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MATERNAL OBESITY SURGERY: EFFECTS IN WOMEN, SPOUSES AND OFFSPRING

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Maternal obesity surgery: Effects in women, spouses and offspring

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Till **Viola Berglind**

”Om Jag förlorar dig

Så förlorar Jag synen

Om Jag förlorar dig

Då har Jag ingenting kvar

Förlåt mig men Du är allt Jag har”

Jocke Berg

ABSTRACT

Introduction

Bariatric surgery is an important treatment for the worldwide increasing epidemic of obesity. However, the effects of such surgery on offspring epigenetic profile and effects on objectively measured physical activity and sedentary behavior in women undergoing bariatric surgery and family members are essentially unknown.

Aim

The aim of this thesis was to investigate possible effects of maternal weight loss after bariatric surgery and effects on differences in maternal gestational weight gain in repeated pregnancies of the same women on sibling body size in childhood, epigenetic profile and physical activity among siblings and spouses.

Methods

Longitudinal studies with repeated objective measures in the same women, spouses and offspring before and after maternal bariatric surgery.

Results

There were positive associations, in women undergoing bariatric surgery (n = 124), between differences in total and second trimester gestational weight gain and differences in offspring birth weight.

Maternal bariatric surgery, with subsequent weight loss between pregnancies, is associated with overrepresentation of differences in methylated sites in genes involved in inflammation and type-2 diabetes signaling when comparing offspring born before (n = 31) and after (n = 31) maternal bariatric surgery.

There were no significant differences in objectively measured physical activity or time spent sedentary from three months before to nine months after surgery in women undergoing Roux-en-Y Gastric Bypass (n = 56), despite substantial weight loss.

Objectively measured physical activity and time spent sedentary three months before and nine months after the women's Roux-en-Y Gastric Bypass did not differ significantly in spouses (n

= 33). However, during the same period, physical activity decreased significantly in children (n = 75), while time spent sedentary increased significantly.

Conclusion

Interpregnancy differences in gestational weight gain, in women undergoing bariatric surgery, are associated with increased differences in offspring birth weights and differences in the epigenetic regulation of obesity-related genes in comparison of siblings born before and after maternal bariatric surgery. Furthermore, large weight loss after maternal Roux-en-Y Gastric Bypass is not associated with significant change in physical activity or time spent sedentary in women or spouses, while children decrease physical activity and increase time spent sedentary from three months before to nine months after maternal Roux-en-Y Gastric Bypass.

LIST OF SCIENTIFIC PAPERS

I. Daniel Berglind, Mikaela Willmer, Erik Näslund, Per Tynelius, Thorkild I. A. Sørensen and Finn Rasmussen

Differences in gestational weight gain between pregnancies before and after bariatric surgery: Correlation with birth weight but not childhood BMI
Pediatr Obes. *2013 Dec 11. doi: 10.1111/j.2047-6310.2013.00205.x.*

II. Daniel Berglind, Patrick Müller, Mikaela Willmer, Indranil Sinha, Per Tynelius, Erik Näslund, Karin Dahlman-Wright and Finn Rasmussen

Differential methylation levels in genes involved in inflammation and type 2 diabetes in siblings born before and after maternal bariatric surgery
Submitted manuscript

III. Daniel Berglind, Mikaela Willmer, Ulf Eriksson, Andres Thorell, Magnus Sundbom, Joanna Uddén, Mustafa Raof, Jakob Hedberg, Per Tynelius, Erik Näslund and Finn Rasmussen

Longitudinal assessment of physical activity in women undergoing Roux-en-Y gastric bypass
Obes Surg. *2014 Jun 17. doi: 10.1007/s11695-014-1331-x*

IV. Daniel Berglind, Mikaela Willmer, Per Tynelius, Ata Ghaderi, Erik Näslund and Finn Rasmussen

Women undergoing Roux-en-Y Gastric Bypass surgery: Family resemblance in pre- to post-surgery physical activity and sedentary behaviour in children and spouses
SOARD. *(Accepted for publication. 2014 October)*

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LIST OF ABBREVIATIONS

ACSM	American College of Sports Medicine
AMS	After maternal surgery
ATP10A	ATPase, class V, type 10A
BIA	Bioelectric impedance
BMI	Body mass index
BMS	Before maternal surgery
BPD	Biliopancreatic diversion
CpG-islands	Regions of DNA where a cytosine occurs next to a guanine
CPM	Counts per minute
DMS	Differently methylated sites
EWAS	Epigenome-wide association studies
FTO	Fat mass and obesity-associated protein
FACS	Fluorescence activated cell sorting
GWAS	Genome-wide association study
GWG	Gestational weight gain
HLA-DQA1	Major histocompatibility complex, class II, DQ alpha 1
HLA-DQB1	Major histocompatibility complex, class II, DQ beta 1
IGF1	Insulin-like growth factor 1
IGF2	Insulin-like growth factor 2
IL1	Interleukin 1
IL6	Interleukin 6
INSR	Insulin receptor
IOM	Iinstitute of medicine
IPA	Ingenuity pathway analysis

MC4R	Melanocortin receptor 4
MET	Metabolic equivalent
MVPA	Moderate to vigorous physical activity
PA	Physical activity
PDK4	Pyruvate dehydrogenase kinase, isozyme 4
PGC1-α	Peroxisome proliferator-activated receptor, 1 alpha
POMC	Proopiomelanocortin
RYGB	Roux-en-Y gastric bypass
SB	Sedentary behavior
SD	Standard deviation
SDS	Standard deviation score
TMEM18	Transmembrane protein 18
TNF	Tumor necrosis factor
WHO	World Health Organization

1 INTRODUCTION

1.1 DEFINITIONS

Overweight and obesity

In order to internationally measure and monitor overweight and obesity in a standardized manner the Department of Nutrition and Health and Development, together with the World Health Organization (WHO), developed the WHO Global Database on body mass index (BMI) [(1)]. BMI is non-invasive, easy to use and at present frequently used to measure overweight and degree of obesity. However, BMI has its limitations: it does not measure body fat or muscle distribution, and does not take into account the changes in body composition that occur with age [(2)].

BMI is calculated by dividing the individual's weight in kilograms by the square of their height in meters. The WHO definition of BMI, often used to calculate excess body weight and excess body weight loss, is presented in Table 1 [(1)].

Table 1. Classification of BMI according to the World Health Organization (WHO)

Classification of BMI according to WHO	
BMI	***Classification***
18.5 – 24.9	Normal weight
25 – 29.9	Overweight
30 – 34.9	Class I obesity
35 – 39.9	Class II obesity
≥ 40	Class III obesity

Since BMI in childhood changes substantially with age [(3)], cut-off points related to age are necessary to define child obesity. Therefore, an international standard definition, connected to adult cut-off points, is used when defining child overweight and obesity [(4)].

Gestational weight gain

Gestational weight gain (GWG) is a product of the growing fetus and placenta, increasing volume of maternal blood and water, and gain in adipose tissue [(5)]. Table 2 demonstrates the 2009 Institute of Medicine (IOM) published and revised GWG guidelines, which are based on pre-pregnancy BMI ranges for underweight, normal-weight, overweight, and obese women [(6)].

Table 2. Institute of Medicine guidelines for GWG depending on pre-pregnancy BMI

	Pre-pregnancy BMI (kg/m^2)	**Total GWG (kg)**
Underweight	<18.5	12.7 – 18.2
Normal weight	18.5 – 24.9	11.4 – 15.9
Overweight	25.0 – 29.9	6.8 – 11.4
Obese	≥ 30.0	5.0 – 9.1

Physical activity

Physical activity (PA) is defined as bodily movement produced by skeletal muscles that results in energy expenditure [(7)]. Energy expenditure has three components: basal metabolic rate, the thermic effect of food, and energy expenditure due to PA which is a major modifiable variable for an individual's regulation of energy expenditure. PA is a behavior that can be further divided into different categories: leisure-time activity, occupational activity, automatic movements, and exercise, which is a type of leisure-time activity that is planned, structured and repetitive and done to improve or maintain physical fitness [(8)].

Intensity, frequency and duration are the three components that determine the effect of PA. The intensity of PA is often described in terms of MET (metabolic equivalent), which is the ratio of work metabolic rate during an activity to resting metabolic rate. MET describes the energy cost of physical activities, and therefore the rate of energy consumption during a specific physical activity to a reference metabolic rate. One MET is set as the resting metabolic rate obtained during quiet sitting. Light PA (LPA) is considered to range from 1.5 to 3.0 METs, and Moderate-to-Vigorous PA (MVPA) to be at 3.0 METs or more [(9)].

Sedentary behavior

Sedentary behavior (SB) is defined as waking behavior resulting in energy expenditure lower than 1.5 METs while in a sitting or reclining position [10]. SB implies muscular inactivity within the large muscle groups of the body, and is thereby characterized by low energy expenditure.

Epigenetics

The term epigenetics was originally described in the early half of the 20th century in terms of causal interactions by genes and their products affecting the phenotype [11]. Nowadays, epigenetics is generally accepted as *''the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence''* [12]. Epigenetic modifications include DNA methylation of cytosine residues at the carbon five positions, histone modifications (e.g., acetylation, ubiquitylation), and also the effects of non-coding RNAs [13]. DNA methylation was the first epigenetic modification described at a molecular level [14] and has since then been the most widely studied to date, especially in the context of molecular epidemiology [15].

1.2 BACKGROUND

The co-morbidities of overweight and obesity, such as cardiovascular disease, several cancer forms and type-2 diabetes (T2D), kill at least 2.8 million adults worldwide each year [16]. During the last few decades, the prevalence of overweight and obesity in Sweden has increased, with approximately 50% of men, 40% of women and 10% of children now overweight, and 14% of both men and women and 5% of children obese [17] [18], which reflects a more than 50% increase from 1980 to 2012 in men and women [19]. Moreover, the prevalence of overweight and obesity among women in early pregnancy has increased rapidly in Sweden, from 10.6% and 2.1%, respectively, in 1982 to 24.8% and 12.6% in 2010 [20]. This is of special concern as obesity during pregnancy is a major risk factor for complications in pregnancy and delivery, including gestational diabetes, and increased offspring birth weight, which in turn are risk factors for childhood obesity [21]. In addition, large maternal GWG is associated with increased birth weight and risk of obesity in offspring [22]. However, the etiology of childhood obesity, which is an established major public health problem [23], is complex and influenced by inheritance of the parents' obesity-related genes, their eating and PA behaviors, and possibly also by structural conditions such as family factors and the community physical activity environment [24].

Causes of obesity

An increase in size and most likely also the number of fat cells in adults are vital to the development of obesity [25]. The simple explanation for this phenomenon is an anomaly in energy balance [26]. However, it is not known whether it is a decrease in PA or an increase in energy intake, over the past decades, which explains the majority of the increases seen in the prevalence of obesity in large parts of the world. There seems to be evidence for both reduced expenditure of intake and increased energy intake [27-29]. The imbalance itself can be further subdivided into the genetic, epigenetic and environmental factors, and is most likely a combination of them all [30]. The gene-environment factor is generally seen as one of the major contributors to increases in obesity [30], and the link between genes and environment may be explained by epigenetic variations due to environmental influences on gene expression [31]. Furthermore, twin-data from Silventoinen et al. indicate that PA modifies the degree of genetic influence on obesity where individuals who are at greatest genetic risk of developing obesity would benefit the most from increasing their PA [32].

Co-morbidities of obesity

Only a few of the numerous co-morbidities that accompany obesity, with different strengths of association, are included in this thesis. T2D is a common co-morbidity strongly associated with obesity [33], which seems to increase with severity of obesity [34]. High blood pressure is another common co-morbidity associated with obesity that likewise increases with severity of obesity [34]. Furthermore, cardiovascular disease [35] and increased cardiovascular-related mortality are strongly associated with obesity [36]. Several cancer forms, such as pancreatic, colorectal, and postmenopausal breast and kidney cancer, are associated with obesity [16]. With all these co-morbidities and their associations with obesity, it is not surprising that mortality is elevated by increasing obesity [37]. The increased risk of BMI-related mortality seems to be higher among younger individuals, although the risk is elevated, in both men and women, until 75 years of age [38]. As an example, a 20 year-old white man with a BMI of 45 or more is predicted to lose 13 years of his predicted 78 years of life [39, 40].

Childhood obesity is associated with numerous risk factors for later heart disease, such as hyperlipidaemia, hyperinsulinaemia, atherosclerosis, and hypertension [41]. However, it is not fully known whether these risk factors operate through the association between child and adult obesity or if they act independently [42]. Nonetheless, WHO has defined childhood obesity as an established major public health problem, stating the importance of the world wide epidemic [23].

