The first 1000 days and beyond

Küpers, Leanne Karen

CHAPTER 1

GENERAL INTRODUCTION

CHILDHOOD OBESITY: DEFINITION, PREVALENCE AND CONSEQUENCES

"One of the biggest public health challenges of this century" is how the World Health Organisation (WHO) described the worldwide obesity epidemic in May 2015. This report estimated that overweight would still increase in 41 of the 53 Euro-region countries until at least 2030[1]. Previously, the WHO estimated that in 2014 worldwide, almost 2 billion adults were overweight and of these over 600 million (13%) were obese. In 2013, 42 million children (<5 years) were overweight or obese[2]. Also in the Netherlands in 2009, childhood prevalence of overweight and obesity was alarming, with up to 15% for overweight and 2% for obesity, in 2-21 year-olds. For 2-5 year-olds, this prevalence of overweight and obesity was already 8-18%, depending on age and gender[3].

In adults, overweight is commonly defined using the Body Mass Index (BMI), which is calculated as weight divided by height squared (kg/m^2). In adults we define overweight as a BMI≥25 and obesity as BMI≥30. This definition is extrapolated to internationally acceptable cut-offs for overweight and obesity in children, taking into account differences in growth, dependent on age and gender (**Figure 1**)[4].

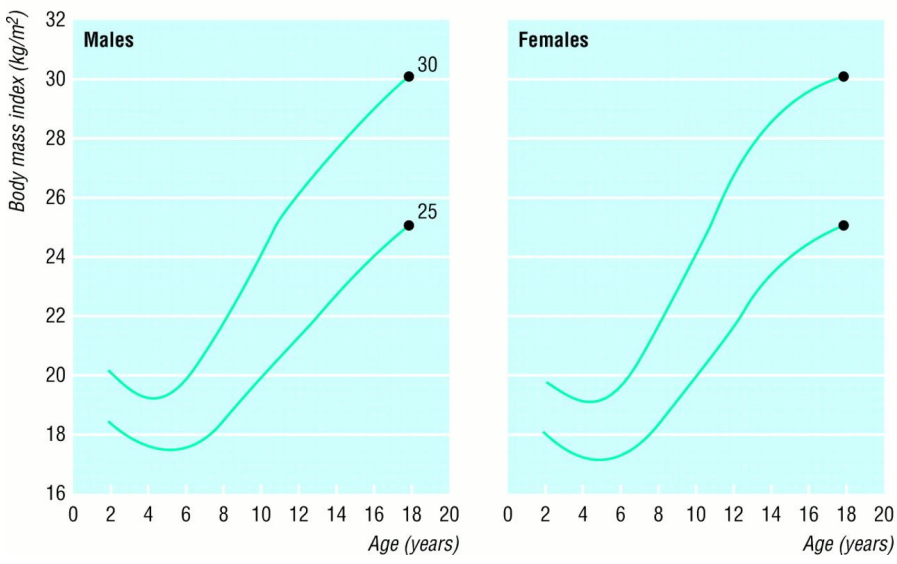

FIGURE 1. International BMI cut-off values for childhood overweight and obesity extrapolated from BMI of 25 and 30 kg/m^2 in adulthood [4].

Childhood overweight is known to track into adulthood, thus overweight children are at increased risk of becoming overweight adults[5]. Childhood overweight also increases the risk of cardiovascular disease and diabetes mellitus in adulthood[6]. Additionally, childhood overweight can have many serious health consequences already early in life, e.g. cardiometabolic problems (high blood pressure and dyslipidemia), insulin resistance, respiratory problems and psychosocial problems (this is mainly related to bullying and a low self-esteem)[7].

CHILDHOOD OBESITY: A MULTIDIMENSIONAL PROBLEM WITH FOETAL AND INFANT ORIGINS

It is well-known that overweight is caused by an imbalance between energy intake and energy expenditure. However, a between-person variation in the energy balance complicates this understanding. Additionally, overweight in children is more complex, because of the growth during childhood. This growth could cause changes in the proportions of weight and height and their contribution to adiposity.

