ARTICLE

Differential effects of age and sex on insulin sensitivity and body composition in adolescent offspring of women with type 1 diabetes: results from the EPICOM study

Zuzana Lohse^{1,2} · Sine Knorr^{3,4} · Birgitte Bytoft^{5,6,7} · Tine D. Clausen⁸ · Rikke B. Jensen⁹ · Peter Oturai¹⁰ · Henning Beck-Nielsen¹ · Claus H. Gravholt^{3,4} · Peter Damm^{5,6,7} · Kurt Højlund^{1,11} · Dorte M. Jensen^{1,2,12}

Received: 5 June 2017 / Accepted: 15 August 2017 / Published online: 3 October 2017
© Springer-Verlag GmbH Germany 2017

Abstract

Aims/hypothesis The aim of this study was to investigate the influence of age and sex on insulin sensitivity and insulin secretion in the adolescent offspring of women with type 1 diabetes, compared with the background population.

Methods This was a prospective nationwide follow-up study (Epigenetic, Genetic and Environmental Effects on Growth, Cognitive Functions and Metabolism in Offspring of Women with Type 1 Diabetes [EPICOM]) in a Danish population. We examined 278 offspring of women with type 1 diabetes from the Danish Diabetes Association Register born during 1993–1999 (index offspring) and 303 control offspring, identified through the Danish Central Office of Civil Registration and matched to the index offspring with respect to date of birth, sex and postal code. The offspring had an overall mean age of 16.7 years (range 13.0–20.4 years). The main outcomes were age-related

changes in fasting OGTT-derived indices for insulin sensitivity (BIGTT-SI_{0–30–120}, Matsuda index, HOMA-IR) and insulin secretion (acute insulin response [BIGTT-AIR_{0–0–30–120}], insulinogenic index, HOMA of insulin secretory function [HOMA-β], disposition index) and physical activity (International Physical Activity Questionnaire). In addition, we determined total body fat (TBF) percentage using dual-energy x-ray absorptiometry.

Results We observed significantly lower insulin sensitivity in index offspring compared with control offspring, increasing with age. The differences were attenuated after adjustment for TBF percentage, but were still significant at 17 and 18 years of age. We also observed decreased disposition index and insulin secretion-sensitivity index-2 in index offspring at the same age, but we found no significant differences in other indices of insulin secretion compared with control offspring. With

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00125-017-4458-1>) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

✉ Dorte M. Jensen
Dorte.Moeller.Jensen@rsyd.dk

¹ Department of Endocrinology, Odense University Hospital, Klørvænget 6, 4th floor, 5000 Odense C, Denmark

² The Department of Clinical Research, Faculty of Health and Medical Sciences, University of Southern Denmark, Odense, Denmark

³ Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, Denmark

⁴ Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark

⁵ Center for Pregnant Women with Diabetes, Rigshospitalet, Copenhagen, Denmark

⁶ Department of Obstetrics, Rigshospitalet, Copenhagen, Denmark

⁷ The Institute of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

⁸ Department of Gynecology and Obstetrics, Hilleroed Hospital, Hilleroed, Denmark

⁹ University Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark

¹⁰ Department of Clinical Physiology and Nuclear Medicine, Rigshospitalet, Copenhagen, Denmark

¹¹ Section of Molecular Diabetes & Metabolism, Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark

¹² Department of Gynaecology and Obstetrics, Odense University Hospital, Odense, Denmark

age, TBF percentage became increasingly more divergent between index and control offspring, and was consistently higher among female but not male index offspring.

Conclusions/interpretation Differences in insulin sensitivity between the offspring of women with type 1 diabetes and control offspring increased with age. This was only partially explained by higher adiposity in the index offspring.

Trial registration: [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01559181) NCT01559181

Keywords Adolescent offspring · Beta cell function · Fetal programming · Insulin secretion · Insulin sensitivity

Abbreviations

BIGTT-AIR	BIGTT acute insulin response
BIGTT-SI	BIGTT sensitivity index
DEXA	Dual-energy x-ray absorptiometry
DI	Disposition index
EPICOM	Epigenetic, Genetic and Environmental Effects on Growth, Cognitive Functions and Metabolism in Offspring of Women with Type 1 Diabetes
GDM	Gestational diabetes mellitus
HOMA- β	HOMA of insulin secretory function
IGT	Impaired glucose tolerance
IPAQ	International Physical Activity Questionnaire
ISSI-2	Insulin secretion-sensitivity index-2
MET	Metabolic equivalent of task
TBF	Total body fat

Introduction

The incidence of both type 2 diabetes and obesity is increasing among adolescents, with wide variations worldwide [1, 2]. Newly diagnosed adolescents with type 2 diabetes have similar abnormalities to newly diagnosed adults, including decreased insulin sensitivity and impaired insulin secretion relative to their degree of insulin sensitivity [3]. Results from longitudinal studies indicate that both insulin sensitivity and insulin secretion decrease early in the development of type 2 diabetes and during the transition from normal to impaired glucose tolerance (IGT) [4]. The most common cause of decreased insulin sensitivity in childhood and adolescence is obesity [5]. Impaired glucose metabolism has also been described in individuals with an increased risk of diabetes, such as those born with low birthweight [6], first-degree relatives of individuals with type 2 diabetes [7], and women with polycystic ovarian syndrome [8] or previous gestational diabetes mellitus (GDM) [9]. Recent studies have highlighted that intrauterine exposure to a diabetic environment also increases the risk of impaired glucose tolerance, impaired fasting glucose and type 2 diabetes [10–15]. These long-term adverse

effects on glucose metabolism have been observed in the offspring of women with pregestational diabetes (type 1 and type 2 diabetes) and those with GDM. While adiposity in offspring exposed to type 1 diabetes in utero can be observed at a very young age [16], impairment of glucose metabolism is often first reported in adolescence [11] and adulthood [14, 17, 18].

