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FETAL PROGRAMMING AND SUBSEQUENT RISKS IN ADULTHOOD: ARE THE ASSOCIATIONS CONFOUNDED BY GENETIC AND/OR ENVIRONMENTAL FACTORS?

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To Signe and Anna, you are what I'm all about.

ABSTRACT

The fetal origins hypothesis proposes that fetal growth is inversely associated with subsequent adult disease risk. The objective of this thesis was to investigate whether familial (shared environmental and genetic) factors confound the association between birth weight and risks of hypertension, low intellectual performance, and type-2 diabetes.

We performed three large population-based studies of singleton born boys conscripted for military service (Papers I-III) to investigate the association between birth characteristics and risk of high systolic blood pressure and low intellectual performance in early adulthood. We found that males born light for gestational age (<-2 standard deviation scores [SDs]) were at a 14 % increased risk of high systolic blood pressure and 22 % increased risk of low intellectual performance, after adjusting for social and maternal factors (Papers I and II). In sibling analyses we found that the association between birth weight for gestational age and risk of high systolic blood pressure was similar within and between full siblings. In contrast, the associations between birth weight, birth length, and head circumference and risks of low intellectual performance were weaker within siblings, compared with between siblings. Thus, whereas the association between birth weight for gestational age and risk of high systolic blood pressure is independent of familial factors, the associations between measurements of fetal growth and risk of low intellectual performance are at least partly confounded by familial factors. Furthermore, it appears that during early stages of gestation, birth length and head circumference is of greater concern for intellectual development than birth weight, whose importance may increase with gestation (Paper III).

To investigate if the association between birth weight and risk of hypertension in adulthood is confounded by shared environmental or genetic factors, we performed a study on the association in a sample of middle aged and elderly Swedish like-sexed twins. We found that a 500-g decrease in birth weight was associated with a 42 % increased risk of hypertension in the whole cohort of twins. Co-twin control analyses showed that corresponding risks within dizygotic and monozygotic twin pairs were 34 % and 74 %, respectively. The results suggest that the inverse association between birth weight and hypertension is independent of shared familial environment and genetic factors.

To assess whether there is a familial link between birth weight and type-2 diabetes, we studied the association between offspring birth weight for gestational age and parental risk of type-2 diabetes in the twin sample used in Paper IV. Decreasing offspring birth weight for gestational age (with 1 SDs) was associated with a 72 % increased risk of type-2 diabetes among fathers, and 57 % decreased risk among mothers, independent of measured social factors. Furthermore, we found that both the mother's and father's risk of type-2 diabetes associated with decreasing offspring birth weight was similar within and between twin pairs, although slightly smaller within pairs effects were found among fathers. Thus, the well established association between paternal type-2 diabetes and offspring birth weight seems to primarily be due to unique environmental factors experienced by each twin and its offspring.

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LIST OF PUBLICATIONS

I. Bergvall N, Iliadou A, Tuvemo T, Cnattingius S.
Birth characteristics and risk of high systolic blood pressure in early adulthood: socioeconomic factors and familial effects.

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II. Bergvall N, Iliadou A, Tuvemo T, Cnattingius S.
Birth characteristics and risk of low intellectual performance in early adulthood: are the associations confounded by socioeconomic factors in adolescence or familial effects?

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III. Bergvall N, Iliadou A, Johansson S, Tuvemo T, Cnattingius S.
Risks for low intellectual performance related to being born small for gestational age are modified by gestational age.

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IV. Bergvall N, Iliadou A, Johansson S, de Faire U, Kramer MS, Pawitan Y, Pedersen NL, Lichtenstein P, Cnattingius S.
Genetic and shared environmental factors do not confound the association between birth weight and hypertension: a study among Swedish twins.

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V. Bergvall N, Lindam A, Pawitan Y, Lichtenstein P, Cnattingius S, Iliadou A.
Importance of familial factors in associations between offspring birth weight and parental risk of type-2 diabetes.

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CONTENTS

1 INTRODUCTION

In 1977, Forsdahl found an association between poor living conditions during childhood in Norwegian counties and increased risks of atherosclerotic heart disease later in life.[1] In 1986, Barker demonstrated a strong association between infant mortality in the early 1920s and coronary heart disease mortality between 1968 and 1978.[2] Similarly, geographical differences in maternal mortality rates between 1911 and 1914 were closely correlated with later death rates from stroke in England and Wales among individuals born around that time.[3] Migration studies have also provided insights into the association between environment early and late in life and subsequent risk of disease. Among interregional migrants in England and Wales, it was found that both place of birth and place of later residence played a role in mortality from cardiovascular disease.[4]

During the last two decades a growing field of research has found associations between measures of size at birth and risks in adulthood, foremost cardiovascular diseases.[5] The hypothesis formulated following the findings has been given several names, including "the fetal origins hypothesis", "the fetal programming hypothesis" and "the Barker hypothesis", after its originator.[6]

Barker found that low birth weight was associated with an increased risk of coronary heart disease.[7] Following results from this cohort study, Barker hypothesized that cardiovascular disease are associated with specific patterns of disproportionate fetal growth that results from fetal undernutrition from middle to late gestation.[6] Insufficient energy supply for organ development could then permanently alter organ development and functioning, thereby increasing disease susceptibility later in life. Malnutrition can permanently change the number of cells in the body, change the distribution of cell types, hormone secretion pattern, metabolic activity and organ structure.

Numerous studies have replicated Barkers original findings,[5] providing strong evidence that there exists an inverse association between size at birth and risk of coronary heart disease. The fetal origins hypothesis has gained support in several fields; systematic reviews provide evidence for an association between birth weight and blood pressure,[8] and type-2 diabetes.[9] In addition, birth characteristics have also been associated with other long-term outcomes, including intellectual development later in life.[10]. However, most of the evidence supporting associations between birth characteristics and subsequent risks later in life comes from observational studies, which often suffer from a number of methodological difficulties, foremost random error and confounding. Random error can be dealt with by aggregating quantitative results in a meta-analysis.

Confounding is a serious concern, and there are at least two alternative pathways, which could explain associations between size at birth and risks in adulthood. Socioeconomic and genetic factors influence both fetal growth and birth weight,[11] risks of cardiovascular diseases,[12] and intellectual performance.[13] Thus, it is possible that the association between fetal growth and subsequent adult risks may be confounded by genetic and/or socioeconomic factors.

In the present thesis and in the included papers, we have studied associations between measures of fetal growth and risks in adulthood, including cardiovascular phenotypes and measures of cognition. To answer if such associations are confounded by familial (shared environmental and genetic) factors, we have studied the associations in different study bases with varying degree of relatedness of family members:

- In Papers I and II we used a large sample of full brothers conscripted for military service to study if associations between birth characteristics and risk of high systolic blood pressure and low intellectual performance in early adulthood are confounded by familial factors.

- In Paper III, we used the same study population as in Papers I and II to study if the association between birth characteristics and risk of low intellectual performance in early adulthood was modified by gestational age.

- In Paper IV, we studied the association between birth weight and risk of hypertension in a sample of middle aged and elderly twins born in Sweden between 1926 and 1958. Using the different degrees of relatedness between dizygotic and monozygotic twins, we studied if the association was confounded by shared environmental and/or genetic factors.

- Finally, in Paper V, using the same twin sample as in Paper IV, we studied if the association between offspring birth weight and parental risk of type-2 diabetes is confounded by factors which the twin parents share.

2 BACKGROUND

2.1 HISTORY OF THE FETAL ORIGINS HYPOTHESIS

The idea that early environment may be a predictor of health in adulthood was proposed already during the 1930s.[14] Kermak et al. showed that death rates in Britain and Europe over the last two centuries fell with each successive year-of-birth cohort.[14] They concluded that "results are consistent with the hypothesis that the important factor from the point of view of the health of the individual during his whole life is his environment up to the age of say 15 years, and that improved conditions at later ages have little direct effect." In 1964, Rose found that the siblings of patients with coronary heart disease had stillbirth and infant mortality rates twice those of individuals with healthy siblings.[15] Rose concluded that "ischaemic heart disease tends to occur in individuals who come from a constitutionally weaker stock", which fits well with the hypothesis that an unfavorable childhood environment predisposes to later cardiovascular disease. In a Norwegian sample, Forsdahl found a geographical correlation between coronary heart disease mortality during the 1960s and infant mortality rates 70 years earlier.[1] Forsdahl suggested that the high infant mortality rates indicated a poor childhood environment, and that growing up in such an environment caused permanent damage which left people with a vulnerability to aspects of a sedentary adult lifestyle.

The studies which formally launched the fetal origins hypothesis were conducted during the 1980s by David Barker and his colleagues at Southampton University. In 1986, Barker et al., found a strong correlation between ischaemic heart disease mortality rates in 1968 to 1978 and infant mortality rates in 1921 to 1925 in England and Wales.[2] Furthermore, they found that mortality from ischaemic heart disease was correlated with both neonatal and post-neonatal mortality. While postnatal mortality may reflect the postnatal environment, they concluded that neonatal mortality is related to exposures during prenatal and early postnatal life. They discussed that the correlations may be caused by a factor independently related to ischaemic heart disease and infant mortality.

Barker and Osmond suggested that intrauterine environment, foremost fetal nutrition, programmed adult cardiovascular disease.[2] However, to that point all the findings came from ecological studies, which are based on groups and not individuals. As such, it could not be concluded if the infants who had suffered from fetal malnutrition or poor living conditions were the same individuals that developed heart disease in adulthood. Furthermore, the studies had no information on potential confounders, and it was impossible to pinpoint the exact timing of the proposed insults. An equally probable explanation was that the correlations were due to the fact that a disadvantaged environment in infancy leads to a deprived environment in adulthood.

Migration studies have also identified similar patterns as those found in the ecological studies. For example, several studies have investigated migration patterns in the United Kingdom and risk of coronary heart disease, as there is a strong geographical gradient of heart disease. Risk of coronary heart disease was associated with geographical locations in adulthood, and with birth place.[4,16] However, similar to ecological studies, migration studies also suffer from methodological flaws. Foremost, individuals that migrate may not represent the general population.

The first cohort study which assessed the association between the intrauterine environment and heart disease in adulthood, was conducted by David Barker in 1989.[7] They used historical midwifery data from a cohort of women and men born between 1911 and 1930 in Hertfordshire, UK, which was individually linked to information on death from ischaemic heart disease from national registers. They found that mortality from ischaemic heart disease declined with increasing birth weight, both among men and women. Furthermore, they found no association between birth weight and risk of lung cancer, which was interpreted that the obtained association with ischaemic heart disease was not confounded by smoking or socioeconomic factors. Although they were unable to adjust the analysis for social class in adulthood, they found that social class at death was not associated with birth weight. Subsequent analysis using material from the Hertfordshire cohort also established that low birth weight is associated with increased blood pressure and increased risk of type-2 diabetes.[17,18]

Following the findings from the Hertfordshire cohort, numerous studies have replicated the original findings of an association between low birth weight and increased risk of coronary heart disease,[5] increased blood pressure,[19] and type-2 diabetes.[9]

2.2 THE FETAL ORIGINS HYPOTHESIS

Barker and colleagues formulated the "fetal origins hypothesis", which postulates that coronary heart disease, type-2 diabetes, stroke and hypertension originate in developmental plasticity, in response to undernutrition in utero and during infancy.[6] They suggested that fetal malnutrition in middle to late gestation, leading to disproportionate growth, can result in permanent alterations in physiology and metabolism that lead to an increased risk of cardiovascular diseases in adulthood. It is thought that such adaptations to malnutrition may be beneficial for short-term survival, but detrimental to health in adulthood.

Although the mechanisms causing associations between size at birth and cardiovascular disease in adulthood are largely unknown, several processes have been proposed. One mechanism is alternations in the kidney, leading to reduced numbers of nephrons and resulting glomerular hyperfiltration, and ultimately hypertension.[20] Others have proposed structural changes in the vasculature tree, leading to impaired endothelial function and arterial stiffness, resulting in an increased risk of hypertension and stroke.[21] Furthermore, fetal growth restriction may also result in alterations in the pancreas, and resetting of the hypothalamic-pituitary-adrenal axis.[22]

The thrifty phenotype hypothesis was formulated by Hales and Barker to account for the association between birth weight and type-2 diabetes.[23] They suggested that fetuses experiencing malnutrition make adaptive responses which, if faced with an affluent postnatal environment, increase the risk of type-2 diabetes, and ultimately cardiovascular diseases. A growing field of research has also found that the effects of low birth weight on risks of cardiovascular diseases and type-2 diabetes are dependent on postnatal growth and obesity later in life.[24,25]

Recently is has been highlighted that whereas structural changes in key organs can explain the consequences in low birth weight infants, it cannot explain the continuous relationship

between birth weight and later disease.[26] To explain how nutritional stimulus could exert influence on risk of disease across the whole spectrum of birth weights, and not only among those born growth restricted, they suggested that a mismatch between nutrition in early and later life could result in a increased risk of disease in adulthood.[27] Nutritional stimulus in utero would not only lead to adaptive responses for short-term survival but also adaptations influencing nutritional demands in adulthood. Furthermore, the authors suggested that epigenetic changes in response to environmental stimulus in utero, including nutritional factors, are the primary cause of the association between birth weight and later disease, and that structural changes in organs are secondary to changes in gene expression.[26]

2.3 CRITICISM OF THE FETAL ORIGINS HYPOTHESIS

Although evidence has accumulated which suggests that there is an inverse association between size at birth and risks in adulthood, critics have argued that methodological flaws or alternative pathways, including social and genetic factors, may explain the observed associations.[19,28-32]

2.3.1 Genetic confounding

Several researchers have suggested that genetic factors may confound the association between size at birth and cardiovascular disease in adulthood.[31,33] The basis for such a hypothesis is that genetic factors are of substantial importance for birth weight,[11] and a number of outcomes found to be associated with birth weight, including blood pressure,[34] coronary heart disease,[12] type-2 diabetes,[35,36] and intelligence.[13] Hattersley et al. suggested that genes affecting fetal insulin secretion, a key determinant of fetal growth, may also affect insulin resistance, and lead to increased risk of type-2 diabetes and cardiovascular diseases in adulthood.[33] They proposed that certain fetal genes are pleiotropic, and would result in two phenotypes, one being a small thin baby and the other an adult with increased vulnerability to cardiovascular diseases (Figure 1).[33,37]

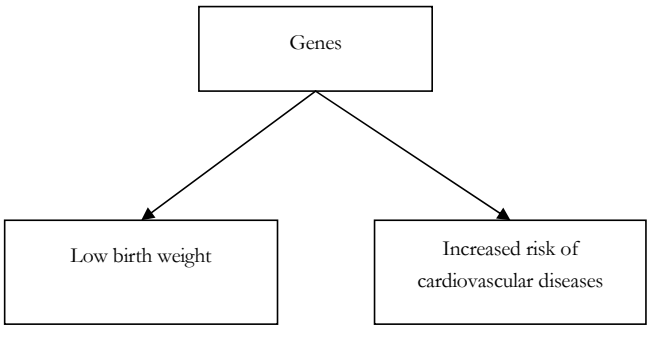

Figure 1. Genetic confounding of the fetal origins hypothesis.

However, identifying such genes is difficult, and attempts so far have been inconsistent, due to the polygenic nature of most outcomes.[38] Instead, evidence of common genetic factors for restricted fetal growth and increased risk of cardiovascular diseases come from a number of studies which have found associations between offspring birth weight and

paternal risk of cardiovascular diseases and type-2 diabetes,[39,40] suggesting that the two traits have common familial components.

Furthermore, a study found that offspring birth weight is associated with maternal blood pressure measured many years after pregnancy.[41] Maternal hypertension during pregnancy is associated with offspring birth weight.[42] Thus, if offspring inherits the maternal tendency to be hypertensive, the association between birth weight and hypertension, and ultimately cardiovascular diseases, is likely to at least partly be confounded by genetic factors or maternal hypertension rather than fetal nutrition.

Twin studies provide the opportunity to disentangle familial (shared environmental and genetic) factors into shared environmental and genetic factors.[43] Results from twin studies suggest that at least part of the association between size at birth and risk of cardiovascular disease may be explained by genetic factors.[31,44,45] Similar results have also been found with respect to size at birth and intelligence.[46]

2.3.2 Socioeconomic confounding

Confounding by social environment is another mechanism which has been put forward as an alternative explanation of the fetal origins hypothesis.[28-30] Socioeconomic factors are associated with both birth weight and chronic adult disease.[47,48] Low birth weight infants are also more likely to experience later socioeconomic disadvantaged than infants with higher birth weight.[49] Thus, it is reasonable to assume that the association between size at birth and risks in adulthood may, partly or substantially, be explained by such factors (Figure 2). Although some studies have adjusted for socioeconomic factors in adulthood and at birth, most studies have not. Generally, studies which have adjusted for social factors, found that associations between size at birth and risks in adulthood are independent of social environment.[50,51] However, measured factors of the social environment are only crude proxies of underlying social mechanisms. If unmeasured social confounding is of importance, we would expect to find attenuated associations within families, as they at least share socioeconomic exposures during childhood.

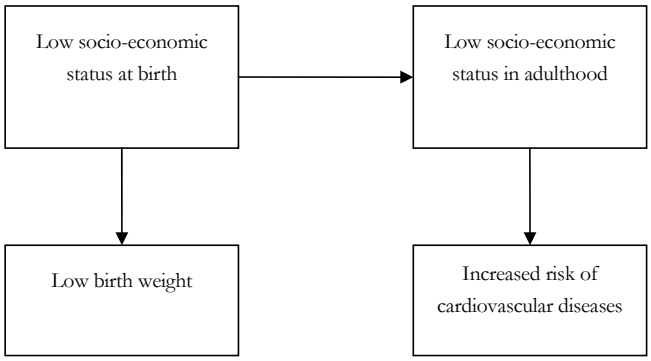

Figure 2. Socio-economic confounding of the fetal origins hypothesis.

2.3.3 Resolving confounding

As illustrated, there are a number of environmental and genetic factors which could be independently associated with birth weight and later disease, creating an apparent (non-causal) association between the two phenotypes. Studying the association between intrauterine factors and later disease in family-based studies provides the opportunity to assess the importance of such confounding factors. Conventionally, relatives have been used to assess the importance of familial (shared environmental and genetic) factors on traits. In addition, comparing concordance of disease in dizygotic and monozygotic twins has been used to separate the importance of environmental from genetic causes in the development of diseases. However, the different degrees of relatedness among dizygotic and monozygotic twins can also be used to identify causal pathways behind the fetal origins hypothesis.