GWG and obesity

Excess GWG (as defined by IOM) is a risk factor for short- and long-term post-partum weight retention, and thus for overweight and obesity in women [43]. Furthermore, maternal obesity and excess GWG may have intergenerational effects, via fetal epigenetic programing of metabolic function, which may perpetuate obesity in the next generation [44]. This vicious circle may have serious long-term negative consequences for both mothers and their offspring. It is speculated that maternal obesity and excess GWG result in epigenetic modifications, which predict the need for efficient fat storage in postnatal life [45, 46]. The negative long-term effects on offspring of maternal obesity during pregnancy is supported by epidemiological data indicating that children born to obese women before maternal bariatric surgery (BMS) are at higher risk of obesity than those born after maternal surgery (AMS) [47]. Results from the same series of obese women undergoing bariatric surgery and their offspring indicate differential methylation patterns in glucoregulatory, inflammatory and vascular disease genes in BMS siblings compared with AMS siblings [48]. Further results emphasizing the importance of the intrauterine environment come from survivors of the 1944 Dutch Hunger Winter. Individuals exposed to famine when in utero showed a higher incidence of coronary heart disease and dyslipidaemia later in life compared with siblings who had grown up under "normal" in utero conditions. The same individuals who were prenatally exposed to famine during the 1944 Dutch Hunger Winter had, six decades later, less DNA methylation of gene promoter of the imprinted insulin-like growth factor 2 gene (*IGF2*) compared with their unexposed, same-sex siblings [49]. Excessive fetal growth, from excessive GWG and high dietary fat and sugar intake during pregnancy, which may lead to altered glucose metabolism, are associated with intergenerational effects. Human studies indicate that excessive fetal growth may predispose offspring to develop obesity, T2D and cardiovascular disease [50-52]. Further data supporting the effect of fetal environment on offspring susceptibility to disease later in life come from studies on epigenetic heritability in mice. Expression of the agouti viable yellow (A_{vy}) locus, which is associated with a yellow coat and obese phenotype, differ among offspring depending on DNA methylation and whether the mother was fed a diet rich in methyl donating compounds (e.g., folate), or not [53]. This obese phenotype has been shown to be transmitted across two subsequent generations [54], and in the third generation only females maintained the obese phenotype [55]. This finding highlights the possibility of an imprinting mechanism in the heritability of obesity, at least in certain species [30]. Imprinting is a phenomenon where epigenetic markers, such as methylation, affect gene expression according to their maternal or

paternal origin [56]. Taken together, these data support the hypothesis that changes in the availability of nutrients in fetal life may lead to epigenetic changes that are maintained throughout life [45]. Consequently, there is increasing support in the literature for the need of dietary interventions for obese mothers during and perhaps even before pregnancy in order to, via possible epigenetic mechanisms, decrease the risk of obesity in offspring [57].

DNA methylation

DNA methylation involves covalent attachment of a methyl group to a cytosine at the five-carbon position of its pyrimidine ring. This occurs primarily in CpG dinucleotides, where a cytosine (C) base is positioned adjacent to a guanine (G) base in the DNA sequence. The human genome is estimated to contain approximately thirty million CpGs [58], which are overrepresented in regions referred as CpG islands, often present in the promoter region of a gene [59]. Approximately eight to 12% of gene promoters are methylated under normal conditions [60]. Methylation of the promoter region inhibits the binding of transcription factors, and is typically associated with transcriptional inactivation. Furthermore, recent evidence suggests that methylation of DNA sequences outside but within a two-kilo base-pair distance of a CpG island is associated with transcriptional repression of gene activity [61]. In contrast to promoter methylation, methylation in the gene body may lead to increased transcriptional activity [62]. Thus, activation or repression of gene activity is not only dependent on total methylation status, but also highly dependent on the specific sites throughout the gene where the methylation takes place.

Genetic and epigenetic control of obesity

The etiology of obesity is complex, influenced by genetic and epigenetic changes in combination with reduced levels of PA and increased consumption of energy-dense foods [63]. Currently, approximately 150 genetic loci, identified in genome-wide association studies (GWAS), are linked to obesity, each accounting for only a small proportion of the predicted heritability of obesity. For BMI, the strongest genetic factors that appear in virtually all GWAS studies are fat and obesity associated protein (*FTO)*, mealnocortin 4 receptor (*MC4R*), and transmembrane protein 18 (*TMEM18)* [63]. Approximately two percent of the observed variance in BMI can be explained by these loci [64], whereas twin and adoption studies have reported 45% to 85% heritability of obesity [65]. Considering that the proportions of gene variants (single nucleotide polymorphism) that have been found to be associated with occurrence of obesity are modest, this highlights the question of whether some of familiar aggregation as well as many of

the effects of environmental exposures on weight differences may reflect epigenetic processes. Epigenetic mechanisms can, via alterations in gene expression, predispose individuals to a particular phenotype, which is more or less susceptible to gains in fat mass. These mechanisms can operate in fetal life, early childhood, and also throughout adult life [66]. In short, the increases in obesity over the past decades [17] can potentially be explained by genetic and epigenetic changes to the genome, together with reduced levels of PA and increased consumption of energy-dense foods, causing an energy imbalance [63].

It is speculated that environmental factors can alter the epigenetic regulation of genes involved in appetite regulation, thereby increasing the risk of development of obesity among individuals with an obesogenic genotype [67]. Leptin is a central hormone of importance for appetite regulation and metabolism control, which is positively correlated with increased body fat deposits in humans [68]. It has been speculated that the degree of methylation in specific promoter regions of the leptin gene could be used as epigenetic biomarkers for a tendency to respond with advantageous (less obesogenic) epigenetic regulation of the leptin gene from a low calorie diet [69]. Another gene associated with obesity and important for appetite regulation is the *MC4R* gene, which has been shown to be hypomethylated in the promoter region, leading to increased gene expression, and consequently increased appetite and feeding behavior, in mice given a high-fat diet [70]. However, the exact mechanism and impact on obesity of the genes involved in appetite regulation are not known [30, 71].

Nutritional compounds may modify expression of genes involved in the development of obesity through epigenetic mechanisms such as DNA methylation [72]. Lomba et al. found that a high-fat diet given to rats is associated with subsequent weight gain and altered DNA methylation patterns in genes involved in the development of obesity, e.g., fatty acid synthase which is a gene coding for a key enzyme involved in lipogenesis [73]. Thus, a high-fat diet may effect the expression of obesogenic genes, which are regulated by epigenetic mechanisms, thereby increasing the risk of developing obesity [74].

Low-grade systemic inflammation is associated with excessive adiposity, and may be subject to epigenetic influences [75]. However, it is not known whether the excessive adiposity is either cause or consequence of a chronically inflamed state [76]. Diets giving rise to an energy deficiency are associated with altered methylation patterns in the genes involved in inflammatory pathways [77], and severe systemic inflammation is associated with epigenetic

alterations in pro-inflammatory genes [78]. In short, several inflammatory genes are under the epigenetic control of gene activity.

Physical activity and obesity

The dramatic increase in obesity worldwide during the past decades is associated with, and has been partly ascribed to, lower levels of PA [79]. Furthermore, clinical practice today for obesity treatment and long-term weight management includes PA counseling [80]. Higher levels of PA should be an integrated part of any treatment plan for obese individuals regardless of weight loss goals since high levels of PA are inversely associated with cardiovascular disease, T2D and all-cause mortality [81]. In fact, numerous epidemiological studies suggest that high levels of PA attenuate the health risk of obesity [82-84], although the likelihood of having high PA and high fitness is to a great extent influenced by BMI. A study by Duncan et al. [85] showed that only nine percent of obese individuals had high cardiorespiratory fitness, whereas 17% of overweight and 30% of normal-weight individuals showed similarly high levels of fitness.

Physical activity recommendations

The current American College of Sports Medicine (ACSM) recommendations for PA to maintain health [86] and promote weight loss [87] are summarized in Table 3. The intensity of PA should be at least MVPA, performed in bouts of at least 10 minutes duration [88].

Table 3. American College of Sports Medicine recommendations for physical activity

Recommendations for Physical Activity	Minutes per week in MVPA 10 min-bouts
Maintaining and improving health	At least 150
Prevention of weight gain	150 - 250
Promotion of clinically significant weight loss	225 - 420
Prevention of weight gain after weight loss	200 - 300

Physical activity and weight change

Weight change is affected by the amount of energy expended versus the amount of energy consumed [89]. There is evidence that high levels of PA can attenuate weight gain in people at risk of obesity [90]. Weight loss interventions, involving increased PA and exercise training

programs without a dietary plan, produce, on average, a modest weight loss of two kilograms according to Donnelly and colleagues [87].

Objectively measured cross-sectional data support strong associations between lower levels of PA and higher risks of obesity in children, adolescents and adults [91, 92]. However, the cross-sectional study design makes it impossible to draw conclusion about causality. There is currently no firm evidence for any longitudinal association between PA and the risk of developing obesity [93, 94]. Most previous longitudinal observational and intervention studies assess PA by questionnaires or interviews that are susceptible to misclassification and recall bias [95]. In short, PA at baseline is not a strong predictor of weight change in children, adolescents or adults. However, high levels of PA are consistently and strongly associated with several positive health outcomes, such as beneficial changes in, for example, cholesterol [96], and interventions including exercise training programs to promote PA confer health benefits on overweight individuals even in the absence of weight loss [97, 98]. Thus, high levels of PA should be promoted for public health, although the impact of PA on weight change should not be exaggerated.

As indicated in ACSM recommendations for PA (Table 3), obese individuals require a substantial amount of PA to maintain their weight after successful weight loss [87]. Unfortunately, research on weight regain after subsequent weight loss mostly relies on observational studies using retrospective data [99]. However, some studies of weight regain with data on PA are worth mentioning. Andersen et al. [100] monitored weight and PA at one year follow-up after an intervention weight loss study extending over 16 weeks. Two different groups were evaluated with a low fat diet and exercise training program, and a low-fat diet and "regular" PA counseling. After 16 weeks of intervention, both groups lost approximately eight kilograms in weight. At one-year follow-up, people who were the most active (exercise training program) lost an additional 1.9 kg, whereas the less active group regained 4.9 kg. Another study of weight maintenance observed a dose-response association between the amount of self-reported PA and success with weight loss at 18 months after an intervention comprising caloric restriction and exercise training. Participants who exercised more than 200 minutes per week lost more weight (-13.1 kg) than those who exercised less than 150 minutes per week (-3.5 kg) [101]. Lastly, Jakicic et al. [102] observed similar findings in a 12-week long intervention comprising caloric restriction and exercise training in women. At 12-month follow-up, women who exercised more than 200 minutes per week had maintained a significantly greater percentage of their weight loss (13.6%) compared with those who had exercised less than 150

minutes per week (4.7%). A recent review exploring the effects on long-term weight loss and risk of chronic-disease risk factors when diet is combined with exercise, compared with either diet or exercise alone, found no advantage in minimizing weight regain for any weight loss method [103]. In summary, PA may have a role in preventing weight regain after successful weight loss from interventions including high levels of PA and caloric restriction, although the effect of PA on minimizing weight regain should not be exaggerated.

1.3 BARIATRIC SURGERY

The American Heart Association's scientific statement on obesity and weight loss recommends weight loss in obese patients to reduce several obesity-related co-morbidities and increase longevity [104]. Unfortunately, traditional weight loss regimes, based on dietary restrictions and PA counseling, have modest long-term effects [105]. With these facts in mind, it is not surprising that the use of bariatric surgery has increased sharply in the last few decades. The number of patients treated for severe obesity by surgery in Sweden has increased from 790 in 1997 to over 7900 in 2012 [106]. Laparoscopic Roux-en-Y Gastric Bypass (RYGB) has been standardized in Sweden [107], and accounts for approximately 92% of all bariatric surgery carried out in Sweden today [106]. The laparoscopic approach reduces the risk of 30-day in-hospital morbidity and mortality compared with open surgery, and is further associated with shorter in-hospital stays [108].

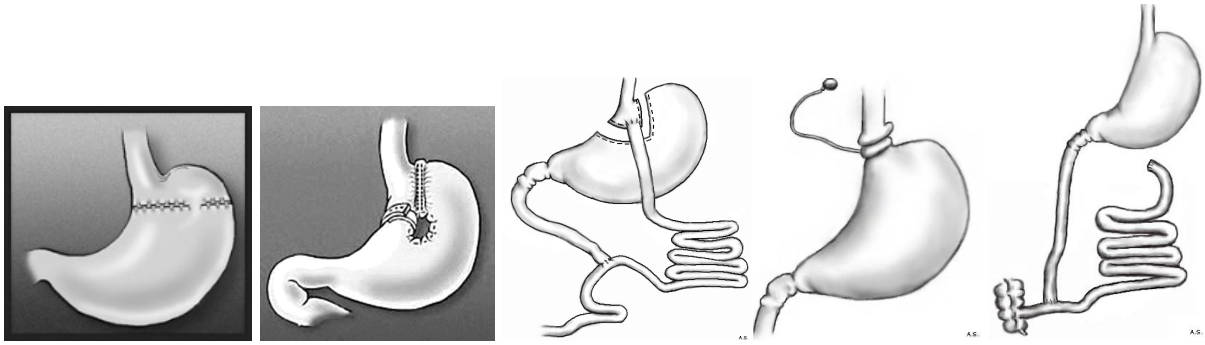
Indications

Swedish recommendations for bariatric surgery are for people with BMI of 35 or more, with or without co-morbidity [109]. The patient should have made serious attempts to lose excess weight prior to primary bariatric surgery. Surgery is performed in the age span 18 to 60 years, although there are exceptions. Patients under the age of 18 may be treated by surgery if they are enrolled on a scientific program, and in patients over the age of 60 a risk-benefit evaluation is of importance [109, 110]. Contraindications for bariatric surgery include non-compliant patients with cognitive or mental disorders, alcohol or drug abuse or severe mental illness [111]. Although obesity prevalence in Sweden is fairly equally prevalent in men and women [17], approximately 70% of bariatric surgery candidates are women [106]. This may reflect society's views on obesity differing according to gender [112].

Surgical methods

The currently used bariatric surgery procedures can be divided into restrictive and gut peptide altering surgery [113]. Some examples of restrictive procedures are horizontal gastroplasty (1), vertical banded gastroplasty (2) and gastric banding (4), while examples of gut peptide altering procedures are RYGB (3) and Jejunoileal bypass (5).

Figure 1. Examples of bariatric surgery procedures



1 **2** **3** **4** **5**

Complications after surgery

Examples of general post-surgery complications are gastric leak, bleedings, internal hernias, and dumping syndrome (RYGB) [114]. Furthermore, nutritional deficiencies, which are more common after gut peptide altering compared with restrictive procedures, may follow bariatric surgery [115]. The post-surgery mortality risk is generally low after bariatric surgery. According to the Scandinavian Obesity Surgery Registry, which has been linked to the Swedish total population register, 30-day and 90-day mortality between 2007 and 2012 were 0.05% and 0.08%, respectively [106].

