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SHORT COMMUNICATION

R. Singh · E. Pearson · P. J. Avery · M. I. McCarthy · J. C. Levy · G. A. Hitman · M. Sampson · M. Walker · A. T. Hattersley

Reduced beta cell function in offspring of mothers with young-onset type 2 diabetes

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Abstract *Aims/hypothesis:* Animal models indicate that even exposure to mild maternal hyperglycaemia in utero is detrimental to the beta cell function of the offspring, but evidence of this in humans is limited. In Europids who are diagnosed with type 2 diabetes before the age of 50 years, the risk of diabetes in the offspring of the diabetic mothers is greatly increased compared with the risk in those born to diabetic fathers. We hypothesised that offspring born to mothers with young-onset type 2 diabetes would have been exposed to mild hyperglycaemia in utero, so we studied the impact of this on their beta cell function. *Subjects and methods:* We measured beta cell function using early insulin response (EIR) after oral glucose; insulin resistance using HOMA; and HbA_{1c} in 568 non-diabetic adult offspring born to parents with type 2 diabetes (mean age 55.8 years), split according to which parent was affected (in 327 it was the mother) and parental age of diagnosis: <50 years (n=117) or ≥50 years. To reduce the impact of genetic susceptibility, the offspring of affected fathers were used as control subjects. *Results:* Offspring of mothers with young-onset type 2 diabetes had lower EIR (log EIR 4.32, 95% CI [4.14–4.51] vs 4.63 [4.43–4.83] p=0.02) and higher HbA_{1c} (4.89% [4.79–4.99] vs 4.68% [4.57–4.79] p=0.02) than the offspring of fathers with young-onset type 2 diabetes. Insulin sensitivity was similar in the two groups. There were no differences in EIR or HbA_{1c} between the offspring born to mothers and fathers who were diagnosed after the age of 50 years. *Conclusions/interpretation:* We conclude that the offspring of mothers with young-onset type 2 diabetes have a reduction in beta cell function. This is consistent with exposure to mild maternal hyperglycaemia programming beta cell function.

Keywords Beta cell function · Diabetes · Hyperglycaemia in utero · Programming

Abbreviations EIR: early insulin response · HOMA: homeostatic model assessment · MODY: maturity-onset diabetes of the young · OGTT: oral glucose tolerance test

R. Singh · E. Pearson · A. T. Hattersley (✉)
Institute of Biomedical Sciences,
Peninsula Medical School,
Barrack Road,
Exeter EX2 5AX, UK
e-mail: A.T.Hattersley@ex.ac.uk
Tel.: +44-1392-406806
Fax: +44-1392-403027

P. J. Avery
School of Mathematics and Statistics,
University of Newcastle upon Tyne,
Newcastle, UK

M. I. McCarthy
Wellcome Trust Centre for Human Genetics,
University of Oxford,
Oxford, UK

M. I. McCarthy · J. C. Levy
Oxford Centre for Diabetes, Endocrinology and Metabolism,
University of Oxford,
Oxford, UK

G. A. Hitman
Centre for Diabetes and Metabolic Medicine,
Barts and the London, Queen Mary's School of Medicine
and Dentistry, University of London,
London, UK

M. Sampson
Department of Diabetes and Endocrinology,
Norfolk and Norwich University Hospital,
Norwich, UK

M. Walker
School of Clinical Medical Sciences,
University of Newcastle upon Tyne,
Newcastle, UK

Introduction

In animal models the intrauterine exposure to hyperglycaemia is a risk factor for hyperglycaemia in the offspring, which is predominantly mediated through reduced offspring beta cell function. Studies in Pima Indians have shown that the offspring of mothers who are diabetic

during pregnancy have the highest prevalence of young-onset type 2 diabetes [1]. Similar findings indicating that in utero hyperglycaemia has an adverse effect on the diabetes risk of offspring were obtained in studies of families with *HNF-1α* mutations [2], and of offspring born to parents with type 1 diabetes [3].

