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ARTICLE

Increased metabolic risk in adolescent offspring of mothers with type 1 diabetes: the EPICOM study

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Abstract

Aims/hypothesis We aimed to investigate metabolic risk factors, insulin sensitivity and insulin secretion in adolescent offspring of mothers with type 1 diabetes compared with offspring of non-diabetic mothers.

Methods During 1993–1999, pregnancies of women with type 1 diabetes in Denmark were prospectively reported to a central registry in the Danish Diabetes Association. Data included information on maternal demography, diabetes status and pregnancy outcome. We invited 746 eligible children from this cohort (index offspring) to a follow-up examination. Control offspring were identified through The Danish Central Office of Civil Registration and matched with respect to date of birth, sex and postal code. Anthropometric measurements and blood sampling for metabolic characterisation, including an oral glucose tolerance test, were performed.

Results We examined 278 index offspring (mean age 16.7 years; range 13.0–19.8 years) and 303 control offspring (mean age 16.8 years; range 13.5–20.4 years). Index offspring had higher BMI SD score (0.44: 95% CI 0.21, 0.66) compared with controls, after adjustments for pubertal development and maternal pre-pregnancy BMI. Furthermore, index offspring had a higher prevalence of components included in metabolic syndrome and prediabetes (impaired fasting glucose and/or impaired glucose tolerance), with reduced insulin sensitivity and relative insulin secretion deficiency, compared with controls. Maternal HbA_{1c} levels in pregnancy were not directly associated with offspring metabolic outcomes.

Conclusions/interpretation Adolescent offspring of mothers with type 1 diabetes had a less favourable metabolic profile and higher frequency of prediabetes than the background population. Significant associations between these outcomes and

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maternal HbA_{1c} levels in pregnancy could not be demonstrated.
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Keywords Adolescent offspring · Beta cell function · Disposition Index · Fetal programming · Insulin sensitivity · Intrauterine hyperglycaemia · Metabolic syndrome · Prediabetes

Abbreviations

AC	Abdominal circumference
BIGTT-AIR	OGTT-derived index of acute insulin response
BIGTT-IS	OGTT-derived index of insulin sensitivity
DI	Disposition index
DBP	Diastolic BP
EPICOM	Epigenetic Genetic and Environmental Effects on Growth, Metabolism and Cognitive Functions in Offspring of Women with Type 1 Diabetes study
GDM	Gestational diabetes mellitus
HOMA-β	HOMA of insulin secretion
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
MetS	Metabolic syndrome
NGT	Normal glucose tolerance
p-glucose	Plasma glucose
SBP	Systolic BP
SDS	SD score
s-insulin	Serum insulin

Introduction

The metabolic syndrome (MetS) is a cluster of risk factors for cardiovascular disease and type 2 diabetes [1]. It is estimated that around 20–25% of the world's population meet the criteria for MetS [2]. The prevalence of the MetS increases with age [1], but along with a rising prevalence of overweight among children and adolescents MetS is becoming more prevalent in young age groups [3]. Overweight in childhood and adolescence tends to persist into adulthood and is associated with subsequent adverse health outcomes [4]. Recently, it has been suggested that environmental exposures during fetal life and early infancy may influence metabolic risk in later life [5].

The offspring of mothers with diabetes may display excess fetal growth or macrosomia [6] as initially suggested in the so-called Pedersen hypothesis [7]; maternal hyperglycaemia results in fetal hyperglycaemia, hyperinsulinaemia and thus overgrowth. Other contributing factors to fetal macrosomia are maternal overweight/obesity [8].

The potential clinical implication of intrauterine hyperglycaemia and fetal overgrowth became apparent after studies revealed that exposure to maternal diabetes could have long-term effects on offspring in terms of obesity [9–16], hypertension [10, 17] and impaired glucose metabolism [18–21]. However, a number of previous studies examined offspring of women with gestational diabetes mellitus (GDM) [9–11] and type 2 diabetes [14], where maternal obesity is a prominent feature. Studies in childhood of the offspring of women with type 1 diabetes reported an increased risk of overweight/obesity [12, 13] and inflammatory markers [22], whereas studies in adulthood also found defects in glucose metabolism [19, 21, 23]. However, two recent studies of pre-pubertal children of women with type 1 diabetes reported no direct contribution of maternal type 1 diabetes to childhood overweight [24, 25].

Studies of adolescent offspring are scarce and, to our knowledge, associations between maternal HbA_{1c} levels during pregnancy and long-term metabolic outcomes in adolescent offspring of mothers with type 1 diabetes in a prospective setting have not previously been studied.