Weight outcome after bariatric surgery

The general criterion for successful bariatric surgery is set at 50% or more excess weight loss sustained for five years after surgery (excess weight equals total pre-surgery weight minus ideal weight) [116]. A Cochrane review from 2009 showed that two-year follow-up weight loss was greater following RYGB compared with vertical banded gastroplasty and adjusted gastric banding [117]. The Swedish Obese Subjects (SOS) study shows a maximum weight loss, one to two years after surgery, of 32% in RYGB, 25% in vertical banding, and 20% in gastric banding [118]. In the SOS study 13% of all surgical procedures were RYGB and the mean post-surgery weight losses after 10, 15 and 20 years were 17%, 16% and 18%, respectively [119]. However, data from the Scandinavian Obesity Surgery Registry shows that all surgical procedures are

associated with a slight weight regain (approximately 5%), which often occurs between two and five years post-surgery [106].

1.4 BEFORE AND AFTER BARIATRIC SURGERY

1.4.1 Gestational weight gain and offspring weight

Weight-loss before conception is an effective way of reducing the risk of obstetric complications in women. Thus, bariatric surgery has become an important alternative for obese women planning pregnancy [120]. Studies that have examined pregnancy outcomes after bariatric surgery have reported lower rates of adverse perinatal outcomes after surgery [121], and similar outcomes for the different procedures [122]. Moreover, compared with obese women not undergoing bariatric surgery, the risk of obesity-related gestational complications decreased significantly after bariatric surgery [123]. However, data on GWG and offspring weight development in early childhood based on paired pregnancies with siblings born before and after bariatric surgery are sparse. Aricha-Tamir et al. showed similar GWG before (3.9 BMI units) and after (3.9 BMI units) bariatric surgery in 144 paired pregnancies. However, offspring birth weight was significantly lower after bariatric surgery. A study from 2013 with paired data on 109 women who gave birth before and after bariatric surgery (only restrictive bariatric procedures were included) showed similar GWG across three pregnancies, while offspring birth weight was significantly lower in the two pregnancies subsequent to bariatric surgery compared with offspring birth weight before surgery [124]. Although parity is a risk factor for weight gain and visceral obesity [125], the women had essentially the same pre-pregnancy BMI and GWG in both pregnancies following surgery. Bearing in mind the fact that women retain significant weight postpartum [125], contributing to the vicious circle of obesity, it is of importance that women undergoing bariatric surgery do not gain weight between pregnancies following bariatric surgery [124].

1.4.2 Physical activity

The vast majority of bariatric surgery candidates are known to engage in low levels of PA [126], and fail to increase objectively measured PA post-surgery despite substantial weight loss [127, 128]. On the contrary, subjectively measured PA increase after surgery [129-131], which clearly

reflects the problem of the over-reporting of self-reported PA among obese individuals [132]. Moreover, time spent sedentary [133] and PA through the activities of daily living (e.g., house-work) have been shown to be more difficult to accurately recall compared with planned exercise [134]. This is of concern since PA among bariatric surgery candidates mostly derives from daily living and not planned exercise [135]. Previous studies, assessing pre- to post-bariatric surgery changes in objectively measured PA, have used pedometers or accelerometers, to assess PA after surgery [128] [127]. A recent study of 40 patients used the Sense Wear Armband and the Step Watch Activity Monitor when assessing PA and SB six to 18 months after bariatric surgery. Based on accelerometer data, the study reported that patients spend more than 70% of their waking time in SB, and as little as five percent in MVPA. However, in an assessment based on average daily step counts form pedometers, 39% (9108 SD 4360 steps per day) of patents were classified as active [136]. Nevertheless, almost all current results on objectively measured PA after bariatric surgery [127, 128, 136] indicate that post-surgery PA levels following bariatric surgery may not be sufficient to optimize weight loss and prevent weight regain over time [86, 87].

1.4.3 Epigenetics

Bariatric surgery, especially RYGB, dramatically improves insulin sensitivity and may lead to the clinical remission of T2D, often before weight loss has occurred [137]. Epigenetic regulation via DNA methylation has been suggested to regulate the activity of genes involved in metabolic regulation in T2D [138], and change in DNA methylation at specific sites within a gene may be one of the underlying mechanisms contributing to rapid metabolic improvements after RYGB. Barres and colleagues have reported differences in DNA methylation within the promoter region of genes peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (*PGC-1α*) and pyruvate dehydrogenase lipoamide kinase isozyme 4 (*PDK4)*, which are involved in the regulation of lipid metabolism and associated with improvements in insulin sensitivity and weight loss following RYGB [139]. RYGB but not caloric restriction alone, results in alterations in the promoter methylation of genes involved in inflammation and metabolic regulation, such as *PDK4,* interleukin 1 (*IL1),* interleukin 6 (*IL6)* and tumor necrosis factor (*TNF)*. Thus, alterations in promoter specific methylation following RYGB may be elicited by factors other than mere caloric restriction [140].

Maternal obesity and diet in pregnancy may impact on epigenetic control, via regulation of the accessibility of the transcription machinery to the chromatin, and may predispose offspring to a particular phenotype, which may be or less susceptible to gains in fat mass and the development of T2D [66]. The large weight loss that often follows bariatric surgery may contribute to the exposure of a fetus to a less obesogenic intrauterine environment, which may decrease the risk of obesity in offspring by epigenetic mechanisms [141]. Previous studies have shown that children born after biliopancreatic diversion (BPD) have a lower prevalence of obesity [47], greater insulin sensitivity, and an improved lipid profile [142] in comparison with children born before maternal BPD. By contrast, other researchers failed to detect an effect of bariatric surgery on weight development in offspring in an analysis of 164 children born before and 176 children born after maternal surgery [143]. The discrepancies seen in offspring BMI after bariatric surgery in studies by Kral et al. and Willmer et al. may be due to different surgical methods used in the two studies (BPD compared with mostly restrictive surgery) with different effects on both post-surgery weight loss and other biological aspects such as the epigenetic regulation of gene activity [144].

Bariatric surgery has been shown to have an impact on the methylation levels of genes in glucose regulation, immune response and inflammatory signaling in comparison with BMS and AMS siblings [48, 145]. Moreover, a pilot study from 2011 that focused on epigenetic analyses, using whole blood in patients undergoing RYGB, showed changes in the gene expression of T2D and obesity-related pathways [146]. Collectively, these results demonstrate powerful effects of RYGB and BPD on the epigenetic and transcriptional levels of the genes involved in inflammation and metabolism, both in patients undergoing surgery and in offspring born after maternal surgery.

2 AIMS OF THE STUDIES

The overall aim was to (i) investigate the possible effects of maternal weight loss after bariatric surgery, and the effects of differences in maternal gestational weight gain in repeated pregnancies of the same women on sibling body size in childhood, epigenetic profile and (ii) to objectively characterize physical activity and sedentary behavior among women, children and spouses, before and after maternal Roux-en-Y gastric bypass surgery.

Specific research questions:

- Are differences in maternal gestational weight gain in repeated pregnancies of the same women before and after bariatric surgery associated with differences in offspring birth weight and BMI at four and six years of age? (Study one)

- Are there epigenetic methylation differences in obesity-related genes between siblings born before and after maternal weight loss after bariatric surgery? (Study two)

- Are obese women treated by Roux-en-Y gastric bypass who lose a large amount of weight more physically active nine months after surgery? (Study three)

- Are the children and spouses of obese women treated by Roux-en-Y gastric bypass who lose a large amount of weight more physically active nine months after surgery? (Study four)

3 MATERIAL AND METHODS

3.1 DATA COLLECTION ONE

Data collection one was used for studies one and four.

Data-registers

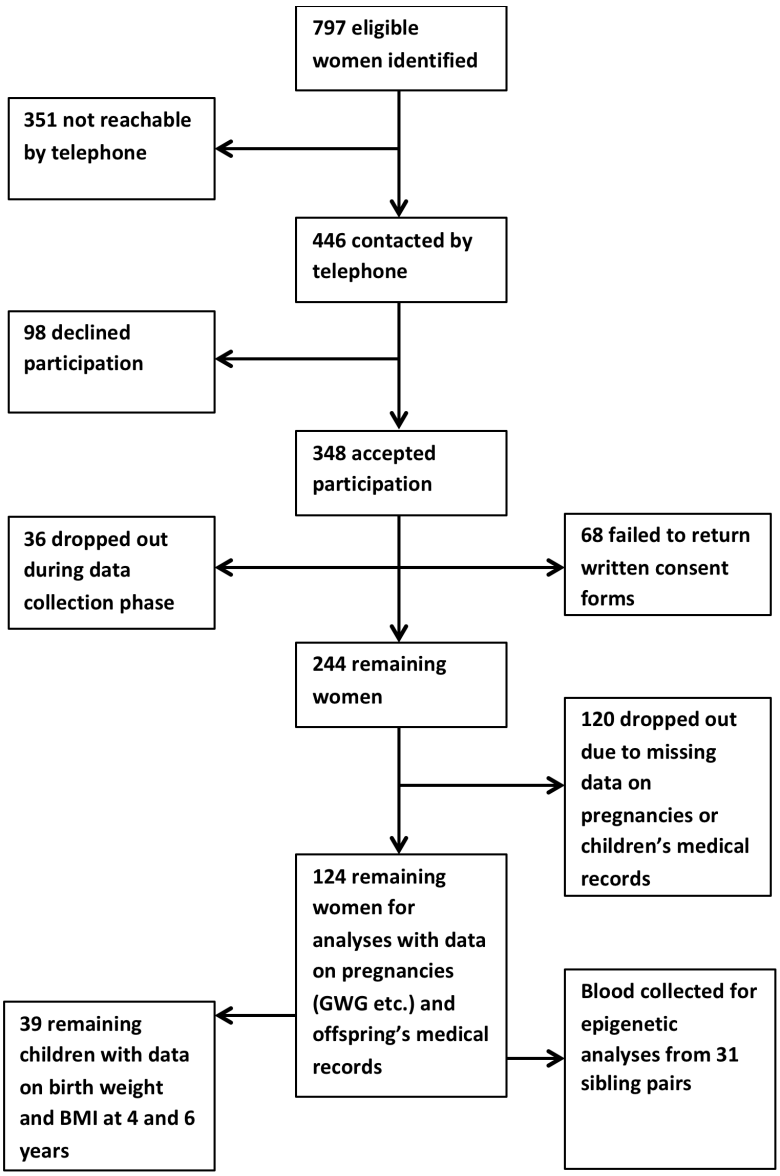
The personal identification number (ID) unique to each Swedish resident enabled us to create a database by record linkage between the Swedish Medical Birth Register, which covers 99% of all births in Sweden [147], the Hospital Discharge Register, the Cause of Death Register ,which contains information about all bariatric operations performed in Sweden, the Cancer Register, and the Register of the Total Population, which contains data on all current residents in Sweden.

The data set finally comprised 797 women who had undergone bariatric surgery between 1980 and 2006, and had given birth both before and after surgery. All 797 women received an information letter by mail, and were then contacted by telephone. The women who wished to participate and their adult children signed a general consent form and a specific form that allowed our team to collect information from the registers described above (including ID numbers).

Data collection on GWG and offspring weight

We then collected measured data from hospital archives on the women's BMI from first antenatal visit (approximately in gestational week ten) and throughout pregnancy, and about complications during pregnancy. Moreover, we collected data on BMI at the time of their bariatric surgery, and all available follow-up BMI data. For children, we collected growth charts from birth to six years of age from child health centers, school health services, and county council and municipal archives. In total, we collected GWG and birth-weight data on 124 women and their 124 offspring sibling-pairs (248 siblings in total), and also measured data on height and weight from birth to six years of age for 39 offspring sibling-pairs (Figure 2).

Figure 2. Overview of the data collection one procedure



Furthermore, we collected information from medical birth records, which contained the following data: maternal age, weight, height, gestational week, parity, offspring birth weight and length, gestational diabetes, pre-eclampsia, breast-feeding, and smoking status.

Blood collection

The 124 women and their offspring were contacted via telephone and mail and asked to visit their nearest health center for their blood (2 x 5ml) to be collected. Blood samples were collected during an overnight fast from an antecubital vein into tubes containing EDTA (Qiagen). The final study sample (comprising women who had lost at least one BMI unit between pregnancies) encompassed blood samples from 31 women with offspring born before and after their mothers' bariatric surgery (62 siblings). The blood collection followed standard

procedures, and the collected blood was sent for storage at -80° to the biobank held by Karolinska Institutet.

DNA methylation analysis

Recent technological advances have made it possible to map DNA methylation genome-wide, at high resolution in large samples [148]. The recent and rapidly growing field of epigenetic epidemiology [149] has made it possible to map DNA methylation patterns in large cohorts. This type of study is referred to as an epigenome-wide association study (EWAS), which requires robust normalization algorithms for microarray-based DNA methylation data and rigid statistical tests for the identification of differently methylated sites (DMS) between exposed and unexposed subjects [150].

There are several experimental methods developed for genome-wide DNA methylation mapping [148]. The most accurate method known today is high-throughput methylome sequencing, which cover the whole genome at a single base resolution. However, this method is extremely costly and time-consuming, which is why array-based methods (assembly of microscopic DNA segments attached to a solid surface) have become increasingly popular for use in EWAS studies [151]. For the epigenetic methylation analyses of obesity-related genes in Study two, the most frequently used array-based method was used, namely Illumina Human Methylation 450 Bead Chip platform, which quantifies DNA methylation levels in slightly more than 485.000 regions (96%) of DNA where a cytosine nucleotide occurs next to a guanine nucleotide (CpGs). There array covers an average of 17 CpG sites per gene region in 99% of known genes throughout the whole genome [152].