The development of childhood overweight is believed to start already before birth[8,9]. The theory of developmental origins of health and disease is based on the hypothesis that predisposition to many chronic diseases during adulthood is already established during childhood, and often even already during pregnancy[10]. This process is also known as foetal programming, because external influences can affect the development of the foetus and the foetus adapts to the expected outside world. This can have lifelong effects, especially if the outside world differs from what the child was programmed for in the womb; the mismatch theory[11]. This problem of vulnerability in early life has gained worldwide attention within the concept of "the first 1000 days of life" [12,13]. This concept is recognized by the World Health Organization[12] and the website www.ThousandDays.org has been promoted by Hillary Clinton[14]. These 1000 days are seen as a critical window of development, from the start of pregnancy until the baby is two years of age, in which all organs are formed. Therefore, it can also be interpreted as a window of opportunity for nutritional or lifestyle intervention programs[12-14]. The development of overweight can (partly) be explained by this foetal programming hypothesis. If a foetus is exposed to an adverse intrauterine environment (e.g. parental famine, as in the well-known Dutch hunger winter studies) during a "critical" period of development, the foetus could be programmed for a comparable later environment[15]. This could lead to a mismatch in a

later energy-rich environment during childhood and could cause a disturbance in the energy balance, which in turn leads to overweight[11].

DNA METHYLATION AND CHILDHOOD WEIGHT STATUS

The underlying biological mechanism through which the previously explained long term effects of foetal programming could be explained is still not completely unravelled. However, epigenetics could play an important role[11]. Epigenetics is the science of DNA alterations that lead to differences in gene expression, without changing the DNA sequence, thus it can be seen as the bridge between genotype and phenotype[16]. The term "epigenetics" could be translated to "the study of what is upon the DNA", since the Greek words *epi* means "upon or among". The two best-studied epigenetic processes are histone modifications and DNA methylation, in this thesis we only focus on DNA methylation.

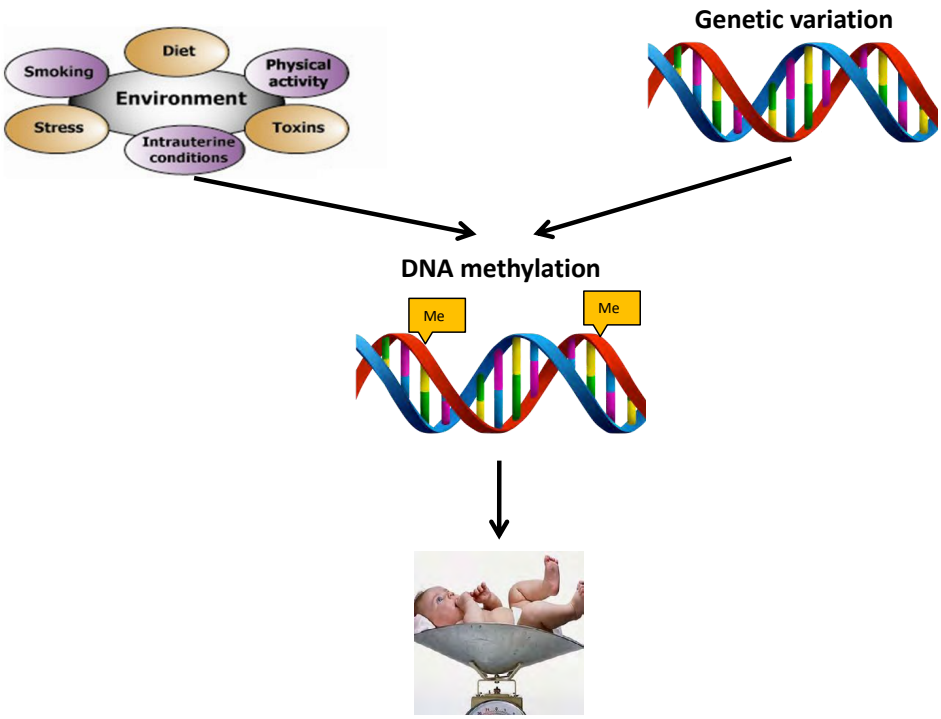

FIGURE 2. Schematic overview of gene-environment interaction and the role of DNA methylation, adapted from van Vliet-Ostaptchouk et al.[17]