In cohorts of twins, size at birth is related to factors which both fetuses share, including socioeconomic and genetic factors, and unique factors specific to each individual fetus. However, birth weight differences within twin pairs cannot be attributable to shared factors. Thus, comparing associations between birth weight and later diseases in cohort of twins and within twin pairs provides the opportunity to assess to importance of shared factors on the fetal origins hypothesis (Figure 3, Altered version from Morley et al.[43]).

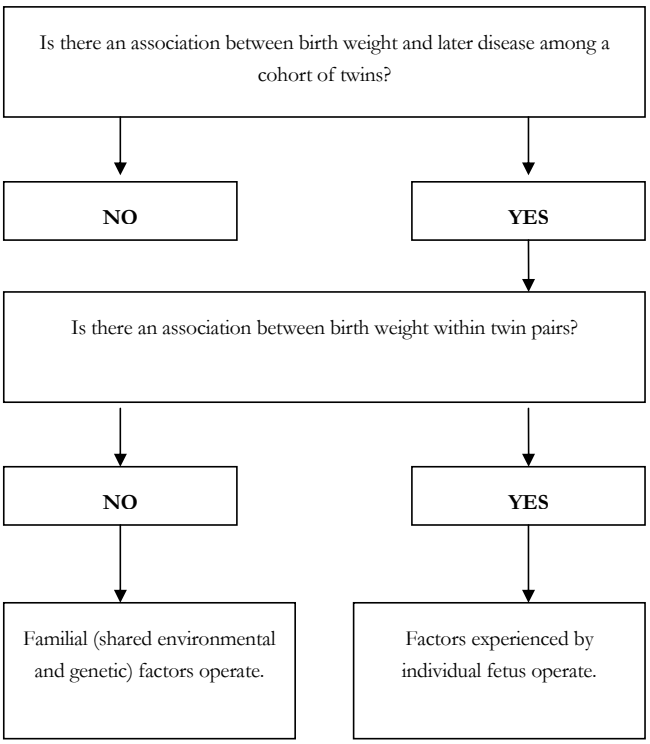

Figure 3. Importance of unique and shared factors in twins with respect to the fetal origins hypothesis.

If associations between birth weight and later disease remain within pairs of twins, factors specific to each individual fetus must be operating. In contrast, if there is no association within twin pairs, then factors which twin pairs have in common are of importance. However, it is likely that both common and unique factors are of importance, in which case we would see a reduction in associations within twin pairs, rather than no association.

Stratifying analyses of birth weight and later disease by zygosity provides the opportunity to shed light on the importance of genetic factors. Dizygotic twins share half of their segregating genes whereas monozygotic twins share all of their segregating genes. Thus, if associations are seen both in cohort of twins and within dizygotic twin pairs, but not within monozygotic pairs, genetic factors are of substantial importance. If associations are diminished or reduced both within dizygotic and monozygotic twin pairs, shared environmental or maternal factors are of importance.

Several twin studies have used this approach to assess the importance of familial (shared environmental and genetic) factors on associations between birth weight and later disease.[31] However, the method has also been criticized by researchers, who claim that findings in twins cannot be generalized to the general population.[52]

Further, other groups of relatives can also be used to assess the importance of familial factors. Studies have used samples of full siblings, and contrasted associations within full sibling pairs with that found in cohorts of singletons to address the issue of confounding by familial factors.[53,54] Although the issue of generalizibility is resolved, the separate effects of genetic and environmental factors cannot be investigated in studies of full siblings.

2.3.4 Birth weight, a proxy of what?

The fetal origins hypothesis was formulated and based on findings of associations between birth weight and later disease.[6] The associations were extrapolated to implicate that maternal and fetal malnutrition during pregnancy influence later risk of disease. Whereas researchers would have wanted measurements on specific factors affecting fetal nutritional uptake, they were left with birth weight, at best available in medical records and registries.

Birth weight is a complex trait which is influenced both by numerous environmental and genetic factors.[11,47] Although important factors influencing birth weight, including gestational age and measured variables of the social environment, have been taken into account, there are numerous social and genetic factors which could be related to both birth weight and cardiovascular diseases. To date, studies assessing the importance of familial factors have often been underpowered and inconclusive.

Animal studies have found that alterations in maternal diet may result in cardiovascular programming of the offspring, without necessarily affecting birth weight, suggesting that birth weight may fail to reflect intrauterine factors associated with later disease.[55] However, although studies have shown that nutritional factors influence later disease risk in animals, they do not provide any evidence of the strength of the effect in humans. The fetal origins hypothesis is formulated based on the association between birth weight and later disease in humans.[6] If such an association would be largely attenuated after controlling for genetic or socioeconomic factors, remaining factors influencing birth weight, including unique fetal

and placental factors that affect fetal nutrition, may have small impacts on risks in adulthood.

2.4 SOCIOECONOMIC AND GENETIC DETERMINANTS

A necessary prerequisite for socioeconomic or genetic confounding of the fetal origins hypothesis, is that there is significant influence of familial (shared environmental and genetic) factors on the studied exposures and outcomes. Several methods have been used to disentangle the effect of shared environmental factors within families from shared genetic factors. Family studies, and correlation of phenotypes between relatives, indicate if familial factors are of importance.

2.4.1 Birth weight

Size at birth is a complex entity, which depends on a number of environmental and genetic factors. Wilcox said that birth weight is "one the most accessible and most misunderstood variables in epidemiology".[56]

Genetically related family members tend to have similar birth weights.[57] Offspring birth weight is associated with both maternal and paternal birth weight, but weaker with respect to fathers birth weight,[58] suggesting that both maternal factors, including genotype, and inherited fetal genotypes operate.

Measures of fetal growth have also been found to have a genetic component. A recent Swedish study found that almost half of the variability in small for gestational age births was accounted for by genetic factors,[59] which corroborated well with previous studies that found genetic effects for birth weight, fetal growth and gestational age.[11,60] However, the most recent study was able to disentangle the effect of maternal and fetal genotype, and found that the genetic variation in small for gestational age was foremost due to the fetal genotype.[59] Other measures of size at birth have also been found to have a genetic component, including birth length and head circumference.[57]

However, resemblance in birth weight between family members may also be influenced by shared environmental factors. Socioeconomic factors influence both intrauterine growth and gestational age.[47]

2.4.2 Cardiovascular diseases

Researchers have for a long while been aware that coronary heart disease tends to cluster within families.[15] Twin studies have shown that the familial clustering of coronary heart disease is at least partly explained by genetic factors.[12]

Monozygotic twins, compared to dizygotic twins, have higher within pair correlations with respect to systolic blood pressure, a pattern which is consistent with a genetic component for blood pressure.[61] In a recent study of six different twin populations, heritability estimates ranged from 52 % to 66 %, suggesting that at least half of the variability in systolic blood pressure is accounted for by genetic factors.[34]

However, although it is well established that genetic factors influence the risk of cardiovascular diseases, socioeconomic factors are also of importance. Socioeconomic factors explain differences in risk of coronary heart disease and type-2 diabetes.[48,62] Interestingly, socioeconomic status in childhood influences the risk of cardiovascular disease independently of socioeconomic status in adulthood.[48]

2.4.3 Intelligence

Social environment has a strong influence on intellectual performance, where individuals from deprived socioeconomic environments perform worse on test of intelligence and cognition than individuals from affluent environments.[63] Evidence of genetic factors influencing intelligence in adulthood was published already in the 1960s, when correlations within relatives followed a pattern which was consistent with polygenic inheritance.[64] Later reviews of studies assessing the influence of genetic factors on adult intelligence have found that at least half of the variability in intelligence in adults is attributed to genetic differences.[13] Furthermore, it was found that the influence of genetic factors increases with age, and that the influence of shared environmental factors within families decreases with age, following early adulthood.[13]

2.5 FETAL ORIGINS OF CARDIOVASCULAR DISEASES

Barker and colleagues originally found that low birth weight was associated with an increased risk of cardiovascular disease and stroke in the Hertfordshire cohort.[7,65] However, the associations were initially criticized for the large loss of follow up, and lacking control for socioeconomic circumstances and smoking.[66] This criticism was largely answered in a study of 15 000 Swedish men, with a 97 % follow-up rate.[50] They found that men with birth weights in the highest quartile had a 26 % reduction in risk of ischaemic heart disease compared to men with birth weight in the lowest quartile.[50] Furthermore, the association was, if anything, only slightly attenuated after adjusting for socioeconomic factors in adulthood. Since Barker's original work, several studies have replicated the association between birth weight and coronary heart disease,[50,67-70] with few exceptions.[71] A recent review, which included 18 studies, found that a 1 kg higher birth weight was associated with a 16 % reduction in risk of ischemic heart disease.[5]

Although atherosclerosis and lipid profile are predictors and risk factors of coronary heart disease, they are only weakly associated with birth weight.[72-74] However, one study showed that the association between birth weight and cardiovascular mortality was not mediated through blood pressure, suggesting that intermediates of cardiovascular disease may not always be present.[75]

The most studied outcome in the field of fetal origins of cardiovascular diseases is by far blood pressure. The association between size at birth and blood pressure is well established, and has been replicated in a vast number of studies, both when measuring systolic blood pressure in childhood, adolescence, and in adulthood.[8] A systematic review early on reported that a majority of the published studies found an inverse association between birth weight and systolic blood pressure.[76] Subsequent meta-analyses have also found to a varying degree, that decreasing birth weight is associated with decreasing systolic blood pressure.[8,19] Although debated,[19] it has been suggested that the association between birth weight and systolic blood pressure amplifies with increasing age.[77]

2.5.1 Environmental confounding

2.5.1.1 Ecological studies

Forsdahl speculated that the ecological associations between infant mortality and arteriosclerotic heart disease in adulthood was due to permanent damage in infancy caused by nutritional deficits.[1] However, the possibility of socioeconomic confounding was also discussed, after finding an association between infant mortality and malignant neoplasms, including lung cancer.[1] He suggested that part of the association with heart disease may be caused by an increased tendency to smoke cigarettes.[1] The same concern was discussed by Barker and Osmond,[2] who judged the association between infant mortality and lung cancer as inconsistent, and hence discarded the possibility of confounding by smoking. In contrast, Ben-Shlomo et al.,[78] showed that the association between infant mortality rates and ischaaemic heart disease was null or attenuated, after adjusting for social class in adulthood. Thus, results from ecological studies are inconsistent with respect to environmental confounding of associations between early life exposures and subsequent risks of cardiovascular diseases.

2.5.1.2 Cohort studies

Several cohort studies of the association between birth weight and coronary heart disease have adjusted for measures of the socioeconomic environment, both at birth, and in adulthood. The most recent meta-analysis of the association between birth weight and ischeamic heart disease, found that the association was unchanged after adjusting for measures of social class, either at birth or in adulthood.[5] However, the authors argued that residual confounding was possible. Measures of social class do not capture the full range of social differences, and its relationship with relevant risk factors, such as smoking, diet and physical activity.[5] In fact, using social class as a proxy of socioeconomic position underestimates the association between social risk factors and mortality.[79] However, of the reviewed studies with information on social class,[50,70,80,81] only one study found that adjusting for social class attenuated the association between birth weight and ischemic heart disease.[50] However, the attenuation was, if anything, trivial, questioning the concern about residual social confounding. Studies have also found that socioeconomic factors or education do not explain the association between birth weight and myocardial infarction.[68,70] If social factors were of importance, adjusting for social factors, albeit crude measures, would at least partly attenuate the association between birth weight and cardiovascular disease.

The same authors who published the meta-analysis on the association between birth weight and ischeamic heart disease, have previously published a meta-analysis with respect to systolic blood pressure.[19] Only seven of the 55 reviewed papers had adjusted for any measures of the social environment, and the authors concluded that the association between birth weight and blood pressure could likely be due to residual confounding.[19] Although some have suggested that part of the association between birth weight and blood pressure could be attributed to socioeconomic factors,[82] most studies which have examined the effect of socioeconomic factors have, if anything, only found small effects. Koupilova et al. found that adjusting for socioeconomic measures in adulthood did not alter the association, whereas adjusting for social environment at birth slightly reduced the

association.[51] However, another study found that the association between birth weight and blood pressure was not confounded either by mother's education, or childhood and adult social class.[83] A recent study in five different European samples, found that adjusting for paternal social class if anything only slightly attenuated the association between birth weight and systolic blood pressure, and adjusting for mother's education had no effect on the associations.[84]

Studies of the association between birth weight and blood pressure within full siblings, which provide control for unmeasured social factors in childhood and fixed maternal factors, have found that shared environmental factors do not confound the association. Leon et al. found that the association was in fact stronger within full siblings than between unrelated individuals.[54]

Maternal smoking is causally associated with restricted fetal growth, and the weaker association between smoking and preterm birth is probably also causal.[85] Although inconsistently,[86] maternal smoking during pregnancy has also been found to be associated with offspring blood pressure.[87] Thus, some have suggested that the association between birth weight and later cardiovascular disease may to some extent be due to maternal smoking.[88] However, a recent study found that both the associations between paternal and maternal smoking during pregnancy and offspring blood pressure were of similar magnitude and attenuated when controlling for social factors.[89] The authors concluded that observed increase in offspring blood pressure among mothers that smoked during pregnancy seen in crude models were not caused by intrauterine mechanisms but rather by familial (shared environmental and genetic) factors. This suggests that mothers who smoke during pregnancy provide a different social environment for the offspring than mothers who do not smoke, which would be consistent with the hypothesis proposed by Brion et al.[89]

Irrespective of mechanisms, maternal smoking has been found to influence both offspring birth weight and blood pressure.[85,87] However, studies which have adjusted or accounted for maternal smoking during pregnancy, have found that it does not explain the inverse association between birth weight and blood pressure.[54,90] Furthermore, the association between birth weight and blood pressure has primarily been observed in cohorts, born at a time when maternal smoking during pregnancy was unlikely to be prevalent.[17]

In summary, results from cohort studies do not generally support the hypothesis that the association between birth weight and subsequent risks of cardiovascular diseases are confounded by environmental factors.

2.5.2 Genetic confounding

Several researchers have highlighted the possibility that the association between birth weight and cardiovascular diseases is confounded by genetic factors.[44,45,91,92] There is some evidence that low birth weight and hypertension may be inherited together. Studies have found that adjusting for parental blood pressure attenuates that association between birth weight and blood pressure.[41,54] Authors have argued that low birth weight is at least partly influenced by the inherited predisposition for hypertension.[41,54] Another study found that individuals with at least one close hypertensive relative, have both higher blood pressure

and lower birth weight, compared to individuals with normotensive relatives, suggesting that fetal growth and hypertension have a common genetic component.[93]

2.5.2.1 Twin studies

2.5.2.1.1 Cardiovascular diseases

As highlighted by a recent review,[5] only two studies have assessed the importance of genetic factors on the association between size at birth and risk of cardiovascular disease. Furthermore, both studies were conducted by the same research group in Sweden. In the first study they found an association between low birth weight and increased risk of acute myocardial infarction in the whole cohort of twins, which was diminished in the within pair analysis.[44] In the second study, they found that low birth weight was associated with and increased risk of angina pectoris within dizygotic but not within monozygotic twin pairs.[45] Both studies concluded that genetic factors may confound the association between size at birth and risks of cardiovascular diseases.

2.5.2.1.2 Blood pressure

Several studies have assessed the association between birth weight and blood pressure within twin pairs, where some have supported,[91,92] and others refuted the hypothesis that genetic factors contribute to the association.[94,95] The potential confounding by familial (shared environmental and genetic) factors of the association between birth weight and systolic blood pressure was recently scrutinized in a meta-analysis of twin studies.[31] The review covered ten studies and included 3901 twin pairs. Among studies which provided both unpaired (between twin pairs) and paired (within twin pairs) estimates on the effect of a 1 kg increase in birth weight on systolic blood pressure, the pooled unpaired estimate (-2 mm hg/kg) was similar to that found in singletons. However, the paired estimate was not significantly different from zero (-0.4 mm hg/kg), suggesting that factors which twin siblings share confound the association. Similar results were obtained if studies using self-reported information on birth weight were excluded, and analysis was restricted to studies which had obtained information on birth weight from obstetric or midwife records. However, when stratifying the analysis by zygosity, the estimates were not significantly different from each other, or from zero. Thus, the authors were unable to conclude if it was genetic or environmental factors which explained the attenuation of the paired effect among all twins.

2.5.2.1.3 Cardiovascular risk factors

Barker et al. found an association between size at birth and raised serum cholesterol concentrations.[96] Findings have been interpreted as evidence that fetal growth restriction causes permanent damage in the function and structure of organs, which leads to an adverse lipid profile in adulthood, a known risk factor of cardiovascular diseases. However, a recent meta-analysis concluded that birth weight does not influence total blood cholesterol to the extent that it has material impact on vascular disease risk.[73] A previous review similarly concluded that there does not exist a strong association between birth weight and blood lipid levels.[74]

The first twin study to assess the influence of genetic factors on the association between birth weight and lipid profiles, suggested that genetic factors accounted for the association

between low birth weight and high levels of total cholesterol, LDL cholesterol, and apolipoprotein B, whereas factors in utero may account for the association with HDL cholesterol.[97] In contrast, a recent twin study found associations between birth weight and total cholesterol and low-density lipoprotein, in unpaired, but not in paired analysis, both among dizygotic and monozygotic twin pairs.[98] The authors concluded that the association between birth weight and lipid levels are confounded by shared environmental or maternal factors. However, another recent twin study did not replicate any of the findings from the previous studies, either in unpaired or paired analyses.[99]

Increased levels of plasma fibrinogen concentrations have been identified as a risk factor for cardiovascular diseases.[100] Three twin studies on the association between birth weight and fibrinogen concentrations have been published.[101-103] The most recent study supported the possibility that genetic factors contribute to the association between birth weight and clottable fibrinogen levels, as a weaker association was found within monozygotic twin pairs compared to dizygotic twins pairs.[103] However, the unpaired analysis showed that the association was similar and non-significant between and within monozygotic twins. The second study found a weaker association within monozygotic pairs compared to dizygotic pairs,[101] which is consistent with genetic confounding of the association. The third twin study found no association between birth weight and fibrinogen.[102]

In summary, results from these studies are difficult to interpret, most likely because of insufficient statistical power. Furthermore, one of the studies did not present any results on the association in the overall cohort, which makes it impossible to infer whether the null finding within twin pairs is caused by confounding by familial (shared environmental and genetic) factors or not.[102]

2.5.2.2 Intergenerational studies

Following findings that adjusting for parental blood pressure attenuates the association between birth weight and blood pressure in adulthood, it was suggested that low birth weight is a phenotype of an inherited predisposition to cardiovascular diseases.[41] If common genetic factors underlie both fetal growth and risk of cardiovascular disease, there should be an intergenerational association between the two phenotypes. Associations between father's and offspring phenotypes supports common genetic factors, as fathers can only directly affect offspring phenotypes through inherited fetal genes. Mothers, on the other hand, can also influence offspring phenotypes through environmental risk factors during pregnancy.

