Exposure in utero to maternal diabetes leads to glucose intolerance and high blood pressure with no major effects on lipid metabolism

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Abstract

Aim. – Recent evidence shows that adult metabolic disease may originate from an adverse fetal environment that can alter organ development and function in postnatal life. This study aimed to analyze the effect of exposure in utero to maternal diabetes on the development of the metabolic syndrome in the offspring.

Methods. – Pregnant rats were made diabetic (blood glucose was 20 mM) with a single streptozotocin injection on day 0 of gestation. Offspring from diabetic mothers (DMO) and control mothers (CMO) were followed from birth to 12 months of age. In these animals, metabolic parameters, such as glucose tolerance, insulin sensitivity and plasma lipid levels, as well as pancreatic insulin and morphology were studied.

Results. – Compared with controls, DMO offspring had normal birth weights, but impaired postnatal growth that persisted throughout life. Metabolic tests revealed that DMO offspring also showed impaired glucose tolerance at six months associated with decreased insulin sensitivity and low insulin secretion. In older animals (12 months old), this phenotype persisted, but to a lesser extent. The DMO offspring also presented with high blood pressure and decreased levels of fasting plasma triglycerides, but normal plasma NEFA, and HDL and total cholesterol.

Conclusion. – Altogether, these results show that our model of exposure in utero to maternal diabetes led to normal birth weights, and induced transient glucose intolerance and increased blood pressure with no major effects on lipid metabolism. It also suggests that a hyperglycaemic fetal environment may be able to ‘programme’ hypertension and glucose intolerance, but not alter lipid metabolism.

Keywords: Maternal diabetes; The metabolic syndrome; Fetal programming; Blood pressure; Glucose tolerance

Résumé

L’exposition in utero à un diabète maternel induit une intolérance au glucose sans effet majeur sur le métabolisme lipidique.

Objectif. – Des données récentes ont montré que les maladies métaboliques adultes peuvent être programmées par un environnement fœtal délétère qui altère le développement et la fonction de certains organes. Nous avons étudié ici, chez le rat, l’influence de l’exposition in utero à un diabète maternel sur l’apparition du syndrome métabolique dans la descendance.

Méthodes. – Un diabète modéré a été induit chez des rates gestantes (glycémie à 20 mM) par une injection unique de streptozotocine au jour 0 de gestation. Les animaux issus de mères diabétiques (DMO) et de mères témoins (CMO) ont été suivis de la naissance à 12 mois. La tolérance au glucose, la sensibilité à l’insuline et les niveaux plasmatiques de lipides ont été analysés.

Résultats. – Comparés aux témoins, les animaux issus de mères DMO ont un poids de naissance normal mais une croissance post-natale réduite. À six mois, ils présentent une intolérance au glucose, leur sensibilité à l’insuline est altérée et leur sécrétion d’insuline est diminuée. À 12 mois, ce phénotype persiste mais dans une moindre mesure. Ces animaux issus des mères DMO présentent également une hypertension artérielle et un taux de triglycérides bas. En revanche, leurs taux d’acides gras non estérifiés, d’HDL-cholestérol et de cholestérol total sont normaux.

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Mots clés : Diabète maternel ; Syndrome métabolique ; Programmation fœtale ; Pression artérielle ; Tolérance au glucose

1. Introduction

The metabolic syndrome (MetS) refers to a cluster of metabolic risk factors related to type 2 diabetes, cardiovascular disease, chronic kidney disease and mortality (for a review, see Symonds et al. [1]). The MetS is an established risk factor for cardiovascular morbidity and mortality and, in humans, is defined as a combination of three or more of the following five factors: large waist circumference; elevated triglycerides; reduced high-density lipoprotein (HDL) cholesterol; raised blood pressure; and increased fasting glucose. The prevalence of the MetS is estimated to be nearly 15% among adults in Europe, reaching 30% in some European countries. Epidemiological studies have clearly identified maternal health as a major factor for the development of MetS in offspring. Thus, factors related to the development of MetS in children include maternal diabetes, maternal obesity, neonatal macrosomia and childhood obesity. In addition, maternal hyperglycaemia and insulin resistance during pregnancy are associated with an increased incidence of markers of the MetS during childhood [2].