DNA methylation can be explained as the binding of methyl groups on cytosine-phosphor-guanine (CpG) dinucleotides. This binding of methyl groups to the DNA is essential in the process of cell division, because it causes cells to differentiate from embryonic stem cells to specific tissues. DNA methylation influences gene expression, which in turn translates into differences in health outcomes, e.g. birth weight in the example of **Figure 2**. DNA methylation is a mechanism of developmental plasticity[11] and both environmental exposures and genetic variation could affect DNA methylation, as shown in **Figure 2**. An example of such an environmental exposure is maternal lifestyle during pregnancy, e.g. maternal smoking, alcohol consumption or pre-pregnancy BMI. Most population-based epigenome-wide association studies (EWAS) in epigenetic epidemiology use the Illumina HumanMethylation450 BeadChip array to perform high-throughput DNA methylation profiling[8,18,19]. This array quantifies the methylation level of >485,000 CpG sites across the genome; with an average of 17 CpGs per gene region the array covers 99% of RefSeq genes. This chip has a high-throughput; with 12 samples per array it enables analysis of 96 samples per run, while it requires only 500 nanogram input.

INFANT LIFESTYLE AND CHILDHOOD WEIGHT STATUS

As explained previously, within the foetal programming hypothesis, DNA methylation could program a child as resilient or as susceptible to the obesogenic environment and an unhealthy lifestyle. Thus a combination between adverse foetal programming and unhealthy exposures later in life could be detrimental for the health of the child.

As previously mentioned, it is well known that an imbalance between energy intake and energy expenditure increases fat storage, which leads to overweight. Several theoretical models are developed to explain this imbalance; one example is the Ecological Systems Theory of Davison and Birch[20]. This model can be found in **Figure 3**, slightly adjusted specifically for the foetal programming focus of this thesis. In this model many determinants of childhood weight status are introduced, e.g. nutrition, physical activity, foetal and childhood environment, parental lifestyle, socioeconomic factors etc. Currently, no clear consensus exists about the most important determinants for this energy imbalance causing childhood overweight.

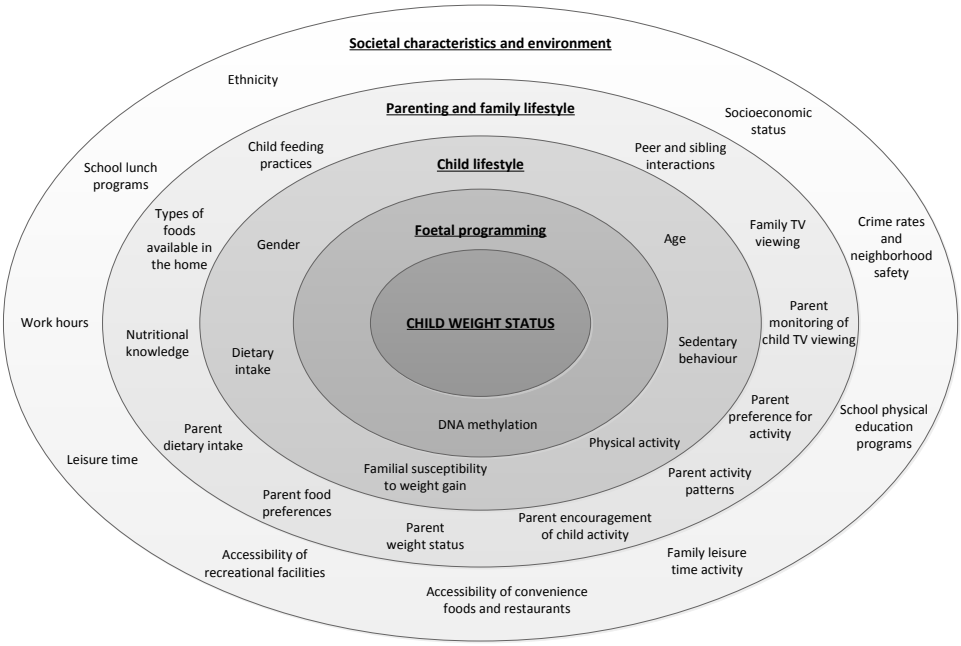

FIGURE 3. Ecological Systems Theory for explanation of child weight status, adapted from Davison and Birch[20].

