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Decreased Uterine Blood Flow in the Diabetic Pregnant Rat Does Not Modify the Augmented Glucose Transfer to the Fetus

M. Palacín, M.A. Lasunción, A. Martín, E. Herrera

Departamento de Investigación, Centro Ramón y Cajal, Madrid, y
Departamento de Bioquímica, Facultad de Medicina, Universidad de Alcalá de Henares, Madrid, España

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Abstract. To determine whether changes in uterine blood flow affect placental glucose transfer in the diabetic pregnant rat, on the 7th day of gestation rats were intravenously treated with either streptozotocin (45 mg/kg) (diabetics) or buffer (controls). On the 20th day of gestation, fetal body weight and uterine blood flow appeared reduced whereas fetal/maternal plasma glucose was enhanced and lactate ratios were unchanged in diabetics versus controls. After 20 min of (U-^{14}C)-*D*-glucose infusion through the maternal left uterine artery, plasma values of fetuses from left and right uterine horns were higher for ^{14}C-glucose and lower for ^{14}C-lactate in diabetics versus controls, and placental glucose transfer was greatly augmented in diabetics whether or not uterine blood flow was included in its calculation. Whereas a linear correlation existed between placental glucose transfer and maternal plasma glucose concentration, transferred glucose conversion into lactate remained stable even when the maternal glucose level was high. It was concluded that enhanced placental glucose transfer in the pregnant diabetic rat is not modified by reduced uterine blood flow. The limited capacity of the fetus to handle the great incoming flux of glucose through the placenta of a severely diabetic mother produces permanent hyperglycemia which may impair fetal growth.

Introduction

Fetal growth depends on the quality and quantity of metabolic fuels crossing the placenta, which may be used directly as building blocks for fetal accretion and also modulate fetal β-cell insulin release which is recognized as an important growth factor for the fetus [2, 19]. In a previous study in the diabetic pregnant rat [8] we reported that placental metabolite transfer to the fetus is independent of the severity of maternal diabetes, being mainly modulated by the respective concentration of these metabolites in maternal cir-

culation. While placental glucose transfer is therefore directly related to maternal glycemia, from studies in guinea pigs it is known that changes in blood flow in the placenta may modify the transfer rate of diffusible substances [11] and that there is a linear relationship between placental blood flow and fetal weight [15]. As it has been recently reported that placental blood flow decreases in the diabetic pregnant rat [6], the present study was performed to determine whether this change affects fetal/maternal glucose relationships in the streptozotocin-diabetic late pregnant rat. For this purpose, placental glucose transfer was measured in diabetic rats using our recently reported in situ placental preparation [13], and placental blood flow was estimated by the infusion of labelled microspheres to other animals [18]. This study was extended to evaluate the proportional conversion of placental transferred glucose into lactate by the fetus of the diabetic rat, since we had previously proposed that the rat fetus has a limited capacity to handle the incoming load of glucose from a highly hyperglycemic mother [8], and that placental lactate formation from maternal substrates constitutes an important factor for fetal metabolic economy and development [14].

Materials and Methods

Female Wistar rats maintained in a light (12 h on-off cycles) and temperature (22 ± 2 °C) controlled room and fed ad libitum a purina chow diet were mated when weighing 160–180 g. On day 7 of gestation (estimated by the appearance of spermatozoids in vaginal smears), rats were made diabetic by a single intravenous injection of streptozotocin (kindly donated by Dr. *A.Y. Chang,* Upjohn Co., Kalamazoo, Mich.) (45 mg/kg body weight) dissolved in 50 m*M* citrate buffer pH 4.5. Other pregnant rats were injected with only the buffer and used as normal controls. All animals were studied on day 20 of gestation.