The method is based on bisulfate conversion where an epigenetic code (methylation) is translated into a genetic code by conversion of unmethylated cytosine residues into uracil while leaving methylated cytosine unaltered, which enables detection of methylation levels through DNA sequencing techniques. The Illumina Human Methylation 450 Bead Chip platform uses two different probe types (fragment of DNA used to detect the presence of nucleotide sequences) to distinguish between methylated and unmethylated DNA. Using two probe types, binding to different sites within methylated and unmethylated DNA, enables coverage of large parts of the human genome. However, the two different probe types have slightly different distributions. Therefore, normalization, by adjusting values measured on different scales to a general common scale, is necessary [153]. A recent study showed that more sophisticated normalization by Beta-mixture Quantile dilation might improve data quality and reduce signal

bias between the two probe types [154]. The normalization subtracts background noise and uses a positive (100% methylation) and a negative (no methylation) control probe. The main results of the normalization process are a table of b-values, an alternative term for the absolute DNA methylation levels. The b-value is often transformed into a M-value, which is a logistically transformed b-value used in several common statistical tests [155].

The most common goal of DNA methylation mapping is to identify systematic differences between groups of samples, such as BMS and AMS siblings in our study. Next, the typical step is to identify DMS between sample groups. It is widely known that DMS can control cell-type specific transcription of a gene [156]. However, it is difficult to assess the practical influences of susceptibility to disease from observed differences in methylation patterns in specific regions of a gene. Therefore, researchers often apply gene set pathway analysis tools to identify biologically meaningful effects from differently methylated regions in genes [157].

Ethical committee approval was obtained from the Stockholm Regional Ethical Review Board (no. 2009/709-31/2).

3.2 DATA COLLECTION TWO

Data collection two was used for studies three and four.

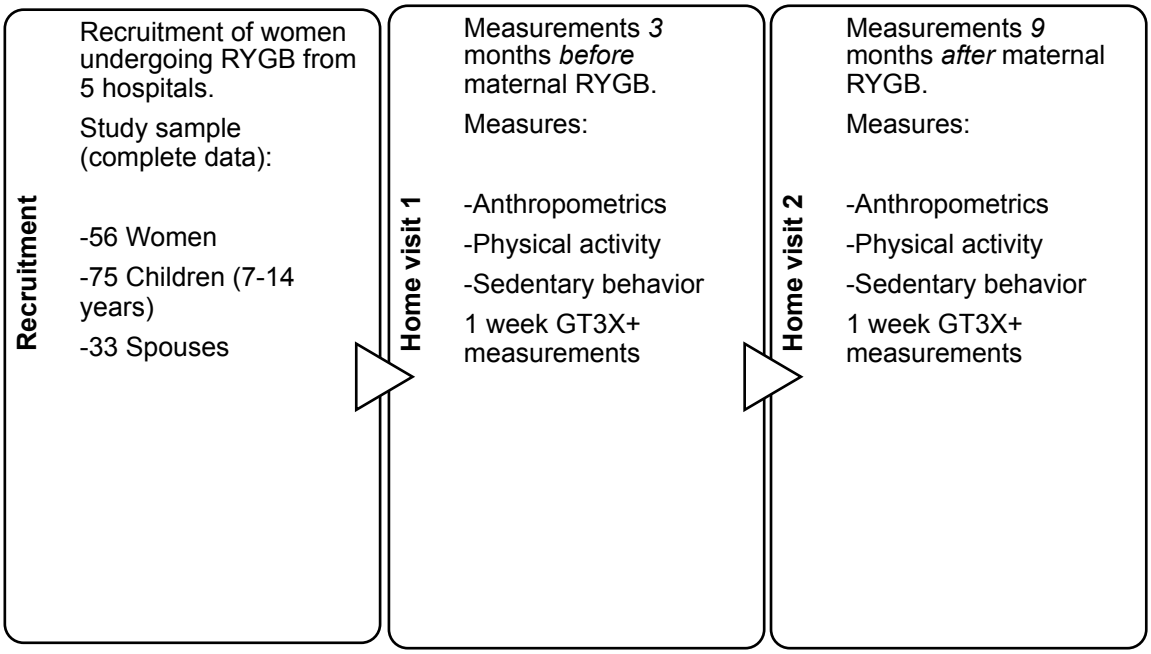
Participants

We recruited women living with or without a spouse, with children between the ages seven to14 years on RYGB-surgery waiting lists (primary bariatric surgery) at five Swedish hospitals; Danderyd Hospital, Ersta Hospital, Uppsala University Hospital, Örebro University Hospital, and St:Görans Hospital. The women underwent laparoscopic RYGB surgery between June 2012 and January 2013. We were able to recruit 69 families for the study where 56 women (81%) had data on PA both pre- and post-surgery. Only families where the women undergoing RYGB had PA data before and after surgery were included in Study four. Baseline descriptive (e.g., BMI) in families (before and after maternal surgery) with complete PA data did not differ significantly from those with incomplete data.

A research visit was made by the current and a fellow PhD student (Mikaela Willmer) to the participants' homes three months before and nine months after maternal RYGB surgery in order to limit seasonal effects on PA and SB (one year between measurements). The mean time interval between pre- and post-surgery assessments was 12.8 (SD 2.3) months.

For Study three, we analyzed 56 women, while, for Study four, the final study sample encompassed 56 families, comprising 56 women, 33 spouses, and 75 children between the ages seven and 14 years. Of 69 recruited families, 13 (16%) had incomplete PA data before and after maternal surgery and five families (7%) were lost at the follow-up measurement nine months after surgery for different reasons (e.g., lack of time or interest to participate).

Figure 3. Overview of data collection two



Anthropometrics

Anthropometric measures were taken in the participants' home using high quality and calibrated electronic scales for weighing and a stadiometer for the measurement of height. The two PhD students took all measures at both research visits. Fat free mass (FFM) and fat mass (FM) were derived from bioelectric impedance analysis (BIA) (Quantum II, RJL Systems, Clinton Township, MI, USA), and were calculated using an equation by Kyle et al. [(158)].

Physical activity assessment

PA was measured using the Actigraph GT3X+ monitor (Pensacola, FL), a tri-axial accelerometer assessing acceleration on the vertical, antero-posterior, and medio-lateral axes. We analyzed vector magnitude (V_m) activity counts, calculated as the square root of the sum of the three axes. The GT3X+ accelerometer is a valid tool for estimating PA and most types of human daily activities [(159)].

Participants were asked to wear the monitor at their right hip during all waking hours for seven consecutive days. To further increase compliance, three standardized text messages were sent to the participants' cell phones during the seven-day measurement period. The number of high-intensity minutes occurring in bouts of 10 minutes or more was computed by using an algorithm developed by Choi et al. [160], while non-wear time was defined as 60 minutes of consecutive zero recordings, allowing for two minutes of non-zero interruptions [161]. Bouts and wear time were computed using R-packages: *PhysicalActivity* and *'Accelerometry*. All participants with at least ten hours per day of monitor wear time for three days or more [128] at both the before and after maternal surgery assessments were included in the analyses. There is evidence in the literature of the use of age specific cut-offs to assess different levels of PA [162]. Hence, for our analyses we chose to use cut-offs based on the validation studies of Santos-Lozano [163] et al. and Hanggi et al. [164], since the study populations used in those studies matched our study population with regard to age (Table 4).

Table 4. The accelerometer cut-offs for GT3X+, expressed as counts per minute (cpm), used in studies three and four

Adults *Santos-Lozano et al.* [163]	**Children** *Hanggi et al.* [164]
SB < 100 cpm	**SB** < 180 cpm
LPA 101 - 3208 cpm	**LPA** 180 - 3360 cpm
MVPA > 3208 cpm	**MVPA** > 3360 cpm

Ethical committee approval was given by the Stockholm Regional Ethical Review Board (no. 2009/1472-31/3).

3.3 OVERVIEW OF THE DATA COLLECTION

Table 5. Overview of the data collections that took place between the years 2010 and 2014

Data collection	**Study (1-4)**	**2010**	**2011**	**2012**	**2013**	**2014**

Contacting 797 women and offspring sibling-pairs via telephone and letter	1 and 2	X	X			
Collecting antenatal medical records for 348 women and growth charts from offspring sibling-pairs	1 and 2		X	X		
Collecting blood samples from 124 women and offspring sibling-pairs	1 and 2		X	X	X	
Recruiting 69 women with family members from 5 different hospitals	3 and 4			X	X	
Research-visit in home environment in 69 families before and after maternal Roux-en-Y Gastric Bypass	3 and 4			X	X	X

4 OVERVIEW OF THE FOUR STUDIES

Table 6. Overview of the characteristics of studies one to four

	Study one	Study two	Study three	Study four
Design	Longitudinal study mainly based on existing routine data in archives	Longitudinal study with retrospective data and blood samples	Prospective longitudinal study	Prospective longitudinal study
Participants	124 women, 248 sibling-pairs, 39 sibling-pairs with BMI at 4 and 6 years	31 sibling-pairs (62 siblings)	56 women undergoing RYGB	56 Families: 56 women, 33 spouses, and 75 children
Methods	Measured objective data from medical journals and growth charts	Measured objective data from medical journals and growth charts. Epigenetic data from Illumina 450k analyses	Accelerometer data (GT3X+) and objectively measured anthropometrics	Accelerometer data (GT3X+) and objectively measured anthropometrics
Data	Objective data (secondary data)	Objective data (secondary data and self-collected)	Objective data (self-collected)	Objective data (self-collected)
Outcome measures	Differences in GWG, offspring birth weight and BMI at 4 and 6 years	Differences in epigenetic regulation of obesity-related genes	Changes in objectively measured PA and SB	Changes in objectively measured PA and SB

Statistical analyses	Mixed linear models, fixed effect regression	Paired t-tests, McNemar's tests	Linear regression, fixed effect regression, paired t-tests, McNemar's tests	Linear regression, fixed effect regression, paired t-tests, McNemar's tests

5 STATISTICAL ANALYSIS

All statistical analyses for papers one to four were performed with STATA versions 12.1 and 13.1 (STATA Corp, College Station, Texas, USA) software.

We used data from subjects who were repeatedly measured over time in studies with a longitudinal design. Statistical analyses for such a study design must take into account that data from an individual at different times are very unlikely to be independent, and most likely are correlated. If correlation is ignored, it may negatively impact on parameter estimation, hypothesis testing, and the efficacy of the study design [165]. There are several issues when conducting statistical analyses of longitudinal data, such as correlation between repeated outcome measurements, missing data, irregularly timed data, and a mixture of static and time-varying covariates (the word covariate is sometimes used synonymously with independent variable, predictor variable, explanatory variable, and risk facts).

5.1 STUDY ONE

In Study one we used a mixed model (*"xtmixed"* command in STATA) that primarily focuses on relationships restricted to observations at individual level. The mixed model is used when modeling a continuous outcome measure as a function of fixed effects while simultaneously modeling individual parameters as random effects. Hence, the mixed model can handle both time-dependent and static covariates [166]. Fixed effect describes the impact of known covariates (e.g., genetic factors) and is assumed to be constant between measurement points. Random effect measures the impact of known variables where effects are assumed to vary within the study population (e.g., environmental factors). We used the mixed model in Study one to estimate the extent to which associations, between differences in GWG and differences in birth weight and BMI at four and six years, were driven by shared genetic and lifestyle factors (fixed effects) or by non-shared factors (between effects).

5.2 STUDY TWO

In Study two the data were expressed as means ± SD for unadjusted values, and the differences in descriptive characteristics in mothers and BMS and AMS siblings were assessed using a within-subject paired t-test for continuous variables and McNemar's test for dichotomous variables. Since BMI was rarely measured at exactly four years of age, we predicted BMI at four and six years, and also maternal pre-pregnancy weight and GWG using a non-parametric regression method, so-called kernel smoothing [167], with the *"lokern"* package in R-software (http://www.r-project.org).

Epigenetic analyses

GenomeStudio software version 2011.1 (Illumina Inc.) was used for processing epigenetic methylation data. All samples were adjusted for color bias and normalized using Quantile normalization method using the *Lumi* package in R *Bioconductor* (http://www.bioconductor.org). The Illumina 450k methylation array uses two different probe types (Infinitum one and two) with different characteristics (colors red and green), thus requiring cautious normalization to reduce technical bias [153]. We used BMIQ normalization as proposed by Marabita F et al. to improve data quality and reduce technical variation [154]. Methylation levels (b-values) were estimated as the ratios of the signal intensity of the methylated alleles to the sum of methylated and unmethylated intensity signals. The b-values vary from zero (no methylation) to one (100% methylation). Log2 ratios known as M-values were generated and were used for further analysis. Sites with a p-value of 0.05 or more were removed and not analyzed further. Differences in M-value between groups (<0.5 or >0.5) were taken as significant differences in methylation, and used as the main statistical results for comparison between BMS and AMS siblings [155].

We used the ingenuity pathway analysis (IPA) platform to analyze potentially altered gene functions and pathways between BMS and AMS siblings. Results from the differential methylation analysis provided a list of probes with significant DMS and corresponding gene identification numbers. Differently methylated probes present at CpG sites with the highest DMS were selected, and thereafter used in an IPA analysis. IPA measures the likelihood that these genes participate in a particular function or pathway, and calculates a p-value using right-tailed Fisher's exact test for each unique pathway and gene function.