Barker et al. found an association between mother's birth weight and offspring blood pressure, which was independent of maternal blood pressure.[104] As they found no association between father's birth weight and offspring blood pressure, they concluded that the association with mother's birth weight was not caused by genetic factors, but rather that fetal growth restriction of female fetuses result in permanent changes in physiology which lead to raised blood pressure in the next generation.[104] However, as pointed out by Ijzerman et al,[105] if Barker et al. had used the same birth weight categories as in their original article,[17] which described the study sample, it could be seen that offspring blood pressure increased with decreasing paternal birth weight, suggesting that genetic factors may partly account for the associations.

Several studies have examined the risk of cardiovascular disease among mothers and fathers with respect to offspring birth weight.[40,106-108] There was only one study with null findings.[108] The other three studies found that decreasing offspring birth weight was associated with an increased risk of cardiovascular disease among parents.[40,106,107] Furthermore, all three studies found that the associations were independent of measured confounders, including social environment.[40,106,107]

Studies assessing only mother's risks have all found that low birth weight among offspring is associated with an increased risk of cardiovascular [109-111] and cerebrovascular disease.[112] Several individual studies concluded or hypothesized that the associations found between offspring birth weight and parental risk of cardiovascular disease are at least partly due to common genetic factors.[40,106,109,112] However, in a recent review, which included a meta-analysis of published results, pooled estimates revealed that there was a significantly stronger association between offspring birth weight and mother's risk of cardiovascular disease.[113] The authors suggested that the stronger association between offspring birth weight and mother's risk of cardiovascular disease, compared to father's risk, was consistent with an intergenerational effect on intrauterine growth among mothers, and that fathers risk was due to residual socioeconomic confounding.

In summary, studies investigating genetic confounding of the association between birth characteristics and risks of cardiovascular disease are scarce. Although hampered by statistical power, results from some studies indicate a role for genetic confounding.

2.6 FETAL ORIGINS OF TYPE-2 DIABETES

The increasing prevalence of type-2 diabetes is a global public health challenge, largely due to the strong association between type-2 diabetes and cardiovascular disease.[114] During the last two decades a growing field of research has found associations between restricted fetal growth and an increased risk of type-2 diabetes.[9]

The first large cohort study to investigate the association between birth weight and later risk of type-2 diabetes was conducted by Hales et al. in 1991.[18] Using a cohort of men aged 64 years from Hertfordshire, England, they found that those born with lowest birth weight had more than 6-fold increased risk of type-2 diabetes compared to those with highest birth weight.[18] Furthermore, they found that the highest plasma glucose concentrations following loading were found among men born with low birth weight and with the highest body mass index at age 64.[18] The authors concluded that factors in early life combine with an adult lifestyle which favors obesity, to increase the risk of type-2 diabetes.[18] The findings have also been replicated in numerous epidemiological studies,[9] including a study from Sweden, where the authors found that reduced fetal growth was associated with an increased prevalence of type-2 diabetes.[115]

The first systematic review found that individuals born with a low birth weight generally had an adverse profile of later glucose and insulin metabolism.[116] Thirteen of 16 papers reported an inverse relationship between birth weight and risk of type 2 diabetes.[116] A second review was published in 2007, and included a meta-analysis of published results.[9] The pooled results showed that individuals born with low birth weight (<2500 g) or high

birth weight (>4000 g) had an increased risk of type-2 diabetes compared to individuals that were born with normal birth weight (2500-4000 g).[9] They suggested that the association between birth weight and later risk of type 2 diabetes was not linearly inverse, but U-shaped.[9] They found that this conflicts with almost all the qualitative reviews of the association, since 46 out of 47 reviews suggested that the association was inverse and linear.[9] The authors also highlighted that only ten studies adhered to basal standards of study quality to perform a quantitative analysis of the association between birth weight and risk of type 2 diabetes.[9] Furthermore, even though infants born preterm have decreased insulin sensitivity compared to term controls,[117] only two of the studies included in the meta-analysis were adjusted for gestational age, and all included studies used different confounders to calculate adjusted odds ratios.[9] The authors could therefore not draw any conclusions about the impact of potential confounders on the association between birth weight and risk of type 2 diabetes.[9] Despite some inconsistencies, there is overwhelming evidence that there exists an association between low birth weight and an increased risk of type-2 diabetes. However, the mechanisms of the associations are unknown. During the last decade, two competing hypothesis have been formulated to account for the observed association between fetal growth and type-2 diabetes and insulin resistance, namely the "thrifty phenotype hypothesis" and the "fetal insulin hypothesis".

2.6.1 The thrifty phenotype hypothesis

The thrifty phenotype hypothesis suggests that the association between restricted fetal growth and increased risk of type 2 diabetes is due to maternal malnutrition, or other maternal or placental abnormalities influencing fetal nutrition, and subsequent permanent changes in glucose and insulin metabolism (Figure 4, Altered version of Hales et al.[118]).[23,118] The hypothesis was originally formulated following the observed association between low birth weight and increased risk of type-2 diabetes in the Hertfordshire cohort.[18]

Under circumstances of intrauterine malnutrition, a fetus will adopt a number of adaptive strategies which enhance survival. Whilst favoring nutritional distribution to key organs, such as the brain, insufficient nutrition to insulin and glucose metabolizing organs will result in alterations in the functional capacity of such organs, including decreased islet function and impaired β-cell function, and as a consequence metabolic programming.[23] In conjunction with other metabolic and hormonal consequences of fetal growth restriction,[118] the programming would result in a state which confers advantage in a nutritionally restrained postnatal environment. The hypothesis postulates that such metabolic adaptations will favor thrift (i.e., cells and tissues harness energy more efficiently), which if faced with an affluent diet in childhood, adolescence or adulthood, will increase the risk of type-2 diabetes. Furthermore, it has been suggested that an increased risk of type-2 diabetes may not only be due to malnutrition in utero followed by postnatal overnutrition, but to any mismatch between the prenatal and postnatal nutritional environment.[27]

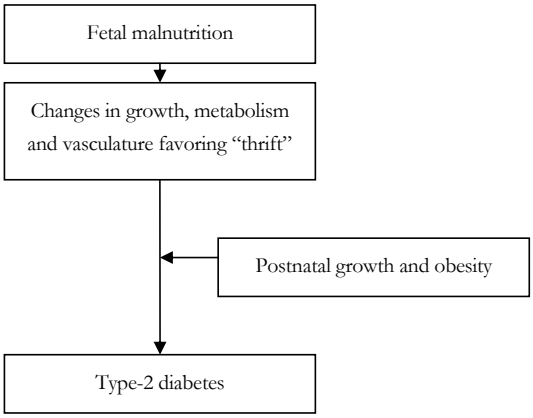

Figure 4. The thrifty phenotype hypothesis.

2.6.2 Fetal insulin hypothesis

According to the fetal insulin hypothesis, associations between restricted fetal growth and symptoms of the metabolic syndrome, including type-2 diabetes, are at least partly caused by an insulin resistant prone genotype.[33] The hypothesis postulates that genetic factors increasing insulin resistance in utero and in adult life produce two phenotypes: a growth restricted infant and an adult with insulin resistance and increased risk of type-2 diabetes.

Fetal insulin secretion is a strong determinant of fetal growth, acting foremost during the third trimester. The most obvious clinical illustration of this are the increased rates of large for gestational age infants found among diabetic mothers compared to non-diabetic mothers. However, Hattersley et al.[33] suggested that fetal insulin mediated growth is not only caused by fetal insulin secretion in response to maternal glucose, but also due to genetic factors in the fetus which regulate insulin secretion and the sensitivity of fetal tissues to the effects of insulin (Figure 5, Altered version of Hattersley et al.[33] and Frayling et al.[37]).

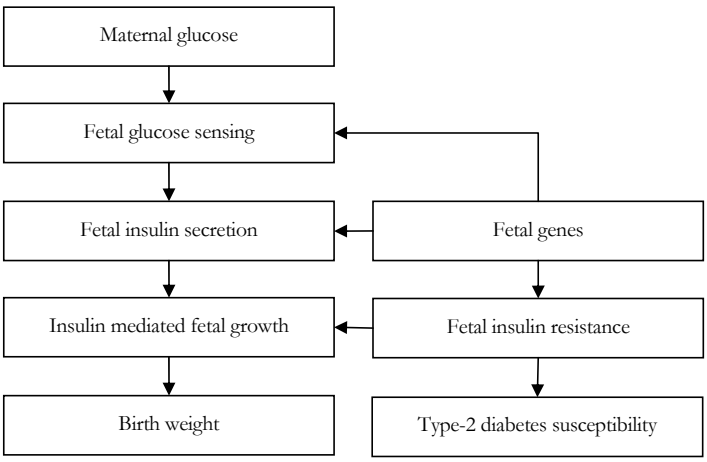

Figure 5. The fetal insulin hypothesis

Genetic abnormalities influencing insulin secretion and insulin resistance, have strong effects on birth weight.[37] Although these conditions have large effects on fetal growth, they are rare, and cannot explain associations between fetal growth and type-2 diabetes in the general population. However, they have established the principle that fetal genes can alter insulin mediated growth. A recent study found that paternal insulin resistance was associated with umbilical cord insulin concentrations in their offspring, even after controlling for maternal confounders.[119] Whereas mothers can affect the growth of fetus both through genetic factors and the intrauterine environment, fathers can only affect fetal growth of their offspring through genetic factors. Thus, the findings suggest that insulin mediated growth may be affected by genetic factors also in normal pregnancies, independent of the intrauterine environment.

2.6.3 Genetic confounding

2.6.3.1 Genetic association studies

To confirm the fetal insulin hypothesis, candidate genes which are associated both with fetal growth and insulin resistance need to be identified. Although several genes involved in insulin metabolism have been studied with respect to their association with birth weight, results have so far been inconsistent.[38]

Hattersley et al. suggested the insulin gene (INS) as a candidate gene as insulin is both a promoter of fetal growth, and insulin secretion and action, which are important in the pathogenesis of type-2 diabetes.[33] Several studies have found associations between allelic variations in the INS gene (INS-VNTR), type 2 diabetes, and measures of insulin,[120,121] whereas others have not.[122] Similarly, some studies have found associations between the INS gene and fetal growth,[123,124] whereas others have not.[125] Lindsay et al. found that common allelic variation in the INS gene (INS-VNTR) was associated both with reduced birth weight and increased prevalence of type-2 diabetes.[126] However, others have failed to replicate these findings, and reporting only weak associations with type-2 diabetes related intermediate traits and no association with fetal growth.[127]

Insulin-like growth factor I (IGF-I), which is regulated both by metabolic and genetic factors, is an important promoter of both pre- and postnatal growth. Several studies have found associations between low IGF-I serum levels and increased risk of type 2 diabetes, making the IGF-I gene a prime candidate for evaluating the fetal insulin hypothesis. Vaessen et al. found that individuals who did not have the wild-type allele of a polymorphism in the promoter region of the insulin-like growth factor I gene were lighter than those homozygous for the allele.[128] They had previously shown that those missing the same wild-type had a 70 % increased risk of type-2 diabetes and myocardial infarction.[129] However, another study was unable to replicate the findings.[130] A recent study found an association between a polymorphism in the IGF-I gene and low serum IGF-I among children born small for gestational age.[131] The authors suggested that polymorphisms in the IGF gene may partly explain the association between low birth weight and risk of type 2 diabetes.[131]

Finding specific genes which are associated with both fetal growth and risk of type-2 diabetes is difficult due to the heterogeneous nature of the disease.[38] Hattersley et al.

suggested that the most probable pathway is that polygenic genetic factors explain the association between low birth weight and type-2 diabetes.

2.6.3.2 Twin studies

Twin studies provide a possibility for additional insights in the fetal programming hypothesis. Examining the association between birth weight and risk of type 2 diabetes within twin pairs is equivalent to controlling for factors that are shared by twin siblings. Within monozygotic twin pairs fetal genes are fully controlled for as they are genetically identical.

To date, seven papers have addressed the topic of genetic confounding of the association between birth weight and type-2 diabetes (or measures of insulin and glucose metabolism). In a study by Poulsen et al. published in 1997, birth data were available for 14 disease-discordant monozygotic pairs and 14 discordant dizygotic twin pairs. In both zygotic groups, birth weights were lower in diabetic twins compared with their non-diabetic co-twins.[132] The authors concluded that the association between birth weight and type-2 diabetes was at least partly independent of a type 2 diabetes susceptibility genotype.[132] However, the authors did not find any difference in birth weight in twin pairs discordant for impaired glucose tolerance, which they themselves interpreted as a theoretical possibility that the association between birth weight and impaired glucose intolerance coincides with a specific genotype.[132] In contrast, in a study of 13 monozygotic and 8 dizygotic twin pairs with increased susceptibility to type 2 diabetes and discordant for oral glucose tolerance, the authors found that twins with abnormal oral glucose tolerance test results were significantly lighter than their co-twin with normal glucose tolerance tests.[133] They concluded that intrauterine environmental conditions may be responsible for metabolic abnormalities in adulthood.[133]

Three twin studies found neither a within-pair nor a between-pair association between birth weight and insulin-glucose metabolism and insulin resistance.[52,134,135] The authors discussed the possibility that the null finding between birth weight and measures of insulin and glucose, both between and within twin pairs, is that low birth weight in twins does not have the same effect as that seen in singletons. Two of the studies also concluded that common genetic determinants for fetal growth and glucose tolerance are not likely to be prevalent or powerful.[52,135]

However, in a sample of 11 162 Swedish twins, an association between birth weight and type 2 diabetes was found both between and within twins pairs, albeit smaller in the within pair analyses.[136] This suggests that the association between birth weight and type-2 diabetes is reproducible in twin samples. Moreover, the results indicate that the association between birth weight and type-2 diabetes is partly caused by growth restriction in utero and partly by common factors shared by twins, including common environment and genetic factors. In contrast, another twin study found that the inverse association between birth weight and insulin secretion and action in elderly twins was strengthened after controlling for genetic factors (i.e., within monozygotic twin pairs).[137] However, although strengthened, the association in young twins was not inverse but positive linear, and results in dizygotic twins were inconsistent.

2.6.3.3 Intergenerational studies

The etiology of type-2 diabetes is heterogeneous. To study if the association between low birth weight and increased risk of type-2 diabetes is confounded by genetic factors, Hattersley et al.[33] suggested that one could study if there is an association between offspring birth weight and paternal risk of type-2 diabetes. If there is a common genetic factor for fetal growth and type-2 diabetes susceptibility, an individual's birth weight should not only be associated with their own risk of type-2 diabetes, but also with that of their parents. Associations between offspring birth weight and father's risk of type-2 diabetes strengthen the fetal insulin hypothesis, as fathers can only directly affect offspring fetal growth through inherited fetal genes.

The most recent intergenerational study, which was conducted by the authors who suggested the fetal insulin hypothesis, found no association between offspring birth weight with paternal insulin resistance, HDL cholesterol concentrations or triglycerides concentrations.[138] Other research groups have also failed to find associations between offspring birth weight and parental risk of type-2 diabetes.[108] However, several studies have found associations between offspring birth weight and parental risk of type-2 diabetes.[39,139-141]

The association between offspring birth weight and mothers' risk of type-2 diabetes has yielded conflicting results. Some studies have found that low birth weight increases the risk of type-2 diabetes and insulin resistance in mothers,[140,142] whereas others have found that high birth weight increases the risk.[141,143] This is probably because women with type-2 diabetes are more likely than non-diabetic women to have experienced gestational diabetes,[144,145] which increases the risk of giving birth to a large for gestational age infant.[146,147]

Associations between low offspring birth weight and increased risk of parental type-2 diabetes may also be explained by other mechanisms than genetic factors. There are at least two additional mechanisms which could explain this association. First, fetal malnutrition may cause permanent physiological alterations, which could result in an adverse intrauterine environment for the individual's offspring.[148-150] This cannot explain associations between low offspring birth weight and father's risk of type-2 diabetes, as fathers can only directly influence offspring birth weight through fetal genetic effects. However, it has been hypothesized that nutritional insults could also induce epigenetic modifications of the fetal genome, affecting later disease risk.[149,151] If this epigenetic state is also associated with factors regulating growth and inherited, the offspring may also be at increased risk of fetal growth restriction. Second, adverse environmental factors may be triggers of both fetal growth restriction and type-2 diabetes, and act across the life course of generations. Socioeconomic status is one of the universally most well known factors associated both with fetal growth,[47] cardiovascular morbidity and mortality in adulthood,[152] including diabetes.[48] Furthermore, parental socioeconomic status strongly predicts offspring socioeconomic status in adulthood.[153]

In conclusion, twin studies are inconsistent with respect to supporting an environmental or genetic basis for the fetal origins of type-2 diabetes. Several studies are small, and many have not found any association, either between or within twin pairs. Although

intergenerational studies generally are consistent with a common genetic component for fetal growth and type-2 diabetes, intergenerational correlations may also be caused by environmental factors.

2.7 FETAL ORIGINS OF INTELLIGENCE

Nutrition plays an important role in the maturation and functional development of the central nervous system.[154] Restricted nutrition in utero may hamper the developing brain in several ways, ultimately resulting in reduced intelligence and cognitive function later in life.[154] Numerous studies have found that low birth weight, a marker of fetal malnutrition, is associated with behavioral problems, impaired cognitive function, and low intellectual performance.[155-158] However, several studies have assessed intellectual outcomes among individuals born very preterm or with very low birth weight, where mechanisms between restricted fetal growth and increased risk of low intellectual and cognitive performance may be accounted for by pregnancy and neonatal complications.[159] However, a recent systematic review found a positive association between birth weight in the normal range (<2500 g) and intelligence in childhood, suggesting that normal variation in fetal growth is also associated with intelligence later life.[10]

There are three factors which are important for brain development: genetic, social, and nutritional factors. Insults resulting from any of these factors may result in varying degrees of cognitive dysfunction and reduced intellectual performance. Several studies suggest that intrauterine malnutrition is especially detrimental among the socially disadvantaged.[154] For example, individuals from low socioeconomic environments are not only at risk of malnutrition,[47] but also unfavorable social factors, such as inadequate education and impaired familial relationships. Studies have found that individuals from disadvantaged environments have remarkably lower intellectual performance than those from affluent environments.[63] Thus, there is higher risk of fetal malnutrition and adverse social factors among individuals from low socioeconomic groups. Given the genetic variability in intelligence and fetal growth, it is reasonable to suspect that at least part of the association between birth weight and cognitive function may be confounded by familial (shared environmental and genetic) factors.