Perinatal programming may be defined as the phenomenon whereby a stimulus that occurs during a critical window of development can bring about lifelong changes in bodily structure and function. Early developmental programming leading to later dysfunction and disease is the result of a combination of mechanisms acting on organs and tissues on both cellular and molecular levels. Various metabolic abnormalities have been demonstrated in the offspring in animal models of perinatal programming, such as low-calorie [3] or low-protein maternal diets [4], or placental insufficiency [5]. These models all lead to profound impairment of fetal growth, with intrauterine growth retardation (IUGR) that is associated, or not, with postnatal catch-up growth.

The aim of the present study was to assess the metabolic features of the offspring in a rat model of moderate maternal diabetes without IUGR. In earlier studies, we developed a rat streptozotocin-induced model of fetal exposure to maternal diabetes that was characterized by moderate levels of maternal hyperglycaemia (20 mM) throughout the entire gestational period, with normal pregnancy, no IUGR and the delivery of healthy, normotrophic pups. Previously, we also showed that the offspring of diabetic mothers (DMO) develop hypertension in adulthood [6]. The present study was focused on the programming of glucose and fat/lipid metabolism abnormalities.

2. Design and methods

2.1. Experimental model of maternal diabetes

Pregnant Sprague–Dawley rats (Charles River, France), weighing 250–300 g, were made diabetic on day 0 of gestation by a single intraperitoneal injection of streptozotocin (Sigma, 35 mg/kg). The diabetic state was confirmed by measuring plasma glucose concentrations (ACCU-CHEK®, Roche). Only pregnant females with plasma glucose levels of 15–20 mmol/L were included in the study, and their diabetic status was confirmed every two days until delivery. On the day of delivery, the newborn pups were weighed, and each litter reduced to ten pups and restricted to male offspring only. All animals were maintained in a temperature- and light-controlled room at 21°C with a 12-hour light cycle. All had free access to food (A03 chow; SAFE-Diets, Augy, France, Villemoisson-sur-Orge, France) and tap water. All experiments were conducted in accordance with our institutional guidelines, and the recommendations for the care and use of laboratory animals as proposed by the French Ministry of Agriculture (protocol number p3/2008/027). Altogether, 12 rats issued from five control mothers (CMO) and ten rats issued from five DMO were studied. The animals were followed from age one to 12 months and sacrificed at 18 months of life.

2.2. Insulin tolerance test

Insulin sensitivity was evaluated with an insulin tolerance test (ipITT). After a four-hour fast, a single intraperitoneal injection (1 IU/kg of body weight) of human recombinant insulin (Actrapid®, Novo Nordisk) was delivered. Blood glucose was then measured from the tail vein just before, and 15, 30, 60 and 120 minutes after insulin injection, using test strips and reader (ACCU-CHEK Go®, Roche). For insulin sensitivity, the first-order rate constant for glucose disappearance (kITT) was estimated from the slope of regression line of ln plasma glucose compared with time, according to the formula kITT = ln2t1/2 × 100, at 0–30 minutes after delivery of the insulin bolus.

2.3. Glucose tolerance test

After an overnight fast, a glucose tolerance test (ipGTT) was performed, using a single intraperitoneal injection of a glucose solution (100 mg/100 g of body weight). Rats were weighed, and
blood samples collected from the tail were placed in Eppendorf tubes containing heparin for determination of fasting glycaemia, fasting insulinaemia and lipid profiles. Blood glucose levels were measured just before, and 15, 30, 60 and 120 minutes after glucose injection, while blood samples were collected at the same time for measurement of plasma insulin by enzyme-linked immunosorbent assay (Elisa; Mercodia).

2.4. Pancreatic insulin content

Pancreata were crushed in an acid–alcohol solution (75% ethanol, 1.5% hydrochloric acid), and extraction was performed overnight at −20°C. Tissues were then centrifuged, and supernatants stored at −20°C. The total pancreatic insulin contents were assayed using a Mercodia Elisa kit.