5.3 STUDIES THREE AND FOUR

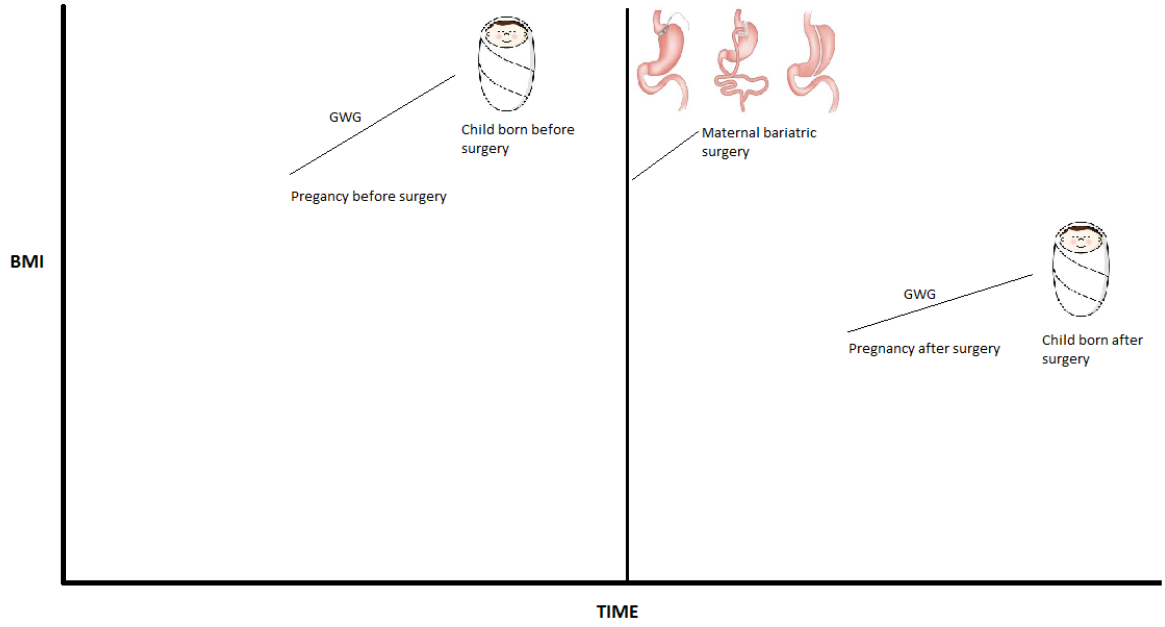
In studies three and four we used linear regression to estimate associations between baseline PA, SB and anthropometric measures, with post-surgery PA and SB, and pre- to post-surgery change in PA as outcomes. Furthermore, we created difference variables (post-surgery minus pre-surgery) for PA, SB and the anthropometric measures, which enabled us to control for all effects that are constant (e.g., genetic factors) from before to after surgery (fixed-effects regression). Thus, we conducted a linear regression on differences in several variables from three months pre- to nine months post-surgery. To account for slight deviations from the normal distribution, we used robust standard errors to estimate confidence intervals. As well as using robust variance, also ran robust regression models (STATA command '*rreg'*) to see whether outliers had any impact on the results. However, the results remained stable and did not change any of the conclusions drawn in Study three or Study four.

6 RESULTS

6.1 STUDY ONE

We used a sibling-pair design to examine the associations of differences in total and trimester specific GWG in repeated pregnancies of the same woman (n = 124), before and after bariatric surgery, with differences in offspring birth weight and BMI at four and six years of age.

Figure 4. Schematic overview of the design of Study one



The main findings in this study were that there were positive associations, in women undergoing bariatric surgery, between differences in total GWG and GWG in the second trimester and differences in offspring birth weight when maternal characteristics (e.g., genetic factors), fixed from one pregnancy to a later pregnancy, were taken into account. Furthermore, women undergoing bariatric surgery had lower GWG before surgery, at 11.3 (SD 7.2), compared with after surgery, at 8.3 (6.4), giving a p-value of less than 0.001. Time between pregnancy and bariatric surgery was 4.1 years (SD 2.7 years) with a range 0.7 to 12.8 years, and 3.7 years (SD 2.3 years) with a range 0.9 to11.5 years, before and after surgery, respectively.

After adjusting for pre-pregnancy BMI, we found that the greater differences in total GWG and GWG in the second trimester were associated with increased differences in birth weight both

within siblings and between non-siblings: for total GWG within siblings, a 0.041 standard deviation score (SDS) (one birth-weight SDS score corresponds to approximately 0.55 kg or 1.43 BMI units) per 1-kg increase in weight (95% CI, 0.014, 0.069), and for the second trimester a 0.96 SDS for each 1-kg greater weight change per week (95% CI, 0.32, 1.61). The associations within siblings and between non-siblings did not differ from each other (p = 0.81 and p = 0.79, respectively) (Table 7).

Table 7. Within-pair and between-pairs effects of 1-kg greater gestational weight gain and mean growth rate (kg/week) during each trimester on child's birth weight (n=124)

	Total gestational weight gain (GWG)	**Mean increase in kg/week during trimester 1**	**Mean increase in kg/week during trimester 2**	**Mean increase in kg/week during trimester 3**
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Model 2 (adjusted for maternal age, pre-pregnancy weight and height)				
Within	0.041 (0.014, 0.069)	0.44 (-0.085, 0.97)	0.96 (0.32, 1.61)	0.49 (-0.19, 1.17)
Between	0.036 (0.0043, 0.068)	0.32 (-0.38, 1.01)	0.83 (0.068, 1.59)	0.74 (-0.074, 1.56)
Test for difference	P=0.81	P=0.78	P=0.79	P=0.65

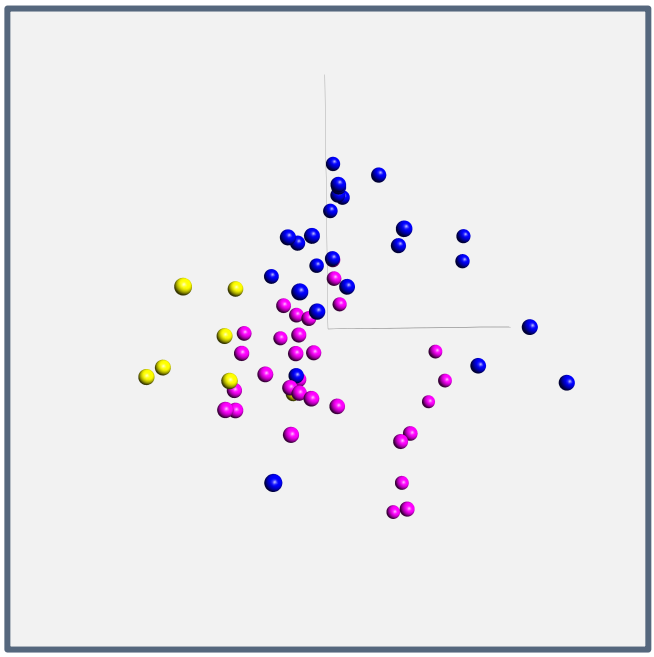
In contrast to the above, data associations of GWG with differences in offsprings' BMI SDS at birth and at four and six years for a subset of 39 mothers and 78 children with complete data on all three occasions were weak and non-significant.

6.2 STUDY TWO

We used a sibling-pair design to examine the effects of maternal weight loss due to bariatric surgery between two pregnancies on methylation levels of genes associated with T2D and obesity in comparisons between BMS and AMS siblings. The main findings of this study were that maternal bariatric surgery, with subsequent weight loss between pregnancies, was

associated with overrepresentation of DMS in the genes involved in cytokine inflammatory signaling and T2D signaling pathways. Additionally, we show that the genes involved in T1D, T2D and obesity, such as major histocompatibility complex, class II, DQ beta 1 (*HLA-DQB1)*, proopiomelanocortin (*POMC)*, *IGF2*, insulin receptor (*INSR)*, fat mass and obesity associated protein (*FTO)* and *TNF* are either hypermethylated or hypomethylated when comparing DMS between BMS and AMS siblings. A total of 4230 genes, with 27 310 corresponding methylation sites, of which 8036 within CpG islands, were differently methylated. Furthermore, we found differences in global methylation distribution patterns when comparing all siblings born before maternal bariatric surgery with siblings born after gut peptide altering maternal surgery and restrictive maternal surgery (Figure 5). Siblings born after maternal restrictive surgery exhibited hypermethylation of *HLA-DQA1* major histocompatibility complex, class II, DQ alpha 1 (*HLA-DQA1)* and *HLA-DQB1* (the two most significantly differently methylated genes), by contrast with siblings born after maternal gut peptide altering surgery.

Figure 5. Principal component analysis (PCA) of global methylation patterns in differently methylated genes of all siblings born before (n = 31) and siblings born after restrictive maternal surgery (n = 21) and siblings born after gut peptide altering maternal surgery (n = 10). Each of the circles in the figure represents a sample. The PCA analysis and visualization were performed using the Qlucore Omics Explorer.



- Siblings born before maternal surgery
- Siblings born after restrictive maternal surgery
- Siblings born after gut peptide altering maternal surgery

To address the aim of Study two with possible effects on offspring methylome from alterations in the maternal intrauterine environment, we selected women who had lost at least one BMI unit between pregnancies. This created a somewhat different study population compared with that used in Study one (tables 8 and 9).

Table 8. Descriptive characteristics of women included in studies one and two

Characteristic	Delivery before bariatric surgery, Study one (SD)	Delivery before bariatric surgery, Study two (SD)	Delivery after bariatric surgery, Study one (SD)	Delivery after bariatric surgery, Study two (SD)
Maternal age at delivery (years)	25.5 (3.3)	24.2 (2.5)	33.3 (4.3)	32.2 (3.5)
Maternal BMI in gestational week 10	36.5 (5.0)	38.5 (5.2)	31.2 (6.2)	31.0 (6.5)
Maternal BMI at birth	40.7 (4.5)	42.6 (5.2)	34.2 (6.3)	34.3 (7.3)
Total GWG (kg)	11.3 (7.2)	10.6 (8.2)	8.3 (6.4)	9.4 (7.9)
Birth weight (kg)	3.7 (0.7)	3.8 (0.5)	3.5 (0.6)	3.4 (0.6)
BMI 4 years	17.4 (2.8)	17.4 (1.7)	18.2 (3.6)	16.7 (1.9)
BMI 6 years	17.0 (1.8)	18.1 (2.7)	17.7 (2.3)	16.9 (2.2)
Girl (%)	41.0	41.2	41.0	52.9

Table 9. Differences in surgical procedures and year of surgery for women included in studies one and two.

Surgical procedure	Women included in Study one	Women included in Study two
Vetrical banded gastroplasty (%)	39.7	45.0
Gastric banding (%)	30.0	15.0
RYGB (%)	13.3	25.0
Jejunoileal bypass (%)	11.5	9.0
Horizontal gastroplasty (%)	5.5	6.0
Mean year for surgery (range)	1994 (1980 - 2006)	1995 (1986 - 2006)

Maternal descriptive characteristics, surgical procedures and the year at which the surgery was performed differ between the populations for Study one and Study two. Thus, it is uncertain whether the results found in Study four would have been the same if we had used the study population in Study one. Moreover, effects other than altered intrauterine environment between pregnancies may explain differences in the methylome between BMS and AMS siblings found

in Study two. For example, AMS siblings have grown up in a later time period and possibly in environments that differed somewhat with regard to eating and physical activity behaviors compared with BMS siblings.

6.3 STUDY THREE

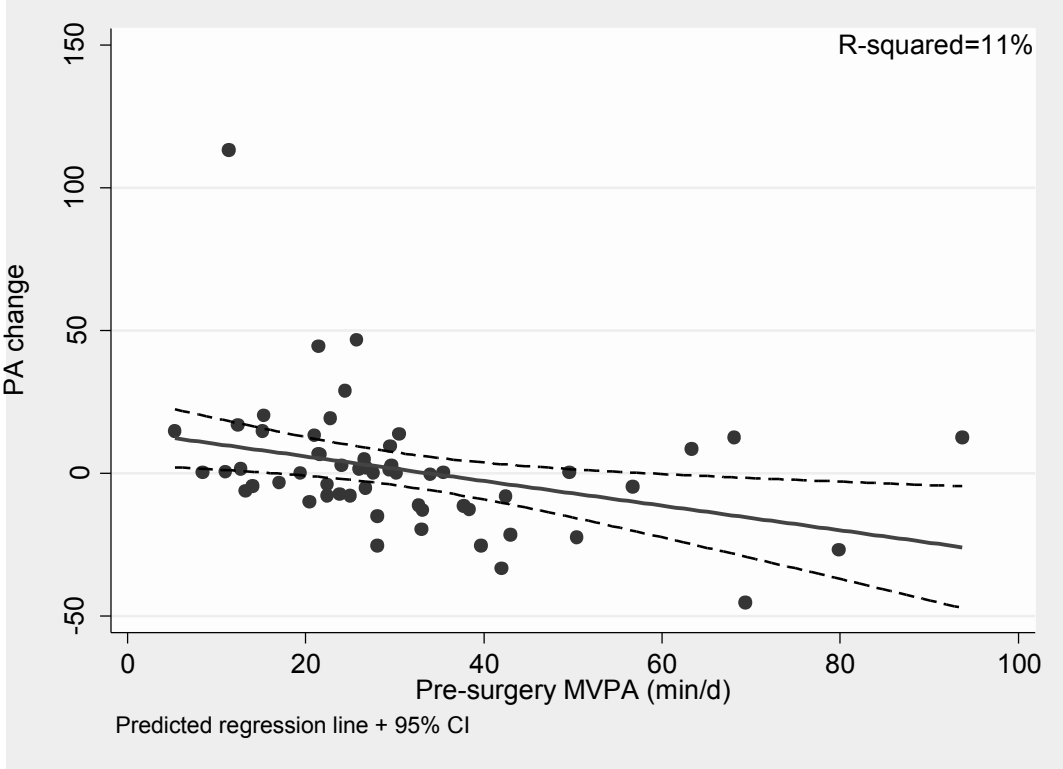
We characterized three months before to nine months after surgery changes in PA and SB in 56 women undergoing RYGB, using the GT3X+ accelerometer. Additionally, we examined the associations between pre- respectively post-surgery PA and SB on anthropometric measures. Our main findings indicate no significant differences in MVPA, MVPA in 10-minute bouts, LPA or SB from three months before to nine months after surgery in women undergoing RYGB, despite substantial weight loss (Table 10).

Table 10. Changes in PA and SB from three months before to nine months after surgery in women undergoing RYGB

Characteristics	Before RYGB surgery (SD)	After RYGB surgery (SD)	Pre- to post-surgery change (SD)	p-value
BMI	39.1 (3.2)	27.5 (3.1)	-11.7 (2.7)	<0.001
MVPA (min/day)	30.9 (17.7)	32.1 (23.5)	1.2 (22.6)	0.71
LPA (min/day)	441.0 (92.1)	441.8 (92.5)	0.08 (79.4)	0.99
SB (min/day)	430.2 (141.5)	420.7 (128.8)	-9.5 (129.4)	0.58
MVPA 10-min bouts (min/week)	65.8 (81.6)	88.9 (116.5)	23.5 (17.1)	0.15

However, women with higher PA before surgery decreased their PA after surgery while women with lower PA increased their PA post-surgery. This is illustrated in Figure 6 where pre-surgery MVPA shows a negative association with change in MVPA from before to after surgery.