These findings could indicate that offspring obesity is an early consequence of intrauterine hyperglycaemia, whereas impairment of insulin sensitivity and insulin secretion occurs later in life. However, it is not clear at what age abnormalities in glucose metabolism appear, and whether they worsen with age.

We have previously reported increases in BMI, the prevalence of components of the metabolic syndrome and the occurrence of impaired glucose tolerance and impaired fasting glucose in the adolescent offspring of women with type 1 diabetes compared with the background population [11]. In the present study, we aimed to further investigate differences in insulin sensitivity and secretion between the two groups of offspring, and to evaluate the influence of age and sex on insulin sensitivity and insulin secretion in adolescents.

Methods

This study is a part of a prospective nationwide follow-up study (Epigenetic, Genetic and Environmental Effects on Growth, Cognitive Functions and Metabolism in Offspring of Women with Type 1 Diabetes [EPICOM]) that has been described previously [11]. The protocol was in accordance with the Declaration of Helsinki and approved by the regional ethics committee (M-20110239). All participants assented with the participation in the study. Written informed consent was obtained either from the parents (if the participants were aged < 18 years) or the participants themselves.

The study design has been described previously [11]. In short, the study group consisted of adolescent offspring from the Danish Diabetes Association Birth Register (index offspring) born to mothers with type 1 diabetes between 1993 and 1999. The registry contains detailed information on maternal demography, diabetes status and pregnancy outcome. Only singletons and only the first child per mother were included. Control offspring were identified through the Danish Central Office of Civil Registration and matched with respect to date of birth, sex and postal code. We examined 278 index offspring with a mean age of 16.7 years (range 13.0–19.8 years) and 303 control offspring with a mean age of 16.8 years (range 13.5–20.4 years).

Clinical examinations of the offspring took place at three different university hospitals in Denmark (Copenhagen, Odense and Aarhus) from April 2012 to October 2013. All procedures were identical across the three centres for both index offspring and control offspring. Participants were studied after an overnight fast and the following examinations were performed.

Anthropometric measurements

All measurements except for height were performed three times and the mean value was used for 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 scale in kilograms.

Examination of pubertal development

Pubertal development was assessed according to the method of Tanner based on breast development in girls [19] and genital development and measurement of testicular volume in boys [20].

Measurement of total body fat percentage

Total body fat (TBF) percentage was determined using dual-energy x-ray absorptiometry (DEXA). A trained laboratory technician performed DEXA scans after OGTT. The DEXA scans were performed using a GE Healthcare Lunar Prodigy whole-body scanner (model DF+350646; GE Medical Systems, Madison, WI, USA) in Copenhagen; and a Hologic (Bedford, MA, USA) whole-body scanner model Discovery A (Odense) or Discovery W (Aarhus).

Physical activity

Data regarding physical activity were obtained using the self-reported short version of the International Physical Activity Questionnaire (IPAQ) [21], which is recommended by the WHO as a valid tool to estimate physical activity. The short IPAQ form provides separate scores on walking and moderate- and vigorous-intensity activity during the last 7 days.

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 the glucose administration to determine plasma glucose and serum insulin levels. In addition, at time 0, venous blood was drawn to measure HbA_{1c}. We used the WHO 1999 criteria to determine the presence of diabetes and impaired glucose tolerance and/or impaired fasting glucose.

OGTT was not performed if the child had already been diagnosed with diabetes ($n = 2$). One index offspring fulfilled the criteria for diabetes during OGTT testing. The index offspring with type 1 diabetes were excluded from the study. We found no individuals with diabetes among the control offspring.

Biochemical analyses

Glucose was measured in venous plasma using a hexokinase-glucose-6-phosphate dehydrogenase assay (Abbott, Chicago, IL, USA). Serum insulin was measured by ELISA utilising dual-monoclonal antibodies (ALPCO, Salem, NH, USA), and HbA_{1c} levels by cation-exchange HPLC (G8 analyser, Tosoh Bioscience, Tokyo, Japan).

Calculations

Height, weight and BMI SD scores were calculated using normal Danish reference material [22]. Indices of insulin sensitivity were obtained from the OGTT-derived model for assessment of insulin sensitivity index (BIGTT-SI_{0–30–120}) [23], the Matsuda index [24] and HOMA-IR [25]. To assess beta cell function, we calculated the OGTT-derived index of acute insulin response (BIGTT-AIR_{0–30–120}) [23] and the fasting-derived HOMA of insulin secretory function (HOMA- β) [25], and estimated the first-phase insulin responses to glucose challenge (the insulinogenic index) as the ratio of the increment in the plasma insulin level to that in the plasma glucose level during the first 30 min of OGTT [26]. To evaluate insulin secretion corrected for insulin sensitivity, we calculated the disposition index (DI) as $\text{BIGTT-SI}_{0-30-120} \times \text{BIGTT-AIR}_{0-30-120}$, and the insulin secretion-sensitivity index-2 (ISSI-2) defined as the product of insulin secretion measured by the ratio of the total area under the insulin curve to the area under the glucose curve and the Matsuda index [27]. Estimates of physical activity were calculated according to the IPAQ scoring protocol and are reported as the metabolic equivalent of task (MET)-min per week [28].