We aimed to investigate: (1) metabolic risk factors, prevalence of the MetS and pre-diabetes (defined as the presence of impaired fasting glucose [IFG] and/or impaired glucose tolerance [IGT]) in adolescent offspring of mothers with type 1 diabetes in comparison with offspring of non-diabetic mothers; and (2) insulin sensitivity and insulin secretion in these two groups.

Methods

Study design

The Epigenetic, Genetic and Environmental Effects on Growth, Metabolism and Cognitive Functions in Offspring of Women with Type 1 Diabetes (EPICOM) study is a prospective nationwide follow-up study in Denmark focusing on the long-term effects of intrauterine diabetic environment. The EPICOM study group consists of adolescent offspring born to mothers with type 1 diabetes between the years 1993 and 1999. During this period, all pregnancies in women with type 1 diabetes in Denmark were prospectively reported to a national register in the Danish Diabetes Association. The register contains detailed information on maternal demography, diabetes status and pregnancy outcome. The women delivered in eight centres: four university hospitals (Copenhagen, Aarhus, Aalborg, Odense) and four county hospitals (Esbjerg, Fredericia, Herning, Hilleroed) with a special interest in diabetes and pregnancy. Data were collected after each delivery by one to three caregivers per centre. The inclusion criterion was delivery after 24 completed weeks of gestation. The women entering the study were all judged as having type 1 diabetes by their caregivers and were on insulin treatment

before conception. Most of the women were normal weight and the mean diabetes duration was 12 years. The coverage of the cases reported from the centres was 75–93% evaluated by alternative local data sources [6].

The current study is a follow-up of the offspring from this register (index offspring), compared with a group of offspring of non-diabetic mothers from the background population (control offspring). The study protocol was in accordance with the Declaration of Helsinki and approved by the regional ethics committee (M-20110239). Written informed consent was obtained either from the parents (if the participants were below 18 years of age) or the participants themselves.

Study participants

The Danish Diabetes Association register consists of 1,215 records of index offspring. For this follow-up study, only singletons and only the first child per mother were included (n=965). Among the cases fulfilling the inclusion criteria we were not able to identify either the mother or the child in 111 cases. Reasons for this were missing personal identification numbers (n=30) or missing offspring birth date in the Danish Diabetes Association register (n=10), unlisted addresses in The Danish Central Office of Civil Registration or residence outside of Denmark (n=40), or that either the mother or child were not alive at the time of recruitment (n=31). Furthermore, 108 women had chosen a so-called research protected status. Thus, 746 index offspring from the original cohort were eligible for the follow-up examination and invited to participate in the study (Fig. 1). Index offspring were invited with a letter addressed to the mother. Control offspring were identified through The Danish Central Civil Registration System and matched with respect to date of birth, sex and place of residence (postal code) as an indirect marker of the socioeconomic status. Between one to five control offspring per all eligible index offspring were invited to participate in the study either with a letter directly (if they were over 18 years of age) or with a letter addressed to the mother. In case of no response two reminders were sent within 6–8 weeks.