Potential risk factors for aberrant growth and the development of overweight in childhood are unhealthy dietary habits and family lifestyle during infancy. Important dietary habits could be breastfeeding, correct timing of introduction of complementary feeding and sugar sweetened beverages[21-23]. Other lifestyle factors that could play a role in the development of overweight could be sleep, screen time and physical activity[24,25]. However, also socioeconomic factors, that could be seen as a proxy for an obesogenic environment, could be important, e.g. ethnicity, educational level and possibly family structure[20].

Additionally, certain time windows have been suggested as critical for the risk of overweight, if accelerated growth occurs. One of those periods takes place in the adiposity rebound phase[26], which can be seen as the dip in the growth curves in **Figure 1** around the age of five years. However, the period before this adiposity rebound is also very interesting. Accelerated growth during infancy has been identified to be associated with later childhood overweight[27,28], as well as the growth between two and seven years of age[29]. However, it is not clear whether specific smaller time windows during infancy and childhood are particularly important here.

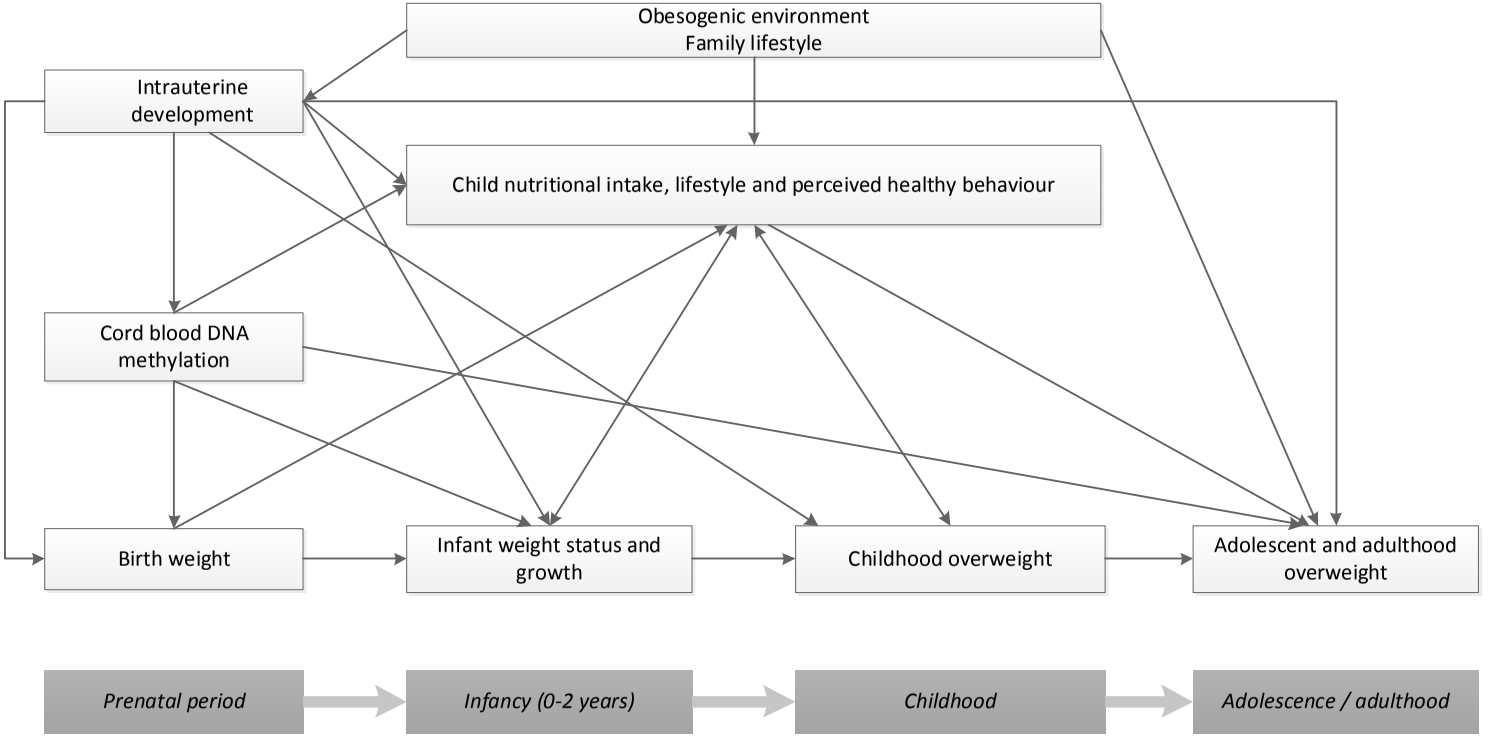

FIGURE 4. Theoretical model for life course trajectories in the development of overweight.

Overall, obesity is known as a multidimensional health problem with many biological, lifestyle and environmental factors playing a role in the development. **Figure 4** shows the life course trajectories that are hypothesized to play a role in the development of overweight during childhood.

AIMS OF THIS THESIS

The main aim of this thesis was to investigate determinants of childhood overweight. First, we investigated whether DNA methylation could explain (part of) the association between maternal lifestyle during pregnancy and offspring health and development. Thus we determined underlying biological pathways that could program a newborn as susceptible or resilient to the obesogenic environment. Second, we examined whether this obesogenic environment and lifestyle during pregnancy and infancy could explain differences in childhood weight status.

GENERAL DESIGN

The studies in this thesis were performed in a population-based birth cohort in the Netherlands and within the context of a large international consortium of multiple birth cohorts.

GECKO DRENTHE BIRTH COHORT