2.7.1 Environmental and genetic confounding

The association between birth weight and intelligence has been observed in many studies.[10] However, adjusting for socioeconomic factors partly attenuates the association.[160-162] As socioeconomic factors are only crude measures of the social environment, which do not capture all social variability, residual confounding of unmeasured familial factors may still be a problem. As maternal and family characteristics, including genotype and numerous social factors, are concordant between sibling pregnancies, sibling analyses can be used to control for such factors.

To date, five studies have assessed the association between birth weight and intelligence within full siblings.[53,162-165] The methodology of using within sibling analysis is to assess the importance of familial factors on the association. Record et al. found an association between birth weight and verbal reasoning scores in the overall cohort but not within full siblings, concluding that socioeconomic factors confound the association.[165] Another study found that birth weight was associated with verbal ability in adolescence in the overall

cohort, but not within sibling pairs.[163] Recently, these findings were replicated by Lawlor et al. who found an association between birth weight and intelligence between unrelated siblings, which was attenuated within full siblings.[162] However, Lawlor et al. had previously, in a different yet small sample, found an association between birth weight and verbal comprehension and general intelligence in childhood, within sibling pairs, concluding that fixed maternal and socioeconomic factors do not confound the association.[164] This conclusion was also supported by Matte et al., who had previously found a within sibling association between birth weight and full IQ, at least for boys, and argued that the association is independent of familial (shared environmental and genetic) factors.[53]

Although within sibling analyses provide the opportunity to control for fixed maternal factors and shared environmental factors, including socioeconomic environment, full siblings only share half their genes. Thus, within sibling analyses do not provide full control for genetic factors, and gives no possibility to study separate effects of genetic and shared environmental confounding. Thus, such analyses do not permit researchers to assess if it is mainly genetic or environmental factors causing the attenuation in within sibling analyses.

A recent study found that the association between birth weight and intelligence was attenuated up to two-thirds, after taking mother's IQ into account, suggesting that the association may be partly explained by genes.[166] Four twin studies have used within twin pair analyses stratified by zygosity, to determine if the association between birth weight and intelligence is primarily of environmental or genetic origin.[46,167-169] All studies have been small and interpretation of the findings is difficult. The largest of the four studies found that birth weight differences within twin pairs was associated with differences in verbal IQ, within dizygotic but not monozygotic twin pairs, suggesting that the association between birth weight and verbal IQ is confounded by genes.[167] In contrast, they found that birth weight was associated with full IQ, both within dizygotic and monozygotic twin pairs.[167] A recent study found no association between birth weight and IQ at 7 and 10 years of age within monozygotic twin pairs, suggesting that the association was confounded by genetic factors.[46] However, at age 5 birth weight differences within monozygotic twin pairs was significantly associated with differences in IQ.[46] The latter finding had been seen in two other twin studies, which found a positive association between birth weight and intelligence within monozygotic twin pairs.[168,169] However, the samples were small, 25[168] and 27[169] sets of monozygotic twin pairs.

In summary, although several recent family studies suggest that the shared factors between relatives are of importance for the association between birth weight and intellectual performance, results are inconsistent and twin studies, which provide full control for genetic and maternal factors, are small and underpowered.

3 AIMS

The overall objective of this thesis was to study if associations between measures of fetal growth and adult health are confounded by familial (shared environmental and genetic) factors, including social environment and genetic factors.

The specific aims of the included studies were:

- To study if the association between birth weight for gestational age and risk of high systolic blood pressure in adolescence is confounded by familial factors shared between full siblings, including socioeconomic, environmental and genetic factors (Study I).

- To study if the association between birth characteristics and risk of low intellectual performance in adolescence is confounded by familial factors shared between full siblings (Study II).

- To study if the association between birth characteristics and risk of low intellectual performance in adolescence is modified by gestational age (Study III).

- To study if the association between birth weight and risk of hypertension in middle-aged and elderly twins is confounded by environmental and/or genetic factors shared within dizygotic and monozygotic twin pairs (Study IV).

- To study if the association between offspring birth weight and parental risk of type-2 is confounded by factors shared within twin pairs (Study V).

4 MATERIAL AND METHODS

4.1 SETTING

The included studies were all based on data from Swedish population-based registers. As health registers include information on individual's national registration number, a unique identifier assigned to each resident in Sweden (at birth or immigration), we have been able to individually link information from several data sources.

4.2 DATA SOURCES

4.2.1 The Medical Birth Register

The Birth Register, held by the National Board of Health and Welfare, was established in 1973. The Birth Register contains data on essentially all births in Sweden. Starting with the first antenatal visit, information is prospectively collected from all births, including demographic data, reproductive history, and complications during pregnancy, delivery, and the neonatal period. When the mother and child leave the hospital, such information is forwarded to the Birth Register, where it is computerised. All births and deaths reported to the Birth Register are validated every year against the Register of the Total Population, by use of the mother's and infant's unique national registration numbers. Information about the mother includes maternal age, parity, citizenship or country of birth, complications during pregnancy and delivery, and mode of delivery. Information about the infant includes if he/she is stillborn or born alive, single or multiple birth, birth weight, birth length, head circumference, gestational age, sex, Apgar score, and infant diagnoses.

4.2.2 The Conscript Register

The Swedish Conscript Register includes information about Swedish males, conscripted for military service. Conscription is mandatory and enforced by law. Most men are conscripted at 18-25 years of age, but those with known severe handicaps, congenital malformations or chronic diseases are not generally conscripted (about 2.4 % in each birth cohort). At conscription, all males undergo a thorough health examination, including height, weight, and blood pressure measurements, and a number of cognitive tests.

4.2.3 The Population and Housing Census of 1990

Statistics Sweden has conducted population censuses since 1860 (housing census since 1945) as an effort to highlight changes in the Swedish society. Medical research has benefited greatly from such censuses, as it has enabled researchers to study associations between both the home environment and working conditions and later disease. In the present thesis, the Populations and Housing census was used to collect information on individual's socioeconomic status, education and family structure.

4.2.4 The Multi-generation Register

The Multi-Generation Register was created by Statistics Sweden in the early 1990s by linkage of several different data sources, providing information on all first-degree relatives for residents born in Sweden 1932 or later. To be included in the register, index persons had to be alive in 1960 or born thereafter. Nine million index persons and their parents are included in the register and adoptions and other non-biological relations are flagged.

4.2.5 The Swedish Twin Register (STR)

The STR is a unique resource for the scientific community. It was first established in the late 1950s to study the importance of smoking and alcohol consumption on cancer and cardiovascular diseases while controlling for genetic propensity to disease. Today the STR includes 170,176 twins in 85,088 pairs, in principle all twins born in Sweden between 1886 and 2000.

The STR database is regularly updated with current addresses, as well as information about cancer diagnoses, hospital discharges and inpatient diagnoses, cause of death, conditions during birth, and vital status (all this information is passed on from the relevant national health care registries). Zygosity is assigned based on questions about intrapair similarities in childhood.

In 1973, all like-sexed pairs born 1926-1958 were contacted through a questionnaire. The questionnaire contained information on zygosity, anthropometry, socio-demographic factors (residential history, occupation, education), health-related behaviors (such as alcohol, tobacco and caffeine consumption, diet, physical activity, and stress), and health status (particularly cardiovascular diagnoses, respiratory disorders, asthma and allergy, and headache).

From 1998 to 2002, all living twins born 1958 or earlier, regardless of gender composition of the pair, were subjected to a telephone interview. The study is called the Screening Across the Lifespan Twin Study (SALT). Data were collected over the telephone by trained interviewers (with medical background), using a computer-based data collection system. A number of items were asked to all twins, including information on birth order (first vs. second twin), zygosity, anthropometric data (height and weight), socio-demographic factors (residential history, education, past and present occupation), health-related behaviors (such as alcohol, tobacco and caffeine consumption, diet, physical activity, and stress), and prescription and non-prescription medication use. The interview collected sufficient information to screen for most complex diseases. Special emphasis was put on diagnostic items that can determine whether a twin is likely to have the disease (rather than simply asking the twin whether they have a disease). Items are presented in a "branching" format such that individuals are asked follow-up items (within the item domain) if they respond positively to key introductory items.

4.2.6 Birth records

Information about pregnancy and birth characteristics is routinely filled out at birth in individual birth records. Previous to the initiation of the Medical Birth Register such information was available only in birth records, located in more than 200 archives, all over

Sweden. The birth records in this thesis were collected from delivery hospital records for hospital deliveries, and from midwives' record books for home deliveries. These records include information about the mothers, including maternal age, age at menarche, marital status, occupational status (father's or single mother's profession), residence, parity, and disease during pregnancy. Available information about the infants includes Christian name (if baptized at birth), birth date, exact time of birth, gestational age, birth weight, birth length, head circumference, presentation at birth, and type of (spontaneous or instrumental) delivery. For multiple births, the exact time of birth and birth order of each infant are registered. Moreover, birth order of each twin within a pair is reported to the parish of birth, where Christian names are noted by birth order, which facilitates correct identification of birth characteristics of each twin within like-sexed twin pairs.

4.3 STUDY DESIGN AND SUBJECTS

Table 4.1 provides an overview of the design of the included papers in this thesis. It describes the subjects, data sources, key comparisons, outcome measures, risk factors, and covariates included in the respective papers.

4.3.1 The studies of conscripted males (Papers I-III)

We performed three large population-based cohort studies (Papers I-III) to assess the association between birth characteristics and risks of high systolic blood pressure and low intellectual performance by linking the Swedish Medical Birth Register, Swedish Conscript Register, Swedish Multi-Generation Register and the Population and Housing Census of 1990. The Swedish Birth Register included information on 458,371 live born male infants, born between 1973 and 1981. To achieve higher homogeneity within the studied cohort, we excluded men born to mothers of non-Nordic nationality, men with congenital malformations, men born before 28 weeks or after 43 weeks, and multiple births. Of the remaining 401 264 males, 377 527 (94%) were conscripted between the years 1991 and 2000.

From the Medical Birth Register we retrieved information on birth weight, birth length and head circumference for gestational age, gestational age, and maternal age and parity. Anthropometric birth measurements were standardized according to the Swedish standards, and were expressed in standard deviations (SDs) for gestational age. More than 2 SDs below the mean birth weight for gestational age was defined as light for gestational age, between -2 and +2 SDs as appropriate weight for gestational age, and above 2 SDs as heavy for gestational age. Definitions for birth length and head circumference followed the same procedure. Gestational age was estimated from the date of the last menstrual period and stratified to very preterm (≤31 completed weeks), moderately preterm (32–36 weeks), term (37–41 weeks), and post-term births (≥42 weeks). Parity was defined as the number of births (1,2,3, or ≥4). Maternal age was defined as age in completed years at the time of delivery (≤19, 20-24, 25-29, 30-34, or ≥35).

Table 1. Overview of included papers in the thesis.

Paper	I	II	III	IV	V
Subjects	Singleton boys, born 1973 through 1981			Twins born 1926 through 1958	Twins born 1926 through 1958, and their offspring born 1973 and onwards.
N	330,768	357,768	352,125	16,265	11,411
Data sources	Medical Birth Register, Conscript Register, Population and housing Census 1990, Multi-Generation Register.			Twin Register and Birth records	Twin Register, Medical Birth Register, Multi-Generation Register, and Birth records.
Outcome	Hypertension	Low intellectual performance		Hypertension	Parental diabetes
Risk factors	Birth weight-, and birth length for gestational age, and gestational age.	Birth weight-, birth length-, and head circumference for gestational age, and gestational age.		Birth weight	Offspring birth weight for gestational age.
Covariates	Height, BMI, year of conscription. Maternal age and parity. Household socioeconomic status, education and family structure.	Growth in height, BMI and year of conscription. Maternal age and parity. Household socioeconomic status, education and family structure.		Gestational age, sex, birth year, maternal age and parity, parental SES, SES in adulthood, BMI, height, smoking, alcohol consumption.	Maternal age and parity, parental birth year, parental BMI in adulthood, parental smoking in adulthood, parental SES in adulthood, grand parental SES, and parental birth weight.
Key comparison	Cohort analysis Analysis between and within full siblings		Cohort analysis Stratified analysis by gestational age	Cohort analysis Co-twin control analysis within dizygotic and monozygotic twins.	Cohort analysis Analysis between and within twin pairs.

At the time of military conscription, Swedish men undergo a thorough health examination that includes measurements of weight, height, and blood pressure, and tests of intellectual performance. Weight is measured in kilograms (in light indoor clothes) and height is measured in centimeters (without shoes). Body mass index (BMI) was calculated as the ratio between adult weight and squared adult height (kg/m^2). Blood pressure (BP) is measured after 5 to 10 minutes rest in the supine position. If the measurement is considered elevated (systolic BP ≥135 mm Hg or diastolic BP ≥85 mm Hg), a second measurement is performed and the lowest measurement is recorded. We defined systolic blood pressure as high if it was ≥140 mm Hg. Intellectual performance is measured in 4 dimensions (logical/inductive, verbal, spatial, and theoretical/technical) and conducted through a time-limited test. The test consists of 160 questions, 40 for each dimension, and has been computerized since 1994, which precludes observer bias. The results on intellectual performance are presented as standard 9 (stanine) scores. Low intellectual performance was defined as a score of 2 or less. Individuals scoring 2 or less in intellectual performance can be expected to have difficulties in coping with basic education programs. Measurements of systolic blood pressure and intellectual performance were available for 330 768 and 357 768 of the conscripted boys, respectively.

The variables coming from the Population and Housing Census were classified according to recommendations set fourth by Statistics Sweden. Socioeconomic category was classified in the following manner: unskilled blue-collar workers; skilled blue-collar workers; low-level white-collar workers; intermediate-level white-collar workers; high-level white-collar workers; and self-employed. Households' highest education was similarly classified into 9-year compulsory school, upper secondary school 2 years, upper secondary school 3 years, higher education ≤3 years, and higher education >3 years. The family structure of the household was categorized as living with both biological parents, only living with biological mother, only living with biological father, and living with neither biological parent.

The Multigeneration register was used to identify full brothers in the defined study population. In all we identified 106 513 boys which had one or more brothers in the defined study population. Among these, 89 856 and 96 189 had information on high systolic blood pressure and low intellectual performance, respectively.

4.3.1.1 Papers I-II

In the first two population-based studies of conscripted males, we studied whether familial (shared environmental and genetic) factors confounded the association between birth weight for gestational age and risk of high systolic blood pressure (Paper I), and the association between birth characteristics (birth weight-, length-, and head circumference for gestational age) and risk of low intellectual performance (Paper II). Two methodological approaches were used to elucidate the importance of familial factors: First, to estimate the overall effect of birth characteristics on the studied outcomes we used regular cohort analyses. This type of analysis provides limited possibilities to adjust for measured factors of socioeconomic environment and no possibilities to adjust for genetic factors. Secondly, we restricted the study population to boys who had at least one brother in the defined study population, and performed analysis of the association between birth characteristics on the studied outcome between and within pairs of full brothers.

Comparisons within pairs of full brothers provided control for familial factors (brothers share environment in childhood and adolescence and also share on average 50% of their segregating genes).

4.3.1.2 Paper III

In the third paper we studied whether the associations between birth characteristics (birth weight-, length-, and head circumference for gestational age) and risk of low intellectual performance (Paper III) was modified by the gestational age of the boys. Cohort analysis of the association between birth characteristics and risk of low intellectual performance was stratified by subgroups of gestational age: preterm (28-36 completed weeks of gestation), term (37-41 completed weeks of gestation) and post-term (42-43 completed weeks of gestation).

4.3.2 The studies of Swedish twins (Papers IV and V)

We performed two large prospective cohort studies on the association between birth weight and risk of hypertension and type-2 diabetes among Swedish like-sexed twins. The purpose of the studies was to investigate whether the association between birth weight and cardiovascular diseases in adulthood are confounded by familial (shared environmental and genetic) factors.

Eligible participants in the studies were like-sexed twins born in Sweden from 1926 to 1958, who are included in the Swedish Twin Registry (n=37 392). In 1998, 24 295 twins were invited and responded to the SALT interview. The response rate was 74 percent. In this study, we restricted the cohort to twins with known zygosity (n=23 547), as determined by questions regarding childhood resemblance.

In SALT, all participating twins were asked questions regarding their medical history and current use of prescription medication. In Paper IV, individuals were diagnosed with hypertension if they answered yes to both of the following two questions: "Do you have or have you had high blood pressure?" (**Q1**) and "Do you take any medication daily?" (**Q2**), and also named an antihypertensive drug. An antihypertensive drug was identified as a medication having an Anatomical Therapeutic Chemical Classification System (ATC) code of C02, C03, C07, C08 or C09, and listed in the Swedish Drug Compendium (FASS) during the years 1997-2002. In Paper IV, type-2 diabetes was diagnosed if an individual, when asked what type of diabetes they had according to their doctor, responded old-age diabetes, type-2 diabetes or non-insulin dependent diabetes mellitus. If an individual did not know when asked what type of diabetes they were diagnosed with, type-2 diabetes was considered present if they reported that their present or past treatment of diabetes was diet or tablets, or if the age of onset of their diabetes was over 45 years. A schematic view of the algorithm to diagnose type-2 diabetes has been previously presented.[136]

Measurements on birth weight of the twins were abstracted from birth records. For the 23 547 like-sexed twins with known zygosity, birth records, with correct identification of individual twins, were obtained for 18 572 (79 percent) individuals, of which 16 265 also had information on hypertension.

Information on adult weight, height, smoking and alcohol consumption were collected through the 1973 postal questionnaire. Body mass index was calculated as the ratio between weight and squared height (kg/m^2). Smoking status was dichotomized into those who had ever smoked (current and previous smokers) versus those who had never smoked. Alcohol consumption was classified according to recommendations by WHO as low consumption (woman: 0-19 gr alcohol/day; men: 0-39 gr alcohol/day), medium consumption (woman: 20-39 gr alcohol/day; men: 40-59 gr alcohol/day) and high consumption (woman: 40- gr alcohol/day; men: 60- gr alcohol/day).

