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FETAL/MATERNAL PLASMA AMINO ACID RELATIONSHIPS

IN THE STREPTOZOTOCIN DIABETIC RAT

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INTRODUCTION

Gestational diabetes mellitus (GDM) affects placental composition and intrinsic metabolism both in humans (1) and rats (2), and causes abnormalities in maternal-fetal metabolite transfer (3). These changes may actively contribute to the altered fetal growth that occurs in GDM, which directly depends on the quality and quantity of metabolic fuels crossing the placenta. These fuels may be used as building blocks for fetal accretion and also modulate fetal beta-cell insulin release which is recognized as an important growth factor for the fetus (4).

A major factor that modulates the placental transfer of any metabolite is its concentration in maternal circulation. We previously found that whereas plasma concentration of total amino acids in streptozotocin-diabetic pregnant rats was unaffected, the level of the same parameter was intensely reduced in their fetuses indicating either an impaired placental transfer or an enhanced utilization in the fetal side.

The aim of the present study was to establish whether maternal diabetes during late pregnancy in the rat impairs placental amino acid transfer, and to elucidate the mechanism through which this effect is produced. For this purpose, on day 7 of gestation, rats were made diabetic by a single intravenous injection of 45 mg streptozotocin/Kg dissolved in citrate buffer pH 4.5. Other rats were injected with only buffer and used as controls. All the animals were studied on day 20 of gestation. Placental amino acid transfer was measured using an *in situ* placental preparation (5), and placental blood flow was estimated by infusion of ^{99m}Tc labelled microspheres to other animals under the same experimental condition (6). The *in vitro* uptake and utilization of ($\text{U-}^{14}\text{C}$)-L-alanine and ($\text{U-}^{14}\text{C}$)- α -aminoisobutyric acid was studied in placental slices from diabetic and control animals.

RESULTS

As reported previously (3), on the 20th day of gestation, streptozotocin diabetic pregnant rats show reduced maternal body weight

free of conceptus structures, conceptus and fetus weights as well as plasma levels of radioimmunoassayable insulin both in mothers and fetuses. Litter size and placental weight was not modified in diabetics as compared with controls. Maternal plasma glucose concentration was approximately 6 times higher in diabetics than in controls. Plasma levels of glucose in fetuses were below values in their respective mothers. Glycemia in fetuses of diabetic mothers was about 11 times greater than in those of controls. Consequently, the fetal/maternal plasma glucose ratio was significantly enhanced in diabetic rats.

In spite of the intense diabetic condition, maternal plasma amino acid levels were modified very slightly, with significant reductions in glutamine and serine, and increments in glycine and ornithine, giving an unmodified value for total plasma amino acid levels. Different to the mothers, in fetuses from diabetic rats there was a reduction in the plasma level of most individual amino acids as compared with controls. Consequently, as shown in Figure 1, the fetal/maternal plasma amino acids ratio was significantly reduced in diabetic rat as compared to controls, with the exception of alanine, glutamine and glutamate which did not differ between the two groups. As expected, fetal/maternal plasma ratio was above 1 for most amino acids (Figure 1).

Due to the stable fetal/maternal plasma alanine ratio in diabetic rats and the fact that placental alanine transfer seems to take place by both the ASC and A transport systems proposed by Christensen, which are the same as those used by most amino acids, we decided to study how the transfer of L-alanine is affected in the diabetic rat. For comparison, the study was also extended to determine the placental transfer of glucose in animals kept under similar conditions.

Placental alanine and glucose transfer was determined by infusion of either (U-¹⁴C)-L-alanine or (U-¹⁴C)-D-glucose through the maternal left uterine artery, following our already validated technique (5). Animals were anesthetized with sodium pentobarbital (33 mg/Kg, intravenously) and they were infused for 20 min with medium containing the ¹⁴C-labelled tracer through a cannula placed counter-current into the left external iliac artery to the level of the left uterine artery with the collateral vessels clamped. 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. Results of radioactivity were always corrected by considering 1×10^6 dpm as the total infused radioactivity per rat. Comparison of radioactivity present in fetal plasma from the left and right uterine horns, specific radioactivity of the tracer in the left uterine artery and uterine blood flow were used to calculate the actual placental transfer (equation shown in Figure 2). Left uterine blood flow was determined by infusing other experimental rats with ^{99m}Tc labelled albumin microspheres, (6) through a cannula placed in the carotid artery and determining the amount of radioactivity appearing in the left uterine horn as compared to that present in blood withdrawn from the femoral artery. As show in Table 1, 20 min after constant infusion, maternal plasma ¹⁴C-glucose values were similar in diabetic and control rats, whereas plasma ¹⁴C-glucose specific radioactivity was significantly lower in diabetics, and the difference of total radioactivity between left minus right fetuses was significantly higher in these animals. However, when L-alanine was the infused tracer neither of these parameters differed between the two groups (Table 1). Left uterine blood flow was significantly reduced in diabetic rats (Table 1). When these parameters are used to calculate maternal-fetal transfer (Figure 2), it appears that, as expected, the transfer of glucose is intensely enhanced, whereas that of L-alanine is significantly decreased in diabetic rats as compared to controls. When the infused tracer was alanine,