2.5. Pancreatic morphometric analysis

Pancreata from adult rats were dissected (five from each group), and fixed, sectioned and prepared for immunohistochemistry, using a Dako Autostainer (Trappes, France) and anti-insulin antibody (Sigma-Aldrich), followed by incubation with secondary antibodies coupled with peroxidase (Immunotech, Marseilles, France) and revealed using DAB. Islet parameters (mean islet size, islet numbers, and percentages of small, medium and large islets), as described elsewhere [7], were quantified, using a Leica Qwin 500 imaging software, as previously described [8].

2.6. Plasma lipid measurements

Triglycerides, non-esterified fatty acids (NEFA), HDL cholesterol and total cholesterol were measured with an Olympus chemistry analyzer at the biochemistry Core Laboratory of the Bichat–Claude-Bernard Hospital in Paris.

2.7. Statistical analysis

All results are presented as means ± SEM values. Statistical analysis was performed using the unpaired Student’s t test, the Mann-Whitney non-parametric test and Anova (analysis of variance) when appropriate. Statistical significance was defined as P < 0.05.

3. Results

3.1. Impaired postnatal growth

Despite normal gestational and delivery conditions, the DMO group exhibited impaired postnatal growth. Glycaemia in the five DMO was constant throughout gestation (20.3 ± 1.3 mM) and the rats delivered spontaneously at term (21 days) after normal gestation. The number of pups per litter was similar in both the CMO and DMO groups (14.3 ± 3.6 and 11.4 ± 4.5, respectively). At birth, the DMO pups appeared to be healthy, with birth weights similar to those of the controls (Fig. 1). However, despite having similar birth weights, the DMO rats exhibited impaired postnatal growth starting at day 7. This change in weight gain persisted after weaning and throughout their entire life (Fig. 1).

3.2. Regulation of glucose metabolism

Fasting glycaemia and insulinaemia were similar in both DMO and CMO groups at age three, six and 12 months (Supplementary data, Table S1; see supplementary material associated with this article online). To investigate DMO ability to regulate glucose metabolism, ipGTT and ipITT were performed in the same set of animals at three, six and 12 months of age. At three months (young adults), the DMO presented with normal responses to glucose infusion (ipGTT) and normal sensitivity to insulin (ipITT; Fig. 2A and B). In more mature animals (six months of age), DMO exhibited impaired ipGTT (Anova; group effect, P = 0.008) compared with CMO, whereas ipITT was similar in both groups (Fig. 2C and D). Finally, in older rats (12 months of age), both ipGTT (P < 0.05) and ipITT (P < 0.05) were altered in DMO compared with CMO (Fig. 2E and F). This progressive, age-dependent impairiment of glucose metabolism regulation in the DMO is illustrated in Fig. 3, which shows that the ipGTT area under the curve (AUC) was increased in DMO after six months of age. In addition, plasma insulin concentrations measured at the t15-min peak were reduced in 12-month-old DMO (Fig. 3C), while the AUC for insulin levels tended to be decreased in six-month-old DMO, but was clearly decreased in the 12-month-old DMO (Fig. 3D). Overall, glucose intolerance is more pronounced in six-month-old DMO vs 12-month-old DMO, suggesting a transient phenotype. These results indicate impaired insulin secretion in response to glucose infusion.

To determine whether or not this deficit of insulin secretion revealed by ipGTT was linked to pancreatic structural abnormalities, morphometric analysis was performed in pancreata from 12-month-old animals after insulin immunostaining. As shown in Supplementary data, Fig. S1A–C (see supplementary

Fig. 1. Total body weights of the rat offspring of control mothers (CMO, open bars) and diabetic mothers (DMO, black bars) from birth to day 30 (left) and from three to 18 months (right). Values are means ± SEM for eight to 12 animals issued from five litters. Comparisons were made using two-way Anova (results shown in the graph) and Student’s t test for CMO and DMO rats of the same age. **P < 0.01, ***P < 0.001, ****P < 0.0001.
material associated with this article online), the number of islets, mean size of islets and percentages by size of endocrine islets were similar in both groups, suggesting a normal endocrine mass in DMO. Pancreatic insulin concentration during the postabsorptive period was also in the same range for both CMO and DMO (Supplementary data, Fig. S1D; see supplementary material associated with this article online).

