 7. "Co-sibling control analysis" with children differentially exposed to maternal smoking in utero. The odds of stillbirth for the exposed sibling compared to the unexposed sibling.

Maternal smoking during pregnancy	Crude OR (95 % CI)	Adjusted* OR (95 % CI)
Moderate smoker vs. non-smoker	1.43 (1.14-1.80)	1.21 (0.93-1.58)
Heavy smoker vs. non-smoker	2.57 (1.90-3.48)	2.17 (1.53-3.07)

*Adjusted for maternal age, cohabitation with the child's father and birth order.

Study II: Maternal smoking during pregnancy and adolescent offspring blood pressure

The mean SBP in the cohort was 130.7 mm Hg, and the mean DBP was 69.3 mm Hg. Mean blood pressure was positively correlated with maternal smoking during pregnancy, conscript's height and BMI. Birth weight for gestational age and gestational age showed a negative association with mean blood pressure. The prevalence of prenatal exposure to maternal smoking in the cohort was 26.2% (16.8% had mothers who were moderate smokers and 9.3% had mothers who were heavy smokers).

There was a small positive association between maternal smoking during pregnancy and late adolescent offspring SBP and DBP (Table 8). For SBP, the estimate was statistically significant among sons whose mothers had been smoking 10 or more cigarettes per day [β: 0.53 (95% CI: 0.26 to 0.80)] and non-significant for sons whose mothers had smoked 1-9 cigarettes per day [0.12 (-0.09 to 0.33)]. Point estimates for the increase in DBP were higher and statistically significant for sons of both moderate and heavy smokers. The strongest attenuations in these associations were seen after adjustment for BMI and parental education.

Table 8. Regression coefficients (β with 95% CI) for the association of maternal smoking during pregnancy and offspring blood pressure in late adolescent in a cohort of Swedish males born 1983-1988.

	Crude	Adjusted *
Systolic blood pressure		
Non-smoker†	0	0
Daily smoker	0.35 (0.17-0.52)	0.26 (0.09-0.44)
1-9 cig/day	0.18 (-0.02-0.39)	0.12 (-0.09-0.33)
≥10 cig/day	0.64 (0.38-0.90)	0.53 (0.26-0.80)
Diastolic blood pressure		
Non-smoker†	0	0
Daily smoker	0.57 (0.44-0.70)	0.45 (0.31-0.59)
1-9 cig/day	0.43 (0.27-0.58)	0.33 (0.17-0.49)
≥10 cig/day	0.82 (0.62-1.02)	0.67 (0.47-0.88)
Total number	87 223	85 438

* Adjusted for age, height, BMI, parental education and cohabitation status, parity and maternal blood pressure disease during pregnancy.
†Reference group

In the within brother analysis, the regression coefficients represent the expected change in offspring blood pressure (mm Hg) for a one unit change in maternal smoking habits (from smoking to non-smoking or the other way around). Within brother pairs, the expected change was 0.69 mm Hg (95% CI: -0.67 to 2.04) for SBP and 1.14 mm Hg (95% CI: 0.10 to2.17) for DBP if the mother had smoked during pregnancy compared with if she had not. Adjustments for potential confounders resulted in a loss of significance for the DBP estimate, but the point estimates were only slightly changed (Table 9).

Table 9. Crude and adjusted estimates (β with 95% CI) of the expected change in blood pressure (mm Hg) in male full siblings discordant for maternal smoking during pregnancy following a change in smoking status between pregnancies.

	Crude	Adjusted*
Systolic blood pressure	0.69 (-0.67-2.04)	0.81 (-0.56-2.19)
Diastolic blood pressure	1.14 (0.10-2.17)	0.85 (-0.19-1.90)
Total number of discordant brothers	780	758

*Adjusted for age at conscription, height, BMI, parental cohabitation status at time of pregnancy, parity and maternal blood pressure disease during pregnancy.

Study III: Intergenerational social mobility and the risk of hypertension

The overall prevalence of hypertension in the cohort was 15.8%. The prevalence increased with increasing BMI and alcohol consumption and was inversely associated with birth year, socioeconomic status (both parental and adult), birth weight and height. Compared to those who had parents with high SES, those whose parents had low SES had 55% increased odds of hypertension and those who had self-employed parents had 23% increased odds of hypertension (borderline significant). These estimates were essentially unchanged after adjustments for both birth and adult characteristics, while further adjustment for adult SES slightly attenuated the odds. There was no interaction between parental SES and sex (p=0.95). However, between adult SES and sex (p=0.03) there was a significant interaction and the analyses were therefore stratified by sex. Among men no association between adult SES and hypertension was seen. Among women with low adult SES the odds of hypertension was increased by 31% compared to women with high adult SES. In the social mobility analysis, information about parental SES and adult SES was combined. Compared to those with high SES in both generations, those with low SES in both generations, the upward mobile group, and the downward mobile group (not statistically significant for the last group) had increased odds of hypertension (Table 10). These results were slightly attenuated after adjustments for birth and adult characteristics. In the co-twin case-control analysis, we could only include those with low parental SES as the variation within twin pairs was too small to yield meaningful estimates in the group with high parental SES. Although not statistically significant, the point estimates indicated decreased odds of hypertension for the upward mobile twin compared to the stable low SES co-twin.

Table 10. Adjusted ORs (95% CI) for the intergenerational social mobility among Swedish like-sexed twins born from 1926 to 1958 and risk of hypertension.

Social mobility over two generations	Study population	Hypertensive	Odds ratios (95% CI)	
	N	%	Model 1*	Model 2**
Stable lower SES	4995	15.4	1.72 (1.36-2.17)	1.67 (1.29-2.17)
Upward mobile	2676	12.5	1.46 (1.14-1.87)	1.39 (1.05-1.83)
Downward mobile	577	10.8	1.26 (0.89-1.77)	1.23 (0.84-1.79)
Stable higher SES†	1191	8.0	1.00	1.00
Self employed	2591	11.5		
Missing	9184	19.6		
Total	21214	15.8	12030	9316

*Model 1: Adjusted for birth year and sex.
**Model 2: Adjusted for birth year, sex, birth characteristics; birth weight, gestational age, mother's age at delivery and parity, and adult characteristics height, BMI, smoking and alcohol consumption.
† Reference group

Study IV: Intergenerational influence on birth size – genes or environment?

