A nationwide follow-up study of children of women with type 1 diabetes mellitus

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A nationwide follow-up study of children of women with type 1 diabetes mellitus

Een landelijk vervolgonderzoek naar de ontwikkeling van kinderen van vrouwen met type 1 diabetes mellitus

(met een samenvatting in het Nederlands)

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Aan mijn ouders

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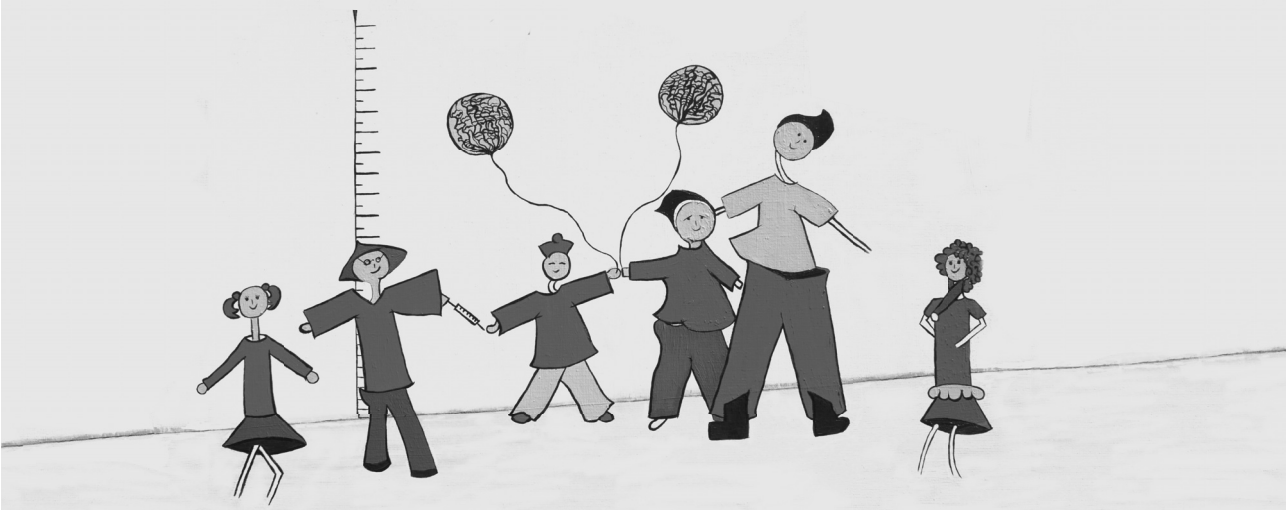
Introduction and outline of the thesis



Type 1 diabetes mellitus is the result of a T-cell mediated autoimmune destruction of pancreatic beta cells. Patients are therefore unable to maintain adequate insulin secretion in order to prevent hyperglycemia. Treatment of type 1 diabetes has been possible since the discovery of insulin by Banting and Best in the early twenties of the last century, and over the years outcome of patients with type 1 diabetes has improved because of (self) monitoring of blood glucose levels, together with advanced treatment technologies. However, until today, no definitive cure has been developed.[1] Therefore, it remains of utmost importance to keep improving care, treatment and outcome in patients with type 1 diabetes, the more so since the incidence of type 1 diabetes is increasing worldwide.[2] The prevalence of type 1 diabetes in women of fertile age (15-44 years) in The Netherlands anno 2007 is approximately 4.5 per 1000 women.[3]

Type 1 diabetes and pregnancy

Before the discovery of insulin many women with type 1 diabetes who became pregnant died during pregnancy. Maternal and neonatal outcome of type 1 diabetic pregnancies has improved dramatically over the last decades, mainly due to improvements of glycemic control and obstetric and neonatal care.[4] However, despite these improvements, pregnancy in women with type 1 diabetes still remains a high risk situation for both mother and child. Several studies have shown higher maternal complication rates during pregnancy, and children appear not only at risk for adverse neonatal outcome, but also for impaired long term development.

Complications – short term

In a previous nationwide study on outcome of 323 type 1 diabetic pregnancies, Evers et al.[5] found a higher prevalence of maternal and neonatal complications despite the fact that pre-pregnancy care was good (with 84% planned pregnancies and 70% preconceptional folic acid supplementation) and overall glycemic control during pregnancy was adequate (mean HbA1c 6.2%). Maternal complications included episodes of severe hypoglycemia (40%), preeclampsia (13%) and cesarean section (44%). Fetal complications included congenital anomalies (9%), prematurity (32%), perinatal mortality (3%), neonatal macrosomia (45%), and other neonatal morbidity (14% shoulder dystocia, 64% neonatal hypoglycemia, 26% hyperbillirubinemia, 15% respiratory disorders, 5% clinical signs of hypertrophic cardiomyopathy).[5] Similar rates of complications have been found in other nationwide studies in Denmark,[6] the United Kingdom[7] and Sweden.[8]

There is now a substantial body of evidence that children born after a type 1 diabetic pregnancy are also at risk for adverse effects regarding their long term development, which is believed to be mainly due to the altered intrauterine circumstances. The Pedersen hypothesis states that

maternal hyperglycemia results in an increased placental transport of glucose, thereby causing fetal hyperglycemia as maternal insulin does not cross the placenta.[9] After 20 weeks of gestation, the fetus has a functioning pancreas and fetal hyperglycemia results in hypertrophy of fetal pancreatic islets and subsequent hyperinsulinemia. Insulin promotes storage of excess nutrients (glucose), thereby acting as a fetal growth factor. Simultaneously, levels of insulin-like growth factors also increase. These mechanisms are thought to be responsible for fetal overgrowth (macrosomia), which typically is asymmetric and characterized by an enlarged thoracic and abdominal circumference, thereby increasing complications during delivery such as shoulder dystocia and asphyxia. The excess fetal glucose is also held responsible, at least in part, for the occurrence of long term cardiovascular and metabolic effects.

Complications – long term

Intrauterine and early postnatal environment may affect health later in life. However, the mechanisms by which exposure to diabetes in utero increases the risk of later health problems in the offspring are not fully understood. The concept of 'developmental origins of health and disease' describes the effect of the environment during human development on the risk of chronic disease later in life. This field of research has gained worldwide knowledge since the discovery that low birth weight is associated with increased cardiovascular and metabolic diseases later in life (Barker's 'thrifty phenotype hypothesis').[10] However, a general etiological concept of perinatal 'programming' of the functioning of fundamental regulatory systems was already postulated in the early 1970's by Dörner[11] and some years later by Freinkel[12]. Dörner described the principle of 'functional teratology', based on the results of studies that showed that hormones, when present in non-physiological concentrations because of alterations of the intrauterine and/or early postnatal environment, can 'program' the neuro-endocrine-immune network, leading to developmental disorders and diseases throughout life.[11] Freinkel's hypothesis of 'fuel-mediated teratogenesis' proposed that intrauterine exposure to an excess of fuels (mainly glucose in case of a diabetic pregnancy) causes embryopathy (early in pregnancy) or alterations in fetal organ development (during the entire length of pregnancy), such as in brain cells, pancreatic beta cells, adipose and muscle cells and nephron development.[12] These alterations may eventually result in health problems, such as obesity, type 2 diabetes, hypertension and neurocognitive problems.

The current understanding is that early developmental programming of later dysfunction and disease results from a combination of mechanisms acting at organ, tissue and cellular levels. Epigenetic mechanisms of altered gene expression (changes in gene transcription through for example DNA methylation, in the absence of changes in the gene DNA sequence) may also be involved.[13]

Cardiovascular and metabolic development

Several studies in offspring of diabetic women have shown that they are at risk for the development of obesity and type 2 diabetes later in life[13]. Two prospective studies that contributed largely to this area of research are the Pima Indian Study[14] and the Diabetes in Pregnancy Study from the Northwestern University in Chicago.[15] The Pima Indians from Arizona have the world's highest incidence of type 2 diabetes, and are therefore widely studied regarding the role of the intrauterine diabetic environment on growth and development in the offspring. Several studies in this high-risk population have shown that intrauterine exposure to maternal diabetes renders the offspring at risk for later obesity and type 2 diabetes, independent of their genetic predisposition.[14] The Diabetes in Pregnancy Study from Chicago studied the development from birth into late adolescence of offspring born between 1977 and 1983 of women with pregestational (type 1 and type 2) diabetes and gestational diabetes. They found that neonatal macrosomia in the offspring resolved within the first year of life, but that obesity recurred in childhood, and that one third of the children showed impaired glucose tolerance already in adolescence.[15] Other studies have shown that children of diabetic women have a higher incidence of other cardiovascular and metabolic risk factors, such as altered lipid parameters,[16-19] and elevated blood pressure.[16,19,20] A recent study in Denmark has shown that adult offspring of type 1 diabetic women are also at risk for the development of the metabolic syndrome, which comprises an association of obesity, hypertension, dyslipidemia and insulin resistance or glucose intolerance.[21]

Neurocognitive development

Two principle mechanisms have been proposed for the possible long term effects of a diabetic pregnancy on neurocognitive development in the offspring.[22] The first links maternal metabolism and child development in a direct teratogenic way as described earlier. Because fetal brain development occurs during the entire length of pregnancy,[23] altered antepartum glucose levels may have effects on the developing brain. According to the second mechanism, the effects of maternal diabetes on neurocognitive development are mediated by perinatal complications and morbidities. In this view, a diabetic pregnancy increases the risk of adverse perinatal outcome, which in turn may affect subsequent cerebral development primarily through tissue damage and developmental arrest. It is likely that these two models operate in tandem.[22] Neurocognitive development in the offspring of diabetic women has been investigated in several studies, among which are two well described study cohorts. One study described the development of 57 children at 5-12 years of age of women with pregestational (type 1 or type 2) diabetes.[24-26] The Diabetes in Pregnancy Study from the Northwestern University in Chicago prospectively studied the development of approximately 200 children of pregestational as well as gestational diabetic women from the neonatal period through adolescence.[22,27-30] Both studies found that overall intellectual ability in offspring born after a diabetic pregnancy was within the normal range, but that they showed more

subtle neuropsychological impairments, such as impaired fine motor function, inattention and hyperactivity.

Other fields of interest

As illustrated in the previous section, evidence is accumulating that in utero exposure to maternal diabetes may have long lasting effects on the offspring's development. Most studies in children of women with diabetes so far have focused on traditional cardiovascular, metabolic and neurocognitive aspects of development. However, possible effects on other aspects of long term development may also be of interest.

Immune function

Cytokines are the 'hormones' of the immune system and are produced by a variety of cells, including lymphocytes, monocytes and macrophages. Cytokines are signaling proteins which mediate immune responses and enable communication between the immune system and other organ systems.[31] Many cytokines appear to share similar functions, but they may functionally be categorized into pro-inflammatory cytokines (e.g. Tumor Necrosis Factor alpha (TNF-α) and gamma Interferon (IFN-γ)) and anti-inflammatory cytokines (e.g. Interleukin 4 (IL-4) and IL-10). Pro-inflammatory cytokines promote cellular immunity, anti-inflammatory cytokines promote humoral immunity (antibody responses). Cytokines in one of these two subsets tend to inhibit the effects of those in the other, and thus it is important to maintain a balance between these subsets.[32,33]

The immune response is highly integrated with metabolic regulation, and a proper function of each is dependent on the other. There is increasing evidence that chronic (or 'low-grade') inflammation is a key feature of metabolic and cardiovascular disturbances.[34] Maternal type 1 diabetes has been shown to induce a pro-inflammatory intra-uterine environment and to impair placental and embryo development which may lead to long term effects on the immune response in the offspring.[35] Furthermore, increased inflammatory markers have been shown at birth in umbilical cord blood of infants of mothers with type 1 diabetes.[36,37] However, studies regarding immune response and inflammatory parameters at later ages in offspring of diabetic women are scarce.

Hypothalamo-pituitary-adrenal axis

The hypothalamo-pituitary-adrenal (HPA) axis is a neuroendocrine system that is involved in many processes including metabolism, immune response and regulation of stress and emotions.[38] In reaction to a stressor, the hypothalamus produces corticotropin-releasing hormone (CRH), which releases adrenocorticotropic hormone (ACTH) from the pituitary gland. ACTH stimulates the excretion of cortisol, the major effector of the HPA-axis, from the adrenal glands. Cortisol inhibits its own production by inhibiting the release of CRH (negative

feedback). Epigenetic programming of neuroendocrine systems regulating metabolism such as the HPA-axis has also been suggested to be involved in the etiology of early programming of later disease.[11] The HPA-axis has been shown to be susceptible to programming during fetal and neonatal development, and has been suggested to be linked to the development of cardiovascular disease, insulin resistance and type 2 diabetes in adult life.[38] Increased HPA-axis activity in patients with type 1 diabetes has been reported,[39] but studies regarding HPA-axis functioning in their offspring are lacking.

Cardiac function

With current diabetes treatment regimens, structural cardiac defects occur in 2-3% of the offspring of diabetic women.[5-7] Hypertrophic cardiomyopathy (mainly interventricular septal hypertrophy) can be demonstrated in 25-45% of the newborn infants.[40-44] It may be associated with functional cardiac changes in the fetus during pregnancy as well as in the newborn.[44-49] Although hypertrophic cardiomyopathy usually normalizes within the first six months after birth,[42] it may be hypothesized that, in analogy with the above mentioned long term effects of the diabetic intrauterine environment, cardiac function may be affected. However, follow-up studies regarding cardiac function in offspring of women with type 1 diabetes have not been performed.

Early vascular alterations

As mentioned before, previous studies in offspring of diabetic women have shown that they are at risk for cardiovascular health problems later in life. Therefore, it is important to recognize and prevent cardiovascular disease risk factors early in the course of life and even, if possible, already in childhood. One of the underlying pathologies of cardiovascular disease is atherosclerosis. Although clinical complications of atherosclerosis normally do not occur until adult life, the atherogenic process begins early in life, and precursors of atherosclerotic cardiovascular disease may be present already at young age.[50]

Maternal diabetes has previously been associated with alterations in cholesterol and lipid concentrations in the offspring already at young age.[17,18,51] Hence, this may play a role in the pathogenesis of atherosclerosis in adult life. Carotid intima-media thickness and to a lesser extent carotid artery distensibility are well-known markers for atherosclerosis in adults.[52] They can be assessed non-invasively by ultrasound and are therefore suitable markers to investigate in children,[53] but studies regarding such parameters in the offspring of diabetic women are scarce and limited to the neonatal period.[54]

Aims and outline of this thesis

Several studies in offspring of diabetic mothers have shown that intrauterine exposure to maternal diabetes may be associated with an increased risk of long term cardiovascular, metabolic and neurocognitive effects, not only at later age, but already during childhood. Direct comparison of these studies with each other is hampered by the fact that many studies considered offspring of mixed cohorts of women with pregestational type 1 and type 2 diabetes and/or gestational diabetes, and lacked information regarding maternal glycemic control during pregnancy or comparison with a control group of offspring of non-diabetic women. Furthermore, most studies considered small study groups and/or children who were born more than 20 years ago, when prepregnancy care and glycemic control during pregnancy were far less optimal.

Current prepregnancy care and glycemic control during pregnancy has been shown to be near-optimal in The Netherlands.[5] Although this apparently is not good enough to prevent short term neonatal morbidity,[5] one may hypothesize that it may nevertheless have beneficial effects on the long term development in the offspring. Therefore, we conducted a follow-up study in the offspring of this nationwide cohort of women with type 1 diabetes to evaluate physical, cardiovascular, metabolic and neuropsychological aspects of their development at school age (6-9 years of age). Additionally, we investigated whether outcome in these children was related to common complications of type 1 diabetic pregnancy, such as suboptimal maternal glycemic control during pregnancy and neonatal macrosomia. The results from this follow-up study are described in this thesis.

In **chapter 2** we described body composition, prevalence of childhood overweight and BMI growth trajectories.
In **chapter 3** we studied other cardiovascular and metabolic parameters, such as blood pressure, parameters of fasting glucose regulation and lipid metabolism and components of the metabolic syndrome.
In **chapter 4** we studied mitogen-induced immune response, inflammatory parameters and HPA-axis function.
In **chapter 5** several aspects of neurodevelopmental outcome (intellectual function, working memory, visual-motor integration ability and executive function) were studied.
In **chapter 6** cardiac function (especially ventricular function) was evaluated using echocardiography in a subgroup of participating children.
In **chapter 7** we investigated early atherogenic markers using novel ultrasound-based technology in a small sample of participating children.
In **chapter 8** the findings from this follow-up study are summarized and discussed.

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CHAPTER

2

Risk factors for childhood overweight in offspring of type 1 diabetic women with adequate glycemic control during pregnancy: nationwide follow-up study in The Netherlands

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ABSTRACT

Introduction

Pregnancy in type 1 diabetic women remains a high-risk situation for both mother and child. In this study, we investigated long term effects on body composition, prevalence of overweight, and insulin resistance in children of type 1 diabetic women who had had adequate glycemic control during pregnancy (mean HbA1c 6.2%), and we related their outcome to perinatal factors, including macrosomia (birth weight >90th percentile).

Methods

Anthropometric measurements were performed at 6–8 years of age in 213 offspring of type 1 diabetic mothers who participated in a previous nationwide study. Homeostasis model assessment of insulin resistance (HOMA-IR) was determined from a fasting blood sample in 155 of these children. In addition, we studied BMI standard deviation score (SDS) growth trajectories. Results were compared with national reference data.

Results

The prevalence of overweight in the study population was not different from that in the reference population. However, children who were born macrosomic showed twice as much overweight as non-macrosomic children. Macrosomia and maternal overweight were independent predictors of childhood overweight. Overweight children showed an increase in BMI SDS starting already after 6 months of age and had a significantly increased HOMA-IR.

Conclusions

In type 1 diabetic women with adequate glycemic control during pregnancy, long term effects on body composition and overweight in their offspring at school age are limited and related mainly to macrosomia at birth. Possible targets for prevention of childhood overweight are fetal macrosomia, maternal overweight, and an increase in BMI SDS during the first years of life.

INTRODUCTION

Perinatal outcome in diabetic pregnancies has improved dramatically over the past decades, mainly due to improvements in maternal glycemic control and in obstetric and neonatal care.[1] However, despite these improvements, pregnancy in women with type 1 diabetes remains a high-risk situation for both mother and child as we have shown in a Dutch nationwide prospective study.[2] The incidence of maternal and neonatal complications such as congenital malformations (9%) and macrosomia (45%) was still high despite good prepregnancy care and overall adequate glycemic control during pregnancy (mean HbA1c 6.2%). Similar rates of complications have been found in Denmark[3] and in the U.K.[4] and have also been found in type 2 diabetic pregnancies.[4-6]

Evidence is accumulating that an altered intrauterine environment has long term effects on the offspring's development. Previous studies have shown the effects of a diabetic pregnancy on several aspects of development in the offspring such as body composition and glucose homeostasis (see ref. 7 for an overview). However, most studies included small or mixed study cohorts concerning offspring of women with pregestational type 1 and type 2 diabetes as well as gestational diabetes mellitus. Furthermore, most studies considered offspring within a wide range of ages or those born >20 years ago when glycemic control was not as good as in current times. Therefore, we conducted a follow-up study in our nationwide Dutch cohort of type 1 diabetic women to investigate the long term effects of current (adequate) control and treatment during pregnancy on body composition, childhood overweight, and BMI growth trajectories in their offspring at school age. Furthermore, we related these outcomes to perinatal factors, including macrosomia at birth, and investigated insulin resistance to determine whether possible effects on body composition would have metabolic consequences already at this young age.

RESEARCH DESIGN AND METHODS

The study group consisted of offspring of type 1 diabetic mothers (ODM) who participated in our nationwide study on pregnancy outcome in the Netherlands. In that study, type 1 diabetic women presenting for antenatal care were recruited by gynecologists, internists, and diabetes nurse educators from all 118 Dutch hospitals between 1 April 1999 and 1 April 2000. Included were 324 infants born after 24 weeks of gestation. All children were born between July 1999 and December 2000.[2] There were six stillbirths and three neonatal deaths, and two infants died in the first month after birth, leaving 313 children eligible for follow-up. Elements of this follow-up study protocol were a home visit for anthropometric measurements and neurocognitive tests, a fasting blood sample on a separate occasion after the home

visit, and the collection of data concerning growth patterns (results from neuropsychological evaluations will be described elsewhere).

From 313 ODM eligible for follow-up, 213 (99 boys and 114 girls) participated in the investigations performed at home and 155 of them agreed to additional blood sampling. Parents of another 33 ODM provided only growth data, resulting in a total participation rate of 79% (246 of 313), with 17 children lost to follow-up and 50 non-participants. The most frequent reason for parents not participating in the investigations at home and/or blood sampling was the consideration that their children were too young to be subjected to (invasive) medical research. Mean age of ODM at the time of measurements was 6.6±0.2 years (range 6.2-7.3 years) and at the time of blood sampling was 7.4±0.4 years (range 6.5-8.5 years). Mean time between the home visit and blood sampling was 0.8 year (range 0.1-2.1 years).

At the time of the measurements, the investigator was unaware of specific characteristics of the pregnancy and neonatal outcome. Information concerning pregnancy outcome, baseline maternal characteristics, and diabetes treatment during pregnancy was subsequently obtained from the previous study,[2] and parents provided information regarding their current height and weight and breast-feeding in the neonatal period. Information concerning growth in the offspring was derived from child welfare clinics, which monitor growth during childhood at 1, 2, 3, 4, 6, 7½, 9, 11, 14, 18, 24, 36, and 45 months of age. BMI was calculated from height and weight at these ages. This study was approved by the Medical Ethics Committee of the University Medical Center Utrecht, and parents gave written informed consent.

Measurements

The children's height, weight, and waist and hip circumference were measured twice and skinfolds (biceps, triceps, suprailiac, and subscapular) were measured three times at the right side of the body, and the averages were used for analysis. BMI and waist-to-hip ratio were calculated. All measurements were performed by one investigator (M.R.) to exclude interobserver variability. A fasting blood sample was taken to determine glucose and insulin levels. All blood samples were analyzed at one research laboratory.

Definitions

Macrosomia was defined as birth weight >90th percentile corrected for gestational age, sex, and parity according to the Netherlands Perinatal Registry data from 2001 (available at http://www.perinatreg.nl). To investigate the possible effects on outcome of different levels of macrosomia, we defined moderate macrosomia as birth weight between the 90th and 97.7th percentile and severe macrosomia as birth weight >97.7th percentile. Maternal and paternal educational level was categorized as low, intermediate, or high according to international standards.[8] Childhood overweight and obesity were defined according to the International Obesity Task Force cut-off points for BMI-for-age, which are incorporated in the Dutch refer-

ence BMI growth diagrams.[9] The homeostasis model assessment (HOMA) formula was used to estimate fasting insulin resistance (HOMA-IR) from fasting glucose and insulin levels.[10]

Statistical methods

Anthropometric measurements at 6–8 years of age and BMI data from the child welfare clinics were converted into a standard deviation score (SDS) according to the Dutch age- and sex-specific growth diagrams[11] using Growth Analyser 3.5 software (2007, Dutch Growth Foundation). An SDS of 0 equals the age- and sex-specific mean (or 50th percentile) of the national reference population. For normally distributed variables, means±SD were used, and differences between groups were tested by *t* test; otherwise, median and interquartile range and the Mann-Whitney *U* test were used. For categorical variables, group differences were tested by χ^2 analysis or Fisher exact test as appropriate. The prevalence of overweight in ODM was compared with the national reference data using a χ^2 goodness-of-fit test. Differences between non-macrosomic and macrosomic ODM and between moderately and severely macrosomic ODM were analyzed with analysis of variance using age and sex (unless otherwise stated) as covariates. If residuals were not normally distributed, log-transformed geometric means were compared and then back-transformed. Multiple logistic regression analysis was performed to determine independent predictors of childhood overweight with macrosomia, sex, parity, current maternal and paternal overweight, low maternal and paternal educational level, mean pregnancy HbA1c >7%, and breast-feeding during the first week as predictor variables. Results are expressed as odds ratios (ORs) with 95% CIs. Data were analyzed using SPSS 15.0 for Windows (SPSS, Chicago, IL). P <0.05 was considered to be statistically significant.

RESULTS

Maternal age, parity, prepregnancy maternal BMI, highest achieved educational level, first/second/third trimester HbA1c and mean HbA1c during pregnancy, duration of diabetes and prevalence of diabetic complications, preeclampsia, severe hypoglycemia during pregnancy, and cesarean section in participating mothers were not significantly different from those of non-participating mothers. There were no statistically significant differences in gestational age, sex, birth weight, or prevalence of macrosomia, congenital malformations, and neonatal morbidity between participating and non-participating children.

Compared with the national reference population, mean SDS in ODM was -0.05±1.05 for height, 0.15±1.12 for weight, 0.26±1.01 for weight-for-height, 0.24±0.98 for BMI, 0.53±1.05 for waist circumference, 0.58±0.99 for hip circumference, and -0.01±0.96 for waist-to-hip ratio. The prevalence of both overweight and obesity in the ODM group was not significantly higher than that in the reference population at 7 years of age[9] (15.2 vs. 13.5% for overweight [p=0.8] and 3.8 vs. 2% for obesity [p=0.2]). Because of the low prevalence

Table 1. Possible risk factors for development of childhood overweight in ODM.

	Normal weight	Overweight	p^a	OR (95% CI)
n	171[b]	40		
Parity (multiparity)	47	48	0.8	0.8 (0.4-2.0)
Sex (female)	51	63	0.2	0.9 (0.4-2.2)
Mean pregnancy HbA1c >7%	15	21	0.5	1.4 (0.5-3.8)
Birth weight >90th percentile	47	70	0.01	4.4 (1.6-11.8)
Current maternal BMI ≥25 kg/m^2	37	59	0.02	2.8 (1.2-6.6)
Current paternal BMI ≥25 kg/m^2	49	63	0.1	1.6 (0.7-3.8)
Low maternal education	21	23	0.9	0.8 (0.2-2.4)
Low paternal education	18	30	0.1	2.7 (0.9-8.1)
Breast-feeding at 1 week	66	59	0.4	0.7 (0.3-1.6)

Data are % or adjusted ORs (95% CIs) for multiple logistic regression analysis.

[a] χ^2 test. [b] Data on weight (and thus BMI) are missing for 2 children.

of obesity at this age, we considered overweight and obesity together as "overweight" for further analyses. Univariate analysis with possible predictors of childhood overweight showed that the prevalence of macrosomia at birth and of current maternal overweight was significantly higher in ODM who developed overweight at 6-8 years of age (Table 1). Multiple logistic regression analysis showed that macrosomia and maternal overweight were independent predictors of childhood overweight (adjusted OR 4.4 [95% CI 1.6-11.8] and 2.8 [1.2-6.6], respectively) (Table 1). HOMA-IR in ODM (mean±SD 1.05±0.56; median 0.96 [interquartile range 0.71-1.38]) was not higher than that of healthy 7-year-old Dutch children (1.10±0.53; no median mentioned).[12] There were no statistically significant differences in mean SDS for

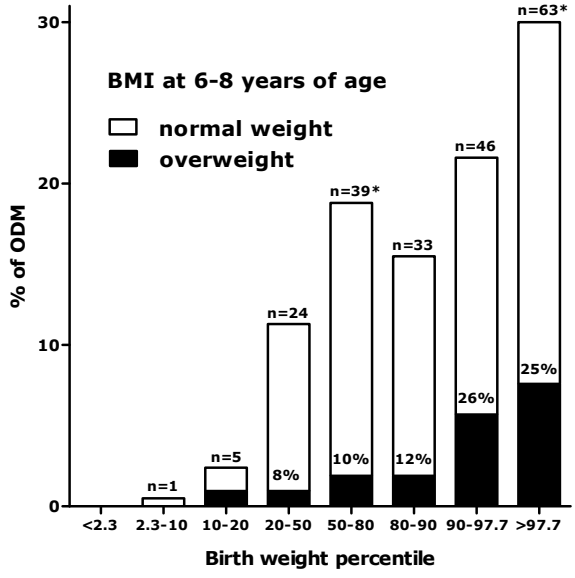

Figure 1. Prevalence of childhood overweight according to birth weight percentile. The total bars represent the percentage of ODM born per birth weight percentile group; the black areas represent the percentage of ODM with overweight at school age (*weight and thus BMI are missing for one child in these birth weight percentile groups).

Table 2. Anthropometric measurements and HOMA-IR in ODM according to level of macrosomia at birth (birth weight ≤90th vs. >90th percentile and birth weight 90th-97.7th vs. >97.7th percentile).

	BW ≤90th percentile	**BW >90th percentile**	*p*
n	103	110	
Height (cm)	122.5 (121.6-123.4)	124.0 (123.0-124.9)	0.03
Weight (kg)	24.3 (23.7-24.8)	25.4 (24.8-26.0)	<0.01
BMI (kg/m^2)*	15.7 (15.3-16.0)	16.6 (16.3-17.0)	<0.01
Waist (cm)*	55.3 (54.3-56.3)	57.8 (56.8-58.8)	<0.01
Hip (cm)	63.0 (61.9-64.0)	65.7 (64.7-66.7)	<0.01
WHR	0.88 (0.87-0.89)	0.88 (0.87-0.89)	0.9
S4S (mm)*	27.9 (26.0-29.9)	31.9 (29.8-34.2)	<0.01
HOMA-IR*	0.93 (0.79-1.09)	0.89 (0.79-1.01)	0.6

	BW 90th-97.7th percentile	**BW >97.7th percentile**	*p*
n	46	64	
Height (cm)	124.6 (123.2-126.0)	124.2 (123.1-125.3)	0.7
Weight (kg)	25.7 (24.7-26.6)	26.1 (25.3-27.0)	0.5
BMI (kg/m^2)*	16.4 (15.9-17.0)	16.7 (16.3-17.2)	0.4
Waist (cm)*	57.4 (55.8-59.0)	58.0 (56.7-59.4)	0.5
Hip (cm)	65.6 (64.0-67.2)	65.7 (64.3-67.0)	0.9
WHR	0.88 (0.87-0.89)	0.89 (0.88-0.90)	0.4
S4S (mm)*	31.0 (28.0-34.3)	32.7 (29.9-35.6)	0.4
HOMA-IR*	0.93 (0.73-1.18)	0.85 (0.68-1.05)	0.6

Numbers represent adjusted means with 95% CI or *back-transformed geometric means with 95% CI if data were log transformed for analysis. All means were adjusted for age and sex, height was also adjusted for maternal and paternal height, and weight was also adjusted for height. BW: birth weight; S4S: sum of four skinfolds; WHR: waist-to-hip ratio.

anthropometric measurements, prevalence of overweight, and mean HOMA-IR between boys and girls (data not shown).