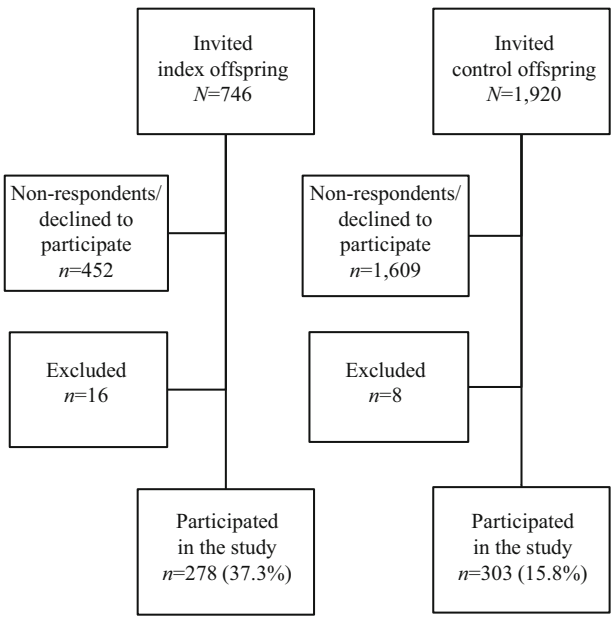

Fig. 1 Flowchart of the study

Variables

Main outcomes Height and weight SD scores (SDS) were calculated using a Danish normal reference material [26]. BMI was calculated as weight in kilograms divided by height in metres squared and BMI-SDS was calculated using the Danish normal reference curves [26]. Abdominal circumference (AC) SDS was calculated using British normal reference material [27], which provides reference curves for adolescents up to 17 years of age. AC for offspring aged 17 years and older was used directly. Indices of insulin sensitivity and secretion were derived using data obtained from the OGTT. Insulin sensitivity was evaluated by the OGTT-derived index of insulin sensitivity (BIGTT-IS) [28] and fasting-derived HOMA-IR [29]. To evaluate insulin secretion, we calculated OGTT-derived index of acute insulin response (BIGTT-AIR) [28] and the fasting-derived HOMA of insulin secretion (HOMA-β) [29]. Insulin secretion corrected for insulin sensitivity – the disposition index (DI) – was calculated as BIGTT-IS multiplied by BIGTT-AIR. Details are given in electronic supplementary material (ESM) Table 1.

We defined prediabetes and diabetes according to the WHO 1999 criteria [30]. Prediabetes was defined as the presence of IFG (fasting plasma [p-]glucose ≥6.1 and <7.0 mmol/l with 2-h p-glucose <7.8 mmol/l) and/or IGT (fasting p-glucose <7.0 mmol/l with 2-h p-glucose ≥7.8 and <11.1 mmol/l). Diabetes was defined as fasting p-glucose ≥7.0 mmol/l and/or 2-h p-glucose ≥11.1 mmol/l.

MetS in offspring <16 years of age was diagnosed according to the International Diabetes Federation 2007 criteria [31], which include abdominal obesity (assessed by sex- and age-specific AC percentiles) plus any two or more of four additional metabolic risk factors: elevated triacylglycerols ≥1.7 mmol/l, low HDL-cholesterol <1.03 mmol/l, high BP (≥130 systolic and/or ≥85 diastolic) and increased fasting plasma glucose (≥5.6 mmol/l). Offspring who were aged 16 years and older were diagnosed with the adult MetS criteria [2].

Exposure variables The main exposure variable was exposure to the intrauterine diabetic environment (defined as the offspring of women with type 1 diabetes – the index offspring).

Potential confounders and effect modifiers Offspring age and sex were included in the analyses unless the studied

outcomes were presented as age- and sex-specific SDS. Pubertal development was assessed by the Tanner method based on breast development in girls [32] and genital development and measurement of testicular volume in boys [33]. Maternal pre-pregnancy BMI was taken either from the Danish Diabetes Association register (index offspring) or was found retrospectively from the obstetric medical records (control offspring). We used peri-conceptional HbA_{1c} (first trimester HbA_{1c} and in case of missing value the latest pre-pregnancy HbA_{1c}), second trimester HbA_{1c} and the third trimester HbA_{1c} to investigate the effect of maternal glycaemic control on long-term metabolic outcomes in the offspring. All participants and their parents were asked to fill in a questionnaire addressing social aspects and education levels. Educational level of the mother was derived as the sum of years in school plus years of higher education.

The laboratory staff member responsible for the analyses of blood samples was blinded to the status of diabetes exposure in the offspring. The caregivers performing the clinical examinations were responsible for the recruitment of the participants and were, therefore, not blinded.

Clinical examination

The clinical examinations took place in three different university hospitals in Denmark (Copenhagen, Odense, and Aarhus) from April 2012 until October 2013. All procedures were identical for index offspring and control offspring and across the three centres. Participants were studied after an overnight fast and the examinations detailed below were performed.

Anthropometric measurements All measurements except height were performed three times and the mean value was used for the analyses. Height was measured to the nearest 0.1 cm without shoes with a permanently affixed stadiometer in centimetres. Weight was measured to the nearest 0.1 kg on a calibrated personal weight in kilograms. Measurement of AC was done using a tape measure to the nearest 0.5 cm midway between the arcus costae and crista iliaca after exhalation. Blood pressure was measured in the supine position after 5 min of rest using a digital BP metre (Omron 705 IT; Omron Healthcare, Hoofddorp, the Netherlands).

Biochemical testing and standard 2 h OGTT OGTT was performed with a glucose load of 1.75 g/kg body weight up to a total of 75 g. Venous plasma was drawn from an antecubital vein at 0, 30 and 120 min after glucose administration to determine plasma glucose and serum insulin levels. Furthermore, at 0 min, venous blood was drawn to measure plasma lipids and HbA_{1c}. OGTT was not performed if the child had already been diagnosed with diabetes.