In this cohort, the mean difference in birth weight within dizygotic twin pairs was 349 g (range 10-1990 g) and within monozygotic twin pairs the mean difference was 310 g (range 10-1610 g). Offspring's birth weight and birth length generally increased with mother's birth weight, gestational age, birth weight for gestational age, birth length, birth year and education. No associations were seen between mother's zygosity or age at delivery and offspring's size at birth. Maternal smoking during pregnancy was inversely associated with offspring birth weight and birth length.

In the full cohort of twins (both dizygotic and monozygotic), an increase of 500 g in mother's birth weight was associated with an increase in offspring birth weight of 101 g (95% CI: 89 to 113) and an increase in offspring birth length with 0.34 cm (0.29 to 0.40) in the adjusted analyses. An increase in mother's birth length of 1cm was associated with an increase in offspring birth weight with 32 g (28 to 37) and an increase in offspring birth length with 0.15 cm (0.13 to 0.17). When stratified by zygosity, monozygotic twins had lower point estimates than dizygotic twins.

Within dizygotic twin pairs, a one unit increase (500 g or 1 cm) in maternal birth weight/birth length difference from the twin pair mean was associated with statistically significant increases in offspring's birth weight and birth length (Table 11). Estimates were generally slightly lower than those seen in the cohort analyses of dizygotic twins. Within monozygotic twin pairs, neither a change in mother's birth weight, nor a change in mother's birth length deviance from the twin pair mean influenced birth weight or birth length in offspring.

Table 11. Within twin pair analysis estimating the expected change in offspring birth weight according to a one unit change (500 g) of twin mother's deviance from the twin pair mean size at birth, among 1479 dizygotic and 1526 monozygotic twin pairs.

| | Offspring birth weight (g) | |
| | Crude | Adjusted* |
Mother	β (95% CI)	β (95% CI)
Dizygotic twins		
500 g increase	74 (35 to 113)	70 (35 to 106)
1 cm increase	26 (10 to 42)	26 (12 to 40)
Monozygotic twins		
500 g increase	-12 (-49 to 26)	8 (-25 to 41)
1 cm increase	-4 (-19 to 10)	3 (-11 to 16)

*Adjusted for offspring gestational age and year of birth and mother's education.

6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

Study design

Before discussing the findings from the four studies, some general methodological considerations will be addressed. All four studies in the thesis are *cohort studies* with a nested within twin/sibling comparison. The co-twin case-control analysis (in Study III) has large similarities with a *case-control study*.

Cohort study

The word cohort comes from the Latin cohors, which in the Roman army constituted a group of soldiers, more exactly one tenth of a legion. In epidemiology, a cohort has been defined as "any designated group of persons who are followed or traced over a period of time" [127]. In a cohort study, exposed and unexposed individuals are followed over a period of time. Then the exposed group is compared to the unexposed group in terms of occurrence of the outcome. The idea is that, if the exposure does not have an effect on the outcome, there should be no differences in occurrence of the outcome between the exposed and the unexposed group. In prospective cohort studies, the exposure is assessed at the start, and new exposure information may be collected during follow-up. At the end of follow-up, the cohort is evaluated with regards to the outcome. Experiments, such as clinical trials, are prospective cohort studies where the exposure has been randomly assigned within the cohort. In retrospective cohort studies the follow up time has already past when the study is initiated. This is the case when historical data is used. If the information about exposure was collected before start of the follow up time, the data collection is still prospective, although the study is retrospective. The studies in this thesis are all retrospective, but with prospectively collected data.

Cohort studies have several advantages, especially when the cohort is large and population based and data are prospectively collected. Prevalence, incidence and relative risks can be estimated; rare exposures can be studied; time between exposure and outcome can be assessed; and multiple outcomes can be studied. For many research questions, where an experimental design is not possible, a cohort study may be the best option. However, drawbacks for prospective cohort studies are that they often are expensive and in case of a long follow up time, it can take decades until results can be evaluated. Retrospective cohorts do not have these limitations to the same extent. Cohort studies can further be hampered by a large loss to follow up. Register based cohort studies are limited to the information on exposures and confounders that the registers contain. In this thesis this problem has partly been dealt with through register linkage.

Case-control study

In the case-control study, the approach is somewhat reversed compared to the cohort study. Participants who have the outcome (cases), are compared to subjects who are free of the outcome (controls) with respect to exposure information before disease development. It is essential that the controls should come from the population that generated the cases. Case-control studies are suitable when studying rare diseases,

diseases with long latency periods and multiple exposures. Case-control studies can be quicker to perform and cheaper than cohort studies. However, prevalence, incidence or multiple outcomes cannot be measured; the selection of controls can be difficult and introduce bias; if the exposure is common it might be difficult to find a difference between cases and controls; further, recall bias and reverse causation might be a problem.

A case-control study is nested when the cases and the controls are taken from the population in a cohort study. In this thesis, nested within twin/sibling comparisons were used in all studies (not published in the article for Study I). The strength of these analyses is the possibility to adjust for unmeasured familial confounding.

Internal validity

Epidemiological studies are based on measurements [128]. They can, for example measure how common a disease is, if an exposure is a risk factor for a disease or which treatment that is the most effective for a disease. How reliable the measurement is depends on the validity and the precision. Validity is the absence of *systematic error*, and precision is the absence of *random error*. There are different types of systematic errors, so called biases. Random error is caused by chance. While random error can be dealt with through increasing the study population, systematic errors should be taken into consideration, both at the design and the data analysis stages.

Selection bias

Selection bias can occur in the process of selecting study participants, or through factors that influence study participation. There can be selection bias if the association between exposure and outcome is different between those who are selected/participate and those who theoretically would be able to participate, including those who were excluded/chose not to participate. Selection bias can be caused by, for example, differential loss to follow up, self-referral (self-selection bias) and diagnostic bias [129]. In Study II we only had blood pressure measurements for those who were considered suitable for military service (43% of the conscripts). Hence, this is a selection of men who were likely to be healthier than those not included in the study. Further, participants had been exposed to maternal smoking less often than non-participants (29.2% vs. 26.2%); their parents had higher education and were more often cohabiting during pregnancy. However, this selection has probably resulted in a more homogenous population with higher internal validity and less confounding from socio-economic factors. In Study III, there was a large part (42%) of the final study population who lacked information about parental socio-economic status (SES). This group also had higher prevalence of hypertension (19.5% vs. 15.8%) than the part of the cohort with information about parental SES. This difference was however entirely explained by differences in age between the groups; those who lacked information about parental SES were older and had somewhat lower adult SES than the part of the cohort with full information. This selection should rather have led to an underestimation of the association between parental SES and hypertension. In Study IV, only voluntary participants could be included as the zygosity determination depends on response to questionnaires. However, the response rates were rather high (Q73: 83% SALT: 73% STAGE: 66%) and probably not related to the exposure or outcome (birth weights).

