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PREGNANCY WEIGHT GAIN

Family studies on the effects on offspring's body size and blood pressure

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THESIS FOR DOCTORAL DEGREE (Ph.D.)

by

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To my network of beautiful friends and family, and to myself. The relationships and connections we have built mean the world to me. All my love.

ABSTRACT

Introduction: Increasing maternal weight gain during pregnancy, gestational weight gain (GWG), is associated with several adverse outcomes in the child, e.g. high birth weight, childhood overweight and obesity, as well as adult blood pressure (BP). Studies have also shown that specific periods of pregnancy might be more sensitive in terms of influencing these outcomes. However, the aforementioned associations could be explained by genetic and/or environmental factors which are shared between the mother and child. As far as we know, no studies have examined to what extent these genetic and environmental factors explain the variation in GWG.

Aims: The overall aim of this PhD thesis was therefore to investigate the possible associations between GWG and the children's birth weight and body mass index (BMI) during childhood (study 2), and BP during early adulthood (study 3), while taking environmental and genetic factors shared between the mother and child into account (within the twin/sibling pairs). The current thesis also aimed at exploring how much of the variation in GWG which is determined by genetic (the heritability) and both unique and common environmental factors (study 1).

Methods and Results: Study 1 was a register-based twin study with Swedish female monozygotic (MZ) and dizygotic (DZ) twin pairs with children born 1982-1989 and 1992-2010. Genetic factors accounted for 43% of the variation in GWG in the first pregnancy (N = 694 twin mother-pairs) and 26% in the second pregnancy (N = 465 twin mother-pairs). Unique environmental factors explained the remaining variation in GWG. **Studies 2 and 3** were both prospective cohort studies, where study 2 was based on a data-collection of Swedish MZ twin mothers born 1962 to 1975 and their children (N = 82 twin mother-pairs), and study 3 was register-based and included Swedish male sibling pairs born 1982-1989 (N = 4908 brother pairs). In **study 2**, the results indicated that total, and possibly also second and third trimester weight gain, were associated with birth weight in the offspring within the twin pairs in the fully adjusted model. In terms of GWG and offspring weight and BMI during infancy and childhood, no associations were found. In **study 3**, no significant associations were found between GWG and systolic BP, or diastolic BP, or the offspring's risk of hypertension, neither within nor between the sibling pairs.

Conclusions: This thesis shows that the total GWG, and specifically weight gain during the second and third trimester, seem to be positively associated with offspring birth weight, but no effects were seen for BMI during infancy and childhood. However, due to the limited sample size, this requires further investigation. Moreover, no association was found between total GWG and the male sibling pairs' BP at the age of 18 years. The variation in GWG seems to be largely explained by the mother's unique environment during pregnancy and to a smaller degree by genetic factors.

LIST OF SCIENTIFIC PAPERS

1. Elina Scheers Andersson, Karri Silventoinen, Per Tynelius, Ellen Aagaard Nohr, Thorkild I.A. Sørensen and Finn Rasmussen. Heritability of gestational weight gain – a Swedish register-based twin study. *Twin Res Hum Genet* 2015 Aug 18(4): 410-418.

2. Elina Scheers Andersson, Karri Silventoinen, Per Tynelius, Ellen Aagaard Nohr, Thorkild I.A. Sørensen and Finn Rasmussen. Total- and trimester-specific gestational weight gain and offspring birth and early childhood weight: A prospective cohort study on monozygotic twin mothers and their offspring. *Twin Res Hum Genet* 2016 Aug 19(4): 367-376.

3. Elina Scheers Andersson, Per Tynelius, Ellen Aagaard Nohr, Thorkild I.A. Sørensen and Finn Rasmussen. No association of maternal gestational weight gain with offspring blood pressure and hypertension at age 18 years in male sibling-pairs: a prospective register-based cohort study. *PLoS ONE* 2015 March 20. 10(3) (Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0121202)

These papers will be referred to by their numbers (1-3) in the text.

CONTENTS

LIST OF ABBREVIATIONS

BMI	Body Mass Index
GWG	Gestational Weight Gain
GDM	Gestational Diabetes Mellitus
WHO	World Health Organization
MBR	Medical Birth Register
IOM	Institute of Medicine
LMP	Last Menstrual Period
ppBMI	Pre-pregnancy Body Mass Index
BMR	Basal Metabolic Rate
PA	Physical Activity
LGA	Large-for-gestational-age
CI	Confidence Interval
BP	Blood Pressure
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
RCT	Randomized Controlled Trial
MZ	Monozygotic
DZ	Dizygotic
STR	Swedish Twin Registry
MGR	Multi-Generation Register
MSCR	Military Service Conscription Register
SRE	Statistic's Sweden's Register for Education
RTP	Register of Total Population
SEM	Structural Equation Modeling
GEE	Generalized Estimating Equations
SALT	Screening Across the Lifespan Twin study
STAGE	Study of Twin Adults: Gene and Environment
N	Number
mmHg	Millimeter of Mercury
RR	Relative Risk

A	Additive genetic factors
C	Common environmental factors
D	Dominance genetic factors
E	Unique environmental factors
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism

1 BACKGROUND

1.1 GESTATIONAL WEIGHT GAIN (GWG)

1.1.1 Historical background

The concept of foetal and infant growth being sensitive periods for later development of adult disease, due to maternal undernutrition *in utero* or in early postnatal life, was originally introduced in the early nineties by D J P Barker and C N Hales. Their initial review proposed a novel hypothesis which they named the "thrifty phenotype hypothesis" or "foetal origins hypothesis"(1). They believed that their findings, that impaired foetal or infant growth (e.g. low birth weight) was associated with adult cardiovascular disease and Type 2 diabetes mellitus, were due to programming effects of undernutrition on the foetus.

The first studies to link foetal undernutrition to adult obesity were carried out in offspring of women who were exposed to famine during the so called Dutch Hunger Winter in the last part of the Second World War (2, 3). The findings from these studies showed that the male offspring of the mothers with severe undernutrition during early gestation, followed by adequate nutrition for the remaining course of the pregnancy, were more likely to become obese in young adulthood (2). Their results also showed that the exposed female offspring had a higher body mass index (BMI (weight (kg)/ (height $(m)^2$)) and waist circumference than the non-exposed (3). The authors also postulated that programming effects of the foetus could be the mechanism behind their observations, e.g. through dysregulated hypothalamic function affecting later appetite-control.

Due to the current disease burden from the global obesity epidemic, the focus in most upper-middle and high income countries today is on overnutrition rather than undernutrition. In order to possibly prevent obesity and its related co-morbidities, much attention has been given to understanding the mechanisms behind obesity and its possible origins in foetal life. What is known today as the "developmental overnutrition hypothesis" or "foetal overnutrition hypothesis", suggesting that greater supply of nutrients (such as glucose) during pregnancy could result in later obesity in the offspring (4), was originally developed during the 1950's by a researcher named Jørgen Pedersen (5). He proposed in his study that the mechanism behind mothers with diabetes during pregnancy, gestational diabetes (GDM), delivering larger babies, was that the foetus was being overfed during pregnancy. Pedersen speculated that this was due to an increased supply of maternal glucose, resulting in increased foetal insulin (acting as a growth stimulating factor). It has later been hypothesized that the effects of overnutrition on the foetus could be mediated through epigenetic changes (e.g. DNA methylation) taking place in utero (6). This theory is however still much debated as evidence of its effects in humans is scarce (7).

A large amount of studies have been published since Pedersen's work, supporting the developmental overnutrition hypothesis in terms of GDM resulting in increased birth weight through the intrauterine mechanisms previously explained (e.g. (8, 9)). More recent studies have extended this hypothesis to also include more long-term effects of diabetes during pregnancy on the offspring in terms of overweight and obesity and its related diseases (10) (8,

11). It has also been suggested that it is not only GDM which could predispose the offspring to an increased risk of overweight and obesity, but also the mother's pre- or early-pregnancy BMI-status and/or her weight gained during pregnancy, gestational weight gain (GWG) (12-14). All of the latter circumstances are associated with increased adiposity and possibly also an increased *in utero* supply of circulating glucose levels (as well as other nutrients), resulting in foetal overnutrition (15, 16). The mechanisms by which these effects on the foetus could be passed on to the next generation are not yet well understood, but the foetal overnutrition hypothesis is proposed to be a key driver in the intergenerational cycle of obesity (see **Figure 1**) (17, 18).

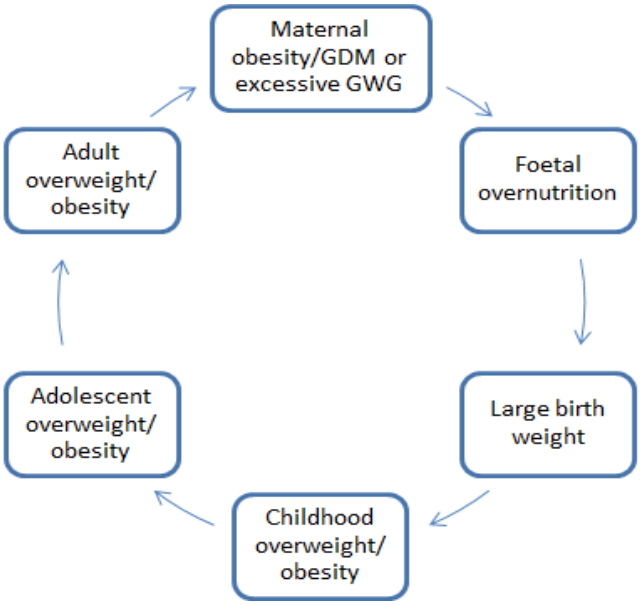

Figure 1. **The intergenerational cycle of obesity.** Note: GDM = Gestational Diabetes Mellitus.

1.1.2 Descriptive epidemiology

Overweight and obesity (defined as a BMI $\geq$ 25 kg/m^2 or $\geq$ 30 kg/m^2 respectively) were once considered an issue in high-income countries only, but the epidemic is currently also on the rise in low- and middle-income countries (19). According to recent data from the World Health Organisation (WHO), around 40% of women above the age of 18 years were categorized as overweight and around 15% as obese, worldwide (20).

As a consequence of the obesity epidemic, the prevalence of women entering their pregnancy being overweight or obese is increasing. In fact, the European perinatal network, Euro-Peristat, reported that around 30-37% of women in most European countries were categorized as overweight or obese according to their early-pregnancy BMI (recorded at the first antenatal visit in most countries), with the exception of Poland, France and Slovenia (which reported lower percentages) and Scotland (which reported a higher proportion of overweight and obese women) (21). However, the latter data was only available for 13 out of 29 European countries which had < 10% missing data. Due to this incompleteness in systematic reporting of data on pre-or early-pregnancy BMI and GWG in most European

countries, the overall burden of maternal overweight and obesity is unclear (22). In Sweden, maternal weight and height started to be recorded in 1992, at the time of registration at the antenatal care clinic (around gestational weeks 8-12). Since then, the prevalence of early-pregnancy overweight and obesity has increased from 25% in 1992 to 38% in 2013 (23).

In terms of GWG, as previously stated, nationally representative data is lacking for the majority of countries (22). In Sweden, data on early-pregnancy weight and delivery weight (used to calculate GWG) from the national Medical Birth Register (MBR) is available for 76% of the women who delivered between 1982 and 1989, and for 33% of the women who delivered between 1992 and 2010. The prevalence of GWG is usually reported according to the guidelines for healthy weight gain in pregnancy (to promote optimal health for the mother and infant), published by the United States (US) Institute of Medicine (IOM) in 1990 and revised in 2009 (see **Table 1** below for the latest version) (24). Many of the studies including a large amount of data on GWG come from the US. According to two of these more recent studies, most women gained above the IOM recommendations (excessive GWG) according to their early-pregnancy BMI (25, 26). In fact, these two latter studies found that between 44-68% of the women gained above the guidelines. They also found that overweight and obese women were more likely to gain an excessive weight compared to normal weight women (25, 26). A large register-based study from Sweden (N ≈ 163,000) recently reported that, for mothers who had their first or second child between 1982 and 2010, around 33-37% had an excessive GWG according to the IOM classification (27). A recent systematic review suggested that the majority of women who were obese in early pregnancy gained above the IOM recommended weight (28). Johnson et al. (25) also observed a trend from 2000 to 2009 (according to their US cohort with data from 14 states), in terms of a statistically significant increase in the proportion of women with excessive GWG.

According to a recent article, the range of GWG observed in a low-risk group of Swedish women was considerably broader than the range currently advised by the IOM (29). The latter study, and another recent study, both found that the weight gain of the women who were overweight or obese in early-pregnancy was well above the IOM guidelines (30). These results are also in line with the findings from other studies (25). The IOM guidelines have received criticism for being too generous for overweight and obese women and it has been suggested that especially obese women could benefit from gaining less weight than what is currently recommended (31, 32).

Table 1. IOM 2009 Recommendations for total and rate of gestational weight gain, by pre-pregnancy Body Mass Index (BMI) for women with singleton foetuses (24).

	Total weight gain	**Rates of weight gain 2nd and 3rd trimester***
Pre-pregnancy BMI (kg/m^2)	**Range in kg**	**Mean (range) in kg/week**
Underweight (< 18.5 kg/m^2)	12.5 – 18	0.51 (0.44 – 0.58)
Normal weight (18.5 – 24.9 kg/m^2)	11.5 – 16	0.42 (0.35 – 0.50)
Overweight (25.0 – 29.9 kg/m^2)	7 – 11.5	0.28 (0.23 – 0.33)
Obese (≥ 30.0 kg/m^2)	5 – 9	0.22 (0.17 – 0.27)

* Calculations assume a 0.5 – 2 kg weight gain in the first trimester.

1.1.3 Components and pattern

Gestational weight gain is a very complex trait influenced by both maternal and foetal environmental and physiological factors, with its primary function being to support the growth and development of the foetus (24). As opposed to maternal weight before pregnancy (pre-pregnancy weight), which reflects the woman's nutritional status and fat accumulation, GWG reflects the growth of the foetus as well as the different components of the weight gain.

In a theoretical model of a normal weight woman (with no oedema or leg oedema) delivering a baby with an average birth weight (≈ 3.4 kg), total GWG is assumed to be approximately 12.5 kg (33). The foetal components of the total weight gain at term (on average 40 weeks), i.e. foetus, placenta and amniotic fluid, comprise approximately 3.4 kg (with large variation), 0.65 kg and 0.80 kg respectively. The maternal components, including tissue (uterus and mammary glands), blood volume, fat stores and extracellular extravascular fluid, make up around 1.38 kg, 1.45 kg, 3.35 kg and 1.48 kg respectively of the total GWG (33). **Figure 2** gives an illustration of the different components. The components with the largest variation are birth weight, maternal fat stores and water (due to a high variability in water retention possibly resulting in oedema) (33, 34). According to the review from the IOM committee (who developed the most recent version of the GWG guidelines), a large part of the variation in GWG is due to the increase in fat mass. Just as for GWG, the fat mass during gestation is inversely associated with pre-pregnancy BMI (24).

The pattern of the weight gain during pregnancy, in normal weight women, is usually described as slightly sigmoidal or linear (29, 35). A recent study by Ismail and colleagues found that GWG increased in a linear fashion in a population of normal weight women from eight different countries around the world (35). Interestingly, they also observed that the patterns of GWG were strikingly similar across their cohort, regardless of culture, ethnicity, behaviours and clinical practice. Hutcheon et al. observed that the weight gain of overweight and obese women seemed minimal until 15-20 weeks gestation and then increased in a linear pattern until term (36). As for the rate of weight gain during pregnancy, women tend to gain more weight during the second (on average 0.45 kg/week) and third trimester (on average 0.40 kg/week) (37) compared to the first (on average 0.24 kg/week) (38). The rate of weight gain during the second trimester is generally higher than the third, except for women who were obese before pregnancy who had a lower weight gain during the second compared to the third trimester (37, 38).

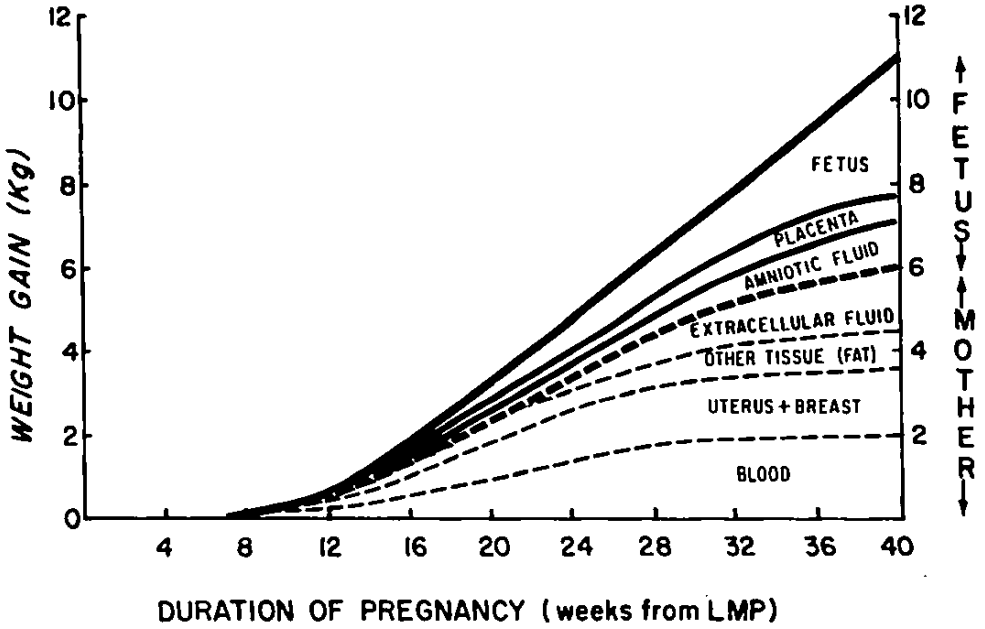

Figure 2. Components of gestational weight gain. Note: LMP = Last Menstrual Period. Reprinted with permission from (39).

1.1.4 Determinants

The weight gained during pregnancy is influenced by several complex factors such as maternal behavioural, physiological, sociodemographic and genetic factors (24), which in turn also influence the foetal environment (intrauterine environment). Some of the major factors, which were considered important in terms of the maternal influence on GWG in the studies included in this thesis, have been summarized below. These determinants are also important to consider as potential confounding factors. This is discussed further in chapter 3.2.3. See **Figure 3** for an overview of these possible determinants of GWG.

The paternal influence on GWG is for natural reasons more difficult to elucidate, thus research in this areas is scarce. However, in terms of foetal growth, paternal factors (e.g. paternal size at birth and adult BMI) have also been shown to have an impact, although to a much lower extent than maternal factors (40). For these reasons the following chapter is focused on the maternal influence on GWG.

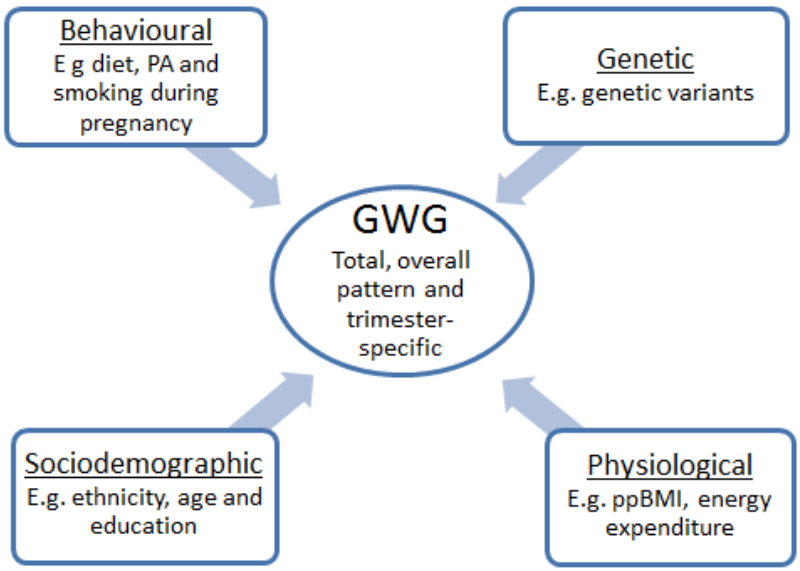

Figure 3. Schematic summary of potential determinants of gestational weight gain. Note: ppBMI, pre-pregnancy BMI; PA, Physical activity. Note that the arrows indicate associations with GWG and not causal influences on GWG.