Statistical analyses

Continuous variables with symmetric distribution are presented as means $\pm$ SD, and continuous variables with skewed distribution are presented as medians (interquartile range). Insulin sensitivity and secretion indices were analysed as continuous variables using a linear regression model, with separate age effects for index and control offspring, and controlled for offspring sex, stage of pubertal development, maternal prepregnancy BMI and TBF percentage. The effect of age on insulin sensitivity and secretion indices was modelled continuously by restricted cubic spline with three degrees of freedom [29]. Estimated differences between index and control offspring were computed from the continuous model at selected ages (13, 14, 15, 16, 17, 18 and 19 years) and are reported with 95% CIs and p values. Curves adjusted for offspring sex, Tanner stage and physical activity and maternal prepregnancy BMI were modelled first. Curves additionally adjusted for TBF percentage were then modelled. The assumption of normality of the residuals was checked. Variables that showed skewed distribution of residuals were log-transformed before

analysis, and covariate effects were reported as per cent changes in the variable. Predicted values were computed for female offspring in Tanner stage 5, physical activity of 2500 MET-min/week, maternal prepregnancy BMI of 25 kg/m² and TBF of 30%. All statistical analyses were performed by using the statistical program R version 3.0.3 (R-Core Team, URL <https://www.r-project.org/>).

Results

The baseline anthropometric characteristics of the participants, together with estimates of insulin sensitivity and secretion, are given in Table 1. Some of these data have previously been presented [11].

There were no statistically significant differences in self-reported level of physical activity between index and control offspring (Table 1).

Age- and sex-related changes in glucose metabolism

Insulin sensitivity Estimates of insulin sensitivity (BIGTT-SI_{0–30–120} and Matsuda index) diverged between index and control offspring, being lower in index offspring, and the divergence increased with age: from a difference of 0.01 in BIGTT-SI_{0–30–120} at 13 years to −2.05 at 19 years; and from a difference of −2.0% in the Matsuda index at 13 years to −19.4% at 19 years (data not shown). Differences between the groups became significant at 16 years of age in the case of BIGTT-SI_{0–30–120} and at 17 years in the case of the Matsuda index (data not shown). These analyses were adjusted for offspring sex, Tanner stage and physical activity, as these are important modulators of insulin sensitivity. When additionally adjusting the analyses for offspring TBF percentage, the differences between the groups were attenuated and remained significant only at 17 and 18 years of age for both measures (Table 2; Fig. 1; electronic supplementary material [ESM] Table 1; ESM Fig. 1). We observed a similar, although non-significant, difference between index and control offspring in HOMA-IR (ESM Table 2; ESM Fig. 2).

Insulin secretion In the previously published results from the EPICOM study, we found decreased overall oral DI in index offspring compared with control offspring [11], but when investigating age-specific differences between index and control offspring, no significant differences were found (except borderline significant difference at 18 years of age). In the current analyses, the differences in DI between index and control offspring seemed to be more pronounced at 13 and 14 years of age, and then again at 17 and 18 years in the fully adjusted model, although the difference was only significant at 18 years (Table 3; Fig. 2). Similar differences were also observed in the analyses of ISSI-2 as another estimate of insulin secretion

corrected for insulin sensitivity, and differences were significant at 17 and 18 years (ESM Table 3; ESM Fig. 3). The age-specific differences in other estimates of insulin secretion (BIGTT-AIR, insulinogenic index, HOMA-β) were less obvious and non-significant (data not shown). Although the overall difference in BIGTT-AIR was significant in index offspring compared with control offspring [11], we found no significant age-specific differences in BIGTT-AIR between index and control offspring (data not shown).

Body composition Our analyses have clearly shown that TBF percentage and BMI, as measures of body composition, are important independent modulators of especially insulin sensitivity. We therefore studied the influence of age on these variables. With age, TBF percentage became increasingly more divergent in index and control offspring (Fig. 3a; ESM Table 4), with higher TBF percentages among index offspring with increasing age, although at age 13 years the TBF percentage was slightly lower among index offspring. A similar picture was observed with BMI (ESM Fig. 4a; ESM Table 5). Furthermore, it is clear that important sex differences are present and, while TBF percentage was not consistently higher in male index offspring, TBF percentage in female index offspring was significantly higher than in control offspring throughout puberty (Fig. 3b, c; Table 4), as was BMI (ESM Fig. 4b, c).

Maternal prepregnancy BMI In correlation analyses, maternal prepregnancy BMI was an important modulator of insulin secretion, insulin resistance and offspring body composition (data not shown). However, since we did not have complete data on this variable, with missing data on 53 index mothers and 117 control mothers, we chose not to include the variable in the presented adjusted models, as this would have led to a significant loss of power. However, in fully adjusted models that included offspring sex, TBF percentage and Tanner stage, and then also prepregnancy maternal BMI, the inclusion of this significant contributory variable did not materially change the models (results not shown).