GECKO is an abbreviation for Groningen Expert Center for Kids with Obesity. The GECKO Drenthe birth cohort is a population-based birth cohort that focuses on risk factors associated with the development of overweight from birth until adulthood[30]. In this cohort we collected environmental, social, nutritional, biomedical and epigenetic data of up to 2874 children and their parents. These 2874 children were born between April 2006 and April 2007 in Drenthe, a province in the Netherlands. Mothers were invited to participate in the third trimester of their pregnancy. Trained nurses at municipal health services performed the anthropometric measurements at regular intervals, which were routinely performed in the Dutch health care system. Following each regular measurement the parents were asked to answer a questionnaire. Additionally, directly after delivery, umbilical cord blood was collected for buffy coats from 1565 children and stored at -80˚C for DNA isolation. Based on these questionnaires and measurements the

children were extensively phenotyped on parental lifestyle characteristics, pregnancy and delivery, child lifestyle, nutritional intake, health and development (**Table 1**).

TABLE 1. Overview of measurements in the GECKO Drenthe birth cohort.

Age (months)	**0**	**0.5**	**1**	**2**	**3**	**4**	**6**	**7**	**9**	**11**	**14**	**18**	**24**	**36**	**45**	**60**
Organisation																
Well Baby Clinics		x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Youth Health Services																x
Questionnaires																
Nutritional intake		x	x	x	x	x	x	x	x	x	x	x	x		x	x
FFQ														x	(x)	
Physical activity parents	x				x										x	(x)
Physical activity child									x	x	x	x	x		x	x
Health	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x
Behaviour		x	x	x	x	x	x		x			x	x		x	x
Environment	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
SDQ																x
Measurements																
Height			x	x	x	x	x	x	x	x	x	x	x	x	x	x
Weight			x	x	x	x	x	x	x	x	x	x	x	x	x	x
Head circumference			x	x	x	x	x	x	x	x						
Waist circumference			x	x	x	x	x	x	x	x	x	x	x	x	x	x
Hip circumference			x	x	x	x	x	x	x	x	x	x	x	x	x	
Blood pressure													x			x
Movement sensor														x	x	x
BIA (subgroup)				x				x								
Ultrasound (subgroup)				x				x								
HbA1c (subgroup)									x							
Biomaterials																
Umbilical cord blood	x															
Umbilical cord tissue	x															
Blood sample									x							
Urine (albumin and creatinine)													x			

FFQ: Food Frequency Questionnaire. SDQ: Strengths and Difficulties Questionnaire. BIA: Bioelectrical Impedance Analysis. HbA1c: glycated haemoglobin (A1c), average long-term plasma glucose concentration.