Biochemical analyses

Glucose was measured in venous plasma with a hexokinase-glucose-6-phosphate dehydrogenase assay (Abbott Diagnostics, Abbott Park, IL, USA). Serum insulin was measured by ELISA using dual-monoclonal antibodies (ALPCO Diagnostics, Salem, NH, USA). Lipids were measured by enzymatic calorimetric analysis, end-up reaction (Abbott) and HbA_{1c} was measured by cation-exchange HPLC (G8 analyzer; Tosoh Bioscience, San Francisco, CA, USA). Analyses of maternal HbA_{1c} between 1993 and 1999 were measured on local assays. Correction was made to a common standard by multiplying the HbA_{1c} value with a correction factor (mean of the reference values for a standard assay divided by the mean of the reference values for the given assay). The assays were subjected to thorough centralised national quality control during this period.

Statistical analyses

Continuous variables with symmetric distribution are presented as means and SDs; continuous variables with skewed distribution are presented as medians and interquartile ranges. We fitted a linear model for each of the outcomes with index/control status as an independent variable reporting the differences between the groups with 95% CIs and p values. The assumption of normality of the residuals was checked. Data with skewed distribution were $\log_e$-transformed and relative differences between the groups are given as a percentage difference. Analyses were adjusted for sex, age (except for the SDS-corrected indices), Tanner stage and maternal pre-pregnancy BMI. Both systolic BP (SBP) and diastolic BP (DBP) were additionally adjusted for height, which is a strong predictor for BP in childhood and adolescence [34].

BIGTT-AIR and BIGTT-IS were not adjusted for sex, because sex (along with BMI) is incorporated in the calculations of these indices. Calculation of HOMA-IR and HOMA-β does not include BMI and these two variables were, therefore, additionally adjusted for offspring BMI. We analysed the effect of maternal HbA_{1c} levels on metabolic outcomes in the offspring by multiple regression using a linear model with HbA_{1c} as an independent continuous variable. Analyses were done for peri-conceptional second and third trimester HbA_{1c}. Results are reported as change per percentage of HbA_{1c}.

Comparison of categorical variables was done using Fisher's exact test. All statistical analyses were performed using the statistical program R, version 3.0.3 [35].

Results

Maternal/fetal baseline characteristics and offspring anthropometrics and metabolic characteristics at follow-up are shown

in Tables 1 and 2, and in Fig. 2. Additional information is available in the ESM.

A total of 278 index offspring (37.3%) with mean age 16.7 years (range 13.0–19.8 years) agreed to participate in the study, while 452 (60.6%) either did not respond or did not wish to participate. Sixteen index offspring (2.1%) were excluded due to the following reasons: maternal diagnosis of type 1 diabetes was later reclassified to either MODY or type 2 diabetes (n=12), the mother had no contact with the child (n=2), drug abuse (n=1) or pregnancy at the time of recruitment (n=1). Baseline data on index participants and non-participants are given in ESM Table 2. Of 1,920 invited matched control offspring, 303 (15.8%) with mean age 16.8 years (range 13.5–20.4 years) participated in the study, while 83.8% either did not respond or did not wish to participate. We excluded eight control offspring due to the following reasons: they were adopted and had no contact with their biological mothers (n=4), their place of birth was outside of Denmark (n=1) or obstetric medical records revealed that their mothers had gestational diabetes (n=3).

All participants were born in Denmark and most of them were of white European ethnicity. Only 1.2% (three index and four control offspring) belonged to other ethnic groups. Baseline characteristics for both index and control offspring are shown in Table 1. Index offspring had on average 1.8 higher birthweight SDS (corrected for sex and gestational age) than control offspring but there were no significant differences as regards maternal age and BMI.

Metabolic and anthropometric characteristics of the participants by index/control status at follow-up are shown in Table 2. Data on anthropometrics are 100% complete. The OGTT data are >95% complete.

Index offspring had on average 0.44 higher BMI-SDS after adjusting for pubertal development and maternal pre-pregnancy BMI compared with controls. Both SBP and DBP were increased in index offspring (1.9 mmHg and 1.3 mmHg, respectively) compared with controls. Furthermore, index offspring had lower HDL-cholesterol levels than controls in crude analyses, but the differences disappeared in adjusted analyses.

During the OGTT, index offspring had higher levels of p-glucose and s-insulin in both fasting and postload stage. Only levels of fasting p-glucose remained significantly increased in adjusted analyses. Levels of s-insulin remained significantly increased during the whole OGTT, even after adjusting for confounders, and were 15% higher than those in control offspring.