Discussion

To our knowledge, this is the first study to investigate changes in insulin sensitivity and secretion with age and sex in the adolescent offspring of women with type 1 diabetes. We observed significant differences in insulin sensitivity compared with a control group, and these differences increased with age and furthermore were modulated by sex. The attenuated insulin sensitivity remained significant only in 17- and 18-year-old offspring, when the analyses were additionally adjusted for TBF percentage of the offspring. In fact, it seems that changes in body composition, with increased fat mass content, are a

Table 1 Basic characteristics of the study participants

Variable	Index offspring		Control offspring		<i>p</i> value
	<i>n</i> = 278	No. missing values	<i>n</i> = 303	No. missing values	
Age (years)	16.7 ± 1.7	—	16.8 ± 1.8	—	
Age dispersal					
13 years	16		16		
14 years	42		40		
15 years	45		54		
16 years	48		43		
17 years	57		62		
18 years	44		53		
≥ 19 years	26		35		
Female sex	164 (59)	—	182 (60)	—	
Height SDS	0.06 ± 1.13	—	0.05 ± 0.99	8	0.871
Weight SDS	0.60 ± 1.33	—	0.16 ± 1.15	8	< 0.001
BMI SDS	0.69 ± 1.27	—	0.24 ± 1.14	8	< 0.001
TBF (%)					
Combined	28.7 ± 10.7	—	26.1 ± 9.1	1	0.001
Female offspring	35.1 ± 7.4	—	31.3 ± 6.3	1	< 0.001
Male offspring	19.6 ± 7.7	—	18.4 ± 7.0	—	0.229
Pubertal stage ^a		25		19	
I	2		0		
II	2		3		
III	16		23		
IV	74		100		
V	159		158		
Physical activity (MET-min/week)					
Combined	2584 (1168–4124)	4	2560 (1302–4876)	1	0.245
Female offspring	2079 (1120–3600)	3	2248 (1215–4542)	—	0.438
Male offspring	3276 (1370–5064)	1	2853 (1833–5238)	1	0.350
Prepregnancy maternal BMI (kg/m ²)	23.5 ± 3.2	53	23.3 ± 4.0	117	0.569
HbA _{1c} (mmol/mol)	33.7 ± 3.0	19	33.3 ± 3.0	6	0.102
HbA _{1c} (%)	5.23 ± 0.27	19	5.19 ± 0.27	6	0.102
OGTT fasting p-glucose (mmol/l)	5.39 ± 0.41	6	5.33 ± 0.36	5	0.021
OGTT fasting s-insulin (pmol/l)	56 (42–72)	6	52 (40–68)	5	0.012
HOMA-IR	2.22 (1.62–2.95)	7	2.04 (1.56–2.73)	7	0.006
BIGTT-SI _{0–30–120}	8.12 ± 3.36	21	9.53 ± 3.19	11	< 0.001
Matsuda index	5.14 (3.72–6.77)	21	5.78 (4.30–7.45)	11	< 0.001
HOMA-β	101.4 (76.0–130.3)	7	97.4 (73.8–128.3)	7	0.123
BIGTT-AIR _{0–30–120}	1927.6 (1562.4–2509.5)	21	1755.6 (1443.4–2158.7)	11	0.002
Insulinogenic index	1.03 (0.69–1.78)	18	1.02 (0.69–1.66)	10	0.526
DI	16,415 ± 6554	23	17,344 ± 5286	11	0.056
ISSI-2	1.90 (1.52–2.31)	23	1.92 (1.62–2.26)	11	0.201

Data are means ± SD if normally distributed, medians (interquartile range) if skewed distributed, or *n* or *n* (%)

^aData on pubertal stage are presented in five groups, as described by Tanner (see Methods)

SDS, SD score

consequence of being born to a mother with type 1 diabetes. This was most pronounced for female offspring, while male

offspring did not have a consistently higher TBF percentage or BMI. We observed increased differences in DI and ISSI-2 in

Table 2 Estimated differences in insulin sensitivity, evaluated by BIGTT-SI_{0–30–120}, between index and control offspring at selected ages

Age (years)	Index vs control ^a	95% CI	<i>p</i> value
13	−0.63	−4.24, 2.97	0.731
14	−0.27	−1.66, 1.13	0.707
15	−0.24	−1.23, 0.75	0.639
16	−0.71	−1.52, 0.10	0.086
17	−1.20	−2.04, −0.37	0.005
18	−1.23	−1.97, −0.49	0.001
19	−0.94	−1.91, 0.04	0.060

Data are adjusted for offspring sex, stage of pubertal development, physical activity and TBF percentage

^a Computed using linear regression models at selected ages

the index offspring at the same ages, but no significant age-specific differences in other insulin secretion indices. Thus, the current study adds more information to the previously published results [11] and, in addition, provides new findings on differences in insulin sensitivity and secretion in the adolescent offspring of women with type 1 diabetes and differential effects of age and sex on these variables.