ODM who were macrosomic at birth had increased height, weight, BMI, waist and hip circumference, and thicker skinfolds and showed more than twice as much overweight compared with non-macrosomic ODM (26 vs. 12%, p=0.01) (Figure 1, Table 2). Waist-to-hip ratio and HOMA-IR did not differ between those groups. There were no statistically significant differences in anthropometric measurements, prevalence of overweight, and HOMA-IR between children who had been moderately macrosomic or severely macrosomic at birth (Figure 1, Table 2).

Figure 2A shows the BMI SDS growth trajectories for macrosomic and non-macrosomic ODM. A course along the baseline in this figure equals growth along the 50th percentile line according to the reference BMI growth diagram.[11] In non-macrosomic ODM, the course of the BMI SDS was continuously around the reference population's mean (i.e., the baseline). In macrosomic ODM, however, BMI SDS initially declined in the first months after birth but started to rise at ~7 months of age. The course of the BMI SDS growth trajectories in children who had been moderately macrosomic or severely macrosomic at birth was similar (data

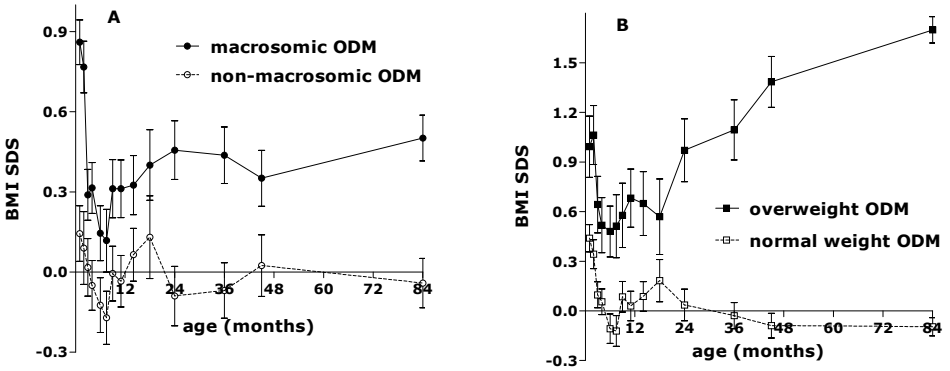

Figure 2. BMI SDS growth trajectories in macrosomic and non-macrosomic ODM (A) and in overweight and normal weight ODM (B). Data are means±SEM.

not shown). Furthermore, we determined HOMA-IR and the BMI SDS growth trajectory in ODM who developed overweight to investigate how these differed from ODM with a normal weight at 6-8 years of age. HOMA-IR was higher in overweight ODM than in normal weight ODM (adjusted means 1.20 [95% CI 0.92-1.57] and 0.85 [0.76-0.95], respectively, p=0.019), and the BMI SDS trajectory in overweight ODM showed (after an initial decline) an increase starting already at 6 months of age (Figure 2B).

CONCLUSIONS

The results of this nationwide follow-up study showed that, with adequate control and treatment during type 1 diabetic pregnancies, the long term effects on body composition in offspring at 6-8 years of age were limited and that fasting HOMA-IR and the prevalence of overweight were not different compared with those for the reference population. Fetal macrosomia and maternal overweight were independent predictors of childhood overweight, and the BMI SDS growth trajectory in children who developed overweight showed an increase already very early in life. Despite an increased prevalence of childhood overweight and increased anthropometric measurements, ODM who were macrosomic at birth showed no increase in insulin resistance. However, insulin resistance was significantly higher in overweight children than in children with a normal weight at 6-8 years of age.

Previous studies in offspring of diabetic mothers have shown that they are at risk for long term effects such as overweight and type 2 diabetes.[7] Most of these studies concerned mixed cohorts of women with pregestational type 1 and type 2 diabetes as well as gestational diabetes mellitus. Our cohort concerned offspring of exclusively type 1 diabetic women who were well prepared before pregnancy (84% planned pregnancies and 70% prepregnancy

folic acid supplementation) and achieved adequate glycemic control during pregnancy (mean HbA1c 6.2%).[2] By extrapolating Freinkel's hypothesis on fuel-mediated teratogenesis,[13] it could be hypothesized that with adequate glycemic control during pregnancy, long term outcome in the offspring should be better. Indeed, the prevalence of childhood overweight in ODM was not higher, and the effects on body composition were minimal compared with those for the reference population, especially in the children who were born with a birth weight appropriate for gestational age. Further follow-up and comparison with more recent reference data (as the reference growth diagrams date from 1997) should show whether the slightly higher SDS of some anthropometric measurements in ODM is a result of a continuing positive secular growth change in the Netherlands[14] or should be attributed to the diabetic pregnancy.

There seemed to be a clear cut-off increase in the prevalence of overweight in infants with a birth weight >90th percentile (Figure 1). Multiple logistic regression analysis with possible predictors for childhood overweight showed that fetal macrosomia, together with maternal overweight, was indeed an independent predictor for overweight in ODM. These results are in accordance with other studies showing that childhood overweight was associated with fetal macrosomia in children of type 1 diabetic women[15,16] and with maternal overweight as well in children of women with gestational diabetes mellitus.[17,18] The number of infants with a birth weight <20th percentile (*n*=6) (Figure 1) was too small to determine whether there was also an increased prevalence of overweight in the low birth weight categories, as has been found in some studies.[19] Despite differences in body composition between macrosomic and non-macrosomic ODM, there was no difference in fasting HOMA-IR. Further follow-up may show whether these children will develop insulin resistance or impaired insulin secretion later in life.

Interestingly, anthropometric measurements, the prevalence of childhood overweight, and HOMA-IR in ODM who were severely macrosomic at birth (birth weight >97.7th percentile) were not increased compared with those of moderately macrosomic ODM (birth weight 90th-97.7th percentile). A possible explanation for this observation could be that glycemic control during pregnancy in mothers of severely macrosomic children was not different from that in mothers of moderately macrosomic children (HbA1c 6.37±1.02 and 6.43±0.81, respectively, *p*=0.7). Follow-up is necessary to show possible differences in the further development between severely macrosomic and moderately macrosomic children.

Breast-feeding has recently been shown to protect against later overweight in children of type 1 diabetic mothers.[15] In contrast, others have found that ingestion of breast milk from diabetic mothers, especially in the first week of life, may increase the risk of becoming overweight.[20] In our cohort, we did not find an effect of early breast-feeding on overweight at 1 year of age[21] nor at 6-8 years of age, although our study lacked detailed information on volume of breast milk ingested, as was used by Rodekamp et al.[20]

The BMI SDS growth trajectory in ODM who had developed overweight at school age showed an initial decline after birth, followed by a steep rise after ~6 months of age. Eriksson et al.[22] found a similar rise in the BMI *z* score growth pattern in the first years of life in individuals who developed type 2 diabetes later in life, especially if they had a higher birth weight. In addition, in our study infants with a high birth weight showed an initial decline in BMI SDS followed by an increase after ~6 months of age (although this increase was smaller than that in the ODM who developed overweight at school age). Touger et al.[23] and Silverman et al.[24] showed comparable growth patterns in ODM. Based on the findings by Eriksson et al.,[22] the BMI SDS growth trajectories in our cohort may suggest that these children are at risk for developing type 2 diabetes later in life, although this suggestion has to be substantiated in our population at further follow-up. Despite the latter limitation, we hypothesize that these findings may be helpful in identifying those children of diabetic mothers who are at risk for future health problems.

In summary, our findings suggest that in our cohort of type 1 diabetic women with adequate glycemic control during pregnancy, the long term effects on body composition of their offspring at 6-8 years of age are limited. The prevalence of overweight is comparable to that in the reference population, provided that the child is born with a birth weight appropriate for gestational age. However, because of the high prevalence of macrosomia and its clear association with the development of childhood overweight, the prevention of macrosomia remains important, more so because overweight children showed increased insulin resistance at 6-8 years of age compared with that in normal weight children. It is possible that continuous glucose monitoring during pregnancy may be an effective tool to reduce the risk of fetal macrosomia.[25] In addition, reducing maternal overweight could be a target for the prevention of childhood overweight in ODM, and close monitoring of the infants' BMI SDS growth trajectory in the first years of life may be helpful in identifying those ODM at risk for developing overweight at school age. Further research is needed to assess the possible influence of such interventions on the prevalence of childhood overweight in ODM.

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CHAPTER

3

Cardiovascular and metabolic outcome in 6-8 year old offspring of women with type 1 diabetes with near-optimal glycemic control during pregnancy

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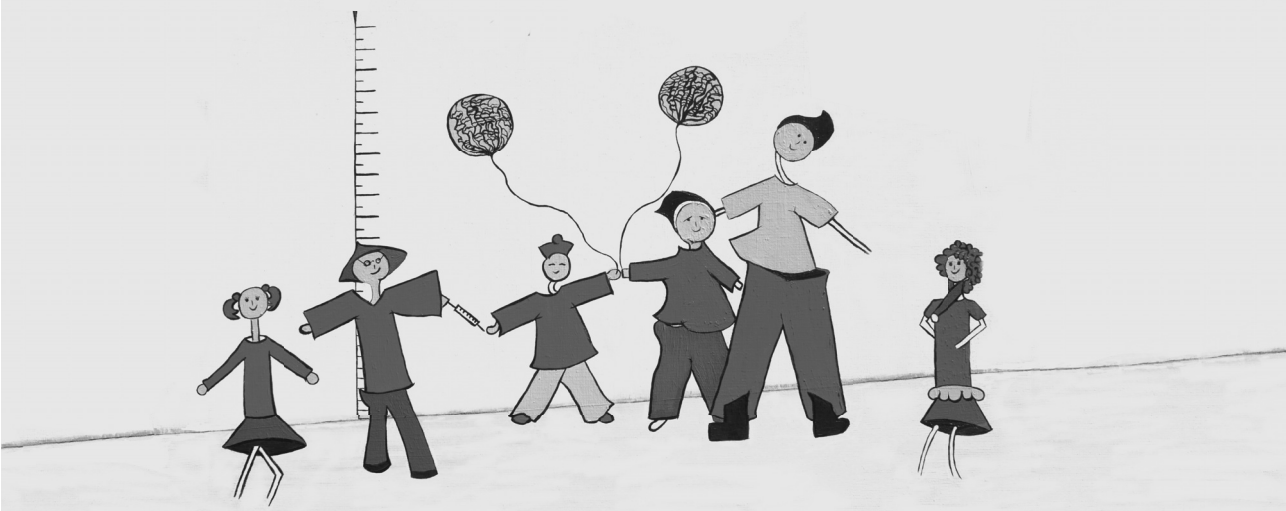

ABSTRACT

Introduction

High maternal glucose concentrations during diabetic pregnancy may lead to health problems in the offspring later in life. We showed in a previous nationwide study on pregnancy outcome in type 1 diabetic women that prepregnancy care was good and a near-optimal glycemic control during pregnancy was achieved (mean HbA1c 6.2%). In this study, we investigated to what extent current care and treatment of pregnant women with type 1 diabetes were related to cardiovascular and metabolic disturbances in the offspring at school age. Additionally, we studied the influence of level of maternal glycemic control, preterm birth and neonatal macrosomia (birth weight >p90).

Methods

BMI, blood pressure, parameters of fasting glucose regulation and lipid metabolism, components of the metabolic syndrome (overweight, hypertension, impaired fasting glucose, dyslipidemia) were determined in 6-8 year old offspring of women with type 1 diabetes (ODM, n=213) and a control group of children of non-diabetic women (n=79).

Results

Parameters of fasting glucose regulation and lipid metabolism and the frequency of components of the metabolic syndrome did not significantly differ between ODM and controls. Systolic blood pressure was slightly higher in ODM. The influence of level of maternal glycemic control, preterm birth and neonatal macrosomia on outcome in ODM was limited.

Conclusions

Current care and treatment of pregnant women with type 1 diabetes result in cardiovascular and metabolic outcome in the offspring at 6-8 years of age that is comparable to that in children of non-diabetic women. Further follow-up should substantiate these results at later age.

INTRODUCTION

The theory that an aberrant maternal fuel metabolism during diabetic pregnancy might influence long term outcome in the offspring was originally postulated by Freinkel almost 30 years ago.[1] There is now a substantial body of evidence for his theory of 'fuel-mediated teratogenesis', which postulates that high glucose concentrations during diabetic pregnancy make the developing tissues in the offspring more vulnerable to functional alterations later in life. This may lead to the development of type 2 diabetes and the metabolic syndrome.[2,3]

In a previous nationwide study on pregnancy outcome in women with type 1 diabetes we showed that prepregnancy care in these women was good (84% planned pregnancies and 70% preconceptional folic acid supplementation) and that adequate maternal glycemic control was achieved during pregnancy (mean HbA1c 6.2%).[4] Despite this adequate level of control and treatment before and during pregnancy, the prevalence of perinatal complications was still high compared with national data (9% congenital malformations, 32% preterm birth, 45% neonatal macrosomia (birth weight >p90)). Near-optimal glycemic control (HbA1c <7.0%) apparently is not good enough to prevent short term (neonatal) morbidity.[4]

Extrapolating Freinkel's theory, good glycemic control during diabetic pregnancy may result in improved long term developmental outcome. To investigate to what extent current care and treatment of pregnant women with type 1 diabetes are related to long term effects on the development in the offspring, we performed a follow-up study in the children of our nationwide cohort of women with type 1 diabetes at school age.

We have recently shown that long term effects on body composition and growth in these children were limited.[5] In the present study we investigated several cardiovascular and metabolic parameters at 6-8 years of age, and compared them to those in a control group. Additionally, we studied the influence of common complications of a type 1 diabetic pregnancy (suboptimal maternal glycemic control, preterm birth and neonatal macrosomia) on cardiovascular and metabolic outcome.

METHODS

Study population

The study group consisted of offspring of type 1 diabetic mothers (ODM) who participated in our nationwide study on pregnancy outcome.[4] In that study, women with type 1 diabetes presenting for antenatal care were recruited by gynecologists, internists and diabetes nurse educators from 118 Dutch hospitals between 1 April 1999 and 1 April 2000. A total of 324 infants born after 24 weeks of gestation were included. There were six stillbirths, three neonatal deaths, and two infants died in the first month after birth, leaving 313 children eligible for follow-up. The follow-up study consisted of a home visit for anthropometric measurements,

blood pressure recordings and neurocognitive tests (results from neurocognitive tests will be described elsewhere). A fasting blood sample was taken on a separate occasion and data regarding growth were collected.

From 313 children eligible for follow-up, 17 were lost to follow-up, and 83 children did not participate in the home visit or blood sampling. From the 213 children who participated in the home visit, 155 agreed to additional blood sampling. The most frequent reason for parents for not participating in the follow-up study was their consideration that the child was too young to be subjected to (invasive) medical research. Mean age of the ODM group at time of measurements was 6.6 years (range 6.2-7.3) and at time of blood sampling 7.4 years (range 6.5-8.5).

For the control group we invited randomly selected offspring of non-diabetic women without severe maternal disease during pregnancy, who were born at the University Obstetric Center, Utrecht, The Netherlands, in the same period as the ODM. Seventy-nine children participated in the home visit and 67 of them agreed to additional blood sampling. Mean age of the controls at time of measurements was 6.8 years (range 6.4-7.2) and at time of blood sampling 7.4 years (range 6.8-8.7).

Measurements

Blood pressure was measured three times on the right arm in sitting position after five minutes of rest with a two minute interval period, using an automated oscillometric device (DINAMAP®, Critikon, Tampa, FL). The average of the last two measurements of systolic (SBP) and diastolic (DBP) blood pressure were used for analysis. All measurements were performed by one investigator (M.R.) to exclude inter-observer variability. A fasting venous blood sample was taken on a separate occasion after the home visit to determine glucose, insulin, glycated hemoglobin (HbA1c), total cholesterol, high- and low-density lipoprotein cholesterol (HDL, LDL), triglycerides and high sensitivity C-reactive protein (hsCRP). The homeostasis model assessment (HOMA) formula was used to estimate insulin resistance (HOMA-IR) and beta cell function (HOMA-B) from fasting glucose and insulin levels.[6] All blood samples were analyzed at the same research laboratory.

The investigator was unaware of specific characteristics of the pregnancy and neonatal outcome, and information from the study group regarding pregnancy outcome, maternal characteristics and diabetes treatment during pregnancy was subsequently obtained from our previous study on pregnancy outcome.[4] Information concerning maternal characteristics and pregnancy outcome from the control group was obtained from hospital records and from additional questionnaires. This study was approved by the Medical Ethics Committee of the University Medical Center Utrecht, The Netherlands. All parents gave written informed consent.

Definitions

Preterm birth was defined as birth before 37 weeks of gestation. Maternal glycemic control during first, second and third trimester of pregnancy was divided into three categories, based on our previous study on pregnancy outcome:[4] mean HbA1c ≤6.0% (i.e. within the normal range, 'optimal'), mean HbA1c 6.1-7.0% (i.e. 2-4 SD from normal, 'good') and mean HbA1c >7.0% ('non-optimal'). Neonatal macrosomia was defined as birth weight >90th percentile for gestational age, sex and parity according to the Netherlands Perinatal Registry data.[7]

We defined components of the metabolic syndrome (MetS) in line with Boney et al.,[8] based on modifications of the adult criteria from the National Cholesterol Education Program. Childhood overweight was defined according to the International Obesity Task Force cut-off points for BMI-for-age[9] which are incorporated in the Dutch reference BMI growth diagrams.[10] Hypertension was defined as SBP or DBP >95th percentile for age, sex and height-percentile according to recent data from the USA[11] (for accurate usage of these data, we defined height-percentiles according to the U.S. Centers for Disease Control and Prevention growth charts[12]). Impaired fasting glucose (IFG) was defined as glucose >6.1 mmol/l.[13] Low HDL and high triglycerides level were determined as <5th and >95th percentile for age and sex, respectively.[14] Additionally, we determined high cholesterol and high LDL level as >95th percentile for age and sex.[14]

Statistical methods

General characteristics were compared between groups using independent *t* test for normally distributed variables, Mann-Whitney *U* test for not normally distributed variables and χ^2 test (or Fisher's exact test if appropriate) for categorical variables. Differences in measurements between ODM and controls and between subgroups of ODM were analyzed using analysis of variance with post-hoc Bonferroni correction for multiple comparisons if necessary. Means were adjusted for sex and age at measurement. If data were not normally distributed, they were log-transformed prior to statistical analysis. Prevalence of components of MetS was compared between groups using χ^2 test (or Fisher's exact test as appropriate). Data were analyzed using SPSS 15.0 for Windows (SPSS, Chicago, IL). A *p*-value <0.05 was considered to be statistically significant.

RESULTS

General characteristics

In mothers of participating ODM, mean age at delivery was higher and prevalence of pre-eclampsia was lower compared with mothers of non-participating ODM (mean±SD 31.1±3.5 vs. 30.1±4.0 years for age at delivery [*p*=0.022] and 10.8 vs. 19.0% for preeclampsia [*p*=0.047]). Other characteristics did not significantly differ between the participating and

non-participating ODM group (data not shown). Two boys in the participating ODM group and one girl in the non-participating ODM group had developed type 1 diabetes (the two boys did not participate in blood sampling). In the participating ODM group mean maternal age at delivery and gestational age were significantly lower compared with the control group (Table 1). As expected, prevalences of cesarean section and neonatal macrosomia were higher in the ODM group compared with the control group.

Table 1. General characteristics in participating ODM and controls.

	Controls	**ODM**	*p*
n	79	213	
Maternal characteristics			
Race: Caucasian	78 (98.7)	209 (98.1)	1.0
Age at delivery (years)	33.8±4.1	31.1±3.5	<0.01
Parity (multiparity)	46 (58.2)	99 (46.7)	0.08
Duration of diabetes (years)	-	12 [5-18]	-
Pregnancy characteristics			
Mean pregnancy HbA1c (%)	-	6.21±0.95	-
Preeclampsia	7 (8.9)	23 (10.8)	0.6
Gestational age (days)	278 [270-284]	264 [255-269]	<0.01
Preterm birth	2 (2.5)	66 (31)	<0.01
Cesarean section	22 (27.8)	94 (44.3)	0.01
Neonatal characteristics			
Sex (male)	40 (50.6)	98 (46.2)	0.5
Birth weight (grams)	3736±601	3555±735	0.063
Macrosomia	30 (38.0)	110 (51.9)	0.035
Congenital malformations	3 (3.8)	10 (4.7)	1.0

Data represent adjusted mean±SE, median with interquartile range or number with percentage.

Measurements

Parameters of fasting glucose regulation and lipid metabolism, hsCRP, and the prevalence of components of MetS did not significantly differ between ODM and controls (Table 2, Figure 1a). Mean SBP was slightly higher in ODM compared with controls. Additional adjustment for BMI and gestational age did not change the significance of the difference in mean SBP (adjusted p=0.018).

ODM who were born macrosomic had a significantly higher BMI and showed more childhood overweight compared with appropriate-for-dates ODM (Table 3, Figure 1b). All other measurements did not significantly differ between macrosomic ODM and appropriate-for-dates ODM. There were no significant differences in outcome in the ODM group regarding preterm birth (Table 3).

Mean HbA1c level in children of mothers with a non-optimal glycemic control during first trimester of pregnancy (mean HbA1c >7.0%) was significantly higher compared with children of mothers with better glycemic control (Table 4). Other measurements and the prevalence of components of MetS did not significantly differ regarding maternal glycemic control during

Table 2. Cardiovascular and metabolic parameters in ODM and controls.

	Controls	ODM	*p*
n	79	213	
BMI (kg/m^2)	15.4 [14.9-16.8]	15.9 [14.9-17.2]	0.1
Overweight	10 (12.8)	40 (19.0)	0.2
SBP (mmHg)	96.5±0.9	100.4±0.6	<0.01
SBP >p95	1 (1.3)	9 (4.4)	0.3
DBP (mmHg)	58.1±0.6	58.8±0.4	0.3
DBP >p95	0 (0)	0 (0)	-
Glucose (mmol/l)	4.51±0.04	4.41±0.03	0.07
IFG	0 (0)	0 (0)	-
Insulin (mIU/L)	5.11 [3.83-7.48]	5.11 [3.83-6.39]	0.2
HbA1c (%)	5.14±0.05	5.07±0.03	0.2
HOMA-IR	1.04 [0.78-1.44]	0.96 [0.70-1.38]	0.1
HOMA-B (%)	103 [79-141]	112 [77-150]	0.5
Chol (mmol/l)	4.24±0.08	4.05±0.06	0.06
Chol >p95	8 (12.1)	19 (13.1)	1.0
HDL (mmol/l)	1.34±0.03	1.31±0.02	0.5
HDL <p5	2 (3.0)	7 (4.8)	0.7
LDL (mmol/l)	2.59±0.07	2.46±0.05	0.1
LDL >p95	6 (9.1)	15 (10.3)	0.8
TG (mmol/l)	0.58 [0.46-0.78]	0.57 [0.46-0.74]	0.4
TG >p95	5 (7.7)	10 (7.2)	1.0
HsCRP (mg/l)	<0.10 [<0.10-0.30]	<0.10 [<0.10-0.42]	0.1
≥1 components MetS	16 (20.5)	59 (28.0)	0.2
≥2 components MetS	2 (2.6)	7 (3.3)	1.0
≥3 components MetS	0 (0)	0 (0)	-

Data represent adjusted mean±SE, median with interquartile range (if data were log-transformed prior to analysis) or number with percentage (of the total number of children per measure). IFG: impaired fasting glucose; TG: triglycerides.

first, second or third trimester of pregnancy (Table 4; data on second and third trimester are not shown, all *p*-values >0.1).

DISCUSSION

The results of this nationwide follow-up study in offspring of women with type 1 diabetes showed that measures of fasting glucose regulation and lipid metabolism and prevalence of components of MetS at 6-8 years of age were comparable to those in a control group of children of the same age, but that SBP was slightly higher in ODM. Despite an increased prevalence of childhood overweight, ODM who were macrosomic at birth showed no increase in adverse cardiovascular or metabolic outcome parameters compared with appropriate-for-dates ODM. Non-optimal glycemic control during early pregnancy was related to a higher HbA1c level in the offspring. The level of glycemic control during second and third trimester

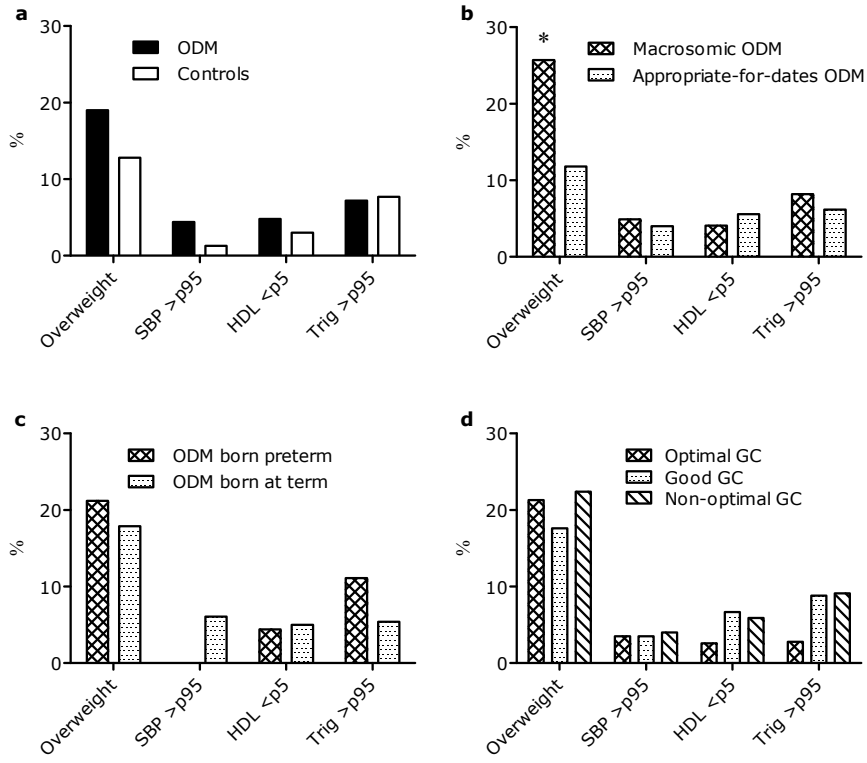

Figure 1. Prevalence of components of metabolic syndrome in (a) ODM vs. controls, (b) macrosomic ODM vs. appropriate-for-dates ODM, (c) preterm ODM vs. ODM born at term, and (d) ODM regarding different levels of maternal glycemic control (GC) during first trimester of pregnancy (graphs regarding second and third trimester were comparable). Diastolic blood pressure >p95 and impaired fasting glucose did not occur and are therefore not shown. * $p<0.05$. SBP: systolic blood pressure; Trig: triglycerides.

of pregnancy and occurrence of preterm birth were not related to cardiovascular or metabolic outcome in ODM at this age.

There is a seemingly discrepancy between these favorable results and the adverse short term perinatal outcome in ODM as was described in our previous study on direct pregnancy outcome.[4] We hypothesize that the adverse short term neonatal outcome is a direct effect of the fetal hyperglycemia and hyperinsulinemia, and therefore may only be improved by very strict maternal glycemic control during pregnancy. On the other hand, long term effects on outcome in the offspring may be mediated by a different underlying mechanism, such as programming of neuroendocrine systems regulating body weight and metabolism.[15] It may be that good prepregnancy care and near-optimal glycemic control during pregnancy may not be good enough to prevent short term (neonatal) complications, but may be good enough

Table 3. Cardiovascular and metabolic parameters in ODM regarding neonatal macrosomia (birth weight >p90) and preterm birth (gestational age <37 weeks).

	BW >p90	**BW ≤p90**	**GA <37 wk**	**GA ≥37 wk**
n	110	113	66	147
BMI (kg/m^2)	**16.4 [15.4-17.7]**	**15.4 [14.5-16.6]**	15.7 [15.2-17.1]	15.9 [15.2-17.0]
Overweight	**28 (25.7)**	**12 (11.8)**	14 (21.2)	26 (17.9)
SBP (mmHg)	99.7±0.8	100.8±0.8	100.2±1.1	100.2±0.7
SBP >p95	5 (4.5)	4 (3.9)	0 (0)	9 (6.1)
DBP (mmHg)	57.8±0.5	59.6±0.5	58.4±0.7	58.8±0.4
DBP >p95	0 (0)	0 (0)	0 (0)	0 (0)
Glucose (mmol/l)	4.37±0.05	4.44±0.05	4.40±0.06	4.41±0.04
IFG	0 (0)	0 (0)	0 (0)	0 (0)
Insulin (mIU/L)	5.11 [3.83-6.39]	5.11 [3.83-6.39]	5.11 [3.32-6.39]	5.11 [3.83-7.67]
HbA1c (%)	5.02±0.05	5.11±0.05	5.01±0.06	5.10±0.04
HOMA-IR	0.96 [0.70-1.39]	0.96 [0.70-1.36]	0.95 [0.65-1.23]	0.96 [0.73-1.42]
HOMA-B (%)	111 [78-155]	114 [77-147]	94.0 [71.0-134.6]	114.9 [80.5-150.4]
Chol (mmol/l)	4.04±0.08	4.06±0.08	4.06±0.10	4.06±0.07
Chol >p95	9 (12.2)	10 (14.1)	4 (8.9)	15 (15.0)
HDL (mmol/l)	1.30±0.03	1.32±0.03	1.29±0.04	1.32±0.03
HDL <p5	3 (4.1)	4 (5.6)	2 (4.4)	5 (5.0)
LDL (mmol/l)	2.45±0.07	2.46±0.07	2.48±0.09	2.45±0.06
LDL >p95	8 (10.8)	7 (9.9)	4 (8.9)	11 (11)
TG (mmol/l)	0.57 [0.45-0.77]	0.58 [0.48-0.73]	0.54 [0.41-0.86]	0.58 [0.48-0.68]
TG >p95	6 (8.2)	4 (6.2)	5 (11.1)	5 (5.4)
HsCRP (mg/l)	0.17 [<0.10-0.55]	<0.10 [<0.10-0.40]	<0.10 [<0.10-0.55]	<0.10 [<0.10-0.42]
≥1 components MetS	**37 (33.6)**	**22 (21.4)**	20 (30.3)	39 (26.5)
2 components MetS	5 (4.5)	2 (1.9)	1 (1.5)	6 (4.1)

Data represent adjusted mean±SE, median with interquartile range (if data were log-transformed prior to analysis) or number with percentage (of the total children per measure). Significant differences are presented in bold. BW: birth weight; GA: gestational age; IFG: impaired fasting glucose; TG: triglycerides.

to prevent the increased risk of obesity, diabetes and its consecutive risks later in life. Further follow-up will show if this assumption was right or not.