BIGTT-S was decreased (1.6 units) and HOMA-IR was increased (by 10%) in index offspring, as was BIGTT-AIR. No significant differences in HOMA-β were observed between the two groups. The DI was lower in index offspring compared with controls.

All components of the MetS were more prevalent in index offspring than in control offspring (ESM Table 3). Overall, the MetS tended to be more frequent in index offspring than in controls (2.8% vs 0.7%; p=0.054). The distribution of glucose tolerance groups was less favourable among index offspring, with 15.4% prediabetes vs 8.1% in controls (p=0.011; ESM Table 4). Two index offspring were previously diagnosed with type 1 diabetes. One index offspring was diagnosed with diabetes during OGTT (fasting p-glucose 8.3 and 2-h p-glucose 13.9 mmol/l). There was no significant association of BMI-SDS with prediabetes; offspring with prediabetes had on average 0.06 higher BMI-SDS than offspring without

Table 1 Baseline characteristics of mothers and their offspring by index/control status

Characteristic	Index mothers and their offspring n=278 Mean±SD	Control mothers and their offspring n=303 Mean±SD	Differences between mothers and their offspring by index/control status		
			Difference	95% CI	p value
Parity	1.54	1.75			
Maternal pre-pregnancy age (years)	29.2±4.3	29.2±4.1	0.0	(−0.7 to 0.7)	0.946
Maternal pre-pregnancy BMI (kg/m^2)	23.5±3.2	23.3±4.0	0.2	(−0.5 to 0.9)	0.577
Duration of diabetes (years)	12.4±8.3				
Peri-conceptional HbA_{1c} (%)	7.3±1.1				
HbA_{1c} in second trimester (%)	6.6±1.0				
HbA_{1c} in third trimester HbA_{1c} (%)	6.7±1.0				
Peri-conceptional HbA_{1c} (mmol/mol)	56.0±12.2				
HbA_{1c} in second trimester (mmol/mol)	48.7±10.7				
HbA_{1c} in third trimester (mmol/mol)	49.8±10.8				
Birthweight SDS	1.83±2.05	0.01±0.95	1.82	(1.53 to 2.12)	<0.001

Data are presented as means±SD

Differences between the groups are reported as estimates from linear regression with 95% CI and p values

Table 2 Anthropometrics and metabolic characteristics of the offspring by index/control status