The impact of mild hyperglycaemia on offspring beta cell function in humans is less certain. In all the examples described above, the mother had established diabetes at the time the offspring was in utero. The large, prospective Framingham Offspring Study [4] showed that the odds ratio for type 2 diabetes and abnormal glucose tolerance in offspring whose mothers were diagnosed with diabetes

Table 1 Baseline and adjusted offspring characteristics according to parental age at diabetes diagnosis

Offspring characteristics	Offspring with parents diagnosed with diabetes <50 years			Offspring with parents diagnosed with diabetes ≥50 years		
Parent with type 2 diabetes	Mother	Father	*p* value	Mother	Father	*p* value
Number of offspring	62	55		265	196	
Number of families	28	23		103	86	
Offspring excluded because of diabetes	4	1	0.2	14	15	0.3
Parental age at diabetes diagnosis	45.5±3.4	46.3±2.6	0.1	58.5±5.6	58.1±6.0	0.5
Interval between parental diabetes diagnosis and birth of offspring (years)	19.8±7.2	17.7±5.6	0.1	31.5±7.4	28.2±8.3	<0.001***
Sex (M:F)	1:1.5	1:1.6	0.7	1:1.5	1:1.6	0.06
Age (years)	37.2±7.8	33.0±5.2	0.001**	40.9±7.4	38.7±7.7	0.002**
BMI (kg/m^2)	28.5±5.3	27.7±5.3	0.3	27.6±5.2	27.6±5.9	0.9
WHR	0.86±0.1	0.88±0.1	0.2	0.86±0.1	0.86±0.1	0.8
Body fat (%)	31.2±8.9	28.2±9.5	0.1	31.3±9.5	29.8±9.8	0.1

Offspring characteristics	Offspring of parents diagnosed with diabetes <50 years				Offspring of parents diagnosed with diabetes ≥50 years			
Parent with type 2 diabetes	Mother *n*=64	Father *n*=55	*p* value	GEE *p* value	Mother *n*=265	Father *n*=196	*p* value	GEE *p* value
Log EIR[a]	4.33±0.09	4.63±0.10	0.03*	0.02*	4.42±0.04	4.46±0.05	0.52	0.61
HbA_{1c}[b] (%)	4.89±0.05	4.68±0.06	0.006*	0.02*	4.72±0.03	4.70±0.03	0.58	0.60
Fasting glucose[b] (mmol/l)	5.07±0.07	5.17±0.08	0.16	0.28	5.06±0.03	5.09±0.04	0.62	0.69
Log fasting insulin[b]	0.92±0.03	0.94±0.03	0.92	0.93	0.91±0.01	0.91±0.01	0.25	0.26
Fasting C-peptide (nmol/l)	0.65±0.03	0.70±0.04	0.61	0.64	0.65±0.02	0.63±0.02	0.60	0.59
30-min glucose[b] (mmol/l)	8.51±0.22	8.20±0.24	0.21	0.29	8.25±0.11	8.1 ±0.12	0.40	0.46
Log 30-min insulin [b]	1.70±0.04	1.76±0.04	0.31	0.38	1.68±0.02	1.70±0.02	0.41	0.48
30-min C-peptide[b] (nmol/l)	2.21±0.14	2.40±0.15	0.44	0.49	2.07±0.07	1.98±0.08	0.45	0.51
Body fat[b] (%)	31.3±0.88	29.7±0.97	0.24	0.29	30.5±0.43	30.5±0.49	0.97	0.98
Log HOMAS[b]	2.04±0.03	2.00±0.03	0.92	0.93	2.04±0.01	2.04±0.01	0.95	0.95

Offspring were only included in this study if they had a uniparental history of type 2 diabetes. Data are shown as means±SD in the top part of the table. The *p* values are derived from an unpaired Student's *t*-test comparing means. Adjusted values are given as means±SEM, and *p* values obtained from comparing means by ANCOVA (using age, sex and percentage body fat) as covariates in the lower part of the table. Generalised estimating equation (GEE)-corrected *p* values were obtained by applying the GEE to take family relationships into account in the lower part of the table.

EIR Early insulin response, *F* female, *GEE* generalised estimating equation, *HOMAS* homeostatic model assessment for insulin sensitivity, *M* male

[a]Corrected for age, sex and body fat across all four groups studied; [b]corrected for age and sex across all four groups studied

before the age of 50 years was 9.0, compared with an odds ratio of 1.8 if paternal diabetes was diagnosed before 50 years [4].

We hypothesised that in Europids, the offspring of mothers with young-onset type 2 diabetes, diagnosed before 50 years, would have been exposed to mild hyperglycaemia in utero, while those born to fathers with type 2 diabetes diagnosed at a similar age would not have this exposure. If there was a programming effect of mild maternal hyperglycaemia in utero we expected to see a reduction in the beta cell function of offspring in adulthood.

Subjects and methods

Subjects for clinical studies

The subjects were 578 adult offspring, unaffected by diabetes, of sibling pairs with type 2 diabetes [5] collected by six UK centres that are members of the Warren 2 Consortium, established by Diabetes UK. All offspring were over the age of 18 years and had only one parent with diabetes. The study protocol was approved by Local Research Ethics committees and the study was performed in accordance with the Declaration of Helsinki. All participants gave written informed consent.