Although the definition of MetS in pediatric medicine is controversial,[16,17] we selected components of MetS which were based on adult criteria in order to assess possible clinical signs of cardiovascular and metabolic disturbances (overweight, hypertension, impaired fasting glucose, dyslipidemia) at this young age. Recently, we described that the prevalence of overweight in our cohort of 6-8 year old ODM was comparable to that in the Dutch reference population.[5] In this study we found that the prevalence of hypertension, impaired fasting glucose and dyslipidemia were also comparable to those in a control group and that only 3% of ODM had more than two components of MetS.

Before cardiovascular and metabolic disturbances such as impaired fasting glucose, dyslipidemia or hypertension become clinically evident, it is likely that they are preceded by

Table 4. Cardiovascular and metabolic parameters in ODM according to level of maternal glycemic control during first trimester of pregnancy.

	HbA1c ≤6.0%	HbA1c 6.1-7.0%	HbA1c >7.0%
Maternal HbA1c			
1st trim. (n / mean±SD)	57 (5.44±0.48)	85 (6.44±0.27)	49 (7.76±0.90)
2nd trim. (n / mean±SD)	97 (5.20±0.65)	76 (6.46±0.28)	16 (7.62±0.68)
3rd trim. (n / mean±SD)	68 (5.25±0.69)	79 (6.45±0.30)	32 (7.66±1.08)
BMI (kg/m^2)	16.1 [14.8-17.0]	15.6 [14.9-17.2]	16.0 [15.0-17.6]
SBP (mmHg)	99.1±1.1	99.8±0.9	101.5±1.1
DBP (mmHg)	57.8±0.7	58.3±0.6	59.3±0.8
Glucose (mmol/l)	4.46±0.07	4.36±0.05	4.44±0.07
Insulin (mIU/l)	5.11 [3.83-6.39]	4.28 [3.32-6.39]	5.11 [3.83-7.03]
HbA1c (%)	4.98±0.06	5.03±0.05	5.27±0.06 *
HOMA-IR	0.94 [0.73-1.39]	0.89 [0.65-1.23]	1.00 [0.77-1.44]
HOMA-B (%)	93.9 [65.6-153.1]	111.2 [78.3-144.1]	126.0 [89.4-156.2]
Chol (mmol/l)	3.99±0.11	4.17±0.08	3.91±0.11
HDL (mmol/l)	1.32±0.05	1.33±0.04	1.31±0.05
LDL (mmol/l)	2.41±0.09	2.56±0.07	2.30±0.1
TG (mmol/l)	0.56 [0.45-0.68]	0.57 [0.46-0.70]	0.56 [0.47-0.87]
HsCRP (mg/L)	<0.10 [<0.10-0.43]	<0.10 [<0.10-0.37]	<0.10 [<0.10-0.78]

Data represent adjusted mean±SE or median with interquartile range (if data were log-transformed prior to analysis) and regard glycemic control during first trimester (all comparisons between subgroups regarding the second and third trimester were not significant). * p<0.01 (after Bonferroni correction) compared with the HbA1c 6.1-7.0% and HbA1c ≤6.0% subgroups. Trim.: trimester; TG: triglycerides.

subclinical changes in glucose regulation, lipid metabolism or blood pressure. In our cohort of ODM at school age, we did not find differences in parameters of glucose regulation or lipid metabolism. This is in contrast to several other (smaller) studies in offspring who were in utero exposed to maternal diabetes, which showed changes in glucose regulation[18-21] or lipid metabolism[19,20,22,23] already at school age. Although it is reassuring that we did not find differences in parameters of fasting glucose regulation, it would be of additional value to perform a glucose tolerance test at a later age to evaluate the response after a glucose load.

Some previous studies in children of women with pregestational and/or gestational diabetes showed a higher blood pressure in the offspring at young age,[20,23,24] but others did not.[22,25,26] We found that mean SBP was slightly higher in ODM than in controls and additional adjustment for gestational age and BMI did not change this result. However, the prevalence of hypertension did not significantly differ between ODM and controls. As mean SBP of the ODM group was within the normal range according to European reference data,[27] and these results are based on blood pressure recordings performed on only one occasion, these results should be substantiated in future studies.

Although previous studies indicated that preterm born children may show altered body composition, hypertension and decreased insulin sensitivity later in life,[28,29] we did not find this in our study. Likewise, data from previous studies have shown that macrosomic offspring

of women with diabetes are at increased risk for developing overweight and other cardiovascular diseases.[8,18,21,30,31] In our study, however, parameters of glucose regulation, lipid metabolism and blood pressure in ODM who were macrosomic at birth were comparable to those who were born with a birth weight appropriate for gestational age, despite a higher prevalence of overweight in macrosomic ODM. Boney et al.[8] have previously shown in offspring of gestational diabetic women that the effect of a high birth weight on the development of components of MetS did not became evident until after the age of seven, so clinical cardiovascular and metabolic disturbances may be a later phenomenon in this population, and the same may account for those who were born preterm.

Extrapolating Freinkel's theory on fuel-mediated teratogenesis,[1] better outcome may be expected in offspring of diabetic women who had achieved better glycemic control during pregnancy. Indeed, we found a lower mean HbA1c level in the offspring of women who had achieved good glycemic control during early pregnancy. Furthermore, we consider the lack of clinical cardiovascular and metabolic disturbances in the ODM group promising, and we suggest that the overall favorable outcome in ODM may be the result of the near-optimal mean maternal glycemic control during pregnancy. However, we have to consider that the lack of finding significant differences may be due to a lack of statistical power. As we were not able to include all children who were eligible for follow-up, future follow-up studies including more ODM and/or a larger control group should substantiate our findings. Another consideration is that maternal HbA1c may not be an accurate tool for the classification of level of glycemic control during pregnancy. Comparison with continuous glucose monitoring data has shown that HbA1c level does not reflect the complexities of glycemic control in pregnant diabetic women.[32]

As we mentioned before, several studies in offspring of diabetic mothers have shown that intrauterine exposure to maternal diabetes is associated with an increased risk of long term effects such as overweight and impaired glucose tolerance already during young childhood and adolescence. However, comparison with each other or with our results is hampered by the fact that many studies a) considered offspring of mixed cohorts of women with pregestational type 1 and type 2 diabetes and/or gestational diabetes, b) lacked information regarding maternal glycemic control during pregnancy or comparison with a control group of offspring of non-diabetic women, and/or c) considered small study groups, offspring within a large age range or offspring who were born more than 20 years ago, when prepregnancy care and glycemic control during pregnancy were far less optimal. This follow-up study should be a valuable addition to the previous literature, as it provides important developmental data of offspring at school age from a large, well defined, well prepared and well controlled cohort of Caucasian women with type 1 diabetes. Furthermore, we investigated the children within a small age range and compared their outcome with that of a control group of children who were in the same age range of mothers without diabetes. The study group described in this study was a representative sample of the total cohort of ODM that was eligible for follow-up

at the start of the study, as maternal and neonatal characteristics in the participating group were overall comparable to those in the non-participating group. A limitation of this study was the fact that the control group was not as large as the study group. The control group may, therefore, not reliably represent the normal population. However, we consider the control group of great value to our study because of the lack of recent age-specific reference data for many of the measurements.

In conclusion, our findings indicate that with current (prepregnancy) care and treatment in type 1 diabetic pregnancies, cardiovascular and metabolic disturbances in the offspring at 6-8 years of age do not occur more often compared with a control group of children of non-diabetic mothers. The influence of common complications of type 1 diabetic pregnancies (suboptimal maternal glycemic control, preterm birth and neonatal macrosomia) was limited. Neonatal macrosomia was only related to a higher prevalence of childhood overweight, and non-optimal maternal glycemic control during early pregnancy was related to a slightly higher HbA1c level in the offspring. We suggest that the favorable long term outcome in ODM is the result of good prepregnancy care and an overall adequate (near-optimal) maternal glycemic control during pregnancy. Clinicians who are involved in the care and/or treatment of (pre-) pregnant women with type 1 diabetes may use these results in their counseling regarding glycemic control and pediatric outcome. Further follow-up of our cohort of ODM and comparison with a larger control group should substantiate our results, and should show whether blood pressure in these children will continue to develop within the normal range, or that the slightly higher SBP in ODM may nonetheless be indicative of early maladaptive programming of renal or vascular mechanisms as has been suggested by others.[2]

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CHAPTER

4

HPA-axis and immune function in 6-8 year old offspring of women with type 1 diabetes

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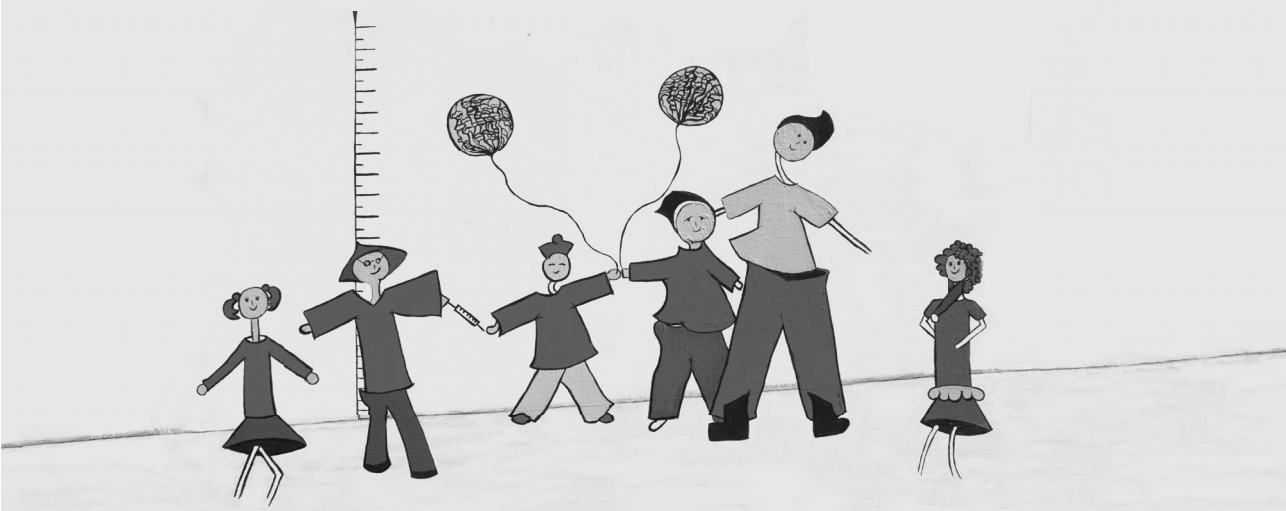

ABSTRACT

Introduction

Intrauterine exposure to maternal type 1 diabetes induces long term cardiometabolic disorders in the offspring, but the underlying mechanisms are not fully understood. Programming of the hypothalamo-pituitary-adrenal (HPA) axis or the immune system may be involved. In this study, we investigated immune and HPA-axis function in children of type 1 diabetic women and the influence of neonatal macrosomia and level of maternal glycemic control during pregnancy.

Methods

In vitro mitogen-induced cytokine production, plasma level of high sensitivity CRP (hsCRP) and basal as well as dexamethasone-suppressed salivary cortisol parameters were measured in offspring of type 1 diabetic mothers (ODM; *n*=155) and 67 control children at 6-8 years of age.

Results

ODM showed higher pro-inflammatory TNF-α and IL-8 production, but lower IP-10 and anti-inflammatory IL-5 production compared with controls. HsCRP level and HPA-axis function in ODM were comparable to those in controls. Neonatal macrosomia and level of maternal glycemic control during pregnancy had no influence on HPA-axis or immune function, except for lower stimulated TNF-α and IL-1β production in children of mothers with non-optimal glycemic control during third trimester.

Conclusions

Our results of a higher mitogen-induced pro-inflammatory cytokine response in addition to a lower anti-inflammatory cytokine response may indicate the first signs of a pro-inflammatory phenotype in ODM at school age. We suggest that this may be the result of epigenetic programming of the immune system during diabetic pregnancy, and that this change in inflammatory activity may contribute to the susceptibility to later cardiometabolic disease. Neonatal macrosomia was not related to HPA-axis or immune function at school age, but level of maternal glycemic control during pregnancy may have consequences for later immune function. Longer follow-up should show whether our findings of a higher mitogen-induced pro-inflammatory response and lower IL-5 and IP-10 production in ODM may be of clinical relevance.

INTRODUCTION

In utero exposure to maternal type 1 diabetes induces long term cardiovascular and metabolic disorders in the offspring, including obesity, hypertension and type 2 diabetes.[1] The mechanisms by which in utero exposure to diabetes increases the risk for these cardiometabolic disorders are not fully understood.

One of the proposed mechanisms is 'fuel-mediated teratogenesis', which describes an adverse direct effect of excess maternal (and thereby fetal) glucose on fetal organ development.[2] Another (or complementary) suggested mechanism is epigenetic 'programming' of neuroendocrine systems regulating body weight and metabolism.[3] The hypothalamo-pituitary-adrenal (HPA) axis is a candidate neuroendocrine system, as it has been shown to be susceptible to programming during fetal development in studies regarding prenatal stress, antenatal glucocorticoids and reduced fetal growth.[4] However, studies regarding HPA-axis function in offspring of diabetic women have not yet been performed.

There is also increasing evidence that cardiovascular and metabolic disorders are associated with and preceded by a mild chronic inflammatory state, even already at young age, demonstrating the interaction between the metabolic and immune system.[5,6] Increased inflammatory markers (C-reactive protein (CRP), intracellular adhesion molecule (ICAM)-1) have been found in umbilical cord blood of newborns of mothers with type 1 diabetes,[7,8] but little is known about inflammatory activity at later age. Moreover, there is a bidirectional interaction between the HPA-axis and the immune system, and a dysfunction in this communication could also lead to an altered modulation of the inflammatory response.[9]

In this study we investigated immune and HPA-axis function in 155 children at 6-8 years of age from a nationwide Dutch cohort of type 1 diabetic women and in a control group of 67 children of non-diabetic women. In addition, we investigated the possible influence of neonatal macrosomia (birth weight >p90) and suboptimal maternal glycemic control during pregnancy on immune response and HPA-axis function.

METHODS

Participants

The study group consisted of 6-8 year old offspring of type 1 diabetic mothers (ODM) who participated in a Dutch nationwide study on type 1 diabetes and pregnancy outcome.[10] This study showed that prepregnancy care in these women was good (84% planned pregnancies and 70% preconceptional folic acid supplementation), and that adequate maternal glycemic control was achieved during pregnancy (mean HbA1c level 6.2%). From 313 children eligible for follow-up, 17 were lost to follow-up, 213 children agreed to participate in saliva sampling for salivary cortisol assessment, and from them 155 agreed to blood sampling. The most

frequent reason for not participating was that parents considered their children too young for (invasive) medical research. The control group consisted of randomly selected offspring of non-diabetic women without severe maternal disease during pregnancy, born in the same period as the ODM at the University Obstetric Center, Utrecht, The Netherlands. From the 79 children who participated in saliva sampling, 67 agreed to blood sampling.

Procedures

This study was approved by the Medical Ethics Committee of the University Medical Center Utrecht, The Netherlands. All parents gave written informed consent. During a home visit the saliva sampling procedure was explained to child and parent(s). A fasting blood sample was taken on a separate occasion. General characteristics were obtained from our previous study[10] for the ODM group, and from hospital records and additional questionnaires for the control group.

Saliva sampling / HPA-axis function parameters

Saliva samples from 132 ODM and 60 controls were obtained. Saliva sampling using cotton wool swabs (Salivette®; Sarstedt, Etten-Leur, The Netherlands) occurred immediately after awaking (T0), 15 and 30 minutes thereafter (T15 and T30) and at bedtime, followed by oral administration of 0.25 mg dexamethasone. On the next morning, samples were taken again at the same time points. Children were instructed to stay in bed and not to eat or drink during the period of sampling. Frozen saliva samples were returned by mail. The most frequent reasons for not returning saliva samples were a lack of time and rejection by the child. Saliva samples were analyzed using luminescence immunoassay.[11]

We analyzed basal morning cortisol level, cortisol awakening response (CAR) during the first 30 minutes after awakening with area under the curve with respect to ground (AUC_G, reflecting total cortisol secretion) and area under the curve with respect to increase (AUC_I, indicating the increase of cortisol production),[12] dexamethasone-suppressed cortisol level and cortisol suppression ratio (cortisol at T0 on day 1 divided by cortisol at T0 on day 2). We excluded cortisol data from 29 children who had been awake for more than ten minutes before sampling and from two children because their cortisol levels were extremely high (>13 SD).

Immune response

High sensitivity CRP (hsCRP) was measured in plasma. Circulating numbers of peripheral blood cells were assessed in whole blood using dual color fluorescence analysis. Absolute numbers of cells were calculated based on a total leukocyte count. T-cell proliferation and cytokine production were measured in whole blood diluted with antibiotics (1:10 RPMI-1641 (Gibco, Grand Island, NY), 100 U/ml penicillin, 100 mg/ml streptomycin and 2 mM L-glutamine). T-cell mitogen-induced proliferation and cytokine production were measured in supernatants of whole blood cultures, after stimulation for 72 hours with anti-CD2/CD28

monoclonal antibodies (Sanquin, Amsterdam, The Netherlands; final concentration anti-CD2.1/anti-CD2.2 0.33 mg/ml and anti-CD28 1.33 mg/ml) at 37 ^{0}C / 5% CO_2 in 96 wells round-bottom plates. Cultures were pulsed after 72 hours with 1 mCI/well [^{3}H]-thymidine (Amersham, Buckinghamshire, UK), and 16-18 hours later [^{3}H]-thymidine incorporation was measured using a liquid scintillation beta-counter. T-cell proliferation data are presented as count per minute (cpm). LPS-induced cytokine production was measured in supernatants of whole blood cultures after stimulation for 24 hours with lipopolysaccharide (LPS, Escherichia Coli 0127:B8, Sigma, final concentration 2 ng/ml) at 37 ^{0}C / 5% CO_2 in 96 wells flat-bottom plates. Supernatants were stored at -80 ^{0}C and cytokine levels were measured using a multiplex bead-based assay or ELISA as described earlier.[13,14] For cytokine levels above the upper detection limit level we imputed the upper detection limit, for cytokine levels below the lower detection limit we imputed the lower detection limit divided by $\sqrt{2}$.[15]

Blood sampling failed in ten children. Four children who reported to have (had) fever on the day of blood sampling or the days before were excluded from analysis, as well as four children with hsCRP $\geq$10 mg/l because of the probability of underlying clinical infection.

Definitions

Neonatal macrosomia was defined as birth weight >90th percentile for gestational age, sex and parity.[16] Maternal glycemic control during pregnancy was divided into three categories: mean HbA1c $\leq$6.0% (i.e. within the normal range, 'optimal'), mean HbA1c 6.1-7.0% (i.e. 2-4 SD from normal, 'good') and mean HbA1c >7.0% ('non-optimal'). Atopia was defined as having asthma, eczema and/or allergy.

Statistical analysis

General characteristics were compared between groups using independent *t* test for normally distributed variables, Mann-Whitney *U* test for not normally distributed variables or χ^2 test for categorical variables. Group differences regarding cortisol measures, peripheral blood cell population and cytokine production were analyzed using analysis of covariance (ANCOVA) with post-hoc Bonferroni correction for multiple comparisons if necessary. Mean cortisol measures were adjusted for age, sex and sampling day (week- or weekend day), means of peripheral blood cell population and cytokine production were adjusted for age, sex and season of blood sampling. Differences in CAR were analyzed using repeated measures analysis with time after awakening as within-factor, group, sex and sampling day (week- or weekend day) as between-factors and age as covariate. Group differences in cytokine response were additionally analyzed using multivariate ANCOVA with LPS-induced pro-inflammatory cytokines, T-cell mitogen induced anti-inflammatory cytokines or chemokines (see Table 3) as dependent variables, group, sex and season of blood sampling as between-subjects factors and age as covariate, with additional discriminant analysis to evaluate which of the dependent variables mainly discriminated the groups. Data were square root- or log-transformed prior

to analysis if necessary to yield normal distributions. Data were analyzed using SPSS 15.0 for Windows (SPSS, Chicago, IL). A *p*-value <0.05 was considered to be statistically significant.

RESULTS

Maternal/neonatal characteristics

Maternal and neonatal characteristics of the participating ODM group did not significantly differ from those in the non-participating group (data not shown). General characteristics of participating ODM and controls are presented in Table 1. Mean maternal age at delivery and mean gestational age were significantly lower in the ODM group compared with the control group, and prevalence of neonatal macrosomia was significantly higher in ODM.

HPA-axis function

Basal cortisol parameters and dexamethasone-suppressed cortisol parameters did not significantly differ between ODM and controls (Table 2). ODM and controls showed a significant rise in salivary cortisol level during the first 30 minutes after awakening (data not shown, $p<0.01$ for both groups on both days). There were no significant differences in CAR between ODM and controls ($p=0.3$ for day 1, $p=0.6$ for day 2). Basal and dexamethasone-suppressed

Table 1. Maternal, pregnancy and child characteristics in the participating ODM group and control group.

	Controls	ODM	*p*
n	66	138	
Maternal characteristics			
Race: Caucasian	66 (100)	136 (98.6)	1.0
Age at delivery (years)	33.9±4.0	31.0±3.3	<0.01
Pregnancy characteristics			
Glycemic control 1st trim. (O/G/NO)[a]	-	37 / 58 / 30	-
Glycemic control 2nd trim. (O/G/NO)[a]	-	66 / 51 / 9	-
Glycemic control 3rd trim. (O/G/NO)[a]	-	46 / 52 / 23	-
Mean HbA1c during pregnancy (%)	-	6.18±0.86	-
Gestational age (days)	278 [270-284]	264 [256-269]	<0.01
Child characteristics			
Sex (male)	29 (43.9)	64 (46.4)	0.8
Birth weight (grams)	3729±553	3596±608	0.1
Neonatal macrosomia	24 (36.4)	73 (52.9)	0.03
BMI at school age (kg/m^2)[b]	15.4 [14.9-16.7]	15.9 [15.1-17.1]	0.1

Data represent mean ± standard deviation, median with interquartile range or number with percentage, and are based on children participating in blood sampling (comparisons between ODM and controls who participated in saliva sampling yielded similar results). [a] O/G/NO: number of children of mothers with optimal (O), good (G) or non-optimal (NO) glycemic control during 1st, 2nd and 3rd trimester of pregnancy. [b] Determined at home visit.[26]

Table 2. HPA-axis function parameters in ODM and controls at 6-8 years of age.

	Controls	ODM	*p*
n	60	132	
Age at sampling (years)	7.41±0.38	7.37±0.39	0.4
Day 1			
Number of samples	56	127	
Sampling day (weekend day)	37 (61.6)	70 (53.0)	0.2
Awakening time (hr)	7:27 [6:55-8:00]	7:35 [7:01-8:13]	0.6
Cortisol at T0 (nmol/l)	9.7 [7.0-12.9]	9.0 [6.8-11.6]	0.8
Positive cortisol rise	37 (66.1)	77 (60.6)	0.4
AUC_G	349.4 [284.7-468.0]	323.6 [263.9-412.5]	0.4
AUC_I	83.6 [-9.1-128.8]	36.5 [-17.3-127.1]	0.5
Evening sampling time (hr)	20:15 [19:44-21:15]	20:30 [19:50-21:05]	0.4
Cortisol at evening (nmol/l)	0.63 [0.41-0.88]	0.65 [0.45-0.90]	0.5
Day 2 (after DST)			
Number of samples	48	110	
Sampling day (weekend day)	35 (72.9)	62 (56.4)	0.06
Awakening time (hr)	7:10 [6:54-8:08]	7:35 [6:50-8:15]	0.5
Cortisol at T0 (nmol/l)	1.2 [0.7-4.0]	1.0 [0.5-2.9]	0.3
Cortisol suppression rate	6.9 [2.8-15.3]	8.6 [2.6-19.3]	0.4
Cortisol suppression rate >1	57 (93.7)	128 (97)	0.5

Data represent mean ± standard deviation, median with interquartile range or number with percentage. CAR: cortisol awakening response; AUC_G: area under the curve with respect to ground; AUC_I: area under the curve with respect to increase; DST: dexamethasone suppression test.

cortisol parameters in ODM were not significantly influenced by neonatal macrosomia or level of maternal glycemic control during first, second or third trimester of pregnancy.

Inflammatory markers / immune response

Mean plasma level of hsCRP did not significantly differ between ODM and controls (Table 3). To investigate immune function, we measured adaptive immune function parameters (T-cell mitogen-induced proliferation and cytokine production) as well as innate immune function parameters (LPS-induced cytokine production). T-cell mitogen-induced cell proliferation did not significantly differ between ODM and controls (median with interquartile range 27310 [15908-40494] cpm in ODM and 23262 [16015-37809] cpm in controls, p=0.8).

LPS-induced pro-inflammatory TNF-α and IL-8 production were significantly higher in ODM compared with controls (Table 3). Multivariate analysis also showed a trend towards a higher LPS-induced pro-inflammatory response in ODM (p=0.07) with additional discriminant analysis confirming the main contribution of these cytokines. T-cell mitogen-induced IL-5 and IP-10 production were significantly lower in ODM compared with controls, and there was a trend towards a lower IL-4 production in ODM (Table 3). Additional multivariate analysis confirmed a significant lower T-cell mitogen-induced chemokine response in ODM (p=0.03, mainly due to the lower IP-10 production), but the difference in anti-inflammatory cytokine response was not significant (p=0.18).

Table 3. Plasma hsCRP, composition of peripheral blood cell population and stimulated cytokine production in ODM and controls.

	Controls	ODM	*p*
n	66	138	
Age at sampling (years)	6.93±0.37	6.83±0.40	0.1
Plasma			
HsCRP (mg/l)	0.00 [0.00-0.32]	0.17 [0.00-0.52]	0.1
Blood cell population			
Leukocytes	5.9 [5.1-6.9]	5.6 [4.9-6.9]	0.6
Granulocytes	3.4 [2.7-4.1]	3.1 [2.5-4.0]	0.7
Lymphocytes	2.4 [1.9-2.7]	2.3 [1.9-2.7]	0.5
Monocytes	0.23 [0.15-0.28]	0.25 [0.18-0.29]	0.2
T-cells	1.7 [1.4-2.0]	1.6 [1.3-1.9]	0.3
$CD4^+$ T-cells	0.91 [0.76-1.09]	0.84 [0.71-1.07]	0.1
$CD8^+$ T-cells	0.49 [0.39-0.57]	0.48 [0.39-0.59]	0.8
B-cells	0.30 [0.24-0.40]	0.29 [0.23-0.37]	0.6
NK-cells	0.23 [0.17-0.36]	0.25 [0.16-0.35]	0.8
Cytokine production[a]			
LPS-induced			
***p* TNF-α**	**1145 [876-1491]**	**1420 [966-2029]**	**0.029**
p IL-1α	29.5 [19.60-42.8]	29.5 [19.6-48.3]	0.9
p IL-1β	360.3 [253.9-464.3]	364.4 [227.5-554.3]	0.5
p IL-6	2305 [1725-2968]	2481 [1674-3347]	0.3
***p* IL-8**	**1433 [1274-1618]**	**1570 [1347-1854]**	**0.046**
a IL-10	17.4 [8.4-24.9]	17.1 [8.2-30.4]	0.8
T-cell mitogen induced			
p TNF-α	474.2 [328.5-766.9]	432.2 [266.7-729.7]	0.7
p IFN-γ	3347 [1124-8800]	3060 [853-8124]	0.9
p IL-2	370.0 [122.9-834.5]	237.2 [117.6-622.8]	0.3
p IL-6	174.8 [88.1-290.8]	137.5 [54.2-252.2]	0.5
a IL-4	97.5 [52.4-170.3]	70.4 [41.3-117.1]	0.069
***a* IL-5**	**786.9 [377.9-1106.5]**	**378.8 [163.1-829.6]**	**0.027**
a IL-10	297.7 [177.7-508.3]	237.7 [126.7-452.3]	0.2
c MCP-1	7206 [4345-11263]	7336 [4185-11296]	0.5
***c* IP-10**	**5089 [2544-8355]**	**3494 [1731-5469]**	**0.043**
c RANTES	3314 [1607-5036]	3284 [1914-5423]	0.3

Data represent mean ± standard deviation or median with interquartile range for transformed data. Significant differences are presented in bold. Levels of peripheral blood cells are presented in 10^9/l, levels of induced cytokine production in pg/ml.

[a] *p*: pro-inflammatory, *a*: anti-inflammatory, *c*: chemokine.

Neonatal macrosomia did not significantly influence mean plasma hsCRP level, peripheral blood cell populations, cell proliferation or cytokine responses. LPS-induced TNF-α and IL-1β production were significantly lower in children of mothers with non-optimal glycemic control during third trimester (mean HbA1c >7.0%) compared with those of mothers with good glycemic control (mean HbA1c 6.0-7.0%; p=0.03 for TNF-α and p=0.04 for IL-1β production (Table 4); p=0.04 for multivariate analysis). Maternal glycemic control during first or second

trimester was not related to cytokine responses in ODM. Plasma hsCRP level, peripheral blood cell population and cell proliferation did not significantly differ between children of mothers with different levels of glycemic control during first, second or third trimester of pregnancy.