Information bias

Information bias, or misclassification, occurs when information about exposure or outcome for the study participants is incorrect. This can for example happen when study participants do not remember their exposure or outcome correctly (recall bias) or when study participants underreport or deny an exposure or outcome. Misclassification can be differential, i.e. related to other variables, or non-differential, i.e. unrelated to other variables [129]. Differential misclassification can either overestimate or underestimate an effect, while non-differential misclassification usually causes a dilution of the effect. Misclassification of confounding factors hampers the ability to control for the confounder in the analysis [129]. In Study I and II, the information about maternal smoking was self-reported by the women in early pregnancy. Self-reported smoking during pregnancy has been shown to have an acceptable validity [116,130]. However, it is known that some women underreport the number of cigarettes they smoke or completely deny the habit [116,131,132]. This might be an increasing problem for later years as smoking has become less and less socially acceptable. Further, 20-40% of women who report that they smoke in early pregnancy quit smoking later in pregnancy [5]. Therefore, it is probable that there is misclassification of exposure in Studies I and II, especially in the groups who reported a change in smoking habits between pregnancies. Assuming non-differential misclassification, it can have led to an underestimation of the associations between maternal smoking during pregnancy and stillbirth, and maternal smoking during pregnancy and offspring blood pressure, respectively. In Studies III and IV twin data was used. For like-sexed twins there is a risk that their birth characteristics have been mixed, so that the characteristics of twin A has been assigned to co-twin B, causing misclassification [123]. In Study III, this would be misclassification of a confounder (birth weight and gestational age) and in Study IV this would be misclassification of exposure. To minimize this misclassification the cohorts were restricted to twins with known birth order, using different algorithms. In Study IV, these algorithms partly built on self-reported birth weight, which is open to some recall bias. In Study III, the prevalence of hypertension is probably underestimated, due to misclassification of the outcome. In the cohort, 16% were classified as hypertensive (aged 40-76). In Swedish samples, a prevalence of hypertension of 26-27% has previously been reported [89,133]. Self-reported hypertension has been shown to have a high specificity (80-95%) but low sensitivity (43-82%) [134]. Assuming non-differential misclassification, this should have led to an underestimation of the association between socio-economic status and hypertension. Further, if the diagnosis and treatment of hypertension are socially patterned, those with low social status would probably be less likely to report hypertension than those with high social status; again, this would have led to an underestimation of the association between social status and hypertension.

Confounding

Confounding is a mixing of effects that distorts the association between exposure and outcome. Through a confounding factor (a confounder), an association between the exposure and the outcome can be underestimated, overestimated, created or even reversed. For a factor to be a confounder it has to: [135]
1. Covariate with the exposure.
2. Be a cause (or marker of a cause) of the outcome.

3. Not be in the causal pathway between the exposure and the outcome (i.e. a confounder should not be an effect of the exposure).

Confounding occurs when the exposed and the unexposed groups differ in terms of such a third factor. It is possible to assess confounding at the design stage of a study through, for example, randomization or restriction. Through randomization all confounding factors, including the unknown, are randomly assigned between the exposed and the unexposed groups. If differences between the groups remain after randomization they are due to chance, hence, confounding is a random error in experimental studies. Through restriction, study participants who have the same, or nearly the same value on the confounder are selected into the study. This method can be used in all study designs. At the analysis stage confounding can be assessed through stratification or through adjustment in regression models, this requires that data on the confounders have been collected. In the stratified analyses different estimates are presented for different levels of the confounder. Compared to the multivariate regression analysis, it is easier to get an overview of what has happened to the data in the stratified analysis. In the multivariate analysis, on the other hand, it is possible to adjust for several confounders at the same time.

Restriction was used in Studies II and IV. In Study II, the study population was restricted to men without congenital malformations, who had been singletons and whose mothers had been born in a Nordic country. As blood pressure data was only available for those who had been considered suitable for military service, the study population was in practice also restricted on the basis of that. In Study IV, the study population was restricted to include only the twin's first born offspring, this was done in order to avoid effects from parity.

In all studies in this thesis confounding was assessed through adjustment in regression models. In Study I, information about maternal BMI, alcohol consumption, passive smoking and illicit drug use was lacking and could not be controlled for. In Study II, information about postnatal smoking was lacking. A large part of the parents who smoke during pregnancy also smoke after the baby has been born. It is, in Study II, not possible to separate effects of antenatal and postnatal smoking. In Study III, information about the confounders was obtained long before the outcome was assessed and they can therefore have changed over time. This can have led to an insufficient adjustment for confounders. However, we prioritized to use data collected before the occurrence of the outcome in order to avoid reverse causality. Further, considering the association between socio-economic status and hypertension, all covariates could also be thought of as mediators. Adjusting for them then becomes an attempt to investigate how much of the association between socio-economic status and hypertension that can be attributed to the specific covariates. All these adjustments require that the confounders are both known and measured. The idea of the twin/sibling pair comparisons is to further adjust for familial confounding, i.e. adjustment for environmental and genetic factors that might not be measured or even known.

Effect modification/interaction
A biological interaction is when the effect of one factor is dependent on the presence of another factor, this kind of interaction either exist or do not exist. An example of a

biological interaction is the metabolic disease phenylketonuria (PKU) [136]. To develop the disease (manifested as mental retardation), both the genetic mutation and exposure to the amino acid phenylalanine in the diet is required. Through diet restrictions the disease development can be prevented. A statistical interaction, or effect modification, occurs when the effect changes over values of a third variable, for example, when an exposure has different effects in young and old people or in men and women. Effect modification can be present on one scale but not another, e.g. there can be a statistical interaction on the risk ratio scale but not on the risk difference scale. Effect modification should be looked for if there is a biological or social hypothesis behind it. If detected, stratified analyses should be presented. In Study III there was a significant interaction between sex and adult socio-economic status, the analyses where therefore stratified by sex. Low adult socio-economic status was associated with hypertension in women, but not in men.