Variable	Index offspring	Control offspring	Difference between index and control offspring					
			Crude			Adjusted		
	n=278	*n*=303	Difference	95% CI	*p* value	Difference	95% CI	*p* value
Age (years)	16.7±1.7	16.8±1.8						
Sex (female %)	164 (59%)	182 (60%)						
Weight-SDS	0.60±1.33	0.16±1.15	0.44	(0.24, 0.64)***	<0.001	0.35	(0.10, 0.59)**	0.005
Height-SDS	0.06±1.13	0.05±0.99	0.01	(−0.16, 0.19)	0.871	−0.14	(−0.36, 0.07)	0.196
BMI-SDS	0.69±1.27	0.24±1.14	0.45	(0.25, 0.65)***	<0.001	0.44	(0.21, 0.66)***	<0.001
AC-SDS (<17 years)	0.84±1.11	0.42±1.07	0.41	(0.17, 0.66)**	0.001	0.34	(0.03, 0.65)*	0.031
AC (cm) (≥17 years)	77.3±9.3	73.1±7.3	4.2	(2.2, 6.1)***	<0.001	4.2	(2.1, 6.4)***	<0.001
SBP (mmHg)	119.8±11.0	117.7±10.1	2.2	(0.5, 3.9)*	0.013	1.9	(0.0, 3.9)*	0.048
DBP (mmHg)	65.5±7.6	64.0±6.5	1.6	(0.4, 2.7)**	0.008	1.3	(−0.1, 2.7)	0.062
LDL-cholesterol (mmol/l)	2.2±0.6	2.2±0.6	0.0	(−0.1, 0.1)	0.558	0.1	(−0.0, 0.2)	0.134
HDL-cholesterol (mmol/l)	1.4±0.3	1.4±0.3	−0.1	(−0.1, 0.0)*	0.039	−0.0	(−0.1, 0.0)	0.294
Triacylglycerol (mmol/l)[a]	0.8 (0.6–1.0)	0.7 (0.6–1.0)	3.5%	(−3.1, 10.5)	0.418	5.5%	(−2.8, 14.5)	0.198
HbA_{1c} (mmol/mol)	33.7±3.0	33.3±3.0	0.4	(−0.1, 0.9)	0.102	0.4	(−0.2, 1.0)	0.219
HbA_{1c} (%)	5.23±0.27	5.19±0.27	0.04	(−0.01, 0.08)	0.102	0.03	(−0.02, 0.09)	0.221
OGTT fasting p-glucose (mmol/l)	5.4±0.4	5.3±0.4	0.1	(0.0,0.2)*	0.021	0.1	(0.0, 0.2)**	0.008
OGTT 30 min p-glucose (mmol/l)	8.1±1.4	7.8±1.3	0.3	(0.1, 0.5)*	0.011	0.3	(−0.0, 0.5)	0.064
OGTT 120 min p-glucose (mmol/l)	6.4±1.3	6.1±1.2	0.3	(0.0, 0.5)**	0.009	0.2	(−0.1, 0.4)	0.136
OGTT fasting s-insulin (pmol/l)[a]	56 (42–72)	52 (40–68)	9.7%	(2.1, 17.9)*	0.012	15.2%	(5.6, 25.7)**	0.001
OGTT 30 min s-insulin (pmol/l)[a]	352 (252–548)	320 (222–447)	15.0%	(4.6, 26.3)**	0.004	14.4%	(1.7, 28.7)*	0.025
OGTT 120 min s-insulin (pmol/l)[a]	238 (150–344)	206 (142–297)	13.7%	(2.7, 25.9)*	0.013	14.9%	(1.7, 29.8)*	0.025
BIGTT-IS	8.12±3.36	9.53±3.19	−1.41	(−1.96, −0.86)***	<0.001	−1.58	(−2.27, −0.89)***	<0.001
HOMA-IR[a]	2.22 (1.62–2.95)	2.04 (1.56–2.73)	11.3%	(3.1, 20.1)**	0.006	10.4%	(0.8, 21.0)*	0.037
BIGTT-AIR[a]	1,917(1,562–2,497)	1,756 (1,443–2,159)	11.5%	(4.0, 19.6)**	0.002	9.7%	(0.7, 19.5)*	0.035
HOMA-β[a]	101.4 (76.0–130.3)	97.4 (73.8–128.3)	5.8%	(−1.5, 13.6)	0.123	4.1%	(−3.8, 12.7)	0.321
DI	16,377±6,570	17,345±5,284	−968	(−1,962, 26)	0.056	−1,473	(−2,732, −214)*	0.022

Data are presented as means±SD if normally distributed or as medians and interquartile range if skewed distributed

Differences between the groups are reported as estimates from linear regression with 95% CI and *p* values

Data with skewed distribution are log-transformed and differences are given as percentage difference

Models are adjusted for sex and age (excluding SDS indices), Tanner stage and maternal pre-pregnancy BMI

HOMA indices are further adjusted for offspring BMI

SBP and DBP are further adjusted for height

[a] log transformed

*p<0.05,**p<0.01,***p<0.001

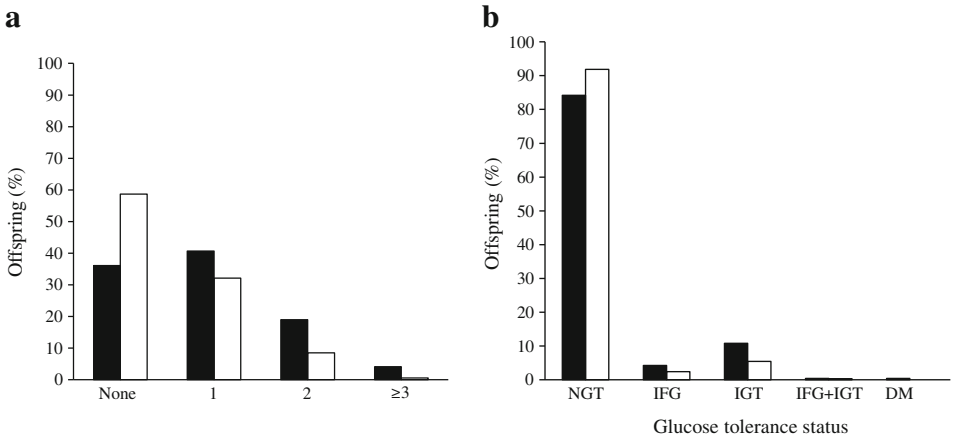

Fig. 2 Percentage of index offspring (black bars) and control offspring (white bars) by the presence of (**a**) components of MetS and (**b**) glucose tolerance status

(p=0.730). The lack of association of BMI-SDS with prediabetes was similar for index and control offspring.