Additional correction for reported viral complaints and/or atopia at time of blood sampling did not affect any of the results reported above.

Table 4. LPS-induced TNF-α and IL-1β production in ODM regarding level of maternal glycemic control during third trimester of pregnancy.

	n	**ln TNF-α**	**ln IL-1β**
HbA1c ≤6.0%	46	7.19±0.08	5.87±0.09
HbA1c 6.0-7.0%	52	**7.37±0.07**	**6.01±0.09**
HbA1c >7.0%	23	**7.02±0.11**	**5.63±0.13**

Data represent adjusted log-transformed (ln) means ± standard error. Significant differences (after Bonferroni correction) are presented in bold.

DISCUSSION

To the best of our knowledge we are the first to describe HPA-axis function and cytokine production capacity in offspring at school age of women with type 1 diabetes. Despite the fact that overall maternal glycemic control during pregnancy was adequate, ODM showed higher stimulated pro-inflammatory TNF-α and IL-8 production, but lower IP-10 and anti-inflammatory IL-5 production compared with children of non-diabetic mothers. Mean plasma hsCRP level and HPA-axis function parameters in ODM were comparable to those in controls. Neonatal macrosomia and level of maternal glycemic control during pregnancy had no influence on HPA-axis or immune function, except for lower stimulated TNF-α and IL-1β production in children of mothers with non-optimal glycemic control during third trimester of pregnancy.

Previous studies have shown alterations in the distribution of lymphocyte subsets and/or increased cord blood inflammatory parameters (CRP, IL-6) in newborn offspring of diabetic mothers.[7,8,17-22] In this study we measured the *in vitro* cytokine production capacity after stimulation with a mitogen. We found a significantly higher production of LPS-induced pro-inflammatory cytokines TNF-α and IL-8, and a lower production of T-cell mitogen-induced anti-inflammatory cytokines IL-5 and IL-4 in ODM at 6-8 years of age. In the human immune system, a dynamic balance exists between pro-inflammatory and anti-inflammatory components, and the net effect of these interactions determines the nature of the immune response.[23,24] It may therefore be hypothesized that a higher pro-inflammatory response and a reduced anti-inflammatory response may render ODM susceptible to effects of prolonged inflammation, which in turn may put them at risk for the development of metabolic diseases at later age.[5,25] We recently showed in our cohort of ODM that insulin resistance and prevalence of overweight at school age were not significantly higher compared with controls.[26]

In addition, we showed here that mean plasma hsCRP level was not higher in ODM, and multivariate analyses including other pro- and anti-inflammatory cytokines did not yield significant results. Therefore, we hypothesize that the possible effects of fetal programming of the immune response during a type 1 diabetic pregnancy on cardiometabolic diseases in ODM may not become evident until later age. To substantiate this hypothesis, metabolic, cardiovascular and immune function parameters should be re-evaluated at later age.

T-cell mitogen-induced IP-10 production in ODM was significantly lower than in controls. IP-10 is one of the C-X-C chemokine superfamily members, which are generally involved in promoting acute, neutrophil-driven inflammatory reactions.[27] However, IP-10 is a unique member of the C-X-C family, since it appears to be devoid of effects on neutrophil-function. Although accumulating reports indicate that it is a pleiotropic molecule involved in various processes such as delayed-type hypersensitivity response and even regulation of feeding, its biological functions are still unclear.[27] Therefore, it is difficult to speculate on a possible effect of the lower T-cell mitogen-induced IP-10 production in ODM.

Early programming of the HPA-axis has been suggested to be a pathway by which adverse intrauterine circumstances may predispose the offspring to later metabolic and cardiovascular disease, due to an altered exposure to endogenous glucocorticoids (the end product of HPA-axis activation) throughout life.[4] Salivary cortisol sampling is a well-established non-invasive technique for measuring HPA-axis activity in children.[28] In this study we did not find any differences in basal cortisol or in the response of the HPA-axis during awakening, nor did we observe an alteration in the cortisol levels after dexamethasone-suppression in ODM at 6-8 years of age compared with controls, indicative of a normal HPA-axis function in ODM. However, we did not measure stress-induced HPA-axis activity or additional (possibly more sensitive) parameters of HPA-axis function such as adrenocorticotropic hormone (ACTH). Therefore, we cannot exclude the possibility that minor changes of HPA-axis function may nevertheless be present in ODM at this age. It would be of additional value to evaluate HPA-axis functioning in more detail by investigating the stress-induced HPA-response (including cortisol as well as ACTH levels) under controlled circumstances at later age.

Higher pro-inflammatory cytokines (IFN-γ, IL-2) have previously been found in cord blood of macrosomic newborns of gestational diabetic women, and it was suggested that this might contribute to the development of later cardiovascular morbidity in these infants.[29] We have recently shown that ODM who were macrosomic at birth are at risk for developing childhood overweight,[26] but in this study we did not find alterations in immune response or HPA-axis function in children who were macrosomic at birth to support the former suggestion.

Extrapolating Freinkel's theory of 'fuel-mediated teratogenesis',[2] one might hypothesize that poorer maternal glycemic control during pregnancy may be related to poorer cardio-metabolic outcome and therefore to a more pro-inflammatory phenotype in the offspring. However, we found that mitogen-induced pro-inflammatory TNF-α and IL-1β production was higher in children of mothers with good glycemic control (mean HbA1c 6.0-7.0%) during

third trimester of pregnancy as compared to children of mothers with non-optimal glycemic control (mean HbA1c >7.0%). One might speculate that (the risk of) maternal severe hypoglycemic events when aiming for better glycemic control may cause a form of maternal stress and thereby alter later immune function in the offspring, as prenatal stress has been shown to lead to immune alterations in the offspring.[30] However, it should be mentioned that maternal HbA1c may not be an accurate tool for the classification of level of glycemic control, because it does not reflect the complexities of glycemic control in pregnant diabetic women.[31]

CONCLUSIONS

In conclusion, our findings of a higher stimulated pro-inflammatory cytokine response in addition to a lower stimulated anti-inflammatory cytokine response may indicate the first signs of a pro-inflammatory phenotype in the offspring of women with type 1 diabetes at school age. We suggest that, despite the fact that overall maternal glycemic control during pregnancy was adequate, this may be the result of early epigenetic programming of the immune system during pregnancy, and that this may contribute (at least in part) to the susceptibility to cardiometabolic diseases later in life. There were no signs of altered HPA-axis function in ODM at this age, but a more detailed evaluation under controlled circumstances at a later age should substantiate this. Neonatal macrosomia was not related to HPA-axis or immune function at school age, but the level of maternal glycemic control during pregnancy may have consequences for later immune function. Longer follow-up should show whether this pro-inflammatory phenotype in ODM at school age may develop into metabolic or cardiovascular abnormalities at later age, and if the lower mitogen-induced IP-10 production in ODM may be of any clinical relevance.

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CHAPTER

5

Neurocognitive functioning in 6 and 7 year old offspring of women with type 1 diabetes

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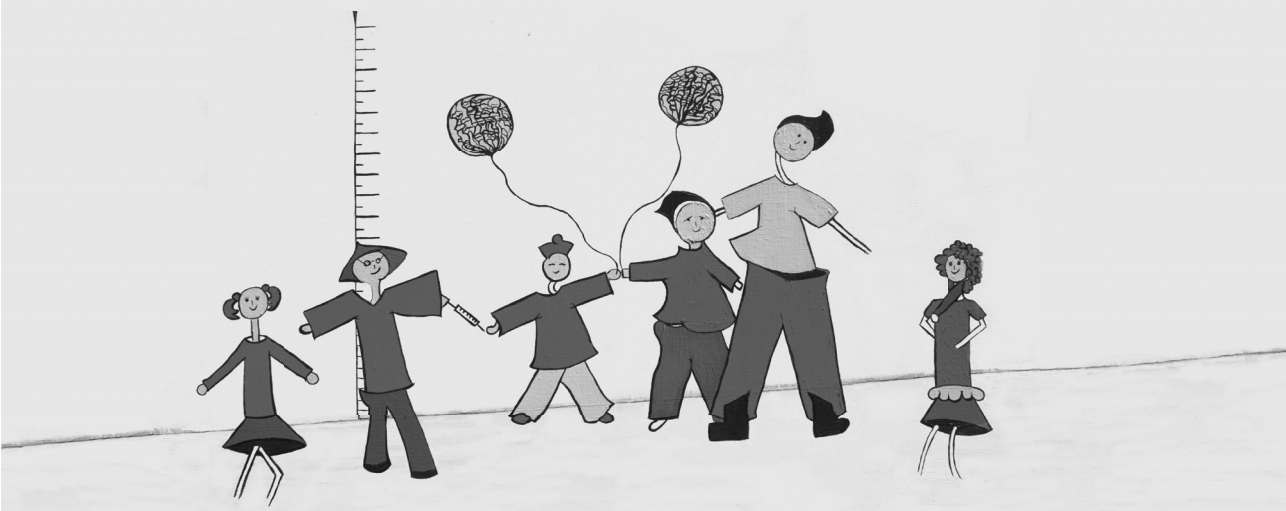

ABSTRACT

Introduction

Previous studies on effects of a type 1 diabetic pregnancy on later neurocognitive development in the offspring are inconclusive. In this study, we investigated neurocognitive functioning in the offspring of a recent nationwide cohort of type 1 diabetic women in relation to maternal glycemic control and hypoglycemia during pregnancy, neonatal hypoglycemia and macrosomia (birth weight >p90).

Methods

Measures of intellectual functioning, visual-motor integration abilities and information processing (response speed, accuracy, response speed variability and working memory) were investigated in 6-7 year old offspring of type 1 diabetic mothers (ODM, n=212) and compared with those in 79 age-matched controls.

Results

Estimated IQ did not significantly differ between ODM and controls, but ODM showed significant poorer performance on working memory, visual-motor integration abilities and several aspects of information processing, mainly due to a lower mean gestational age at birth. Maternal hypoglycemia during pregnancy and neonatal macrosomia were not related to neurocognitive functioning in ODM, but poorer maternal glycemic control and severe neonatal hypoglycemia were related to poorer neurocognitive functioning at 6-7 years of age.

Conclusions

Overall intellectual functioning in 6-7 year old ODM was comparable with that in controls, but ODM showed poorer performance on more subtle domains of neurocognitive functioning, mainly due to a lower mean gestational age at birth. Poorer maternal glycemic control during pregnancy and neonatal hypoglycemia were related to poorer neurocognitive functioning, but maternal hypoglycemia and neonatal macrosomia were not. Future follow-up studies should show whether these findings may be related to learning difficulties in ODM.

INTRODUCTION

In type 1 diabetic pregnancy, high glucose concentrations make the developing fetal tissues more vulnerable to cardiometabolic alterations later in life, such as obesity and type 2 diabetes.[1] Since human brain development is a dynamic process that continues throughout the entire length of pregnancy and into the postnatal period,[2] the developing fetal brain may also be susceptible to adverse long term effects of intrauterine metabolic disturbances or common complications of a type 1 diabetic pregnancy, such as maternal or neonatal hypoglycemia. However, previous studies on long term effects of neonatal hypoglycemia in offspring of diabetic women have shown conflicting results,[3,4] and studies on possible effects of severe maternal hypoglycemia during pregnancy on the development in the offspring are scarce.[5]

Most previous studies concerning neurocognitive outcome in children of diabetic mothers showed normal overall intellectual functioning at school age,[6-8] but lower intelligence scores have also been described.[9] Some studies showed neurocognitive dysfunctions such as lower gross and fine motor abilities,[8] but other studies reported normal neurocognitive functioning.[10] These results are inconclusive, which at least in part is due to methodological differences between studies. Most studies considered offspring of different mixed cohorts of women with pregestational and/or gestational diabetes, and offspring of different ages. Furthermore, in most studies the children were born more than 20 years ago when prepregnancy care and glycemic control during pregnancy were far less optimal.

In this study we investigated possible effects of a type 1 diabetic pregnancy on neurocognitive functioning in 6 and 7 year old offspring from a recent and well defined nationwide cohort of women with type 1 diabetes, and we compared them with control children of non-diabetic women. Additionally, we studied possible influences of maternal and neonatal hypoglycemia, neonatal macrosomia (birth weight >90th percentile) and maternal glycemic control during pregnancy.

METHODS

Study population

The study group consisted of offspring of type 1 diabetic mothers (ODM) who participated in a previous nationwide study on type 1 diabetes and pregnancy outcome in The Netherlands.[11] Details of this cohort have been described elsewhere.[12] From 313 children eligible for follow-up, 17 children were lost to follow-up and 83 children did not participate, resulting in 213 participating children. The most frequent reason for parents for not participating was the consideration that their children were too young to be subjected to medical research. We excluded one boy from analysis because of a cerebral hemorrhage in the neonatal period due to extreme prematurity. Mean age of the ODM was 6.6 years (range 6.2-7.3).

The control group consisted of randomly selected offspring of non-diabetic women without severe maternal disease during pregnancy. These children were born in the same period as the ODM group at the University Obstetric Center in Utrecht, The Netherlands. Seventy-nine children participated in the control group. Mean age of the controls was 6.8 years (range 6.4-7.2).

Neurocognitive measures

Based on previous literature, a broad spectrum of neurocognitive measures was selected to evaluate intellectual functioning, working memory, visual-motor integration abilities and information processing capacities. Details on tests and selected measures are provided in Table 1.

Intellectual functioning. Verbal, performance and total IQ were estimated using a shortened version of the 3rd edition of the Dutch Wechsler Intelligence Scale for Children (WISC-III-NL),[13] including two verbal subtests (Information, Similarities) and two performance subtests (Picture Arrangement, Block Design). Additionally, Digit Span was performed to assess verbal working memory.

Visual-motor integration abilities. These were assessed using the 5th edition of the Beery-Buktenica Developmental Test of Visual-Motor Integration (Beery-VMI).[14]

Information processing. Aspects of information processing were assessed by measures from the Amsterdam Neuropsychological Tasks (ANT) computer test battery, which is designed to evaluate basic processes underlying more complex neurocognitive skills in a standardized and systematic way.[15] From three tasks (Baseline Speed (BS), Sustained Attention Objects-2 keys (SA) and VisuoSpatial Sequencing (VSS)) measures were selected that assess response speed (dependent variables: mean reaction time of BS and SA, mean series completion time of SA), accuracy (percentage missings and false alarms of SA), response speed variability (standard deviation of mean reaction time of BS and SA) and visual working memory (number of identified targets and number of identified targets in the correct order of VSS), see also Table 1 (BS and VSS have previously been described in more detail elsewhere[16]).

Procedure

Because of their young age, children were visited at home to create a comfortable atmosphere. Neuropsychological testing was performed in a quiet room without the direct presence of family members. Task instructions were given prior to each task and a practice session was performed in order to ensure that children had understood the task instructions. All participants were tested by one well-trained investigator (M.R.) to exclude interobserver variability. Scoring of the Beery-VMI and WISC tests occurred afterwards and under supervision of a clinical child neuropsychologist (D.S.). The investigator was unaware of specific characteristics of the pregnancy or neonatal outcome at time of the home visit. General characteristics were obtained from our previous study on pregnancy outcome[11] for the ODM group and from

Table 1. Overview of neurocognitive tasks and selected measures.

Task/domain	Aims of test	Measures
WISC-III-NL		
Information (IN)	Measures general cultural knowledge and acquired facts.	Age-specific norm score
Similarities (SI)	Measures abstract logical thinking and reasoning; concept formation is also required.	Age-specific norm score
Picture Arrangement (PA)	Measures the ability to interpret actions as depicted by pictures, to recognize their sequence in a story, and to arrange them in sequential order.	Age-specific norm score
Block Design (BD)	Measures the ability to analyze an abstract design, and then reproduce the design with colored plastic blocks.	Age-specific norm score
Digit Span (DS)	Numerical digits to be repeated forward or backward; measures verbal working memory and attention.	Age-specific norm score
Beery-VMI	Geometric forms to be copied using pencil and paper; measures the ability to integrate visual and motor abilities (eye-hand coordination).	Age-specific standard score
ANT		
Baseline Speed (BS)	A simple information processing task; measures reaction time on a visual cue.	RT, SDRT
Sustained Attention Objects - 2 keys (SA)	A visual continuous performance task; the right button should be pressed depending on the stimulus shown; measures the ability to sustain attention.	RT, SDRT, SCT, PM, PFA
VisuoSpatial Sequencing (VSS)	Several circles, pointed at in a 3x3 matrix of circles, have to be identified in the correct temporal order; measures visuo-spatial working memory.	NIT, NITCO
Domains of information processing		
Speed	Measures speed of information processing on a simple (BS) and a complex (SA) task.	BS_RT, SA_RT, SA_SCT
Accuracy	Measures accuracy of information processing during a complex task.	SA_PM, SA_PFA
Response speed variability	Measures variability in reaction time during a simple (BS) and a complex (SA) task.	BS_SDRT, SA_SDRT
Visual working memory	Measures visuo-spatial working memory.	VSS_NIT, VSS_NITCO

RT: mean reaction time; SDRT: standard deviation of mean reaction time; SCT: mean series completion time; PM: percentage of missings; PFA: percentage of false alarms; NIT: number of identified targets; NITCO: number of identified targets in correct order; BS_RT: mean RT of Baseline Speed task (etc.)

hospital records and additional questionnaires for the control group. This study was approved by the Medical Ethics Committee of the University Medical Center Utrecht, The Netherlands. All parents gave written informed consent.

Definitions of maternal characteristics

Neonatal macrosomia was defined as birth weight >90th percentile for gestational age, sex and parity according to the Netherlands Perinatal Registry data.[17] Severe maternal hypoglycemia was defined as an episode for which external help had been needed (data were available for first and third trimester of pregnancy).[11] Moderate neonatal hypoglycemia was defined as blood glucose level <2.6 mmol/l, severe neonatal hypoglycemia as blood glucose level <2.0 mmol/l.[11] Parental educational level was categorized as low, intermediate or high according to international standards.[18]

Statistical analysis

General characteristics were compared between groups using independent ,*t* test for normally distributed variables, Mann-Whitney *U* test for not normally distributed variables and χ^2 test (or Fisher's exact test as appropriate) for categorical variables. Group differences regarding estimated IQ scores, Digit Span score and Beery-VMI test score were analyzed using univariate analysis of variance (ANOVA), with adjustment for sex and highest achieved parental educational level (norm scores on these tests are already age-specific). Group differences regarding information processing domains were analyzed using multivariate ANOVA with the measures per information processing domain as dependent variables, group and sex as between-subjects factors and age as a covariate, and additional discriminant analysis in case of significant results to evaluate which of the dependent variables mainly discriminated the groups. If data were not normally distributed, they were log-transformed prior to statistical analysis. The correlation between maternal HbA1c level during pregnancy and neurocognitive measures in the offspring were evaluated using Pearson's (or Spearman's, if appropriate) correlation coefficient. To determine effect sizes of the analyses, partial eta squared (η_p^2) was calculated. Data were analyzed using SPSS 15.0 for Windows (SPSS, Chicago, IL). A *p*-value <0.05 was considered to be statistically significant.

RESULTS

1. General maternal and neonatal characteristics

In mothers of participating ODM, mean age at delivery was significantly higher and prevalence of preeclampsia was lower compared with mothers of non-participating ODM (mean±SD 31.1±3.5 vs. 30.1±4.0 years [*p*=0.02] for age at delivery, and 10.8 vs. 19.0% [*p*=0.047]

for preeclampsia). All other maternal and neonatal characteristics did not significantly differ between the participating and non-participating ODM group (data not shown).
In the ODM group, mean maternal age and gestational age at delivery were significantly lower compared with those in the control group (p<0.01, Table 2). Parents from the control group were higher educated compared to parents from the ODM group (p<0.01). The prevalence of neonatal macrosomia in the ODM group was higher compared with that in the control group (p=0.04, Table 2).

Table 2. Maternal and neonatal characteristics in ODM and controls.

	Controls	**ODM**	*p*
n	79	212	
Maternal characteristics			
Race: Caucasian	78 (98.7)	209 (98.6)	1.0
Age at delivery (years)	33.8±4.1	31.1±3.5	<0.01
Parity (nulliparity)	46 (26.1)	113 (53.3)	0.08
Duration of diabetes (years)	-	12 [5-18]	-
Educational level L/I/H (%)[a]	7 / 17 / 76	8 / 45 / 47	<0.01
Pregnancy characteristics			
Mean pregnancy HbA1c (%)	-	6.21±0.95	-
Preeclampsia	7 (8.9)	23 (10.8)	0.6
Gestational age (days)	278 [270-284]	264 [255-269]	<0.01
Neonatal characteristics			
Sex (male)	40 (50.6)	98 (46.2)	0.5
Birth weight (grams)	3736±601	3555±735	0.06
Macrosomia (BW >p90)	30 (38.0)	110 (51.9)	0.04
Congenital malformations	3 (3.8)	10 (4.7)	1.0

Data represent mean±SD, median with interquartile range or number with percentage.
[a] Highest achieved parental educational level (Low/Intermediate/High). BW: birth weight.

2. Neurocognitive measures

2.1 ODM vs. controls

There were no significant differences between ODM and controls regarding intellectual functioning (WISC-III-NL; Table 3). ODM had a significantly lower score on verbal working memory (Digit Span) and visual-motor integration abilities (Beery-VMI) compared to controls ($F_{(1,275)}$=7.31, p<0.01, η_p^2=0.026 for Digit Span, and $F_{(1,277)}$=4.44, p=0.04, η_p^2=0.016 for Beery-VMI). Multivariate analyses showed that ODM performed significantly poorer than controls on ANT-response speed ($F_{(1,268)}$=2.82, p=0.039, η_p^2=0.031; mainly due to the SA-measures), response speed variability ($F_{(1,268)}$=3.73, p=0.025, η_p^2=0.027; mainly due to the SA-measure) and visual working memory ($F_{(1,269)}$=3.58, p=0.029, η_p^2=0.026).
Because gestational age at delivery was significantly lower in ODM than in controls, we repeated the analyses with additional adjustment for gestational age. After adjustment for gestational age, the differences in scores on verbal working memory, visual-motor integration

Table 3. Neurocognitive measures in ODM and controls at 6-7 years of age.

	Controls	ODM	*p*
n	79	212	
WISC-III-NL			
Estimated Verbal IQ	106.1±1.9	103.9±1.3	0.3
Estimated Performance IQ	104.7±2.1	102.0±1.4	0.2
Estimated Total IQ	106.3±1.9	103.4±1.3	0.1
Digit Span	10.7±0.4	9.6±0.3	<0.01
Beery-VMI			
Standard score	101.5±1.5	98.3±1.0	0.04

Data represent adjusted means±SE (means were adjusted for sex and highest achieved parental educational level).

abilities, ANT-response speed variability and visual working memory were no longer significant. Additional adjustment for gestational age did not change the significant difference in response speed.

2.2 Severe maternal hypoglycemic events

No significant differences in neurocognitive functioning (including information processing) were found between children of mothers who had endured severe hypoglycemic events during pregnancy and children of mothers who had not (Table 4).

2.3 Maternal glycemic control during pregnancy

There was a (near) significant negative correlation between maternal mean HbA1c level during pregnancy and visual-motor integration abilities (Beery-VMI) in the offspring (Pearson's correlation coefficients were 0.14 for first trimester [p=0.06], -0.19 for second trimester [p=0.01], and -0.17 for third trimester [p=0.02], Table 4). Correlations between maternal HbA1c level during pregnancy and other neurocognitive measures were non-significant.

2.4 Neonatal macrosomia

No significant differences in neurocognitive functioning (including information processing) were found between ODM who were macrosomic at birth and those with a birth weight appropriate for gestational age (Table 4).

2.5 Neonatal hypoglycemia

ODM who had endured severe neonatal hypoglycemia had a lower estimated performance IQ compared with those who had not ($F_{(1,189)}$=4.00, p=0.047, η_p^2=0.021; Table 4). Multivariate analyses showed that visual working memory was significantly lower in ODM who had endured moderate ($F_{(1,195)}$=4.52, p=0.012, η_p^2=0.044) or severe ($F_{(1,185)}$=3.38, p=0.036, η_p^2=0.035) hypoglycemia compared with those who had not.

Table 4. Influence of maternal glycemic control and severe maternal hypoglycemia during pregnancy, macrosomia and neonatal hypoglycemia on neurocognitive functioning in ODM at 6-7 years of age.

		WISC-III-NL				Beery-VMI
	n	VIQ	PIQ	TIQ	DS	SS
Maternal hypoglycemia						
1st trim. (yes)	83	105.9±2.0	103.7±2.1	105.6±2.0	9.4±0.4	98.3±1.5
1st trim. (no)	109	104.9±1.6	101.7±1.8	103.8±1.6	9.7±0.3	97.2±1.2
3rd trim. (yes)	32	103.8±2.7	100.0±3.0	102.2±2.7	10.1±0.6	97.0±2.1
3rd trim. (no)	147	104.7±1.5	102.5±1.7	104.3±1.5	9.7±0.3	98.5±1.2
Glycemic control						
1st trim. HbA1c	191	-0.07	0.03	-0.03	0.05	-0.14
2nd trim. HbA1c	189	-0.05	0.02	-0.02	0.02	**-0.19**
3rd trim. HbA1c	181	-0.05	-0.02	-0.04	0.03	**-0.17**
Neonatal macrosomia						
BW >p90	99	105.1±1.7	102.1±1.9	104.3±1.7	9.8±0.3	98.8±1.3
BW ≤p90	107	103.8±1.6	102.3±1.8	103.5±1.6	9.7±0.3	97.8±1.3
Neonatal hypoglycemia						
Moderate (yes)	127	105.1±1.6	101.8±1.8	104.0±1.6	9.8±0.3	98.5±1.3
Moderate (no)	80	103.6±1.7	102.8±1.9	103.6±1.8	9.6±0.4	97.8±1.4
Severe (yes)	82	103.6±1.8	**99.7±2.0**	102.0±1.8	9.7±0.4	98.7±1.5
Severe (no)	115	104.9±1.6	**104.1±1.7**	105.2±1.5	9.7±0.3	98.1±1.2

Numbers represent correlation coefficients or adjusted means±SE (means were adjusted for sex and highest parental educational level). Significant findings are represented in bold. VIQ: estimated Verbal IQ; PIQ: estimated Performance IQ; TIQ: estimated Total IQ; DS: Digit Span; SS: standard score; trim: trimester; BW: birth weight.

DISCUSSION

The results of this nationwide follow-up study in offspring of women with type 1 diabetes showed that their overall intellectual functioning at school age did not significantly differ from that in a control group of children of non-diabetic women. However, ODM showed significant poorer performance on verbal and visual working memory, visual-motor integration abilities and information processing response speed and response speed variability. Severe maternal hypoglycemic episodes during pregnancy and neonatal macrosomia showed no effects on later neurocognitive functioning in ODM at school age. Poorer maternal glycemic control during pregnancy was related to poorer visual-motor integration abilities, and severe neonatal hypoglycemia was related to a lower performance IQ and poorer visual working memory.

Our results are in accordance with previous studies in offspring of type 1 diabetic women in that overall intellectual function was normal at school age.[6-8] However, we found significant differences between ODM and controls on specific neurocognitive functioning domains.

Although the present findings should be interpreted in light of some methodological limitations that will be discussed in the last paragraph, a preliminary conclusion could be that children of type 1 diabetic women may be at risk for specific neurocognitive dysfunctioning and learning problems at school age.

ODM showed significantly poorer verbal and visual working memory than controls. There is growing evidence that working memory, defined as a temporary storage of information that supports ongoing neurocognitive processes, may be a crucial aspect of neurocognitive functioning, and that poor working memory skills may underlie learning difficulties, independent of related neurocognitive skills.[19] A second interesting finding regarding neurocognitive functioning in ODM that might have possible consequences for learning abilities is the lower visual-motor integration ability that was found in ODM compared with controls. This finding may indicate that these children have more difficulties in eye-hand coordination and fine motor movements, which have been postulated as key factors in neurocognitive and perceptual development.[20] Furthermore, poorer performance on the Beery-VMI test has been related to poorer academic performance (for example math and reading achievements) in 7-10 year old children.[21] As the children in our study had just entered elementary school at time of assessment (or even not yet), we are not able to report on possible learning difficulties in our cohort of ODM. Replication studies and follow-up studies are needed to investigate working memory performance and possible consequences with respect to learning difficulties in these children.

The present study showed a significant weaker performance on some aspects of information processing in ODM compared with controls concerning response speed and response speed variability (visual working memory is already discussed in the previous paragraph). The difference in response speed between the groups was largely caused by the more complex information processing task SA, as opposed to the simple reaction time task BS. Furthermore, ODM showed higher response speed variability, indicating instability of information processing. These results may suggest that complex information processing in ODM may be disturbed. Daily life puts high demands on information processing abilities in terms of response speed and response speed stability, and we showed that performance on these aspects was weaker in ODM.

Most differences in neurocognitive outcome between ODM and controls were no longer significant after adjustment for, and may therefore be explained by, a lower mean gestational age at birth in ODM. Preterm birth occurs in approximately 30% of type 1 diabetic pregnancies.[11] Previous studies have shown that children who were born preterm (including late preterm infants) show more neurocognitive impairments and academic difficulties compared with full term controls.[22] Although most preterm deliveries in type 1 diabetic pregnancies occur due to a medical cause such as maternal preeclampsia or fetal distress[11] and therefore will be hard to prevent, obstetricians should keep in mind that preterm birth, even late preterm birth, may have long term neuropsychological consequences for the child. As fetal

macrosomia may be a cause of preterm birth (mainly induced delivery due to fetal distress or high estimated birth weight), it may also be hypothesized that differences in neuropsychological functioning between ODM and controls are due to an underlying difference in occurrence of fetal macrosomia. However, as we showed no effects of macrosomia on neuropsychological functioning in ODM, this is not a likely hypothesis.