Random error
Rothman & Greenland describes random variation as the part of our experience that we cannot predict [129]. Often this is attributed to chance. Estimates from small study samples are more sensitive to the influence of random error, i.e. they often have lower precision. The primary way to increase precision/reduce random error in a study is therefore to increase the study size [129]. The confidence interval around a point estimate gives information about the amount of random error in the analysis. The interpretation of 95% confidence intervals is that; if the study could be repeated an unlimited number of times, the confidence interval would include the true value with a frequency of at least 95% [129]. This assumes that there is no systematic error in the study and that the statistical model used fits the data. As this most often is not completely true, the confidence intervals can rather be seen as a guide to the uncertainty in an epidemiologic result [129]. The width of the confidence interval reflects the precision of the estimate. Further, the width is influenced by the arbitrarily chosen significance level; the most common choice is 95%. As the studies in this thesis are all based on large cohorts, the precision should in general be good. However, some investigated groups within the cohorts can still be small and therefore have a larger statistical uncertainty; for example, the group of women who were non-smokers in their first pregnancy and smokers in the second pregnancy (starters) in Study I, and the group with downward social mobility in Study III. Furthermore, in Study II relatively few brother pairs were discordant for exposure to maternal smoking during pregnancy, and in Study III very few twin pairs were discordant for adult socio-economic status, which hampered the co-twin case-control analysis.

External validity
While internal validity is about how reliable the results are within the studied population, external validity refers to the generalizability of the results to other non-studied populations or if the findings could be expressed as a scientific theory or hypothesis more or less separated from time and place [129]. This is often a matter of judgement and discussion. If the internal validity of a study is poor, external validity has no meaning. In Study II, only men were included and it is therefore not known whether the results would have been the same for women. Hence, these findings are not generalizable to women.

External validity of twin studies

Twin studies have been pointed out as a way to investigate causal pathways underlying the association between birth size and adult disease, especially through between and within twin pair analyses [137]. McGue et al. even argues that discordant monozygotic twin pairs provide an analog to the counterfactual design [138]. However, the generalizability of findings among twins to the general population (which mostly consists of singletons) has been questioned [1,139,140]. Twins are in general smaller at birth and born after shorter gestations than singletons. In accordance with the DOHaD hypothesis, twins were therefore hypothesized to have increased risk of cardiovascular disease later in life. However, no such differences between twins and singletons have been found [141-143]. Furthermore, no difference in size at birth has been reported for offspring of twins compared to offspring of singletons [144]. It has therefore been argued that foetal growth in twins is biologically different from that in singletons and perhaps of limited use in investigating later health outcomes [1]. However, the association between low birth weight and later health among twins have shown similar results to those seen among singletons [54,140,145]. Further, in Study IV, we have seen that there is an intergenerational association in size at birth also among twin mothers and their children. Morley has argued that the general constraint in twins might not be associated with later health outcomes, while differences in foetal growth *among* twins might be [146]. That is, the association between birth weight and later health might differ quantitatively but not qualitatively, at least not for later blood pressure [147]. However, factors such as placentation and factors related to assisted reproductive technologies need to be considered [147].

Other aspects of twin studies

As all differences between monozygotic and dizygotic twins are interpreted as a genetic effect, twin studies usually overestimate the importance of genes. However, potential influences from gene-environment interactions, epigenetic differences and environmental factors that are more correlated within monozygotic twins cannot be ruled out [110].

Studies III and IV were both based on twin data. In Study III, the twins were mainly analysed as a cohort since the co-twin case-control analysis was hampered by low precision and could not be stratified by zygosity. The described concerns about twin data is therefore of less importance in Study III and twins are probably representative for the whole population with regards to socio-economic status and social mobility. In Study IV, where intergenerational birth size was investigated, these concerns are of greater importance. We did not have information about chorionicity (which leads to a shared or separate placenta) and could hence not investigate if this could explain differences between monozygotic and dizygotic twins. Twin-to-twin transfusion syndrome (TTTS) could also be a cause of differences in birth weight between monozygotic and dizygotic twins, and above all within monozygotic monochorionic twin siblings. However, due to the poor prognosis of untreated TTTS [148], it is unlikely to have had an impact on our results.

6.2 FINDINGS AND IMPLICATIONS

Maternal smoking during pregnancy and the risk of stillbirth

Study I, shows that women who quit smoking between two consecutive pregnancies have the same risk of stillbirth in the second pregnancy as women who never smoked when pregnant. Women, who were smokers in one pregnancy and non-smokers in one pregnancy, had an increased risk of giving birth to a stillborn infant in the pregnancy where they smoked, compared to the pregnancy when they did not. There was also a dose-response relationship where risks increased with amount smoked. These results support that maternal smoking during pregnancy is causally related to stillbirth.

However, no increased risk was seen among women who stated that they were non-smokers in their first pregnancy and smokers in their second pregnancy (starters). This somewhat complicates the interpretation, but could be due to other causes touched upon previously, namely: misclassification, confounding and random error. First, smoking was only measured in early pregnancy and 20-40% quit smoking later during pregnancy [5]. Successful smoking cessation is related to degree of addiction and women who managed to sustain from smoking in the beginning of their first pregnancy are probably less addicted and more likely to quit later in pregnancy (which causes misclassification of exposure). Second, women who take up smoking between their pregnancies may differ from persistent smokers with respect to other factors influencing stillbirth risk such as BMI, weight change between pregnancies and obstetric history (i.e. confounding) [78,149,150]. Third, this group is smaller than the others and stillbirth is relatively uncommon, so even though the study population is large, this could be a chance finding (random error). Further, A.L.V. Johansson has shown that women who did not smoke in the first pregnancy but were heavy smokers ($\geq$10 cigarettes per day) in the second pregnancy had an increased risk of stillbirth in the second pregnancy (unpublished data). This was done by survival analysis using data from the Medical Birth Register.