1.1.4.1 Physiological factors

A woman's pre-pregnancy BMI is the strongest predictor of excessive GWG (gaining above IOM's recommended weight ranges), where women with a high BMI (overweight or obese) before pregnancy had significantly higher odds of having an excessive GWG compared to normal weight women, according to two studies by Brawarsky et al. and Krukowski et al. (41, 42). Abrams et al. found that the influence of pre-pregnancy BMI on GWG varied according to trimester, and it seemed to be most predictive of the second trimester weight gain (43).

A woman's weight gain during pregnancy is also clearly determined by her energy expenditure (the sum of the basal metabolic rate, physical activity and thermal effects of food), and her energy intake (44). The energy cost of pregnancy (energy expenditure and the energy from fat deposited in maternal and foetal tissues) increases gradually throughout pregnancy, and seems to be dependent on the woman's pre-pregnancy BMI (44). According to a recent study by Gilmore et al., to support a healthy weight gain within the IOM guidelines, their calculations recommended an extra intake (above their cohort's estimated energy requirement of 2368 kcal/day) of 129 kcal/day during the first trimester, an additional 249 kcal/day during the second trimester and an additional 108 kcal/day during the third trimester (45). They also found that weight gain above IOM's guidelines was due to an increased energy intake among the women in their study, rather than reduced or maintained energy expenditure (45).

1.1.4.2 Sociodemographic factors

The maternal sociodemographic factors, which according to the literature appear to be most influential in determining weight gain during pregnancy, will be the focus of the following sub-chapter, namely: maternal socioeconomic status, race, ethnicity, age and parity.

Several studies have looked at how maternal socioeconomic status (an index combining an individual's educational level, occupation and income) or educational level can affect GWG (27, 46, 47). Chu et al., e.g. found that women with the highest educational level gained more than those with < 12 years of education, independent of pre-pregnancy BMI (47). Holowko et al. observed that, for Swedish women with a normal pre-pregnancy BMI, education was protective against gaining excessively during both first and second pregnancies, however for pre-pregnant overweight or obese women this was not the case (27). These latter results are also in line with another Swedish study by Bjermo et al. (30). In fact, these authors also observed that overweight or obese women of higher education had a somewhat higher risk of excessive GWG (even after adjusting for pre-pregnancy BMI).

According to Krukowski et al., although African American and Hispanic women were more likely to be overweight or obese before pregnancy, they found that these groups of women were less likely to have an excessive GWG than white women, regardless of their pre-pregnancy BMI (42). Similar results were also found in the study by Pawlak et al., where Hispanic women had significantly lower odds of gaining an excessive weight during pregnancy compared to white women (48). On the contrary, Ismail et al. described in their study that, despite ethnical differences in their cohort of healthy women, similar patterns of GWG were observed, although the authors did not investigate potential differences within the GWG categories according to the IOM guidelines (35).

As for maternal age, women ≥ 30 years of age were more likely to gain less weight during pregnancy than younger women (49). Perry et al. and Chu et al. both found that gaining excessively decreased with age (47, 50). As for parity, a study by Harris et al. found that parity was independently associated with GWG (adjusted for several covariates such as age), and this association was not explained by the pre-pregnancy weight difference between mothers of different parities (51). The latter study also found that mothers who were pregnant for the first time (nulliparous) gained most weight during their pregnancy compared to mothers of higher parity. Nulliparous mothers also seemed to have higher odds of gaining an excessive amount of GWG compared to women of higher parity (42, 47, 48).

1.1.4.3 Behavioural factors

The current literature is unclear about the association between diet and GWG, as well as the effect of diet in limiting GWG. Several studies have found significant associations between e.g. total energy intake and greater GWG (52, 53), whereas others have failed to find any relationship between diet and GWG (or excessive GWG) (54, 55). A recent randomized control trial from the UK (the UPBEAT trial) targeting both physical activity and diet, was effective in reducing GWG in women who were obese at the beginning of their pregnancy (BMI ≥ 30 kg/m^2), however this did not result in a reduction in their primary outcomes (large-for-gestational age infants and GDM) (56). Several well-designed systematic reviews

have been conducted on the effect of diet and/or exercise in limiting GWG, with conflicting conclusions (57, 58). The Cochrane review from 2012 included 28 studies with varying degrees of quality. As the studies had quite small effects sizes, the authors concluded that not enough evidence was available to recommend specific interventions (both in terms of targeting diet and exercise) to limit GWG (57). In the review by Skouteris et al., some of the studies included reported significantly less GWG in the intervention group, however there was no consistency in limiting the weight gain across all pre-pregnant BMI categories, or which factors should be targeted to reduce GWG (58). In relation to exercise specifically, a recent systematic review found that only 38% of the included interventions had statistically significant reductions in GWG, mainly among normal weight populations (59). Another randomized trial by Dekker et al. focused on providing exercise help to increase physical activity among obese women (at the beginning of their pregnancy). These authors did not find any difference between the intervention and the control group (60).

According to national statistics in Sweden, around 6% of the mothers reported to be smoking at their first visit to the antenatal clinic (around gestational week 12) (23). Smoking during pregnancy is associated with a lower weight gain in a dose-response manner, due to the harmful effects of smoking on the growth of the foetus (foetal growth retardation) (61-63). Alcohol drinking during pregnancy is also associated with decreased foetal growth even at moderate levels (64). There are few studies on the impact of alcohol consumption on GWG, and one study observed that alcohol consumption during pregnancy (both for women who drank 0.5-3 drinks/week and who drank > 3 drinks/week) was more common among women with lower GWG than women with medium GWG (65).

1.1.4.4 Genetic factors

As seen above, many studies are focusing on the environmental determinants of GWG, however in terms of the genetic influences on GWG, evidence is scarce. There have been very few studies assessing the genetic influences on GWG, and most of them have focused on investigating the effects of single nucleotide polymorphisms (SNPs) on GWG (66-69). Two studies found that obesity- and diabetes-related SNPs were also related to GWG (67, 68), whereas another study did not find that the common obesity-related SNPs were associated with GWG (69). In the case of obesity however, despite its generally high heritability (the extent to which the variation in obesity is explained by genetic factors) of around 50-80% (70, 71), according to a recent large genome wide association study, only a small part of the genetic variation could be explained by specific genes (72). As far as we are aware, no previous study has investigated the heritability of GWG.

1.1.5 Health consequences

Gestational weight gain, and especially excessive GWG, is associated with both short- and long-term health consequences for both the mother and the child (e.g. (73-75)). As for the mother, gaining more weight than what is recommended by the IOM guidelines can increase the risk of e.g. GDM (76, 77) (defined as glucose intolerance with onset during the second or third trimester (78)), gestational hypertension and/or preeclampsia (defined as a persistent high blood pressure (BP) and protein in the urine developed during pregnancy) (32, 79) and postpartum weight retention (80). Gestational diabetes and preeclampsia can also increase the

mother's and the child's risk of later disease development, such as Type 2 diabetes (increased risk for the mother) and obesity (increased risk for the child) (81, 82).

In terms of the child, larger GWG has been found to be positively associated with increased birth weight (74, 83) and/or being born large-for-gestational age (LGA) (>90th percentile for that gestational age) (65, 84), as well as with more long-term effects such as child and adult overweight and obesity (75, 85, 86). Gaining weight above the IOM guidelines has also been associated with a shorter duration of breastfeeding or failure to initiate breastfeeding (87, 88), although being overweight or obese before pregnancy seems to be a stronger risk factor for a having a shorter length of breastfeeding (24, 88). As a longer breast-feeding duration can be protective against childhood overweight and/or obesity (89), the relationship between breastfeeding behaviour and GWG is important to recognize.

The studies included in this PhD thesis investigate the possible effects of GWG on the child, and in particular on the offspring's birth weight, weight/BMI during infancy and childhood and BP in early adulthood. Previous studies focusing on these particular short- and long-term outcomes will therefore be discussed in more detail below.

1.1.5.1 Offspring weight and BMI

As previously mentioned, GWG, and especially weight gain above the recommended guidelines, is associated with larger birth weight and/or being born LGA (65, 90). The effects of GWG on birth weight might differ depending on the specific time of the weight gain. Most of the studies which have looked at trimester-specific effects of GWG found effects for all trimesters on offspring birth weight or adiposity at birth (91-95). However, some studies indicated stronger, or specific, effects during the second trimester (92, 93, 95, 96), whereas others suggested that weight gain during the first and second trimesters had stronger effects on birth weight or adiposity at birth, compared to the third trimester (94, 97).

Numerous studies have also investigated more long-term effects of GWG in terms of children's weight and BMI. Body mass index is a crude measure of adiposity and is commonly used to define overweight and obesity in both children and adults (98). Even though BMI has been criticized for not being able to detect differences in body composition (fat mass vs muscle mass) (99), it is a simple and practical tool which can be used on a group level. The same cut-off values for BMI which are applied in adults (see **Table 1**) cannot be used for children, as e.g. growth development at different ages need to be considered. Instead, reference values from the International Obesity Task Force have been established which are based on sex, age, weight, height and BMI (100).

The majority of studies investigating child BMI as an outcome have found an association both between absolute GWG (measured as a continuous variable) and offspring childhood overweight and obesity (101), and between relative GWG (excessive GWG, measured as a categorical variable) and offspring childhood overweight and obesity (e.g. (102-104)). Evidence also exists for a possible association between GWG and offspring adult overweight or obesity, both during young adulthood (18-21 years old) (12-14, 93) and later adulthood (> 30 years old) (85, 105, 106).

Several recent systematic reviews and meta-analyses have also proposed GWG to be a potential modifiable factor to prevent childhood overweight and obesity (107-111). One of the two identified meta-analyses found a pooled odds ratio of 1.38 (95% confidence interval (CI): 1.21-1.57) for the association of excessive GWG and childhood overweight (110) and the second study found an overall relative risk (RR) of 1.40 (95% CI: 1.23-1.59) between excessive GWG and obesity during child- and adulthood (111).

Just as for birth size, different effects of GWG on childhood overweight or obesity have also been detected depending on the trimester of the weight gain. These studies consistently showed stronger effects of weight gain during first and/or second trimester (or early- and mid-pregnancy), compared to third trimester or late pregnancy weight gain (91, 101, 112-115).

1.1.5.2 Offspring blood pressure

As many studies have demonstrated that BP (both systolic and diastolic) is strongly and significantly associated with several cardiovascular disease outcomes, regardless of sex, age and ethnicity (116), it is from a public health perspective important to determine whether the aforementioned associations of GWG and offspring BMI may also increase BP and/or the risk of hypertension in the offspring.

Most of the studies investigating the association of GWG with childhood BP have found positive associations for childhood systolic BP (SBP) (101, 113, 117). However, two studies found an inverse association between GWG and SBP (118, 119), although one of them only investigated GWG during mid-pregnancy and their findings indicating an inverse association were not statistically significant (119).

In terms of longer follow-up into adulthood, few studies have examined the association of GWG and offspring BP with inconsistent results (13, 120-123). Hrolfsdottir et al. and Hochner et al. both found weak statistically significant positive associations of GWG and offspring SBP at 20 and 32 years respectively (120, 122). Only the smaller study by Hrolfsdottir et al. found a significant association with DBP, but only in the male offspring (122). On the contrary, Laor et al., Wander et al. and Mamun et al. failed to identify any significant associations between GWG and SBP or DBP (13, 121, 123). The latter study by Mamun et al. also examined if there was an association in the higher ranges of BP (offspring's risk of hypertension), but found no significant associations (13).

1.2 FAMILIAL CONFOUNDING

One important limitation with the majority of the observational studies discussed in the previous chapter is confounding from shared genetic and/or environmental factors, so called familial confounding. The observed associations between GWG and e.g. childhood BMI or adult BP may be explained by various shared (family-based) socio-demographic, lifestyle-related and genetic factors.

Well conducted randomized controlled trials (RCTs) are considered to be the "gold standard" of study designs to reduce confounding, but they are often very costly and time-consuming. There are however several non-experimental study designs (so called quasi-experimental designs) which can be used in order to take confounding from shared environmental and/or genetic factors into account. Some of these study designs are family-based, meaning that the offspring are related to different degrees, such as full-siblings, half-siblings and offspring of monozygotic (MZ) twins (124). More specifically, the associations between the exposures and outcomes of interest are compared in the offspring and if the association remains after adjusting for these shared genetic and/or environmental characteristics, the association could be due to intrauterine mechanisms (supporting the foetal overnutrition hypothesis, explained in chapter 1.1.1). On the contrary, if the association disappears, it is most likely due to shared genetics and/or environment. **Figure 4** illustrates the possible associations between GWG and the short- and long-term negative outcomes in the offspring studied in this thesis, and the possible confounding from shared genes and/or environment.

In terms of weight at birth and weight and/or BMI during childhood, as discussed earlier, studies have found effects across all trimesters for birth weight and stronger effects for first and/or second trimester (or early- and mid-pregnancy) weight gain for childhood weight and/or BMI, but confounding by shared genetic and/or environmental factors cannot by excluded. The studies which have attempted to adjust for these shared factors using sibling pairs, found that the association between total GWG and offspring birth weight remained after adjustment (12, 125, 126), where one of the studies found trimester-specific effects for the second trimester after controlling for shared genes and/or environment (125). Studies which have used similar study designs with longer follow-up of weight and/or BMI into childhood are very few (125, 127, 128). In the study by Branum and colleagues, no association was found between GWG and offspring BMI at age 4 years (127), while Ludwig et al. detected a positive association between GWG and BMI in the children at around 12 years of age – after controlling for shared genetic and environmental factors (128). Berglind and colleagues did not detect any significant associations between total, or trimester-specific, GWG, neither within nor between the sibling pairs (125).

As far as I am aware, no family-based studies have been able to investigate the association between GWG and adult BP. However, one study by Wander et al. attempted to take maternal genetic variation into account in the models by adjusting for genetic risk scores, and they failed to find any associations between GWG and SBP or DBP at 32 years of age both before and after the adjustment (121).

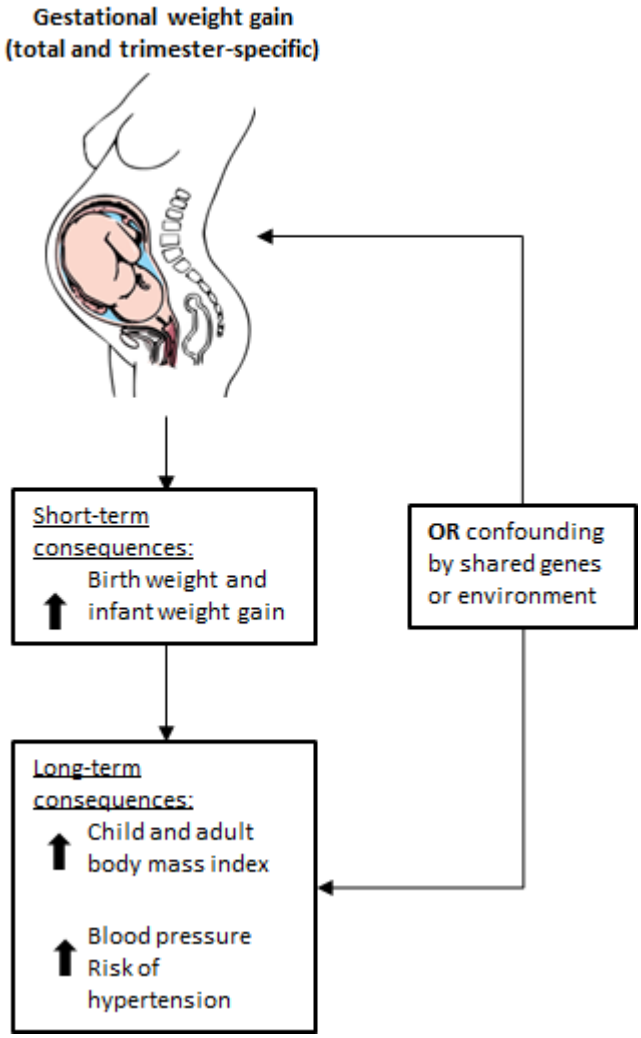

Figure 4. The possible associations between gestational weight gain and the short- and long-term health consequences in the offspring (which are studied in this thesis), and possible confounding by shared genetic and/or environmental factors.

2 AIMS

2.1 OVERALL AIMS

The overarching aim of this thesis was two-fold:

- The first objective was to explore the extent to which genetic and environmental factors influence the variation in GWG.
- The second objective was to investigate if total GWG (and trimester-specific GWG) are associated with health outcomes in the offspring at birth, during early childhood (weight/BMI) and early adulthood (BP/hypertension), when taking shared genetic and environmental factors into account.

2.2 SPECIFIC RESEARCH QUESTIONS

The specific research questions of this thesis were:

1. To which extent is the variation in GWG determined by genetic and environmental factors, and does it differ between two consecutive pregnancies (study 1)?
2. Is there an association between total and trimester-specific GWG and weight/ BMI in the offspring at birth and during childhood, when taking genetic and environmental characteristics, shared between the mother and child, into account (Study 2)?
3. Is there an association between GWG and BP, and the risk of hypertension, in the offspring during early adulthood, when taking shared genetic and environmental factors into account (study 3)?

See **Figure 5** for an overview of the different outcome variables of the three studies in a timeline.

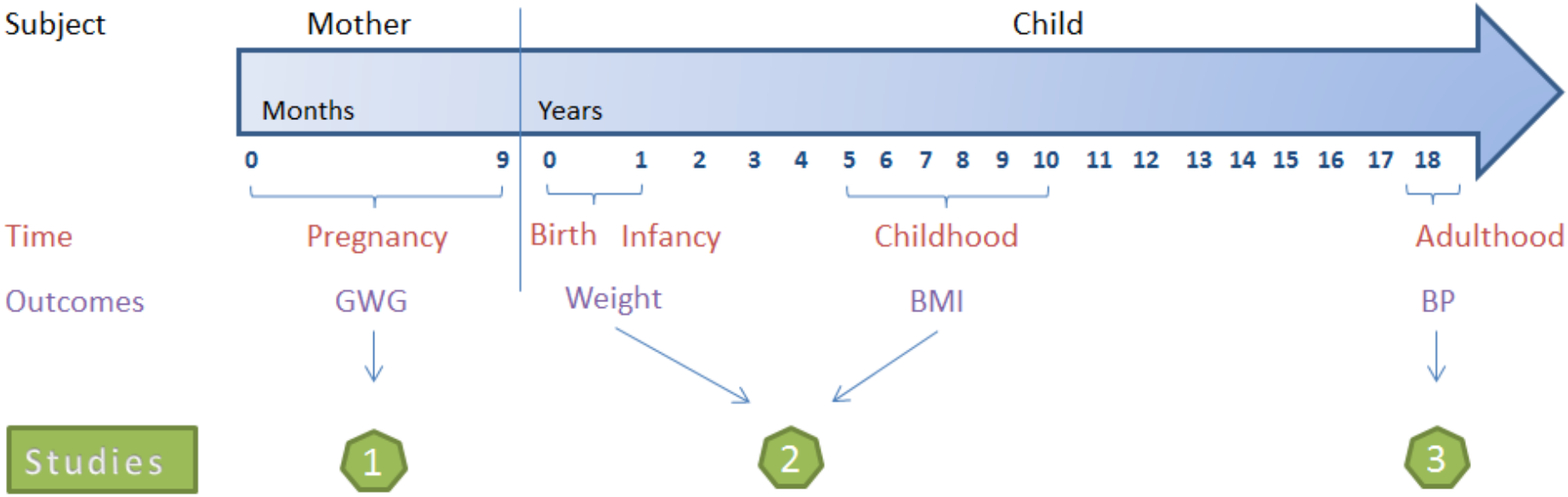

Figure 5. Timeline of the outcome variables of the three studies included in the thesis

3 MATERIAL AND METHODS

3.1 STUDY POPULATIONS

The current thesis consisted of three different materials: study 2 was based on a data collection procedure (described in chapter 3.1.2), whereas studies 1 and 3 were based on two separate register-linkages of several national Swedish registers (described in chapter 3.1.1 below). The study population of study 1 consisted of female MZ and dizygotic (DZ) twin pairs, where each woman had given birth to at least one child (univariate analysis), or two children (bivariate analysis), born 1982-1989 and 1992-2010. In terms of study 2, a register-linkage was carried out to identify women born on the same day (i.e. twins) between 1962 - 1975, and who had given birth to at least one child. These women could later be contacted for recruitment to the study. As for study 3, the study population was based on mothers who had given birth to at least two male children between 1982 -1989. An overview of the three studies, and the materials and methods used, can be found in **Table 2**.