In Paper V, we studied the association between offspring birth weight and parental (twins) risk of type-2 diabetes. We used the Medical Birth Register to collect data on maternal age at delivery, parity, birth weight and gestational age of the offspring. Birth weight for gestational age was calculated among offspring born between 28 and 43 weeks of gestation by standardizing birth weight according to the Swedish birth weight standards, birth weight for gestational age was expressed in standard deviations (SDs) for gestational age.

4.3.2.1 Paper IV

In Paper IV, we studied if the association between birth weight and hypertension was confounded by familial (shared environmental and genetic) factors within twin pairs. Similarly to Papers I and II, two methodological approaches were used to elucidate the importance of familial factors. First, we performed a cohort analysis, which facilitates comparisons with results from previous investigations (mainly performed on singletons). Cohort analysis does not provide the possibility to test genetic confounding, but permitted us to study the influence of several potential confounders, including socioeconomic status, both at birth and in adulthood. Second, in nested case control analysis stratified by zygosity, we estimated the effect of birth weight and risk of hypertension among hypertensive twins and their healthy (dizygotic or monozygotic) co-twins. Twins are brought up together, share 50-100 % of their genes, and the co-twin control method therefore provides a very effective tool to minimize confounding by familial effects. Analyses within monozygotic twin pairs provide perfect matching for genetic factors, since monozygotic twins share all of their segregating genes.

4.3.2.2 Paper V

In Paper V, we used an intergenerational approach to investigate whether the association between fetal growth and type-2 diabetes is confounded by familial factors. One way to study if the birth weight and type-2 diabetes association may be influenced by familial factors is to study if there is an association between low offspring birth weight and parental risk of type-2 diabetes.[142] If there are common familial factors for fetal growth and type-2 diabetes susceptibility, offspring birth weight should not only be associated with their own risk of type-2 diabetes, but also with that of their parents. Similarly to previous papers in this thesis, we first analyzed the effect of offspring birth weight on parental risk of type 2 diabetes in the whole cohort of twins. Thereafter, we analyzed the association within twin pairs to elucidate the importance of familial factors.

4.4 STATISTICAL METHODS

4.4.1 Controlling for familial factors

Clustered and correlated data occur in a wide variety of situations, including school-based research (where classes or schools form natural clusters), dental research (where teeth from one mouth belong to a cluster), longitudinal study designs (where repeated measurements are taken on a single individual) and family-based research (where relatives form clusters). In the present thesis, clusters have been formed by full siblings (Papers I-III), twin pairs (Papers IV & V), and parents and offspring (Paper V).

Whereas regular cohort analysis can only control for measured factors, for example socioeconomic status, using the different degrees of relatedness within clusters, paired analysis within clusters allows us to control for several factors that family members share (Table 2). As presented in the study design section, several methods were used to assess whether, and if so, which familial (shared environmental and genetic) factors confound the association between fetal characteristics and health in adulthood.

Table 2. Could an association between birth characteristics and health in adulthood be confounded by….?

	Maternal factors	Familial environmental factors	Genetic factors
Type of analyses:			
Cohort	Partly	Partly	Yes
Within pairs of:			
full siblings	Partly	No	Partly
dizygotic twins	No	No	Partly
monozygotic twins	No	No	No

4.4.1.1 Between and within design

In Papers I, II and V we used the "between and within" design to assess whether association between fetal growth and risks in adulthood are confounded by familial factors. Birth weight (and other birth characteristics) generally assume a different value for each member of a family, and the mean of the measurements varies between families. Therefore, birth weight has both a between family component and a within family component. Separate between and within family effects of birth weight can be estimated by partitioning the measurement of fetal growth into a between-cluster ($\overline{X}_i$) and within cluster component ($X_{ij} - \overline{X}_i$). The between coefficient measures the effect of fetal growth on risk in adulthood based on comparisons between families (i.e., unrelated individuals), whereas the within coefficient measures the effect of fetal growth within families (i.e., related individuals).

Differences in birth weight between unrelated individuals (cohort analyses and between analyses), are influenced both by familial (shared environmental and genetic) factors, and unique factors specific for each individual. In contrast, differences in birth weight within related individuals (for example siblings or twins) cannot be influenced by environmental or genetic factors shared by such relatives. Thus, if associations between birth weight and risk in adulthood found between unrelated individuals also remains within relative pairs (i.e., identical between and within estimates), shared factors are not of importance. Thus, unique factors experienced by each individual must be involved. In contrast, if the association is null within related pairs, then factors shared by such pairs explain the association seen between unrelated individuals. However, the most probable situation is that both familial and unique factors are involved, in which case we would see a weaker effect within than between effect. Thus, by contrasting the within with the between effect, we can asses the importance of environmental and genetic factors shared by relatives on the association between birth weight and risks in adulthood. Stratifying the analysis by degrees of relatedness (for example by zygosity) will provide insight as to which familial factors are operating (see next section)

4.4.1.2 Co-twin control design

The second methodology used in the present thesis to control for familial factors, is the co-twin case control design. In these analyses we used a healthy co-twin (in both monozygotic and dizygotic twin pairs) as a control for the case twin. Twins are brought up together, share 50-100 % of their genes, and the co-twin control method therefore provides a very effective tool to minimize confounding by familial effects (i.e., differences in parental socioeconomic environment, childhood or adolescent environment, and genetic factors). If estimates in cohort analysis show associations between birth measurements and disease, and the risk remains similarly high in the co-twin control analyses, the results will provide evidence for a direct effect of fetal growth on the adult conditions. However, if there is no association in the co-twin control analyses, this will indicate the importance of shared factors within twin pairs (environmental and/or genetic).

We performed matched analyses stratified by zygosity. This design is ideal for controlling for genetic risk. Restricting the study to monozygotic twins and comparing twins to their twin siblings will allow us to study differences in birth characteristics in a population in which cases and controls are genetically identical. Using this approach one is therefore confident that risk and protective factors are not confounded by genetic predisposition.

The hypothesis of a causal association between fetal growth and risk of chronic disease will be supported if the twin with indications of reduced fetal growth (e.g. low birth weight) more often develops the disease within monozygotic pairs and within dizygotic twin pairs.

- The hypothesis that genetic factors may have confounded the association between reduced fetal growth and risk of chronic disease is supported if an association is found in the cohort analyses, to a lesser extent among discordant dizygotic twin pairs, but not among discordant monozygotic twin pairs.

- The hypothesis that socioeconomic, environmental or life-style factors early or later in life may have confounded the association between reduced fetal growth

and risk of chronic disease is supported if an association is found in cohort analyses, but not within discordant twin pairs, regardless of zygosity.

- The hypothesis that background (parental) socioeconomic environment may have confounded the association is supported if, in the cohort analyses, the association decreases when adjusted for background socioeconomic environment.

- The hypothesis that socioeconomic environment or life-style factors in adulthood may have confounded the association between birth characteristics and risks of cardiovascular diseases is supported if the associations decrease or disappear after adjustments for these factors, in cohort analyses, and among monozygotic and dizygotic co-twin controls.

In the present thesis we used a nested case-control design to assess the importance of shared factors in twin siblings on the association between birth weight and risk of hypertension (co-twin case control design). Although we had the opportunity to assess the influence of familial (shared environmental and genetic) factors by decomposing birth weight into a between and within twin pair component, such analyses, although conducted in the whole cohort is not more efficient than conditional logistic regression. Logistic mixed effects regression is more efficient than conditional logistic regression when the covariates do not have a perfect negative correlation within pairs.[170] However, such assumptions are based on a common between and within cluster effect. In fact, conditional likelihood logistic regression estimate the same within effect as that obtained in mixed effects models, and are equally efficient.[171]

4.4.2 Papers I-III

Multivariate logistic regression models were used to estimate the risk for high systolic blood pressure and low intellectual performance, in relation to birth characteristics. Odds ratios (OR) with 95 % confidence intervals (CI) were used to estimate relative risk. Due to the clustered nature of the data, risk estimates were fitted with generalized estimating equations (GEE) fitted with a logit link using SAS PROC GENMOD.

In Papers I and II, associations between birth characteristics and risk in adulthood were initially analyzed in the whole cohort of boys. These analyses were adjusted for selected covariates. In Paper I, variables were included in the multivariate analyses if they were judged a priori to be potential confounders (maternal age, maternal parity, household socioeconomic category, household education, household family structure, and conscription year). In addition, since we wanted to study whether the effect of our main exposure (birth weight for gestational age) was independent of birth length for gestational age, height at conscription, and BMI at conscription, these factors were also included as covariates. In Papers II-III, the same criteria as in Papers I-II were utilized for inclusion of variables in the multivariate analysis. However, in addition to the variables discussed above, head circumference for gestational age was also included in the models. Furthermore, in Papers II-III all birth characteristics (birth weight-, length, and head circumference for gestational age) were independently assessed as main exposures, and studied with and without the inclusions of remaining birth characteristics in the models.

4.4.3 Paper IV

In paper IV, data was analyzed both using a cohort and nested case-control approach. The association between birth weight and hypertension was initially analyzed in the twin cohort (n=16 265). Generalized linear mixed models were used to correct for the fact that we have correlated data, with random intercepts that vary from pair to pair and assuming a logit link function fitted with PROC NLMIXED. OR with 95 % CI were used to estimate relative risk.

Covariates were included in multivariate models if they were judged to be potential confounders (birth year, gestational age, sex, mothers age at birth, maternal parity, socioeconomic status at birth and in adulthood). In addition, to study whether the effect of birth weight on risk of hypertension was independent of factors that may be considered in the causal pathway (BMI, height in adulthood, smoking and alcohol consumption), these covariates were included as covariates in additional analyses. Due to varying number of individuals in the adjusted models, we also fitted models which were restricted to individuals which had information on all selected covariates.

The effect of birth weight on risk of hypertension, controlling for common genetic and shared environmental factors, was estimated in a nested co-twin case control analyses. To infer confounding by genetic and shared environmental factors on the association between birth weight and hypertension, the co-twin control analysis was stratified by zygosity. Whereas the cohort analysis utilizes the entire cohort of twins, the co-twin case control analysis was restricted to the 594 dizygotic and 250 monozygotic twin pairs discordant for hypertension. Due to the matched nature of the design, multivariate models only included covariates which varied within a twin pair (BMI, height in adulthood, smoking and alcohol consumption). The paired effects in the co-twin case control analysis were estimated by conditional logistic regression in SAS with PROC PHREG.

In the co-twin case control analysis, healthy co-twins were used as matched controls for the cases. Since twins share intrauterine exposures, maternal factors, 50 percent (dizygotic) or 100 percent (monozygotic) of their segregating genes, and generally childhood and adolescent environment (97 % of the twins responded that they lived with their co-twin until age 15), the matched nature of the co-twin control design minimizes confounding by these factors.

4.4.4 Paper V

First, the association between mean offspring birth weight and parental type-2 diabetes was analyzed in the whole cohort of twins (n=11 411). Second, to study if there was any association between parental and offspring birth weight that was independent of familial (shared environmental and genetic) factors, we analysed if the twin with lowest birth weight within monozygotic and dizygotic twins pairs also had offspring with lower birth weight. Differences, with confidence intervals, in mean offspring birth weight for gestational age were calculated. These analyses were restricted to intact twin pairs discordant for birth weight and with information on offspring birth weight for gestational age (2 841 pairs).

Third, we wanted to estimate the effect of offspring birth weight for gestational age on risk of parental type-2 diabetes, controlling for common genetic and shared environmental factors. This analysis was restricted to intact twins pairs (n=3 369), and estimated the effect of offspring birth weight for gestational age on risk of parental type-2 diabetes between- and within twin pairs.

Due to the clustered nature of our data we fitted random effects linear models assuming a log-linear distribution in order to obtain the estimates from the cohort, as well as the between- and within-twin pair effects of offspring birth weight for gestational age on parental risk of type-2 diabetes. All models were fitted with SAS PROC NLMIXED and OR with 95 % CI were used to estimate relative risk.

5 RESULTS

5.1 PAPER I

Men born light for gestational age (<-2 SDs) had a 14 % increase in risk of a high systolic blood pressure (Table 3), compared to men born with normal birth weight for gestational age (-2 to 2 SDs). The association was not confounded by maternal and sociodemographic characteristics, including parental socioeconomic status and education. The inflated risk between the crude and fully adjusted model, was largely attributed to adjustment for BMI at conscription. Adjusting for birth length for gestational age had no effect on the association between birth weight for gestational age and risk of high systolic blood pressure. Crude and adjusted models had varying sample sizes due to missing information on covariates. Thus, in additional, and unpublished, analyses we restricted all the models to subjects without missing values on any covariates. However, near identical results were obtained in the nested analysis, suggesting that adjusting for the selected covariates did not substantially alter the association between birth weight for gestational age and risk of high systolic blood pressure (data not shown).

Table 3. Crude and adjusted odds ratios (95 % confidence intervals) of high systolic blood pressure (≥140 mm hg) in relation to birth weight for gestational age among Swedish men born 1973-1981 and conscripted for military service 1991-2000.

	Model	
	Crude	Adjusted *
	n = 329,363	n = 303,496
Birth weight for gestational age (SDs)		
< -2	1.07 (1.02 – 1.13)	1.14 (1.07 – 1.22)
-2 to +2 †	1.00	1.00
> 2	0.90 (0.85 – 0.95)	0.85 (0.80 – 0.90)

* Adjusted for year of conscription, maternal age and parity, height and BMI at conscription, household socioeconomic category, highest education, and family structure, birth length for gestational age and gestational age.
† Reference category.

Next, we investigated whether the association between birth weight for gestational age and risk of high systolic blood pressure was confounded by familial (shared environmental and genetic) factors. This analysis was restricted to men who had at least one brother in the defined study population, with information on systolic blood pressure and birth weight for gestational age. In the adjusted analysis (83 548 individuals in 47 002 families) we found that the risk of high systolic blood pressure for a 1 SDs decrease in birth weight for gestational age was 5 % between siblings (OR= 1.05, 95 % CI 1.03 – 1.08), and 8 % within siblings (OR= 1.08, 95 % CI 1.04 – 1.12). Similarly to the cohort analysis, adjusting for selected covariates had no effect on the associations (data not shown). Thus, the risk of high systolic blood pressure related to low birth weight for gestational age does not appear to be confounded by familial factors.

5.2 PAPER II

In the cohort analyses, we found that being born light for gestational age (< -2 SDs) was associated with a 59% increased risk of low intellectual performance, whereas born short or with a small head circumference for gestational age were associated with a 62% and a 40% increase in risk, respectively (Table 4, Crude model). In additional unpublished analyses, we analyzed associations between birth characteristics and risk of low intellectual performance after adjusting for groups of covariates. When we adjusted for socioeconomic factors (household socioeconomic status, education, and family structure), these risks were attenuated, suggesting that the associations are partly confounded by social factors (Table 4, Model 2). The associations between each birth characteristic and risk of low intellectual performance were although further attenuated, still significant when including all birth characteristics in the models, suggesting that each individual anthropometric measurement at birth may have an independent association with risk of low intellectual performance (Table 4, Model 4). Similar to Paper I, restricting the models to individuals with no missing values on selected covariates had little effect on the estimates (data not shown).

Table 4. Crude and adjusted odds ratios (95 % confidence intervals) of low intellectual performance in relation to birth characteristics among Swedish men born 1973-1981 and conscripted for military service.

	Model				
	Crude	Model 1 *	Model 2 †	Model 3 ‡	Model 4 §
Birth weight for gestational age (SDs)	n = 356 206	n = 356 113	n = 329 804	n = 321 667	n = 318 857
< -2	1.59 (1.49 – 1.68)	1.65 (1.55 – 1.75)	1.45 (1.36 – 1.55)	1.48 (1.38 – 1.59)	1.22 (1.13 – 1.33)
-2 to +2 ¶	1.00	1.00	1.00	1.00	1.00
> 2	1.01 (0.94 – 1.08)	0.95 (0.89 – 1.02)	0.94 (0.87 – 1.01)	0.87 (0.81 – 0.95)	0.98 (0.90 – 1.06)
Birth length for gestational age (SDs)	n = 355 335	n = 355 242	n = 329 001	n = 321 727	n = 318 857
< -2	1.62 (1.51 – 1.72)	1.62 (1.51 – 1.73)	1.47 (1.37 – 1.58)	1.57 (1.45 – 1.70)	1.33 (1.22 – 1.46)
-2 to +2 ¶	1.00	1.00	1.00	1.00	1.00
> 2	0.89 (0.83 – 0.95)	0.88 (0.82 – 0.94)	0.89 (0.83 – 0.96)	0.78 (0.73 – 0.85)	0.81 (0.75 – 0.88)
Head circumference for gestational age (SDs)	n = 352 650	n = 352 557	n = 326 549	n = 318 899	n = 318 857
< -2	1.40 (1.33 – 1.48)	1.45 (1.37 – 1.53)	1.39 (1.31 – 1.47)	1.39 (1.31 – 1.48)	1.28 (1.20 – 1.37)
-2 to +2 ¶	1.00	1.00	1.00	1.00	1.00
> 2	0.81 (0.75 – 0.88)	0.82 (0.76 – 0.89)	0.83 (0.76 – 0.91)	0.80 (0.73 – 0.87)	0.84 (0.77 – 0.91)

* Adjusted for year of conscription, maternal age and maternal parity.
† Adjusted for variables above and household socioeconomic category, household highest education, and household family structure.
‡ Adjusted for variables above and growth in height (SDs), and BMI at conscription.
§ Adjusted for variables above and additional birth characteristics (birth weight, length and head circumference for gestational age.
¶ Reference category.