Previous studies have consistently reported an association between maternal smoking during pregnancy and stillbirth risk [5,80]. This seems to be true especially for preterm stillbirth [151]. Reported odds ratios range between 1.2 and 1.8 [5] and a dose-response relationship is usually obtained. Wisborg et al have shown that women who quit smoking in the first trimester have the same risk of stillbirth as women who never smoked during pregnancy [152]. However, Dodds et al showed that women who stopped smoking by the 16th week of gestation still had an increased risk of stillbirth, similar to persistent smokers [153]. Further, the increased risk of stillbirth in women who smoke during pregnancy has been explained through increased risks of foetal growth restriction and placental complications [151]. Although smoking during pregnancy has decreased substantially since the 1980s, stillbirth rates have not. This might be explained by the concordant increase in maternal BMI and age, which also are important risk factors for stillbirth [58,154,155].

To conclude, there is good evidence for a causal association between maternal smoking during pregnancy and stillbirth. Although smoking among pregnant women has decreased in Sweden, there are still groups where the prevalence is high. Smoking during pregnancy continues to be an important and preventable risk factor for stillbirth.

Maternal smoking during pregnancy and offspring blood pressure

Study II suggests that maternal smoking during pregnancy may increase offspring blood pressure in late adolescence. In sons of mothers who had smoked during pregnancy, there were small increases of both systolic and diastolic blood pressure. Further, there was also a dose-response relationship where the influence was largest in sons of heavy smokers. Within full brother pairs, where one brother had been exposed to maternal smoking in utero and the other had not, there was a tendency to higher blood pressure in the exposed brother. However, the results from the within-brother comparison were not statistically significant.

Previous studies of maternal smoking during pregnancy and offspring blood pressure have yielded mixed results. Half of the published studies have shown a positive association [87,156-162]. Reported regression coefficients have been in the range from 0.6 to 5.4. Capul-Uicab et al. investigated the association between in utero exposure to maternal smoking and subsequent hypertension, obesity and gestational diabetes among 70 000 Norwegian women aged 14 - 47 [87]. They found an almost 70% increase in odds of hypertension also after adjustments for age, education, personal smoking and BMI. However, the authors also concluded that effects of unmeasured confounding could not be excluded. The other half of the previously published studies have reported null associations for smoking during pregnancy and offspring blood pressure, or associations that disappeared after adjustment for potential confounders [69,163-170]. Brion et al. reported similar influences of maternal and paternal smoking during pregnancy. After further adjustments for social factors these associations decreased towards the null [167]. In a study including 8815 participants aged 45 years, increases in blood pressure, BMI, waist circumference, HbA1c (a marker for hyperglycaemia), and triglycerides (blood lipids) were reported in those who had been exposed to maternal smoking in utero [69]. However, after adjustments for postnatal influences, only associations with BMI and waist circumference remained significant.

As seen, the findings are conflicting and postnatal influences such as the obesity epidemic are likely to have much larger public health implications. However, as seen in studies on low birth weight, the association with hypertension can be substantial despite very modest increases in blood pressure [56]. A recent review concludes that there is an accumulating body of evidence for an association between intrauterine exposure to smoking and later cardiovascular disease, diabetes type 2 and obesity [62]. Smoking during pregnancy is hypothesized to increase offspring blood pressure, at least partly, through IUGR which has been linked to increased arterial resistance, endothelial dysfunction, altered renal and cardiac structure and function, and altered composition and amount of perivascular adipose tissue [62,67,168,171]. Maternal smoking during pregnancy has also been associated with increased carotid intima-media thickness, a marker for atherosclerosis [171].

Further studies are needed to determine if there is an association between maternal smoking during pregnancy and offspring blood pressure, hypertension and cardiovascular disease. These studies will also need to carefully address aetiology and confounding factors.

Intergenerational social mobility and the risk of hypertension

Study III shows that upward social mobility is associated with decreased risk of hypertension compared to the stable low group, and suggests that downward social mobility is associated with increased risk compared to the stable high group. Parental socio-economic status (SES) was associated with increased hypertension risk in both men and women, while adult SES only was associated with hypertension in women. This suggests that social differences in hypertension risk are initiated early in life, but that they are modifiable.

There is a well-known social gradient in the risks of hypertension and cardiovascular disease [9]. Both parental/childhood SES and adult SES have been associated with hypertension and cardiovascular disease [172-174]. A stronger social gradient in hypertension among women vs. men has been suggested to be due to a stronger social gradient in BMI among women [175,176]. In Study III, effects of BMI was adjusted for. However, to avoid possible influence of reverse causation, information on potential confounders were collected long before the outcome was measured. As BMI and other potential confounders can change over time, residual confounding is an issue. Although not all studies have reported an association between social mobility and hypertension [177], the findings from Study III are consistent with several previous reports. James et al found that, compared to the stable high SES group, the upward mobile group had a four times greater risk of hypertension, the downward mobile group had a six fold increased risk and the stable low SES group had seven fold increased risk [178]. Waitzman and Smith reported an increased risk of hypertension in the downward mobile group [179]. Krieger et al investigated blood pressure levels in twin sisters and showed that the working class twin on average had 4.5 mm Hg higher systolic blood pressure than her twin sister with higher SES [180].

The reason to why low SES is associated with hypertension risk is most likely multifactorial and operates over the life course. The social differences in health is to a large extent attributed to social differences in determinants of health, such as smoking, physical inactivity, poor diet and obesity [9]. Up to 90% of the absolute social differences in myocardial infarction have been attributed to the conventional risk factors [181]. Lynch et al. have demonstrated a paradox where conventional risk factors explain the majority of *absolute* social inequity, but only a modest part of the *relative* differences [101]. However, they conclude that if the aim is to reduce both the overall population burden of coronary heart disease and the disproportionate higher burden in some groups, reducing conventional risk factors "will do the job" [101].

The social gradient in hypertension is one example of social inequity in health. Further, it is a cause of the social gradient in cardiovascular disease. Decreasing social gradients in health is a stated goal for both WHO and Swedish politicians. Possible ways to decrease the differences in health could be through interventions targeting special high risk groups, through improved health in the whole population and through a larger improvement in the least affluent groups [9]. Suggested aims have a clear life course approach; a good start in life for all children, good schooling, good possibilities to get a job, good working conditions, healthy minimal living standards etc. [9]. Special taxes and ban on smoking in public environments have also been used to improve public health.