3.1.1 National registers and register-linkages

Sweden has access to several nation-wide registers, and in research they are often referred to as a "gold-mine" (especially in terms of longitudinal studies), since the unique personal identification number, assigned to all Swedish citizens, makes it possible to link detailed high-quality data on a large number of individuals. Statistics Sweden and the National Board of Health and Welfare administer the majority of these national registers.

For study 1, a register-linkage was made between the MBR, the Swedish Twin Registry (STR) and the Multi-Generation Register (MGR). The twin mothers and their offspring were identified in the MGR, while data on the mothers' zygosity was collected from the STR.

The target population of the second study, including the female twins and their children (both boys and girls), was also identified through a register-linkage of the Register of Total Population (RTP), the MGR and the MBR.

In terms of the third study, a register-linkage was carried out between the MBR, MGR, Military Service Conscription Register (MSCR) and Statistics Sweden's Register of Education (SRE) in order to identify and collect data on the biological parents and their male offspring (sibling pairs).

Table 2. Overview of the design and methods of the three studies

	Study 1	Study 2	Study 3
Research questions	To which extent is the variation in GWG determined by genetic and environmental factors, and does it differ between two consecutive pregnancies?	Is there an association between total and trimester-specific GWG and weight/ BMI in the offspring at birth and during childhood, when taking shared genetic and environmental factors into account?	Is there an association between GWG and BP, and the risk of hypertension, in the offspring during early adulthood, when taking shared genetic and environmental factors into account?
Study design	Register-based twin study	Prospective children-of-twins cohort study	Prospective register-based cohort study
Participants	Swedish female twin pairs with children born 1982-1989 and 1992-2010. Univariate analyses: N = 694 pairs (1st pregnancy) N = 465 pairs (2nd pregnancy) N = 143 (bivariate analyses)	Swedish female MZ twin pairs born 1962-1975. N = 82 (birth weight) N = 71 (weight 1yr) N = 69 (BMI 5yrs) N = 57 (BMI 10yrs)	Swedish mothers with two male children born 1982-1989. N = 4908 full brother-pairs.
Data sources	STR, MBR and MGR	TPR, MGR, MBR, STR and self-collected data	MGR, MBR, SRE and MSCR
Exposures	GWG	Total and trimester-specific GWG	Total GWG
Outcomes	–	Offspring birth weight, weight at 1 year and BMI at 5 and 10 years.	Offspring SBP, DBP and risk of hypertension at 18 years.
Methods	Measured objective data from nation-wide registers.	Measured objective data from medical records and growth charts.	Measured objective data from nation-wide registers.
Statistical methods	SEM (A-C-E models) and Cholesky decomposition.	Linear regression (fixed-effects) models with robust variance using GEE.	Linear and Poisson regression (fixed-effects) models with robust variance using GEE.

Note: See the List of abbreviations at the beginning of this thesis for the definitions of the acronyms.

An outline of the registers (as well as the specific variables) used in the studies, can be found on the following pages.

3.1.1.1 Medical Birth Register (MBR)

The MBR, which is administered by the Swedish National Board of Health and Welfare, covers more than 99% of all births in Sweden (129) and includes data surrounding the

pregnancy, delivery and birth of the child (130). The data is registered by health professionals working in the pre- and perinatal care units as part of normal clinical practice. The quality of the data has generally been shown to be good (ranging from acceptable to good for the variables used in this thesis) (131). In studies 1 and 2, data on GWG (or early-pregnancy weight and delivery weight) was collected from the MBR. In terms of using these variables for analysis, it is important to be aware of some of the limitations with the register. All maternal early-pregnancy or delivery weights, during the years 1982-1989, above 98 kg were coded as 99 kg in the MBR. During the years 1990-1991, all values on early-pregnancy weight (from which GWG was calculated) were incorrect and were therefore deleted, hence no data on GWG was available during these two years. Moreover, data on early-pregnancy and delivery weight was available for 76% of the women delivering between 1982 and 1989, compared to only around 33% for the women delivering during the years 1992 to 2010. The missing data during the later years was due to limited resources in the antenatal care, hence not being able to prioritize monitoring and reporting of GWG to the MBR.

The other variables obtained from the MBR include gestational age, date of birth, birth order, birth weight, birth length, mother's parity, maternal age, maternal smoking in early pregnancy, and maternal diseases during pregnancy (preeclampsia and GDM).

3.1.1.2 Swedish Twin Registry (STR)

The STR is an on-going population-based register which collects information on twins born 1886 onwards (132). The register can be divided into five different birth cohorts, with information on twins born 1886 – 1925, 1926 – 1958, 1959 – 1986, 1987 – 1992 and 1992 – 2007 (latest update at the time of writing). The data on zygosity used in this thesis was collected from two studies based on these cohorts, namely Screening Across the Lifespan Twin study (SALT) and Study of Twin Adults: Gene and Environment (STAGE). The SALT study includes twins born 1886-1958 and the response rate of the later part of this cohort, born 1926 – 1958, was 74%. STAGE includes twins born 1959 – 1985 and the response rate for this study was approximately 60% (133).

Zygosity was determined, in both SALT and STAGE, from a set of classical and widely used questions based on the twin pairs' physical resemblance in childhood (134, 135). The first question was: "During childhood, were you and your twin partner as alike as "two peas in a pod" (in Swedish "as alike as two berries") or not more alike than siblings in general?". If both of the individuals in the pair responded "alike as two peas in a pod" they were classified as MZ and if both of them responded "not alike" they were classified DZ. If the twins disagreed or if only one twin responded to the question, the zygosity was considered "not determined". These undetermined pairs were asked a second question: "How often did strangers have difficulty in distinguishing between you and your twin partner when you were children?", and if they answered "almost always or always" or "often" to this question, they were classified as MZ twins. If both twins responded "seldom" or "almost never or never", they were classified as DZ twins. This method has in previous studies, using DNA markers as a validation, shown to have a high accuracy (around 99%) (135, 136).

3.1.1.3 Multi-Generation Register (MGR)

The MGR holds information on the biological parents (97% of the fathers and 95% of the mothers) of almost all individuals born in Sweden from 1932 onwards and those still alive in 1961 (137). The link between the mothers and fathers and their biological children was identified through a record-linkage to this register.

3.1.1.4 Military Service Conscription Register (MSCR)

The military service conscription was mandatory for all Swedish men to participate in until the year 2010. Only those with severe disabilities or disabling conditions were exempted. The Swedish MSCR keeps data from the conscription induction tests which usually took place at the ages of 18 to 19 years. The induction tests were completed in one or two days and included a medical examination by health professionals (weight, height, BP and resting heart measurements), physical strength tests (endurance and muscular strength) and psychological tests (including IQ tests). The main variables from this register which were used in this thesis (study 3) were BP, weight and height (BMI).

Due to reorganization of the Swedish Armed Forces, after around year 2000 fewer men needed to carry out all the medical examinations involved in the military service conscription, which resulted in missing data for BP and BMI. As the cohort of men included in study 3 underwent the military conscription tests between the years 2000 to 2008, we explored the potential bias from this missing data by means of multiple imputation analyses using (among other) data on the men's IQ as well as their fathers' conscription BMI and BP (explained in more detail in the corresponding manuscript).

3.1.1.5 Statistics Sweden's Register of Education (SRE)

Statistics Sweden keeps information on the highest educational level for all citizens registered in Sweden through the SRE. Data on the mothers' highest education was collected from this register for study 3.

3.1.1.6 Register of Total Population (RTP)

The purpose of this register is to collect basic demographic information (e.g. name, sex, age, country of birth and marital status) on the entire Swedish population (138). Data used in this thesis on, e.g., date of birth (the personal identification number), sex and age were drawn from this register.

3.1.2 Recruitment and data collection for study 2

3.1.2.1 Recruitment process

As previously mentioned, a register-linkage was carried out to identify female twin mothers born 1962 – 1975 (who were old enough to have given birth to at least one child who was at least ten years old at the time of carrying out the linkage). The resulting database included 5836 twin individuals (both MZ and DZ twins), to whom information letters were sent out

containing information about the research question, study design and implications of participating in the study. Approximately ten days after the information letters were sent out, an attempt was made to contact the mothers by telephone. They were asked if they wished to take part in a structured telephone interview.

For those women who gave oral consent, the interview began by asking questions to assess the twins' zygosity (based on the STR's validated questions mentioned earlier), as we only wanted to include MZ twins in the study. These questions were "During childhood, were you and your twin partner as alike as "two peas in a pod" (in Swedish "as alike as two berries") or not more alike than siblings in general?" and "How often did strangers have difficulty in distinguishing between you and your twin partner when you were children?". If both twins within a pair responded that they were as alike as "two peas in a pod" to the first question and "almost always" or "often" to the second question, they were classified as MZ. On the contrary, if both twins replied "not alike" to the first question or "seldom", "almost never", or "never" to the second question, they were categorized as DZ. All other twins were considered to be "not determined". To match the twins into pairs, the co-twin was also asked to participate and went through the same interview process.

The self-reported zygosity of the twin sisters was validated by comparing this data to the data on zygosity from the STR (as described earlier). Only around 12% had unknown (undetermined) zygosity in our cohort according to this data. These pairs were categorized as MZ twin pairs according to the answers we retrieved from our own interviews. Almost 21% (17 out of 82 pairs) of the data on zygosity from the STR had been validated by DNA analysis in one of our final cohorts (the cohort with complete data on GWG and birth weight). According to our interviews, all of the twin pairs said that they had been reared together. This was also validated against information from the STR.

As a next step, the women were asked to provide the names of the antenatal clinics where they had been registered with their children (to acquire information on pre-pregnancy weight, gestational weight gain, delivery weight and on any complications/diseases during pregnancy). They were also asked for the names of the hospitals where they had given birth (to gather information on weight and length at birth and possible complications during the birth), as well as the child health care centres and/or schools where their children were registered (for length and weight measurements).

After the interview, the women were sent consent forms along with a questionnaire (or given the option to answer the questionnaire electronically). The questionnaire contained questions about the women's pregnancy/pregnancies. More specifically we asked questions regarding their: physical activity and eating habits, the year before they got pregnant with their first child and the year after their last pregnancy, pre-pregnancy weight, total GWG, smoking habits, complications/diseases during pregnancy (such as GDM and preeclampsia), child's/children's birth weight and length, complications at birth and breastfeeding duration. Separate consent forms were sent to the children who were between 15 and 18 or above 18 years of age. Up to three attempts were made to gather the consent forms. Answering the questionnaire was not a compulsory part of participating in the study, as many women found it too time-consuming and therefore considered not participating. For the same reason, the questions concerning the names of the health care centres, hospitals and schools necessary to

acquire the weight and length data for the study were asked over the phone in the initial interview. The women who did not want to complete the questionnaires were asked to only return the consent forms in order for us to be able to acquire the data necessary for the study (as described below). Unfortunately, as only 48 complete twin pairs (with data on GWG and birth weight on at least one child of each twin) filled in the questionnaire, this data could not be included as covariates in the final analyses.

3.1.2.2 Data collection

The information letters were sent out in November 2012 and the telephone interviews commenced shortly after. The last interviews with the mothers were conducted at the beginning of 2014. At the same time as the interviews were taking place, we also carried out the acquisition of the medical records and growth charts. This data collection process started as soon as we received the first signed consent form at the end of August 2013, and was concluded approximately two years later.

In terms of weight and height data on the children, growth charts were collected from child health care centres, school health care services or from municipality or county archives (depending on the age of the child at the time). Maternal health care centres (where the women had received their antenatal care) were also contacted to acquire the mothers' medical records (for data such as early-pregnancy weight and weight measures throughout the pregnancy). Data regarding the delivery of the child (such as birth weight and length) was acquired from the hospitals.

In order to try to take the fathers' weight and height (BMI) into account in the analyses, we also made an attempt to contact the mothers of the complete twin pairs (164 twin mothers) and ask for their child's/children's father's contact details. Information letters and consent forms were sent to the fathers where we asked for their current weight, as well as for their consent for us to collect data on their BMI at conscription (if they had conscripted) from the MSCR. This data collection was carried out in July 2015. Unfortunately, as we only had complete data on the fathers for 21 twin pairs, this could not be included in the analyses.

When we concluded the data collection in the summer of 2015, we had managed to gather complete data on 82 twin mother pairs and their children (for the outcome birthweight). For a more detailed description of the recruitment and data collection process, including numbers of twin individuals and twin pairs, see **Figure 6** on the following page.

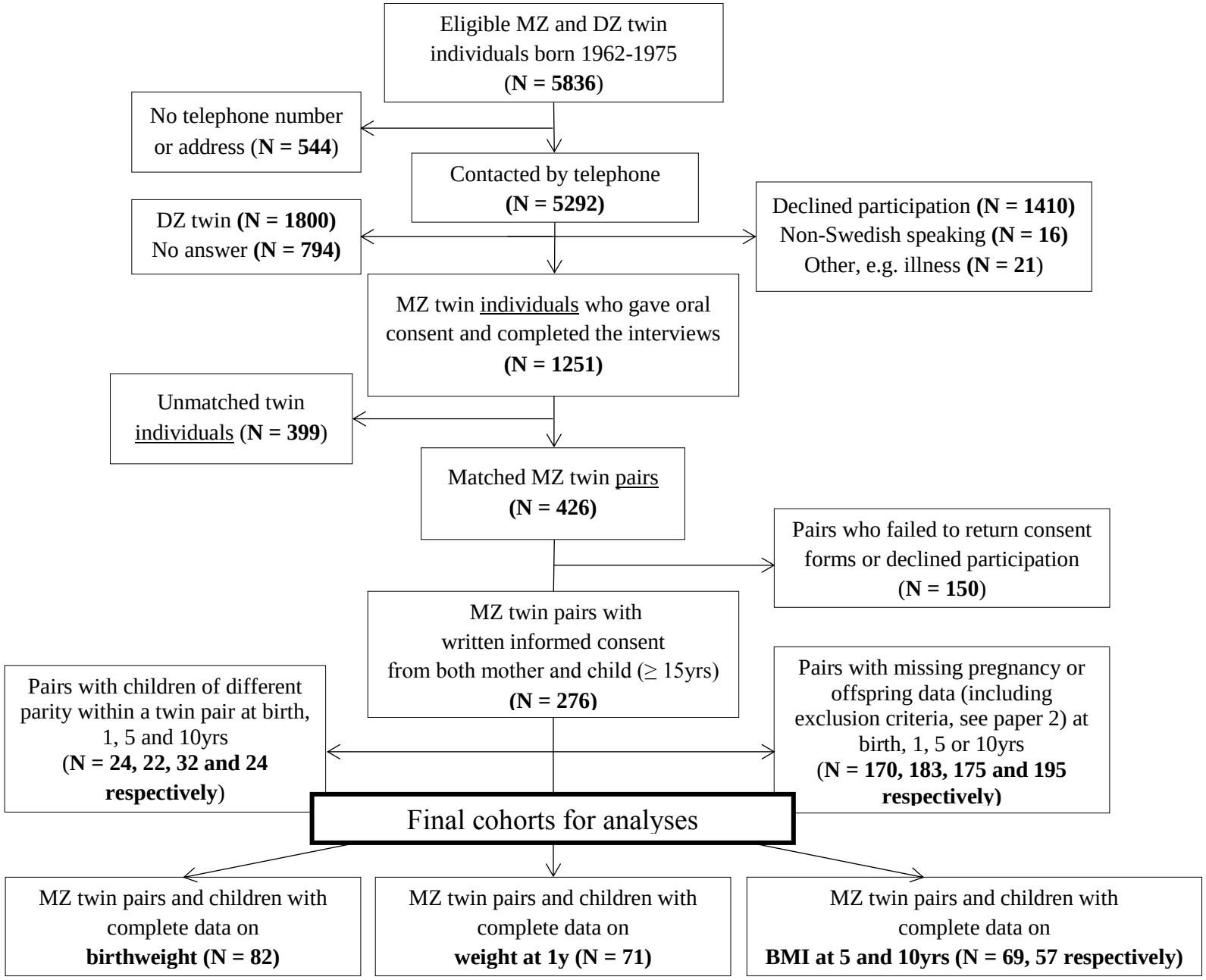

Figure 6. Flow chart of the recruitment and data collection procedure. Note: MZ = Monozygotic twins; DZ = Dizygotic twins

3.2 STUDY VARIABLES AND DEFINITIONS

3.2.1 Exposures

The main exposure in all the studies included in this thesis was total GWG. In study 2 we also measured the average weight gain in each trimester (trimester-specific GWG), at 10-14 gestational weeks (first trimester), 14-27 gestational weeks (second trimester) and at 27 gestational weeks to the week of delivery (third trimester). We defined total GWG as the delivery weight subtracted by the early-pregnancy weight. The delivery weight was measured before and in the same gestational week as delivery and the early-pregnancy weight was measured at the first antenatal visit (on average around week 10 for study 2, and within the first 12 weeks of pregnancy for 90% of the women for studies 1 and 3 (129). Both of these variables were collected either ad hoc, from archived medical records or extracted from the MBR.

3.2.2 Outcomes

As study 1 is a quantitative twin study investigating the heritability of GWG (analyzing the variation of GWG), there is no exposure or outcome defined as in conventional epidemiological studies.

In study 2, the outcome variables were offspring birthweight, weight and BMI (continuous measure) at the ages of 1, 5 and 10 years. As mentioned in the introduction, the cut-off values for categorizing the children's BMI values (only for descriptive statistics) were used according to the International Obesity Task Force's reference values (100). In order to account for the differences in birthweight in terms of sex and gestational age, we created sex-specific and gestational-age adjusted z-scores using a reference from the MBR's nationwide data. Similarly, we also calculated internal sex-specific z-scores using means and standard deviations from predicted growth curves of infant and childhood weight and BMI in our cohort.

The outcome variable for study 3 was BP at conscription (mean age of 18.3 years), which was analyzed both as a continuous and categorical variable (occurrence of hypertension). Hypertension in the offspring was defined as SBP $\geq$ 140 mmHg or DBP $\geq$ 90 mmHg, according to the WHO/International Society of Hypertension (139). The BP was measured in the supine position after 5 – 10 minutes rest with a suitably sized cuff at heart level according to a written protocol. This procedure has for many decades been viewed as the gold standard of measuring BP in clinical settings in Sweden. If the SBP was $\leq$ 145 mmHg and the DBP was 50 - 85 mmHg, it was only measured once. In case the measurements were outside of these limits, the BP was measured again on the next day and this second result was entered in the MSCR (140).

