Hyperglycaemia Induced by Glucose Infusion in the Unrestrained Pregnant Rat: Effect on Body Weight and Lipid Synthesis in Post-Mature Fetuses

A. Ktorza, N. Nurjhan, J.-R. Girard and L. Picon

Laboratoire de Physiologie du Développement, Laboratoire associé au CNRS N° 307,
Université Paris VII and Laboratoire de Physiologie du Développement, Collège de France, Paris, France

Summary. Mild hyperglycaemia was induced in unrestrained pregnant rats from day 20.5 to day 23.5 of pregnancy, using a continuous glucose infusion. Control rats were infused with distilled water. In post-mature fetuses from glucose-infused rats, raised plasma glucose and insulin concentrations were related to increased body weight (6.03 ± 0.07 g) and total carcass fat ($2.02 \pm 0.04\%$ of fresh weight) compared with control fetuses of the same age (5.35 ± 0.07 and 1.5 ± 0.04 g, respec-

tively). Concurrently, the rate of lipogenesis in the carcass, estimated from the incorporation of tritium from tritiated water into fatty acids, was significantly increased in fetuses from glucose infused rats compared with control rats (6.00 ± 0.34 versus 2.62 ± 0.27 and $^3\text{H}_2\text{O} \cdot \text{h}^{-1} \cdot \text{g tissue}^{-1}$, respectively).

Key words: Glucose infusion, pregnant rats, fetus, plasma insulin, fetal body weight, lipid synthesis.

Increased birth weight is a striking feature of infants of diabetic women [4]. Diabetes induced in rats by injecting alloxan or streptozotocin before [9, 17, 18] or during pregnancy [1, 7] has resulted in very conflicting data on fetal weight and fat deposition. Quite different effects were observed when maternal diabetes was either mild or severe. The fetal body weight has been reported to be increased [16], unchanged [9], or decreased [1, 7, 17]. The discrepancy between these results must be related, on the one hand, to the difficulty of controlling hyperglycaemia induced by these diabetogenic drugs, and on the other, to the poor adipose development in the fetal rat at term.

We have used a technique of continuous infusion of glucose in chronically catheterized unrestrained pregnant rats that allows carefully controlled hyperglycaemia for several days in animals living in conditions as close to normal as possible. We have performed this study in 23.5 day-old post-mature fetuses, since it has been reported that fetal adiposity, related to hyperinsulinism, was increased with advancing post-maturity in the rat [15].

Material and Methods

Animals

Albino rats of the Sherman strain, bred in the laboratory, were fed ad libitum with pelleted chow (UAR B-03). Three month-old virgin fe-

males, weighing about 250 g, were caged with a male for one night (17.00–09.00 h) and pregnancy was detected by abdominal palpation 14 days later. In our colony, birth occurred 22 days after mating. Parturition was prevented by injecting 2.5 mg of progesterone daily (Lutogyl-Roussel, Paris, France). The treatment started on day 20.5.

The flexible technique for long-term infusions in unrestrained rats developed by Nicolaïdis et al. [12] was used for glucose infusions as described previously [10]. The infusion period began on 1 day after surgery and lasted for 3 days until removal of the fetuses. Hypertonic 30% sterile glucose (Chaix et Dumarais, Paris, France) was infused at the rate of $30 \mu\text{l}/\text{min}$. Control rats were infused with sterile distilled water. Distilled water was chosen rather than normal saline to avoid sodium overload in the control rats. Since the infusion rate was very slow compared with the blood flow rate in the superior vena cava, haemolysis was theoretically unlikely and were not observed. For each group, six animals were used. The mean $\pm$ SEM litter size was: 8.15 ± 0.06 for control mothers, 8.05 ± 0.1 for glucose-infused mothers.

Samples

The plasma glucose of infused rats was measured daily on blood samples from the tail vein. After maternal anaesthesia by Ketamine ($125 \text{ mg}/\text{kg}$, IP, Imalgène, Mérieux, Lyon, France), the fetuses were exteriorised from the uterus, leaving the placenta and umbilical cord in situ, in order to maintain normal feto-maternal exchanges. Blood was collected from the axillary vessels and the livers were rapidly removed, weighed and frozen in liquid nitrogen for the determination of hepatic lipogenesis. The fetuses were then removed and weighed, the total weight taking into account the weight of the blood and liver samples. The fetuses were kept at -20°C until the lipids were measured. The mothers were infused throughout the period of removal of the fetuses.