Intergenerational influence of birth size - genes or environment?

Study IV, suggests that the intergenerational association in size at birth is due to direct or indirect genetic factors. We found an intergenerational association of size at birth among both dizygotic and monozygotic twin mothers and their offspring. Within dizygotic twin pairs, this association remained. That is, the offspring of the larger twin sister at birth was on average larger at birth than the offspring of the smaller twin sister. Within monozygotic twin pairs, there were no differences in birth size between the sister's children.

There are several previous studies that have demonstrated an association between parent's and offspring's birth weights [19,23,182-191]. Usually, the association between mother and offspring have been stronger than between father and offspring [182,185,186,191], although, one study from India have reported the opposite finding [190]. A one hundred gram increase in mother's birth weight has been estimated to increase offspring birth weight with around 17-27 g [182,185,191] and the corresponding estimate for a one hundred gram increase in father birth weight have been around 9-14 g [182,185,191]. One previous study has reported birth weights in offspring of monozygotic twin pair mothers, who themselves had been discordant in birth weight [192]. They found no differences in offspring birth size within twin pairs. Nordtveit et al. investigated if the mother's birth order influenced her offspring's size at birth [193]. First born infants are usually lighter at birth than their subsequent siblings. Given the intergenerational association in size at birth, it could be hypothesized that those first born would have smaller children than those with higher birth order. However, the authors found that offspring of first born mothers actually were larger at birth than offspring of later born mothers. These differences were entirely explained by socio-economic factors. Furthermore, De Stavola et al. have shown that socio-demographic and behavioural factors moderately, but significantly contribute to the intergenerational correlation in birth size [194].

Birth weight is determined by many different factors and largely influenced by both genes and environment. Furthermore, genes and environment can be correlated. Genes that promote rapid growth can, for example, be present in both the mother and the foetus (as they are related) and *directly* influence growth. Genetic factors can also have caused the high birth weight of the mother and her large adult body size. As the mother's adult height and weight are determinants of offspring birth weight [19], these genes can hence promote foetal growth *indirectly* through the larger body size of the mother. A study of birth weight after ovum donations support an indirect influence, as they found that the size of the infant was correlated with the weight of the woman carrying the pregnancy and not the size of the genetic mother [195].

Genetic, environmental and social factors all contribute to the intergenerational association in size at birth. However, when genetic and, to a large extent, environmental and social factors were held constant (monozygotic twin pairs) no influence of mother's birth size was seen on offspring's birth size. To understand the intergenerational influence of birth weight is of interest in the light of the DOHaD hypothesis.

7 CONCLUSIONS

- Smoking during pregnancy is likely to be causally associated with stillbirth. Primary prevention of smoking is important, as it targets young people who are the future parents, before they develop nicotine dependence. Pregnant women who smoke should receive information about the risks and encouragement and support to quit.

- Smoking during pregnancy may increase offspring blood pressure in late adolescence. Further studies, which carefully addresses confounding, are needed to investigate if this influence remains later in adulthood and if prenatal exposure to smoking increases the risk of hypertension or cardiovascular disease.

- Low socio-economic status in childhood is associated with increased risk of hypertension in middle age. This risk can be modified by adult socio-economic status. This suggests that social differences in hypertension start early, but also that the risk is modifiable.

- The intergenerational association between mother's and offspring's size at birth is suggested to be due to direct or indirect genetic factors.

8 FUTURE CHALLENGES

The negative effects of maternal smoking during pregnancy on several obstetrical and perinatal outcomes have been well documented [5]. The risks are generally also known to the public, at least in western countries [196]. Most women who quit smoking during pregnancy do so because of concern for the foetus [58]. There are also many women who want to quit, but fail in doing so. Lumley et al. have reported that up to 45% of pregnant women stop smoking spontaneously in early pregnancy, however 21% of them relapse already before delivery and only one third of spontaneous quitters are still abstinent after one year [63]. Smoking is declining in western countries, but still increasing in many other populations. WHO has pointed out increased smoking among women in low and middle income countries as one of the most worrying developments in the tobacco epidemic [197].

Although maternal smoking during pregnancy has decreased steadily in Sweden since the 1970's, there are still subgroups where the prevalence is high, for example among teenage mothers and among low educated women. In 2006, there were still municipalities with a smoking prevalence in early pregnancy of 20-30% while other areas had a prevalence of 0-2% [57]. Interventions for smoking cessation during pregnancy were evaluated in a Cochrane review in 2009 [63]. Pooled data from 65 clinical trials showed a reduction in smoking during late pregnancy of 6% in the intervention group compared to the control group (relative risk with 95% confidence interval: 0.94 (0.93-0.96). Further, there was a 17% reduction in the risk of low birth weight, a 14% reduction in the risk of preterm birth and weighted mean birth weight increased with 53 grams [63]. The authors of the Cochrane review conclude that attention to smoking behaviour and support for smoking cessation and relapse prevention should be a routine part of all antenatal care.

Nicotine is an addictive substance. Many pregnant women find it very difficult to quit smoking even though they want to and many women relapse. One very important risk factor for continued smoking during pregnancy is having a partner who smokes [61]. Therefore, primary prevention strategies in the whole community are also very important for obstetric outcomes and neonatal health. Most important is to reduce smoking initiation among young people who are the future parents. Strategies to reduce smoking outlined by the WHO have been prohibition of smoking in public places, increased taxation on tobacco products, preventing sales of tobacco products to young people, bans on tobacco sponsorship of prestigious events and warning texts and pictures on cigarette packages [197]. Advantageous effects of introducing public smoking bans have been shown. In a study from Colorado preterm births decreased by 23% the year after the smoking ban was introduced [198]. In Scotland, the prevalence of smoking in pregnancy decreased from 25% to 19% and the preterm deliveries decreased by 12% after the smoking ban was introduced [199].

As we have seen, smoking during pregnancy is strongly related to socio-economic status and also to psychological factors such as stress and depression [63]. A future challenge is to develop better intervention programs and especially to find ways to help women in these groups. From a public health perspective, smoking and overweight

have been pointed out as the most important modifiable risk factors for pregnancy complications and adverse pregnancy outcomes [58].