Next, we investigated whether the associations between birth characteristics (birth weight, birth length, and head circumference for gestational age), and intellectual performance were confounded by familial (shared environmental and genetic) factors. In addition to the published findings, we also present additional analysis were confounders have been adjusted for in groups. Similarly to Paper I, we restricted the analyses to men who had at

least one full brother in the defined study population, with information on intellectual performance. After adjusting for variables which vary within families, birth characteristics were associated with risk of low intellectual performance, both in analyses between and within families (Table 5, Model 3). However, the associations were weaker in the analyses within families compared with analyses between families (Table 5, Model 3). For example, after adjusting for all variables which vary within families, the between family effect of decreasing birth weight for gestational age, represented by reducing the family's mean birth weight for gestational age with 1 SDs, led to a 16 % increase in risk of low family mean intellectual performance. The corresponding within-family effect showed that a boy born with a birth weight for gestational age 1 SDs below the family mean suffered a lower (11%) increase in risk of low intellectual performance. Similar differences in between-family and within-family effects were found with regard to birth length and head circumference for gestational age. The increases in the within estimates between the crude models and adjusted models 1, were due to the adjustment for parity. Increasing parity was associated with increasing birth weight but decreasing intellectual performance (data not presented). Adjusting for socioeconomic variables, which are fixed within siblings pairs, attenuated the between pair effect for all birth characteristics. However, the within family effect was still smaller, suggesting that the confounding imposed by familial (shared environmental and genetic) factors can not only be attributed to included measures of socioeconomic environment.

Table 5 Crude and adjusted odds ratios * (95 % confidence intervals) of low intellectual performance in relation to a 1 SDs decrease in birth characteristics, between and within families, among Swedish men born 1973-1981 and conscripted for military service.

	Model				
	Crude	Model 1 *	Model 2 †	Model 3 ‡	Model 4 §
Birth weight for gestational age (SDs)	n = 95 846 (50 101 families)	n = 95 806 (50 098 families)	n = 93 546 (49 849 families)	n = 92 769 (49 758 families)	n = 87 035 (46 802 families)
Between families	1.15 (1.12 – 1.18)	1.16 (1.12 – 1.19)	1.19 (1.15 – 1.22)	1.16 (1.13 – 1.20)	1.12 (1.09 – 1.16)
Within families	1.04 (0.99 – 1.09)	1.10 (1.05 – 1.15)	1.13 (1.08 – 1.18)	1.11 (1.06 – 1.17)	1.10 (1.05 – 1.16)
Birth length for gestational age (SDs)	n = 95 647 (50 079 families)	n = 95 607 (50 076 families)	n = 93 557 (49 851 families)	n = 92 769 (49 758 families)	n = 87 035 (46 802 families)
Between families	1.16 (1.13 – 1.20)	1.16 (1.12 – 1.19)	1.20 (1.17 – 1.24)	1.18 (1.14 – 1.22)	1.14 (1.10 – 1.18)
Within families	1.04 (1.00 – 1.08)	1.06 (1.02 – 1.11)	1.11 (1.07 – 1.16)	1.10 (1.05 – 1.15)	1.10 (1.05 – 1.14)
Head circumference for gestational age (SDs)	n = 94 963 (50 014 families)	n = 94 923 (50 010 families)	n = 92 777 (49 760 families)	n = 92 769 (49 758 families)	n = 87 035 (46 802 families)
Between families	1.14 (1.11 – 1.17)	1.12 (1.09 – 1.16)	1.14 (1.11 – 1.17)	1.11 (1.08 – 1.14)	1.10 (1.06 – 1.13)
Within families	1.02 (0.98 – 1.06)	1.06 (1.01 – 1.10)	1.07 (1.02 – 1.11)	1.05 (1.01 – 1.10)	1.05 (1.01 – 1.10)

* Adjusted for year of conscription, maternal age and maternal parity.

† Adjusted for variables above and growth in height (SDs), and BMI at conscription.

‡ Adjusted for variables above and additional birth characteristics (birth weight, length and head circumference for gestational age).

§ Adjusted for variables above and household socioeconomic category, household highest education, and household family structure.

5.3 PAPER III

In the model adjusted for maternal and parental factors, very and moderately low birth weights for gestational age were associated with increased risks for low intellectual performance, irrespective of gestational age (Table 6, Birth weights for gestational age, Adjusted Model 1). However, when we also adjusted for birth length and head circumference for gestational age, very low birth weight for gestational age was no longer associated with an increased risk for low intellectual performance among male individuals who were born preterm. In contrast to men born term or post-term, there appeared to be no linear trend between birth weight for gestational age and risk of low intellectual performance.

Among male individuals who were born very short for gestational age, risk for low intellectual performance was more pronounced among preterm compared with term- and postterm-born male individuals, irrespective of whether we adjusted only for maternal factors or also adjusted for birth weight and head circumference for gestational age (Table 6, Birth length for gestational age, Adjusted Models 1 & 2).

A very small head circumference for gestational age was, similar to a very short birth length for gestational age, foremost associated with risk for low intellectual performance in male individuals who were born preterm (Table 6, Head circumference for gestational age, Adjusted Model 2). Compared with males born preterm with an appropriate head circumference for gestational age, those who were born preterm with a very small head circumference for gestational age had a near doubled increase in risk for low intellectual performance. In contrast, among males born at term, those born with a very small head circumference for gestational age experienced only a 24% increase in risk.

5.4 PAPER IV

We found that the risk of hypertension increased with decreasing birth weight in the cohort of twins. Using birth weight as a continuous variable, we found that a 500 g decrease was associated with a 42 % increased risk of hypertension in the fully adjusted model (n=9 294). Adjusting for covariates did not substantially alter the association between birth weight and risk of hypertension.

In the co-twin case control analyses, nested within the twin cohort, we found both among dizygotic and monozygotic twins, that rates of low birth weight (≤1 999 g) were higher among hypertensive twins compared with their healthy co-twin controls (Table 7). Among dizygotic twins, a 500 g difference in birth weight within a pair was in the adjusted analysis associated with a 34 % increased risk of hypertension. Contrary to the hypothesis of genetic confounding, we found that the corresponding risk within monozygotic twin pairs was 74 %.

Table 6. Adjusted odds ratios of low intellectual performance in relation to birth characteristics stratified by gestational age, among Swedish men born 1973-1981 and conscripted for military service.

	Preterm (28-36) N = 13 141	Term (37-41) N = 274 149	Postterm (42-43) N = 38 782
Birth weight for gestational age (SDs)		Adjusted Model 1 *	
< -2	1.50 (1.09 – 2.08)	1.46 (1.35 – 1.57)	1.54 (1.29 – 1.85)
-2 to -1	1.58 (1.34 – 1.87)	1.23 (1.18 – 1.27)	1.13 (1.02 – 1.24)
-1 to 1	1.00‡	1.00‡	1.00‡
1 to 2	1.04 (0.88 – 1.23)	0.90 (0.86 – 0.94)	0.92 (0.83 – 1.03)
> 2	1.29 (1.00 – 1.67)	0.94 (0.86 – 1.02)	0.85 (0.67 – 1.09)
		Adjusted model 2 †	
< -2	0.77 (0.49 – 1.19)	1.15 (1.05 – 1.27)	1.35 (1.08 – 1.69)
-2 to -1	1.34 (1.09 – 1.64)	1.11 (1.06 – 1.16)	1.04 (0.93 – 1.16)
-1 to 1	1.00‡	1.00‡	1.00‡
1 to 2	0.99 (0.82 – 1.20)	0.96 (0.92 – 1.01)	0.93 (0.83 – 1.05)
> 2	1.15 (0.82 – 1.62)	1.06 (0.97 – 1.17)	0.87 (0.67 – 1.14)
Birth length for gestational age (SDs)		Adjusted Model 1 *	
< -2	2.25 (1.67 – 3.03)	1.49 (1.37 – 1.62)	1.40 (1.19 – 1.65)
-2 to -1	1.26 (1.09 – 1.47)	1.22 (1.17 – 1.27)	1.23 (1.13 – 1.34)
-1 to 1	1.00‡	1.00‡	1.00‡
1 to 2	1.25 (1.06 – 1.48)	0.88 (0.85 – 0.91)	1.07 (0.93 – 1.24)
> 2	1.36 (1.03 – 1.82)	0.88 (0.81 – 0.95)	0.86 (0.69 – 1.06)
Adjusted model 2 †		Adjusted model 2 †	
< -2	1.78 (1.21 – 2.61)	1.23 (1.11 – 1.36)	1.21 (0.99 – 1.48)
-2 to -1	1.05 (0.88 – 1.25)	1.11 (1.06 – 1.16)	1.19 (1.08 – 1.30)
-1 to 1	1.00‡	1.00‡	1.00‡
1 to 2	1.34 (1.10 – 1.63)	0.91 (0.88 – 0.95)	1.11 (0.95 – 1.29)
> 2	1.47 (1.02 – 2.12)	0.92 (0.84 – 1.00)	0.90 (0.72 – 1.13)
Head circumference for gestational age (SDs)		Adjusted Model 1 *	
< -2	2.16 (1.63 – 2.86)	1.40 (1.31 – 1.49)	1.20 (0.96 – 1.50)
-2 to -1	1.30 (1.09 – 1.55)	1.19 (1.14 – 1.24)	1.07 (0.98 – 1.16)
-1 to 1	1.00‡	1.00‡	1.00‡
1 to 2	0.88 (0.74 – 1.06)	0.93 (0.90 – 0.97)	0.97 (0.89 – 1.05)
> 2	0.95 (0.65 – 1.39)	0.82 (0.74 – 0.90)	0.99 (0.77 – 1.28)
Adjusted model 2 †		Adjusted model 2 †	
< -2	1.89 (1.36 – 2.62)	1.24 (1.16 – 1.33)	1.00 (0.79 – 1.26)
-2 to -1	1.21 (1.00 – 1.47)	1.12 (1.08 – 1.17)	1.00 (0.91 – 1.09)
-1 to 1	1.00‡	1.00‡	1.00‡
1 to 2	0.80 (0.66 – 0.97)	0.98 (0.94 – 1.02)	1.01 (0.92 – 1.11)
> 2	0.77 (0.50 – 1.17)	0.86 (0.78 – 0.95)	1.09 (0.84 – 1.42)

* Adjusted for year of conscription, mother's age at delivery, parity, household's highest socio-economic status, household's highest education and household's family structure.

† Adjusted for all variables in Adjusted Model 1 and remaining birth characteristics (birth weight, birth length, and head circumference for gestational age.

‡ Served as reference group

Table 7. Adjusted odds ratios of hypertension in relation to birth weight in the co-twin control analysis.*†

	Dizygotic twins	Monozygotic twins
	n = 766	n = 356
Birth weight (g)		
≤1999	1.12 (0.50 – 2.52)	3.21 (1.07 – 9.63)
2000 – 2499	1.11 (0.72 – 1.72)	2.27 (1.17 – 4.39)
2500 – 2999 ‡	1.00	1.00
3000 – 3499	0.81 (0.52 – 1.25)	1.03 (0.41 – 2.61)
≥3500	0.27 (0.12 – 0.62)	0.93 (0.18 – 4.89)
Per 500 g decrease	1.34 (1.07 – 1.69)	1.74 (1.13 – 2.70)

* Twin pairs are matched for shared environmental and common genetic factors
† Adjusted for socioeconomic status in adulthood, BMI, height, smoking status and alcohol consumption in 1973.
‡ Reference group

5.5 PAPER V

In the cohort analysis, decreasing offspring birth weight for gestational age was associated with a reduced risk of type-2 diabetes among mothers, and increased risk among fathers (Table 8). Adjustment for covariates decreased sample size considerably; however, nested analysis showed that potential confounders did not substantially influence these risks.

Table 8. Crude and adjusted odds ratios (95 % confidence intervals) of parental type-2 diabetes in relation to a 1 SDs decrease in mean offspring birth weight for gestational age.

	Model			
	Crude	Adjusted 1*	Adjusted 2†	Adjusted 3‡
Mothers §	n = 3 402	n = 3 402	n = 3 402	n = 3 402
	0.28 (0.11 – 0.74)	0.43 (0.22 – 0.84)	0.24 (0.08 – 0.68)	0.30 (0.12 – 0.75)
Fathers §	n = 3 081	n = 3 081	n = 3 081	n = 3 081
	1.49 (1.04 – 2.14)	1.49 (1.03 – 2.15)	1.45 (1.01 – 2.07)	1.48 (1.03 – 2.13)

* Adjusted for parental birth year, parental BMI in 1973, parental smoking status in 1973 and parental SES in adulthood.
† Adjusted for parental birth weight for gestational age.
‡ Adjusted for grand parental SES.
§ All models were restricted to parent and offspring pairs with information on all covariates.

In the within twin pair analyses (1 749 female pairs and 1 620 male pairs) we found that decreasing birth weight for gestational age among offspring decreased mothers' risk of type-2 diabetes, and the risks were of similar magnitude both between and within twin pairs. In contrast, among fathers, corresponding risks were increased, and the within effect (OR= 1.71, 95 % CI 1.10 – 2.67), was slightly lower than the between effect (OR= 1.90, 95 % CI 1.10 – 3.28).

6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

6.1.1 Study design

In this thesis, two common epidemiological study designs were utilized, the cohort study and the case control study. Briefly, the cohort study focuses on the distribution of disease in exposed and non-exposed cohorts, and reversibly, the case-control study assesses the distribution of exposure in cases and controls, i.e., individuals with and without a certain disease.

6.1.1.1 Cohort studies (Papers I-V)

A cohort study is a study in which individuals with a certain exposure (or treatment) are followed over time and compared with another group which is not exposed. In a prospective cohort study, exposures are documented before start of follow-up and occurrence of disease. Internal validity in prospective cohort studies is good as the risk of selection bias, with respect to exposure assessment, is null. Drawbacks of cohort studies included the necessity to obtain data from large populations, as only a minority of those followed develop the diseases. This limitation does not apply to retrospective register-based cohort studies. However, with register-based cohort studies we are restricted to information on exposures and confounders included in the registers. In the present thesis, all included studies used a cohort design, with prospectively collected information on birth characteristics, to assess their association with risks in adulthood.

6.1.1.2 Case-control studies (Paper IV)

In cohort studies, information on exposures and outcomes must be collected on large samples. With certain outcomes, only a small portion of the population develop the disease of interest, in which circumstances a case control study may offer a cost efficient alternative, as information only needs to be collected on cases and their matched controls. However, it is important that the exposure among controls is representative of the source population from which the cases are obtained. In Paper IV, we performed a nested case-control study among twins discordant for hypertension. Normotensive co-twins were individually matched to hypertensive twins, implying that controls are indeed representative of the source population. In case control studies, overmatching for confounding factors may limit the range of exposure. In co-twin case control studies, twins are by definition matched for genetic and maternal (and other environmental) factors. However, limiting the range of birth weight is the sole purpose of the study design. This is done in order to assess if remaining differences in birth weight (caused by unique factors not shared between the twin siblings) are distributed unevenly between cases and their co-twins.

The primary difference between cohort and case-control studies is that whereas cohort studies use the whole source population, case-control studies sample from the source population. Thus, many methodological aspects apply to both study designs. In Paper IV, we had the option to assess the within twin pair association between birth weight and risk of hypertension using a cohort design, instead of a nested co-twin case control design. However, using the appropriate statistical methodology (see statistical methods), both

produce identical within twin pair effects, with equal efficiency. Thus, selection of analysis is much more a matter of presentation.

6.1.2 Internal validity

Two types of errors are common in epidemiological studies, *random errors* and *systematic errors*. Systematic errors are often referred to as biases, and can arise from the way the study subjects have been selected (selection bias), they way variables have been measured (observation bias), or due to some factor that has not been controlled for in the analysis (confounding). Internal validity can be defined as the absence of systematic errors, including bias and confounding, and chance. Whereas random errors can be addressed by increasing sample sizes, systematic errors are independent of the size of the study. In a study which suffers from bias, internal validity may be severely hampered.

6.1.2.1 Selection bias

Selection bias is generally less of a concern in cohort studies compared to case-control studies. Since study participants are selected before the outcome occurs, there is no opportunity for disproportionate sampling by the researcher with respect to disease. Even if disproportionate sampling of exposed and non-exposed occurs, it will not result in selection bias when forming measures of association. Thus, initial selection of study participants is not the most common form of selection bias in cohort studies. Instead, selection bias in cohort studies is often related to the length of participation of the study participants. Selection bias occurs when those lost to follow up have different probabilities of disease compared to those that remain in the cohort, and when the frequency of loss to follow-up is different across exposure groups (i.e., when loss to follow-up is associated both with exposure and disease status). In studies of fetal origins of disease large loss to follow-up may often be a problem due to the very long follow-up periods.[29]

In our studies of conscripted males (Papers I-III), exposure status was related to participation rate. Among those included in the first study, 2.5 % were born small for gestational age, whereas among those lost to follow-up, 3.1 % were born small for gestational age. Similar differences could also be seen in Papers II and III. However, selection bias would not occur unless rate of participation was also associated with disease status. If those lost to follow-up have higher systolic blood pressure (or lower intellectual performance) we likely underestimated the associations, whereas if they have lower systolic blood pressure (or higher intellectual performance) we likely overestimated the associations. However, in all three studies we were able to obtain high rates of follow up. In Paper I, 82 % of the original cohort was analyzed, and corresponding rates in Papers II and III were 89 % and 88 %, respectively. This suggests that the selection bias was, if anything, small.

Similars problem could be observed in Paper IV. However, in contrast to the first three papers, we had some information on birth weight among those with no information on hypertension, and vice versa. Compared to the analysed cohort (n=16 265), we found that excluded individuals had lower birth weight and higher rates of hypertension (Table 9). Given the inverse association between birth weight and risk of hypertension obtained in Paper IV, this suggests that we have, if anything, underestimated the association between birth weight and risk of hypertension.

In Paper V, we found, both among men and women, that those not included in the study had higher rates of type-2 diabetes than those included (Table 9). The number of excluded individuals with information on offspring birth weight was low. However, if (as suggested by the results in Table 9), birth weight is in fact higher among those not included in the study we may have overestimated the association between offspring birth weight and risk of type-2 diabetes in fathers, and underestimated the association in mothers.

Table 9. Means and rates of exposures and outcomes among those with and without data in the defined twin study population (n=23 547).

	Study cohort		Excluded individuals		P-value
	n	Mean or rate (%)	n	Mean or rate (%)	
Paper IV					
Birth weight	16 265	2 647	2 179	2 617	0.01
Hypertension	16 265	14.6 %	3 894	20.1 %	<0.01
Paper V					
Offspring birth weight (SDs)					
Women	5 659	0.40	39	0.71	0.05
Men	5 752	0.43	66	0.52	0.48
All	11 411	0.41	105	0.59	0.08
Type-2 diabetes					
Women	5 659	1.4 %	6 732	4.3 %	<0.01
Men	5 752	2.9 %	5 099	6.5 %	<0.01
All	11 411	2.2 %	11 831	5.2 %	<0.01

6.1.2.2 Observation bias

Systematic errors and erroneous conclusions can occur if information on study participants is inaccurate. In all of our papers, information on main exposures (birth characteristics) were recorded prospectively, thus, it precludes recall bias. In the present thesis all information on birth characteristics was collected from the Swedish Medical Birth Register or from birth records (before 1973).