To determine glucose placental transfer, rats were anesthetized with nembutal (33 mg/kg body weight) and subjected to our previously described surgical procedure [13]. A PE-10 cannula (Intramedic, USA) was introduced countercurrent into the left external iliac artery to the level of the left uterine artery. The superior vesical artery, the superior gluteal artery and the hypogastric trunk of the left side were clamped. A solution of 0.9% NaCl (250 µl) containing 10 µCi of (U-^{14}C)-*D*-glucose (spec. act. 257 mCi/mmol; Radiochemical Centre, Amersham, UK) was infused for 20 min through the cannula. In this way the infusion medium becomes diluted with maternal blood reaching the left uterine artery, and the left uterine horn receives the tracer directly before it becomes diluted in the mother's general circulation. After infusion, blood was collected from the abdominal aorta into heparinized syringes and fetuses from the left and the right uterine horn were immediately excised. They were decapitated and blood collected into heparinized receptacles. Blood from all fetuses from left and right uterine horns was pooled separately. Maternal and fetal plasma aliquots were used for counting total radioactivity and deproteinized with 10% $HClO_4$ and neutralized with saturated $KHCO_3$. Aliquots of protein-free supernatants were used for glucose assay [14] and for ^{14}C-glucose and ^{14}C-lactate purification by ion exchange column chromatography [20]. Other methodological details were as previously described [8]. Radioactive values were corrected by considering 1×10^6 dpm as the total radioactivity infused per rat.

Blood flow in the uterine horn was studied in other animals kept under the same environmental and experimental conditions as above, using a slightly modified version of the procedure described by Rosso and Kava [18]. Rats were anesthetized with nembutal and polyethylene catheters were inserted into the right femoral and the right carotid arteries. ^{99m}Tc-labelled albumin microspheres (Sovin Biomedica SpA, Saluggia, Italy) with a diameter of 23–45 µm were suspended in 10% dextran containing 0.1% Tween-80. One milliliter of the suspension containing approximately 100,000 microspheres was injected per rat via the carotid catheter in 30 s by means of a multichannel peristaltic pump. Simultaneously, blood was withdrawn from the femoral artery for 90 s at a rate of approximately 0.8 ml/min, after which rats were

killed. The left uterine horn was excised and after removing the fetuses the whole horn was processed to count its total radioactivity which was compared to that present in blood aliquots. Blood flow to the left uterine horn was calculated as described [18].

Placental glucose transfer was calculated as elsewhere [8] whereas glucose conversion into lactate by the fetal-placental unit was calculated by the following formula:

$$\text{Glucose converted to lactate (nmol/ml/min)} = \frac{(\text{Lact*LF} - \text{Lact*RF}) \times G \times \Phi}{G^*},$$

where Lact* denotes the labelled lactate (dpm) in left and right fetal plasma (LF and RF, respectively), G is maternal glucose concentration in arterial plasma, G* is injected ^{14}C-glucose per rat (corrected to 1×10^6) and Φ the blood flow to the uterine horn.

Results and Discussion

As shown in table I, streptozotocin diabetic pregnant rats show reduced maternal and fetal body weights and blood flow to the uterus, unchanged litter size and maternal and fetal plasma lactate concentrations, and intensely enhanced maternal and fetal plasma glucose levels as well as fetal/maternal plasma glucose ratio, when compared to values present in normal control rats. Reduction in fetal body weight in the diabetic pregnant rat is in agreement with previous findings [3, 5, 6, 8, 17]. This change may be related with reductions in fetal plasma insulin levels present in animals under similar experimental conditions [8], although the decreased uterine blood flow observed could also be responsible for the limited growth of the fetuses. From studies with pregnant guinea pigs under steady-state conditions, it has been proposed that a direct relationship exists between placental blood flow and fetal body weight [15]. Increased fetal/maternal plasma glucose ratio in the presence of unchanged plasma lactate values in the diabetic animals would indicate that placental glucose transfer exceeds fetal capacity to metabolize it.