More research is needed to determine whether maternal smoking during pregnancy has a long term influence on offspring health. Results from some human and animal studies suggest that there is an association [62,67]. However, the influence of socio-economic and familial confounding could be substantial. If maternal smoking during pregnancy can be shown to have long time influences on later health this will raise the question of whether early interventions should be implemented in this group.

Differences in smoking habits and to some extent differences in overweight/obesity have also been suggested to explain a substantial part of the social gradient in health [9,200]. To decrease social inequity in health is a worldwide challenge [201]. Suggested ways to achieve this have for example been to improve health in the whole population and especially target deprived groups. The importance of a life course approach is clear in the given suggestions; to give all children a good start in life which requires good maternal nutrition, antenatal and child care, to provide good schooling, fair and decent working conditions, healthy housing and communities, social protection throughout life and universal health care [9,202].

At present, intrauterine growth restriction is managed by determining aetiology and severity of the growth restriction and selecting time and mode of delivery [12]. There have been several attempts to prevent and treat growth restricted foetuses in utero, for example through low dose aspirin treatment, maternal oxygen therapy, maternal nutritional supplementation and plasma volume expansion [10,21,203]. Some of these interventions, such as low dose aspirin in preventing preeclampsia and intrauterine growth restriction have reported positive results [204]. However, further research on treatment of foetal growth restriction is needed. Considering the many aetiologies, it is unlikely that one intervention will be suitable to all patients.

9   SVENSK SAMMANFATTNING

Avhandlingens syfte

Under fostertiden genomgår vi vår allra största utveckling. På omkring fyrtio veckor blir en enda cell ett fullt utvecklat barn. Det är lätt att tänka sig att faktorer under denna tid, då alla organ och vävnader bildas, kan påverka hälsan på lång sikt, kanske under resten av livet. Denna avhandling, som består av fyra vetenskapliga studier, handlar om just sådana tidiga exponeringar och deras effekter på hälsan. Fokus ligger på moderns rökning under graviditet, födelsevikt och social status. De specifika frågeställningarna har varit följande:

- Förändras risken att föda ett dödfött barn om modern ändrar sina rökvanor mellan två graviditeter? Om förändring av exponering för rökning medför en förändrad risk för dödföddhet stöder detta ett orsakssamband mellan rökning under graviditet och dödföddhet.
- Påverkar moderns rökning under graviditeten avkommans blodtryck i tonåren?
- Förändrar de som gör en social klassresa även sin risk att drabbas av högt blodtryck?
- Får den större tvillingsystern i ett likkönat tvillingpar med olika födelsevikt, i sin tur barn som är större än den mindre tvillingsysterns barn? Eller förklaras sambandet mellan mor och barns födelsevikter av delad genetik/miljö?

Registerbaserade studier

I Sverige finns det ett flertal rikstäckande register, som dessutom kan sammanlänkas via personnumren. Detta sker i anonymiserad form och regleras av lagar som värnar människors integritet. Till exempel måste studier som använder sig av registerdata först godkännas av en etisk kommitté. Denna avhandling bygger på information från framförallt Medicinska födelseregistret, Värnpliktsregistret och Svenska Tvillingregistret.

Sammanfattning av delarbetena

Delarbete I: Moderns rökning och risken att få ett dödfött barn

I denna studie undersöktes moderns rökning under graviditeten som en möjlig orsak till att fostret dör i livmodern. Studiepopulationen innefattade alla kvinnor som födde sina två första barn i Sverige mellan 1983 och 2001. Informationen om kvinnornas rökning tidigt i graviditeten var självrapporterad och kom från mödravårdsjournaler. Definitionen på dödfödd var barn födda utan livstecken efter graviditetsvecka 28.

Resultaten visade att de mödrar som rökte under graviditeten i högre grad riskerade att få dödfödda barn jämfört med icke-rökarna. De kvinnor som rökte mer (10 cigaretter eller mer per dag) löpte en större risk än de som rökte mindre (1-9 cigaretter per dag). Hos kvinnor som slutade röka mellan två graviditeter förelåg ingen ökad risk för att barnet skulle dö i livmodern i den andra graviditeten (då de var icke rökare). För kvinnor som rökte i en av sina två graviditeter var risken att föda ett dödfött barn fördubblad i den graviditet där hon rökte (10 cigaretter eller mer per dag) jämfört med den i vilken hon inte gjorde det.

Delarbete II: Moderns rökning och påverkan på avkommans blodtryck

I denna studie undersöktes om moderns rökning under graviditeten hade någon påverkan på tonårssöners (18 år i genomsnitt) blodtryck. Studiepopulationen bestod av män födda i Sverige mellan 1983 och 1988, som mönstrade mellan 2001 och 2006. Resultaten visade en mycket liten men statistiskt signifikant ökning av blodtrycket (både över- och undertrycket) hos söner till mödrar som rökt under graviditeten. Denna ökning var störst hos de vars mor hade rökt mest. Samma tendens sågs mellan brödrapar. Det vill säga, brodern som hade exponerats för moderns rökning i livmodern verkade ha något högre blodtryck än brodern som inte hade exponerats. Detta resultat kunde dock inte statistiskt säkerställas.

Delarbete III: Social mobilitet över två generationer och högt blodtryck

I denna studie undersöktes om en klassresa mellan föräldrars socialgrupp och egen socialgrupp i vuxen ålder förändrade risken för högt blodtryck. Studiepopulationen bestod av likkönade tvillingar födda i Sverige mellan 1926 och 1958. Information om yrke användes som indikator för socialgrupp. Tvillingarna delades in i låg status (facklärd och icke-facklärd arbetare samt tjänsteman på låg nivå) och hög status (tjänsteman på mellan eller hög nivå). Den sociala mobiliteten - eller klassresan - definierades som byte av social status jämfört med den status föräldrarna hade haft. Resultaten visade att låg social status som vuxen var kopplat till högt blodtryck hos kvinnor, men inte hos män. Att ha haft föräldrar med låg social status var dock kopplat till 40% högre risk för högt blodtryck hos både män och kvinnor, jämfört med dem som hade haft föräldrar med hög social status. De som gjort en klassresa från låg till hög social status hade 20% lägre risk för högt blodtryck jämfört med de som stannat i gruppen med lägre social status. De som gjort en omvänd klassresa - från hög till låg social status - hade en tendens till ökad risk för högt blodtryck. I jämförelsen inom tvillingpar, där bara den ena tvillingen hade gjort en klassresa uppåt, sågs samma tendens till minskad risk för högt blodtryck hos den tvilling som klättrat jämfört med den som inte hade gjort det. Den statistiska säkerheten i analyserna påverkas emellertid av att endast få tvillingpar där bara ena gjort en klassresa kunde identifieras.