PREGNANCY AND CHILD EPIGENETICS (PACE) CONSORTIUM

For the meta-analysis of epigenome-wide association studies we have formed an international consortium, combining many birth cohorts and childhood cohorts. With the GECKO Drenthe birth cohort we actively contribute to this consortium by leading one of the projects on the association between DNA methylation and birth weight (**Chapter 4**) and with our GECKO data we contribute to many other projects within the consortium. The aim of this Pregnancy and Childhood Epigenetics (PACE) consortium is to assess the association between intrauterine exposures, DNA methylation and health outcomes in newborns.

Outline of this thesis

PART I – DNA methylation and childhood weight status.

In **Chapter 2** we performed an epigenome-wide association study to identify genes with differential DNA methylation in children of smoking mothers compared to children of non-smoking mothers. Additionally, we studied whether DNA methylation mediated the effect of maternal smoking on birth weight.

In **Chapter 3** we performed a meta-analysis of multiple epigenome-wide association studies in newborns to identify of which genes the DNA methylation in cord blood was associated with maternal smoking during pregnancy.

In **Chapter 4** meta-analysed epigenome-wide association studies in multiple birth cohorts to identify of which genes the DNA methylation in cord blood was associated with birth weight.

In **Chapter 5** we created a pipeline for automating the quality control of one or multiple results files prior to the meta-analysis of epigenome-wide association studies: QCEWAS.

PART II – Infant lifestyle and childhood weight status.

In **Chapter 6** we studied the determinants of body weight growth rate during the first two years of life, grouped in four domains: prenatal, nutrition, lifestyle and socioeconomic domains.

In **Chapter 7** we assessed whether skipping breakfast in 2- and 5-year olds was associated with childhood overweight.

In **Chapter 8** we tested several dietary habits (e.g. breastfeeding, fruit, vegetables, sweetened beverages) during infancy to predict growth between two and six years of age.

In **Chapter 9** we assessed whether physical activity of parents was associated with the physical activity of their children, measured with tri-axial accelerometers from Actigraph.

Chapter 10 provides a discussion of the main results in this thesis, methodological considerations and future perspectives for research of childhood overweight.

REFERENCES

1 Breda J, Jewell J, Webber L, Galea G. WHO projections in adults to 2030. *Obesity Facts - Eur J Obesity* 2015; 8: 17.

2 World Health Organization. Fact sheet No 311: Obesity and overweight. January 2015;.

3 Schonbeck Y, Talma H, van Dommelen P, et al. Increase in prevalence of overweight in Dutch children and adolescents: a comparison of nationwide growth studies in 1980, 1997 and 2009. *PLoS One* 2011; 6: e27608.

4 Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* 2000; 320: 1240-3.

5 Singh AS, Mulder C, Twisk JW, van Mechelen W, Chinapaw MJ. Tracking of childhood overweight into adulthood: a systematic review of the literature. *Obes Rev* 2008; 9: 474-88.

6 Reilly JJ, Methven E, McDowell ZC, et al. Health consequences of obesity. *Arch Dis Child* 2003; 88: 748-52.

7 Dietz WH. Health consequences of obesity in youth: childhood predictors of adult disease. *Pediatrics* 1998; 101: 518-25.

8 Sharp GC, Lawlor DA, Richmond RC, et al. Maternal pre-pregnancy BMI and gestational weight gain, offspring DNA methylation and later offspring adiposity: findings from the Avon Longitudinal Study of Parents and Children. *Int J Epidemiol* 2015;.