In univariate analyses, BMI-SDS increased with peri-conceptional HbA_{1c} levels: BMI-SDS 0.14 (95% CI −0.01, 0.28) per % HbA_{1c}. The effect of peri-conceptional HbA_{1c} weakened when adjusting for stage of pubertal development and maternal pre-pregnancy BMI: BMI-SDS 0.06 (95% CI −0.09, 0.21) per % HbA_{1c}. Analyses using third trimester HbA_{1c} showed increased BMI-SDS with increasing HbA_{1c} levels: 0.18 (95% CI 0.02, 0.34) per % HbA_{1c}, but this was no longer statistically significant when adjusting for stage of pubertal development and maternal pre-pregnancy BMI: 0.13 (95% CI −0.06, 0.31) per % HbA_{1c}. Likewise, second trimester HbA_{1c} did not significantly affect the results (data not shown). There were no significant associations between HbA_{1c} levels in pregnancy and offspring BP, lipids, insulin sensitivity and insulin secretion (ESM Tables 5 and 6). Adjusting the data for maternal education did not significantly change the results.

Discussion

This well-characterised, large and prospectively identified cohort of offspring of mothers with type 1 diabetes had a higher frequency of metabolic risk factors included in the MetS than offspring of the same age born to non-diabetic mothers. Furthermore, index offspring had decreased insulin sensitivity and insufficient compensatory insulin secretion compared with offspring in the control group, resulting in a higher prevalence of prediabetes. Significant associations between these outcomes and maternal HbA_{1c} levels in pregnancy could not be demonstrated.

Several studies have previously reported an association between maternal diabetes and increased risk of overweight and other metabolic disorders in the offspring. Studies of Pima Indians showed that both diabetes and elevated 2-h blood glucose during OGTT in pregnancy were strong predictors of overweight and type 2 diabetes in the offspring [14]. Pima Indians have specific genetic traits with a high prevalence of overweight and type 2 diabetes, which might have influenced the results. However, within the same family, offspring born after the mother was diagnosed with diabetes had a much greater risk of being obese and of developing type 2 diabetes at an early age than offspring born before the mother was diagnosed with diabetes [36]. This finding could indicate that exposure to the intrauterine diabetic environment is an important determinant of obesity and type 2 diabetes in addition to the genetic predisposition. It has been suggested that associations between maternal diabetes and effects on the offspring may be related to intrauterine hyperglycaemia per se and not to the type of maternal diabetes. A similar prevalence of IGT in offspring aged 1–9 years of mothers with both type 1 diabetes and GDM has been reported [18]. In a Danish study of young adults, the prevalence of prediabetes/type 2 diabetes was >10% in the offspring of mothers with type 1 diabetes and >20% in offspring born to mothers with GDM [19].

In cohorts consisting exclusively of offspring of women with type 1 diabetes, Rijpert et al [25] found a similar prevalence of overweight in offspring aged 6–8 years (n=213) of women with type 1 diabetes with adequate glycaemic control during the pregnancy and a reference population. Lindsay et al [12] reported increased BMI, AC and adiposity at 7 years (n=100), but no differences in plasma glucose or insulin levels. In a large study by Hummel et al [24] of 578 offspring of mothers and 636 offspring of fathers with type 1 diabetes, aged up to 8 years of age, no evidence was found for maternal type 1 diabetes to be an independent predictor for overweight in childhood. In the study by Weiss et al [13], offspring aged 5–15 years (n=75) of mothers with type 1 diabetes had significantly higher BMI and incidence of risk factors predictive for type 2 diabetes, including increased postload glucose, fasting and postload insulin, and insulin resistance, than children born to metabolically healthy mothers. These findings are in accordance with our results. A summarised overview of the previously published studies in offspring of women with type 1 diabetes is given in the ESM Table 7. It could be

speculated that impairment in insulin sensitivity and insulin secretion in offspring exposed to diabetic intrauterine environment appear later in life. Silverman et al [20] reported an increase in the prevalence of IGT in a mixed cohort of offspring exposed to the intrauterine diabetic environment first after 10 years of age.