Figure 6. Associations between change in MVPA from three months before to nine months after surgery and pre-surgery MVPA in women undergoing RYGB



The recommendations in national PA guidelines, accumulating 150 min per week MVPA in periods of 10 minutes or more [86], were met by 14.2% of women before and 16.1% after RYGB surgery. Depending on the PA intensity, 46.4 to 58.9% of the women decreased their PA and 51.8% increased time spent sedentary from before to after RYGB surgery. After stratification into two groups, we found that women who increase MVPA from before to after RYGB have lower pre-surgery weight, less MVPA minutes per day, and spend more time sedentary compared with women who decrease MVPA from before to after surgery. After surgery, women who increase MVPA from before to after surgery have lower weight, more MVPA minutes per day, and spend less time sedentary compared with women who decrease MVPA from before to after RYGB (Table 11).

Table 11. Descriptive characteristics of women who decrease or increase their MVPA from three months before to nine months after RYGB

Characteristics	Women who increased MVPA (n = 29). Before RYGB (SD)	Women who decreased MVPA (n = 27). Before RYGB (SD)	Women who increased MVPA (n = 29). After RYGB (SD)	Women who decreased MVPA (n = 27). After RYGB (SD)

Weight (kg)	107.2 (11.6)	112.3 (10.7)	72.3 (9.3)	80.5 (8.5)
BMI	39.1 (2.9)	40.2 (2.8)	26.5 (3.0)	28.9 (2.2)
MVPA (min/day)	27.4 (18.5)	32.8 (14.6)	38.2 (21.8)	19.9 (11.3)
MVPA 10 min bouts (min/week)	22.7 (28.2)	29.0 (23.5)	38.1 (34.1)	13.5 (11.8)
LPA (min/day)	440.8 (124.5)	441.4 (94.5)	452.9 (100.8)	432.4 (91.1)
SB (min/day)	433.8 (124.5)	412.6 (159.5)	406.8 (114.9)	452.3 (140.7)

We also found a trend (non-significant) towards an overall increase in MVPA in periods of 10 minutes or more from before to after surgery, suggesting that participants may be able to perform work of higher intensity for longer sustained periods of time after RYGB. However, there is great variability in changes in PA and SB, and it is important to remember that a large proportion of women undergoing RYGB decrease their PA and increase their SB from before to after RYGB surgery.

6.4 STUDY FOUR

We aimed to (i) objectively characterize three months pre- to nine months post-surgery changes in the PA and SB of children (seven to 14 years) and spouses of women who have undergone RYGB, and (ii) to explore the associations between pre- and post-surgery changes in PA and time spent sedentary among family members. The main findings were that objectively measured PA three months before and nine months after the women's RYGB surgery showed no significant differences in PA intensity or SB in spouses. However, in children, MVPA and LPA decreased significantly, while SB increased significantly, between the before and after maternal surgery measurements. There were no significant associations between the women's pre-surgery PA or change in PA and change in PA for spouses or children. Moreover, 43.8% of spouses and 38.4% of children decreased time spent on MVPA, and increased SB between the before and after maternal RYGB measurements.

There were no significant pre- to post-surgery differences for MVPA between children or spouses who were normal weight, overweight or obese before their mother's surgery. However, there were large variations in pre- and post-surgery PA and SB among children and spouses, and also in their differences over time (as illustrated in figures 7 and 8).

Figure 7. Change in MVPA and SB in children (n = 75) from three months before to nine months after maternal RYGB, stratified according to pre-surgery weight status

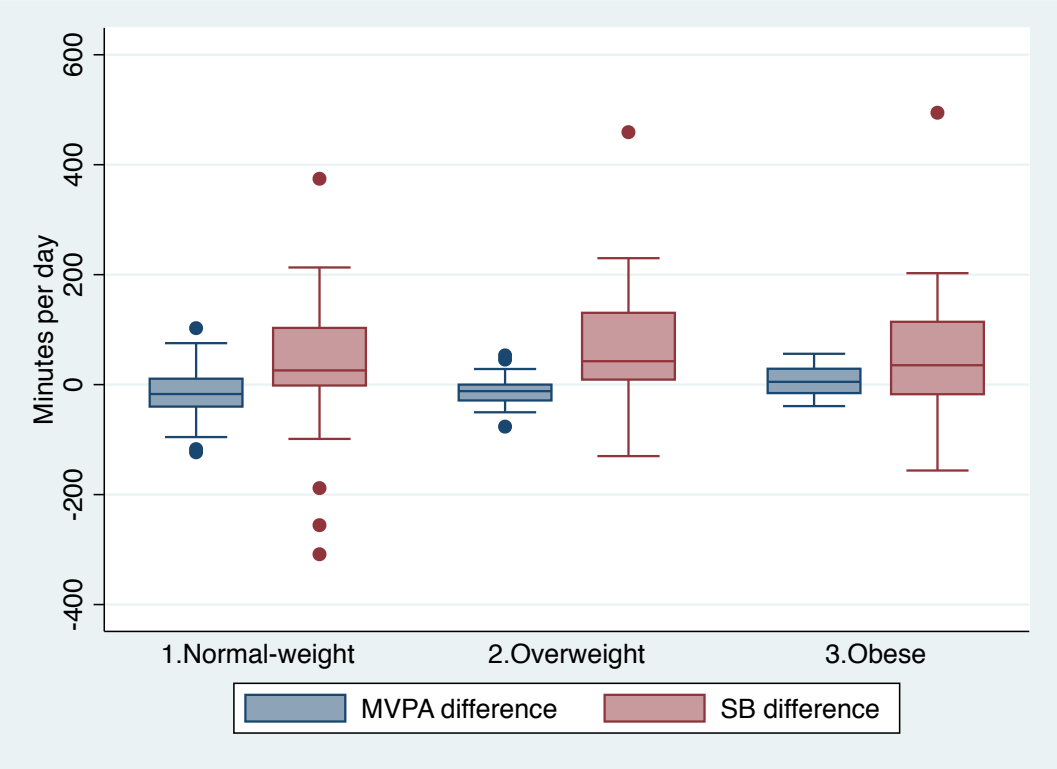
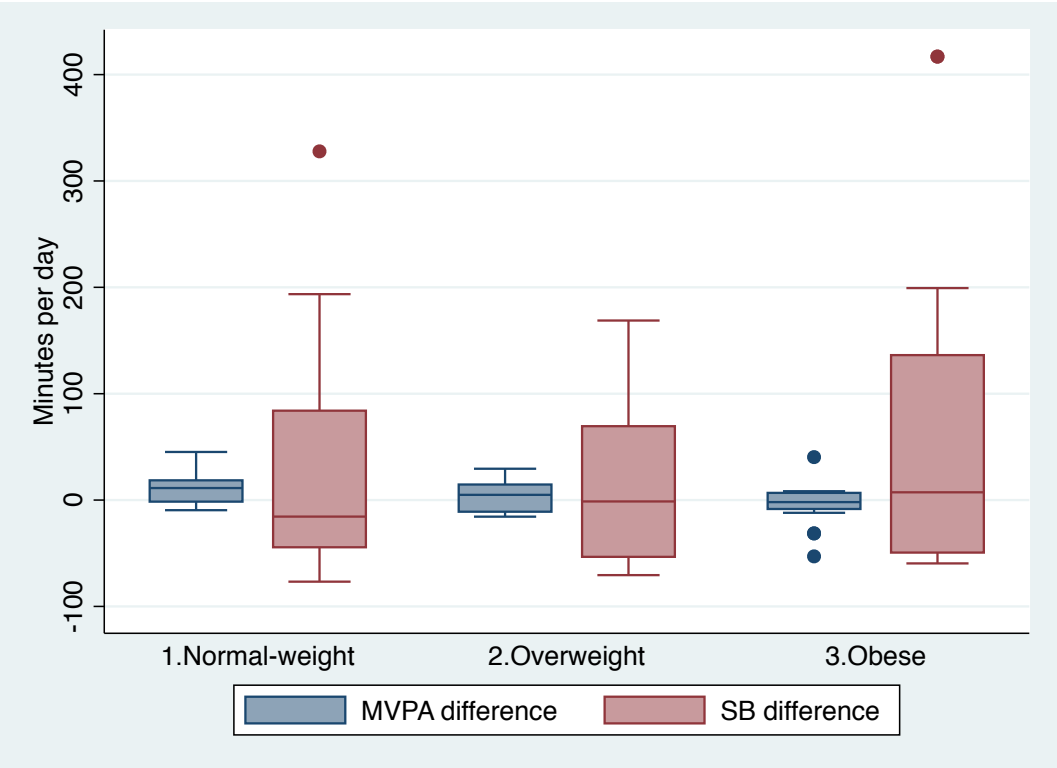


Figure 8. Change in MVPA and SB in spouses (n = 33) from three months before to nine months after maternal RYGB, stratified according to pre-surgery weight status



As shown in Table 12, Children under ten years are more active and spend less time sedentary compared with children over ten years at both three months before and nine months after maternal RYGB. Furthermore, girls are less active compared with boys both before and after maternal RYGB. However, it seems that boys increase the time they spend sedentary more than girls from before to after maternal RYGB. In fact, boys spend more time sedentary compared with girls after maternal RYGB.

Table 12. Descriptive statistics on children, stratified by age and sex, from three months before to nine months after maternal RYGB

Characteristics	Children under 10 years of age (n = 44) (SD)	Children over 10 years of age (n = 31) (SD)	Girls all ages (n = 37) (SD)	Boys all ages (n = 38) (SD)
3 months before maternal RYGB surgery				
Weight (kg)	34.6 (7.2)	57.0 (16.6)	41.6 (12.3)	44.0 (18.7)
BMI	18.9 (2.8)	22.8 (4.4)	20.3 (3.4)	20.5 (4.4)
MVPA (min/day)	85.5 (41.3)	63.1 (36.0)	62.9 (32.2)	90.3 (44.0)
MVPA 10min bouts (min/week)	42.4 (33.5)	30.7 (24.6)	26.1 (21.2)	49.3 (34.4)
LPA (min/day)	486.2 (65.7)	389.1 (78.6)	460.0 (78.6)	437.6 (90.6)
SB (min/day)	260.0 (110.6)	408.2 (24.6)	322.3 (146.2)	312.5 (142.0)
9 months after maternal RYGB surgery				
Weight	39.5 (8.9)	61.2 (16.1)	47.6 (12.4)	48.5 (19.1)
BMI	19.6 (3.2)	23.0 (4.2)	20.9 (3.6)	20.8 (4.4)
MVPA (min/day)	74.3 (32.2)	52.6 (25.7)	56.5 (25.8)	75.2 (34.1)
MVPA 10min bouts (min/week)	36.0 (25.6)	24.3 (17.0)	23.4 (15.7)	39.3 (26.3)
LPA (min/day)	447.9 (70.4)	365.2 (83.1)	435.5 (89.8)	396.9 (77.1)
SB (min/day)	322.2 (131.7)	446.9 (156.0)	332.5 (107.9)	407.3 (181.0)

7 DISCUSSION

7.1 METHODOLOGICAL CONSIDERATIONS

The different study designs used in the four studies are shown in Table 6. In all four studies cohorts of individuals were followed or traced over a period of time [168]. The longitudinal study design can establish the temporal order of events and sometimes allow causal interpretation of results, which is not possible when cross-sectional study design is used [169]. In studies one and two, most primary data were collected and registered in the past, and collected ad hoc in present time from routine information sources, which make these studies historical (or retrospective) cohort studies. However, the methylation data used for Study two is primary data collected for this specific study. The objective nature of data for all four studies eliminated potential recall bias (for studies one and two), which is often seen in studies where the study subjects report events from the past [169]. Nevertheless, we may have introduced selection bias as people who are more positive to participate in research may have agreed to participate in the two data collections to a greater extent than people who have a negative attitude towards participating in research.

7.1.1 Data collection one

Since Sweden has a long tradition of well-kept registers in combination with the personal identification number, we were able to find a large proportion of women who had undergone bariatric surgery between the years 1980 and 2006, and had given birth both before and after surgery. Unfortunately, we were unable to obtain information on all available women since some archives had taken out some of the medical records collected at child care centers. We also found it difficult to come into contact with some of the women and their children by telephone or mail to ask for their informed consent. Furthermore, 98 women declined participation after the initial telephone interview. Perhaps these women were dissatisfied with the outcome after bariatric surgery, which may have resulted in a selection bias that potentially could affect the results presented in studies one and two. Further selection bias may have occurred in Study two where we collected blood from siblings born before or after their mother's bariatric surgery. Siblings did not leave blood for different reasons, e.g., lack of time

or interest, or because they were afraid of needles. Thus, we may have ended up with a study population subset of women who were relatively more satisfied with post-surgery outcomes and in general were positive to participate in research.

Epidemiological wide association studies (EWAS)

EWAS studies provide new opportunities for understanding associations between epigenetic changes in gene activity and several complex diseases. However, the many different techniques used for epigenetic analysis together with their somewhat different outputs make it challenging to interpret results from EWAS studies.

Finding an adequate sample size to achieve necessary statistical power to address the specific research question is a potential problem in EWAS studies since this is a newly emerging field with limited available preliminary data compared with GWAS studies [150]. Via array-based platforms it is now possible to interpret methylation data from millions of CpGs. Thus, multiple comparisons should be accounted for in order to reduce the risk of false positive (type 1 error) detections of differences in methylation patterns. One additional fundamental aspect that is of importance when interpreting methylation data from EWAS studies is that the studied differences in methylation patterns are dynamic by nature and can change over time, compared with genomic data that is static [149]. Furthermore, it is important to note that the lllumina Human Methylation 450 Bead Chip platform used in Study two is not sensitive enough to detect all methylation differences. The platform can, approximately, detect differences of 20% in methylation with 99% confidence [152].