3.2.3 Covariates

As described earlier in the introduction, confounding from shared unmeasured environmental and genetic factors were adjusted for by the "fixed effects" design used in studies 2 and 3. There are, however, a number of other measured covariates which should be considered when

investigating the associations between GWG and weight/BMI and BP in the offspring, as well as when estimating the heritability of GWG. We have, based on *a priori* knowledge, decided to include the covariates outlined on the following page in the analyses of each study. As written in the introduction (chapter 1.1.4), there are more covariates which could possibly influence GWG (possible determinants), however the variables on the following pages were the ones for which there were data available.

3.2.3.1 Physiological factors

Maternal early-pregnancy BMI (weight and height were recorded at the first assessment at the antenatal clinic), parity, birth order, gestational age (assessed by ultrasound scans since the 1980's in the majority of cases, with an accuracy of ± 7 days (23)), maternal age at birth, birth year (date of birth), GDM and preeclampsia were all measured by midwives, obstetricians, or medical doctors as part of normal clinical practice. These variables were attained from the MBR for the register-based studies, or directly from the medical records for study 2. The brothers' age at conscription and conscription centre, accounted for in study 3, were obtained from the MSCR.

We decided not to adjust for birthweight and BMI at conscription in the final analyses of study 3 as we believe that they were most likely mediating factors (on the causal pathway between GWG and offspring blood pressure at age 18). For the same reason, we did not include birthweight as a covariate in the analyses of study 2.

3.2.3.2 Sociodemographic factors

Maternal education (highest level obtained for the years 1990-2010) was extracted from the SRE for studies 2 and 3. The variable was categorized into five levels: 1) primary or lower secondary (≤ 10 years), 2) secondary (< 12 years), 3) full secondary (12 years), 4) higher education < 15 years, and 5) higher education ≥ 15 years.

3.2.3.3 Behavioural factors

The mothers' smoking habits in early pregnancy were recorded by the midwives at the first antenatal visit (within the first 12 weeks of pregnancy for the majority of women). The mothers were asked if they had been smoking three months before pregnancy and whether or not they were currently smoking (if they were smoking, they were asked how many cigarettes per day they smoked). Data on maternal smoking was obtained for studies 1 and 2.

As previously mentioned, we asked the mothers about their pre-pregnancy and post-partum physical activity and dietary habits in the questionnaire, however due to the low response rate we were unable to use these variables in the analyses.

Data on the mothers' breast-feeding duration was also collected from the child health centre records for study 2. Data on possible diseases during pregnancy (preeclampsia and GDM) were also collected from the antenatal records for study 2 and extracted from the MBR for study 3. However, due to a big percentage of missing data on breast-feeding, and due to very few cases being diagnosed with diseases during pregnancy, we did not include these covariates in the analyses.

3.3 STATISTICAL ANALYSIS

3.3.1 Family studies

As explained in the introduction, shared genetic and environmental factors can confound the associations found in previous studies (based on unrelated individuals) between GWG and birthweight and childhood weight/BMI and BP. Studies which are family-based, e.g. studies of mothers and their offspring, offer the possibility to take shared mother-child genetic and environmental factors into account. More specifically, within and between associations can be compared to quantify possible effects. The focus, on the following pages, will be on explaining the two methods used in this thesis to address these types of confounding, as well as the methods used to analyze the heritability of GWG.

3.3.1.1 Offspring-of-MZ-twins design

Based on the assumption that MZ twins are genetically identical, many studies have analyzed offspring of MZ twins discordant for a certain disease or disorder, to understand how the environment and the genetics influence the intergenerational transmission of the trait (see e.g. (141, 142)). In this thesis (study 2), we took advantage of both the genetic similarity of the offspring of MZ twins (sharing 25% of their segregating genes) and the environment which the mothers shared during their upbringing, as well as prenatally, to study the relationship between the GWG of the MZ twin mothers and the weight and BMI of their children.

In more detail, to understand if any possible associations detected between GWG and offspring birthweight/childhood BMI were due to shared environmental or genetic factors (within-pair effects) or to non-shared factors (between-pair effects), we used generalized estimating equations (GEE) to account for the correlation between co-twins. The between-pair association was estimated by calculating the means of the GWG of the twin pairs and the means of the offspring birth weight or infants'/childhood weight/BMI. The within-pair association on the other hand, was based on the difference from the mean of the exposure and the outcome variables.

In terms of interpretation, the within-pair estimate takes all factors (both genetic and environmental) which the twins within a pair share/shared into account, e.g. lifestyle patterns established during childhood (such as physical activity and eating habits) which can later influence both their GWG pattern and their children's weight/BMI. Therefore, if an effect observed in the between-pair analysis remains in the within-pair analysis, this could indicate that the association between GWG and birthweight or childhood weight/BMI might have a foetal origin (due to an *in utero* programming of the foetus) (143-145). It should however be mentioned that, as the offspring only share 25% of their segregating genes, genetic confounding cannot be ruled out completely. Other limitations/assumptions of the offspring of MZ twins-design (as well as of the sibling pair design) are described on the following pages (chapters 3.3.1.4 and 3.3.1.5).

3.3.1.2 Sibling-design

Full siblings share, on average, 50% of their segregating genes and, due to the high number of sibling pairs easy accessible in national registers (in contrast to twin pairs), sibling-comparison design is a popular and powerful method used in family-based quasi-experimental design studies to account for shared genetic and environmental confounding (124, 146).

In this thesis, we applied sibling-comparison design to study the association between GWG (in the same mother in two separate pregnancies) with BP in her male offspring (full-sibling pair), at approximately 18 years of age (study 3). We applied the same linear regression analysis as described for study 2, comparing between sibling pair (based on the mean values of the exposure and outcome variables) and within sibling pair regression estimates (based on the difference from the mean values of exposure and outcome variables) (147).

Similar to the offspring-of-twins design previously explained, the within-pair estimate adequately controls for all fixed factors in the mother and factors which are shared by both siblings in the pair. These fixed factors are factors which do not change from the first pregnancy to the second (e.g. maternal education, maternal and paternal genetics, and possibly pregnancy and postnatal eating and physical activity habits). This means that, if we would observe within-pair effects it would suggest that the association of GWG and later BP in the offspring reflects intrauterine environmental factors (factors unique to each individual must be involved). Conversely, if we observed between-pair effects and these would diminish or disappear in the within-pair analysis, factors common to the sibling pair would be responsible for the association (148).

In the third study, we also adopted the fixed effects regression design as detailed above, using modified Poisson regression with robust variance to estimate the association of GWG and the RR of hypertension in the male sibling pairs.

3.3.1.3 Quantitative genetic analyses of twins

Human quantitative genetic methods are applied to estimate how much the variation in a specific trait of a population is due to genetic or environmental differences between individuals. These are based on comparing the resemblance (co-variance) of MZ and DZ twins (due to the underlying assumption that MZ twins share 100% of their segregating genes and DZ twins share 50% of their segregating genes). The total variation of a trait, in our case GWG (study 1), was decomposed into the following latent variables: A (additive genetic factors), C (common/shared environmental factors, D (dominance genetic factors) and E (unique environmental factors and random measurement error) using structural equation modelling (SEM) (as seen in the univariate model in **Figure 7**). With our MZ and DZ data with twins raised together, the SEM models cannot simultaneously estimate the D and C components (149) which is why we started off by estimating ACE and ADE models separately in the first and second pregnancies.

We also estimated the covariance of genetic and/or environmental factors between GWG of two consecutive pregnancies (**Figure 8**). This was carried out in order to detect possible

genetic or environmental overlapping or distinct effects, using a bivariate approach by means of the Cholesky decomposition model (150).

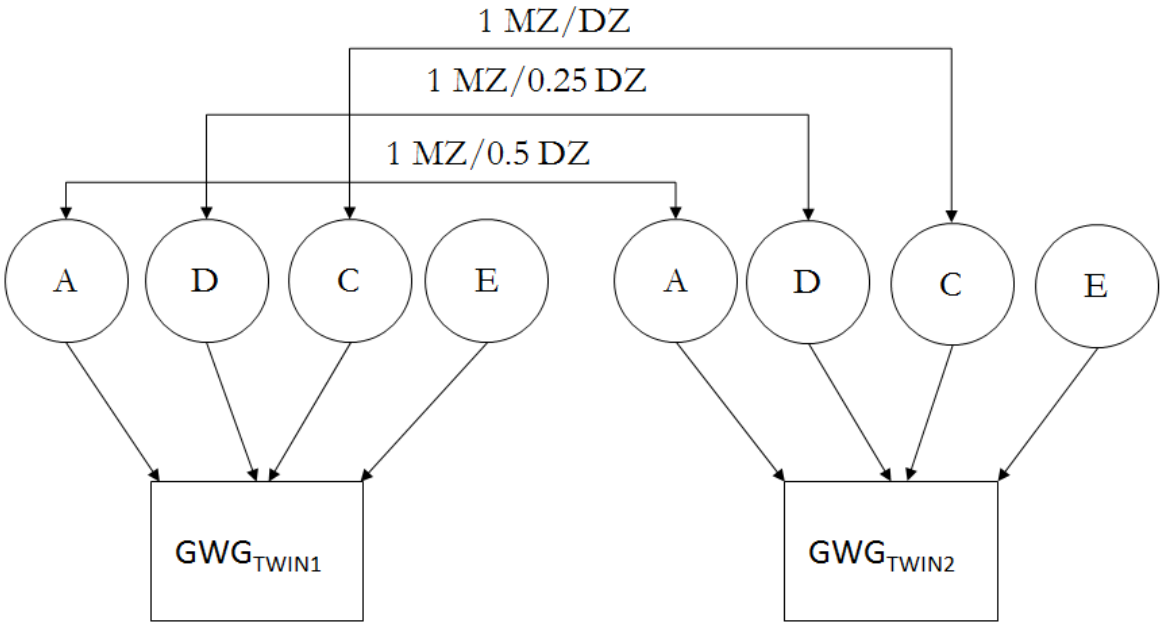

Figure 7. Schematic presentation of the univariate model. Note: A = additive genetic factors; D = dominance genetic factors; C = common environmental factors; E = unique environmental factors

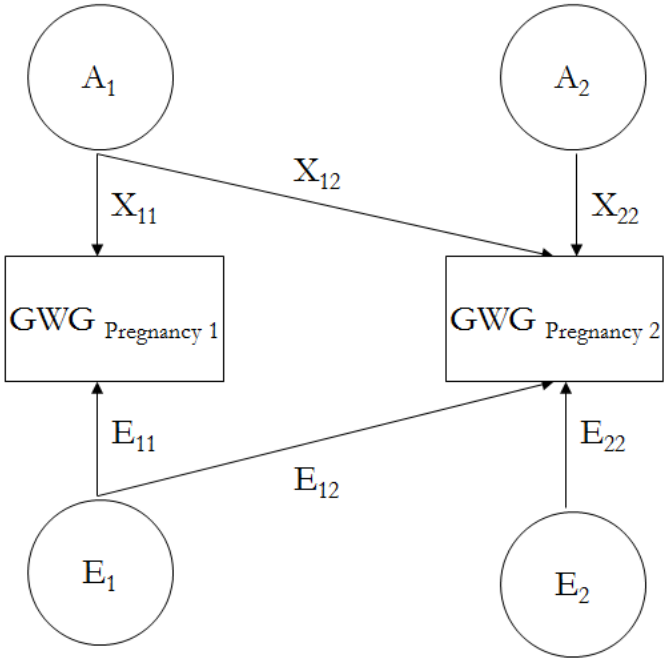

Figure 8. Schematic presentation of the bivariate Cholesky decomposition and its path coefficients for one twin only. Note: A = additive genetic factors; E = unique environmental factors; E_{11}, $_{12}$ and $_{22}$ = path coefficients of the unique environmental factors; X_{11}, $_{12}$ and $_{22}$ = path coefficients of the additive genetic factors

3.3.1.4 Assumptions of quantitative genetic twin studies

The assumptions made in quantitative genetic twin studies can be divided into technical (based on the way the model is specified) and interpretative (do not affect the model but the interpretation of the results) assumptions. The following assumptions were considered most important in terms of the studies included in thesis.

The most essential technical assumption is that means and variances are the same for both MZ and DZ twins, as well as for both the first and the second twin within the pair. This was tested by carrying out X^2 tests, where a non-significant result ($p > 0.05$) would indicate that these assumptions have not been violated. The other core assumptions of twin modelling include equal environment assumption, random (non-assortative) mating, no gene-environment interaction and that the results from twin studies can be generalized to the general population (151).

The equal environment assumption means that MZ and DZ twins (where the twin pairs are reared together) experience equally similar environments (both non-shared and shared), i.e. the size of the environmental variation is similar in both MZ and DZ twins. This assumption can be tested by comparing the fit of the current model to the fit of the saturated model. If the fit of the suggested model is not statistically significantly worse than that of the saturated model, this assumption is fulfilled.

Assortative (as opposed to random) mating exists in two ways. The first type is based on genetic relatedness (inbreeding), which is not so common in Western countries today. The second type is the tendency for people to choose a partner who is similar to themselves (phenotypic assortment), or who come from a similar environment (social homogamy). Normal weight women e.g., could be more likely to choose partners who also are of normal weight, with similar eating and physical activity habits. This similarity however, does not necessarily have to be due to assortative mating but rather due to social interaction (partners tend to become more similar with time). Phenotypic assortment and inbreeding would increase the genetic correlation of DZ twins, more than the assumed 0.5, which would make them more similar compared to MZ twins. As the twin design is based on the comparison of the genetic and environmental correlations of MZ and DZ twins, assortative mating would result in an overestimation of the C component and underestimation of the A or D components (152).

The assumption of lack of gene-environment interaction in twin studies would mean that genetic and environmental factors do not interact with each other and therefore have separate effects on the trait. However, lack of gene-environment interaction is not an issue in terms of the model itself, as gene-environment interaction is modelled as part of the additive genetic component if the environment is shared or as part of the unique environmental component if the environment is not shared. Due to this, any effects of a gene-environment interaction cannot be separated from the effects of the A or E components. Therefore, this assumption is difficult to test, and should rather be considered when interpreting the results.

Generalizing the results from twin studies and assuming that twins are representative of the general population is more or less correct depending on the trait. As twins are, on average,

lighter than singletons and are born around three weeks pre-term, the generalizability of the results from a heritability study on birth weight is more limited than studies carried out in adult traits. In the case of this thesis, we made the assumption that the twins included in studies 1 and 2 are representative of singletons as there is no evidence that twins are different to singletons for most traits after early childhood (153).

3.3.1.5 Assumptions of sibling-comparison and offspring-of-MZ-twins studies

Like quantitative genetic studies, there are also several assumptions and limitations to fixed effects regression design when estimating within- and between-pair effects using both siblings and offspring of MZ twin pairs. To my knowledge, there have been more articles written on the assumptions of sibling-comparison design compared to offspring-of-MZ-twin design, which is why this sub-chapter will focus on the former. Nonetheless, as we have used the same statistical method for both designs (studies 2 and 3), the following assumptions can also be applied to the within-pair design using offspring of MZ twins.

As the within-pair design controls for all factors which are fixed within the sibling pair (e.g. home environment), it is assumed that these family factors are stable and that the pair shared a similar environment as they were reared together (154). Factors which were not completely shared by the sibling pair or were not stable, e.g. birth order and maternal age, are most often controlled for in the analyses. However, other non-shared factors (e.g. pregnancy-related lifestyle habits, if these have changed from one pregnancy to the next) are more difficult to control for (often due to lack of data) (146, 155). A relatively short pregnancy interval between the two pregnancies (e.g. 2 to 4 years) reduces the likelihood of a change in the environment (both pre- and postnatally) and therefore also the introduction of bias from such non-shared factors. According to Frisell et al. (146), a limitation to the possible within-pair estimate (if a within-pair association is observed) is that bias from any measurement error (or misclassification) in the data is increased compared to the unpaired (between) estimate.

3.4 ETHICAL CONSIDERATIONS

All three studies were granted ethical approval by the Regional Ethical Review Board in Stockholm (ref no 2011/691–31/2 and 2011/666-31/3).

With regards to the two register-based studies (1 and 3), the unique identity number of each individual was replaced by unique numbers by which we could link the mothers to their children and the twins to their co-twins (de-identification). This was done by Statistics Sweden before the datasets were delivered to the research team. All subjects were therefore anonymous and no individual consent was possible to obtain (or needed), in accordance with the Public Access to Information and Secrecy Act and the Personal Data Act.

When it comes to the data collection for study 2, written informed consent was obtained for all participating mothers and their children ≥ 15 years of age (the mothers also consented for their children < 15 years of age with their consent form). The majority of women who chose

to participate did not express any concern about granting us access to their medical records. However, a couple of participants did not want us to retrieve their entire antenatal and delivery records (the pages where the inscription date, gestational age, and weights at the different time points during pregnancy are filled in), but only the specific data we requested. As they still wanted to participate, they acquired the medical records themselves and sent us only the information we had requested, which we naturally respected. It should also be added that the majority of women we were in contact with at the time of recruitment showed a genuine interest in our research questions and wanted to contribute with their data. The possible risks of participating in this study must therefore be considered minimal, and the potential benefits of the research for the society were thought to outweigh any such risks.

4 RESULTS

In this chapter I will present the main findings of each study included in this PhD thesis.

4.1 HERITABILITY OF GWG (STUDY 1)

To which extent is the variation in GWG determined by genetic and environmental factors, and does it differ between two consecutive pregnancies?

For the univariate analyses, we had complete data on GWG and the relevant covariates of the first pregnancy for 380 MZ and 314 DZ twin mother pairs, and for the second pregnancy we had complete data on 242 MZ and 223 DZ twin pairs. As for the bivariate analysis, 143 twin pairs (81 MZ and 62 DZ twin pairs) had complete data on both the first and the second (consecutive) pregnancies.

In terms of model fit, the univariate ADE and ACE models showed very similar results in both pregnancies. However, the dominance genetic component of the ADE model was zero in both the first and second pregnancies and was therefore discarded. Subsequently, we tested the model fit of the AE and CE models and compared these to the fit of the ACE models (see the corresponding manuscript for detailed data on the model fit (156)). We found that the AE models had a better fit compared to the saturated ACE model, meaning that additive genetic factors and unique environmental factors seemed to explain the variation of GWG in both the first and the second pregnancy in the univariate analyses.

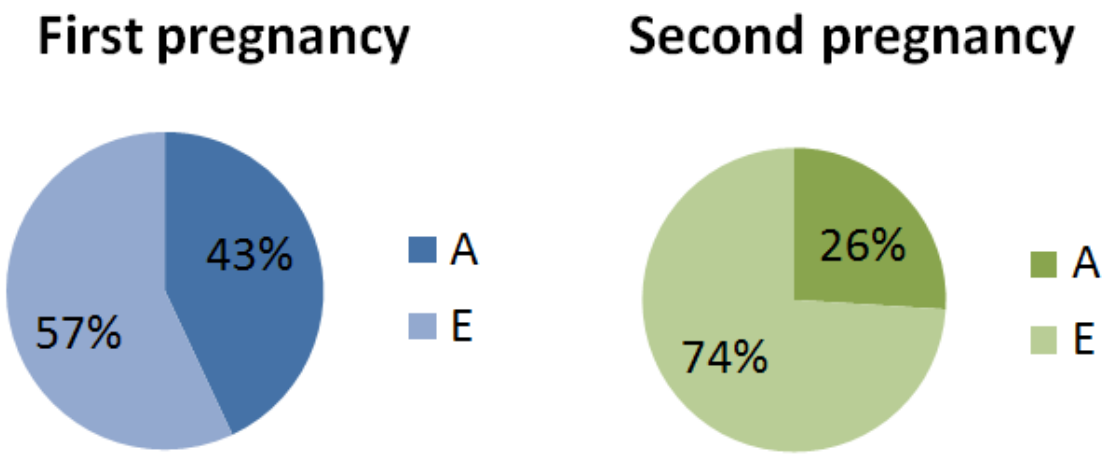

Figure 9. Variance component estimates of the first and the second pregnancy. Note: A = additive genetic factors; E = unique environmental factors

As shown in **Figure 9** above, according to the adjusted AE models, 43% (95% CI: 36-51%) of the variation in the mothers' GWG in the first pregnancy and 26% (95% CI: 16-36%) in the second pregnancy, was explained by additive genetic factors, and consequently the remaining variation was explained by unique environmental factors (first pregnancy, 57%, 95% CI: 50-64% and second pregnancy, 74%, 95% CI: 64-84%).