To evaluate the rate of lipogenesis in maternal and fetal livers and fetal carcasses in vivo, 23.5-day pregnant rats were injected, via the in-

Table 1. Plasma glucose, immunoreactive insulin, α -amino nitrogen levels, oral food intake and hepatic lipogenesis in control and glucose-infused rats

	Plasma glucose (mmol/l) (n = 6)	Immunoreactive insulin (mU/l) (n = 6)	Plasma α -amino nitrogen (mmol/l) (n = 6)	Oral food intake (Kcal/day) (n = 6)	Hepatic lipogenesis ($\mu\text{mol} \cdot ^3\text{H}_2\text{O} \cdot \text{h}^{-1} \cdot \text{g}$ of liver ⁻¹) (n = 5)
Control rats	5.41 $\pm$ 0.47	36 $\pm$ 5	5.40 $\pm$ 0.26	63.6 $\pm$ 1	9.72 $\pm$ 0.7
Glucose-infused rats	12.74 $\pm$ 0.60 ^a	361 $\pm$ 30 ^a	5.10 $\pm$ 0.24	17.2 $\pm$ 0.6 ^a	110.8 $\pm$ 22.7 ^a

Values are expressed as mean $\pm$ SEM; ^a $p < 0.001$: glucose-infused versus control rats

Table 2. Plasma glucose, immunoreactive insulin, α -amino nitrogen levels, body weight, carcass fat and lipogenesis in fetuses from control and from glucose-infused rats

	Plasma glucose (mmol/l)	Plasma immuno- reactive insulin (mU/l)	Plasma α -amino nitrogen (mmol/l)	Body weight (g)	Total carcass fat (percentage of fresh weight)	Lipogenesis ($\mu\text{mol} \cdot ^3\text{H}_2\text{O} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$)	
						Carcass	Liver
23.5 day-old fetuses from control rats	3.11 $\pm$ 0.28 (31)	158 $\pm$ 20 (31)	12.1 $\pm$ 0.6 (12)	5.35 $\pm$ 0.07 (31)	1.58 $\pm$ 0.04 (31)	2.62 $\pm$ 0.27 (20)	7.48 $\pm$ 0.38 (22)
23.5 day-old fetuses from glucose infused rats	9.00 $\pm$ 0.55 ^a (32)	499 $\pm$ 45 (30)	11.9 $\pm$ 0.6 (12)	6.03 $\pm$ 0.07 ^a (32)	2.02 $\pm$ 0.04 ^a (32)	6.00 $\pm$ 0.34 ^a (23)	7.33 $\pm$ 0.49 (24)

Values are expressed as mean $\pm$ SEM, number of determinations in parentheses. ^a $p < 0.001$: fetuses from glucose-infused rats versus fetuses from control rats

fusion system, with 10 mCi $^3\text{H}_2\text{O}$, 1 h before fetal removal. Lipogenesis was estimated according to the method of Fain et al. [3] and expressed as μmol of $^3\text{H}_2\text{O}$ incorporated into lipids per g of tissue per hour. Total lipid content of the carcass was measured in the whole fetus according to the method of Folch et al. [5].

Aliquots of plasma from three or four fetuses of the same litter were deproteinized in cold 5% (v/v) trichloroacetic acid. The trichloroacetic acid supernatants were used for colorimetric determination of α -amino nitrogen using serine as standard [11]. Plasma glucose was determined by the glucose oxidase technique on 10 μl samples using a glucose-analyser (Beckman, Palo Alto, USA). Plasma immunoreactive insulin was estimated using purified rat insulin as standard (R171, Novo, Copenhagen, Denmark), antibody to human insulin [20] and porcine monoiodinated ^{125}I -insulin [6]. Silicate was used to separate free from bound hormone [19]. The method allowed the determination of 6 mU/l (0.25 ng/ml) with a coefficient of variation within and between assays of 10%.

The results are expressed as mean $\pm$ SEM and the significance of difference between means was evaluated by Student's unpaired t-test. Statistical correlations were performed by the classical least squares procedure.