There are numerous distinct cell types in the human body, all composed by their identical genetic code. All somatic cells in the body have a stable genotype, but the epigenetic pattern of a cell and tissue is varied [170, 171]. This may have great impact on interpretations of EWAS data since some or all observed epigenetic variation in a biologic sample may be attributed to differences in cell-type distribution in the sample. Blood is often used as a surrogate tissue to examine systemic alterations in DNA methylation in response to exposure or disease. When analyzing blood in EWAS studies, it is of importance to consider that epigenetic alterations detected in blood may not necessarily reflect changes occurring in other tissues. Another issue when analyzing blood is the heterogeneity of blood. Blood is composed of a broad mixture of multiple cell types, and therefore the proportion of cell types in a blood sample should be taken into account as each cell type holds its own specific DNA methylation pattern [172]. Thus, inter-individual variation in blood cell proportion can potentially confound the observed relationship between methylation patterns and the exposure of interest. A technique to quantify blood cell

proportions is fluorescent activated cell sorting (a.k.a. FACS) which is an expensive method that requires fresh blood samples. Another approach to account for the problem with blood heterogeneity is to use a reference epigenome to identify differently methylated regions [173]. While the reference epigenome is a project under development, the analogue human genome map is well-established [173]. Inter-individual variation in DNA methylation appears to be common among individuals, and may be heavily influenced by underlying genetic differences [174]. Until there is a reference epigenome available, it is a challenge to give biological meaning to observed differences in methylation patterns, e.g., between siblings born before and after maternal bariatric surgery.

The genotype is typically the same for an individual throughout the life span. However, the epigenetic profile of an individual may change over time [149]. Changes to the epigenome may occur during aging and in response to exogenous exposures (e.g., nutrition and smoking). This increases the risk of misclassification or other types of bias, and thus caution is required when interpreting results from EWAS studies. Additional problems arise when interpreting results from cross-sectional EWAS studies, where the study design makes is impossible to establish whether epigenetic dysregulation leads to disease or vice-versa, thus highlighting the issue of reverse causality.

Study one, strengths and limitations

The major strength of Study one was in reducing observed and unobserved confounding due to maternal genetic, social and life style factors that were constant between pregnancies of the same woman. However, even in a sibling study, residual confounding can never be excluded, due to genetic differences between full siblings (who share approximately 50% of their segregating genes), or due to changes in environmental or life style circumstances between pregnancies. Unobserved environmental changes affecting GWG, intrauterine environment and offspring weight may have occurred in between pregnancies since the mean time interval between pregnancy and bariatric surgery was large. Although maternal genetics are fixed between pregnancies, the contributions of the maternal and paternal genomes to the child genome differ between siblings. It is strength that we had access to detailed measured data on GWG, which made it possible to assess the effects of GWG in different trimesters. Nevertheless, using data from national registers that were collected routinely may lead to some degree of non-differential misclassification.

The main limitations to Study one were the small sample size that reduced statistical power, and changes in surgical procedures over a long period of time (between 1980 and 2006), from

the less effective to the more effective surgical procedures, which resulted in greater weight loss after surgery later in the study period. The small sample size made stratification on surgery procedure impossible, so we were unable to draw conclusions on effects from specific bariatric surgery procedures in studies one and two. Moreover, differences in maternal illness, medication and behaviors, e.g., smoking or use of alcohol, between pregnancies are not accounted by study design or taken into account by adjustments since we had no access to this information.

Study two, strengths and limitations

A major strength of Study two was in reducing observed and unobserved confounding, by study design, due to maternal genetic, social and lifestyle factors, at least to the extent that they are held constant between pregnancies of the same woman. In addition, we had access to detailed objectively measured data on both maternal GWG, pre-pregnancy BMI, BMI at surgery and follow-up, and offspring birth weight and BMI at four and six years (same data as for Study one).

This study also has some limitations. Although the size of our population may appear small, it is of comparable size to previous research with a similar design involving 25 siblings born before and after maternal BPD [48, 145]. We used DNA extracted from whole blood, as it is more convenient and acceptable than tissue biopsies, especially in children. As previously described blood is a heterogeneous tissue composed of a broad mixture of multiple cell types. Thus, blood may not necessarily reflect changes occurring in other tissues. However, the overall impact of tissue-specific DNA methylation is minor given the known similarities in methylation patterns across tissues [175]. We did not stratify for sex in our gene methylation analyses as previous research has failed to detect sex-specific methylation patterns [176]. Due to the before-after design of our study, the age differences between the two sibling groups were large. Although DNA methylation is relative stabile over time [175], age-related differential methylation has been reported among twins [177-179]. However, age-related differential methylation has been shown to represent a small proportion of CpG sites (1.3%; 360 sites on 27 578 CpGs analyzed) [180] and to be site- and location-specific [181]. Given the lack of sex-specific methylation patterns and the limited impact of age on gene methylation, it is reasonable to assume little impact of sex and age in the present study.

7.1.2 Data collection two

RYGB surgery is a well-established surgical procedure in Sweden, and is conducted in the same way in the five participating surgical centers [107]. Nevertheless, the hospitals have somewhat different pre- and post-surgery counseling regarding diet and PA, which may have had some impact on participants' PA before and after surgery. Furthermore, discrepancies in hospital routines for identifying possible study participants made it impossible to collect information on patients who declined to participate in the study. Thus, we may have ended up with a study sample of people who were more positive to participating in research, and may have somewhat different baseline characteristics compared with individuals who declined participation.

Studies three and four, strengths and limitations

The major strength of these studies are the longitudinal design with standardized three months pre- and nine months post-surgery objectively measured PA, SB and anthropometrics, enabling control, by design, of all measured effects that are constant from pre- to post-surgery. Anthropometrics on weight, height, waist circumference, and body composition were collected with standardized best practice methods and measured by the current PhD students before and after surgery. Furthermore, PA and SB were measured with a high quality tri-axial accelerometer, accurately estimating PA [159], and the time frame between home visits gave limited room for seasonal differences in PA and SB [182]. However, recent Swedish data indicate that the seasonal differences in PA and SB are marginal [183]. In addition, both the monitor wear time protocol and data processing protocol followed best practices [184]. The accuracy of the accelerometer in measuring different types of physical activities, e.g., weight lifting and bicycling is unknown. However, research has shown that activity types are performed with similar frequencies before and after bariatric surgery [128].

BIA is useful in describing mean body composition for groups of individuals, but bias at individual level may limit its usefulness, especially among the obese [185]. Further problems with BIA analyses are its dependence on hydration status [186], and that there are a lot of different equations used to interpret the results from BIA outputs [187, 188]. For severely obese individuals undergoing bariatric surger, the BIA method and suggested equations for analyzing body composition show large fluctuations and limited comparability when compared with more accurate dual-energy X-ray absorptiometry measurements [189].

Accelerometers

Accelerometers, such as the Actigraph GT3X+, have been shown to accurately estimate PA [159], while estimates of really high intensity work is a well-known limitation of accelerometers, described as the "ceiling" effect [190]. Data for cut-offs developed using tri-axial data (V_m) from the GT3X+ accelerometer or other tri-axial accelerometers are sparse [163]. Thus, it is challenging to compare our data on time spent in different PA intensities with other studies using older uni-axial accelerometers, such as the Actigraph GT1M accelerometer [164]. Furthermore, the cut-offs developed for GT3X+ (V_m) to assess PA in different intensities differ considerably between studies. For example, MVPA was defined as >2690 cpm in one validation study of 28 men and 22 women (mean age = 26.9 SD 7.7 years) [191], whereas another validation study of 16 men and 15 women (mean age = 47.1 SD 3.5 years) defined MVPA as >3208 cpm [163]. In children, a recent validation study of 19 boys and 12 girls (mean age = 14.7 and SD 1.0 years) defined MVPA as > 2114 cpm [163], whereas a validation study of 27 boys and 22 girls (mean age = 10.8 and SD 1.9 years) by Hanggi et al. defined MVPA as > 3360 cpm [164]. This discrepancy clearly demonstrates the problem of using cut-offs to define different levels of PA. Furthermore, it highlights the importance of developing standardized specific cut-offs for each accelerometer model and age range owing to the importance of PA for health and the wide use of PA as exposure or outcome in epidemiological research [192]. Most published studies on the assessment of PA with accelerometers use uni-axial data from the vertical axis (V_t). For example, the Actigraph GT1M has been used for data collection in the well-known NHANES study [193]. However, data can be comparable between the GT1M and GT3X+ when both accelerometers are set to collect data in the vertical axis plane [191]. In theory, analyzing all three axes from the GT3X+ should provide a more accurate assessment of PA. Nevertheless, data show that the additional information obtained from analyzing three axes is not more efficient or accurate than one axis when compared with oxygen consumption [194]. On the other hand, there are studies indicating that tri-axial accelerometers are more accurate than uni-axial accelerometers when assessing PA and energy expenditure in children and adults [195, 196]. There may be several reasons why the GT3X+ does not seem to assess PA more accurately than the GT1M. First, there is evidence to support the notion that the vertical axis is the most important axis when assessing PA [195]. Second, most validation studies are performed on a treadmill, with no or very little incline, and therefore very little opportunity for antero-posterior and medio-lateral axis movements. Thus, the treadmill may not elicit enough force to detect motion in the antero-posterior or medio-lateral axis [194]. Perhaps accuracy of assessing PA is more accurate with the GT3X+ than with the GT1M under free-living conditions, which involve all three-dimensional movement patterns.

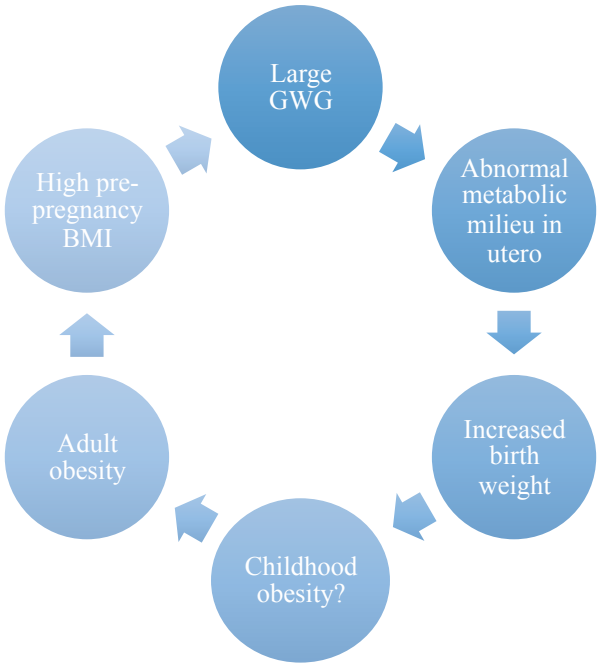
The most recent definition of SB is *"waking behaviour characterized by an energy expenditure less than 1.5 MET:s while in sitting or reclining posture"* [197]. In the analyses in studies three and four, intensity lower than 1.5 MET:s but no information on posture was applied to define SB. Thus, it cannot be excluded that some of the time spent sedentary has been misreported as standing time, which is not covered by the definition of SB. However, this potential misreporting is most likely equivalent in the before and after RYGB surgery measurements. The GT3X+ accelerometer has an inclinometer that can be used to detect posture. However, research has shown limited validation of the inclinometer [164]. For example, in a sample of 49 children, Hanggi et al. [164] showed that the standing position was misclassified 75% of the time as sitting and correctly identified only 20% of the time as the standing position. Hence, posture classification by the inclinometer should be interpreted cautiously. For more valid assessment of postures, the activPal™ has been recommended [198].

8 IMPLICATIONS OF RESULTS

8.1 STUDY ONE

This study shows that there are positive associations, in women undergoing bariatric surgery, between differences in total GWG and GWG in the second trimester and differences in offspring birth weight. In accordance with previous research [199], we did find a significant decrease in birth weight in AMS siblings compared with BMS siblings. On the other hand, no associations were found between differences in GWG and differences in children's BMI in preschool age, which may be due to low statistical power (39 sibling-pairs). One possible explanation for the positive associations between differences in GWG from one pregnancy to a later pregnancy of the same women with differences in offspring birth weight might be that higher GWG in total or in the second trimester before rather than after surgery may be related to higher delivery of glucose and fatty acids to the developing fetus, and thus higher birth weight before than after surgery [200]. Given the obesity epidemic and the fact that a one kilogram increment in birth weight increases the risk of overweight in adolescence by 30% to 50% [201], evidence that both total GWG and second trimester GWG are associated with increased differences in birth weight may be of importance when considering interventions to reduce the intergenerational vicious cycle of high maternal GWG and childhood obesity in the next generation (Figure 9).

Figure 9. Schematic figure over the vicious circle of high maternal GWG and childhood obesity in the next generation



Similar to findings presented by Aricha-Tamir et al. [199], we detected a significant decrease in gestational-age adjusted birth weight in offspring born after maternal surgery compared with siblings born before such surgery. The timing of GWG and birth weight is not fully understood. We found an association between GWG in the second trimester and birth weight, which is in line with previous findings indicating that GWG in the second trimester is most strongly associated with birth weight [202]. Although intervention research on how to modify GWG during pregnancy is sparse, there are data indicating that excessive GWG may be preventable [203]. Thus, our results indicating an association between greater second trimester GWG and increased birth weight may be important to consider for future interventions to diminish excessive GWG.