Methods for clinical studies

Measurements were taken according to a common protocol across the six recruitment centres and techniques were standardised. Subjects fasted from 22.00 h the night before assessment, and avoided excessive exercise and alcohol for the previous 24 h. Fasting samples for insulin, C-peptide and glucose were taken and 447 subjects underwent a shortened OGTT. Fifteen minutes after venous cannulation, three fasting blood samples were taken at 5-min intervals before a 75-g oral glucose load, after which blood was sampled at 10, 25 and 30 min. Body composition was assessed by bioelectrical impedance (Bodystat 1500, Douglas, Isle of Man, UK), and was available in 558 subjects. Waist and hip measurements were taken.

The primary endpoint was early insulin response (EIR) following OGTT, calculated using the formula (30-min insulin–mean fasting insulin)/(30-min glucose) [6]. Secondary endpoints were HbA_{1c} and insulin sensitivity measured by the homeostasis model assessment (HOMAS).

Offspring characteristics were compared depending on whether they had a mother or father affected with type 2 diabetes, dichotomised by parents' age at diagnosis of diabetes as <50 or ≥50 years.

Sample analysis

Plasma glucose was measured locally using the glucose oxidase method. HbA_{1c} was analysed in a single laboratory in Exeter, UK. All other samples were spun immediately, frozen and transferred for analysis in Newcastle.

Statistical analysis

Data were analysed using SPSS version 11 for Windows and presented as means with SD. Baseline means were compared using the unpaired Student's *t*-test. All other means were compared by ANCOVA using age and sex as covariates, except for EIR and HbA_{1c}, which were adjusted for age, sex and body fat. Adjusted values are reported with SEM. Data that were skew distributed were log transformed and expressed as geometric means and 95% CIs. All tests were two-tailed and the significance level was 0.05. Since many offspring were full siblings, the general estimating equation SPSS macro (http://www.ori.org/methodology/gee/gee.txt, last accessed in April 2006)

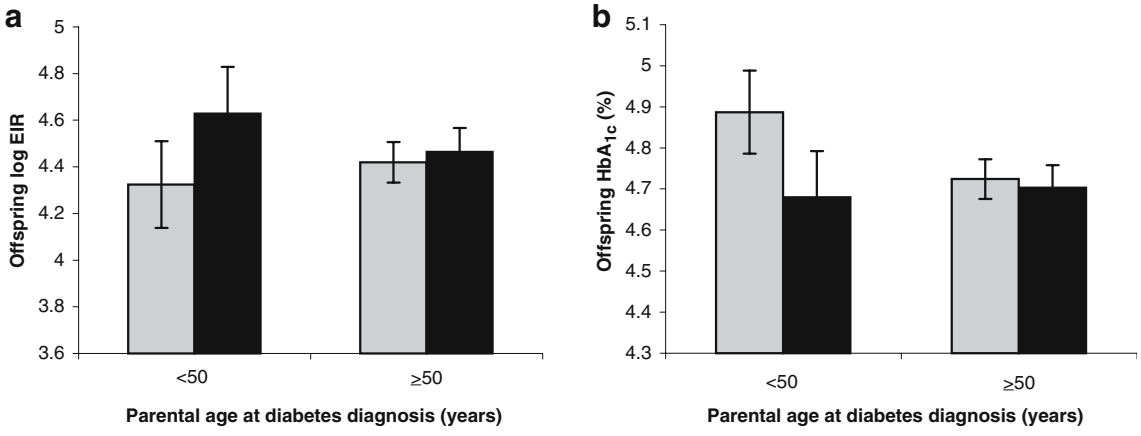

Fig. 1 Mean log EIR (**a**) and mean HbA_{1c} (**b**) in non-diabetic offspring according to which parent had diabetes. *Light shading* represents maternal diabetes, *dark shading* represents paternal diabetes. Offspring with parents diagnosed before or after 50 years are shown separately. General estimating equation-adjusted *p* values for paternal vs maternal diabetes: log EIR: age <50 years p=0.02; age ≥50 years p=0.61; HbA_{1c}: age<50 years p=0.02, age ≥50 years p=0.60. *Vertical bars* represent 95% confidence limits

was used to control for the non-independence of family members [7].

Results

Out of the 578 offspring, 117 (20%) had a parent diagnosed with diabetes below the age of 50, of whom 62 (53%) had a maternal history of diabetes. All offspring had been born before the mother was diagnosed diabetic, and the mean interval between offspring birth and parent's diabetes diagnosis was 20 years in the young-onset group and 30 years in the late-onset group (Table 1).