We found a negative correlation between maternal glycemic control during pregnancy and visual-motor integration abilities in the offspring at school age. Some previous studies also showed a negative correlation between maternal HbA1c level and later neurocognitive functioning in the offspring,[23] emphasizing the importance of strict maternal glycemic control during pregnancy. The price to pay when aiming for strict glycemic control, however, is an increased incidence of severe maternal hypoglycemic events,[11] but in accordance with a previous study by Rizzo et al.[5] we did not find any long term effects of severe maternal hypoglycemic events during pregnancy on neurocognitive functioning in ODM. We did find, however, that neonatal hypoglycemia was related to poorer visual working memory in ODM and, in case of severe hypoglycemia, also to a lower performance IQ. Results from previous studies regarding long term effects of neonatal hypoglycemia in ODM are inconsistent,[3,4,6,7] which may at least in part be explained by the lack of consensus on the blood glucose level defining (severe) neonatal hypoglycemia. Based on our results, we suggest that close monitoring of neonatal blood glucose level is warranted to prevent neonatal hypoglycemia, and thereby possible negative long term effects on neurocognitive functioning. However, these findings should be interpreted with care, as data on neonatal blood glucose levels in ODM were collected retrospectively from the attending pediatricians through questionnaires, and we do not have additional information on elapsed time after birth, duration and frequency of hypoglycemia and whether hypoglycemia was symptomatic or not.

In conclusion, our results showed that overall intellectual functioning in 6-7 year old children of women with type 1 diabetes was comparable to that in children of non-diabetic women. ODM showed, however, significant poorer performance on more subtle domains of neurocognitive functioning, mainly related to a lower mean gestational age at birth. Maternal hypoglycemic events during pregnancy and neonatal macrosomia did not influence later neurocognitive functioning in the offspring, but poorer maternal glycemic control and neonatal hypoglycemia were related to poorer neuropsychological functioning at school age. This follow-up study may be a valuable contribution to the existing literature, since it describes neurocognitive development in 6-7 year old offspring from a current large and well defined cohort of Caucasian women with type 1 diabetes. The children were studied within a small age range and were compared with age-matched control children of non-diabetic mothers. However, the results from this study should be interpreted in light of some limitations. The number of participating children in the control group was not as large as in the study group, and parents in the control group were higher educated than parents in the ODM group. However, as these factors can be accounted for in statistical analysis, we consider the

control group nevertheless of great value to our study. Furthermore, intellectual function was estimated based on only four subtests of the WISC-III-NL. It should also be mentioned that effect sizes of the significant differences were only small (η_p^2<0.06).[24] These limitations do not allow firm conclusions, but future follow-up studies with a more extensive neurocognitive test battery and comparison with a larger control group may substantiate our results.

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CHAPTER

6

Cardiac function in 7 year old offspring of women with type 1 diabetes

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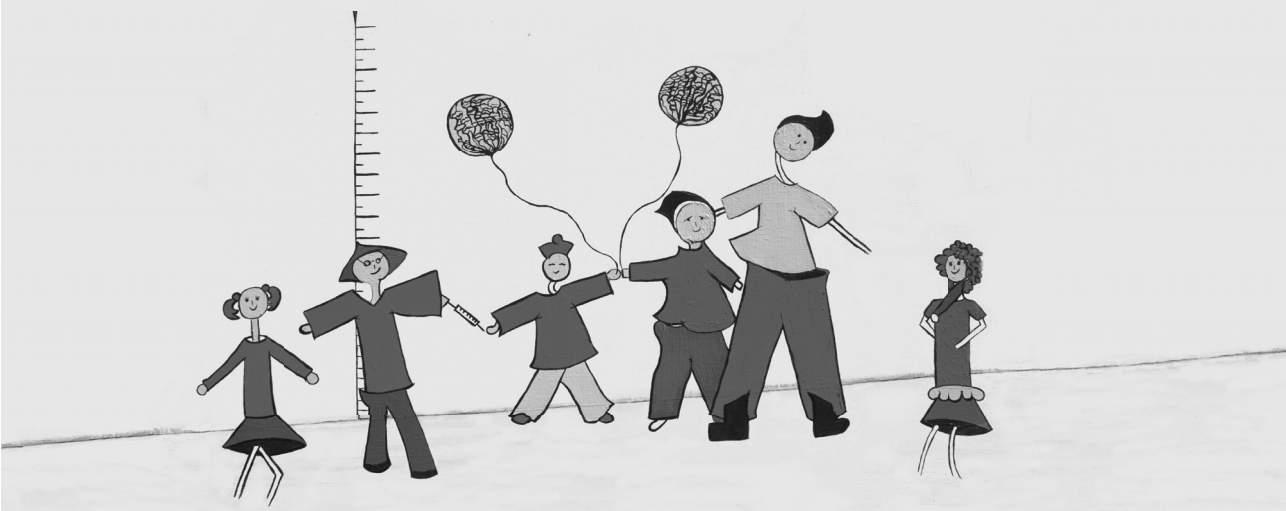

ABSTRACT

Introduction

Offspring of type 1 diabetic mothers (ODM) are at risk for short term (neonatal) complications, such as congenital malformations, neonatal macrosomia (birth weight >90th percentile) and hypertrophic cardiomyopathy (HCM). There is increasing evidence that these children are also at risk for cardiovascular morbidity later in life, but no studies have yet been performed in ODM regarding cardiac structure or function at later age. The objectives of this study were to investigate cardiac dimensions and function in ODM at school age in relation to neonatal macrosomia and maternal glycemic control during pregnancy.

Methods

ODM (*n*=30) underwent full echocardiographic evaluation at 7 years of age (including systolic and diastolic function and measurement of cardiac dimensions) and results were compared with those in an age- and birth weight percentile matched control group of 30 children of non-diabetic mothers.

Results

Cardiac dimensions and systolic and diastolic function parameters in ODM at 7 years of age were comparable to those in controls, but systolic blood pressure was slightly higher in ODM and related to maternal HbA1c during third trimester of pregnancy. HCM was present at birth in three out of 30 ODM, but had resolved without any long term sequelae. Neonatal macrosomia and poorer maternal glycemic control during pregnancy were not related to poorer cardiac outcome at 7 years of age.

Conclusions

Cardiac function at 7 years of age in offspring of women with type 1 diabetes is reassuring.

INTRODUCTION

Despite good prepregnancy care and adequate maternal glycemic control during type 1 diabetic pregnancies, the risk of perinatal complications in the offspring, such as preterm birth, neonatal macrosomia (birth weight >90th percentile) and congenital anomalies, is still high compared with the general population.[1] With current diabetes treatment regimens, structural cardiac defects occur in 2-15% of the offspring.[1-4] Hypertrophic cardiomyopathy (HCM, mainly interventricular septal hypertrophy) can be demonstrated in 25-45% of newborn infants of diabetic mothers,[4-8] and may be associated with functional cardiac changes during pregnancy as well as in the neonatal period.[8-13] Interventricular septal hypertrophy usually normalizes within the first six months after birth,[7,8] but to the best of our knowledge no follow-up studies regarding cardiac structure or function have been performed in offspring of women with type 1 diabetes at later age.

There is increasing evidence that children born after a diabetic pregnancy are at increased risk for cardiovascular and metabolic morbidity at later age,[14] especially when macrosomic at birth.[15,16] A possible underlying mechanism for this 'programming' of later disease is the theory of 'fuel-mediated teratogenesis', which states that high glucose concentrations during diabetic pregnancy make the developing tissues in the offspring vulnerable to alterations later in life such as obesity and type 2 diabetes.[17] Because of the high prevalence of neonatal HCM and the risk for later cardiovascular diseases in offspring of type 1 diabetic women, we hypothesized that cardiac dimensions and/or function may also already be altered at later age. The objective of this study was to evaluate cardiac dimensions and function at school age in children who were born after a type 1 diabetic pregnancy in relation to neonatal macrosomia and maternal glycemic control during pregnancy.

METHODS

Study population

The study group consisted of offspring of type 1 diabetic mothers (ODM) who participated in a previous nationwide study on type 1 diabetes and pregnancy outcome in The Netherlands.[1] We performed a follow-up study in 213 of these children at school age, which consisted of a home visit (for anthropometric measurements, blood pressure recordings and neurocognitive tests) and a fasting blood sample on a separate occasion. More details of this cohort and results from anthropometric measurements have recently been described elsewhere.[18] ODM who participated in the follow-up study and lived within 50 kilometers of our hospital (*n*=43) were invited for an additional echocardiogram, and 30 of them participated. Mean age of the ODM at time of the echocardiogram was 7.6 years (range 7.3-8.1). Information regarding maternal characteristics and pregnancy outcome was obtained from the previous study

on pregnancy outcome,[1] which had been provided by the attending gynecologist/internist. Information on neonatal outcome (including diagnosis of HCM) had been provided by the attending pediatrician.

From the control group of offspring of non-diabetic women who participated in the follow-up study (*n*=79), we included 15 macrosomic and 15 non-macrosomic children for this study on cardiac outcome. These children were born in the same period as the ODM at the University Obstetric Center, Utrecht, The Netherlands. Mean age of the controls at time of the echocardiogram was 7.4 years (range 6.9-8.1). Information regarding maternal characteristics and pregnancy outcome was obtained from hospital records and additional questionnaires.

This study was approved by the Medical Ethics Committee of the University Medical Center Utrecht, The Netherlands. All parents gave written informed consent.

Measurements

During a home visit (previous to the echocardiography) blood pressure was recorded three times on the right arm in sitting position after five minutes of rest with a two minute interval period, using an automated oscillometric device (DINAMAP®, Critikon, Tampa, FL). The average of the last two measurements of systolic (SBP) and diastolic (DBP) blood pressure were used for analysis. The children's height and weight were measured on the day of the echocardiography and BMI was calculated.

All patients and controls underwent a full echocardiographic evaluation including a structural echo for cardiac defects and evaluation of systolic and diastolic left ventricular function. Dimensions of the interventricular septum (IVS) and left ventricular posterior wall (LVPW) were measured and p-values (percentiles corrected for weight) were calculated.[19] Systolic left ventricular function was evaluated using shortening fraction (SF) and left ventricular end diastolic dimension (LVEDd). A p-value was calculated for each LVEDd measurement.[19] Cardiac output (CO) was calculated per kilogram body weight as CO=SV·HR (with SV being stroke volume and HR being heart rate). SV was calculated as $SV=LVOT_{area}\cdot VTI(LVOT)$, with $LVOT_{area}$ being left ventricular outflow tract area ($\pi\cdot(diamater/2)^2$), and VTI(LVOT) being Velocity Time Integral of LVOT which was established through averaging three Pulsed Wave Doppler tracings in the LVOT). Systolic right ventricular function was evaluated based on measurement of the tricuspid annular plane systolic excursion (TAPSE). Maximal tricuspid regurgitation pressure gradient (TR max PG) was measured if present. Diastolic left ventricular function was evaluated with Pulsed Wave Doppler signal of the mitral valve inflow pattern and pulmonary vein pattern.[20] E/A and S/D ratio's were calculated (ratio of early/late left ventricular filling speed and ratio of systolic/diastolic pulmonary vein filling speed, respectively). Tissue Doppler Imaging of the IVS and LVPW was performed with measurement of IVS 'S (systolic peak wall motion velocity), 'De (early diastolic peak wall motion velocity), 'Da (late diastolic peak wall motion velocity) and LVPW 'S, 'De, and 'Da values. All examinations were performed using a

GE Vivid 7 Ultrasound Machine (GE Healthcare, UK). The echo technician was blinded for the origin of the participants (ODM or control).

Statistical methods

General characteristics were compared between groups using independent *t* test for normally distributed variables, Mann-Whitney *U* test for not normally distributed variables and χ^2 test (or Fisher's exact test if appropriate) for categorical variables. Differences in measurements between ODM and controls and between subgroups of ODM were analyzed using analysis of covariance with adjustment for sex and age at measurement, unless otherwise stated. If data were not normally distributed, they were log- or square root-transformed prior to statistical analysis. The correlation between maternal HbA1c level during pregnancy and measurements in the offspring was evaluated using Pearson's (or Spearman's, if appropriate) correlation coefficients. Data were analyzed using SPSS 15.0 for Windows (SPSS, Chicago, IL). A *p*-value <0.05 was considered to be statistically significant.

RESULTS

General characteristics

Participating mothers in the ODM group (*n*=30) only significantly differed from the non-participating mothers (i.e. women who had participated in the previous nationwide study on pregnancy outcome, but had not participated in this study, *n*=283) regarding parity (70.0 vs. 49.8% nulliparous women, *p*=0.04). All other maternal and neonatal characteristics did not significantly differ between the participating and non-participating ODM group.

In the participating ODM group, maternal mean age at delivery was significantly lower compared with that in the control group, and the percentage of nulliparous women was significantly higher (Table 1). Mean gestational age at delivery and mean birth weight were lower in ODM compared with controls. The macrosomic and appropriate-for-dates ODM subgroups did not significantly differ regarding maternal or child characteristics, except for a higher mean birth weight in macrosomic ODM. Neonatal HCM was diagnosed in three children from the ODM group (two boys, one girl), none of them had clinical signs attributable to HCM at time of the diagnosis. Maternal and child characteristics of these three patients did not significantly differ from the rest of the ODM group.

At follow-up, mean SBP in ODM (adjusted for sex, height and age at measurement) was slightly higher as compared to controls (Table 1).

Echocardiography

There were no significant differences in cardiac dimensions, and systolic or diastolic cardiac function parameters between ODM and controls at 7 years of age (Table 2). All cardiac

Table 1. Maternal and child characteristics in ODM and controls.

	Controls	ODM	*p*
n	30	30	
Maternal characteristics			
Age at delivery (years)	33.8±3.0	31.4±3.9	0.01
Parity (nulliparity)	10 (33.3)	21 (70.0)	<0.01
Race: Caucasian	30 (100)	29 (96.7)	1.0
Pregnancy characteristics			
Maternal smoking	1 (3.3)	2 (6.7)	1.0
Preeclampsia	2 (6.7)	4 (13.3)	0.7
Mean HbA1c 1st trim. (%)	-	6.51±0.74	-
Mean HbA1c 2nd trim. (%)	-	6.04±0.85	-
Mean HbA1c 3rd trim. (%)	-	6.32±0.93	-
Child characteristics			
Gestational age (days)	277 [270-284]	260 [246-268]	<0.01
Birth weight (grams)	3793±631	3400±646	0.02
Birth weight percentile	87 [20-95]	84 [50-98]	0.2
Neonatal macrosomia[a]	15 (50.0)	14 (46.7)	0.8
Sex (male)	16 (53.3)	14 (46.7)	0.6
Congenital malformation	0 (0)	1 (3.3)[b]	1.0
HCM at birth	0 (0)	3 (10.0)	0.2
Height at age 7 (cm)	126.3±5.0	123.5±5.9	0.053
BMI at age 7 (kg/m^2)	15.3 [14.9-16.9]	16.3 [15.5-17.6]	0.1
SBP at age 7 (mmHg)	95.5 [91.0-99.5]	97.0 [94.0-105.0]	0.04
DBP at age 7 (mmHg)	57.8 [54.5-60.0]	59.5 [55.5-61.0]	0.7

Data represent mean ± standard deviation, median with interquartile range or number with percentage. [a] Birth weight >90th percentile for gestational age, sex and parity according to the Netherlands Perinatal Registry data.[26] [b] Single umbilical artery. Trim.: trimester; HCM: hypertrophic cardiomyopathy; SBP: systolic blood pressure; DBP: diastolic blood pressure.

dimensions and function parameters in the three patients with neonatal HCM were within the normal range and did not significantly differ compared with the other ODM or with controls. Subgroup analyses showed no significant differences in cardiac function or dimensions between macrosomic and appropriate-for-dates ODM or between macrosomic ODM and macrosomic controls (Table 3).

Echocardiographic measurements in ODM were not significantly correlated with maternal glycemic control during pregnancy (assessed by mean HbA1c level during first, second and third trimester), but SBP in ODM was positively correlated with maternal glycemic control during third trimester of pregnancy (Pearson's correlation coefficient 0.40, p=0.04).

Table 2. Echocardiographic measurements in ODM and controls at 7 years of age.

	Controls	ODM	*p*
n	30	30	
Age at echo (years)	7.38±0.05	7.59±0.05	<0.01
Dimensions			
IVSd (percentile)	75 [55-90]	70 [55-90]	0.7
LVPWd (percentile)	54.0±3.7	53.2±3.7	0.9
LVEDd (percentile)	63.2±3.6	55.5±3.6	0.1
Systolic LV function			
SF (%)	34.8±0.9	35.0±0.9	0.9
CO (ml/min/kg)	116 [102-134]	117 [99-131]	0.9
IVS `S (cm/s)	7.4 [7.0-8.0]	7.4 [7.0-8.1]	0.9
LVPW `S (cm/s)	10.6 [9.6-11.6]	10.0 [8.5-11.4]	0.9
Systolic RV function			
TAPSE (cm)	1.99 [1.85-2.21]	1.98 [1.86-2.17]	0.8
TR max PG (mmHg)	15.0 [14.1-17.0]	15.8 [13.1-17.3]	0.8
Diastolic LV function			
E/A ratio	2.2 [2.0-2.37]	2.14 [1.74-2.72]	0.6
S/D ratio	0.83±0.05	0.83±0.05	1.0
IVS `De (cm/s)	13.2 [12.7-14.4]	13.3 [12.0-14.9]	0.3
IVS `Da (cm/s)	5.5 [5.0-6.5]	6.0 [5.4-6.1]	0.7
LVPW `De (cm/s)	17.9 [15.6-19.7]	18.2 [16.5-19.0]	0.8
LVPW `Da (cm/s)	6.2 [5.1-7.0]	6.7 [5.7-7.5]	0.5

Data represent adjusted means ± standard error or median with interquartile range if data were transformed prior to analysis. All means were adjusted for age at echo and sex. IVSd: interventricular septal end diastolic dimension; LVPWd: left ventricular posterior wall end diastolic dimension; LVEDd: left ventricular end diastolic dimension; SF: shortening fraction; CO: cardiac output; TAPSE: tricuspid annular plane systolic excursion; TR max PG: maximum tricuspid regurgitation pressure gradient; E/A: ratio of early and late left ventricular filling speed; S/D: ratio of systolic and diastolic pulmonary vein filling speed; 'S, 'De, 'Da: peak wall motion velocity during systole, early diastole or late diastole.

DISCUSSION

Since (subclinical) HCM can be demonstrated in up to 45% of ODM, and long term cardiovascular sequelae in offspring born after a type 1 diabetic pregnancy may be present already in childhood,[14] we hypothesized that subtle changes in cardiac dimensions or function also might be present in ODM at school age. In this study, we were the first to show that systolic and diastolic function as well as cardiac dimensions in ODM at 7 years of age were comparable to those in an age- and birth weight percentile matched control group. Systolic blood pressure, however, was slightly higher in ODM compared with controls. Although blood pressure was still within the normal range,[21] this finding may be indicative of early programming of renal or vascular mechanisms, as has been suggested by others.[14] However, despite a higher mean SBP, there were no signs of cardiac hypertrophy in ODM (most likely due to the fact that blood pressure was still within the normal range).

Table 3. Echocardiographic measurements in macrosomic ODM, appropriate-for-dates ODM, and macrosomic controls at 7 years of age.

	ODM	ODM		Controls	
	BW >p90	**BW ≤p90**	p^a	**BW >p90**	p^b
n	14	16		15	
Age at echo (years)	7.6±0.08	7.6±0.05	0.9	7.6±0.07	0.7
BMI (kg/m^2)	16.6 [16.0-17.7]	16.0 [15.2-17.0]	0.4	15.3 [15.1-16.9]	0.2
Dimensions					
IVSd (percentile)	77.5 [55-90]	70.0 [52.5-85]	0.4	70 [57.5-80]	0.4
LVPWd (percentile)	52.9±5.5	53.4±4.3	0.9	50.0±5.3	0.8
LVEDd (percentile)	57.1±4.6	54.1±5.2	0.7	68.0±4.5	0.1
Systolic LV function					
SF (%)	34.3±1.4	36.0±1.1	0.4	36.5±1.5	0.3
CO (ml/min/kg)	117 [113-126]	105 [102-136]	0.9	114 [100-133]	0.6
IVS `S (cm/s)	7.2 [7.0-8.0]	8.0 [7.0-8.2]	0.8	8.0 [7.4-8.0]	0.7
LVPW `S (cm/s)	9.8 [8.1-11.2]	10.0 [9.0-11.4]	0.3	10.7 [9.7-12.0]	0.6
Systolic RV function					
TAPSE (cm)	2.00 [1.80-2.30]	1.98 [1.93-2.13]	0.7	1.99 [1.89-2.12]	0.5
TR max PG (mmHg)	15.7 [13.1-17.3]	15.8 [13.3-17.4]	0.9	15.8 [14.5-18.2]	0.9
Diastolic LV function					
E/A ratio	2.14 [1.60-2.69]	2.12 [1.81-2.90]	0.3	2.14 [2.00-2.47]	0.8
S/D ratio	0.86±0.08	0.80±0.07	0.5	0.84±0.09	0.9
IVS `De (cm/s)	13.3 [12.0-15.0]	13.3 [11.8-14.5]	0.6	14.0 [13.0-15.0]	0.3
IVS `Da (cm/s)	6.0 [5.5-6.0]	6.0 [5.4-6.3]	0.7	6.0 [5.0-7.0]	0.9
LVPW `De (cm/s)	19.0 [18.2-20.7]	17.8 [16.3-18.8]	0.2	18.4 [17.0-20.0]	0.7
LVPW `Da (cm/s)	6.2 [5.7-7.1]	7.0 [5.8-7.5]	0.9	6.3 [5.5-7.9]	1.0

Data represent adjusted mean ± standard error (means were adjusted for age at echo and sex) or median with interquartile range if data were transformed prior to analysis.
[a] Macrosomic ODM vs. appropriate-for-dates ODM. [b] Macrosomic ODM vs. macrosomic controls.
BW: birth weight.

In our cohort of ODM only three patients (out of 30) were diagnosed after birth with HCM. However, in newborn ODM from our cohort echocardiography was only performed when HCM was clinically suspected, in contrast to the studies which reported a higher prevalence of neonatal HCM. As fetal cardiac growth is promoted by binding of insulin to the cardiac insulin-like growth factor (IGF)-1 receptor, HCM is believed to resolve within weeks after birth due to normalization of fetal hyperinsulinemia.[22] Here, we showed that cardiac dimensions and function parameters in children with previous neonatal HCM were normal at 7 years of age, indicating that HCM indeed is a transient phenomenon and that routine echocardiographic screening in ODM does not seem necessary (although the small number of ODM with neonatal HCM in our cohort does not allow firm conclusions).

Previous studies have shown that offspring of diabetic women who were macrosomic at birth are at increased risk for developing overweight and other cardiovascular risk factors.[15,16] Therefore, we investigated the possible influence of neonatal macrosomia on cardiac outcome in ODM. We found that neither cardiac dimensions, nor cardiac function significantly

differed between macrosomic ODM and those with an appropriate-for-date birth weight. As some studies have shown more cardiac alterations in macrosomic newborns of diabetic mothers compared with macrosomic newborns of non-diabetic mothers,[23,24] we also compared cardiac outcome of macrosomic ODM with that of macrosomic controls. No significant differences between those subgroups were found, indicating that neonatal macrosomia in ODM has no adverse effects on cardiac function at 7 years of age.

Extrapolating Freinkel's theory on fuel-mediated teratogenesis,[17] one might expect less favorable cardiovascular outcome in offspring of diabetic mothers with poorer glycemic control during pregnancy. Maternal glycemic control during pregnancy was not significantly correlated with cardiac dimensions or function parameters, but we did find a positive correlation between maternal mean HbA1c level during third trimester of pregnancy and SBP in the offspring at 7 years of age. However, this finding should be interpreted with care, since such a correlation was not present in the total ODM group that participated in the follow-up study (n=213, unpublished data). It should further be noted that maternal HbA1c may not be an accurate tool for the classification of level of glycemic control, as it does not reflect the complexities of glycemic control in pregnant diabetic women.[25]

As we are the first to describe cardiac dimensions and function in ODM at school age, this study should be a valuable addition to previous studies on long term effects of a diabetic pregnancy on the development in the offspring. However, the results from this study should be interpreted in light of the limitation that sample sizes were only small. Therefore, we cannot exclude the possibility that the lack of significant differences between ODM and controls or between the subgroups are due to a lack of statistical power.

CONCLUSIONS

We conclude that the results from our study are nevertheless reassuring, showing normal cardiac dimensions and function in offspring of type 1 diabetic women at 7 years of age. Hypertrophic cardiomyopathy was present at birth in three out of 30 ODM, but had resolved without any long term sequelae. Neonatal macrosomia and poorer maternal glycemic control during pregnancy were not associated with adverse cardiac outcome at 7 years of age. Future follow-up studies in larger samples should substantiate our results, and may show whether the slightly higher blood pressure in ODM at 7 years of age may be of influence on later cardiac function.

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CHAPTER

7

Vascular characteristics in 7-9 year old offspring of women with type 1 diabetes; a pilot study

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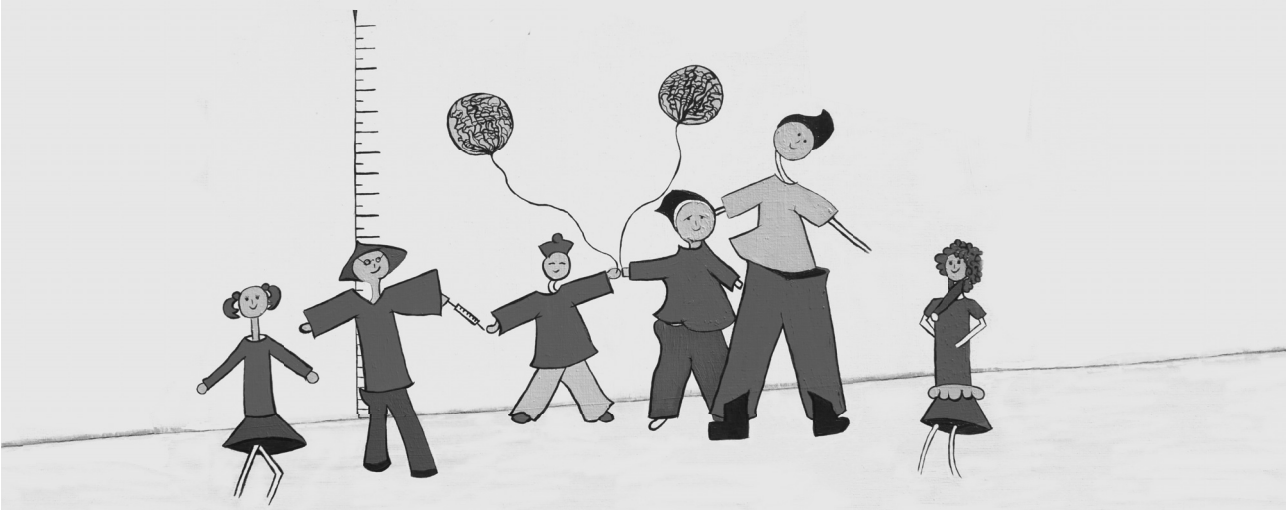

ABSTRACT

Introduction

Children of women with type 1 diabetes have been shown to be at risk for later cardiometabolic diseases. One of the underlying pathologies of cardiovascular disease is atherosclerosis. Precursors of atherosclerotic cardiovascular disease may already be present at young age. In this pilot study we investigated early atherogenic markers in the offspring of type 1 diabetic women at school age.

Methods

Non-invasive measurements of carotid intima-media thickness (CIMT) and carotid artery distensibility (carotid distensibility coefficient, CDC) were performed using a novel ultrasound-based technology in offspring of type 1 diabetic mothers (ODM, *n*=19) and control children (*n*=19) at 7-9 years of age.

Results

Mean CIMT and CDC did not significantly differ between ODM and controls (mean CIMT with 95% CI 386.8 (371.0-402.6) µm in ODM vs. 388.4 (373.1-403.8) µm in controls [*p*=0.8] and mean CDC with 95% CI 85.1 (74.5-95.6) $kPa^{-1} \cdot 10^{-3}$ in ODM vs. 85.0 (74.5-95.6) $kPa^{-1} \cdot 10^{-3}$ in controls [*p*=0.8]). Additional adjustment for known determinants of CIMT did not change the results.

Conclusions

We are the first to describe ultrasound based atherogenic markers in offspring of type 1 diabetic women at school age. Our findings indicate that vascular characteristics indicative of early atherosclerosis in a recent cohort of ODM at 7-9 years of age are not different from those in children of non-diabetic mothers. Although the small sample sizes do not allow us to make firm conclusions, these first results seem favorable. Follow-up studies at later ages including larger samples should substantiate our results.

INTRODUCTION

There is a growing body of evidence that adult cardiovascular disease may have its origin in early (fetal) life, when specific insults during development may alter later body composition and metabolism.[1] During type 1 diabetic pregnancy, maternal hyperglycemia induces fetal hyperglycemia, which in turn makes the developing tissues in the offspring more vulnerable to functional alterations later in life.[2] This may lead to the development of cardiovascular and metabolic diseases such as obesity, hypertension and impaired glucose tolerance.[3] As cardiovascular risk factors tend to track from childhood into adult life, early identification of children at risk for future health problems is important.[4]

One of the underlying pathologies of cardiovascular disease is atherosclerosis. Precursors of atherosclerotic cardiovascular disease may be present already at young age[5] and may be related to several intrauterine risk factors, such as impaired fetal growth and diabetic macrosomia.[6] However, to the best of our knowledge no previous study investigated early atherogenic markers in the offspring of type 1 diabetic women at later age.

Carotid artery intima-media thickness (CIMT) and to a lesser extent carotid artery distensibility (carotid distensibility coefficient, CDC) are well-known markers for atherosclerosis in adults.[7] They can be assessed non-invasively by ultrasound and are therefore suitable markers to investigate in children.[8] In a previous study on pregnancy outcome in a recent cohort of type 1 diabetic women, we showed that prepregnancy care and overall glycemic control during pregnancy were good.[9] In the present study, we investigated whether CIMT and CDC in a small sample of 7-9 year old offspring from this cohort would differ from those in a control group of children of non-diabetic mothers.