Placental glucose transfer was determined by the infusion of (U-^{14}C)-*D*-glucose through

Table I. Effect of streptozotocin diabetes on maternal and fetal weights and circulating glucose and lactate in the 20-day pregnant rat

	Controls	p	Diabetics
Maternal body weight, g	336 ± 7[1]	< 0.01[2]	295 ± 9
Litter size	12.2 ± 0.9	NS	12.3 ± 0.5
Fetus body weight, g	3.79 ± 0.06	< 0.05	3.34 ± 0.12
Left uterine horn blood flow	5.31 ± 0.87	< 0.05	3.16 ± 0.37
Maternal plasma glucose, m*M*	5.1 ± 0.3	< 0.001	24.4 ± 2.3
Fetal plasma glucose, m*M*	2.1 ± 0.2	< 0.001	16.4 ± 1.4
Fetal/maternal plasma glucose ratio	0.42 ± 0.03	< 0.001	0.70 ± 0.03
Maternal plasma lactate, m*M*	2.4 ± 0.5	NS	2.8 ± 0.4
Fetal plasma lactate, m*M*	13.1 ± 1.7	NS	12.8 ± 0.5
Fetal/maternal plasma lactate ratio	7.9 ± 2.7	NS	5.3 ± 0.9

[1] Means ± SEM of 5–7 rats/group.

[2] Statistical comparisons between diabetic and control rats (NS = not significant, $p > 0.05$).

Table II. Effect of streptozotocin diabetes on plasma ^{14}C components, placental glucose transfer and lactate formation in 20-day pregnant rats being infused with (U-^{14}C)-*D*-glucose through the maternal left uterine artery

	Controls	p	Diabetics
Mother			
Plasma ^{14}C-glucose, dpm/ml	6,169 ± 308[1]	NS[2]	6,684 ± 347
Plasma ^{14}C-glucose specific activity, dpm/μmol	1,242 ± 236	< 0.01	310 ± 52
Plasma ^{14}C-lactate, dpm/ml	602 ± 61	< 0.01	286 ± 59
Plasma ^{14}C-lactate specific activity, dpm/μmol	345 ± 87	< 0.01	102 ± 12
Left minus right fetuses			
Plasma radioactivity, dpm/ml	6,119 ± 1,316	NS	10,037 ± 1,422
Plasma ^{14}C-glucose, dpm/ml	2,868 ± 529	< 0.01	7,497 ± 1,092
Plasma ^{14}C-glucose specific activity, dpm/μmol	1,183 ± 207	< 0.01	406 ± 77
Plasma ^{14}C-lactate, dpm/ml	3,088 ± 675	< 0.01	954 ± 214
Plasma ^{14}C-lactate specific activity, dpm/μmol	328 ± 81	< 0.01	55 ± 7
Placental transfer of glucose			
(1/Φ) nmol/ml fetal plasma, min	28 ± 5	< 0.01	233 ± 44
nmol/ml fetal plasma, min	133 ± 11	< 0.01	686 ± 102
Fetoplacental transformation of maternal glucose to lactate			
nmol/ml fetal plasma, min	81 ± 3	NS	78 ± 12
% versus placental transfer	63.3 ± 7.0	< 0.001	14.0 ± 3.1

[1] Means ± SEM of 5–7 rats/group.
[2] Statistical comparisons between diabetic and control rats (NS = not significant, $p > 0.05$).

the maternal left uterine artery, following our already validated technique [13]. As shown in table II, 20 min after constant infusion, maternal plasma ^{14}C-glucose values were similar in diabetic and control rats, whereas plasma ^{14}C-glucose specific activity was significantly lower in diabetics. Both plasma ^{14}C-lactate values and ^{14}C-lactate specific activity were also significantly reduced in diabetic versus control mothers, indicating a reduced glycolytic consumption of labelled glucose by maternal tissues.

A qualitative estimation of maternal glucose transfer to the fetus and its subsequent utilization by fetal tissues may be attained from the difference between ^{14}C-plasma values in fetuses from the left and the right uterine horns. As shown in table II, this value for total radioactivity was higher, although not significantly, in fetuses from diabetic than from control mothers. In fetuses from the diabetic group the main labelled circulating component corresponded to ^{14}C-glucose, this value being significantly higher than in controls, whereas ^{14}C-glucose specific activity was lower in diabetics. Plasma ^{14}C-lactate and its specific activity were significantly lower in fetuses from diabetic than from con-