The baseline characteristics of the offspring according to parental diabetes and age at diabetes diagnosis showed no significant differences other than in age of offspring (Table 1). There was an increased proportion of female to male offspring in the study, which was distributed similarly in all groups studied. All results were therefore corrected for age and gender,

Mean log EIR was reduced in offspring whose mothers had young-onset type 2 diabetes compared with offspring of whom the father had diabetes (p=0.03). The mean HbA_{1c} was also elevated in this group (p=0.006) but fasting glucose was similar (Table 1). After adjusting for the non-independence of family members the EIR remained reduced (p=0.02) and HbA_{1c} was elevated (p=0.02) in offspring born to mothers with young-onset diabetes (Fig 1a,b). There was no difference in insulin sensitivity (p=0.92), BMI or total body fat (Table 1).

In offspring of parents diagnosed with later-onset type 2 diabetes (≥50 years), there were no significant differences seen in any of the outcome variables (Fig. 1a,b, Table 1).

Discussion

We have shown that non-diabetic offspring of mothers with young-onset type 2 diabetes have lower EIR, and higher HbA_{1c} than the offspring of fathers with young-onset type 2 diabetes. No difference was seen in these parameters when offspring of parents with late-onset type 2 diabetes were studied. This could result from the proximity of the onset of type 2 diabetes to the mothers' pregnancy and is consistent with an impact of exposure to mild hyperglycaemia in utero.

There is strong evidence that the offspring of mothers diagnosed as diabetic before they conceived have an increased risk of diabetes and/or beta cell dysfunction [1–3]. The importance of our result is that it suggests an impact of mild hyperglycaemia in utero preceding the diagnosis of diabetes even 20 years later. In Pima Indians 8.6% of offspring developed diabetes before 25 years when born to prediabetic mothers compared with 1.4% of offspring with non-diabetic mothers [1], but this was much less than when mothers were diabetic when the offspring were conceived (45%) [1].

We cannot be certain that the differences seen in the offspring of young-onset diabetic mothers were explained by intrauterine exposure to hyperglycaemia, as other explanations include extragestational environmental factors, mitochondrial diabetes or parental imprinting. Postnatal environmental confounders in offspring born to diabetic mothers versus fathers with diabetes such as feeding behaviour, exercise habits, and grocery choices of mothers, should manifest in differences in obesity, which was not observed, rather than altered beta cell function. A maternally transmitted mutation of the mitochondrial DNA causing a beta cell defect in offspring is possible, however the commonest mitochondrial mutation 3243A→G is too rare to account for our results because it affects <2% of UK diabetic patients. Imprinted genes involved in beta cell dysfunction where only the maternal allele is expressed offer a possible explanation, however no such imprinted gene has been identified to date.

We acknowledge the limitations of our study. Firstly, we do not have measurements of glycaemia in pregnancy, therefore intrauterine exposure to hyperglycaemia cannot be assumed or quantified. We do not have a record of who had gestational diabetes and how many were screened but suspect that in keeping with clinical practice over 30 years ago both were low. None of the mothers with young-onset type 2 diabetes were diagnosed with diabetes before pregnancy, however birthweight data, from other similar cohorts, shows an increase in birthweight by 250 g even when mothers are only diagnosed with type 2 diabetes 20 years later [8]. The increased birthweight is likely to reflect increased foetal insulin secretion in response to a mildly hyperglycaemic intrauterine environment. Secondly, we used age of diagnosis, which is a poor determinant of the age of onset of diabetes. Thirdly, for practical reasons we used EIR derived from a 75-g oral glucose load and HOMAS calculated from fasting insulin and glucose values, which have some limitations compared with the standard methods such as an intravenous glucose tolerance test or euglycaemic clamp, although they are well correlated [6, 9].

In conclusion we have shown that beta cell function is reduced in the offspring of mothers with early-onset type 2 diabetes. This result is consistent with a programming effect of intrauterine exposure to mild hyperglycaemia although there are other explanations. It is likely that the small changes seen in offspring beta cell function reflect the small changes in maternal glycaemia because in previous studies marked hyperglycaemia associated with maternal diabetes greatly increased the type 2 diabetes risk in offspring and markedly reduced their beta cell function [3, 10]. In the future, increasing obesity will lower the mean age of onset of type 2 diabetes and so, if this mechanism is indeed operating, hyperglycaemia exposure in utero will increase, amplifying the effect we have seen.

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