METHODS

Study population

The study group consisted of offspring of type 1 diabetic mothers (ODM) who participated in a previous nationwide study on type 1 diabetes and pregnancy outcome in The Netherlands.[9] We performed a follow-up study in these children at school age, including a fasting blood sample. Details of this cohort have been described elsewhere.[10] ODM who participated in the home visit and lived within 50 kilometers of our hospital (n=43) were invited for an additional carotid ultrasonography. Nineteen children agreed to participate. Median age at time of the ultrasonography was 8.1 years (range 7.6-9.2).

For the control group in our follow-up study, randomly selected offspring of non-diabetic women were invited who were born in the same period as the ODM at the University Obstetric Center, Utrecht, The Netherlands. Thirty-nine of the 79 participating controls who lived within 50 kilometers of our hospital were randomly invited for an additional carotid ultrasonography.

Nineteen children were included in this study. Median age of the controls at time of the ultrasonography was 7.8 years (range 7.1-8.9).

General characteristics were obtained from the previous study on pregnancy outcome for the ODM group[9] and from hospital records and additional questionnaires for the control group. This study was approved by the Medical Ethics Committee of the University Medical Center Utrecht, The Netherlands. All parents gave written informed consent.

Anthropometric measurements

Height and weight were measured with the participants wearing indoor clothes without shoes. Waist and hip circumference were measured twice and the averages were used for analysis. BMI and waist-to-hip ratio were calculated. Abdominal and intra-abdominal fat were measured three times with electronic calipers using ultrasound according to a standardized protocol as was described elsewhere.[11,12] The averages of the three measurements were used for analysis.

Vascular measurements

Blood pressure was recorded three times on the right arm in sitting position using an automated oscillometric device (DINAMAP®, Critikon, Tampa, FL). The averages of systolic (SBP) and diastolic (DBP) blood pressure recordings were used for analysis.

Vascular characteristics of the right common carotid artery (CCA) were assessed using ultrasound, based on high-resolution echotracking technology (Wall Track System, ArtLab, Esaote, Italy), including the use of a 128 radiofrequency line multiarray.[13] Rough radiofrequency data were analyzed online and 6-second cineloops were stored without compression (120 Mbytes) for offline analysis. With this novel technology, vessel wall properties of the carotid artery were obtained by means of the non-invasive assessment of the diastolic diameter (D_d), the change in diameter as function of time, and the CIMT. CIMT was measured with 21-µm resolution and distension was measured with 1.7-µm resolution. All parameters were measured twice and averages were used for analysis. The elastic properties of the carotid artery (distensibility) were assessed through the cross-sectional distensibility coefficient (the relative change in lumen area during systole for a given pressure change), which was defined as $CDC=\Delta A/(A\cdot\Delta P)$, with A being the diastolic lumen area ($\pi D_d^2/4$), ΔA the stroke change in lumen area ($\pi(D_s^2-D_d^2)/4$), and ΔP the carotid pulse pressure (D_s: systolic diameter). Carotid pulse pressure was estimated based on blood pressure recordings in supine position just before and after carotid ultrasonography, and was defined as $\Delta P= ((MAP-DBP)/(D_{mean}-D_d))\cdot(D_s-D_d)$, with MAP being the mean arterial pressure, D_{mean} the mean of D_d and D_s, and assuming (MAP-DBP) constant throughout the large artery tree. All measurements were performed by one investigator (C.G.) who was blinded for the origin of the participants (ODM or controls) at the time of the ultrasonography.

Statistical methods

General characteristics were compared between groups using Mann-Whitney *U* test for interval variables and χ^2 test (or Fisher's exact test as appropriate) for categorical variables. Vascular characteristics were compared between groups using general linear model analysis with CIMT and separately CDC as dependent variable and group (ODM/controls) as independent variable. The same models were used to adjust the associations for possible confounders. The model with CDC as dependent variable was additionally adjusted for sex and age. The model with CIMT as dependent variable was additionally adjusted for sex, age, BMI, SBP and LDL cholesterol level, as these have previously been shown to be determinants of CIMT.[14,15] The relation between maternal HbA1c level during pregnancy and birth weight percentile with CIMT and CDC in ODM was evaluated using Spearman's correlation coefficients. If data were not normally distributed, they were log transformed prior to statistical analysis. Data were analyzed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL). A p-value <0.05 was considered to be statistically significant.

RESULTS

General characteristics

Participating mothers from the study group (n=19) did not significantly differ from non-participating mothers (i.e. women who had participated in the previous study on pregnancy outcome, but did not participate in this study, n=294) regarding age at delivery, parity, prepregnancy BMI, educational level, duration of diabetes, mean HbA1c during 1st, 2nd and 3rd trimester, or occurrence of diabetic complications, preeclampsia and cesarean section (data not shown). Participating ODM did not significantly differ from non-participating ODM regarding mean gestational age, birth weight, neonatal morbidity and occurrence of macrosomia or congenital anomalies.

Mean maternal prepregnancy BMI was significantly higher for the ODM group compared with the control group (Table 1). Mean gestational age and mean total and HDL cholesterol level were significantly higher in children from the control group. Other laboratory parameters, anthropometric measurements, and blood pressure recordings did not significantly differ between children from the ODM group and those from the control group (Table 1).

Vascular measurements

Unadjusted mean CIMT and CDC did not significantly differ between ODM and controls (Table 2). Additional adjustment for sex and age at ultrasonography or sex, age, BMI, SBP and LDL cholesterol level (all known determinants of CIMT) did not change the effect for CIMT (Table 2). The influence of other possible determinants, such as maternal prepregnancy BMI or gestational age, on vascular characteristics is not evident from previous literature. As these variables differed between the ODM and control group (Table 1), they might mask a difference

Table 1. Maternal and child characteristics in ODM and controls.

	Controls	ODM	*p*
n	19	19	
Maternal characteristics			
Prepregnancy BMI	22.7 [20.8-24.0]	24.7 [23.6-25.8]	0.01
Smoking during pregnancy	0 (0)	1 (5)	1.0
Preeclampsia	1 (5)	2 (11)	1.0
Hypertensive disorder[a]	4 (21)	8 (42)	0.2
Age at delivery (years)	33.4 [30.8-37.9]	31.3 [30.0-35.3]	0.2
Educational level L/I/H (*n*)[b]	2 / 4 / 13	1 / 10 / 8	0.1
Neonatal characteristics			
Sex (male)	7 (37)	10 (53)	0.3
Gestational age (days)	279 [275-285]	258 [253-267]	<0.01
Birth weight (grams)	3850 [3500-4238]	3520 [3258-3965]	0.2
Macrosomia[c]	7 (37)	10 (53)	0.3
Measurements at follow-up			
Blood sampling			
Age (years)	7.5 [7.1-7.7]	7.5 [7.4-7.6]	0.4
Cholesterol (mmol/l)	4.43 [3.89-4.76]	3.74 [3.61-4.23]	0.04
HDL (mmol/l)	1.42±0.07	1.21±0.07	0.04
LDL (mmol/l)	2.51±0.10	2.30±0.10	0.1
Triglycerides (mmol/l)	0.57 [0.49-0.75]	0.59 [0.49-0.90]	0.4
HOMA-IR	1.02 [0.78-1.31]	0.97 [0.67-1.26]	0.9
HsCRP (mg/l)	<0.10 [<0.10-0.60]	<0.10 [<0.10-0.23]	0.4
Ultrasonography			
Age (years)	7.8 [7.7-8.3]	8.1 [7.9-8.2]	0.2
BMI (kg/m^2)	16.5±0.4	16.4±0.4	0.9
Waist-to-hip ratio	0.85 [0.83-0.88]	0.85 [0.82-0.88]	0.7
SBP (mmHg)	109.3±2.1	111.2±2.1	0.5
DBP (mmHg)	56.0±1.5	58.2±1.4	0.3
Abdominal sc fat (mm)	6.5 [5.3-12.3]	8.2 [5.6-12.5]	0.7
Intra-abdominal fat (mm)	33.9 [30.8-40.1]	38.8 [34.2-42.3]	0.1

Data represent adjusted mean±SE, median with interquartile range (if data were not normally distributed or transformed prior to analysis) or number with percentage.
[a] Hypertensive disorder during pregnancy (preeclampsia, pre-existent hypertension, pregnancy-induced hypertension). [b] Highest parental educational level (Low/Intermediate/High).[21] [c] Birth weight >90th percentile for gestational age, sex and parity according to the Netherlands Perinatal Registry data from 2001.[22] SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: HOMA insulin resistance; hsCRP: high-sensitivity CRP.

in vascular characteristics between these groups. However, adjustment for maternal prepregnancy BMI and gestational age (additional to adjustment for sex and age) did not change the effects (Table 2). Figure 1 illustrates the dispersal of CIMT and CDC measurements within the ODM group with regard to some complications from type 1 diabetic pregnancy (neonatal macrosomia, maternal hypertensive disorder and poor glycemic control (mean HbA1c >7.0%) during pregnancy). There were no significant correlations between birth weight percentile or maternal glycemic control during pregnancy and CIMT or CDC in ODM (*p*-values >0.7 for

Table 2. Vascular characteristics in ODM compared with controls.

	Controls	ODM		
	mean (95% CI)	mean (95% CI)	*b* (95%CI)	*p*
Unadjusted				
CIMT (µm)	388.4 (373.1 to 403.8)	386.8 (371.0 to 402.6)	-1.7 (-23.7 to 20.4)	0.8
CDC ($kPa^{-1} \cdot 10^{-3}$)	85.0 (74.5 to 95.6)	85.1 (74.5 to 95.6)	0.1 (-14.8 to 15.0)	0.8
Adjusted for sex, age				
CIMT (µm)	388.5 (372.3 to 404.7)	387.0 (370.6 to 403.5)	-1.46 (-24.8 to 21.8)	1.0
CDC ($kPa^{-1} \cdot 10^{-3}$)	84.6 (73.7 to 95.6)	85.7 (74.8 to 96.5)	1.0 (-14.6 to 16.7)	0.7
Adjusted for sex, age, BMI, SBP, LDL				
CIMT (µm)	383.3 (368.8 to 397.9)	386.8 (371.9 to 401.7)	3.5 (-18.0 to 25.0)	0.8
Adjusted for sex, age, mat. BMI, GA				
CIMT (µm)	381.9 (361.5 to 402.3)	394.1 (372.9 to 415.4)	12.3 (-22.5 to 47.0)	0.5
CDC ($kPa^{-1} \cdot 10^{-3}$)	86.0 (72.0 to 100.0)	84.3 (70.3 to 98.3)	-1.7 (-25.0 to 21.5)	0.9

Data represent unadjusted and adjusted means with 95% confidence interval (CI) and regression coefficients (*b*) with 95% CI. SBP: systolic blood pressure; mat. BMI: maternal prepregnancy BMI; GA: gestational age.

correlations with birth weight percentile and >0.3 for glycemic control during first, second and third trimester of pregnancy).

DISCUSSION

To the best of our knowledge, we are the first to describe early ultrasound-based vascular characteristics in the offspring of type 1 diabetic women at school age. We found that early atherogenic markers from the carotid artery in a small sample of ODM at 7-9 years of age were not significantly different from those in children of non-diabetic mothers.

Atherosclerosis is thought to play an important role in the pathogenesis of cardiovascular disease, and asymptomatic atherosclerosis has been shown to be present already at young age.[5] Several studies on the development of children born after a diabetic pregnancy, recently reviewed by Simeoni and Barker,[3] have shown that they are at risk for later cardiovascular and metabolic health problems, such as hypertension and dyslipidemia. Furthermore, alterations in plasma lipid concentrations have been shown in ODM already early in life.[16-18] It may therefore be hypothesized that ODM may also be at risk for increased atherogenic markers already early in life. We did not find, however, an increased CIMT in our cohort of offspring

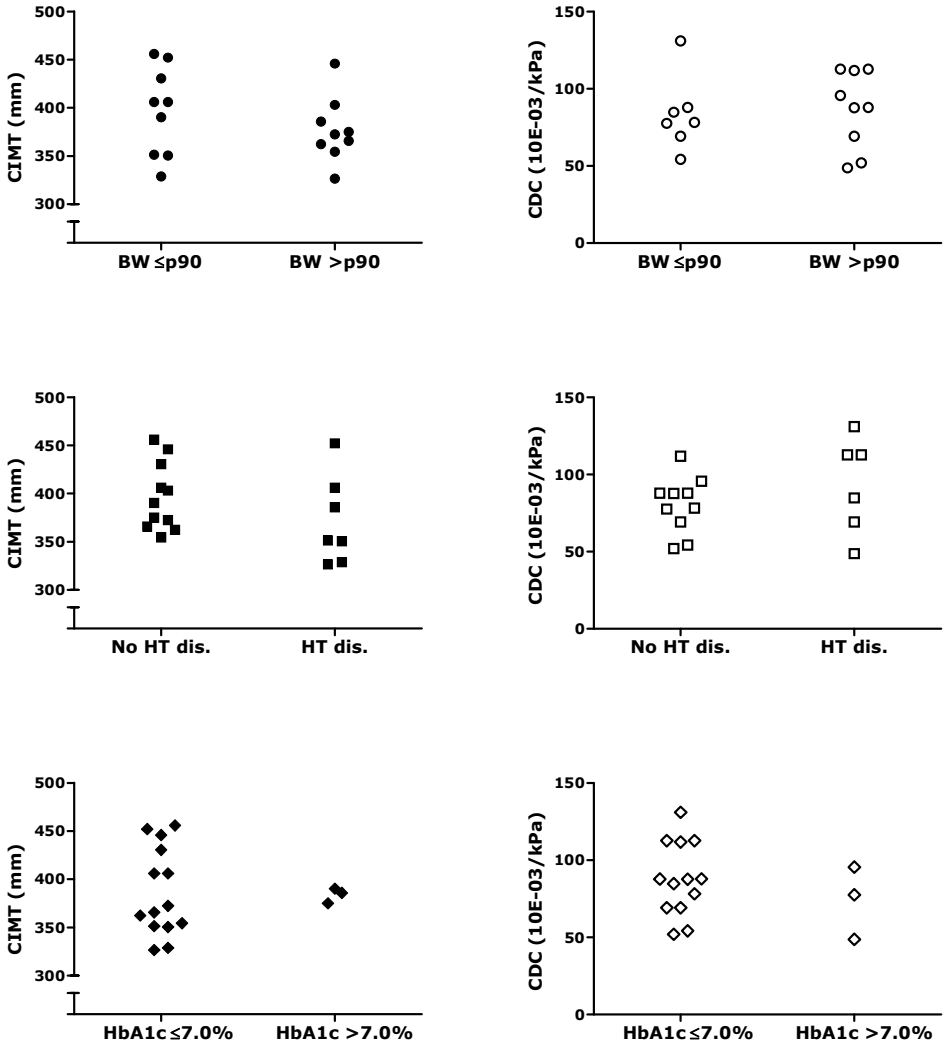

Figure 1. CIMT (black) and CDC (white) in ODM regarding neonatal macrosomia, maternal hypertensive disorder and poor maternal glycemic control during pregnancy.
BW: birth weight; HT dis.: maternal hypertensive disorder during pregnancy (preeclampsia, pre-existent hypertension, pregnancy-induced hypertension).

of type 1 diabetic women at 7-9 years of age, indicating that there are no signs of early atherosclerosis at this age.

Whereas intima-media thickness is a measure of structural vascular wall changes, distensibility is a more functional parameter.[7,8] Arterial distensibility is a measure of the arterial ability to expand and contract with cardiac pulsation and relaxation. A decrease of arterial

distensibility represents increased arterial wall stiffness, which may lead to the occurrence of vascular changes associated with cardiovascular disease. Functional impairment of the arterial wall may occur in an early stage of the atherosclerotic process, even before structural wall changes become detectible.[8] In our study, we did not find any differences in carotid artery distensibility between ODM and controls, indicating that there are no signs of arterial stiffness in ODM at this age.

As the rate of complications in type 1 diabetic pregnancy (such as neonatal macrosomia, maternal hypertensive disorder during pregnancy and poor glycemic control) is still high,[9] it would be of interest to study the possible influence of these complications on early atherogenic markers in the offspring. Previous studies have shown that exposure to maternal preeclampsia is related to an increased risk of cardiovascular disease in the offspring,[19] and that aortic IMT is significantly increased in macrosomic newborns of diabetic mothers.[20] Our cohort, however, was too small to study these subgroups adequately. Although correlation coefficients between CIMT or CDC and birth weight percentiles or maternal HbA1c level during pregnancy were not significant, and there was a considerable overlap between CIMT and CDC measurements in children of mothers with and without these complications (as was shown in Figure 1), a larger sample size is required to study these subgroups in more detail.

We believe this study provides nevertheless valuable data on early atherogenic markers in offspring from a recent, well defined cohort of Caucasian women with type 1 diabetes. We do, however, acknowledge some limitations to our study. As the groups in our study were only small, the lack of finding statistically significant differences may very well be due to a lack of statistical power. Furthermore, because all children in the control group were born at one obstetric center, we cannot exclude the possibility that some selection bias has occurred with respect to the control group. This may be a possible explanation for the difference in mean cholesterol level between ODM and controls. Including larger samples and a more heterogeneous control group may strengthen our results, and would make it easier to study the influence of different determinants of vascular characteristics and of complications such as macrosomia or poor glycemic control.

In conclusion, our findings indicate that early atherosclerosis in a recent cohort of offspring of type 1 diabetic women at 7-9 years of age is not detectable yet by means of non-invasive measurement of carotid vascular characteristics. Although the small sample size does not allow us to make firm conclusions on the presence or absence of early atherogenic markers in ODM at school age, these first results seem favorable. Follow-up studies at later ages including larger samples should substantiate our results and allow us to study the possible influence of common complications of a type 1 diabetic pregnancy, such as neonatal macrosomia and maternal hypertensive disease or poor glycemic control during pregnancy.

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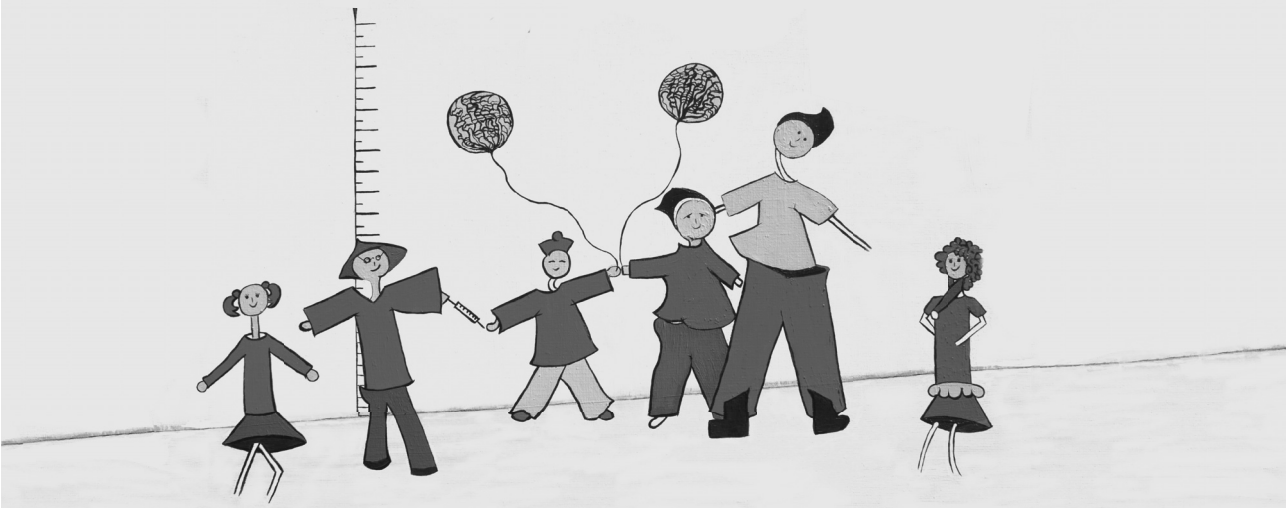
CHAPTER

8

Summary and discussion



SUMMARY

Maternal and neonatal outcome of type 1 diabetic pregnancy has improved considerably over the last decades, mainly due to improvements of glycemic control and of obstetric and neonatal care.[1] However, despite these improvements, pregnancy in women with type 1 diabetes still remains a high risk situation for both the mother and child. Several studies in offspring of diabetic mothers have shown that intrauterine exposure to maternal diabetes may be associated with an increased risk of short term neonatal complications,[2] as well as long term cardiovascular, metabolic and neurocognitive effects.[3,4] However, results from previous follow-up studies on long term outcome in the offspring are inconclusive, and most of these studies were performed in mixed cohorts of pregestational and gestational diabetic women and in children who were born over 20 years ago, when prepregnancy care and glycemic control during pregnancy were far less optimal.

In a previous nationwide study, Evers et al.[5] showed that current prepregnancy care and glycemic control during type 1 diabetic pregnancy were near-optimal in The Netherlands, with 70% preconceptional folic acid supplementation, 84% planned pregnancies, and a near-normal mean HbA1c level during pregnancy of 6.2%. Although this is apparently still not good enough to prevent maternal complications and short term neonatal morbidity (9% congenital malformations, 32% preterm birth, 45% macrosomia (birth weight >90th percentile) and 64% neonatal hypoglycemia),[5-7] one may hypothesize that this may nevertheless be good enough to prevent or lower the incidence of long term adverse effects on the development in the offspring. To investigate the long term effects of current care and treatment in type 1 diabetic pregnancies in The Netherlands, we conducted a follow-up study at school age in the offspring of this nationwide cohort of women with type 1 diabetes. From 313 children who were eligible for follow-up, 246 participated in one or more parts of this follow-up study. Because of the lack of recent age-specific reference data for many of the measurements that were performed, we invited randomly selected offspring of non-diabetic women without severe maternal disease during pregnancy to participate in a control group. These children were born at the University Obstetric Center, Utrecht, The Netherlands, in the same period as the children from the study group. Seventy-nine children participated in the control group.

The objectives of this study were to evaluate various aspects of the development at school age in offspring of diabetic mothers (ODM), and to investigate whether these differed from those in the reference population. An overview of the different aspects of development that were investigated is provided in **chapter 1**. Furthermore, we wanted to investigate the possible influence of common complications of type 1 diabetic pregnancy (such as poor maternal glycemic control during pregnancy and neonatal macrosomia) on the developmental outcome in the offspring.

In **chapter 2** we described body composition, prevalence of overweight and insulin resistance in our cohort of participating ODM. Anthropometric measurements were performed at 6-8 years of age in 213 ODM, and HOMA insulin resistance was determined from a fasting blood sample in 155 of them. We compared the results to national reference data, and we additionally studied BMI standard deviation score (SDS) growth trajectories during childhood. The prevalence of childhood overweight in our study population (19.0%) was not significantly different from that in the Dutch reference population (15.5%),[8] and mean HOMA insulin resistance in the study group was comparable to that in a recent study of healthy 7 year old Dutch children.[9] However, ODM who were macrosomic at birth (birth weight >90th percentile) had a higher BMI and showed twice as much overweight as compared to ODM who were born with a birth weight appropriate for gestational age (26.0 vs 12.0%). Multiple logistic regression analysis with possible risk factors for the development of childhood overweight showed that neonatal macrosomia and maternal overweight were independent predictors of childhood overweight in ODM. We further found that ODM who had developed childhood overweight showed a significantly higher HOMA insulin resistance compared with ODM with a normal weight at 6-8 years of age, and that they showed an increase in BMI SDS starting already at 6 months of age. We concluded that in type 1 diabetic women with adequate glycemic control during pregnancy, long term effects on body composition in their offspring at school age seem limited and relate mainly to macrosomia at birth. Possible targets for prevention of childhood overweight are fetal macrosomia, maternal overweight, and an increase in BMI SDS during the first years of life.

In **chapter 3** we investigated the occurrence of other cardiovascular and metabolic disturbances in ODM. We compared BMI, blood pressure, parameters of fasting glucose regulation and lipid metabolism, and occurrence of components of the metabolic syndrome (overweight, hypertension, impaired fasting glucose, dyslipidemia) in ODM with those in the control group. Parameters of fasting glucose regulation and lipid metabolism and the prevalence of components of the metabolic syndrome did not significantly differ between ODM and controls. Despite the facts that blood pressure was within the normal range and the prevalence of hypertension in ODM was not higher compared with controls, systolic blood pressure was slightly, but significantly, higher in ODM. ODM who were born macrosomic showed no differences in blood pressure or parameters of fasting glucose regulation and lipid metabolism compared with appropriate-for-dates ODM, despite a higher mean BMI and a higher prevalence of overweight. There were also no significant differences within the ODM group regarding preterm birth or poor maternal glycemic control during pregnancy, except for a significantly higher mean HbA1c level at follow-up in children of mothers with poor glycemic control (mean HbA1c >7.0%) during first trimester of pregnancy compared with children of mothers with better glycemic control. We concluded that current care and treatment of pregnant women with type 1 diabetes result in a cardiovascular and metabolic outcome in

the offspring at 6-8 years of age that is largely comparable to that in children of non-diabetic women, and that the influence of preterm birth, neonatal macrosomia and level of maternal glycemic control during pregnancy on cardiometabolic outcome in ODM is limited.

Although there is evidence from previous studies in offspring of diabetic women that they are at risk for later cardiometabolic morbidity,[4] the underlying mechanisms are not fully understood. Besides a direct effect of maternal, and thereby fetal, hyperglycemia on organ development,[10] other mechanisms such as epigenetic 'programming' of the hypothalamo-pituitary-adrenal (HPA) axis or the immune system may be involved.[11] There is also increasing evidence that cardiometabolic disorders are associated with and preceded by a mild chronic inflammatory state, demonstrating the interaction between the metabolic and the immune system.[12] However, studies regarding HPA-axis function in offspring of diabetic women had not yet been performed and little is known about inflammatory activity at later age. In **chapter 4** we described immune and HPA-axis function in ODM at school age. We measured immune function by means of *in vitro* mitogen-induced pro- and anti-inflammatory cytokine production, and HPA-axis function by means of basal and dexamethasone-suppressed salivary cortisol parameters in 155 ODM, and compared these measurements to those in 67 controls. Mean level of high sensitivity CRP (a marker for inflammation) and HPA-axis function did not significantly differ between ODM and controls, but ODM showed a higher production of pro-inflammatory TNF-α and IL-8, and a lower production of IP-10 (a chemokine) and anti-inflammatory IL-5. Since a balance exists between pro-inflammatory and anti-inflammatory components, these findings may indicate the first signs of a pro-inflammatory phenotype in ODM at school age. We suggest that this may be the result of programming of the immune system in ODM and that this may render them susceptible to effects of prolonged inflammation, which in turn may put them at risk for the development of cardiometabolic diseases at later age. We further found that poorer maternal glycemic control during pregnancy and neonatal macrosomia were not related to a pro-inflammatory phenotype or altered HPA-axis function in ODM at school age.

Since human brain development is a dynamic process that continues throughout all stadia of pregnancy and into the postnatal period, the developing fetal brain may also be susceptible to adverse long term effects of the intrauterine metabolic disturbances in type 1 diabetic pregnancy. However, previous studies on neurocognitive outcome in ODM have been inconclusive.[3] In **chapter 5** we investigated neurocognitive functioning in our cohort of ODM by comparing intellectual functioning, working memory, visual-motor integration abilities and several aspects of information processing with those in the control group. Additionally, we investigated possible influences of neonatal macrosomia, neonatal hypoglycemia, maternal hypoglycemia and poorer maternal glycemic control during pregnancy on neurocognitive outcome in ODM. Overall intellectual functioning (estimated total IQ) was normal in ODM,

and total IQ or verbal and nonverbal intellectual functioning were not significantly lower in ODM compared with controls. However, ODM showed significantly poorer performance on working memory, visual-motor integration abilities and several aspects of information processing, mainly due to a lower mean gestational age at birth. Previous studies have shown that poorer performance on these domains may be related to learning difficulties,[13,14] but the children in our study were yet too young to investigate their academic achievement. Severe maternal hypoglycemic episodes during pregnancy and neonatal macrosomia were not related to poorer neurocognitive functioning in ODM, but poorer maternal glycemic control during pregnancy and severe neonatal hypoglycemia (glucose <2.0 mmol/l) were related to poorer neurocognitive functioning at 6-7 years of age.

In type 1 diabetic pregnancy, fetal hyperinsulinemia may lead to fetal hypertrophic cardiomyopathy (HCM; mainly interventricular septal hypertrophy).[15] HCM can be demonstrated in 25-45% of newborn ODM and may be associated with functional cardiac changes during pregnancy as well as in the neonatal period.[16-18] Since ODM have been shown to be at increased risk for later cardiovascular morbidity,[4] one might hypothesize that they might also be at risk for alterations in cardiac function. However, studies on cardiac structure or function in ODM at school age had not yet been performed. Thirty ODM who lived near our hospital underwent full echocardiographic evaluation and results were compared to those in an age and birth weight percentile matched control group of 30 children of non-diabetic mothers (**chapter 6**). Cardiac dimensions and systolic and diastolic function in ODM at 7 years of age were comparable with those in controls. We did find, however, a slightly higher systolic blood pressure in ODM compared with controls, which was still within the normal range but may be of influence on future cardiac function. HCM was present at birth in three out of 30 ODM, but had resolved without any long term sequelae at 7 years of age. Neonatal macrosomia and poorer maternal glycemic control during pregnancy were not related to cardiac outcome at follow-up. We concluded that cardiac function at 7 years of age in offspring of women with type 1 diabetes is reassuring.

Since atherosclerosis is one of the underlying pathologies of cardiovascular disease, we hypothesized in **chapter 7** that precursors of atherosclerotic cardiovascular disease might be present in ODM. Non-invasive measurements of vascular characteristics (carotid artery intima-media thickness and distensibility) were performed using a novel ultrasound-based technology in a small sample of ODM and controls (n=19 for both groups). We found that mean carotid artery intima-media thickness and distensibility did not significantly differ between ODM and controls. Although the small sample size does not allow us to make firm conclusions, these first results seem favorable.