Misclassification bias is the systematic alteration of estimates resulting from faulty measurements or classification of variables. The frequency of misclassification may be the same or similar in all study groups (non-differential misclassification) or may differ between groups (differential misclassification). Non-differential misclassification generally dilutes the estimated effect of the exposure towards the null. Differential misclassification occurs when the frequency of misclassified individuals vary between those study groups.

Differences in misclassification between cases and controls (or exposed and non-exposed) may mask an association or cause a spurious association. However, this type of misclassification is rare when exposures are recorded before the outcome is known (as in cohort design).

In the present thesis, the main source of misclassification is the ascertainment of hypertension in the twin cohort. Both hypertension and type-2 diabetes was ascertained by self-report. Hypertension has low sensitivity and high specificity,[172] implying that cases are often misclassified as controls, and controls seldom misclassified as cases. Thus, if the misclassification is non-differential, the association between birth weight and hypertension will be diluted toward the null. However, as birth weight is associated with several diseases in adulthood, individuals with low birth weight could be more likely to visit health care facilities than individuals with higher birth weight. A recent study found that individuals that recently used health care, were four times more likely to accurately report hypertension compared to those who had not. Thus, low birth weight may increase the likelihood of detecting hypertension. If this would be the case, we have overestimated the association between birth weight and hypertension. However, in the twin cohort, birth weight was not associated with the probability of having blood pressure checked by a doctor or nurse during the five years previous to being interviewed (data not shown). This suggests that the misclassification of hypertension is non-differential and that we have probably underestimated the effect of birth weight on risk of hypertension.

6.1.2.3 Confounding

Most researchers are well aware that an association between exposure and outcome can be biased if a third factor (i.e., confounder) associated with the outcome, is unevenly distributed between exposed and non-exposed. A confounder is associated with both the exposure and outcome, but not an intermediate factor in the causal pathway between exposure and outcome.

The proposed mechanisms causing associations between size at birth and risks in adulthood, and potential confounding by shared environmental and genetic factors, have evoked a lively debate.[19,28-32] Socioeconomic confounding implies that individuals with low birth weight are more often exposed to an adverse social environment, and associated risk factors, which increase the risk of disease in adulthood. Genetic confounding implies that pleoitropic genes cause both slower growth in utero and increase susceptibility of cardiovascular diseases later in life.

Most epidemiological studies view confounding as bias, leading to spurious associations and result in researchers presenting incorrect conclusions. The present thesis and its included papers have studied the presence of confounding to highlight potential mechanisms of the fetal origins hypothesis. Furthermore, whereas most epidemiological studies of the fetal origins hypothesis have use measured familial (shared environmental and genetic) confounders, we have used sets of relatives, with different degrees of genetic and environmental relatedness, to address the issue of confounding by familial factors.

Full siblings share half of their segregating genes, and fixed maternal and postnatal exposures. Thus, the within sibling pair analyses in Papers I and II have controlled for several of the factors, which critics have argued confound the association between birth weight and risks in adulthood, including hypertension and intelligence. However, assessing associations within full siblings do not provide full control for genetic factors. A recent sibling study found that adjusting for maternal and paternal blood pressure, attenuated the association between birth weight and blood pressure, illustrating the importance of genetic

factors.[54] As full siblings do not share all segregating genes, such genes could be distributed differently between siblings, and hence confound the association between birth weight and blood pressure. However, given the matched nature of siblings and genetic relatedness, one would expect attenuating results within sibling pairs.

Twin studies provide a means by which the influence of fetal factors can be separated from shared maternal, environmental, and genetic factors.[43] If associations between size at birth and risk in adulthood are found within dizygotic, but not within monozygotic twin pairs, genetic factors are of importance. On the other hand, if associations are attenuated both within dizygotic and monozygotic twin pairs, compared to between twin pairs (unpaired analysis), shared environmental factors are of importance. If associations are similar both between and within twins, irrespective of zygosity, unique factors influencing fetal growth in each individual twin are of importance. The latter results indicate that fetal nutrition is an important determinant of risks in adulthood.

Numerous studies have assessed the fetal origins hypothesis using twin samples.[31,44,45,136] However, the applied methodology has differed vastly between the individual studies. Some studies have assessed both the between and within twin pair effects, whereas others have only presented within pair effects. The importance of shared factors, including socioeconomic and genetic factors, can only be assessed by contrasting the association found within twin pairs with what is found between pairs. Although some have argued that between pair estimates should be presented by zygosity,[173] this requirement would only be of concern if the (unpaired) association between birth weight and later risks differs by zygosity.

Although twin studies provide a powerful tool for assessing the importance of familial (shared environmental and genetic) factors, results must be interpreted with caution. If methodological obstacles and shortcomings with twin studies have been dealt with appropriately, associations within twin pairs may better capture the influence of fetal nutrition on risks in adult, compared to studying birth weight differences in unrelated singletons. Compared to differences in birth weight between unrelated singletons, birth weight discordances within twin pairs, are independent of familial factors, and studies within monozygotic twin pairs provide full control for genetic factors.

Another method adopted to assess whether familial factors are of importance is studying the association between offspring birth weight and parental risk of cardiovascular diseases (or vice versa).[107] If fetal growth and cardiovascular diseases (or any other diseases) have a common genetic component, then the parents of low birth weight offspring should themselves have an increased risk of cardiovascular diseases. Coherent with this hypothesis, several studies have found that low offspring birth weight is associated with an increased risk of cardiovascular diseases,[40,106,107] and type-2 diabetes,[39,139-141] both in mothers and fathers. Several of the studies interpreted the findings as evidence supporting the hypothesis of genetic confounding. However, the studies have only adjusted the associations for measured confounders, which had little impact, and none have been able to control for genetic factors. Alternative mechanisms in addition to genetic factors may be causing the associations. Mothers influence offspring birth weight both through inherited maternal genes and the intrauterine environment. Thus, the association between low

offspring birth weight and maternal risk of cardiovascular diseases may be caused by environmental precipitants of cardiovascular disease affecting fetal growth in offspring.

Fathers can only directly affect offspring birth weight through inherited paternal genes, thus providing stronger evidence for common genetic components. However, offspring birth weight and paternal risk of cardiovascular diseases may also be confounded by environmental factors. Mothers and fathers share environment, and unhealthy behaviour in fathers may be expressed in the mothers, and directly affect fetal growth of offspring. Studies have found concordance in spouses for coronary risk factors,[174] hypertension,[175] and type-2 diabetes,[176] suggesting that environmental risk factors of cardiovascular disease and type-2 diabetes aggregate within families. Researchers have also suggested that intergenerational association may reflect intergenerational consequences of fetal programming, where restricted fetal growth may have consequences for fetal growth and disease risk in subsequent generations, a perpetuating effect.[149,151]

In Paper V, we studied the association between offspring birth weight for gestational age and parental risk of type-2 diabetes in twin pairs. By using the genetic and environmental relatedness of twin pairs, we were for the first time able to assess whether familial (shared environmental and genetic) factors shared by twins contribute to the association between offspring birth weight and parental risk of type-2 diabetes. Attenuating association within twin pairs would suggest that familial factors, including shared genes, are of importance. If associations remain within twin pairs, the association is likely to be primarily of environmental origin.

In summary, studying associations between size at birth and risks in adulthood within sets of relatives, favour internal validity. In contrast to studies among unrelated individuals, sibling and twin studies provide control for familial factors, and studies within monozygotic twin pairs provide full control for genetic factors. In addition, differences in birth weight within twin pairs reflect differences in fetal growth.

6.1.3 External validity

External validity concerns the generalizability, or applicability, of the findings to other populations and time periods. A necessary prerequisite for external validity is internal validity. If the findings from a study are flawed, external validity becomes irrelevant. However, external validity does not exist per se given internal validity, it is rather a judgement call, and depends on the characteristics of the studied individuals.

The studies of conscripted males (Paper I-III) were all population-based, and covered a large part of adolescent males born in Sweden 1973 through 1981, implying that the findings should be largely transferable to the Swedish male population with the same age distribution.

The last two studies (Papers IV and V) were conducted on a population-based sample of Swedish like-sexed twins. The generalizability of twin studies relies on the assumption that the association between birth weight and later risks in twins are, irrespective of zygosity (or chorionicty), of similar magnitude with that found in cohorts of singletons. If the unpaired associations are vastly different in cohorts of dizygotic and monozygotic twins, unique

fetal factors in monozygotic pregnancies, not found in dizygotic pregnancies, must be involved. Although the within pair effect in monozygotic twins can be contrasted to the corresponding between pair effect, the external validity can be questioned. Several critics have suggested that findings in twins cannot be generalized to singletons. They have argued that the patterns of intrauterine growth are different between singletons and twins, [6] and consequently, that low birth weight in twins does not have the same implication on cardiovascular disease risk.[52]

Twins have shorter mean gestational age than singletons. A recent study suggested that the association between birth weight and blood pressure may be modified by gestational age.[177] However, our twin samples largely consisted of twins born at term or near term.[178] Another concern regarding twins is that they are significantly lighter at birth than singletons.[179]. Twins have a fetal growth curve similar to singletons during the first two trimesters, but down-regulate growth during the last trimester.[179] Such a growth is, according to the fetal origins hypothesis, consistent with an increased risk of cardiovascular diseases later in life.[6] If the third trimester down regulation of twin fetal growth programmes future cardiovascular disease risk, we would expect to see increased risks of cardiovascular morbidity and mortality among twins compared to the general population. However, twins do not differ from singletons with respect to cardiovascular mortality, and blood pressure.[180,181] Therefore, findings from twin studies should be applicable to singletons. In fact, the most recent meta-analysis of the association between birth weight and blood pressure in twins, found pooled estimates that were consistent with those found among singletons.[31] That "maternal constraint" causing reduced fetal growth in twins during the third trimester is not associated with an increased risk of cardiovascular disease, despite dramatically reduced birth weight, highlights the complexity of identifying causal factors causing the association between birth weight and later risks.

All dizygotic and one third of monozygotic twins have separate placentas, whereas two thirds of monozygotic twins share a single placenta. A possible attenuation of the association between birth weight and later cardiovascular diseases in monozygotic twins may be caused by other mechanisms than shared genetic factors. Monochorionic twins with inter-fetal vascular connections are at risk of birth weight discordance of an etiology not experienced by dichorionic twins (or singletons): the twin-to-twin transfusion syndrome (TTTS). However, monozygotic twins suffering from TTTS have very high risk of mortality and preterm birth, despite access to intrauterine interventions and neonatal intensive care. Since twins in our cohort were born 1958 or earlier, when no advanced obstetric or neonatal care was available, complete twin pairs surviving TTTS are likely to be very rare in our sample.

6.2 FINDINGS AND IMPLICATIONS

The fetal origin of adult diseases hypothesis has resulted in a paradigm shift in the way we view determinants of disease. Previously, genetic inheritance and adult lifestyle factors were seen as the primary contributors to disease. The fetal origins hypothesis suggests that diseases also originate in utero, resulting from adaptations in response to variation in fetal nutrition.[6] The hypothesis has attracted a lot of attention and studies have found that size at birth, as a marker of fetal nutrition, is associated with numerous outcomes in adulthood,

including cardiovascular diseases,[5] blood pressure,[8] measures of cognition,[10] and even rates of marriage (men born small are less likely to be married).[182]

The mechanisms proposed to account for these associations are numerous, and include factors prior to conception, during fetal life, and in postnatal and adult life. Studies have suggested that variation in fetal nutrition and restricted fetal growth could result in permanent organ damage, resetting of hormonal axes and metabolism, and epigenetic modifications.[6,20,26,183] It has also been suggested that the resulting consequences interact with postnatal growth and factors in adulthood.[23,24,118,149] The accumulating findings has proved beyond doubt that there is an inverse association between size at birth and risks in adulthood, and also that the associations are modified by factors later in life.

Although animal studies have established that fetal nutrition can influence several phenotypes,[55] the fetal origins hypothesis is formulated following findings in humans. Animal studies do not provide clues about the strength of the association in humans, or if the associations between size at birth and risks in adulthood are largely attributed to other factors. Thus, several of proposed mechanisms could be largely irrelevant (at least with respect to fetal nutrition) if a third factor is causing a spurious or an inflated association between size at birth and risk in adulthood.

In the present thesis and its included papers, we have studied if familial (shared environmental and genetic) factors shared by family members confound the association between birth characteristics and risks of hypertension, low intellectual performance and type-2 diabetes. If familial factors, and especially genetic factors are of importance, proposed mechanisms of fetal origins of adult disease, and modifying factors in adulthood, are more likely to be a consequence of, and interact with inherited fetal genes influencing fetal growth and susceptibility to disease later in life, rather than fetal nutrition per se. Irrespective of the proposed mechanisms, the mechanisms would, although correlated with size at birth, not be causally dependent of fetal nutrition.

Although the idea of familial confounding of the fetal origins hypothesis has been discarded by some,[184] most studies lack information on important confounders. Although studies have established that measured confounders, foremost measures of the social environment, are unlikely to explain the associations,[50,51,68,70,80,81,185,186] few large studies have had the possibility to account for unmeasured familial factors. Thus, although proposed mechanisms of the obtained associations between birth weight and risks in adulthood, and their interactions with adult factors, are both intriguing and possibly also explanatory, it must first be established if they are a consequence of fetal nutrition, or familial factors (Figure 6).

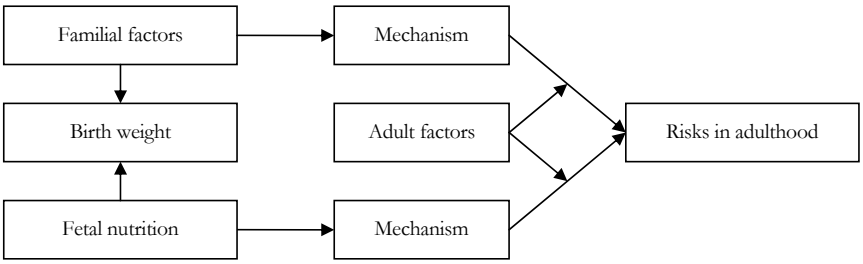

Figure 6. Starting point of mechanisms of the fetal origins of adult diseases: is it familial (shared environmental and genetic) factors or fetal nutrition?

6.2.1 Fetal origins of hypertension

In Paper I, findings suggested that familial (shared environmental and genetic) factors are not of importance for the association between birth weight and risk of high systolic blood pressure (≥140 mm hg) in early adulthood. In a large sample of Swedish (singleton) males, conscripted for military service, we found that the association between birth weight for gestational age and risk of high systolic blood pressure was, although small, significant and independent of measured factors of the social environment (parental educations and socioeconomic status and family constellation). The association between birth weight in kilograms and systolic blood pressure was -1.07 mm hg/kg, which is smaller than what has been found in meta-analyses.[76] Although high readings of systolic blood pressure were rechecked, rates of outcome misclassification may be higher in the present study than previous studies, due to the imprecise measurement of systolic blood pressure at conscription. For example, previous studies have found that blood pressure varies across conscription centres.[187] Furthermore, it has also been suggested that the association between birth weight and blood pressure is amplified with age,[77] which could explain the small effect obtained in our study. Within sibling analyses revealed that the association between birth weight for gestational age and risk of high systolic blood pressure was independent of factors which full siblings share. In fact, the association between birth weight for gestational age and risk of high systolic blood pressure was, if anything, stronger within full siblings than between unrelated individuals.

So which factors can explain that the brother with the lowest birth weight also had the highest systolic blood pressure 18 years later? Several maternal factors regulating fetal growth, including pelvic size and genotype, are fixed between pregnancies, and can therefore not be operating. Furthermore, birth weight discordance between brothers cannot be influenced by maternal nutritional history previous to the first pregnancy, including the mother's own intrauterine exposures. Adverse social factors, some of which are associated with low birth weight and hypertension later in life, are largely shared between siblings, both in early childhood and adolescence. This implies that factors influencing fetal growth which change between pregnancies are of importance. Fetal genes vary between pregnancies and could therefore be one factor, which if pleoitropic, could explain the associations between size at birth and risks in adulthood. A recent Swedish study also found an association between birth weight and systolic blood pressure which

was stronger within than between sibling pairs.[54] They also found that adjusting for parental blood pressure (both mothers or fathers) attenuated the association between sibling pairs, which they interpreted as the possibility of fetal genotype partly explaining the obtained association. However, studying the association between fetal characteristics and later risks within full siblings controls for approximately half the siblings genes, and similar to us, they found that the association was stronger within full siblings, suggesting that controlling for the other half of fetal genotype (not shared) is unlikely to explain the whole association.

However, several studies indicate that birth weight and blood pressure may have a common familial component. Twin studies have found that the association is attenuated within monozygotic twin pairs, suggesting that fetal genes are of importance. [91,92,188] Furthermore, a small Norwegian study found that normotensive men with at least two first or second degree relatives treated for primary hypertension, had lower mean birth weight than normotensive men with no hypertensive first or second degree relatives.[93] Studies have found that adjusting for maternal and paternal blood pressure measured many years after pregnancy attenuates the association between birth weight and blood pressure.[41,54] This could indicate that maternal hypertension during pregnancy and inherited genetic factors may be causing the association. However, one study found that adjusting for maternal blood pressure measured during the third trimester did not attenuate the association.[189] Despite inconsistencies, several studies demonstrate the possibility of genetic confounding. Although intergenerational associations and attenuating associations within related individuals may be caused by shared environmental factors, the influence of social environment has been found to have marginal impact on the association between birth weight and blood pressure. [51]

In Paper IV, we found an association between low birth weight and increased risk of hypertension (self-reported and validated with hypertensive medication) in a cohort of middle aged and elderly twins, which was independent of maternal characteristics, socioeconomic factors, both in childhood and in adulthood, and risk factors of hypertension, including smoking and body mass index. In a nested co-twin case-control analysis of twin pairs discordant for hypertension, we found that the association between birth weight and hypertension was stronger within genetically identical twin pairs, than within fraternal twin pairs, and between unrelated twins. These findings are inconsistent with the hypothesis of genetic and social confounding. In contrast, a recent review of twin studies, found that the association between birth weight and blood pressure was weaker within twin pairs, than between twin pairs.[31] However, the quantitative analysis was unable to determine whether the attenuated association within pairs was attributable to genetic or shared environmental factors. Of the reviewed studies, three concluded or suggested that genetic factors may be influential,[91,92,188] but two of the studies found attenuating results which were partly inconsistent with genetic confounding.[91,188] Shortcomings of twin studies and alternative environmental interpretations, were discussed by the authors of the latter study.[188]

Differences in results in our study and those reported in previous studies may partly be attributable to sample size and definition of outcome. A meta-analysis of twin studies included 7 336 twins with known zygosity from 10 studies, whereas our study included 9 731 dizygotic and 6 534 monozygotic twins, of whom 594 dizygotic and 250 monozygotic

twin pairs were discordant for hypertension.[31] All previous twin studies have compared mean blood pressure measurements among young and middle-aged individuals, whereas our study included middle-aged and elderly twins with diagnosed hypertension. Furthermore, compared to many previous studies which used recalled data on birth weight, our study had prospectively collected information on birth characteristics, and cross-over misclassification of birth weights within twin pairs was minimized. Correct birth identification of each twin was ensured by restriction to twin pairs were both where baptized at birth, or who agreed on birth order in the telephone interview. A validation analyses among twins who were baptized and had information on birth order both in birth records and from the telephone interview, found that 95 % correctly reported birth order in adulthood.