A number of studies have addressed the association between intrauterine hyperglycaemia and offspring metabolic risk at different ages [12, 13, 15, 16, 30]. Bush et al studied the offspring (ages 5–10 years) of women with GDM and reported a significant inverse association between maternal gestational glucose concentrations and the offspring's insulin sensitivity, independent of body composition [30]. On the other hand, a study from Holland by Rijpert et al showed that if adequate control and treatment of women with type 1

Table 3 Estimated differences in DI between index and control offspring at selected ages

Age (years)	Index vs control ^a	95% CI	<i>p</i> value
13	−4519	−12,215, 3177	0.250
14	−1592	−4574, 1390	0.295
15	−8	−2122, 2106	0.994
16	−532	−2263, 1198	0.546
17	−1620	−3411, 171	0.076
18	−1632	−3211, −52	0.043
19	−863	−2947, 1220	0.417

Data are adjusted for offspring sex, stage of pubertal development, physical activity and TBF percentage

^a Computed using linear regression models at selected ages

diabetes was applied during pregnancy then long-term effects on offspring body composition and glucose metabolism at age 6–8 years were limited [31]. Another study from the UK by Lindsay et al [16] found that the children of women with type 1 diabetes had significantly increased abdominal circumference, BMI and adiposity (measured by skin fold) at 7 years of age, but no differences in plasma glucose or insulin levels were observed at this age. An Austrian/German study by Weiss et al reported that the offspring of women with type 1 diabetes had a significantly higher incidence of risk factors predictive for type 2 diabetes at age 5–15 years, including obesity, increased post-load glucose levels and fasting and post-load insulin levels, and decreased insulin sensitivity compared with control individuals [12]. This is in accordance with the results from the EPICOM study [11] and in line with another Danish study by Kelstrup et al, who reported lower

Fig. 1 Estimates of insulin sensitivity evaluated by BIGTT-SI_{0–30–120} for index and control offspring at selected ages. Estimates were modelled continuously by restricted cubic spline with three degrees of freedom and are reported with 95% CIs (thin lines). Black lines, index offspring; green lines, control offspring. The predicted values were adjusted for offspring sex, Tanner stage, physical activity and TBF percentage. The predicted values were computed for female offspring in Tanner stage 5, with physical activity level equal to 2500 MET-min/week and body fat percentage equal to 30%. $p = 0.014$ for the null hypothesis of equality of the two curves

Fig. 2 Estimates of DI for index and control offspring at selected ages. Estimates were modelled continuously by restricted cubic spline with three degrees of freedom and are reported with 95% CIs (thin lines). Black lines, index offspring; green lines, control offspring. The predicted values are adjusted for offspring sex, Tanner stage, physical activity and TBF percentage. The predicted values were computed for female offspring in Tanner stage 5, with physical activity level equal to 2500 MET-min/week and body fat percentage equal to 30%. $p = 0.273$ for the null hypothesis of equality of the two curves

Fig. 3 (a) Estimates of TBF percentage for index and control offspring at selected ages. (b, c) Estimates of TBF percentage for index and control female offspring (b) and index and control male offspring (c) at selected ages. Estimates were modelled continuously by restricted cubic spline with three degrees of freedom and are reported with 95% CIs (thin lines). Black lines, index offspring; green lines, control offspring. In (a) the

predicted values were adjusted for offspring sex, Tanner stage and physical activity; $p < 0.001$ for the null hypothesis of equality of the two curves. In (b) and (c) the predicted values were adjusted for Tanner stage and physical activity; $p < 0.001$ (b) and $p = 0.008$ (c) for the null hypothesis of equality of the two curves

insulin sensitivity and a lower DI in the adult offspring of women with type 1 diabetes, compared with offspring from the background population [17]. Here, we also show that as puberty and age advance, body composition measured both by DEXA (TBF percentage) and BMI becomes increasingly divergent between the offspring of women with type 1 diabetes and the background population, with a higher TBF percentage and BMI among index children, and that these variables are consistently higher in female offspring.

Taken together, the findings of the current study could indicate that the presence of overweight or outright obesity as a consequence of exposure to intrauterine hyperglycaemia precedes the impairment of glucose metabolism. This hypothesis is also supported by the findings of a study by Silverman et al, where the multi-ethnic offspring of mothers with diabetes (mixed type 1 diabetes, type 2 diabetes and GDM) had an IGT prevalence of 5.4% at age 5–9 years and 19.3% at age

10–16 years [13]. In addition, a German study by Plagemann et al reported that the frequency of IGT in the offspring of women with type 1 diabetes increased with age (from 9.4% at 1–4 years to 17.4% at 5–9 years) and was not significantly different from the IGT prevalence among the offspring of women with GDM [15]. Studies of offspring of women with different types of diabetes have shown that the offspring of women with GDM and type 2 diabetes have a higher degree of obesity and glucose intolerance than those of women with type 1 diabetes [14, 32]. This indicates a genetic effect, since the offspring of women with GDM and type 2 diabetes are genetically more prone to developing obesity and hyperglycaemia than the offspring of women with type 1 diabetes. It has therefore been proposed that the timing and degree of prenatal exposure to maternal hyperglycaemia may play an important role in fetal programming. However, in the previously published results of the EPICOM study, estimates

Table 4 Estimated differences in TBF between index and control offspring at selected ages and separately for male and female offspring