9 Lumey LH, Stein AD, Susser E. Prenatal famine and adult health. *Annu Rev Public Health* 2011; 32: 237-62.

10 Barker DJ. The fetal origins of hypertension. *J Hypertens Suppl* 1996; 14: S117-20.

11 Gluckman PD, Hanson MA. Developmental and epigenetic pathways to obesity: an evolutionary-developmental perspective. *Int J Obes (Lond)* 2008; 32 Suppl 7: S62-71.

12 World Health Organization. Essential Nutrition Actions: improving maternal, newborn, infant and young child health and nutrition. 2013;.

13 Koletzko B, Brands B, Chourdakis M, et al. The Power of Programming and the EarlyNutrition project: opportunities for health promotion by nutrition during the first thousand days of life and beyond. *Ann Nutr Metab* 2014; 64: 187-96.

14 1,000 Days Project. www.ThousandDays.org. 2015.

15 Painter RC, Osmond C, Gluckman P, Hanson M, Phillips DI, Roseboom TJ. Transgenerational effects of prenatal exposure to the Dutch famine on neonatal adiposity and health in later life. *BJOG* 2008; 115: 1243-9.

16 Goldberg AD, Allis CD, Bernstein E. Epigenetics: a landscape takes shape. *Cell* 2007; 128: 635-8.

17 van Vliet-Ostaptchouk JV, Snieder H, Lagou V. Gene-Lifestyle Interactions in Obesity. *Curr Nutr Rep* 2012; 1: 184-96.

18 Joubert BR, Haberg SE, Nilsen RM, et al. 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. *Environ Health Perspect* 2012; 120: 1425-31.

19 Markunas CA, Xu Z, Harlid S, et al. Identification of DNA Methylation Changes in Newborns Related to Maternal Smoking during Pregnancy. *Environ Health Perspect* 2014;.

20 Davison KK, Birch LL. Childhood overweight: a contextual model and recommendations for future research. *Obes Rev* 2001; 2: 159-71.

21 Pearce J, Taylor MA, Langley-Evans SC. Timing of the introduction of complementary feeding and risk of childhood obesity: a systematic review. *Int J Obes (Lond)* 2013; 37: 1295-306.

22 Gunnarsdottir I, Schack-Nielsen L, Michaelsen KF, Sorensen TI, Thorsdottir I. Infant weight gain, duration of exclusive breast-feeding and childhood BMI - two similar follow-up cohorts. *Public Health Nutr* 2010; 13: 201-7.

23 Bachman CM, Baranowski T, Nicklas TA. Is there an association between sweetened beverages and adiposity? *Nutr Rev* 2006; 64: 153-74.

24 Sijtsma A, Koller M, Sauer PJ, Corpeleijn E. Television, sleep, outdoor play and BMI in young children: the GECKO Drenthe cohort. *Eur J Pediatr* 2014;.

25 Sijtsma A, Sauer PJ, Stolk RP, Corpeleijn E. Is directly measured physical activity related to adiposity in preschool children? *Int J Pediatr Obes* 2011; 6: 389-400.

26 Brisbois TD, Farmer AP, McCargar LJ. Early markers of adult obesity: a review. *Obes Rev* 2012; 13: 347-67.

27 Claris O, Beltrand J, Levy-Marchal C. Consequences of intrauterine growth and early neonatal catch-up growth. *Semin Perinatol* 2010; 34: 207-10.

28 Monteiro PO, Victora CG. Rapid growth in infancy and childhood and obesity in later life--a systematic review. *Obes Rev* 2005; 6: 143-54.

29 Liem ET, van Buuren S, Sauer PJ, Jaspers M, Stolk RP, Reijneveld SA. Growth during infancy and childhood, and adiposity at age 16 years: ages 2 to 7 years are pivotal. *J Pediatr* 2013; 162: 287,92.e2.

30 L'Abee C, Sauer PJ, Damen M, Rake JP, Cats H, Stolk RP. Cohort Profile: the GECKO Drenthe study, overweight programming during early childhood. *Int J Epidemiol* 2008; 37: 486-9.

PART 1

DNA METHYLATION AND CHILDHOOD WEIGHT STATUS