Fig. 3B shows the variation in insulin-sensitivity index (kITT) scores with ageing. The DMO had lower kITT scores after six months than the CMO, which indicates greater degrees of insulin resistance in ageing DMO. However, Western-blot analysis in tissues from 12-month-old animals showed no differences in the expression of phospho-Akt (Thr308) and phospho-Akt (Ser473) in liver, muscle or adipose tissues (data not shown).

3.3. Diabetic mother rats and the full metabolic syndrome

In addition to glucose metabolism, other components of the MetS were studied. Lipid metabolism was assessed in three-, six- and 12-month-old animals. As shown in Fig. 4, fasting plasma
Fig. 3. Results of glucose tolerance tests (ipGTT), including area under the curve (AUC), kITT and $t_{15}$ insulin levels, in the rat offspring of control mothers (CMO, open bars) and diabetic mothers (DMO, black bars) at three, six and 12 months of age. (A) AUC was calculated from ipGTT values (Fig. 2); (B) kITT (first-order rate constant for glucose disappearance) was estimated at 0–30 minutes after delivery of the intraperitoneal insulin bolus, as described elsewhere (C) insulin peak response, as measured 15 minutes after intraperitoneal glucose load; and (D) AUC of insulin blood levels, as measured during the ipGTT. Values are means ± SEM for eight to 12 animals issued from five litters. Comparisons were made using Anova (results given in each graph) and Student’s $t$ test for CMO and DMO rats of the same age. *$P<0.05$.

total cholesterol, HDL cholesterol and NEFA were similar in both groups, whereas DMO at all ages had lower triglyceride levels than CMO ($P<0.05$).

As a relative increase in fat tissue was observed in the DMO despite their lower body weights, the amounts of perigonadal, perirenal and inguinal fat tissue were also measured at the end of the follow-up period. As shown in Supplementary data, Fig. S2 (see supplementary material associated with this article online), DMO rats were leaner than the CMO, and had lower gonadal and inguinal fat-to-body weight ratios.

4. Discussion

The aim of the present work was to study the metabolic features of rats exposed in utero to moderate maternal diabetes. A follow-up study was also carried out in young-adult (three months of age), mature-adult (six months of age) and aged (12 months of age) animals issued from CMO and DMO mothers. This longitudinal study revealed that six-month-old animals were glucose-intolerant, but had normal insulin sensitivity, whereas 12-month-old animals were still glucose-intolerant, but to a lesser extent, and insulin-resistant. Yet, no major modification of lipid metabolism was observed. Thus, these results demonstrate the effect of ‘programming’ of altered glucose homoeostasis by exposure in utero to hyperglycaemia, but with no changes in plasma lipid levels.

The present findings support — but also extend — the results of other studies concerning the resetting of glucose metabolism in utero exposure to maternal diabetes in rats [2,9,10]. From such studies, it was concluded that: first, the severity of maternal diabetes influences both fetal growth, and endocrine pancreas development and response; and second, that the phenotype appears to vary according to age at the time of analysis — whether during fetal or postnatal periods, or in postweaned or aged animals. However, the results obtained in different studies using different protocols, including various doses of streptozotocin, are difficult to compare. Furthermore, as severe maternal diabetes (high doses of streptozotocin) impairs fetal growth, it also interferes with the well-known postnatal metabolic effects of IUGR.

For this reason, the present study used:

- a model of moderate maternal hyperglycaemia with no fetal hypotrophy;
- dams that were not cross-fostered at birth;
- a longitudinal study design that covered several periods of adulthood, including aged animals.