Delarbete IV: Sambandet mellan mor och barns födelsevikt – gener eller miljö?

I denna studie undersöktes om skillnaden i födelsevikt hos kvinnliga tvillingpar upprepades då de själva fick barn. Det vill säga, fick den vid födelsen större tvillingen större barn än hennes mindre tvillingsyster? Studiepopulationen bestod av kvinnliga tvillingar - födda i Sverige mellan 1926 och 1985 - vilka födde sitt första barn mellan 1973 och 2009.

Resultaten visade att de mödrar som hade varit tyngre och längre vid födseln själva fick större barn. Sambandet fanns kvar inom tvåäggstvillingpar, men försvann inom enäggstvillingarpar. Sambandet försvann alltså inom de par där mödrarna hade samma gener.

Slutsatser

- Om kvinnan slutar röka, innan eller mycket tidigt i graviditeten, reduceras risken till samma nivå som den för kvinnor, vilka aldrig rökt under en graviditet. Dessa resultat stöder hypotesen att rökning kan orsaka dödföddhet.

- Rökning under graviditeten kan eventuellt höja avkommans blodtryck ända upp i tonåren. Huruvida sambandet kvarstår även senare i vuxenlivet och om detta medför en ökad sårbarhet för högt blodtryck och hjärt-kärlsjukdom behöver studeras ytterligare.

- Uppväxten i ett hem med låg social status (föräldrarnas status) är kopplad till en ökad risk för högt blodtryck. Denna risk kan dock modifieras via social status som vuxen. Detta visar att sambandet mellan låg social status och risk för högt blodtryck grundläggs tidigt, men också att effekten är påverkbar.

- Sambandet mellan mor och barns födelsevikter förklaras av gemensamma genetiska faktorer. Dessa kan antingen verka direkt genom att mor och barn till viss del delar samma gener, eller indirekt, genom att mammans gener påverkar hennes fysiologi (t.ex. hennes längd och vikt som vuxen), vilken i sin tur påverkar barnets miljö och tillväxt i livmodern.

10 ACKNOWLEDGEMENTS

When working with anonymous register data, it should not be forgotten that behind the numbers, there are real people. I want to take the opportunity to acknowledge all these Swedish men, women and children who through the registers provide important information and opportunity for research.

In particular I would like to thank:

Anastasia Nyman Iliadou, my main supervisor, for your great support and encouragement; for always being so generous with your time and knowledge; for your flexibility and understanding during the periods when I have been busy with medical studies. You have always known when to push me a little bit (oral presentation in Canada), when to get my feet back on the ground and when to wait for me to find out things by myself. It has been a true privilege to have a supervisor like you!

Sven Cnattingius, my co-supervisor, for taking me in as a summer research student when I was 22 years old and still in the beginning of my medical studies; for giving me all the freedom necessary to combine medical school and PhD studies; for teaching me how exciting research can be. Of all the professors at Karolinska Institutet you were, and still are, my first hand choice.

Gunnar Höglund, my external mentor, for your wise, warm hearted advice in questions concerning both life as a PhD-student and future career planning. After consulting you I have always felt relieved and able to make the right choices, also when we were disagreeing. I hope I will get to keep you as my mentor also after the dissertation.

Cecilia Lundholm, biostatistician and co-author, for your never ending patience in explaining statistical methods and SAS programming to me and for always making me feel welcome when coming to your office. Your help and knowledge have been invaluable.

Sara Öberg, Pär Sparén, Brian D'Onofrio, Niklas Långström, co-authors, for generous contributions and encouragement when preparing, revising and re-revising manuscripts.

Gunnar Petersson, data programmer, for professional help with data base management enabling my fourth study. Without you, I would not have made it in time.

Hatef Darabi, for your friendship throughout my whole time at MEB, for listening to my presentation rehearsals and for always looking out for me.

Present and former fellow PhD-students at MEB: *Stephanie Bonn, Sara Christiansen, Edoardo Colzani, Miriam Elfström, Sandra Eloranta, Adina Feldman, Pia Fernberg, Martin Fransson, Thomas Frisell, Alexander Grankvist, Ralf Kuja-Halkola, Robert Karlsson, Amy Leval, Therese Ljung, Lisa Möller, Iffat Rahman, Maria Sandberg, Amir Sariaslan, Karin Sundström, among others*, for teaching me a lot about epidemiology,

for great support throughout my PhD studies, but most importantly for "fika", celebrations, parties, dinners, sports activities and the most appreciated friendship.

The current and former heads of department: *Henrik Grönberg, Nancy Pedersen and Hans-Olov Adami,* for enabling a good work environment and opportunities for research.

All other staff at MEB, both within and outside research, for making MEB a great place to be working!

Karolinska Institutet. For supporting my research interest, first by LäFo (medical school with orientation towards research) which brought me to MEB in the first place and later by the MD/PhD program which generously financed parts of my PhD-studies.

Jag vill även tacka mina vänner och min familj:

Ann-Marie Olofsson, Tahmineh Pettersson, Wenjing Tao, Malin Thorvaldsson, Maria Undén Morein, Thomas Silfverberg, vänner och kollegor, för delade mödor, såväl som delad glädje, i vår gemensamma strävan mot läkarexamen, alternativt i det dagliga avdelningsarbetet.

Lotti Wicks, för att du drar iväg mig ut på dans.

Ellinor Heiroth och Tove Odland, för långvarig vänskap bortom allt som har med medicin och forskning att göra.

Lisa Moroni, för vänskap sedan barndomen och för illustrationen till omslaget på denna avhandling.

Robert Roxborough, för kärlek, tålamod och språkgranskning.

Märta Högberg, farmor, för att du säger "jaja, forsk'in du bara".

Elias Högberg, min bror, för kloka råd, träffande kommentarer och för att du alltid kan få mig att skratta, till och med när jag vill vara arg.

Ole och Britta Högberg, mina kära föräldrar, för allt, det är er jag brås på...

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