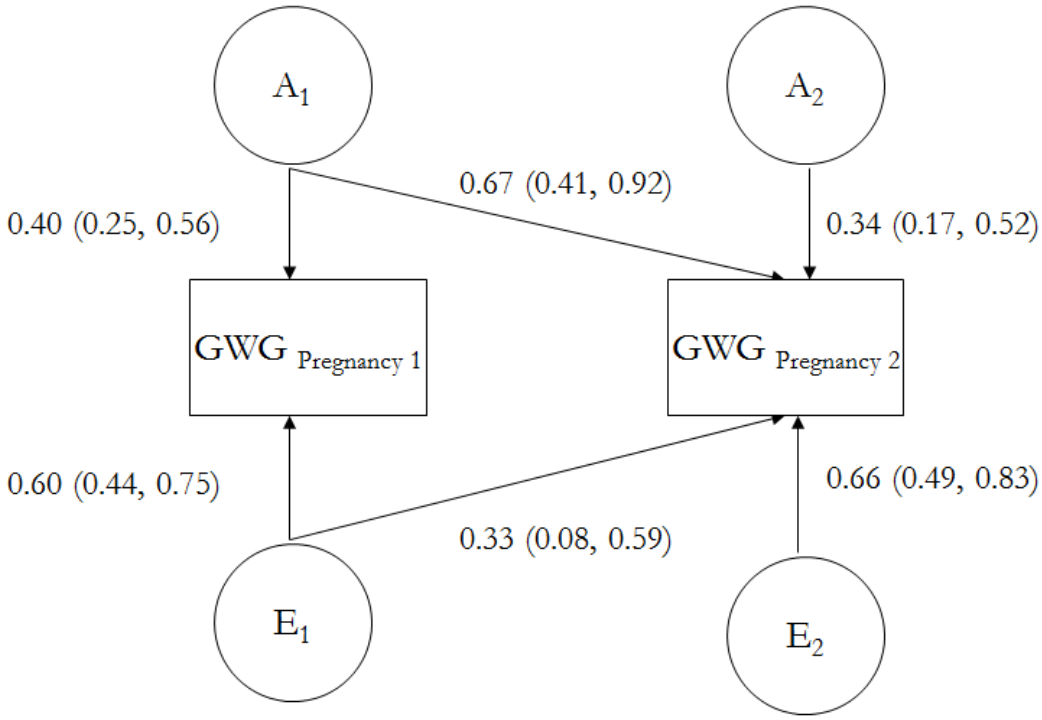

Figure 10. Cholesky decomposition AE model with relative variance and covariance component estimates. Note: A = additive genetic factors; E = unique environmental factors

In the bivariate (best-fitting) AE model, we found that there was an overlap (covariance) in both the additive genetic (A_1) and unique environmental (E_1) factors, although specific additive genetic (A_2) and unique environmental (E_2) components also seemed to influence the variation in GWG in the second pregnancy (as illustrated in **Figure 10** above, where the amount of variance and covariance is also displayed by the relative variance components). The additive genetic correlation (r_A) (standardized covariance) was 0.81 (95% CI: 0.55, 1.08) and the unique environmental correlation (r_E) was 0.24 (95% CI: 0.06, 0.42). The squared correlations (r_A^2 and r_E^2) (indicating the proportion of shared genetic and unique environmental factors) showed that 66% of the genes and 6% of the unique environment were shared between the two pregnancies.

4.2 GWG AND OFFSPRING WEIGHT AT BIRTH AND BMI DURING CHILDHOOD (STUDY 2)

Is there an association between total and trimester-specific GWG and weight/ BMI in the offspring at birth and during childhood, when taking genetic and environmental characteristics, shared between the mother and child, into account?

From the extensive data collection of MZ twin mother-pairs and their children, we managed to retrieve data on GWG on both mothers in the pair, as well as birth weight on the children

for a total of 82 twin pairs. In terms of the later outcomes: weight at 1 year and BMI at 5 and 10 years, complete data was available for 71, 69 and 57 twin pairs respectively. For information on exclusion criteria and details of the data collection, see **Figure 6** in the Methods section.

The mean early-pregnancy BMI of the mothers was 22.5 kg/m^2 (SD = 2.8 kg/m^2) and about 17% were defined as overweight or obese at the beginning of their pregnancy. The mothers' pregnancy weight gain was on average 14 kg (SD = 4.4 kg). As for the children, the mean birth weight was approximately 3600 g (SD = 479 g) and at 5 and 10 years respectively, only around 9% and 11% of the girls, and around 6% at both ages of the boys, were categorized as overweight or obese.

As seen in **Table 3**, the adjusted results from the main analyses showed that each kg of the total GWG resulted in an increase of 0.08 (95% CI: 0.001, 0.17) z-score units (corresponding to approximately 43 g) in birth weight within the twin pairs. In terms of the trimester-specific effects, we found that GWG was statistically significantly associated with BW across all trimesters between the twin mothers, and the strongest effects were seen in the second and third trimester (β = 1.63, 95% CI: 0.68, 2.59 and β = 1.64, 95 % CI: 0.71, 2.58 z-score units per 1-kg mean weight increase per week respectively, corresponding to approximately 782 g and 776 g respectively). However, when adjusting for shared genetic and environmental factors in the within-pair analysis, the results became non-significant. Due to the size of the respective coefficients though, these findings might indicate that the second and third trimester were associated with offspring birth weight (second trimester, β = 1.42 (95% CI: -0.29, 3.14) and third trimester, β = 1.02 (95% CI: -0.50, 2.54) z-score units, corresponding to approximately 682 g and 490 g respectively per 1-kg mean weight increase per week).

The results for total GWG and weight at 1 year in the offspring showed similarly weak effects within (β = 0.04 z-score units, 95% CI:-0.06, 0.15) and between (β = 0.04 z-score units, 95% CI: -0.01, 0.09) the twin pairs. As for the first trimester weight gain and weight at 1 year, no associations were found (see **Table 3**). The beta coefficients of the second and third trimester were overall larger than the first trimester, although with wide confidence intervals. In terms of the later outcomes of BMI at 5 and 10 years of age, we found similar statistically weak (with large confidence intervals) results for both total and trimester-specific GWG, both within and between the twin-mother pairs (see the corresponding manuscript for details (157)).

Table 3. Within- and between-pair effects of 1-kg greater gestational weight gain and mean growth rate (kg/week) during each trimester on children's birth weight (n = 164 children, 82 twin pairs) and weight at 1 year (n = 142 children, 71 twin pairs) in z-scores.

Offspring birth weight (z-scores)				
	Gestational weight gain (kg)	**1st trimester (mean increase in kg/week)**	**2nd trimester (mean increase in kg/week)**	**3rd trimester (mean increase in kg/week)**
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Within	0.08 (0.001, 0.17)	-0.15 (-0.98, 0.67)	1.42 (-0.29, 3.14)	1.02 (-0.50, 2.54)
Between	0.08 (0.04, 0.12)	0.77 (0.07, 1.47)	1.63 (0.68, 2.59)	1.64 (0.71, 2.58)
Wald test for difference	p = 0.91	p = 0.06	p = 0.83	p = 0.49
Offspring weight at 1 year (z-scores)				
	Gestational weight gain (kg)	**1st trimester (mean increase in kg/week)**	**2nd trimester (mean increase in kg/week)**	**3rd trimester (mean increase in kg/week)**
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Within	0.04 (-0.06, 0.15)	0.08 (-0.78, 0.94)	0.78 (-1.21, 2.78)	0.72 (-0.74, 2.18)
Between	0.04 (-0.01, 0.09)	0.12 (-0.51, 0.76)	0.86 (-0.35, 2.07)	0.71 (-0.23, 1.64)
Wald test for difference	p = 0.96	p = 0.94	p = 0.94	p = 0.99

Note: Both analyses were adjusted for maternal age at birth, birth year, parity (only included in the between model), early-pregnancy BMI and maternal education.

4.3 GWG AND OFFSPRING BLOOD PRESSURE IN YOUNG ADULTHOOD (STUDY 3)

Is there an association between GWG and BP, and the risk of hypertension, in the offspring during early adulthood, when taking shared genetic and environmental factors into account?

Data on exposure and outcome variables for all men born in Sweden between 1982 and 1989 was available for 89,829 individuals. These men conducted their military conscription induction tests from 2000 to 2008. After we had applied several exclusion criteria (e.g. limiting the cohort to the mothers' first and second male pregnancies (i.e. sibling pairs) during the study period), we ended up with a study population of 9,816 full brothers for the main analyses who were at a mean age of 18.3 years when carrying out the induction tests.

Descriptive statistics showed that the mean GWG was somewhat lower for the mothers' second pregnancy compared the first (13.8 kg (SD = 4.0 kg) and 14.0 kg (SD = 4.2 kg) respectively). In terms of the sons, most were normal weight at conscription (79%) and had a mean SBP of 131 mmHg (SD = 11.1 mmHg) and a mean DBP of 69 mmHg (8.4 mmHg), and the unadjusted prevalence of hypertension was 17% (SBP mean = 146 mmHg (SD = 5.0 mmHg) and DBP mean = 75 mmHg (SD = 7.7 mmHg)).

In the larger population of men (N = 89,829), we found an overall statistically significant association between GWG and SBP (adjusted as per model 3, as seen in **Table 4** below)

(SBP: β = 0.03 mmHg per 1-kg greater GWG (95% CI: 0.01, 0.04). This motivated further analyses within sibling pairs, as this association might have been confounded by shared genetic and environmental factors. However, as displayed in **Table 4**, no evidence was found of any associations between GWG and BP, or the risk of hypertension, in the offspring at age 18 years, neither within sibling pairs (SBP: β = 0.03 mmHg (95% CI: -0.08, 0.14); DBP: β = -0.03 mmHg (95% CI: -0.11, 0.05) and RR = 1.00 (95% CI: 0.99, 1.01)), nor between unrelated families, after adjustments for several covariates (as per model 3). Similar null-results were also seen when stratifying the BP analyses by the mother's early-pregnancy BMI (see the corresponding manuscript for details (158)).

Table 4. Associations of gestational weight gain with offspring systolic and diastolic blood pressure, as well as the risk of hypertension, at 18 years.

Systolic blood pressure				
Statistical model[a]	**N**	**Within effect[b] β (95% CI)**	**Between effect[c] β (95% CI)**	**P-value[d]**
	9,816			
Model 1		0.03 (-0.06, 0.13)	0.03 (-0.04, 0.09)	0.93
Model 2		0.03 (-0.08, 0.14)	0.02 (-0.05, 0.09)	0.93
Model 3		0.03 (-0.08, 0.14)	0.03 (-0.04, 0.10)	0.95
Diastolic blood pressure				
Statistical model[a]	**N**	**Within effect[b] β (95% CI)**	**Between effect[c] β (95% CI)**	**P-value[d]**
	9,816			
Model 1		-0.05 (-0.12, 0.02)	-0.05 (-0.10, 0.004)	0.91
Model 2		-0.03 (-0.12, 0.05)	-0.05 (-0.10, 0.004)	0.79
Model 3		-0.03 (-0.11, 0.05)	-0.04 (-0.09, 0.01)	0.79
Risk of hypertension				
Statistical model[a]	**N**	**Within effect[b] RR (95% CI)**	**Between effect[c] RR (95% CI)**	**P-value[d]**
	9,816			
Model 1		1.00 (0.98, 1.02)	1.01 (1.00, 1.02)	0.54
Model 2		1.00 (0.98, 1.03)	1.01 (1.00, 1.02)	0.66
Model 3		1.00 (0.98, 1.03)	1.01 (1.00, 1.02)	0.61

[a] Model 1 was adjusted for maternal age at birth, birth year, birth order and gestational age. Model 2 was adjusted as for model 1 plus early-pregnancy BMI, maternal education and parity. Model 3 was adjusted as for model 2 plus offspring's age of conscription and conscription centre.
[b] Difference in offspring SBP or DBP (in mmHg) or risk of hypertension per 1-kg difference in GWG.
[c] Difference in offspring SBP or DBP (in mmHg) or risk of hypertension per 1-kg greater GWG.
[d] P-value to test whether the within and between effects differ, obtained by a Wald test.

5 DISCUSSION

5.1 HERITABILITY OF GWG (STUDY 1)

To which extent is the variation in GWG determined by genetic and environmental factors, and does it differ between two consecutive pregnancies?

5.1.1 Main findings

According to the model with the best fit, data from 380 MZ and 314 DZ twin mother pairs showed that the variation in GWG for the mothers' first pregnancy was explained to 43% by additive genetic factors and to 57% by unique environmental factors. As for the second pregnancy, with data from 242 MZ and 223 DZ twin pairs, the additive genetic component was lower than the first pregnancy and explained 26% of the variation of GWG. The remaining 74% was explained by unique environmental factors. For the bivariate analysis, we had data on a smaller group of twin pairs (143 twin pairs), as they needed to have complete data on both their first and second pregnancies. We found that both additive genetic and unique environmental factors influenced both of the pregnancies, although separate and specific additive genetic and unique environmental factors also seemed to influence the second pregnancy.

5.1.2 Results in relation to previous research

To my knowledge, this is the first study which has investigated the heritability of GWG – direct comparisons with the results from previous research can therefore not be made. The main focus of previous studies has been on examining the environmental determinants of GWG (as discussed in more detail in chapter 1.1.4) (126, 159, 160), very little is therefore known about its influences from genetic factors. A few studies have looked into the relation between specific genetic variants and GWG (or increasing GWG) (67-69). Two studies found that genes which previously have been linked to obesity or Type 2 diabetes were also associated with GWG (67, 68), whereas a study by Lawlor et al. did not find that neither maternal nor foetal adiposity-related genetic variants were related to GWG (69).

In terms of assessing the heritability of other growth-related traits, earlier twin studies have primarily looked at the heritability of BMI and the estimates have generally been quite high, around 50-80% (70, 71, 161-163). Despite the rather high heritability, the located genetic variants related to BMI were, in a recent genome wide association study, shown to explain only < 3% of the total variation in BMI (72). Due to this difficulty in locating genetic variants (even for traits with a high heritability), taking into account that our results suggest that the heritability of GWG is moderate, it is perhaps not surprising that the earlier studies identifying genes related to GWG had conflicting results (67-69). Furthermore, these studies have focused on adiposity- or diabetes-related genetic variants, and as GWG includes other components (e.g. foetus and placenta) than the maternal increase in adipose tissue, GWG is most likely also influenced by other biological and genetic pathways.

5.1.3 Methodological considerations

5.1.3.1 Strengths

The main strength of this study is that we had access to unique data from national registers enabling us to conduct, what we know as, the first study investigating the heritability of GWG. Due to the rather large sample size of the univariate analyses we also had the opportunity to estimate the heritability in two consecutive pregnancies. The data on GWG and on all covariates, apart from maternal height (used to calculate early-pregnancy BMI), was register-based and to a large degree objectively measured. The extent of self-report in terms of data on early-pregnancy and delivery weight from the MBR is unknown, as the time it takes to measure the women highly depends on each individual situation and the priorities made. As for self-reported maternal height, the validity of this measure has previously been reported to be high and the magnitude of the likely overestimation small (164).

The generalizability of our results based on adult MZ twin mothers to a more general population of singleton mothers (with similar demographic characteristics) should be fairly good, as adult twins are highly representative of the general population for almost all traits (153).

5.1.3.2 Limitations

A general limitation with the classic twin design used in this study (SEM models) is that the shared environmental (C) and dominance genetic (D) components cannot be estimated simultaneously (in a hypothetical ACDE model) (165), which might be a reason why we were unable to detect effects from these components in our models. As a consequence of underestimating some of the non-additive (dominance) genetic effects, we might have overestimated the additive genetic component (A) (i.e. the heritability estimate of GWG), as well as underestimating the effects from common environmental effects. In fact, a recent study by Chen et al., which investigated the heritability of anthropometric traits such as weight and BMI by using both the classic twin-based design and Single Nucleotide Polymorphism (SNP)-based models, showed that the role of the dominance genetic effect was greater than previous studies had estimated (166).

If we believe that common environment plays a role in the variation of GWG, one should consider the biological plausibility behind these possible common environmental effects. As the twin mothers have not shared the same environment during their pregnancies (common environment), the possibility of this type of environmental influence is therefore small, if even existing. In order to investigate this issue further, hence simultaneously estimating C and D, future studies would require additional information from other relatives (e.g. adopted siblings and non-twin siblings) or from SNP-based models (although challenging in terms of achieving adequate sample sizes) (166).

We found that the unique environmental component was rather large (compared to other weight traits such as BMI, as discussed in chapter 5.1.2) in both the first and the second pregnancies (57% and 74% respectively). However, due to the fact that any potential measurement error is also being modelled as part of this unique environmental component

(e.g. variation in the measurement procedures and the reporting of the early-pregnancy and delivery weights, such as self-reported weights and different scales being used), the influence from this component might be overestimated. Another aspect which is part of the unique environment (specific to each twin mother) is the genetic contribution, in terms of growth potential, from the father. This has been shown, by taking the paternal birth weight into account, to also influence the offspring's birth weight (which is a proxy for the foetal growth and size) (40, 167).

Although the generalizability of results from studies on adult twin mothers to a more general population of singleton mothers appears to be good (as previously discussed), the external validity in terms of the representativeness of the women included in this specific study is uncertain for several reasons. As mentioned in the introduction, data on GWG was available for 76% of the women who gave birth between 1982 and 1989, compared to only 33% who gave birth between 1992 and 2010, from the national birth register (MBR). As for all issues with missing data, it is difficult to tell whether this underreporting is systematic (i.e. the mothers who gained a healthy amount of pregnancy weight were less closely monitored and therefore lacked data), or non-systematic (i.e. mothers' weight, regardless of BMI or amount of weight gain, was underreported due to e.g. the general time constraint in health care settings). Due to this, the possibility of selection bias cannot be excluded.

5.1.4 Interpretations and Implications

The moderately high heritability estimate of GWG means that unique environmental maternal factors such as diet and physical activity during pregnancy explain more than the majority of the variation in GWG and therefore play a large role in influencing the trait. This could be interpreted as supporting the concept of the pregnancy period often seen as a "window of opportunity" when it comes to being able to change lifestyle habits which could possibly influence the long-term health of both mother and child (168).

Although not statistically ascertained, the heritability estimate of the second pregnancy of the twin mothers was considerably lower than the estimate of the first pregnancy ($h^2 = 0.26$ and 0.43 respectively). As the female body goes through larger physiological adaptations (which could be determined by genetic factors to a large extent), during the first compared to the second and consecutive pregnancies, this could be a possible biological mechanism explaining the lower heritability estimate of the second pregnancy.

In terms of the bivariate results, it should firstly be mentioned that the results need to be interpreted with caution due to the small sample size. One may speculate that the distinct genetic and unique environmental influences, on the observed variation in GWG for the first and second pregnancies, could be due to epigenetic changes from one pregnancy to the next (e.g. genes being switched on or off). As for the unique environmental factors, it is likely that lifestyle factors which influence the variation of weight gain during the first pregnancy (such as maternal dietary habits), are similar to those of the second pregnancy. Therefore they could possibly also play a role in influencing the variation of GWG during the second pregnancy.