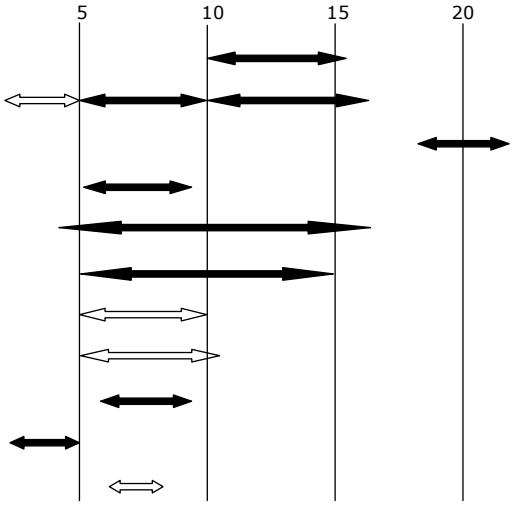
DISCUSSION

It has been concluded from the previous study on pregnancy outcome in women with type 1 diabetes that near-normal glycemic control during pregnancy was not good enough to prevent perinatal and neonatal complications.[5] The results from this follow-up study in the offspring of these women showed that gross developmental outcome at 6-8 years of age is reassuring. Prevalence of childhood overweight in ODM was comparable with that in the Dutch reference population, and prevalence of hypertension, insulin resistance and dyslipidemia (components of the metabolic syndrome) was not higher compared with those in age-matched control children of non-diabetic mothers. Furthermore, overall intelligence and cardiac function in ODM were normal and comparable with those in the control group. Based on these favorable results and on the fact that several previous studies have shown adverse effects of diabetic pregnancy on the offspring's health already at young age (Figure 1), we might conclude that current care and treatment regimens in pregnant women with type 1 diabetes may be good enough to prevent some of the long term adverse effects on the development in the offspring.

However, our results did show some alterations in more subtle developmental parameters in ODM. As some of these parameters may underlie or precede some of the above mentioned gross outcome measures, this may indicate that the ODM from our cohort may nevertheless be at risk for health and/or neurocognitive problems at later age. ODM showed a slightly higher systolic blood pressure and a higher pro-inflammatory cytokine response compared with controls. Blood pressure was still within the normal range, but this finding may nonetheless be indicative of early maladaptive programming of renal or vascular mechanisms as has been suggested by others.[4] Furthermore, the higher pro-inflammatory cytokine response in ODM may be a first sign of a pro-inflammatory phenotype due to programming of the immune system during pregnancy. This may render them susceptible to later cardiometabolic morbidity, since there is increasing evidence that cardiometabolic disorders are associated with and preceded by a mild chronic inflammatory state.[12,19] Whether or not epigenetic mechanisms are involved in programming of these processes may be subject of future research.

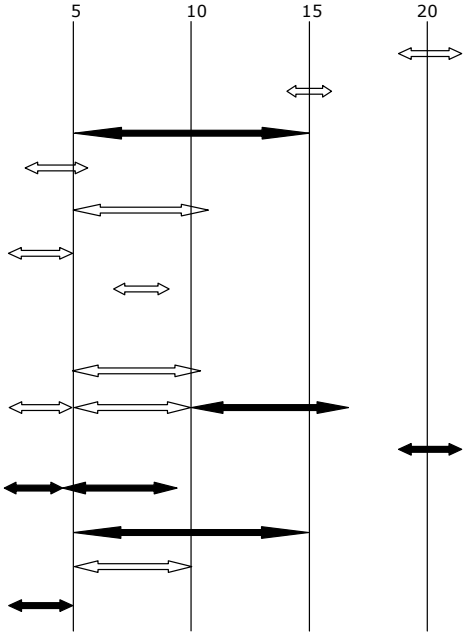
We found that the prevalence of childhood overweight was twice as high in ODM who were macrosomic at birth (birth weight >90th percentile) compared with those with a birth weight appropriate for gestational age. Other cardiometabolic, inflammatory and neurocognitive parameters were comparable between macrosomic and non-macrosomic ODM. However, because of the high prevalence of neonatal macrosomia in type 1 diabetic pregnancies and its clear association with childhood overweight and other long term health risks,[20] we suggest that prevention of neonatal macrosomia remains important. Previous advances, including insulin delivery by continuous subcutaneous infusion, have failed to reduce the

A. Higher BMI / overweight



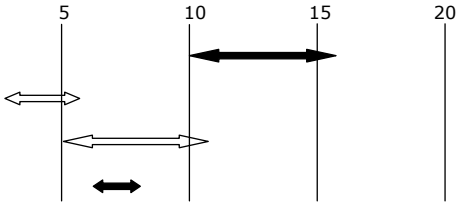
Cho et al.[31]	99 T1/G, 80 Co ('75-'82)
Silverman et al.[32]	124 T1/2/G ('77-'83)
Clausen et al.[33]	160 T1, 128 Co ('78-'85)
Plagemann et al.[34]	31 T1 ('80-'90)
Kostalova et al.[35]	72 T1/2/G, 66 Co ('80-'96)
Weiss et al.[36]	75 T1, 49 Co ('82-'91)
Rodrigues et al.[37]	17 T1, 18 Co ('85-'89)
Manderson et al.[38]	6 1 T1, 57 Co ('90-'96)
Hunter et al.[39]	17 T1, 15 Co ('92-'97)
Buinauskiene et al.[40]	51 T1, 109 Co ('97-'99)
Rijpert et al.[41]	**213 T1, 79 Co ('99-'00)**

B. Impaired fasting glucose regulation (IFGR) / impaired glucose tolerance (IGT)



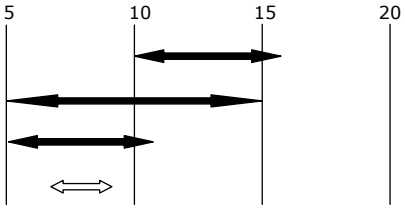
Clausen et al.[42] (IFGR)	160 T1, 128 Co ('78-'85)
Kostalova et al.[35] (IFGR)	21 T1/2/G, 21 Co ('80-'96)
Weiss et al.[36] (IFGR)	75 T1, 49 Co ('82-'91)
Pirkola et al.[43] (IFGR)	16 T1, 25 Co ('90-'95)
Manderson et al.[38] (IFGR)	61 T1, 57 Co ('90-'96)
Buinauskiene et al.[40] (IFGR)	51 T1, 109 Co ('97-'99)
Rijpert et al. (IFGR)	**155 T1, 67 Co ('99-'00)**
Persson et al.[44] (IGT)	53 T1 ('69-'72)
Silverman et al.[32] (IGT)	88 T1/2/G, 80 Co ('77-'83)
Clausen et al.[42] (IGT)	160 T1, 128 Co ('78-'85)
Plagemann et al.[45] (IGT)	129 T1 ('80-'90)
Weiss et al.[36] (IGT)	75 T1, 49 Co ('82-'91)
Hunter et al.[39] (IGT)	17 T1, 15 Co ('92-'97)
Buinauskiene et al.[40] (IGT)	51 T1, 109 Co ('97-'99)

C. Higher blood pressure



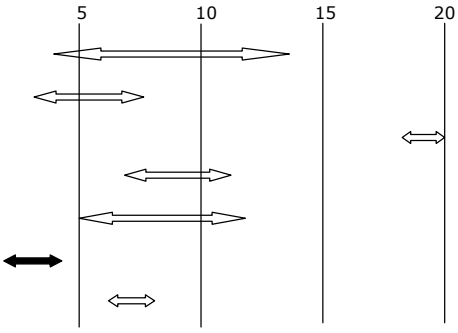
Cho et al.[31]	99 T1/G, 80 Co ('75-'82)
Pirkola et al.[43]	16 T1, 25 Co ('90-'95)
Manderson et al.[38]	61 T1, 57 Co ('90-'96)
Rijpert et al.	**213 T1, 79 Co ('99-'00)**

D. Altered lipid metabolism



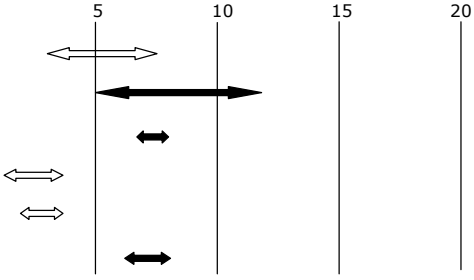
Cho et al.[31]	99 T1/G, 80 Co ('75-'82)
Weiss et al.[36]	75 T1, 49 Co ('82-'91)
Manderson et al.[38]	61 T1, 57 Co ('90-'96)
Rijpert et al.	**155 T1, 67 Co ('99-'00)**

E. Lower IQ



Cummins et al.[22]	51 T1/2/G ('64-'72)
Persson et al.[23]	64 T1 ('69-'72)
Nielsen et al.[46]	227 T1/2/G, 736 Co ('76-'84)
Rizzo et al.[47]	138 T1/2/G, 83 Co ('77-'83)
Ornoy et al.[48]	57 T1/T2, 57 Co ('82-'87)
Yamashita et al.[49]	33 T1/2/G, 34 Co ('87-'89)
Rijpert et al.	**213 T1, 79 Co ('99-'00)**

F. Poorer neurocognitive functioning



Petersen et al.[50]	84 T1, 86 Co ('76-'80)
Ornoy et al.[48]	57 T1/T2, 57 Co ('82-'87)
Stenninger et al.[25]	18 T1, 30 Co ('86-'88)
Sells et al.[51]	109 T1, 90 Co ('88-'91)
Kowalczyk et al.[52]	19 T1, 20 Co ('96-'97)
Rijpert et al.	**212 T1, 79 Co ('99-'00)**

Figure 1. Overview of several studies in offspring of women with type 1 diabetes. Arrows represent the age range (in years) of the children investigated in the mentioned studies. Black arrows represent positive (i.e. the presence of) findings, white arrows represent negative (i.e. the absence of) findings. The number of participants, years of birth and type of maternal diabetes (T1/2/G: type 1, type 2 and/or gestational diabetes) are provided for each study. Co: controls.

risk of macrosomia.[21] New strategies, focusing on reducing postprandial hyperglycemic spikes during second and third trimester of pregnancy, may be more promising. Continuous glucose monitoring (used as a tool to facilitate patient education and shared problem solving, supplementary to standard care of intermittent self monitoring of blood glucose levels) has recently been shown to improve maternal glycemic control during pregnancy, to lower birth weight and to reduce the risk of neonatal macrosomia.[22] Advances in real-time continuous glucose monitoring and the development of closed-loop systems (which are based on the combination of a continuous glucose monitor, a control algorithm, and an insulin pump) may offer additional tools to aim for further reduction of neonatal macrosomia.

Besides fetal macrosomia, preterm birth is also a common complication of type 1 diabetic pregnancy and occurs in approximately 30% of these pregnancies.[5] Although most preterm deliveries in type 1 diabetic pregnancies occur due to a medical cause (such as maternal preeclampsia or fetal distress) and therefore will be hard to prevent, obstetricians should keep in mind that preterm birth, even late preterm birth, may have long term neuropsychological consequences for the child. As fetal macrosomia may be an underlying factor in fetal distress, prevention of macrosomia may, at least in part, contribute to prevention of preterm birth and related neurocognitive deficits in ODM.

Results from previous studies regarding long term effects of neonatal hypoglycemia in ODM are inconsistent.[23-26] In our study, neonatal hypoglycemia was related to poorer performance on some specific neurocognitive functioning domains. Although additional information concerning hypoglycemic episodes (such as duration and frequency) is lacking, we believe these findings emphasize the importance of close monitoring of neonatal blood glucose levels to avoid neonatal hypoglycemia.

Extrapolating Freinkel's theory on fuel-mediated teratogenesis,[10] one might expect less favorable developmental outcome in offspring of diabetic mothers who had poorer glycemic control during pregnancy. Indeed, we found that HbA1c levels were higher and estimated total IQ was lower in children of mothers with a mean HbA1c level >7.0% during first trimester of pregnancy. Poorer maternal glycemic control during pregnancy was also related to poorer visual-motor integration abilities in the offspring at school age (which may be associated with learning difficulties). However, all other outcome parameters in the offspring were not related to the level of maternal glycemic control during pregnancy. A possible explanation could be the fact that overall glycemic control during pregnancy in our cohort of type 1 diabetic women was good, meaning that relatively few women had poor glycemic control. However, it should be mentioned that cut-off points concerning mean maternal HbA1c levels are arbitrary and are not reliable indicators of level of glycemic control during pregnancy, because normal ranges of HbA1c are varying (decreasing) throughout pregnancy.[27] Furthermore, comparison with continuous glucose monitoring data has shown that maternal HbA1c level does not

reflect the complexities of glycemic control in pregnant diabetic women.[28] Therefore, HbA1c may not be an accurate tool to describe maternal glycemic control during pregnancy.

The findings from this study emphasize the importance of strict glycemic control during pregnancy, as near-normal glycemic control in pregnant women with type 1 diabetes seem to prevent some of the adverse effects on later development in the offspring. However, the price to pay when aiming for strict glycemic control is an increase in severe maternal hypoglycemic events, which may induce severe maternal morbidity and even mortality.[5,29] The goal of pregnant diabetic women and their attending physicians should therefore be to achieve the lowest HbA1c level possible without excessive hypoglycemia.[30]

This follow-up study is a valuable addition to the previous literature, as it provides important developmental data of a large nationwide cohort of children at school age of type 1 diabetic women with adequate glycemic control during pregnancy. Furthermore, we investigated the children within a small age range and compared their outcome with that of an age-matched control group of children of non-diabetic mothers. The study group described in this study was a representative sample of the total cohort of ODM that was eligible for follow-up at the start of the study, as maternal and neonatal characteristics in the participating group were overall comparable to those in the non-participating group. However, every study design has its limitations. Limitations of this study were the facts that the control group was not as large as the study group and that parents from the control group were higher educated than parents from the study group. Although these factors can be adjusted for in statistical analyses, the control group may not reliably represent the normal population. Furthermore, because of the young age of the participating children, we were only able to investigate basal, non-invasive physiological parameters. It would be of additional value to perform for example a glucose tolerance test at a later age to evaluate the response after a glucose load, or a stress-test to evaluate the stress-induced HPA-response, which may provide more detailed information on physiological processes in ODM.

CONCLUSIONS

- ODM who were macrosomic at birth showed twice as much childhood overweight compared with those with a birth weight appropriate for gestational age.
- Possible targets for prevention of childhood overweight in ODM are fetal macrosomia, maternal overweight, and an increase in BMI SDS during the first years of life.
- ODM showed poorer performance on several specific aspects of neurocognitive functioning which may be related to learning difficulties, mainly due to a lower mean gestational

age at birth. Obstetricians should keep in mind that preterm birth, even late preterm birth, may have long term neuropsychological consequences for the child.

- Overall intelligence, fasting glucose regulation, lipid metabolism, cardiac function, vascular characteristics and prevalence of childhood overweight and other components of the metabolic syndrome in ODM at 6-8 years of age were comparable with those in offspring of non-diabetic mothers. Near-optimal glycemic control during pregnancy in women with type 1 diabetes may therefore prevent, or at least lower, the incidence of adverse effects on later development in the offspring.
- However, further follow-up should show whether the subtle cardiovascular and immunological differences found in our study (slightly higher systolic blood pressure and higher pro-inflammatory cytokine response) may nonetheless be indicative of later cardiometabolic morbidity in ODM. We suggest that in the mean time the goal of pregnant diabetic women and their attending physicians should be to achieve the lowest HbA1c level possible, without increasing the incidence of severe maternal hypoglycemia.
- Severe maternal hypoglycemia during pregnancy has no proven adverse effects on later neurocognitive functioning in the offspring. However, severe neonatal hypoglycemia (which has a high incidence in the direct neonatal period) has been found to influence later neurocognition and should therefore be avoided.
- Maternal HbA1c during pregnancy does poorly relate to developmental outcome measures in the offspring, possibly because it is not a reliable indicator of glycemic control during pregnancy.

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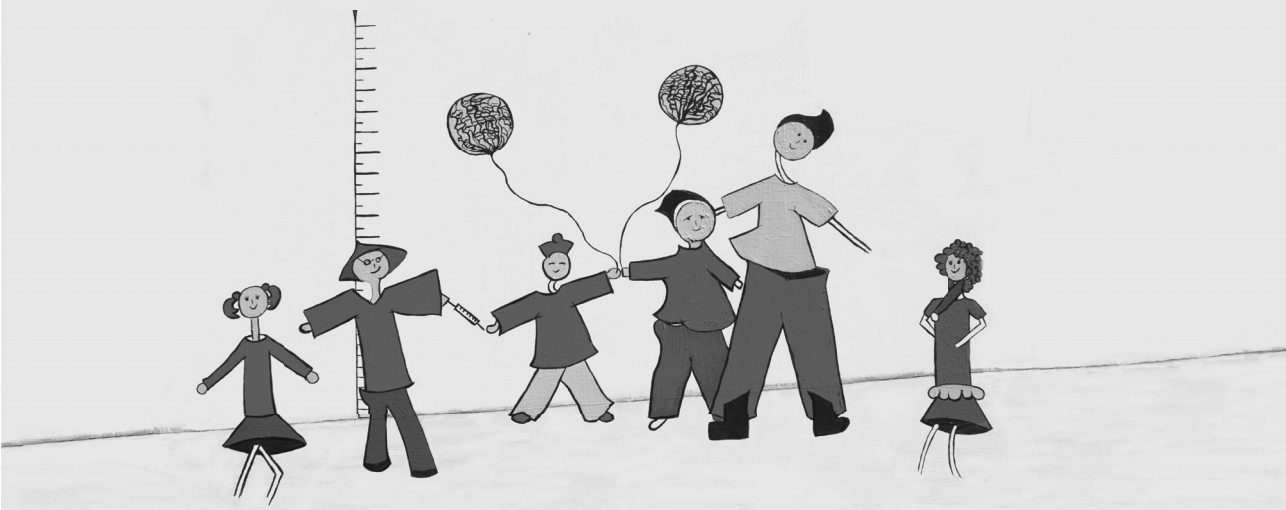
Samenvatting in het Nederlands

(SUMMARY IN DUTCH)



SAMENVATTING

Type 1 diabetes mellitus is een auto-immuun ziekte die veroorzaakt wordt door vernietiging van de insulineproducerende cellen uit de alvleesklier. Patiënten met type 1 diabetes moeten zichzelf daarom insuline toedienen om glucose (suiker) uit het bloed op te kunnen nemen voor de stofwisseling. In Nederland hebben ongeveer 4,5 op de 1000 vrouwen in de leeftijdscategorie van 15 tot 44 jaar type 1 diabetes. Vóór de uitvinding in 1920 van insulinebehandeling konden vrouwen met type 1 diabetes meestal niet zwanger worden, en werden ze al zwanger dan kwamen ze vaak tijdens die zwangerschap te overlijden. De laatste decennia zijn de uitkomsten van een zwangerschap die gecompliceerd wordt door type 1 diabetes aanzienlijk beter geworden, vooral door verbeteringen in de behandeling van diabetes en in de verloskundige en neonatale zorg. Ondanks deze vooruitgang is er echter nog steeds een hoog risico op complicaties voor zowel moeder als kind. Blootstelling van het kind in de baarmoeder aan diabetes van moeder geeft een grotere kans op complicaties zoals aangeboren afwijkingen, vroeggeboorte en macrosomie (een geboortegewicht groter dan de 90^{e} percentiel voor de zwangerschapsduur). Daarnaast hebben verschillende onderzoeken aangetoond dat een diabetische zwangerschap ook effecten kan hebben op latere cardiovasculaire, metabole en neurocognitieve aspecten van de ontwikkeling van het kind (cardiovasculair: met betrekking tot het hart- en vaatstelsel, metabool: met betrekking tot de stofwisseling, neurocognitief: met betrekking tot de hersenfuncties). Kinderen van moeders met type 1 diabetes hebben bijvoorbeeld een hoger risico op het ontwikkelen van overgewicht, hypertensie en type 2 diabetes. De resultaten van deze onderzoeken naar lange termijn effecten zijn echter niet eenduidig en de meeste onderzoeken zijn uitgevoerd in gemengde groepen van vrouwen met type 1, type 2 en/of zwangerschapsdiabetes. Ook zijn de kinderen in deze onderzoeken vaak meer dan 20 jaar geleden geboren, toen de zorg voorafgaand aan en tijdens de zwangerschap van vrouwen met type 1 diabetes minder goed was dan nu.

Een landelijk onderzoek naar type 1 diabetes en zwangerschap, uitgevoerd door Inge Evers en collega's, heeft laten zien dat de huidige zorg voorafgaand aan de zwangerschap en de glucoseregulatie tijdens de zwangerschap van vrouwen met type 1 diabetes zo goed als optimaal zijn. De meeste vrouwen bleken goed op hun zwangerschap voorbereid te zijn: 84% van de zwangerschappen waren gepland, 70% van de vrouwen gebruikte foliumzuur vóór de conceptie en het gemiddelde HbA1c (een maat voor de glucoseregulatie) tijdens de zwangerschap was 6.2% (de streefwaarde tijdens de zwangerschap is <7.0%). Ondanks deze goede voorbereiding en behandeling bleken er toch veel meer complicaties bij zowel moeder als kind voor te komen: 13% van de moeders kreeg pre-eclampsie (zwangerschapsvergiftiging), bij 44% van de moeders werd een keizersnede uitgevoerd, 9% van de kinderen had een aangeboren afwijking, 32% van de kinderen werd te vroeg geboren (bij een zwangerschapsduur korter dan 37 weken), 45% van de kinderen was macrosoom en 64% van de pasgeborenen maakte een hypoglycemie door (te laag glucosegehalte). Eén van de

conclusies van dat onderzoek was dat bijna goed (voor wat betreft de glucoseregulatie tijdens de zwangerschap) toch nog niet goed genoeg is om korte termijn complicaties bij moeder en kind te voorkomen. Desondanks is het denkbaar dat deze verbeteringen wel goed genoeg zijn om nadelige effecten op de lange termijn ontwikkeling van het nageslacht te voorkomen.

Om deze lange termijn effecten te onderzoeken, hebben we een vervolgonderzoek uitgevoerd onder de kinderen van de moeders met type 1 diabetes die aan het eerdere landelijke onderzoek hebben meegedaan. Van de 313 kinderen die in aanmerking kwamen, hebben 246 aan één of meerdere onderdelen meegedaan (de studiegroep). Omdat recente leeftijdsspecifieke referentiegegevens voor veel van de metingen in dit onderzoek ontbreken, hebben we ook kinderen van moeders zonder diabetes onderzocht. De kinderen uit deze controlegroep waren geboren in het Universitair Verloskundig Centrum van het UMC Utrecht en waren even oud als de kinderen uit de studiegroep. Negenenzeventig kinderen hebben deelgenomen aan de controlegroep.

Het doel van dit vervolgonderzoek was om verschillende aspecten van de ontwikkeling op 6-8 jarige leeftijd van kinderen van moeders met type 1 diabetes in kaart te brengen en te onderzoeken of deze verschilden ten opzichte van de referentiepopulatie. In **hoofdstuk 1** worden de betreffende aspecten van de ontwikkeling toegelicht. Daarnaast wilden we onderzoeken wat de mogelijke invloed was van veel voorkomende complicaties van een type 1 diabetische zwangerschap, zoals een slechte glucoseregulatie en macrosomie, op de ontwikkeling van de kinderen.

De resultaten betreffende de lichaamsbouw, de mate van insulineresistentie en de prevalentie (het vóórkomen) van overgewicht bij 213 kinderen uit de studiegroep staan beschreven in **hoofdstuk 2**. Daarnaast brachten we het verloop van het BMI-groeipatroon in de kinderjaren in kaart (BMI: body mass index). Het percentage overgewicht in de studiegroep (19.0%) bleek niet statistisch significant hoger te zijn dan dat in de Nederlandse kinderbevolking (15.5%). Ook de mate van insulineresistentie was niet hoger in vergelijking met gegevens van een onderzoek onder gezonde 7-jarige Nederlandse kinderen. Wel bleek dat overgewicht op 6-8 jarige leeftijd tweemaal zo vaak voorkwam bij kinderen uit de studiegroep die macrosoom waren bij de geboorte (26.0%) als bij de kinderen met een normaal geboortegewicht (12.0%). Van verschillende mogelijke risicofactoren bleken macrosomie en overgewicht van moeder onafhankelijke voorspellers te zijn van overgewicht bij het kind. Verder vonden we dat kinderen die overgewicht hadden ontwikkeld op 6-8 jarige leeftijd een hogere mate van insulineresistentie hadden dan kinderen met een normaal gewicht, en dat hun groeipatroon al een stijging van het BMI vertoonde vanaf een leeftijd van ongeveer 6 maanden. Op basis van deze uitkomsten concludeerden we dat de lange termijn gevolgen van een goed gereguleerde type 1 diabetische zwangerschap op de latere lichaamsbouw van de kinderen beperkt lijken te zijn en vooral gerelateerd zijn aan macrosomie. Mogelijke aangrijpingspunten voor

de preventie van overgewicht bij kinderen van moeders met type 1 diabetes zijn macrosomie, overgewicht van moeder en een stijging van het BMI gedurende de eerste levensjaren.

In **hoofdstuk 3** hebben we verschillende cardiovasculaire en metabole parameters van de kinderen uit de studiegroep vergeleken met die van de kinderen uit de controlegroep, waaronder BMI, bloeddruk, nuchtere glucoseregulatie, vetstofwisseling en componenten van het metabool syndroom (een combinatie van overgewicht, hypertensie, verstoorde nuchtere glucosewaarde en verstoorde vetstofwisseling). Kinderen uit de studiegroep hadden gemiddeld een iets hogere systolische bloeddruk (hoewel deze nog wel binnen de normale grenzen was) dan de kinderen uit de controlegroep, maar de BMI, de nuchtere glucoseregulatie, de vetstofwisseling en de prevalentie van componenten van het metabool syndroom verschilden niet tussen de studiegroep en de controlegroep. De bloeddruk, nuchtere glucoseregulatie en vetstofwisseling van de kinderen uit de studiegroep die macrosoom waren bij de geboorte verschilden niet van die van de kinderen met een normaal geboortegewicht, ondanks het feit dat de macrosoom geboren kinderen gemiddeld een hogere BMI hadden. Opmerkelijk was dat het gemiddelde HbA1c van de kinderen van moeders die een slechte glucoseregulatie hadden tijdens het eerste trimester van de zwangerschap (gemiddeld HbA1c >7.0%) hoger was dan het gemiddelde HbA1c van kinderen van moeders met een betere glucoseregulatie. Vroeggeboorte was niet van invloed op de gemeten parameters. We concludeerden dat met de huidige behandeling van zwangere vrouwen met type 1 diabetes de cardiovasculaire en metabole ontwikkeling van de kinderen op 6-8 jarige leeftijd zo goed als gelijk is aan die van kinderen van moeders zonder diabetes, en dat de invloed van vroeggeboorte, macrosomie en de mate van glucoseregulatie tijdens de zwangerschap beperkt is.

Omdat glucose gemakkelijk de placenta passeert, zorgt een hoge glucosewaarde (hyperglycemie) van moeder voor hyperglycemie bij de foetus. Dit heeft waarschijnlijk een verstorend effect op de ontwikkeling en latere functie van verschillende organen, en wordt gezien als een mogelijke verklaring voor het hogere risico op cardiovasculaire en metabole problemen op latere leeftijd bij kinderen van moeders met type 1 diabetes. Het is echter zeer goed mogelijk dat er ook andere mechanismen een rol spelen, zoals 'programmering' tijdens de zwangerschap van het immuunsysteem (het afweersysteem) of van de hypothalamus-hypofyse-bijnier-as (Engels: hypothalamus-pituitary-adrenal, afgekort HPA). Dit hormoonsysteem is onder andere betrokken bij de stofwisseling en stressregulatie. Het immuunsysteem en de HPA-as hebben niet alleen een interactie met elkaar, maar hebben ook interacties met de stofwisseling. Daarnaast zijn er steeds meer aanwijzingen dat cardiovasculaire en metabole stoornissen (zoals het metabool syndroom) geassocieerd zijn met, en voorafgegaan worden door, een chronische milde vorm van ontsteking. Omdat ontsteking door het immuunsysteem wordt gereguleerd, is het aannemelijk dat er een relatie bestaat tussen veranderingen in het immuunsysteem en latere cardiovasculaire of metabole problemen. In **hoofdstuk 4**

onderzochten we de functie van het immuunsysteem (de immuunrespons) door de natuurlijke afweerreactie na te bootsen. We hebben de hoeveelheid ontstekingsstoffen (cytokinen) gemeten die door de immuuncellen uit het bloed worden geproduceerd. Cytokinen zijn boodschapperstoffen die zorgen voor de communicatie tussen de immuuncellen onderling en tussen de immuuncellen en andere organen. Er zijn pro-inflammatoire (ontstekingsbevorderende) en anti-inflammatoire (ontstekingsremmende) cytokinen, die elkaars productie en werking remmen. Een verstoring van het evenwicht tussen pro- en anti-inflammatoire cytokinen zou kunnen wijzen op een verstoring van de immuunrespons en zou daardoor van invloed kunnen zijn op latere cardiovasculaire of metabole problemen. De functie van de HPA-as werd onderzocht door het eindproduct van de HPA-as te meten (cortisol). Vlak na het ontwaken is er een piek in de cortisolproductie en deze is goed meetbaar in het speeksel. Een andere manier om de functie van de HPA-as te meten is de cortisolproductie te bepalen na toediening van dexamethason (kunstmatig cortisol) dat door een terugkoppelingsmechanisme zorgt voor een afname van de cortisolproductie. Verschillen in de functie van de HPA-as zouden mogelijk ook van invloed kunnen zijn op het latere ontstaan van cardiovasculaire of metabole stoornissen.

Aan dit deel van het onderzoek deden 155 kinderen uit de studiegroep en 65 kinderen uit de controlegroep mee. De functie van de HPA-as verschilde niet tussen de studiegroep en de controlegroep. We vonden wel aanwijzingen voor veranderingen in de immuunrespons bij de kinderen uit de studiegroep. Immuuncellen uit het bloed van deze kinderen lieten een hogere productie zien van de pro-inflammatoire cytokinen tumor-necrose-factor-alfa (TNF-α) en interleukine 8 (IL-8) en een lagere productie van het anti-inflammatoire cytokine IL-5. Deze bevindingen vormen waarschijnlijk een eerste aanwijzing dat er een verschuiving is opgetreden in de balans tussen de productie van pro- en anti-inflammatoire cytokinen. Dit zou het eerste teken kunnen zijn van een chronische milde vorm van ontsteking bij kinderen van moeders met type 1 diabetes, wat de kans op het ontwikkelen van cardiovasculaire of metabole stoornissen op latere leeftijd zou kunnen vergroten. Deze verandering in de immuunrespons zou het gevolg kunnen zijn van programmering van het immuunsysteem van het kind tijdens de diabetische zwangerschap. We vonden geen aanwijzingen dat macrosomie of een slechte glucoseregulatie tijdens de zwangerschap gerelateerd waren aan een veranderde functie van de HPA-as of een hogere productie van pro-inflammatoire cytokinen.