Our results showed the progressive impairment of both insulin secretion and sensitivity with age. Regarding insulin secretion, pancreatic tissue morphometric analysis of 12-month-old animals showed that all parameters (mean endocrine islet
size and number) were similar in both CMO and DMO, indicating that the decreasing insulin response was not related to structural abnormalities of the endocrine pancreas. Likewise, pancreatic insulin contents, as measured in the postabsorptive period in 12-month-old animals, were also similar in both groups.

Beta-cell development in the rat occurs mainly during the last week of fetal growth [11]. DMO rats have normal pancreatic histology and insulin contents, suggesting that moderate maternal diabetes does not alter beta-cell mass. Yet, DMO rats present with impaired insulin secretion, an indication that the beta cells are not functioning properly. In fact, studies have shown that neonatal beta cells lack glucose-induced insulin-secretion capacity [12,13], suggesting that beta-cell function matures after birth and probably before weaning [13]. Interestingly, this is precisely the same period between birth and weaning that our DMO rats presented with growth retardation, a defect probably due to altered nutrition from their diabetic milking dams. This suggests that beta-cell maturation and function may be altered by changes in nutritional conditions during postnatal life when fostering mothers are diabetic. In rats, streptozotocin-induced diabetes led to a reduced ability to synthesize fatty acids in the mammary gland [14] while, in humans, the milk from DMO contains lower amounts of long-chain polyunsaturated fatty acids [15]. Nevertheless, the consequences of such lipid changes in milk on beta-cell function are still, as yet, unknown. However, changes in milk composition could be contributing to the growth retardation observed after birth in offspring.

Our present study showed that beta-cell function was altered, while beta-cell parameters stayed the same. Earlier experimental models also showed that exposure to maternal diabetes induced impaired beta-cell function, while beta-cell mass remained normal [9,10]. However, our present longitudinal design allowed to define the precise sequence of metabolic alterations to be determined by following animals exposed in utero to high glucose. The results clearly show that DMO manifest beta-cell dysfunction and insulin resistance at six months of age while, at 12 months of age, the same alterations are present, but to a lesser extent. This is noteworthy, as the usual aetiology of type 2 diabetes accepts that insulin resistance develops first, thereby imposing an increased insulin demand on beta cells that will eventually lead to insulin exhaustion [16]. One possible explanation is that maternal diabetes alters the development of several organs that will eventually lead to dysfunction in sequence, with beta cells first failing to properly secrete insulin and, later, the peripheral organs failing to take up glucose properly. Interestingly, our DMO rats also presented with decreased fat mass, a feature not usually associated with insulin resistance. At the molecular level, there was no evidence of insulin resistance, as no changes in Akt phosphorylation were observed. This could have been due to the presence of mild insulin resistance in each organ (liver, muscle, adipose tissue) that was too low to be detected by analysis of Akt phosphorylation, but sufficient, when taken as a whole, to induce decreased insulin sensitivity overall.
The DMO rats also presented with increased blood pressure and glucose intolerance, but with no changes in plasma lipids or HDL cholesterol, a reflection of the fact that maternal diabetes can lead to some, but not all, features of the MetS, suggesting that it affects different tissues differently: for instance, fat mass is reduced, but plasma lipids remain normal. However, it should be borne in mind that, unlike human newborns, rat pups have very low stores of adipose tissue at birth, a difference that renders comparison with humans unreliable.

5. Conclusion

The present study showed that in utero exposure to high glucose is a risk factor for any offspring to develop glucose intolerance together with hypertension, as already highlighted in a previous study [6]. The present longitudinal approach demonstrated that beta-cell dysfunction appears before insulin resistance in this rat model, a sequence of events that is not usually seen in the aetiology of type 2 diabetes. However, the question of whether or not these circumstances would also contribute to type 2 diabetes in humans remains to be determined, and needs to be carefully studied in the children born of DMO.

Conflict of interest statement

No potential conflicts of interest relevant to this article was reported.

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Appendix A. Supplementary data

Supplementary data (Fig. S1, Fig. S2 and Table S1) associated with this article can be found, in the online version, at doi:10.1016/j.diabet.2010.10.008 and http://www.sciencedirect.com.

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