However, we also found evidence for separate non-shared environmental factors influencing only the variation in GWG of the second pregnancy.

5.2 GWG AND OFFSPRING WEIGHT AT BIRTH AND BMI DURING CHILDHOOD (STUDY 2)

Is there an association between total and trimester-specific GWG and weight/ BMI in the offspring at birth and during childhood, when taking genetic and environmental characteristics, shared between the mother and child, into account?

5.2.1 Main findings

From the vast data collection on MZ twin mother-pairs and their children, we managed to retrieve complete data on GWG and birth weight, weight at 1 year and BMI at 5 and 10 years for 82, 71, 69 and 57 twin pairs, respectively. We found that birth weight was associated with total GWG (0.08 z-score units per 1-kg mean increase in GWG, corresponding to approximately 43 g) when taking shared genetic and environmental factors into account. As for the effects of GWG specific to each trimester, no statistically significant findings were found once adjusting for genetic and environmental factors shared between the mother and child.

However, due to the magnitude of the effect sizes of the second (approximately 682 g in birth weight per 1-kg mean increase in GWG) and third trimester weight gains (approximately 490 g in birth weight per 1-kg mean increase in GWG), these results could indicate that GWG during these specific trimesters could be associated with children's birth weight. In terms of longer follow-up in the children, although statistically weak, no associations were found between both total and trimester-specific GWG and offspring's weight at 1 year and BMI at 5 and 10 years.

5.2.2 Results in relation to previous research

In line with the results from study 2, the majority of studies have found significant associations between offspring birth weight and weight gain during all trimesters, as well as with total GWG (43, 93-95). A few studies however found that only the effects during the beginning and/or the middle of the pregnancy period were associated with the birth weight (96, 97).

As mentioned in the introduction, some studies have also detected associations between total GWG and offspring birth weight after controlling for shared genetic and/or environmental factors, using within sibling pair analyses (12, 125, 126). The results from our study also confirmed this independent association between total GWG and birth weight. We are only aware of one previous study which has been able to take shared genetic and/or environmental factors into account when investigating the possible trimester-specific effects of GWG on offspring birth weight (125). In a cohort of full siblings born before and after maternal

bariatric surgery, the researchers found stronger effects specifically during the second trimester within the sibling pairs (β = 0.93 z-score units, 95 % CI: 0.26, 1.59). Although not statistically significant, our results also indicated that the weight gained during the second trimester had the strongest effects on offspring birth weight, although our findings also suggested that third trimester GWG could possibly also affect offspring birth weight.

The studies which have attempted to investigate the association of GWG and childhood weight and/or BMI, independent of shared mother-child genetic and/or environmental factors, are very scarce and their findings inconsistent (125, 127, 128). In the study by Ludwig et al. (128), the authors found that total GWG was associated with offspring BMI at around 12 years of age (β = 0.02 kg/m^2 per 1-kg increase in GWG, 95% CI: 0.01, 0.03), even when adjusting for common genetic and/or environmental factors by means of full sibling pairs. On the contrary, Branum et al. (127) failed to detect any within-sibling pair association between total GWG and BMI in the children at 4 years. Berglind and colleagues (125), who also looked at sibling pairs at the ages 4 and 6 years, found no statistically significant associations, neither before nor after adjusting for shared genetic and/or environmental factors. However, as the effect size of the positive within-sibling pair association found in the study by Ludwig et al. (128) was very small and might be explained by unmeasured confounding (from unshared factors), it is difficult to determine whether or not this result could be clinically relevant. Similar to the null-findings of the study by Branum et al. (127), we did not detect any associations of total or trimester-specific GWG and infant or childhood weight/BMI within-sibling pairs, although we could not validate these findings as null-results were also found in the between-sibling pair analyses.

5.2.3 Methodological considerations

5.2.3.1 Strengths

One of the major strengths of this study was that we were able to reduce confounding from shared genetic and environmental factors by analyzing within-pair differences of GWG and child weight/BMI. As the offspring of MZ twin mothers are genetically like half-siblings, i.e. they share only 25% of their genotype, we were unable to eliminate confounding from shared genetics completely. In comparison, studies conducted in full-siblings are able to control for more of the shared genetic confounding as they share 50% of their segregating genes. Yet, the offspring-of-MZ-twins design has a potentially larger exposure variability (generally requiring a smaller sample size (169)), due to the fact that the twin mothers within each pair had separate and unique environments during pregnancy (perhaps including different eating habits and lifestyles). Moreover, we were also able to control for parity, which is a known confounding factor, by restricting our analytical cohort to include only children of the same parity in each twin pair. This is another advantage of using MZ twin mother-pairs compared to full sibling pairs.

As we collected the data ourselves, we had the possibility to extract detailed data on GWG from medical records, which enabled us to investigate both total and trimester-specific effects

of GWG. Although gathering data from registers would have given us an increased power and population-based data, the national registers which we had access to do not offer detailed measurements of GWG, as well as infant and childhood weight and height data in the offspring.

5.2.3.2 Limitations

There are several limitations which should be taken into account when interpreting the results of this study. First of all, due to several difficulties in the recruitment phase, as well as with the tedious data collection, the sample size of the study ended up being relatively small. As seen in the flow chart in **Figure 6**, the largest number of twins whom we lost during the recruitment phase was due to the fact that they declined participation in the study (either actively through a decline during the first telephone contact, or passively by not picking up the phone). Twins, and especially monozygotic twins, are a popular subject group in research and highly sought after by twin researchers. Many twin mothers have already participated in plenty of studies throughout their lifetime and it is therefore likely that the majority of women we contacted did not pick up the phone, or actively declined participation, for this reason. In fact, a large number of the women we spoke to during the telephone interviews reported that they participated in other studies during that time, or simply that they had decided not to participate in any research projects at all (mostly due to time constraints). At the point of retrieving the data from medical records, we had trouble locating the medical records (at the national archives or health care centres), especially for the older children as many of their records had been destroyed or lost by the archives.

We cannot exclude a certain amount of selection bias in terms of the women who chose to participate, as the majority of the mothers participating were of normal weight at the beginning of their pregnancy (~ 80%) and gained a healthy amount of weight during pregnancy (mean weight was ~ 14 kg). For these reasons, it is fair to assume that the participants might generally be healthier than the non-participants. As many studies have found that educational level is inversely associated with weight and BMI (170), we asked the mothers' who did not want to participate about their educational level during the first telephone interviews to find out whether the non-participants differed from the participants in this aspect. However, most women did not want to disclose this information. Nonetheless, according to the descriptive statistics of our cohort, the distribution was fairly equal across all four categories of the highest educational level achieved (see **Table 1** in the corresponding manuscript for details). The twin mothers may therefore be representative of the general population in terms of educational level. As for ethnicity, a recent study found that the association of GWG and birth weight differed by ethnic origin (171), and as the participating women were mostly white Caucasian our findings might therefore not be generalizable to other ethnic groups.

5.2.4 Interpretations and Implications

Although statistically non-significant, our results suggested that both total and trimester-specific GWG during the second and third trimester may be associated with offspring birth weight, partially independent of common genetic and/or environmental factors. An

explanation for these possible trimester-specific associations could be that weight gained during mid- and late pregnancy represents a larger increase in foetal mass, and is therefore more strongly associated with the foetus' weight at birth, compared to the weight gained during early pregnancy which mainly comprises maternal body fat accumulation (73).

The effect sizes of these suggested trimester-specific associations were approximately 682 g and 490 g in birth weight for the second and third trimesters respectively per 1-kg mean weight increase per week in GWG. It has been found that a 1-kg increment in birth weight can increase the prevalence of overweight in childhood and adolescence of about 30% (172). Our findings may therefore be interpreted as both total GWG and weight gain specifically during the second and third trimester could be important for reducing the risk of childhood overweight and possibly obesity in the offspring.

5.3 GWG AND OFFSPRING BLOOD PRESSURE IN YOUNG ADULTHOOD (STUDY 3)

Is there an association between GWG and BP, and the risk of hypertension, in the offspring during early adulthood, when taking shared genetic and environmental factors into account?

5.3.1 Main findings

We found that there was a statistically significant association between GWG and SBP in the offspring at 18 years in a larger population of non-brothers. However, in the smaller cohort of around 10,000 full brothers, no evidence was found of an association between GWG and offspring BP, or the risk of hypertension, between the sibling pairs (158). Similarly, no associations were found when taking shared genetic and environmental factors into account.

5.3.2 Results in relation to previous research

As discussed briefly in the introduction, few studies have investigated the association of total GWG and BP in the adult offspring. These studies were conducted among unrelated individuals and show conflicting results (13, 120-123). Two studies found that GWG was statistically significantly associated with SBP at 20 and 32 years respectively (120, 122), although the effect sizes were rather small and only borderline significant (β = 0.21 mmHg per 1-kg increase in GWG, 95% CI: 0.003, 0.41 and β = 0.3 mmHg, 95% CI: 0.0, 0.6 respectively). Contradictory to these positive findings, the other three studies found no significant associations with offspring SBP/DBP at the ages of 17, 21 and 32 years (13, 121, 123). Mamun et al. (13) also looked into the possible association of GWG and the risk of hypertension at 21 years but found no significantly increased risk. Although we did find an overall association between GWG and BP at 18 years in unrelated individuals, the effect size was very small (β = 0.03, 95% CI: 0.01, 0.04) and only borderline significant. Therefore, in line with these studies which have shown null-findings, our results from the analyses carried out in the offspring who were unrelated also indicated that GWG seemed to have a very small effect on offspring's BP.

To my knowledge, only one study has attempted to take possible confounding from shared genetics into account when studying the relationship between GWG and offspring BP (121). Wander et al. added genetic risk scores to the analyses to adjust for maternal genetic variation (121). These were created by using SNPs which were most predictive of pre-pregnancy BMI, GWG and the outcome of cardiometabolic risk in the offspring. However, no associations were found between GWG and offspring BP (neither SBP nor DBP) at age 32, both before and after this adjustment. Similar to this study, our results also showed that GWG was not related to BP in the children at 18 years, both between and within sibling pairs.

5.3.3 Methodological considerations

5.3.3.1 Strengths

The prospective design with follow-up in the offspring at 18 years, the population-based nature, and the ability to examine the association of total GWG and BP, as well as the risk of hypertension, in the offspring while taking unmeasured shared environmental and genetic factors into account, were the major strengths of this study. The unmeasured confounding which we could, by design, adjust for includes fixed maternal factors (such as height and possibly socioeconomic status and to some extent lifestyle-related factors). It also includes offspring-related risk factors associated with BP and hypertension in young adulthood, (such as a high alcohol intake and low physical activity (173, 174)) which are likely to be more concordant within than between sibling pairs.

In contrast to many of the earlier studies, we obtained objectively measured data (for the majority of variables) on both exposure and outcome measures from national registers, hence reducing possible bias from self-reported data. As written previously, data on GWG (early-pregnancy and delivery weight) extracted from the MBR was available for 76% of the women in our cohort (delivering between 1982 and 1989), compared to around 33% for the women delivering during the later years (1992 to 2010). This implies that the representativeness of this Swedish cohort of mothers seem fairly good in terms of their GWG measures.

5.3.3.2 Limitations

One of the limitations of this study is that we did not know the true values of the early-pregnancy and delivery weights above 98 kg (due to errors in the coding in the MBR, all values above 98 kg have been coded as 99 kg for the children born 1982-1989). As a consequence, the variation in GWG (exposure variability) of our study is likely to be smaller (especially in the higher ranges of GWG and the early-pregnancy weight) than that of other studies, and we can therefore not extrapolate our results beyond our population of mothers weighing up to 98 kg. This limited exposure variability, together with the fact that we generally had a very small discordance in GWG between the two pregnancies (0.2 kg difference in mean GWG), could be possible explanations for the null-associations we observed between GWG and offspring BP and/or risk of hypertension. Additionally, in terms

of external validity, as the study was carried out in a Swedish setting and due to that the military service inscription tests were only mandatory for men, we cannot generalize our results to women and to other ethnicities than white European.

A possible explanation for the rather high unadjusted prevalence of hypertension (~ 17%) (defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg) in this cohort of young men could be the tendency for rounding up the BP measurements to the nearest 5 or 10 digit, which we identified when observing the distribution of the BP measurements of our cohort. As an example, there were around 100-200 individuals who had a SBP of 136 to 139 mm Hg, but 1164 individuals who had a SBP of 140 mmHg. Similar patterns also existed for SBP of 120, 125, 130 and 135 mmHg. However, this limitation is likely to only affect the validity of the specific measurements of the BP (which therefore affects the prevalence of hypertension) and not our main analyses of the associations of GWG with BP.

The sibling-comparison design accounts for all unmeasured shared genetic and environmental factors which are fixed from one pregnancy to the next (as explained in chapter 3.3.1.2). However, the model does not control for unmeasured factors which make the siblings dissimilar (e.g. pregnancy-specific factors such as different lifestyle factors), which is why we further adjusted the analyses for possible confounding factors (such as early-pregnancy BMI, maternal age and parity). However, we cannot exclude the possibility of residual confounding from factors which we lacked data on. Although, as we observed null-associations, and these potential confounding factors are likely to dissimilate any true associations, this limitation seems to be less of an issue.

5.3.4 Interpretations and Implications

The null-associations which we observed between total GWG and young adult BP in the offspring could either be a true null-finding or due to other mechanisms in which GWG is related to offspring BP. The findings from two large studies suggest that one such mechanism could be that cardiometabolic traits, such as BP, are related to the weight gain only during specific trimesters of the pregnancy. In the study by Fraser and colleagues (101), the association between weight gain during pregnancy and cardiovascular risk factors at age 9 in the offspring was only observed during mid-pregnancy (gestational weeks 14-36). Similarly, Gaillaird and colleagues (113) also found an association between GWG and SBP in 6-year old children only during gestational weeks 13.4-29.9 (early- (up to 14 weeks) to mid-pregnancy (around weeks 14-27)). Nonetheless, these studies were only conducted in unrelated children, and at a young age – it therefore remains to be investigated whether these associations exist in offspring of older ages, as well as after adjustment for familial confounding.

In order to understand whether or not GWG is in fact associated with BP and other cardiometabolic risk factors in the adult offspring, it is important to gather a collective understanding from all studies which have been conducted in this field. This is however difficult as an overall understanding requires all findings, regardless of the result, to be published. Publication bias, i.e. studies which have not been published due to their findings of no association between GWG and offspring BP, is therefore an important issue to consider.

6 CONCLUSIONS

The overarching aim of this PhD thesis was to examine the effects of weight gain during pregnancy in terms of the children's body size (during infancy and childhood), and blood pressure (in young adulthood). We also wanted to estimate how much genetic and environmental factors influence the variation in GWG. As an attempt to address these aims we conducted the three studies included in this thesis, from which we may draw some tentative conclusions:

Gestational weight gain seems to have a small effect on the children's birth weight, even after accounting for familial (genetic and environmental) factors. When investigating the weight gain in each trimester of the pregnancy, there might be effects on children's birth weight only during the second and third trimester. There seems to be no effects of GWG in terms of the children's weight and BMI during infancy and childhood (ages 1, 5 and 10 years).

As for more long-term effects, the total weight gain during pregnancy does not seem to be associated with the blood pressure, or the risk of hypertension, in 18-year old male siblings. This is true also when taking genetic or environmental factors shared between the mother and child into account.

Unique environmental factors, such as the mother's lifestyle during pregnancy, seem to explain a large part of the variation in the weight gained during pregnancy. The genetic influence on the variation of GWG appears to be relatively small (around 30-40%).

The aforementioned unique environmental factors seem to influence the variation of the weight gain during mothers' second pregnancy to a larger extent than the first. It also appears that the same environmental and genetic factors affect the variation in GWG during both the first and the second pregnancy (so called overlap). Separate/unique environmental and genetic factors also influence the variation in the weight gain only during the second pregnancy.

Overall, according to the collective evidence from previous studies in unrelated individuals, GWG (both total and trimester-specific) seems to be positively associated with the children's weight and BMI. However, when taking familial confounding into account, evidence is still inconclusive – especially in terms of children's weight and/or BMI after the infancy period and in terms of more long-term effects into adulthood. Although this thesis adds some knowledge to this important area (despite its rather inconclusive results), further large, family-based studies with long follow-up in the children are still needed.

6.1 FUTURE RESEARCH

As mentioned previously, small sample sizes, where we had detailed data on GWG (study 2), and the resulting low statistical power clearly hindered us from achieving our research aims. In order to obtain larger sample sizes with detailed data of good quality, routine measurements of weight gain during pregnancy in the antenatal care is crucial.

However, as written in the conclusion, strong, unbiased evidence on the long-term effects of GWG in the children is still scarce. Due to this, many countries such as the UK, have decided

not to introduce routine weighing during pregnancy as part of their antenatal care services (175). The dilemma is therefore that in order to gather more conclusive evidence from valid data, monitoring and measuring the weight gain during pregnancy at different time points (at least once in each trimester) is crucial. Ideally, to achieve this, detailed and objectively measured data could be gathered in the antenatal care and routinely reported to a national birth register (as is the case for Sweden, although trimester-specific weight measurements are lacking). Alternatively, self-weighing and self-monitoring by the women themselves, e.g. through simple smartphone applications, could be another option to reduce the burden on the already resource-restricted antenatal care and its staff (176, 177).

The same type of objective data on the children's weight and height could also be collected (and reported to a national register) from the child health care centres and schools. Matching and linking these data on both mother and child from national registers would allow researchers to obtain sufficient power in studies with long-term follow-up. This is important in order to be able to provide more robust and valid results, thus more conclusive evidence. Applying methods which can address the genetic and shared environmental confounding, such as the sibling-comparisons' design used in this thesis, is also important to attain a better understanding of the underlying pathways and mechanisms behind the observed associations between GWG and children's weight/BMI and other health outcomes.

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8 REFERENCES

1. Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia. 1992;35(7):595-601. Epub 1992/07/01.

2. Ravelli GP, Stein ZA, Susser MW. Obesity in young men after famine exposure in utero and early infancy. The New England journal of medicine. 1976;295(7):349-53. Epub 1976/08/12.

3. Ravelli AC, van Der Meulen JH, Osmond C, Barker DJ, Bleker OP. Obesity at the age of 50 y in men and women exposed to famine prenatally. Am J Clin Nutr. 1999;70(5):811-6. Epub 1999/10/28.

4. Lawlor DA, Smith GD, O'Callaghan M, Alati R, Mamun AA, Williams GM, et al. Epidemiologic evidence for the fetal overnutrition hypothesis: findings from the mater-university study of pregnancy and its outcomes. Am J Epidemiol. 2007;165(4):418-24. Epub 2006/12/13.

5. Pedersen J. Weight and length at birth of infants of diabetic mothers. Acta endocrinologica. 1954;16(4):330-42. Epub 1954/08/01.

6. El Hajj N, Pliushch G, Schneider E, Dittrich M, Muller T, Korenkov M, et al. Metabolic programming of MEST DNA methylation by intrauterine exposure to gestational diabetes mellitus. Diabetes. 2013;62(4):1320-8. Epub 2012/12/05.

7. Saffery R, Novakovic B. Epigenetics as the mediator of fetal programming of adult onset disease: what is the evidence? Acta obstetricia et gynecologica Scandinavica. 2014;93(11):1090-8. Epub 2014/05/20.

8. Lawlor DA, Fraser A, Lindsay RS, Ness A, Dabelea D, Catalano P, et al. Association of existing diabetes, gestational diabetes and glycosuria in pregnancy with macrosomia and offspring body mass index, waist and fat mass in later childhood: findings from a prospective pregnancy cohort. Diabetologia. 2010;53(1):89-97. Epub 2009/10/21.