Age (years)	Female offspring			Male offspring		
	Index vs control ^a	95% CI	<i>p</i> value	Index vs control ^a	95% CI	<i>p</i> value
13	0.5	−9.8, 10.8	0.924	−3.8	−16.7, 9.2	0.568
14	1.6	−2.5, 5.7	0.434	1.8	−3.3, 6.9	0.492
15	2.6	−0.4, 5.6	0.093	3.6	−0.0, 7.2	0.051
16	3.2	0.8, 5.6**	0.008	−0.2	−3.3, 2.8	0.894
17	3.6	1.2, 6.0**	0.003	−3.6	−7.1, −0.2*	0.040
18	3.8	1.7, 5.9***	< 0.001	−0.9	−4.0, 2.2	0.577
19	3.9	1.3, 6.5**	0.003	6.0	1.7, 10.3**	0.007

Data are adjusted for stage of pubertal development and physical activity

^a Computed using linear regression models at selected ages

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

of insulin sensitivity and beta cell function were not directly associated with maternal HbA_{1c} at different time points during pregnancy [11].

Although decreased insulin sensitivity and DI have previously been reported in the offspring of women with type 1 diabetes [17], no study has previously addressed age-specific changes in insulin sensitivity and secretion. Therefore, in the current study, we meticulously investigated differences between index and control offspring with respect to insulin sensitivity and secretion at selected ages. We observed very few differences in insulin sensitivity between index and control offspring at 13–14 years, but the differences tended to increase from 15 years of age and became significant at 16 years in unadjusted analyses (data not shown) and at 17 years in the fully adjusted model. The age when the differences in insulin sensitivity became significant corresponds with the age at which pubertal development is usually complete. The difference in insulin sensitivity observed at that age could indicate that the index offspring did not regain normal insulin sensitivity, which is physiologically decreased during puberty [33], to the same extent as the control offspring. If insulin sensitivity in offspring first begins to deteriorate during adolescence, this could explain why differences in glucose metabolism are not usually observed in studies of prepubertal offspring. The non-significant differences at 19 years in our study could suggest either that it takes longer for offspring exposed to maternal diabetes to normalise insulin sensitivity after puberty, or simply that our sample size in this age group was too small to demonstrate a difference. DI and ISSI-2 tended to be decreased in the index offspring at 17 and 18 years of age, but we found no significant age-specific differences in other estimates of insulin secretion between the index and control offspring. This could indicate that impairment in insulin secretion is dissociated from the detrimental changes that occur in insulin sensitivity.

We observed increased insulin secretion in index compared with control offspring, but the differences were not statistically significant and the differences were further attenuated after adjusting for offspring TBF. This could indicate that the differences in insulin secretion between the index and control offspring were strongly associated with increased adiposity in the index offspring. Our additional analysis of pubertal changes in body composition likely implicates the important divergence that occurs in both TBF percentage and BMI during puberty between the two groups as an important explanatory variable. It is clear that female index offspring seem to gather far more fat than male offspring as they pass through puberty and beyond in comparison with their control offspring, emphasising that sex is an exceedingly important modulating factor in diabetes research [34]. The underlying mechanisms are presently not clear, but could involve epigenetic as well as societal factors. However, the increased TBF percentage does not entirely explain the differences in insulin

sensitivity between the two groups, indicating that there are other factors involved.

This study was based on a large sample of offspring and conducted in a prospective setting. Investigating the associations between exposure to an intrauterine diabetic environment and long-term outcomes is complicated by the latency of the onset of abnormalities and the multitude of confounding factors that can influence these outcomes at different points in an individual's life. One of the most important confounders is maternal overweight or obesity, which can be directly linked to adverse short-term and long-term outcomes [35]. Another confounding effect that should be considered is the stage of pubertal development in the offspring. Puberty is known to be associated with a reduction in insulin sensitivity. A longitudinal study from the USA showed a 32% reduction in insulin sensitivity during the transition from Tanner stage I to Tanner stage III/IV and an inadequate low increase in the AIR, which suggests an inadequate beta cell response [33]. The participants in our study were examined for pubertal development, and more than 90% of all participants were either Tanner stage IV or V. The euglycaemic–hyperinsulinaemic clamp is widely considered to be the gold standard for measuring insulin sensitivity. In addition, IVGTT with frequent blood sampling has been shown to be a reliable alternative to the clamp in children. However, both methods are labour intensive, relatively invasive and costly, and we therefore opted to use OGTT-derived indices of insulin sensitivity and secretion. BIGTT-SI and BIGTT-AIR models incorporate sex and BMI along with measurements of plasma glucose and serum insulin levels at 0, 30 and 120 min. These indices are highly correlated to indices obtained from IVGTT in adults [23], but have not been validated in adolescents. Therefore, we also used other estimates of insulin sensitivity and secretion to verify our results. The Matsuda index is an insulin-sensitivity measure that has been previously validated in children and adolescents [36], and the ISSI-2 has been shown to be closely correlated with DI obtained from IVGTT and not significantly related to pubertal status [27].