Ook de zich ontwikkelende hersenen zouden vatbaar kunnen zijn voor de effecten van een diabetische zwangerschap, omdat het dynamische proces van de hersenontwikkeling plaatsvindt gedurende de gehele zwangerschap. Resultaten van eerdere onderzoeken naar mogelijke effecten op de neurocognitieve ontwikkeling van deze kinderen zijn echter niet eenduidig. In **hoofdstuk 5** hebben we verschillende neurocognitieve functies (intelligentie, werkgeheugen, oog-hand coördinatie en verschillende aspecten van informatieverwerking) vergeleken tussen 213 kinderen uit de studiegroep en de 79 controlekinderen. Daarnaast

onderzochten we binnen de studiegroep de mogelijke invloed van de volgende factoren op het neurocognitief functioneren van de kinderen: een slechte glucoseregulatie en ernstige hypoglycemieën van moeder tijdens de zwangerschap, macrosomie, en hypoglycemie van het kind na de geboorte. De algemene intelligentie (IQ) van de kinderen in de studiegroep was normaal en het IQ, de verbale en de non-verbale intellectuele vaardigheden verschilden niet tussen de studie- en controlegroep. Wel scoorden kinderen uit de studiegroep relatief minder goed op de tests voor werkgeheugen, oog-hand coördinatie en informatieverwerking dan de controlekinderen. Dit verschil kon grotendeels verklaard worden door het feit dat de gemiddelde zwangerschapsduur van de kinderen uit de studiegroep korter was dan die van kinderen uit de controlegroep. Uit eerder onderzoek is bekend dat kinderen die te vroeg geboren worden, later een zwakkere neurocognitieve functie laten zien. Ernstige hypoglycemieën van moeder tijdens de zwangerschap en macrosomie hadden geen nadelige effecten op het neurocognitief functioneren van het kind. Daarentegen waren een slechtere glucoseregulatie tijdens de zwangerschap en het doormaken van een ernstige hypoglycemie van het kind vlak na de geboorte gerelateerd aan lagere scores op de tests voor respectievelijk oog-hand coördinatie en non-verbale intellectuele vaardigheden. Eerdere onderzoeken hebben een verband aangetoond tussen een mindere prestatie op enkele van de hierboven beschreven tests en leerproblemen op school, maar de kinderen uit ons onderzoek waren nog te jong om hier uitspraken over te doen.

In geval van een diabetische zwangerschap produceert de foetus meer insuline als reactie op de hoge glucosewaarden van moeder. Aangezien insuline ook als groeifactor werkt voor de foetus, kan dit leiden tot onder andere een verdikking van de hartspier (hypertrofische cardiomyopathie), waarbij voornamelijk sprake is van een verdikking van de wand tussen de hartkamers (het interventriculaire septum). Dit kan gepaard gaan met veranderingen in de hartfunctie van het kind tijdens de zwangerschap en in de periode na de geboorte. Hypertrofische cardiomyopathie kan bij 25-45% van de pasgeborenen van moeders met type 1 diabetes worden aangetoond, maar leidt slechts in een klein deel van deze kinderen tot klinische verschijnselen en verdwijnt weer in de eerste maanden na de geboorte. Zoals eerder vermeld, is uit eerdere onderzoeken gebleken dat kinderen van moeders met type 1 diabetes een grotere kans hebben op cardiovasculaire aandoeningen op latere leeftijd. Men zou daarom kunnen verwachten dat ze ook een grotere kans hebben op veranderingen in de hartfunctie. In ons onderzoek hebben we bij 30 kinderen uit de studiegroep en 30 kinderen uit de controlegroep het hart uitgebreid echografisch onderzocht om de dikte van de wanden te meten en de systolische en diastolische hartfunctie te bepalen. De resultaten hiervan staan beschreven in **hoofdstuk 6**. De wanddikte en de hartfunctie verschilden niet tussen de kinderen uit de studiegroep en de controlekinderen. Wel bleek ook uit dit onderzoek dat de kinderen uit de studiegroep gemiddeld een iets hogere systolische bloeddruk hadden dan de kinderen uit de controlegroep. De bloeddruk was nog wel binnen de normale grenzen,

maar het is denkbaar dat een hogere bloeddruk in de toekomst van invloed zou kunnen zijn op de hartfunctie. Bij drie kinderen uit de studiegroep was vlak na de geboorte hypertrofische cardiomyopathie aangetoond, maar we vonden bij deze kinderen geen afwijkingen meer in wanddikte of hartfunctie. Macrosomie en slechtere glucoseregulatie tijdens de zwangerschap waren niet geassocieerd met de wanddikte of hartfunctie op 6-8-jarige leeftijd.

Eén van de onderliggende aandoeningen die kunnen leiden tot latere cardiovasculaire problematiek is atherosclerose (vaatwandverkalking). In **hoofdstuk 7** hebben we bij 19 kinderen uit de studiegroep en 19 kinderen uit de controlegroep echografische metingen verricht aan de vaatwand van de halsslagaders. Op deze manier kunnen de dikte (intima-media dikte) en de elasticiteit (distensibiliteit) van de vaatwand gemeten worden op een niet-invasieve manier. Een grotere intima-media dikte (dikkere vaatwand) en een lagere distensibiliteit (stijvere vaatwand) worden gezien als vroege kenmerken van atherosclerose. De gemiddelde intima-media dikte en distensibiliteit van de halsslagaders verschilden niet tussen de kinderen uit de studiegroep en de controlekinderen. Het aantal kinderen uit de studiegroep was echter te klein om iets te kunnen zeggen over de mogelijke invloed van complicaties van een diabetische zwangerschap zoals een slechte glucoseregulatie of macrosomie. Hoewel we uit deze bevindingen geen harde conclusies kunnen trekken vanwege de kleine aantallen, lijken deze eerste resultaten geruststellend te zijn.

DISCUSSIE

Eén van de conclusies uit het eerdere onderzoek dat tijdens de zwangerschap werd uitgevoerd, was dat een bijna optimale glucoseregulatie tijdens de zwangerschap blijkbaar niet goed genoeg is om perinatale en neonatale complicaties te voorkomen. De resultaten van dit vervolgonderzoek naar de ontwikkeling van de kinderen van deze vrouwen lieten zien dat de ontwikkeling van de kinderen op 6-8 jarige leeftijd over het algemeen geruststellend is. Het percentage kinderen met overgewicht in de studiegroep was vergelijkbaar met dat in de Nederlandse kinderbevolking, en componenten van het metabool syndroom kwamen niet vaker voor dan in de controlegroep. Verder waren de algemene intelligentie en de hartfunctie van de kinderen uit de studiegroep normaal en verschilden deze niet van die uit de controlegroep. Op basis van deze gunstige resultaten en vanwege het feit dat verschillende eerdere onderzoeken al op jonge leeftijd nadelige effecten van een diabetische zwangerschap op de gezondheid van het nageslacht hebben aangetoond (zie Figuur 1 van hoofdstuk 8 [*]), suggereren wij dat de huidige zorg en behandeling van zwangere vrouwen met type 1 diabetes in Nederland mogelijk wel goed genoeg is om sommige nadelige effecten op de ontwikkeling van de kinderen te kunnen voorkomen.

Aan de andere kant echter, lieten onze resultaten wel een wat minder goede uitkomst zien op wat meer subtiele aspecten van de ontwikkeling van kinderen uit de studiegroep. Kinderen uit de studiegroep hadden een iets hogere bloeddruk en een hogere pro-inflammatoire cytokineproductie dan controlekinderen. Hoewel de bloeddruk nog wel binnen de normale grenzen was, zou dit een eerste teken kunnen zijn van programmering tijdens de zwangerschap van de werking van nieren of bloedvaten. De hogere pro-inflammatoire cytokineproductie zou een eerste teken kunnen zijn van programmering van het immuunsysteem met een milde chronische vorm van ontsteking als gevolg, waardoor deze kinderen mogelijk een grotere kans hebben op latere cardiovasculaire aandoeningen.

Overgewicht op de kinderleeftijd kwam bij de macrosoom geboren kinderen uit de studiegroep tweemaal zo vaak voor als bij de kinderen met een normaal geboortegewicht. Andere cardiovasculaire, metabole, ontstekings- en neurocognitieve uitkomstmaten waren vergelijkbaar tussen de macrosoom en niet-macrosoom geboren kinderen. Echter, vanwege het hoge percentage macrosomie onder kinderen van moeders met type 1 diabetes en de duidelijke relatie met overgewicht op latere leeftijd, is naar onze mening preventie van macrosomie toch belangrijk. Eerdere pogingen, zoals behandeling van de vrouw met behulp van een insulinepomp tijdens de zwangerschap, zijn er niet in geslaagd om het percentage macrosomie te verlagen. Nieuwe strategieën die gericht zijn op het verlagen van glucosepieken tijdens de zwangerschap zijn wellicht meer succesvol. Onlangs is er in Engeland onderzoek gedaan naar het gebruik van continue glucosemonitoring tijdens de zwangerschap naast de normale zelfcontrole door middel van vingerprikken. Uit dit onderzoek bleek dat niet alleen de glucoseregulatie verbeterde, maar ook het percentage macrosomie afnam. De verklaring hiervoor is waarschijnlijk dat patiënten door middel van continue glucosemonitoring beter inzicht krijgen in het verloop van hun glucosewaarden over de dag. Nieuwe technologieën zoals real-time continue glucosemonitoring (een continue glucosemonitor gekoppeld aan een insulinepomp) en de closed-loop systemen (de volautomatische variant van real-time continue glucosemonitoring) kunnen in de toekomst het percentage macrosomie wellicht nog verder terugbrengen.

***** Legenda bij Figuur 1: overzicht van onderzoeken naar de ontwikkeling van kinderen van vrouwen met type 1 diabetes. De pijlen stellen de leeftijdsspreiding voor van de kinderen uit de betreffende onderzoeken. Onderzoeken met zwarte pijlen vonden wel verschillen, onderzoeken met witte pijlen vonden geen verschillen. Het aantal deelnemende kinderen, de spreiding van hun geboortejaren en het type diabetes van moeder staan per onderzoek aangegeven (T1/2/G: type 1, type 2 en/of zwangerschapsdiabetes; Co: controlekinderen). Figuur A betreft onderzoeken naar BMI en/of overgewicht; figuur B betreft onderzoeken naar de nuchtere glucoseregulatie (bovenste helft) of glucosetolerantie (onderste helft); figuur C betreft onderzoeken naar de bloeddruk; figuur D betreft onderzoeken naar de vetstofwisseling; figuur E betreft onderzoeken naar de algemene intelligentie; figuur F betreft onderzoeken naar andere aspecten van neurocognitief functioneren.

Behalve macrosomie is vroeggeboorte ook een veel voorkomende complicatie van een type 1 diabetische zwangerschap. Hoewel vroeggeboorte moeilijk te voorkomen is doordat er in de meeste gevallen sprake is van een medische oorzaak (zoals pre-eclampsie of foetale nood), is het voor de betrokken artsen belangrijk om in het achterhoofd te houden dat vroeggeboorte gevolgen kan hebben voor het latere neurocognitief functioneren van het kind. Omdat macrosomie een onderliggende oorzaak van foetale nood kan zijn, zou preventie van macrosomie vroeggeboorte deels kunnen voorkomen en zo een gunstig effect kunnen hebben op de neurocognitieve ontwikkeling van de kinderen.

De uitkomsten van eerdere onderzoeken naar de lange termijn effecten van hypoglycemieën van het kind in de neonatale periode zijn niet eenduidig. In ons onderzoek vonden we een verband tussen het doormaken van een ernstige neonatale hypoglycemie en een minder goede prestatie op non-verbale intellectuele vaardigheden. Hoewel we geen aanvullende informatie hebben over bijvoorbeeld de duur en frequentie van neonatale hypoglycemieën, suggereren wij dat goede glucosecontroles bij het kind in de neonatale periode van belang zijn om hypoglycemieën te voorkomen.

Als we ervan uitgaan dat hoge glucosewaarden tijdens de zwangerschap een negatieve invloed hebben op de ontwikkeling van het kind, zou men kunnen verwachten dat kinderen van moeders met een slechtere glucoseregulatie tijdens de zwangerschap zich minder goed ontwikkelen. In ons onderzoek vonden we inderdaad dat kinderen gemiddeld een hoger HbA1c en een lager IQ hadden als de moeder een gemiddeld HbA1c van >7.0% tijdens het eerste trimester van de zwangerschap had. Ook was er een verband tussen een minder goede glucoseregulatie tijdens de zwangerschap en een minder goede oog-hand coördinatie bij het kind, wat volgens eerder onderzoek negatieve consequenties kan hebben voor de schoolse vaardigheden. Alle andere bestudeerde uitkomsten waren echter niet gerelateerd aan het niveau van glucoseregulatie tijdens de zwangerschap. Een mogelijke verklaring hiervoor is dat over het algemeen genomen de glucoseregulatie in ons cohort van vrouwen met type 1 diabetes goed was, wat betekent dat er relatief weinig vrouwen waren met een slechte glucoseregulatie tijdens de zwangerschap. Hierbij moet echter wel opgemerkt worden dat afkappunten betreffende HbA1c waarden tijdens de zwangerschap arbitrair zijn, en niet erg betrouwbaar het niveau van glucoseregulatie tijdens de zwangerschap weergeven, omdat de normaalwaardes voor HbA1c variëren (afnemen) gedurende de zwangerschap. Ook is uit eerder onderzoek met behulp van continue glucosemonitoring gebleken dat het HbA1c niet goed de complexiteit van de glucoseregulatie van zwangere vrouwen met type 1 diabetes weergeeft.

Aangezien de huidige zorg en behandeling van zwangere vrouwen met type 1 diabetes goed genoeg lijkt te zijn om tenminste sommige nadelige effecten op de ontwikkeling van de kinderen te kunnen voorkomen, lijken de bevindingen uit ons onderzoek het belang van

strikte glucoseregulatie tijdens de zwangerschap te benadrukken. Streven naar strikte glucoseregulatie tijdens de zwangerschap gaat echter gepaard met een toename van ernstige hypoglycemieën van moeder, wat verstrekkende gevolgen kan hebben zoals ziekenhuisopname en in een enkel geval zelfs fataal kan zijn. Zwangere vrouwen met type 1 diabetes en hun behandelaren zouden daarom naar een zo laag mogelijk HbA1c moeten streven zonder dat ernstige hypoglycemieën optreden.

Deze vervolgstudie levert een belangrijke bijdrage aan de reeds bestaande onderzoeken, omdat het de ontwikkeling in kaart brengt van een landelijk geboortecohort van kinderen van vrouwen met type 1 diabetes, van wie we over gedetailleerde gegevens uit de zwangerschap en de neonatale periode beschikken. De leeftijdsspreiding van de kinderen was klein, en we hebben hun ontwikkeling vergeleken met die van kinderen van dezelfde leeftijd van moeders zonder diabetes. Verder was de studiegroep uit dit onderzoek een representatieve steekproef van het totale cohort kinderen uit het vorige onderzoek, omdat de maternale en neonatale eigenschappen van de deelnemende groep over het algemeen niet verschilden van de groep die niet heeft deelgenomen. Echter, elke studieopzet heeft beperkingen. Beperkingen van dit onderzoek waren het feit dat de controlegroep niet even groot was als de studiegroep en het feit dat ouders uit de controlegroep gemiddeld hoger opgeleid waren dan ouders uit de studiegroep. Hoewel we voor deze factoren hebben gecorrigeerd in de statistische analyses, is de controlegroep mogelijk niet een betrouwbare afspiegeling van de Nederlandse bevolking. Daarnaast konden we vanwege de jonge leeftijd alleen niet-invasieve onderzoeken doen en zo alleen basale fysiologische parameters onderzoeken. Op latere leeftijd zouden we ook meer invasieve onderzoeken kunnen uitvoeren zoals een glucose tolerantietest (een suikerbelastingstest) of een stresstest om ook de stress-geïnduceerde respons van de HPA-as te meten. Op die manier kunnen we nog meer gedetailleerde informatie verkrijgen over fysiologische processen bij deze kinderen.

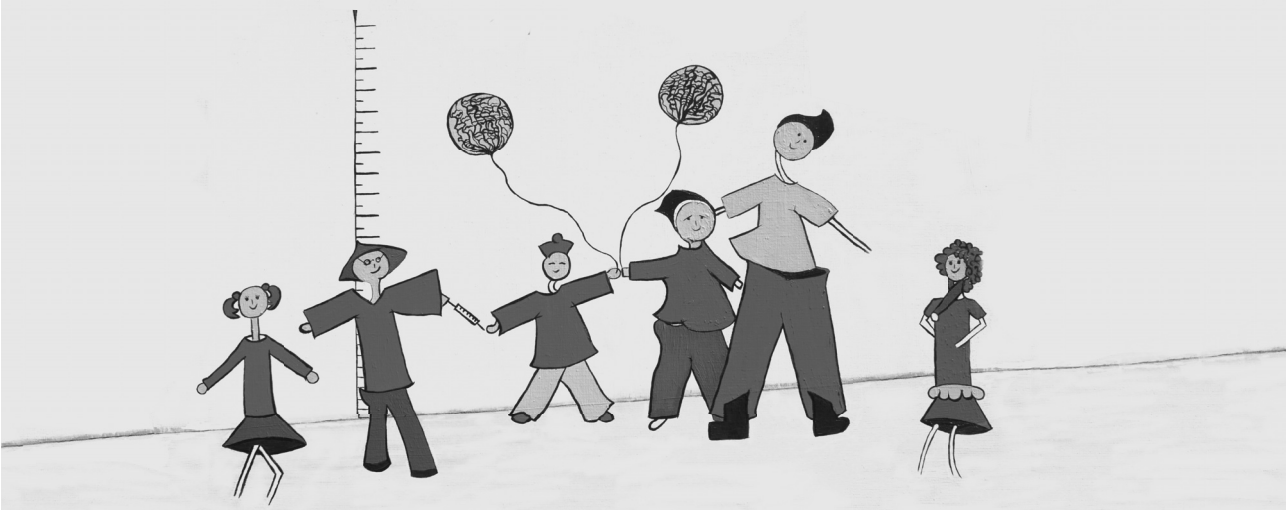
CONCLUSIES

- Bij kinderen van moeders met type 1 diabetes die macrosoom waren bij de geboorte kwam overgewicht op 6-8 jarige leeftijd tweemaal zo vaak voor als bij de kinderen met een geboortegewicht passend bij de zwangerschapsduur.
- Mogelijke aangrijpingspunten voor preventie van overgewicht bij kinderen van moeders met type 1 diabetes zijn macrosomie, overgewicht van moeder en een stijging van de BMI in de eerste jaren na de geboorte.
- Kinderen uit de studiegroep scoorden minder goed dan kinderen uit de controlegroep op enkele specifieke neurocognitieve tests, wat vooral verklaard kon worden door een gemiddeld langere zwangerschapsduur van de kinderen uit de studiegroep. De betrokken

artsen moeten zich ervan bewust zijn dat vroeggeboorte gevolgen kan hebben voor de latere neurocognitieve ontwikkeling van het kind.

- De algemene intellectuele vaardigheden, nuchtere glucoseregulatie, vetstofwisseling, hartfunctie, vaatwandeigenschappen en prevalentie van overgewicht en andere componenten van het metabool syndroom waren vergelijkbaar tussen kinderen van moeders met type 1 diabetes en kinderen van moeders zonder diabetes. Op basis daarvan suggereren we dat de goede glucoseregulatie tijdens de zwangerschap van vrouwen met type 1 diabetes zoals we die kennen in Nederland wellicht goed genoeg is om nadelige effecten op de ontwikkeling van het kind te kunnen voorkomen, of in elk geval te kunnen verminderen.
- Echter, toekomstige onderzoeken zullen moeten uitwijzen of de subtiele cardiovasculaire en immunologische veranderingen bij kinderen van moeders met type 1 diabetes (iets hogere systolische bloeddruk en hogere pro-inflammatoire cytokinerespons) niet toch de eerste tekenen zijn van cardiovasculaire gezondheidsproblemen. Wij adviseren dat tot die tijd zwangere vrouwen met type 1 diabetes en hun behandelaren zouden moeten streven naar een zo laag mogelijk HbA1c zonder dat ernstige hypoglycemieën optreden.
- Hoewel maternale hypoglycemieën tijdens de zwangerschap geen bewezen nadelige effecten hebben op het neurocognitief functioneren van het kind, heeft het doormaken van een ernstige hypoglycemie in de neonatale periode (wat vaak voorkomt bij kinderen van moeders met type 1 diabetes) wel invloed op het latere neurocognitief functioneren en zou daarom vermeden moeten worden.
- Het maternale HbA1c tijdens de zwangerschap vertoont weinig verbanden met de ontwikkelingsparameters van de kinderen op 6-8 jarige leeftijd, waarschijnlijk omdat het een niet betrouwbare weergave is van de mate van glucoseregulatie tijdens de zwangerschap.

List of abbreviations



AN(C)OVA	analysis of (co)variance
AUC_G	area under the curve with respect to ground
AUC_I	area under the curve with respect to increase
BMI	body mass index
CAR	cortisol awakening response
CD	cluster of differentiation
CDC	carotid artery distensibility coefficient
CIMT	carotid artery intima media thickness
CI	confidence interval
CO	cardiac output
'Da	peak wall motion velocity during late diastole (atrial contraction)
'De	peak wall motion velocity during early diastole
DBP	diastolic blood pressure
DS	Digit Span
DST	dexamethasone suppression test
E/A	ratio of early and late (atrial contraction) left ventricular filling speed
FS	fractional shortening
HbA1c	glycated hemoglobin
HDL	high-density lipoprotein
HOMA	homeostasis model assessment
HOMA-B	HOMA beta cell function
HOMA-IR	HOMA insulin resistance
HPA	hypothalamo-pituitary-adrenal
hsCRP	high-sensitivity C-reactive protein
IFG	impaired fasting glucose
IFN-γ	gamma-interferon
IL	interleukin
IOTF	International Obesity Task Force
IP-10	interferon-gamma-induced protein-10
IQ	intelligence quotient
IVS	interventricular septum
IVSd	interventricular septal end diastolic dimension
LDL	low-density lipoprotein
LPS	lipopolysaccharide
LVEDd	left ventricular end diastolic dimension
LVPW	left ventricular posterior wall
LVPWd	left ventricular posterior wall end diastolic dimension
MCP-1	monocytes chemotactic protein-1
MetS	metabolic syndrome

ODM	offspring of type 1 diabetic mothers
OR	odds ratio
PIQ	performance IQ
RANTES	regulated upon activation, normal T-cell expressed and secreted
'S	peak wall motion velocity during systole
S/D	ratio of systolic and diastolic pulmonary vein filling speed
SBP	systolic blood pressure
SD	standard deviation
SE(M)	standard error (of the mean)
SS	standard score
TAPSE	tricuspid annular plane systolic excursion
TIQ	total IQ
TNF	tumor necrosis factor
TR max PG	maximum tricuspid regurgitation pressure gradient
VIQ	verbal IQ
VMI	visual-motor integration
WISC	Wechsler intelligence scale for children

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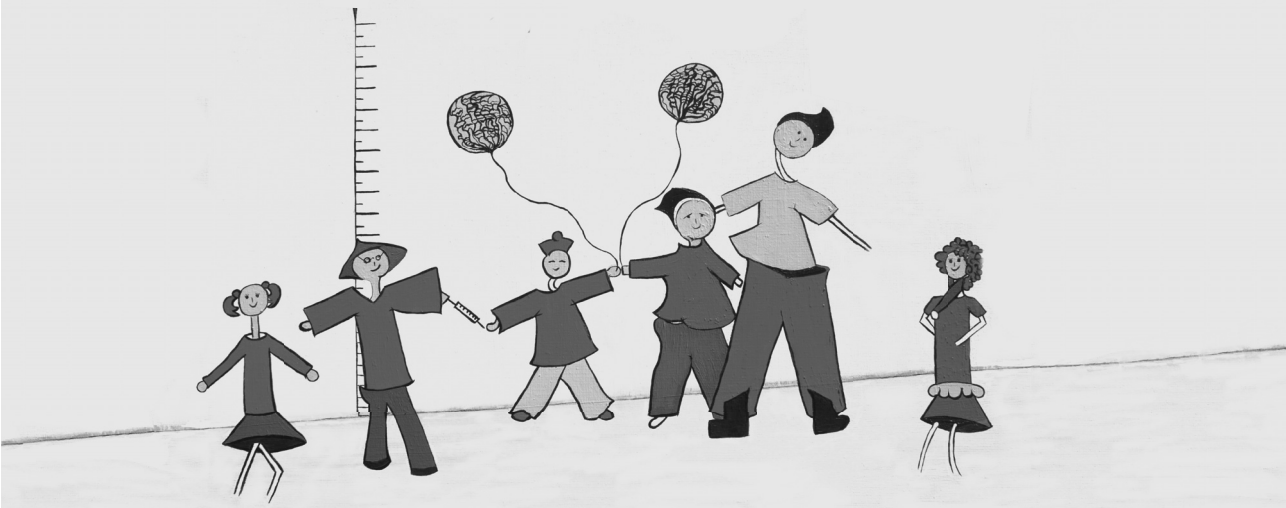

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Dankwoord

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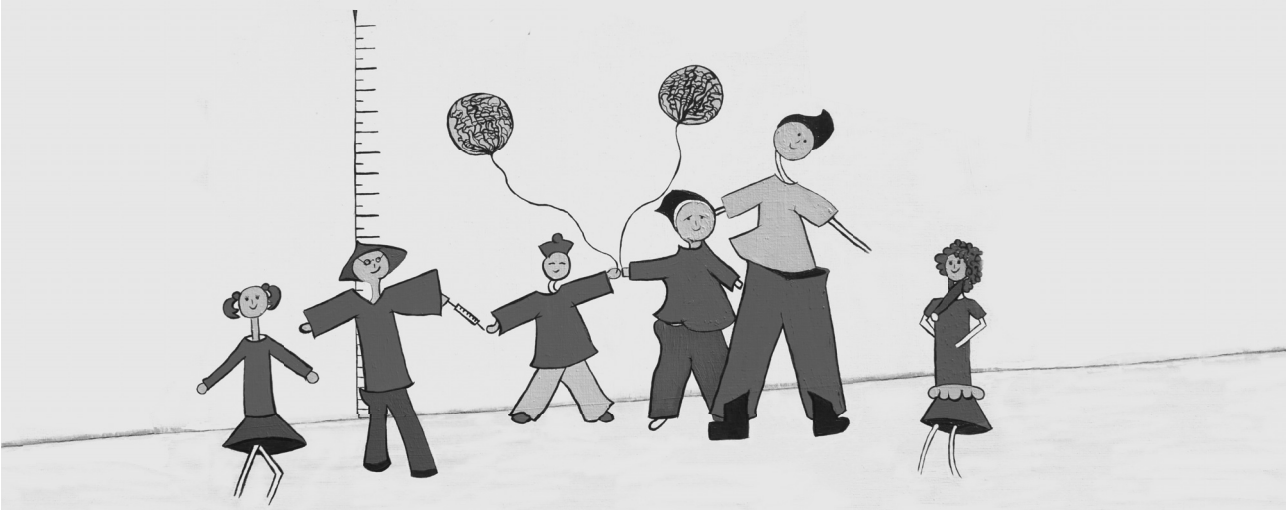
Curriculum vitae



Maarten Rijpert werd geboren op 2 juni 1977 in Roosendaal, en groeide op in Den Bommel op Goeree-Overflakkee. Hij behaalde zijn gymnasiumdiploma in 1995 aan het Erasmiaans Gymnasium in Rotterdam. Omdat hij uitgeloot was voor Geneeskunde in Nederland, studeerde hij twee jaar Geneeskunde in Gent (België). In 1997 werd hij alsnog ingeloot en begon aan zijn studie Geneeskunde in Utrecht. Zijn eerste kennismaking met wetenschappelijk onderzoek was in het vierde jaar van zijn studie. In het kader van zijn wetenschappelijke stage deed hij onderzoek naar de relatie tussen postnatale corticosteroid-toediening en proton magnetische resonantie spectroscopie van de hippocampus bij ex-prematuur geboren kinderen onder supervisie van dr. K.J. Rademaker en dr. F. Groenendaal. Zijn keuzecoschap Kindergeneeskunde bestond uit onderzoek naar meldingen van vermoedens van kindermishandeling in het Wilhelmina Kinderziekenhuis onder supervisie van dr. M.P. L'Hoir en drs. I.M.B. Russel. Na een onderbreking van een jaar, waarin hij Commissaris Onderwijs was in het bestuur van de Medische Studenten Faculteitsvereniging Utrecht "Sams", behaalde hij op 10 december 2004 zijn artsdiploma. Aansluitend deed hij enkele maanden klinische ervaring op als arts-assistent Kindergeneeskunde op de Amalia Kinderafdeling van de Isala Klinieken in Zwolle. Het onderzoek dat geresulteerd heeft in dit proefschrift startte in juni 2005 en werd uitgevoerd onder leiding van prof.dr. G.H.A. Visser, prof.dr. C.J. Heijnen, dr. I.M. Evers en dr. H.W. de Valk. Na een korte periode als arts-assistent Kindergeneeskunde in het Emma Kinderziekenhuis AMC in Amsterdam te hebben gewerkt, is hij op 4 januari 2010 aan de opleiding Kindergeneeskunde begonnen in het St. Elisabeth Ziekenhuis in Tilburg, onder supervisie van dr. J. Frenkel (opleider Wilhelmina Kinderziekenhuis Utrecht) en dr. C.C. Obihara (opleider St. Elisabeth Ziekenhuis Tilburg).