9. Schaefer-Graf UM, Pawliczak J, Passow D, Hartmann R, Rossi R, Buhrer C, et al. Birth weight and parental BMI predict overweight in children from mothers with gestational diabetes. Diabetes care. 2005;28(7):1745-50. Epub 2005/06/29.

10. Aerts L, Van Assche FA. Animal evidence for the transgenerational development of diabetes mellitus. The international journal of biochemistry & cell biology. 2006;38(5-6):894-903. Epub 2005/08/25.

11. Boerschmann H, Pfluger M, Henneberger L, Ziegler AG, Hummel S. Prevalence and predictors of overweight and insulin resistance in offspring of mothers with gestational diabetes mellitus. Diabetes care. 2010;33(8):1845-9. Epub 2010/05/04.

12. Lawlor DA, Lichtenstein P, Fraser A, Langstrom N. Does maternal weight gain in pregnancy have long-term effects on offspring adiposity? A sibling study in a

prospective cohort of 146,894 men from 136,050 families. Am J Clin Nutr. 2011;94(1):142-8.

13. Mamun AA, O'Callaghan M, Callaway L, Williams G, Najman J, Lawlor DA. Associations of Gestational Weight Gain With Offspring Body Mass Index and Blood Pressure at 21 Years of Age Evidence From a Birth Cohort Study. Circulation. 2009;119(13):1720-U52.

14. Stuebe AM, Forman MR, Michels KB. Maternal-recalled gestational weight gain, pre-pregnancy body mass index, and obesity in the daughter. Int J Obes (Lond). 2009;33(7):743-52. Epub 2009/06/17.

15. Lawlor DA, Smith GD, O'Callaghan M, Alati R, Mamun AA, Williams GM, et al. Epidemiologic evidence for the fetal overnutrition hypothesis: Findings from the Mater-University Study of Pregnancy and its outcomes. Am J Epidemiol. 2007;165(4):418-24.

16. King JC. Maternal obesity, metabolism, and pregnancy outcomes. Annual review of nutrition. 2006;26:271-91. Epub 2006/05/18.

17. Dabelea D, Crume T. Maternal environment and the transgenerational cycle of obesity and diabetes. Diabetes. 2011;60(7):1849-55. Epub 2011/06/29.

18. Battista MC, Hivert MF, Duval K, Baillargeon JP. Intergenerational cycle of obesity and diabetes: how can we reduce the burdens of these conditions on the health of future generations? Experimental diabetes research. 2011;2011:596060. Epub 2011/11/24.

19. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. Lancet. 2016;387(10026):1377-96. Epub 2016/04/27.

20. Obesity and overweight. Fact sheet No 311. [Updated January 2015]: World Health Organisation; 2015.

21. Euro-Peristat. The health and care of pregnant women and babies in Europe in 2010. European perinatal health report [Internet]. 2013 June 2016.

22. Devlieger R, Benhalima K, Damm P, Van Assche A, Mathieu C, Mahmood T, et al. Maternal obesity in Europe: where do we stand and how to move forward?: A scientific paper commissioned by the European Board and College of Obstetrics and Gynaecology (EBCOG). European journal of obstetrics, gynecology, and reproductive biology. 2016. Epub 2016/05/11.

23. The Swedish Medical Birth Register. Pregnancies, deliveries and newborn infants. The Swedish Medical Birth Register 1973-2011. Assisted Reproduction, treatment 1991-2010. Sweden: National Board of Health and Welfare 2013.

24. Weight Gain During Pregnancy: Reexamining the Guidelines. Institute of Medicine and National Research Council, 2009.

25. Johnson JL, Farr SL, Dietz PM, Sharma AJ, Barfield WD, Robbins CL. Trends in gestational weight gain: the Pregnancy Risk Assessment Monitoring System, 2000-2009. Am J Obstet Gynecol. 2015;212(6):806 e1-8. Epub 2015/02/01.

26. Deputy NP, Sharma AJ, Kim SY, Hinkle SN. Prevalence and characteristics associated with gestational weight gain adequacy. Obstet Gynecol. 2015;125(4):773-81. Epub 2015/03/10.

27. Holowko N, Chaparro MP, Nilsson K, Ivarsson A, Mishra G, Koupil I, et al. Social inequality in pre-pregnancy BMI and gestational weight gain in the first and second pregnancy among women in Sweden. Journal of epidemiology and community health. 2015;69(12):1154-61. Epub 2015/07/30.

28. Faucher MA, Barger MK. Gestational weight gain in obese women by class of obesity and select maternal/newborn outcomes: A systematic review. Women and birth : journal of the Australian College of Midwives. 2015;28(3):e70-9. Epub 2015/04/14.

29. Johansson K, Hutcheon JA, Stephansson O, Cnattingius S. Pregnancy weight gain by gestational age and BMI in Sweden: a population-based cohort study. Am J Clin Nutr. 2016;103(5):1278-84. Epub 2016/03/25.

30. Bjermo H, Lind S, Rasmussen F. The educational gradient of obesity increases among Swedish pregnant women: a register-based study. BMC public health. 2015;15:315. Epub 2015/04/18.

31. Bogaerts A, Ameye L, Martens E, Devlieger R. Weight loss in obese pregnant women and risk for adverse perinatal outcomes. Obstet Gynecol. 2015;125(3):566-75. Epub 2015/03/03.

32. Cedergren M. Effects of gestational weight gain and body mass index on obstetric outcome in Sweden. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2006;93(3):269-74. Epub 2006/04/22.

33. Hytten F, Chamberlain G. Clinical physiology in obstetrics: Oxford: Blackwell Scientific Publications; 1991.

34. Schrier RW, Cadnapaphornchai MA, Ohara M. Water retention and aquaporins in heart failure, liver disease and pregnancy. Journal of the Royal Society of Medicine. 2001;94(6):265-9. Epub 2001/06/02.

35. Cheikh Ismail L, Bishop DC, Pang R, Ohuma EO, Kac G, Abrams B, et al. Gestational weight gain standards based on women enrolled in the Fetal Growth Longitudinal Study of the INTERGROWTH-21st Project: a prospective longitudinal cohort study. BMJ. 2016;352:i555. Epub 2016/03/02.

36. Hutcheon JA, Platt RW, Abrams B, Himes KP, Simhan HN, Bodnar LM. Pregnancy weight gain charts for obese and overweight women. Obesity (Silver Spring). 2015;23(3):532-5. Epub 2015/02/25.

37. Drehmer M, Duncan BB, Kac G, Schmidt MI. Association of second and third trimester weight gain in pregnancy with maternal and fetal outcomes. PloS one. 2013;8(1):e54704. Epub 2013/02/06.

38. Walter JR, Perng W, Kleinman KP, Rifas-Shiman SL, Rich-Edwards JW, Oken E. Associations of trimester-specific gestational weight gain with maternal adiposity and systolic blood pressure at 3 and 7 years postpartum. Am J Obstet Gynecol. 2015;212(4):499 e1-12. Epub 2014/12/03.

39. Pitkin RM. Nutritional support in obstetrics and gynecology. Clinical obstetrics and gynecology. 1976;19(3):489-513. Epub 1976/09/01.

40. Klebanoff MA, Mednick BR, Schulsinger C, Secher NJ, Shiono PH. Father's effect on infant birth weight. Am J Obstet Gynecol. 1998;178(5):1022-6. Epub 1998/06/03.

41. Brawarsky P, Stotland NE, Jackson RA, Fuentes-Afflick E, Escobar GJ, Rubashkin N, et al. Pre-pregnancy and pregnancy-related factors and the risk of excessive or inadequate gestational weight gain. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2005;91(2):125-31. Epub 2005/10/06.

42. Krukowski RA, Bursac Z, McGehee MA, West D. Exploring potential health disparities in excessive gestational weight gain. J Womens Health (Larchmt). 2013;22(6):494-500. Epub 2013/06/12.

43. Abrams B, Carmichael S, Selvin S. Factors associated with the pattern of maternal weight gain during pregnancy. Obstet Gynecol. 1995;86(2):170-6. Epub 1995/08/01.

44. Butte NF, Wong WW, Treuth MS, Ellis KJ, O'Brian Smith E. Energy requirements during pregnancy based on total energy expenditure and energy deposition. Am J Clin Nutr. 2004;79(6):1078-87. Epub 2004/05/26.

45. Gilmore LA, Butte NF, Ravussin E, Han H, Burton JH, Redman LM. Energy Intake and Energy Expenditure for Determining Excess Weight Gain in Pregnant Women. Obstet Gynecol. 2016;127(5):884-92. Epub 2016/04/08.

46. Samura T, Steer J, Michelis LD, Carroll L, Holland E, Perkins R. Factors Associated With Excessive Gestational Weight Gain: Review of Current Literature. Global advances in health and medicine : improving healthcare outcomes worldwide. 2016;5(1):87-93. Epub 2016/03/05.

47. Chu SY, Callaghan WM, Bish CL, D'Angelo D. Gestational weight gain by body mass index among US women delivering live births, 2004-2005: fueling future obesity. Am J Obstet Gynecol. 2009;200(3):271 e1-7. Epub 2009/01/13.

48. Pawlak MT, Alvarez BT, Jones DM, Lezotte DC. The effect of race/ethnicity on gestational weight gain. Journal of immigrant and minority health / Center for Minority Public Health. 2015;17(2):325-32. Epub 2013/08/13.

49. Prysak M, Lorenz RP, Kisly A. Pregnancy outcome in nulliparous women 35 years and older. Obstet Gynecol. 1995;85(1):65-70. Epub 1995/01/01.

50. Perry GS, Yip R, Zyrkowski C. Nutritional risk factors among low-income pregnant US women: the Centers for Disease Control and Prevention (CDC) Pregnancy Nutrition Surveillance System, 1979 through 1993. Seminars in perinatology. 1995;19(3):211-21. Epub 1995/06/01.

51. Harris HE, Ellison GT, Holliday M. Is there an independent association between parity and maternal weight gain? Annals of human biology. 1997;24(6):507-19. Epub 1997/12/13.

52. Tielemans MJ, Garcia AH, Peralta Santos A, Bramer WM, Luksa N, Luvizotto MJ, et al. Macronutrient composition and gestational weight gain: a systematic review. Am J Clin Nutr. 2016;103(1):83-99. Epub 2015/12/18.

53. Stuebe AM, Oken E, Gillman MW. Associations of diet and physical activity during pregnancy with risk for excessive gestational weight gain. Am J Obstet Gynecol. 2009;201(1):58 e1-8. Epub 2009/05/27.

54. Streuling I, Beyerlein A, Rosenfeld E, Schukat B, von Kries R. Weight gain and dietary intake during pregnancy in industrialized countries--a systematic review of observational studies. Journal of perinatal medicine. 2011;39(2):123-9. Epub 2010/11/13.

55. Herring SJ, Nelson DB, Davey A, Klotz AA, Dibble LV, Oken E, et al. Determinants of excessive gestational weight gain in urban, low-income women. Women's health issues : official publication of the Jacobs Institute of Women's Health. 2012;22(5):e439-46. Epub 2012/07/24.

56. Poston L, Bell R, Croker H, Flynn AC, Godfrey KM, Goff L, et al. Effect of a behavioural intervention in obese pregnant women (the UPBEAT study): a multicentre, randomised controlled trial. The lancet Diabetes & endocrinology. 2015;3(10):767-77. Epub 2015/07/15.

57. Muktabhant B, Lumbiganon P, Ngamjarus C, Dowswell T. Interventions for preventing excessive weight gain during pregnancy. The Cochrane database of systematic reviews. 2012(4):CD007145. Epub 2012/04/20.

58. Skouteris H, Hartley-Clark L, McCabe M, Milgrom J, Kent B, Herring SJ, et al. Preventing excessive gestational weight gain: a systematic review of interventions. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2010;11(11):757-68. Epub 2010/10/01.

59. McDonald SM, Liu J, Wilcox S, Lau EY, Archer E. Does dose matter in reducing gestational weight gain in exercise interventions? A systematic review of literature. Journal of science and medicine in sport / Sports Medicine Australia. 2016;19(4):323-35. Epub 2015/04/08.

60. Dekker Nitert M, Barrett HL, Denny KJ, McIntyre HD, Callaway LK. Exercise in pregnancy does not alter gestational weight gain, MCP-1 or leptin in obese women. The Australian & New Zealand journal of obstetrics & gynaecology. 2015;55(1):27-33. Epub 2015/02/18.

61. Rush D. Examination of the relationship between birthweight, cigarette smoking during pregnancy and maternal weight gain. The Journal of obstetrics and gynaecology of the British Commonwealth. 1974;81(10):746-52. Epub 1974/10/01.

62. Wilcox AJ. Birth weight and perinatal mortality: the effect of maternal smoking. Am J Epidemiol. 1993;137(10):1098-104. Epub 1993/05/15.

63. Secker-Walker RH, Vacek PM. Relationships between cigarette smoking during pregnancy, gestational age, maternal weight gain, and infant birthweight. Addictive behaviors. 2003;28(1):55-66. Epub 2003/01/01.

64. Little RE, Asker RL, Sampson PD, Renwick JH. Fetal growth and moderate drinking in early pregnancy. Am J Epidemiol. 1986;123(2):270-8. Epub 1986/02/01.

65. Nohr EA, Vaeth M, Baker JL, Sorensen T, Olsen J, Rasmussen KM. Combined associations of prepregnancy body mass index and gestational weight gain with the outcome of pregnancy. Am J Clin Nutr. 2008;87(6):1750-9. Epub 2008/06/11.

66. Kwak SH, Park BL, Kim H, German MS, Go MJ, Jung HS, et al. Association of variations in TPH1 and HTR2B with gestational weight gain and measures of obesity. Obesity (Silver Spring). 2012;20(1):233-8. Epub 2011/08/13.

67. Stuebe AM, Lyon H, Herring AH, Ghosh J, Wise A, North KE, et al. Obesity and diabetes genetic variants associated with gestational weight gain. Am J Obstet Gynecol. 2010;203(3):283 e1-17. Epub 2010/09/08.

68. Dishy V, Gupta S, Landau R, Xie HG, Kim RB, Smiley RM, et al. G-protein beta(3) subunit 825 C/T polymorphism is associated with weight gain during pregnancy. Pharmacogenetics. 2003;13(4):241-2. Epub 2003/04/02.

69. Lawlor DA, Fraser A, Macdonald-Wallis C, Nelson SM, Palmer TM, Davey Smith G, et al. Maternal and offspring adiposity-related genetic variants and gestational weight gain. Am J Clin Nutr. 2011;94(1):149-55. Epub 2011/05/20.

70. Elks CE, den Hoed M, Zhao JH, Sharp SJ, Wareham NJ, Loos RJ, et al. Variability in the heritability of body mass index: a systematic review and meta-regression. Frontiers in endocrinology. 2012;3:29. Epub 2012/05/31.

71. Schousboe K, Willemsen G, Kyvik KO, Mortensen J, Boomsma DI, Cornes BK, et al. Sex differences in heritability of BMI: a comparative study of results from twin studies in eight countries. Twin research : the official journal of the International Society for Twin Studies. 2003;6(5):409-21. Epub 2003/11/20.

72. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of body mass index yield new insights for obesity biology. Nature. 2015;518(7538):197-206. Epub 2015/02/13.

73. Hedderson MM, Gunderson EP, Ferrara A. Gestational Weight Gain and Risk of Gestational Diabetes Mellitus (vol 115, pg 597, 2010). Obstet Gynecol. 2010;115(5):1092-.

74. Siega-Riz AM, Viswanathan M, Moos MK, Deierlein A, Mumford S, Knaack J, et al. A systematic review of outcomes of maternal weight gain according to the Institute of Medicine recommendations: birthweight, fetal growth, and postpartum weight retention. Am J Obstet Gynecol. 2009;201(4):339 e1-14. Epub 2009/10/01.

75. Deierlein AL, Siega-Riz AM, Adair LS, Herring AH. Effects of pre-pregnancy body mass index and gestational weight gain on infant anthropometric outcomes. The Journal of pediatrics. 2011;158(2):221-6. Epub 2010/09/25.

76. Hedderson MM, Gunderson EP, Ferrara A. Gestational weight gain and risk of gestational diabetes mellitus. Obstet Gynecol. 2010;115(3):597-604. Epub 2010/02/24.

77. Tomedi LE, Simhan HN, Chang CC, McTigue KM, Bodnar LM. Gestational weight gain, early pregnancy maternal adiposity distribution, and maternal hyperglycemia. Matern Child Health J. 2014;18(5):1265-70. Epub 2013/10/09.

78. Gilmartin AB, Ural SH, Repke JT. Gestational diabetes mellitus. Reviews in obstetrics & gynecology. 2008;1(3):129-34. Epub 2008/11/19.

79. Ruhstaller KE, Bastek JA, Thomas A, McElrath TF, Parry SI, Durnwald CP. The Effect of Early Excessive Weight Gain on the Development of Hypertension in Pregnancy. American journal of perinatology. 2016. Epub 2016/08/05.

80. Davis EM, Stange KC, Horwitz RI. Childbearing, stress and obesity disparities in women: a public health perspective. Matern Child Health J. 2012;16(1):109-18. Epub 2010/11/23.

81. Jovanovic L, Pettitt DJ. Gestational diabetes mellitus. Jama. 2001;286(20):2516-8. Epub 2001/11/28.

82. Duley L. The global impact of pre-eclampsia and eclampsia. Seminars in perinatology. 2009;33(3):130-7. Epub 2009/05/26.

83. Viswanathan M, Siega-Riz AM, Moos MK, Deierlein A, Mumford S, Knaack J, et al. Outcomes of maternal weight gain. Evidence report/technology assessment. 2008(168):1-223. Epub 2008/07/16.

84. Oken E, Kleinman KP, Belfort MB, Hammitt JK, Gillman MW. Associations of Gestational Weight Gain With Short- and Longer-term Maternal and Child Health Outcomes. Am J Epidemiol. 2009;170(2):173-80.

85. Schack-Nielsen L, Michaelsen KF, Gamborg M, Mortensen EL, Sorensen TIA. Gestational weight gain in relation to offspring body mass index and obesity from infancy through adulthood. Int J Obesity. 2010;34(1):67-74.

86. Sridhar SB, Darbinian J, Ehrlich SF, Markman MA, Gunderson EP, Ferrara A, et al. Maternal gestational weight gain and offspring risk for childhood overweight or obesity. Am J Obstet Gynecol. 2014;211(3):259 e1-8. Epub 2014/04/17.

87. Hilson JA, Rasmussen KM, Kjolhede CL. Excessive weight gain during pregnancy is associated with earlier termination of breast-feeding among White women. The Journal of nutrition. 2006;136(1):140-6. Epub 2005/12/21.

88. Bider-Canfield Z, Martinez MP, Wang X, Yu W, Bautista MP, Brookey J, et al. Maternal obesity, gestational diabetes, breastfeeding and childhood overweight at age 2 years. Pediatric obesity. 2016. Epub 2016/03/10.

89. Victora CG, Bahl R, Barros AJ, Franca GV, Horton S, Krasevec J, et al. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. Lancet. 2016;387(10017):475-90. Epub 2016/02/13.

90. Johnson J, Clifton RG, Roberts JM, Myatt L, Hauth JC, Spong CY, et al. Pregnancy outcomes with weight gain above or below the 2009 Institute of Medicine guidelines. Obstet Gynecol. 2013;121(5):969-75. Epub 2013/05/03.

91. Hivert MF, Rifas-Shiman SL, Gillman MW, Oken E. Greater early and mid-pregnancy gestational weight gains are associated with excess adiposity in mid-childhood. Obesity (Silver Spring). 2016;24(7):1546-53. Epub 2016/06/28.