Physical activity is known to be an important determinant of overweight and obesity. A study from Greece that looked at the physical activity and dietary habits of 2000 adolescents found that overweight/obese adolescents spent fewer hours engaged in sports activities outside of school than normal-weight adolescents [37]. Adolescents with overweight/obesity and a sedentary lifestyle are also more likely to have decreased insulin sensitivity [38]. Aerobic physical activity has been reported to increase insulin sensitivity even without changes in body weight or TBF percentage [39]. Levels of physical activity in the current study were evaluated using a short version of the IPAQ and were included in all adjusted analyses. The IPAQ is recommended by the WHO as a valid tool for estimating physical activity [21]. Interestingly, self-reported physical activity was similar between index and

control children, as well as between female and male index and control children, indicating that differences in physical activity were directly linked to the observed changes in body composition and insulin sensitivity.

Social and economic factors might play a role in and modify long-term health outcomes [40]. In this study, we additionally matched index and control children with respect to postal code as an indirect socioeconomic marker. The presented data are not adjusted for dietary habits, however, which might also have potentially influenced the results [37].

This study was performed in a high-income setting and in a population with a relatively low prevalence of obesity. These factors must be taken into account when extrapolating the results to other populations, as pre- and postnatal factors may be different and thus modulate the effects of intrauterine exposure to hyperglycaemia.

In summary, we observed that the reduced insulin sensitivity in the offspring of women with type 1 diabetes compared with control offspring increased with age. However, the differences were attenuated in analyses adjusted for offspring TBF percentage and remained significant only at 17 and 18 years of age, indicating that the relationships between intrauterine conditions, insulin sensitivity, insulin secretion and body composition are complex and difficult to disentangle. We observed decreased DI and ISSI-2 at the same age, but other measures of insulin secretion did not significantly differ between the two groups with advancing age.

Acknowledgements Data collection in the original registry was performed by L. Mølsted-Pedersen, J. Klebe, J. Westergaard, M. Møller, N. Hahnemann, H. Gjessing, J. K. Mostrup, K. H. Frandsen, E. Stage, A. Thomsen, T. Lousen, K. R. Petersen, B. Øvlisen, J. Kvetny H. Poulsen (Working Group for Type 1 Diabetes Pregnancy).

Apart from H. Beck-Nielsen and P. Damm, the original registry working group included A. Frøland, L. Mølsted-Pedersen, J. Klebe and C. E. Mogensen. The statistical analyses were completed with the assistance of senior statistician B. Carstensen (Steno Diabetes Center, Gentofte, Denmark). The Danish Diabetes Association is acknowledged for originally assisting in the creation and funding of the original Danish Diabetes Birth Registry.

Data availability The dataset generated and analysed during the current study is not publicly available due to considerations of the privacy and anonymity of the participants. Full anonymity of the participants in the data is not possible, and individual identification would be a risk. Other researchers can apply for access to the data, if they have the required official approval.

Funding This study was supported by grants from the Danish Council for Independent Research, European Foundation for the Study of Diabetes, Lundbeck Foundation, Danielsens Foundation and Beckett Foundation.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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

References

1. NCD Risk Factor Collaboration (NCD-RisC) (2016) Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. *Lancet* 387:1513–1530
2. Ng M, Fleming T, Robinson M, Thomson B et al (2014) Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 384:766–781
3. Elder DA, Herbers PM, Weis T, Standiford D, Woo JG, D'Alessio DA (2012) β -cell dysfunction in adolescents and adults with newly diagnosed type 2 diabetes mellitus. *J Pediatr* 160:904–910
4. Giannini C, Weiss R, Cali A et al (2012) Evidence for early defects in insulin sensitivity and secretion before the onset of glucose dysregulation in obese youths: a longitudinal study. *Diabetes* 61:606–614
5. Lee JM (2006) Insulin resistance in children and adolescents. *Rev Endocr Metab Disord* 7:141–147
6. Lithell HO, McKeigue PM, Berglund L, Mohsen R, Lithell UB, Leon DA (1996) Relation of size at birth to non-insulin dependent diabetes and insulin concentrations in men aged 50–60 years. *BMJ* 312:406–410
7. O'Rahilly SP, Rudenski AS, Burnett MA et al (2017) Beta-cell dysfunction, rather than insulin insensitivity, is the primary defect in familial type 2 diabetes. *Lancet* 328:360–364
8. Randeva HS, Tan BK, Weickert MO et al (2012) Cardiometabolic aspects of the polycystic ovary syndrome. *Endocr Rev* 33:812–841
9. Ward WK, Johnston CLW, Beard JC, Benedetti TJ, Halter JB, Porte D (1985) Insulin resistance and impaired insulin secretion in subjects with histories of gestational diabetes mellitus. *Diabetes* 34:861–869
10. Knorr S, Stochholm K, Vlachová Z et al (2015) Multisystem morbidity and mortality in offspring of women with type 1 diabetes (the EPICOM study): a register-based prospective cohort study. *Diabetes Care* 38:821–826
11. Vlachová Z, Bytoft B, Knorr S et al (2015) Increased metabolic risk in adolescent offspring of mothers with type 1 diabetes: the EPICOM study. *Diabetologia* 58:1454–1463
12. Weiss PA, Scholz HS, Haas J, Tamussino KF, Seissler J, Borkenstein MH (2000) Long-term follow-up of infants of mothers with type 1 diabetes: evidence for hereditary and nonhereditary transmission of diabetes and precursors. *Diabetes Care* 23:905–911
13. Silverman BL, Metzger BE, Cho NH, Loeb CA (1995) Impaired glucose tolerance in adolescent offspring of diabetic mothers. Relationship to fetal hyperinsulinism. *Diabetes Care* 18:611–617
14. Clausen TD, Mathiesen ER, Hansen T et al (2008) High prevalence of type 2 diabetes and pre-diabetes in adult offspring of women with gestational diabetes mellitus or type 1 diabetes: the role of intrauterine hyperglycemia. *Diabetes Care* 31:340–346
15. Plagemann A, Harder T, Kohlhoff R, Rohde W, Dörner G (1997) Glucose tolerance and insulin secretion in children of mothers with pregestational IDDM or gestational diabetes. *Diabetologia* 40:1094–1100