The mechanism behind glycaemic disorders in offspring exposed to the intrauterine diabetic environment are multifactorial and still only sparsely understood, but impairment of both insulin secretion and insulin sensitivity seem to be two important pathophysiological mechanisms [23, 37]. Low DI is associated with IGT and is predictive of type 2 diabetes development [38] as a result of exhaustion of beta cells and failure to maintain increased insulin secretion. Bush et al [37] found an inverse association between maternal glucose concentration in pregnancy and insulin sensitivity, and a positive association between maternal glucose concentration in pregnancy and beta cell responsitivity in 5–10 years old offspring of mothers with GDM. We observed lower insulin sensitivity and increased insulin secretion along with lower DI in index offspring compared with controls. This is in accordance with the findings of Kelstrup et al [23], though an increase in insulin secretion was not reported in that study, maybe because beta cell exhaustion had taken place in adulthood. How such impairment of insulin sensitivity and insulin secretion comes into action is still unresolved, but fundamental epigenetic changes of the genome may be involved [39]. Based on the developmental origins of disease hypothesis [5], intrauterine development may present a vulnerable time period when the maternal environment can affect the long-term metabolic health of offspring. Our study group has recently reported an association between overall morbidity in index offspring and maternal pre-pregnancy and first trimester HbA_{1c} [40]. However, in the current study we did not find an independent association between HbA_{1c} levels in pregnancy and long-term metabolic outcomes in the offspring.

A major strength of the present study is the large number of offspring of well-characterised mothers with type 1 diabetes, with detailed clinical information available from the pregnancies, including HbA_{1c} levels. Moreover, the longitudinal and prospective study design with a wide range of anthropometric and metabolic outcomes, as well as the large number of matched population controls, make this study unique.

However, some limitations deserve comment. The index offspring had higher participation rate (37%) than control offspring (16%) and if selection is preferential towards healthier controls this may contribute to the type of the results we observed. We did not have information about education and social class for the non-participants, which could be used to evaluate this variable. Furthermore, index mothers might be preferentially interested in health examination of children with potential health problems, which would also bias the study. There is no Danish study addressing the prevalence of prediabetes in children or adolescents, so we do not know whether the prevalence of prediabetes of 8% is the same for the general adolescent population. The participating index children had on average 0.3 higher birthweight SDS than that of non-participants, potentially favouring the former group with a higher metabolic risk. On the other hand, mothers of non-participant index offspring had slightly higher periconceptional HbA_{1c}, which might bias the results towards the null hypothesis. There were no differences in other clinical variables, such as maternal age, BMI, duration of diabetes and gestational age of the offspring (ESM Table 2).

Beside HOMA indexes we used BIGTT indices to evaluate insulin sensitivity and secretion in the offspring. We chose these indices because they incorporate BMI and sex in the calculations, but these indices have not been validated in an adolescent population. The Danish Diabetes Association register does not contain information on maternal pregnancy weight gain and breastfeeding, so we were unable to adjust the linear multivariate analyses for these factors, which could potentially influence the results [11, 24]. Examination of metabolic health in adolescence is challenging since insulin sensitivity decreases during puberty resulting in a compensatory increase in basal and stimulated insulin secretion [41]. Thus, all analyses were adjusted for Tanner stage along with sex and age in order to eliminate influence of different stages of pubertal development on our analyses. Furthermore, all anthropometric measurements are presented as SDS according to the latest Danish reference curves, which makes the calculations more accurate than in most other studies. However, Danish reference curves for AC were available only for children <14 years of age, so the British AC reference curves providing reference data up to 17 years of age were used.

Conclusion

Adolescent offspring of mothers with type 1 diabetes had a less favourable metabolic profile and a higher frequency of prediabetes than the background population. Significant associations between these outcomes and maternal HbA_{1c} levels in pregnancy could not be demonstrated.

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Duality of interest RBJ, HBN, PO, CHG, ERM and PD have given talks for Novo Nordisk (NN). PD, PO and ERM are participating in a multinational study in collaboration with NN, and HBN receives research support from NN. All other authors declare that there is no duality of interest associated with their contribution to this manuscript.

Contribution statement HBN and PD contributed to the establishment of the original birth registry. PD, PO and DMJ contributed with data collection of this registry. All authors contributed substantially to the planning and design of the current study. BB, SK and ZV performed the clinical examinations of the offspring and data collection. ZV analysed the data and wrote the manuscript. DMJ, PD, CHG, TDC, RBJ, ERM, BB and SK contributed to the discussion, and editing and revising the manuscript. HBN, KH and PO reviewed the manuscript. DMJ is the guarantor of the study. All authors approved the final version.

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