92. Abrams B, Selvin S. Maternal weight gain pattern and birth weight. Obstet Gynecol. 1995;86(2):163-9. Epub 1995/08/01.

93. Margerison-Zilko CE, Shrimali BP, Eskenazi B, Lahiff M, Lindquist AR, Abrams BF. Trimester of Maternal Gestational Weight Gain and Offspring Body Weight at Birth and Age Five. Matern Child Hlth J. 2012;16(6):1215-23.

94. Starling AP, Brinton JT, Glueck DH, Shapiro AL, Harrod CS, Lynch AM, et al. Associations of maternal BMI and gestational weight gain with neonatal adiposity in the Healthy Start study. Am J Clin Nutr. 2015;101(2):302-9. Epub 2015/02/04.

95. Wander PL, Sitlani CM, Badon SE, Siscovick DS, Williams MA, Enquobahrie DA. Associations of Early and Late Gestational Weight Gain with Infant Birth Size. Matern Child Health J. 2015;19(11):2462-9. Epub 2015/06/22.

96. Sekiya N, Anai T, Matsubara M, Miyazaki F. Maternal weight gain rate in the second trimester are associated with birth weight and length of gestation. Gynecologic and obstetric investigation. 2007;63(1):45-8. Epub 2006/08/26.

97. Brown JE, Murtaugh MA, Jacobs DR, Jr., Margellos HC. Variation in newborn size according to pregnancy weight change by trimester. Am J Clin Nutr. 2002;76(1):205-9. Epub 2002/06/26.

98. World Health Organization. Body Mass Index - BMI. 2016 [cited 2016 20 Sep]; Available from: http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi.

99. Rothman KJ. BMI-related errors in the measurement of obesity. Int J Obes (Lond). 2008;32 Suppl 3:S56-9. Epub 2008/08/21.

100. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ. 2000;320(7244):1240-3. Epub 2000/05/08.

101. Fraser A, Tilling K, Macdonald-Wallis C, Sattar N, Brion MJ, Benfield L, et al. Association of Maternal Weight Gain in Pregnancy With Offspring Obesity and Metabolic and Vascular Traits in Childhood. Circulation. 2010;121(23):2557-U48.

102. Wrotniak BH, Shults J, Butts S, Stettler N. Gestational weight gain and risk of overweight in the offspring at age 7 y in a multicenter, multiethnic cohort study. Am J Clin Nutr. 2008;87(6):1818-24.

103. Jacota M, Forhan A, Saldanha-Gomes C, Charles MA, Heude B. Maternal weight prior and during pregnancy and offspring's BMI and adiposity at 5-6 years in the EDEN mother-child cohort. Pediatric obesity. 2016. Epub 2016/05/03.

104. van Rossem L, Wijga AH, Gehring U, Koppelman GH, Smit HA. Maternal Gestational and Postdelivery Weight Gain and Child Weight. Pediatrics. 2015;136(5):e1294-301. Epub 2015/10/21.

105. Houghton LC, Ester WA, Lumey LH, Michels KB, Wei Y, Cohn BA, et al. Maternal weight gain in excess of pregnancy guidelines is related to daughters being overweight 40 years later. Am J Obstet Gynecol. 2016. Epub 2016/02/24.

106. Reynolds RM, Osmond C, Phillips DIW, Godfrey KM. Maternal BMI, Parity, and Pregnancy Weight Gain: Influences on Offspring Adiposity in Young Adulthood. J Clin Endocr Metab. 2010;95(12):5365-9.

107. Poston L. Gestational weight gain: influences on the long-term health of the child. Current opinion in clinical nutrition and metabolic care. 2012;15(3):252-7. Epub 2012/03/13.

108. Lau EY, Liu J, Archer E, McDonald SM. Maternal weight gain in pregnancy and risk of obesity among offspring: a systematic review. Journal of obesity. 2014;2014:524939. Epub 2014/11/06.

109. Woo Baidal JA, Locks LM, Cheng ER, Blake-Lamb TL, Perkins ME, Taveras EM. Risk Factors for Childhood Obesity in the First 1,000 Days: A Systematic Review. American journal of preventive medicine. 2016;50(6):761-79. Epub 2016/02/27.

110. Nehring I, Lehmann S, von Kries R. Gestational weight gain in accordance to the IOM/NRC criteria and the risk for childhood overweight: a meta-analysis. Pediatric obesity. 2013;8(3):218-24. Epub 2012/11/23.

111. Mamun AA, Mannan M, Doi SA. Gestational weight gain in relation to offspring obesity over the life course: a systematic review and bias-adjusted meta-analysis. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2014;15(4):338-47. Epub 2013/12/11.

112. Andersen CS, Gamborg M, Sorensen TI, Nohr EA. Weight gain in different periods of pregnancy and offspring's body mass index at 7 years of age. International journal of pediatric obesity : IJPO : an official journal of the International Association for the Study of Obesity. 2011;6(2-2):e179-86. Epub 2010/10/05.

113. Gaillard R, Steegers EA, Franco OH, Hofman A, Jaddoe VW. Maternal weight gain in different periods of pregnancy and childhood cardio-metabolic outcomes. The Generation R Study. Int J Obes (Lond). 2015;39(4):677-85. Epub 2014/10/08.

114. Karachaliou M, Georgiou V, Roumeliotaki T, Chalkiadaki G, Daraki V, Koinaki S, et al. Association of trimester-specific gestational weight gain with fetal growth, offspring obesity, and cardiometabolic traits in early childhood. Am J Obstet Gynecol. 2015;212(4):502 e1-14. Epub 2015/01/06.

115. Margerison-Zilko CE, Shrimali BP, Eskenazi B, Lahiff M, Lindquist AR, Abrams BF. Trimester of maternal gestational weight gain and offspring body weight at birth and age five. Matern Child Health J. 2012;16(6):1215-23. Epub 2011/07/08.

116. Vasan RS, Larson MG, Leip EP, Evans JC, O'Donnell CJ, Kannel WB, et al. Impact of high-normal blood pressure on the risk of cardiovascular disease. The New England journal of medicine. 2001;345(18):1291-7. Epub 2002/01/17.

117. Dello Russo M, Ahrens W, De Vriendt T, Marild S, Molnar D, Moreno LA, et al. Gestational weight gain and adiposity, fat distribution, metabolic profile, and blood pressure in offspring: the IDEFICS project. Int J Obes (Lond). 2013;37(7):914-9. Epub 2013/04/10.

118. Wen X, Triche EW, Hogan JW, Shenassa ED, Buka SL. Prenatal factors for childhood blood pressure mediated by intrauterine and/or childhood growth? Pediatrics. 2011;127(3):e713-21. Epub 2011/02/09.

119. Clark PM, Atton C, Law CM, Shiell A, Godfrey K, Barker DJ. Weight gain in pregnancy, triceps skinfold thickness, and blood pressure in offspring. Obstet Gynecol. 1998;91(1):103-7. Epub 1998/02/17.

120. Hochner H, Friedlander Y, Calderon-Margalit R, Meiner V, Sagy Y, Avgil-Tsadok M, et al. Associations of Maternal Prepregnancy Body Mass Index and Gestational Weight Gain With Adult Offspring Cardiometabolic Risk Factors The Jerusalem Perinatal Family Follow-Up Study. Circulation. 2012;125(11):1381-9.

121. Wander PL, Hochner H, Sitlani CM, Enquobahrie DA, Lumley T, Lawrence GM, et al. Maternal genetic variation accounts in part for the associations of maternal size during pregnancy with offspring cardiometabolic risk in adulthood. PloS one. 2014;9(3):e91835. Epub 2014/03/29.

122. Hrolfsdottir L, Rytter D, Olsen SF, Bech BH, Maslova E, Henriksen TB, et al. Gestational weight gain in normal weight women and offspring cardio-metabolic risk factors at 20 years of age. Int J Obes (Lond). 2015;39(4):671-6. Epub 2014/10/10.

123. Laor A, Stevenson DK, Shemer J, Gale R, Seidman DS. Size at birth, maternal nutritional status in pregnancy, and blood pressure at age 17: population based analysis. Brit Med J. 1997;315(7106):449-53.

124. D'Onofrio BM, Lahey BB, Turkheimer E, Lichtenstein P. Critical need for family-based, quasi-experimental designs in integrating genetic and social science research. American journal of public health. 2013;103 Suppl 1:S46-55. Epub 2013/08/10.

125. Berglind D, Willmer M, Naslund E, Tynelius P, Sorensen TI, Rasmussen F. Differences in gestational weight gain between pregnancies before and after maternal bariatric surgery correlate with differences in birth weight but not with scores on the body mass index in early childhood. Pediatric obesity. 2014;9(6):427-34. Epub 2013/12/18.

126. Ludwig DS, Currie J. The association between pregnancy weight gain and birthweight: a within-family comparison. Lancet. 2010;376(9745):984-90. Epub 2010/08/10.

127. Branum AM, Parker JD, Keim SA, Schempf AH. Prepregnancy body mass index and gestational weight gain in relation to child body mass index among siblings. Am J Epidemiol. 2011;174(10):1159-65. Epub 2011/10/11.

128. Ludwig DS, Rouse HL, Currie J. Pregnancy Weight Gain and Childhood Body Weight: A Within-Family Comparison. Plos Med. 2013;10(10).

129. Källén B, Källén, K., & Otterblad Olausson, P. The Swedish medical birth register: A summary of content and quality (Research report). Stockholm: Epidemiological Center: Swedish National Board of Health and Welfare, 2003.

130. Axelsson O. The Swedish medical birth register. Acta obstetricia et gynecologica Scandinavica. 2003;82(6):491-2. Epub 2003/06/05.

131. Cnattingius S, Ericson A, Gunnarskog J, Kallen B. A quality study of a medical birth registry. Scandinavian journal of social medicine. 1990;18(2):143-8. Epub 1990/06/01.

132. Pedersen NL, Lichtenstein P, Svedberg P. The Swedish Twin Registry in the third millennium. Twin research : the official journal of the International Society for Twin Studies. 2002;5(5):427-32. Epub 2003/01/23.

133. Lichtenstein P, Sullivan PF, Cnattingius S, Gatz M, Johansson S, Carlstrom E, et al. The Swedish Twin Registry in the third millennium: an update. Twin research and human genetics : the official journal of the International Society for Twin Studies. 2006;9(6):875-82. Epub 2007/01/27.

134. Pedersen NaL, P. Scientific Evaluation of the Swedish Twin Registry. Stockholm, Sweden: 2000.

135. Lichtenstein P, De Faire U, Floderus B, Svartengren M, Svedberg P, Pedersen NL. The Swedish Twin Registry: a unique resource for clinical, epidemiological and genetic studies. Journal of internal medicine. 2002;252(3):184-205. Epub 2002/09/25.

136. Silventoinen K, Magnusson PK, Tynelius P, Kaprio J, Rasmussen F. Heritability of body size and muscle strength in young adulthood: a study of one million Swedish men. Genetic epidemiology. 2008;32(4):341-9. Epub 2008/02/14.

137. Statistics Sweden PaWd. Multi-generation register 2010, A description of contents and quality. Örebro, Sweden: 2011.

138. Statistics Sweden. Description of the population in Sweden 2008 Örebro, Sweden: 2009.

139. Whitworth JA. 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. Journal of hypertension. 2003;21(11):1983-92. Epub 2003/11/05.

140. Sundstrom J, Neovius M, Tynelius P, Rasmussen F. Association of blood pressure in late adolescence with subsequent mortality: cohort study of Swedish male conscripts. BMJ. 2011;342:d643. Epub 2011/02/24.

141. D'Onofrio BM, Slutske WS, Turkheimer E, Emery RE, Harden KP, Heath AC, et al. Intergenerational transmission of childhood conduct problems: a Children of Twins Study. Archives of general psychiatry. 2007;64(7):820-9. Epub 2007/07/04.

142. Kringlen E, Cramer G. Offspring of monozygotic twins discordant for schizophrenia. Archives of general psychiatry. 1989;46(10):873-7. Epub 1989/10/01.

143. Carlin JB, Gurrin LC, Sterne JA, Morley R, Dwyer T. Regression models for twin studies: a critical review. International journal of epidemiology. 2005;34(5):1089-99. Epub 2005/08/10.

144. Dwyer T, Blizzard L. A discussion of some statistical methods for separating within-pair associations from associations among all twins in research on fetal origins of disease. Paediatric and perinatal epidemiology. 2005;19 Suppl 1:48-53. Epub 2005/01/27.

145. Dwyer T, Morley R, Blizzard L. Twins and fetal origins hypothesis: within-pair analyses. Lancet. 2002;359(9324):2205-6. Epub 2002/07/02.

146. Frisell T, Oberg S, Kuja-Halkola R, Sjolander A. Sibling comparison designs: bias from non-shared confounders and measurement error. Epidemiology. 2012;23(5):713-20. Epub 2012/07/12.

147. Mann V, De Stavola BL, Leon DA. Separating within and between effects in family studies: an application to the study of blood pressure in children. Stat Med. 2004;23(17):2745-56.

148. Lahey BB, D'Onofrio BM. All in the Family: Comparing Siblings to Test Causal Hypotheses Regarding Environmental Influences on Behavior. Current directions in psychological science. 2010;19(5):319-23. Epub 2010/10/01.

149. Boomsma D, Busjahn A, Peltonen L. Classical twin studies and beyond. Nature reviews Genetics. 2002;3(11):872-82. Epub 2002/11/05.

150. Silventoinen KY, Y. Rasmussen, F. Twin Studies on Anthropometrics: Exploring the Role of Genetic and Environmental Factors. In: Preedy VR, editor. Handbook of Growth and Growth Monitoring in Health and Disease: Springer; 2012.

151. Rijsdijk FV, Sham PC. Analytic approaches to twin data using structural equation models. Briefings in bioinformatics. 2002;3(2):119-33. Epub 2002/07/26.

152. Silventoinen K, Kaprio J, Lahelma E, Viken RJ, Rose RJ. Assortative mating by body height and BMI: Finnish twins and their spouses. American journal of human biology : the official journal of the Human Biology Council. 2003;15(5):620-7. Epub 2003/09/04.

153. Kaprio J, Silventoinen K. Advanced methods in twin studies. Methods Mol Biol. 2011;713:143-52. Epub 2010/12/15.

154. Donovan SJ, Susser E. Commentary: Advent of sibling designs. International journal of epidemiology. 2011;40(2):345-9. Epub 2011/04/01.

155. Keyes KM, Smith GD, Susser E. On sibling designs. Epidemiology. 2013;24(3):473-4. Epub 2013/04/04.

156. Andersson ES, Silventoinen K, Tynelius P, Nohr EA, Sorensen TI, Rasmussen F. Heritability of gestational weight gain--a Swedish register-based twin study. Twin research and human genetics : the official journal of the International Society for Twin Studies. 2015;18(4):410-8. Epub 2015/06/27.

157. Andersson ES, Silventoinen K, Tynelius P, Nohr EA, Sorensen TIA, Rasmussen F. Total and Trimester-Specific Gestational Weight Gain and Offspring Birth and Early Childhood Weight: A Prospective Cohort Study on Monozygotic Twin Mothers and Their Offspring. Twin Research and Human Genetics. 2016;19(4):367-76.

158. Andersson ES, Tynelius P, Nohr EA, Sorensen TIA, Rasmussen F. No Association of Maternal Gestational Weight Gain with Offspring Blood Pressure and Hypertension at Age 18 Years in Male Sibling-Pairs: A Prospective Register-Based Cohort Study. PloS one. 2015;10(3).

159. Dabelea D, Hanson RL, Lindsay RS, Pettitt DJ, Imperatore G, Gabir MM, et al. Intrauterine exposure to diabetes conveys risks for type 2 diabetes and obesity: a study of discordant sibships. Diabetes. 2000;49(12):2208-11. Epub 2000/12/16.

160. Power C, Jefferis BJ. Fetal environment and subsequent obesity: a study of maternal smoking. International journal of epidemiology. 2002;31(2):413-9. Epub 2002/05/01.

161. Herskind AM, McGue M, Sorensen TI, Harvald B. Sex and age specific assessment of genetic and environmental influences on body mass index in twins. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 1996;20(2):106-13. Epub 1996/02/01.

162. Hjelmborg J, Fagnani C, Silventoinen K, McGue M, Korkeila M, Christensen K, et al. Genetic influences on growth traits of BMI: a longitudinal study of adult twins. Obesity (Silver Spring). 2008;16(4):847-52. Epub 2008/02/02.

163. Maes HH, Neale MC, Eaves LJ. Genetic and environmental factors in relative body weight and human adiposity. Behavior genetics. 1997;27(4):325-51. Epub 1997/07/01.

164. Brunner Huber LR. Validity of self-reported height and weight in women of reproductive age. Matern Child Health J. 2007;11(2):137-44. Epub 2006/10/27.

165. Keller MC, Medland SE, Duncan LE. Are extended twin family designs worth the trouble? A comparison of the bias, precision, and accuracy of parameters estimated in four twin family models. Behavior genetics. 2010;40(3):377-93. Epub 2009/12/17.

166. Chen X, Kuja-Halkola R, Rahman I, Arpegard J, Viktorin A, Karlsson R, et al. Dominant Genetic Variation and Missing Heritability for Human Complex Traits: Insights from Twin versus Genome-wide Common SNP Models. American journal of human genetics. 2015;97(5):708-14. Epub 2015/11/07.

167. Magnus P, Gjessing HK, Skrondal A, Skjaerven R. Paternal contribution to birth weight. Journal of epidemiology and community health. 2001;55(12):873-7. Epub 2001/11/15.

168. Shapira N. Prenatal nutrition: a critical window of opportunity for mother and child. Womens Health (Lond). 2008;4(6):639-56. Epub 2008/12/17.

169. Loomis D, Kromhout H. Exposure variability: concepts and applications in occupational epidemiology. American journal of industrial medicine. 2004;45(1):113-22. Epub 2003/12/24.

170. Ball K, Crawford D. Socioeconomic status and weight change in adults: a review. Soc Sci Med. 2005;60(9):1987-2010. Epub 2005/03/04.

171. Lin X, Aris IM, Tint MT, Soh SE, Godfrey KM, Yeo GS, et al. Ethnic Differences in Effects of Maternal Pre-Pregnancy and Pregnancy Adiposity on Offspring Size and Adiposity. The Journal of clinical endocrinology and metabolism. 2015;100(10):3641-50. Epub 2015/07/23.

172. Gillman MW, Rifas-Shiman S, Berkey CS, Field AE, Colditz GA. Maternal estational diabetes, birth weight, and adolescent obesity. Pediatrics. 2003;111(3):e221-6. Epub 2003/03/04.

173. Lurbe E, Cifkova R, Cruickshank JK, Dillon MJ, Ferreira I, Invitti C, et al. Management of high blood pressure in children and adolescents: recommendations of the European Society of Hypertension. Journal of hypertension. 2009;27(9):1719-42. Epub 2009/07/25.

174. Oesterle S, Hill KG, Hawkins JD, Guo J, Catalano RF, Abbott RD. Adolescent heavy episodic drinking trajectories and health in young adulthood. Journal of studies on alcohol. 2004;65(2):204-12. Epub 2004/05/21.

175. National Institute for Health and Care Excellence. Weight management before, during and after pregnancy. 2010.

176. McCarthy EA, Walker SP, Ugoni A, Lappas M, Leong O, Shub A. Self-weighing and simple dietary advice for overweight and obese pregnant women to reduce obstetric complications without impact on quality of life: a randomised controlled trial. BJOG : an international journal of obstetrics and gynaecology. 2016;123(6):965-73. Epub 2016/02/16.

177. Sutton EF, Redman LM. Smartphone applications to aid weight loss and management: current perspectives. Diabetes, metabolic syndrome and obesity : targets and therapy. 2016;9:213-6. Epub 2016/08/04.