16. Lindsay RS, Nelson SM, Walker JD et al (2010) Programming of adiposity in offspring of mothers with type 1 diabetes at age 7 years. *Diabetes Care* 33:1080–1085
17. Kelstrup L, Damm P, Mathiesen ER et al (2014) Insulin resistance and impaired pancreatic β -cell function in adult offspring of women with diabetes in pregnancy. *J Clin Endocrinol Metab* 98:3793–3801
18. Sobngwi E, Boudou P, Mauvais-Jarvis F et al (2003) Effect of a diabetic environment in utero on predisposition to type 2 diabetes. *Lancet* 361:1861–1865
19. Marshall WA, Tanner JM (1969) Variations in pattern of pubertal changes in girls. *Arch Dis Child* 44:291–303
20. Marshall WA, Tanner JM (1970) Variations in the pattern of pubertal changes in boys. *Arch Dis Child* 45:13–23
21. Craig CL, Marshall AL, Sjöström M et al (2003) International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 35:1381–1395
22. Tinggaard J, Aksglaede L, Sørensen K et al (2014) The 2014 Danish references from birth to 20 years for height, weight and body mass index. *Acta Paediatr* 103:214–224
23. Hansen T, Drivsholm T, Urhammer SA et al (2007) The BIGTT test: a novel test for simultaneous measurement of pancreatic β -cell function, insulin sensitivity, and glucose tolerance. *Diabetes Care* 30:257–262
24. Matsuda M, DeFronzo R (1999) Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 22:1462–1470
25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419
26. Hanson RL, Pratley RE, Bogardus C et al (2000) Evaluation of simple indices of insulin sensitivity and insulin secretion for use in epidemiologic studies. *Am J Epidemiol* 151:190–198
27. Retnakaran R, Qi Y, Goran MI, Hamilton JK (2009) Evaluation of proposed oral disposition index measures in relation to the actual disposition index. *Diabet Med* 26:1198–1203
28. IPAQ (2005) Guidelines for data processing and analysis of the International Physical Activity Questionnaire (IPAQ). Available from www.ipaq.ki.se. Accessed 26 February 2017
29. Harrell FE (2001) Regression modeling strategies: with applications to linear models, logistic regression, and survival analysis. Springer Verlag, New York
30. Bush NC, Chandler-Laney PC, Rouse DJ, Granger WM, Oster RA, Gower BA (2011) Higher maternal gestational glucose concentration is associated with lower offspring insulin sensitivity and altered β -cell function. *J Clin Endocrinol Metab* 96:E803–E809
31. Rijpert M, Evers IM, de Vroede MA, de Valk HW, Heijnen CJ, Visser GH (2009) 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. *Diabetes Care* 32:2099–2104
32. Clausen TD, Mathiesen ER, Hansen T et al (2009) Overweight and the metabolic syndrome in adult offspring of women with diet-treated gestational diabetes mellitus or type 1 diabetes. *J Clin Endocrinol Metab* 94:2464–2470
33. Goran MI, Gower BA (2001) Longitudinal study on pubertal insulin resistance. *Diabetes* 50:2444–2450
34. Morselli E, Frank AP, Santos RS, Fátima LA, Palmer BF, Clegg DJ (2016) Sex and gender: critical variables in pre-clinical and clinical medical research. *Cell Metab* 24:203–209
35. Philipps LH, Santhakumaran S, Gale C et al (2011) The diabetic pregnancy and offspring BMI in childhood: a systematic review and meta-analysis. *Diabetologia* 54:1957–1966
36. Yeckel CW, Weiss R, Dziura J et al (2004) Validation of insulin sensitivity indices from oral glucose tolerance test parameters in obese children and adolescents. *J Clin Endocrinol Metab* 89:1096–1101
37. Kosti RI, Panagiotakos DB, Mihas CC et al (2007) Dietary habits, physical activity and prevalence of overweight/obesity among adolescents in Greece: the Vyrionas study. *Med Sci Monit* 13:CR437–CR444
38. Velásquez-Rodríguez CM, Velásquez-Villa M, Gómez-Ocampo L, Bermúdez-Cardona J (2014) Abdominal obesity and low physical activity are associated with insulin resistance in overweight adolescents: a cross-sectional study. *BMC Pediatr* 14:258
39. Nassis GP, Papantakou K, Skenderi K et al (2015) Aerobic exercise training improves insulin sensitivity without changes in body weight, body fat, adiponectin, and inflammatory markers in overweight and obese girls. *Metabolism* 54:1472–1479
40. Kaplan GA, Keil JE (1993) Socioeconomic factors and cardiovascular disease: a review of the literature. *Circulation* 88:1973–1998