8.2 STUDY TWO

The main findings were that maternal bariatric surgery, with subsequent weight loss between pregnancies, is associated with overrepresentation of DMS in genes involved in cytokine inflammatory signaling and T2D signaling when comparing BMS and AMS siblings. Despite the observed differences between BMS and AMS siblings in the methylome, interpretation of these data should be made cautiously. Intragenic methylation may affect gene expression differently, where methylation in the gene body 5'UTR (5' untranslated region) and 3'UTR correlates with increased gene expression, whereas methylation in the promoter region

is associated with decreased gene expression [156]. The influences on risk of development of obesity in offspring through an altered methylome remain uncertain. Nevertheless, this study demonstrated epigenetic effects on sibling methylome of the genes involved in T1D, T2D and obesity from maternal bariatric surgery, with subsequent weight loss between pregnancies. In accordance with our findings, when comparing BMS and AMS siblings whose mothers underwent BPD, Guenard and colleagues found an overrepresentation of pathways involved in inflammation [145], T1D, T2D and *IGF1* signaling [48], and an overrepresentation of differently methylated genes such as *HLA-DQB1* associated with T1D and *FTO* and ATPase, class V, type 10A *(ATP10A)* associated with T2D [48] Hence, alterations in the methylome of offspring born after maternal bariatric surgery may encompass surgical procedures other than BPD.
A maternal high-fat diet in rats has been shown to be associated with increased birth weight, increased leptin levels and hypermethylation of the *POMC* promoter in offspring [204].
Likewise, we observed hypermethylation of the *POMC* promoter in BMS siblings, who were exposed to an obesogenic intrauterine environment during fetal life, compared with AMS siblings, exposed to a less obesogenic intrauterine environment. Our findings are in line with previous data implying increased long-term risk from an obesogenic intrauterine environment, in terms of dysregulation of food intake, energy balance and obesity in offspring, even independent of genetic background [205, 206]. Thus, maternal bariatric surgery, with subsequent interpregnancy weight loss, may constitute a less obesogenic intrauterine environment with less circulating glucose and free fatty acids, which may alter the methylome of obesity and T2D-related genes, and thereby reduce the long-term risk of developing obesity and T2D in offspring.

8.3 STUDY THREE

This study shows that that there were no significant differences in MVPA, MVPA in 10-minute bouts, LPA or time spent sedentary from three months before to nine months after surgery in women undergoing RYGB, despite substantial weight loss. However, women with higher pre-surgery PA decreased their PA post-surgery while women with lower PA increased their PA, which may reflect a regression towards the mean. RYGB surgery enforces caloric restriction with post-surgery reductions in daily-consumed calories and FFM [207]. Data on 48 overweight subjects in a randomized trial, using doubly labeled water, indicate that a caloric deficit is associated with a decrease in PA [208]. Furthermore, whether a person is prone to engage in habitual PA is partly determined by genetic variation [209]. Collectively, these circumstances

may at least partly explain why women undergoing RYGB fail to increase PA post-surgery despite substantial weight loss.

The women in our study are, compared with a Swedish sample of 1172 participants (54% women, mean age 45, 10% obese) measured longitudinally by the GT1M accelerometer between 2001 and 2008 [210], more physically active and spend less time sedentary. In the study by Hagströmer et al. 633 women spent 24 (SD 21) minutes per day in MVPA and 532 (SD 71) minutes per day in SB, whereas women undergoing RYGB in our study spent 30.9 (SD 17.7) and 32.1 (SD 23.5) minutes per day in MVPA, and 430.2 (SD 141.5) and 420.7 (SD 128.8) minutes per day in SB three months before and nine months after surgery, respectively.

In accordance with our results, previous research has failed to detect a significant change in PA from pre- to post-surgery among patients undergoing RYGB when PA is measured objectively [127]. Our findings of pre-surgery PA as an independent factor for post-surgery PA, in addition to data indicating that patients undergoing RYGB fail to increase PA from pre- to post-surgery, despite great weight loss, highlight the importance of pre- and post-surgery interventions aiming to increase PA. Furthermore, awareness should be raised as a large proportion of women undergoing RYGB (depending on the PA parameter, 46.4% to 58.9% of the participants decreased their PA and 51.8% increased SB) decrease PA and increase time spent sedentary from before to after surgery despite substantial weight loss.

8.4 STUDY FOUR

This study shows that that there are no significant differences in PA or SB between three months before and nine months after surgery in spouses or women who undergo RYGB. However, in children, MVPA and LPA decrease significantly, and SB increases significantly, between pre- and post-maternal RYGB surgery. Forty-four percent of spouses and 39% of children decrease time spent on MVPA, and increase time spent sedentary, between pre- and post-surgery. One possible explanation for the observed decrease in PA and the observed increase in SB among children between pre- and post-maternal surgery is the observed decline in PA with age, especially during adolescence [211]. In conflict with our findings, the currently only published study with a similar design and study population reported an increase in PA among adult family members and children from pre- to post-RYGB surgery [212]. However, the researchers used a seven-day PA-recall questionnaire, which is susceptible to misreporting of PA [132], especially among patients undergoing RYGB surgery [127]. Consequently, it is questionable whether the results presented by Woodard et al. [212] would have been the same if PA had been measured objectively. From a policy perspective, given that the PA of the family

members of the women who had undergone RYGB either remained unchanged or decreased post-surgery, there should be an emphasis on interventions designed to increase PA within the family between pre- and post-surgery. Additionally, awareness should be raised that children significantly decrease time spent physically active, and that they increase time spent sedentary between maternal pre- and post-RYGB surgery.

9 FUTURE RESEARCH PERSPECTIVES

The large number of bariatric surgical procedures conducted annually in Sweden and many other developed countries[106], in combination with the limited scientific evidence of long-term effects of such surgery in patients and family members, should motivate researchers to expand this research field in the future. The existing obesity epidemic will most likely maintain the demand for surgical interventions, given that there is no comparable alternative treatment for obesity in the future.

Bariatric surgery has been associated to decrease in adverse obstetric and perinatal effects in women with severe obesity [213]. However, the effects on child and adolescent obesity in comparison s between BMS and AMS siblings are less clear. While Kral et al. [47] have presented results indicating lower rates of obesity in AMS siblings compared with BMS siblings, we were unable to detect such a difference in our sample. It would be of interest to replicate our study in a larger sample to include more data on the BMI of sibling-pairs of six and four years of age stratified according to the surgical procedures most often used in Sweden today, i.e., laparoscopic gastric bypass and, to an increasing extent, also gastric sleeve operations [106]. Such a study would make future results more comparable with the results of Kral et al. who studied the effects of maternal BPD on offspring BMI [47]. In accordance with Aricha-Tamir et al. [199], we reported a decrease in birth weight in AMS siblings compared with BMS siblings. A recent publication by Amsalem et al. [124] reported a significant decrease in pregnancy complications at the second subsequent pregnancy following bariatric surgery. Furthermore, the authors reported that women had essentially unchanged pre-pregnancy BMI for both gestations following surgery. Thus, it would be of interest further to investigate the effects of interpregnancy weight gain, total and trimester-specific GWG, offspring birth weight and BMI at four and six years in multiple pregnancies following bariatric surgery, using our study sample.

Interest in understanding the associations between epigenetic changes and disease outcomes, together with the fast developing EWAS technique, has rapidly increased during the last decade. Knowledge in this area has changed considerably since we started the project. Recent data has indicated that inter-subject variation in leukocyte distribution may confound the observed relationship between methylation and the observed outcome (different methylation of obesity-related genes). Moreover, the use of a surrogate tissue (in our case the use of blood) to examine systemic alterations in DNA methylation may not necessarily reflect changes

occurring in other tissues [149]. In addition, there is recent data indicating epigenetic intra-individual differences after RYGB in muscle tissue [139]. Thus, it would be of interest to conduct a similar data collection to ours for studies one and two, but this time to collect whole blood sorted with FACS as well as biopsies from muscle and fat tissue in BMS and AMS siblings. Moreover, it would be of great interest to see whether the observed methylation differences between BMS and AMS siblings are correlated with actual mRNA expressions [145], and with other epigenetic gene regulating mechanisms such as histone modifications [214]. Another possible approach would be to collect FACS-sorted whole blood and muscle and fat-tissue biopsies from children. Today, several of the mothers included in data collection two have given birth after RYGB surgery, which enables comparisons of epigenetic patterns in obesity-related genes between siblings born before and after maternal RYGB.

In studies three and four, several RYGB patients had difficulties complying with prescribed PA recommendations after surgery, and – even more alarmingly – several women and family members (especially children) decreased PA and increased SB from pre- to post maternal RYGB surgery. Thus, strategies for assisting RYGB patients and family members to increase and maintain higher PA levels from before to after maternal RYGB are of importance. It would be of great interest to study the effects of pre- and post-surgery PA counseling on PA and SB in patients undergoing RYGB and family members. Similar study design and measurements as used in studies three and four could be applied in the PA and SB assessments. However, I would personally like to focus the analyses more on time spent sedentary, and use an inclinometer such as the activPal™ to detect body posture, since SB has recently been recognized as a risk factor for numerous disease states independent of PA [215]. The use of both the GT3X+ and activPal™ to estimate SB would enable us to assess SB with decent validity [164, 198] according to the latest definition of SB [197]. Barriers to engage in PA may be more common among obese adults, and bariatric surgery patients in particular frequently feel too overweight to exercise, experiencing musculoskeletal problems, having past negative experiences, or having fear of exercise-related injuries [216]. Hence, it may be more appropriate to aim at reducing time spent sedentary among patients undergoing RYGB, since reduced SB has beneficial effects on health independent of PA. Furthermore, reduced SB may be easier to achieve in RYGB candidates compared with increased PA, which is associated with both physical, and psychological barriers in RYGB patients [216].

There is preliminary data from the “Bariactive Study” indicating an increase in steps per day from before to after surgery in patients undergoing bariatric surgery. So far, the preliminary results are based on ”standard care” data from the Longitudinal Assessment of Bariatric

Surgery-2 study [128] and PA counseling data from the "Bariactive Study" [217]. If the final results of the "Bariactive Study" show an increase in steps per day from before to after surgery in patients undergoing bariatric surgery, it would be of interest to test a similar intervention design, with a focus on objectively accelerometer-measured PA and SB, in a Swedish sample of patients undergoing RYGB.

10 CONCLUDING REMARKS

- Women undergoing bariatric surgery show a positive association between differences in total and second trimester maternal GWG and differences in offspring birth weight, but no association with offspring BMI at pre-school age.

- Maternal bariatric surgery, with subsequent weight loss between pregnancies, is associated with overrepresentation of differently methylated sites in genes involved in inflammatory signaling and T2D signaling, and differences in the methylation of genes, such as *HLA-DQB1, POMC, IGF2, INSR, FTO* and *TNF,* associated with T1D, T2D and obesity, when comparing siblings born before and after maternal bariatric surgery.

- There are no significant differences in objectively measured PA or time spent sedentary from three months before to nine months after surgery in women undergoing RYGB despite substantial weight loss.

- There are no significant differences in objectively measured PA or SB between three months before and nine months after surgery in spouses or women who undergo RYGB. However, in children, PA decreases significantly, and time spent sedentary increases significantly from before to after maternal RYGB surgery.

11 POLPULÄRVETENSKAPLIG SAMMANFATTNING

Sjukdomar kopplade till övervikt och fetma som hjärtinfarkt, diabetes samt olika cancerformer leder enligt WHO årligen till mer än 2.8 miljoner dödsfall världen over. I Sverige beräknas ungefär 50% av männen, 40% av kvinnorna och 10% av barnen vara överviktiga vilket speglar en ökning med mer än 50% från 1980 till 2012. Därutöver har prevalensen av övervikt hos gravida kvinnor i Sverige ökat markant från 10.6% år 1982 till 24.8% år 2010. Detta är alarmerande eftersom övervikt och kraftig viktuppgång under graviditet är starkt kopplat till förlossningskomplikationer samt ökad risk för fetma och metabola sjukdomar hos avkomman.

Kirurgisk behandling av fetma är idag den mest effektiva behandlingen mot fetma, vilken i majoriteten av fallen resulterar i bestående viktminskning samt positiva effekter på flertalet sjukdomar kopplade till fetma. Nittiotvå procent av all kirurgisk behandling år 2013 i Sverige var s.k. gastric bypass-kirurgi vilken har en kombinerad effekt av minskat födointag genom förminskning av magsäcken samt ett minskat upptag av näringsämnen genom omkoppling av tunntarmen. Effekter av kirurgisk behandling mot fetma på förändring av fysisk aktivitet och stillasittande beteende hos patienter vilka genomgår kirurgi och familjemedlemmar, samt effekter på reglering av gener som är kopplade till fetma i avkomma född efter jämfört med avkomma född före kirurgisk behandling av fetma är till stor del okända.

Avhandlingens syfte var att, via upprepade mätningar inom samma individ, studera effekter av kirurgisk behandling mot fetma hos kvinnor på viktuppgång under graviditet och fostrets födelsevikt samt skillnader i avkommans reglering av gener som är kopplade till fetma beroende av om avkomman är född före eller efter mammans kirurgi. Därutöver studerades förändring i fysisk aktivitet och stillasittande beteende hos kvinnor som genomgår gastric bypass-kirurgi och hennes familj från tre månader före till nio månader efter kirurgi.

Studie ett visade att det finns positiva associationer, hos kvinnor som genomgår kirurgisk behandling mot fetma, mellan skillnader i viktuppgång mellan graviditeter och skillnader i barnets födelsevikt.

Studie två visade att kirurgisk behandling mot fetma hos kvinnor, med medföljande viktnedgång mellan graviditeter, är kopplat till förändring av genaktivitet med kopplingar till inflammation och diabetes vid jämförelse av syskon födda före respektive efter mammans kirurgiska behandling.

Studie tre visade att det inte finns några skillnader i objektivt mätt fysisk aktivitet eller stillasittande beteende från tre månader innan till nio månader efter kirurgi hos kvinnor som genomgår gastric bypass-kirurgi.

Studie fyra visade att objektivt mätt fysisk aktivitet samt stillasittande beteende inte förändras från tre månader innan till nio månader efter kirurgi hos partners medan barn minskar fysisk aktivitet och ökar stillasittande beteende när mamman genomgår gastric bypass-kirurgi.

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