Both our sibling and twin study suggest that fetal genes are not of importance for the association between birth weight and risk of hypertension. The fact that we found that birth weight discordance was associated with hypertension in monozygotic twins, which share all genes and are concordant for all maternal factors both previous to and during pregnancy, strengthens this conclusions. Thus, findings in this thesis suggest that genetic factors, not only fail to explain the association, but they may act as negative confounders: the association between low birth weight and hypertension was stronger within monozygotic twin pairs compared to within dizygotic twin pairs and among unrelated twins. Furthermore, although numerous studies have found socioeconomic disparities in risks of cardiovascular diseases, and increased risks of adverse pregnancy outcomes among socially disadvantaged, including preterm birth and low birth weight, we found that the association between birth weight and hypertension (and variation in blood pressure) was robust, and largely independent of social factors. Although residual confounding may be of concern, both sibling and twin studies control for shared unmeasured environmental factors. Thus, the stronger associations within sibling and twin pairs, further implicate individual variation in fetal nutritional uptake and resulting physiological or epigenetic adaptations as the primary environmental factor influencing risk of hypertension, discarding social factors as potential confounder.

Twin studies may be considered almost ideal in studying associations between birth weight and subsequent risks in adulthood. First, analyses taking zygosity into account allow for varying degress of control for genetic factors. Secondly, twins share all maternal factors, previous to and during pregnancy, and are generally brought up together, thus providing control for unmeasured maternal and social factors. Third, birth weight discordance within monozygotic twin pairs reflects differences in fetal growth. In addition, birth weight differences are common within twin pairs. All dizygotic twins and one third of monozygotic twins are dichorionic. In dichorionic twins, fetal growth is related to both genetic differences (in dizygotic twins) and placental factors (dizygotic and monozygotic twins). Monochorionic twins share one placenta and are genetically identical. In monochorionic pregnancies, discordant fetal growth is related to the vascular architecture of the shared placenta.[190] Although the attenuating results within monozygotic twin pairs may be caused by TTTS, the primary contributor to birth weight discordance in monochorionic twins is unequal placental sharing.[191] Thus, birth weight discordance in monozygotic twins is mainly related to placental factors that influence fetal growth.

Although both our sibling (Paper I) and twin study (Paper IV) indicate that fetal nutrition is the main environmental contributor to the association between birth weight and risk of high blood pressure or hypertension later in life, claims of starting interventions are likely to be difficult, if not impossible. Maternal behaviour and morbidity are factors which influence fetal nutrition and can be altered. Since twins are concordant for maternal factors, our study provides evidence that maternal nutrition is not a necessary component. These results suggest that the association between birth weight and risk of hypertension (and other cardiovascular phenotypes) is influenced by intrauterine factors, including transmission of nutrients to the fetus.

Most studies, including ours (Paper IV), found that birth weight was associated with hypertension also in the upper half of the birth weight distribution, suggesting that the programming effect of fetal nutrition is not only restricted to those who have experienced malnutrition per se, but also to normal variation in fetal nutrition. Although interventions aimed at preventing or terminating severe malnutrition in utero, is ethical and possible, altering transmission of fetal nutrients in an otherwise healthy population, may be detrimental. The ideal birth weight is unknown, and shifting the birth weight distribution to the right will probably lead to increased long-term risks related to high birth weight for gestational age.

6.2.2 Fetal origins of type-2 diabetes

It has been concluded beyond reasonable doubt that size at birth is associated with type-2 diabetes, measures of insulin and glucose metabolism.[9] To account for the observed associations, two main competing hypotheses have been formulated, namely the "thrifty phenotype hypothesis" and the "fetal insulin hypothesis", where the former proposes environmental mechanisms and the latter a genetic mechanisms for the observed association.[23,33]

The hypothesis of genetic confounding of the fetal origins of adult diseases relies on the assumption that pleotropic genes are associated both with restricted fetal growth and increased susceptibility of diseases. The fetal insulin hypothesis forwarded the hypothesis of genetic confounding of the association between size at birth and type-2 diabetes (and insulin metabolism) in adulthood.[33] Support of this hypothesis comes from studies which have found that monogenic diabetes genes are associated with low birth weight.[37] However, such polymorphisms are rare, and other monogenic diabetes genes have been associated with increased offspring birth weight,[37] providing evidence of negative confounding. Contrary to the hypothesis of genetic confounding, a recent study found a copy of common type-2 diabetes gene to be reproducibly associated with increased birth weight.[192] Twin studies have been conflicting with respect to the hypothesis of genetic confounding, where some have found that associations between birth weight and type-2 diabetes (or measures of insulin and glucose metabolism) may be partly explained by genetic factors,[136,137] whereas others have not.[132,133] Several twin studies found no association between birth weight and type-2 diabetes (and insulin and glucose metabolism), either between or within twins pairs.[52,134,135] Furthermore, findings from twin studies have also been questioned regarding generaliziblity to the general population, due to the different intrauterine growth patterns seen in singletons and twins.[52]

However, if the association between size at birth and risks of type-2 diabetes is attributable to fetal genes, then an association between offspring birth weight and parental risk of type-2 diabetes should also be evident. Whereas environmental risk factors of type-2 diabetes in mothers may also influence offspring birth weight, fathers can only directly influence offspring birth weight through inherited fetal genes. Although not all studies have yielded findings which are coherent with this hypothesis,[108] several studies have found that decreasing offspring birth weight is associated with increased risk of paternal type-2 diabetes.[39,139-141] Most have interpreted the inverse association between offspring birth weight and father's risk of type-2 diabetes as evidence supporting the fetal insulin hypothesis. Studies investigating the association between offspring birth weight and mother's risk have yielded conflicting results, finding both linear and inverse linear associations.[140-143] The linear associations have been interpreted that diabetic woman have higher levels of hyperglycemia during pregnancy, and therefore obscure any inverse association. In this context, it is interesting to note that decreasing offspring birth weight was associated with increased maternal risk of type-2 diabetes developed at least 10 years after delivery.[141]

A recent review of studies on the association between offspring birth weight and parental cardiovascular mortality, highlighted that the associations may be stronger in mothers than fathers.[113] They concluded that the association with mother's risk was explained by environmental risk factors of cardiovascular diseases which also cause an adverse intrauterine environment for the offspring, and that father's risk could be attributed to residual social confounding Of the studies which have assessed the association between offspring birth weight and parental risk of type-2 diabetes, none have controlled for genetic factors, and social environment was controlled for using crude measures of parental education and socioeconomic status. Environmental risk factors of type-2 diabetes aggregate within families,[176] providing the possibility of an environmental explanation to the observed association between offspring birth weight and father's risk of type-2 diabetes.

In Paper V, we present a novel approach to study intergenerational associations between birth weight and risk of type-2 diabetes. We studied the association between offspring birth for gestational age and parental risk of type-2 diabetes in twin pairs (twin parents). By utilizing the genetic and environmental relatedness within twin pairs, we were able to assess the influence of familial (shared environmental and genetic) factors on the associations. Similar to previous studies, we found that decreasing offspring birth weight for gestational age was associated with an increased risk of paternal diabetes, but decreased risk of maternal type-2 diabetes. Given then opposing associations in mothers and fathers, this suggests that environmental factors are of importance. Twin pair analyses revealed that the association between offspring birth weight and father's risk was, if anything, only slightly attenuated within twin pairs compared to between pair.

Our findings suggest that although familial factors may partly explain the association between offspring birth weight and risk of paternal type-2 diabetes, they are not of substantial importance. This contradicts previous interpretations of the inverse association between offspring birth weight and father's risk of type 2 diabetes. Instead, findings suggest that it is more likely that unique environmental exposures experienced by each individual twin and its offspring are causing the association. One can only speculate as to

what factors are causing the observed association. Several studies have found that risk factors of type-2 diabetes and cardiovascular diseases, and the diseases themselves, aggregate within families,[174-176] suggesting that adverse environmental factors among fathers are experienced indirectly through the mother. Others have suggested that epigenetic modifications following malnutrition may in fact be inherited.[149,151] In fact, recent studies have found that grandparental prepubertal nutrition predicts longevity and cardiovascular and diabetes deaths in grandchildren. With respect to the fetal orgins hypothesis, the main conclusion that can be drawn from the findings is that familial (shared environmental and genetic) factors are not important. If genetic factors do not operate in intergenerational associations, genetic factors are not likely to operate with respect to an individual's own birth weight and risk of type-2 diabetes.

Our study was hampered by statistical power, and given the different degrees of genetic relatedness among dizygotic and monozygotic twin pairs, analyses stratified by zygosity would have revealed if full control for genetic factors (within monozygotic twin pairs) would have further attenuated the association. Given the inconsistent results found in twin studies, and the small sample sizes, the issue of common genetic factors for fetal growth and risk of type-2 diabetes needs further investigation.

6.2.3 Fetal origins of intellectual performance

Variation in intelligence is influenced both by environmental and genetic factors. Studies have found that approximately half of the variation in adults is accounted for by genetic factors.[13] The influence of genetic factors increases in early adulthood, whereas the influence of familial environmental factors decreases.[13]

The association between size at birth and intelligence is, if anything, only partly explained by measured social factors, whereas the influence of genetic factors for this association has not been studied extensively. Evidence of a common genetic component between fetal growth and intelligence has been seen in adults, where the association between brain volume and intelligence is completed due to genetic factors.[193] Furthermore, adjusting for maternal intelligence attenuates the association between birth weight and intelligence in the offspring.[166] Twin studies have both supported, and refuted the hypothesis that genetic factors explain the association, but have often been small and underpowered.[46,167-169] Similarly, sibling studies have supported both a familial (shared environmental and genetic),[162,163,165] and unique environmental origin of the association between birth weight and intelligence.[53,164] However, the largest and most recent study found that the association was weaker within than between sibling pairs, suggesting that familial factors are of importance.[162]

Our aim was to study if the previously found association between fetal growth and risk of low intellectual performance is confounded by familial factors. In the largest sibling sample to date, we adjusted analyses for measured social confounders, and familial factors shared between full siblings. We found that the association was partly explained by measured factors of the social environment, including socioeconomic status and education of the parents, and familial factors. Although adjusting for social factors attenuated the association between birth weight and risk of low intellectual between full siblings, the within sibling effect remained smaller. This implies that the attenuation

found within full siblings (compared to between siblings) is not accounted for by measured social variables. Thus, consistent with some previous sibling studies,[162,163,165] we found that the associations was at least partly explained by unmeasured familial (shared environmental and genetic) factors.

As discussed previously, association within siblings are accounted for by factors which vary between pregnancies. Conversely, attenuating associations within full siblings are accounted for by factors which are constant between pregnancies. These factors included both social and genetic factors. Full siblings share half of their segregating genes, and one twin study suggested that genetic factors explain the entire association between birth weight and intelligence in childhood.[46] Our results from the cohort and the sibling analyses suggest that both environmental and familial factors are of importance. However, as we could not fully control for genetic factors, what we interpreted as environmental factors may in be fact be residual confounding due to shared fetal genetic factors.

Measuring intelligence is complex, and several of the conflicting results may not only be random and due to small samples, but that studies have captured different aspects of intelligence. Some studies had assessed intelligence with performance measures influenced by behavior, for example school performance,[155,158,161] whereas others have assessed intelligence with standardized tests of intellectual quotient.[156,157] While school performance may often be influenced by social factors, intelligence may be influenced by genetic potential to a greater extent. Furthermore, discrepant results regarding the importance of familial factors may be accounted for by the varying influence of social and genetic factors over time.[13] In our study we measured intellectual performance at conscription, a global measure of results on four separete dimensions of intelligence, logical/inductive, verbal, spatial and theoretical/technical. Besides that, intellectual performance at military conscription is a variable which we unfortunately know little about (test procedures are classified). In addition to intelligence, results on tests of intellectual performance can be influenced by motivation. This hypothesis is supported by the fact that we found that obese boys had an almost two-fold increased risk of low intellectual performance, compared to those with normal weight. The increase may be accounted for by confounding by motivation.

It has been proposed that those born preterm and small for gestational age are at double jeopardy.[194] Both those born small for gestational age, and those born preterm term are at increased risk of cognitive and intellectual impairments.[195] However, the relative contribution and independent effect of prematurity and growth retardation remains unknown. A recent study found that there were no differences in cognitive ability in childhood in preterm infants born small and appropriate for gestational age, when neonatal complications were taken into account.[159] The authors concluded that neonatal complications in preterm infants have larger effects on cognitive function, than if infants are growth restricted or not.

In our study we found that birth weight, birth length and head circumference for gestational age had different impacts on intellectual performance, depending on the gestational age of the individual. When including all measures in multivariate models, we found that birth weight for gestational age was inconsistently (across the birth weight

distribution) associated with low intellectual performance among individuals born preterm, but linearly associated with low intellectual performance among those born at term. Conversely, birth length and head circumference appeared to be important both among those born preterm and at term. It has been hypothesized that fetal length and head circumference increases primarily in the second trimester, whereas fetal weight increases foremost during the third trimester.[196] A recent study found that differences in abdomen circumference in utero between those who are born small and large at birth largely increases with increasing gestational age, whereas differences in femur diaphysis length and head circumference increase mainly during the second and early third trimesters.[197]

These and our results suggest that birth length and head circumference are better proxies of in utero growth restriction than birth weight for gestational age among preterm-born male individuals. The results are also consistent with a critical period of programming. Although the effects of the different measures varied, individuals born preterm appeared more vulnerable to variation in fetal growth. It has been suggested that fetal growth restriction in preterm born infants has specific structural and functional consequence on brain development.[198] Preterm born infants are at increased risk of intra cranial hemorrhage, brain lesions, and abnormalities, which may be further increased if fetal growth is also affected.[199-201]

6.3 CONCLUSIONS

- The association between birth weight for gestational age and risk of high systolic blood pressure in early adulthood is although small, significant and independent of factors which male siblings share, including maternal factors fixed between pregnancies, environmental factors, and common genetic factors.

- The associations between birth weight, birth length and head circumference for gestational, and risk of low intellectual performance are partly confounded by familial (shared environmental and genetic) factors. However, there are remaining influences from size at birth, which are independent of familial factors, and have impact on intellectual performance which are modified by gestational age.

- The association between birth weight and risk of hypertension in twins is similar to that found in singletons, and also independent of familial factors, including socioeconomic status at birth and in adulthood. Birth weight discordance within monozygotic twin pairs was associated with risk of hypertension, suggesting that neither maternal nor genetic factors contribute to the association.

- Decreasing offspring birth weight was associated with a decreased risk of maternal type-2 diabetes, and increased risk of paternal type-2 diabetes. The associations were not attributable to factors which twin siblings share, but rather to unique factors experienced by each parent and offspring.

6.4 FUTURE RESEARCH

The findings from the present thesis and its included papers have both supported and refuted the hypothesis that familial (shared environmental and genetic) factors confound the associations between size at birth and risks in adulthood. The fetal origins and thrifty phenotype hypotheses were formulated to explain the increased risks of cardiovascular diseases and type-2 diabetes among those born with low birth weight. However, the fetal origin of adult diseases has grown to incorporate numerous phenotypes in adulthood, and competing hypotheses supporting a genetic origin of the associations have also been proposed.

Findings in the present thesis have shown that socioeconomic and genetic factors do not appear to confound the association between birth weight and risk of hypertension. We also found that intergenerational associations between offspring birth weight and paternal risk of type-2 diabetes rather than being a phenomenon of inherited pleotropic fetal genes regulating insulin mediated growth and type-2 diabetes susceptibility, appear to be caused by unique environmental factors experienced by each parent and its offspring. In contrast, intellectual performance may share common familial components with fetal growth. Previous findings are also indicative of this conclusion. We were unable to disentangle the effects of shared environmental and genetic factors with respect to the association between birth characteristics and risk of low intellectual performance. However, the attenuation within families suggests that familial factors are of importance. Thus, future research, for example using twin pairs, needs to disentangle and identify which familial components may cause the attenuation. The varying results and conclusions with respect to role of familial factors on fetal origins of adult diseases and risks highlights the complexity of grouping several phenotypes under one unifying hypothesis, and extrapolating conclusions of confounding between adult phenotypes.

For hypertension I propose a number of general and specific questions which should be addressed: are the conclusions applicable to other cardiovascular phenotypes?
What mechanisms explain that normal variation in birth weight influence risks of hypertension? Are mechanisms mainly caused by immediate responses to fluctuations in fetal nutrition, such as structural changes during organ and vasculature development, or are there epigenetic modifications which explain the increased risks?

Future research needs to continue directing attention to structural and regulatory consequences of restricted fetal growth. However, variation in fetal growth in apparently normal fetuses is also associated with risks of cardiovascular diseases in adulthood. This suggests that several distinct processes operate. Fetuses facing fetal malnutrition may respond with permanent detrimental processes which alter physiology, which although beneficial in the short-term, increases susceptibility of cardiovascular disease in adulthood. However, factors influencing variation in fetal growth may not only cause detrimental structural and regulatory responses, but may also lead to changes in gene expression which influence the risk of cardiovascular diseases in adulthood. Most fetuses in western societies are not exposed to fetal malnutrition. Thus, epigenetic modifications in response to mismatch in fetal nutritional supply and demand, may prove to be a far more important pathway than structural effects on organs and metabolic systems following fetal malnutrition.

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