Results

Mild hyperglycaemia (approximately 12 mmol/l) was produced in post-mature pregnant rats infused with glucose (Table 1). Hyperglycaemic pregnant rats had a marked increase in plasma insulin concentration and showed a sharp decrease in oral food intake (Table 1). In these rats, hepatic lipogenesis was increased compared with control rats (Table 1). Plasma α -amino nitrogen was not significantly altered in glucose-infused rats compared with control rats (Table 1).

Maternal hyperglycaemia had a marked effect on glycaemia in the fetuses ($p < 0.001$; Table 2). No signifi-

cant difference was observed in plasma α -amino nitrogen concentration between the fetuses from glucose infused and control rats (Table 2). The level of plasma immunoreactive insulin in 23.5 day-old fetuses from glucose-infused rats was higher than in control fetuses (Table 2). In fetuses from glucose-infused rats, body weight and lipid content of the carcass were significantly increased on day 23.5, compared with control fetuses. When total carcass fat, expressed as a percentage of fresh weight was plotted against fetal body weight, a linear relationship was found between the two parameters ($y = 0.22x + 0.61$; $r = 0.59$; $n = 63$; $p < 0.001$). In fetuses from glucose-infused rats, the rate of lipogenesis in vivo in the carcass was much higher than in control fetuses (Table 2). In contrast, the rate of hepatic lipogenesis was not increased in fetuses from glucose-infused rats.

Discussion

As we have shown previously [10], we were able to induce steady hyperglycaemia in the pregnant rats and their fetuses, using a continuous glucose infusion which led to a dramatically decreased food intake in the mothers. Thus, it was important to know whether this reduced food intake, producing a low protein intake, affected plasma amino acid levels in the mothers. Plasma α -amino nitrogen concentration was not significantly decreased in the glucose-infused pregnant rats and their fetuses when compared with control animals. These data suggest that reduced food intake, during the last

3 days of gestation in glucose-infused rats, did not prevent a normal supply of amino acids to the fetuses, although an imbalance in individual amino acids cannot be excluded. The rate of hepatic lipogenesis was greatly stimulated in glucose infused rats. Possibly, lipogenesis was enhanced by hyperinsulinism and the lack of fat supply resulting from reduced food intake.

Experimentally induced diabetes in rats has led to conflicting results concerning fetal body weight at term. In a previous study [10], we showed that glucose infused into rats during the last 3 days of gestation failed to increase fetal body weight. This is in contrast with the observations made in infants of diabetic women who usually have increased body weight, due mainly to excessive fat deposition [13]. According to Pedersen et al. [14], maternal hyperglycaemia leads to raised fetal blood glucose which stimulates the secretion of insulin and results in increased fat deposition in the human fetus. One explanation for this discrepancy could be the difference in the adipose development between the fetal rat and the human fetus. In the latter, adipose development is important in utero and reaches 16% of the total body weight at birth, whereas, in the rat, adipose development is essentially post-natal (1% of the body weight at birth). Therefore, we assumed that prolonged pregnancy could result in adipose development in the fetal rat, sufficient to demonstrate the effect of insulin. This was suggested by a previous study showing that exogenous insulin injected into fetuses resulted in an increase in fetal body weight and fat deposition which increased with advancing pregnancy [15]. Our results are consistent with this hypothesis, since the lipid content of the carcass and fetal body weight evolved similarly: both were increased in 23.5 day-old fetuses from glucose-infused rats compared with control rats and a close relationship was found between fetal body weight and the percentage of lipids in the carcass. This view is strengthened by the increase in the rate of lipogenesis observed in carcasses of 23.5 day-old fetuses. In contrast, the lack of stimulation of hepatic lipogenesis by increased plasma glucose and insulin concentrations suggests that lipogenesis in the fetal liver is not sensitive to changes in plasma insulin levels. On the other hand, it is not surprising that hyperglycaemia per se, could not stimulate hepatic lipogenesis, since it was demonstrated that glucose poorly contributes to lipid synthesis in the liver in rodents [2, 8].

In conclusion, the present data suggest that the induction of mild hyperglycaemia in the pregnant rat by glucose infusion leads to increased body weight which in post-mature fetuses results from excessive fat accretion related to hyperinsulinism; a situation which may be analogous to that seen in the offspring of diabetic women.

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Dr. A. Ktorza
Laboratoire de Physiologie du Développement
Laboratoire associé au CNRS N° 307
Université Paris 7
Tour 23–33 1er étage
2, Place Jussieu
F-75251 Paris Cedex 05
France