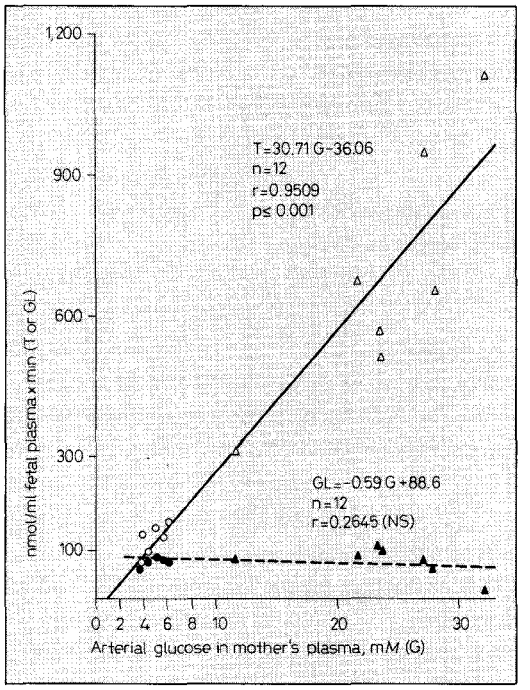

Fig. 1. Relationship between maternofetal glucose transfer (continuous line) or lactate formed from transferred glucose (dotted line) and plasma maternal glucose concentration in diabetic (▲, △) or control (●, ○) pregnant rats.

trol mothers. All these data indicate that placental glucose transfer is enhanced in the diabetic rats whereas glucose conversion into lactate by the fetal-placental unit is reduced in proportion to the transferred glucose. Quantitative estimation of these parameters coincides with these conclusions. As shown in table II, placental transfer of glucose is much greater in diabetics than in normal mothers, and the difference was highly significant whether or not blood flow values were included in the calculations.

Estimation of the transformation of maternal glucose into lactate by the fetal-placental unit gives values which are similar for diabetic and control rats (table II), yielding a proportionally much lower lactate production in the fetal-placental unit from diabetic than from control mothers. These findings are in agreement with our reported results in a similar study in which uterine blood flow was not taken into consideration [8], and besides emphasizing the limited capacity of the fetus to handle the incoming glucose load from a highly hyperglycemic mother, it explains the augmented fetal/maternal plasma glucose ratio found in diabetic pregnant rats (table I).

Since a direct correlation between maternofetal glucose transfer and maternal glycemia has been reported in normal sheep [7] and we also found this type of correlation in rats with maternal diabetes of varying severity [8], it was of interest to establish whether such a correlation also existed in the present study. As shown in figure 1, a linear and highly significant correlation appeared evident between plasma maternal glucose concentration in arterial blood and placental glucose transfer. This finding agrees with the high K_m values (above 12 m*M*) described for placental glucose transfer in other species [1, 4, 10], and demonstrates that in spite of the reduced blood flow to the uterus in diabetics, glucose transfer to the fetus is directly dependent on glucose concentration in maternal circulation. Absolute conversion of transferred glucose into lactate is kept stable at any maternal level of circulating glucose (fig. 1), indicating that the fetus has a limited capacity to metabolize the available glucose. This must contribute to the accumulation of glucose in fetal versus maternal plasma when the mother is diabetic. It remains to be established whether chronic fetal flooding of glucose participates in the teratogenic tendencies of the diabetic mother.

Extrapolation of these findings to human beings should be done with caution, as besides dissimilarities between gestational diabetes in man and streptozotocin diabetes in pregnant rats, a milder form of maternal diabetes is more usual in man since control of glucose homeostasis is usually attempted. Contrary to what occurs in severe diabetes, mild diabetes in the streptozotocin pregnant rat does not affect fetal body weight or fetal/maternal plasma glucose ratio [8] and may even cause fetal macrosomia and hyperinsulinemia [12, 16], more closely resembling the situation in human gestational diabetes. These findings indicate that fetal response to maternal hyperglycemia depends on its severity. Whereas manifest maternal diabetes interferes with fetal pancreas insulin secretion and decreases its ability to metabolize the incoming flux of glucose through the placenta so that normal fetal growth is impaired, mild diabetes enhances fetal β-cell insulin release permitting normal metabolization of available glucose and increasing fetal growth rate.

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E. Herrera, MD,
Departamento de Investigación,
Centro Ramón y Cajal,
Crtra. Colmenar Km 9,
E-28034 Madrid (Spain)