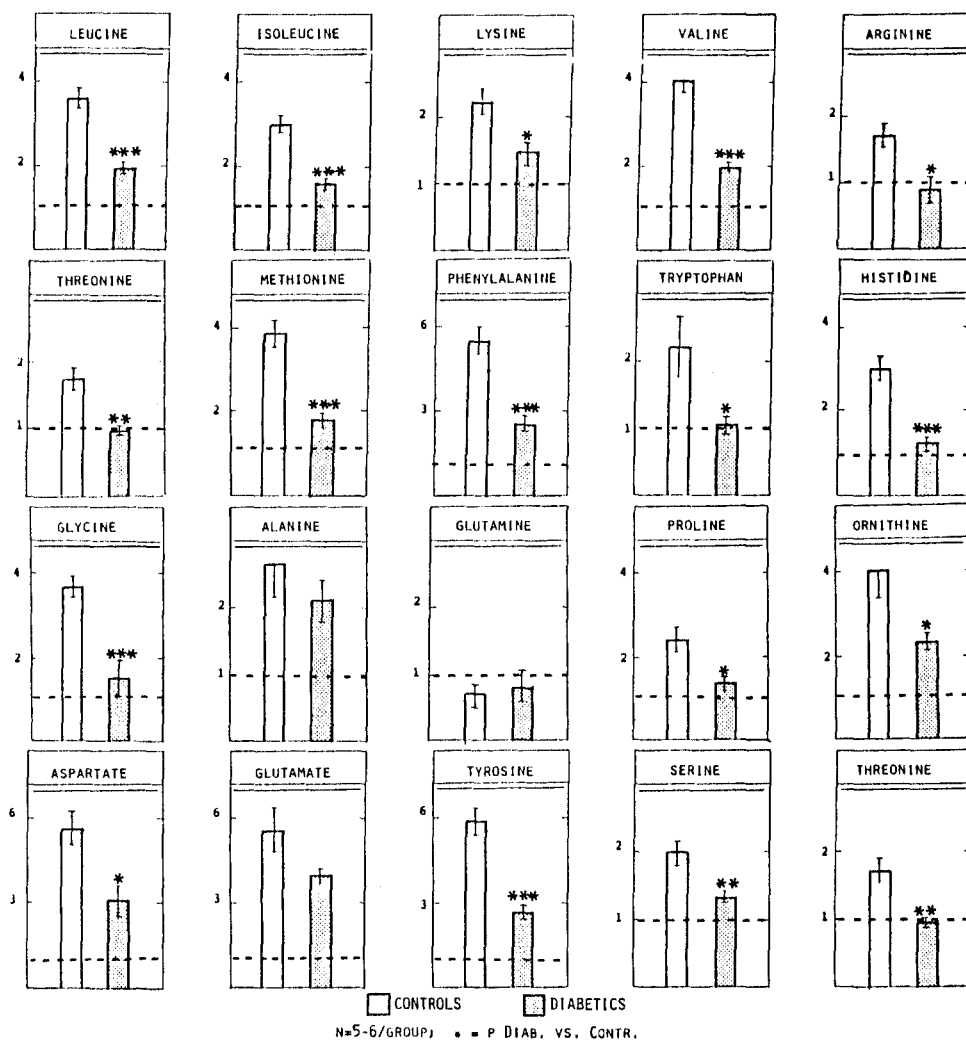


Fig. 1. Fetal/maternal plasma amino acids ratio in control (open bars) and diabetic (shaded bars) 20 day pregnant rats. Means \pm S.E.M. of 5-6 rats/group. Significance of the difference between controls and diabetics is shown by: *, $p < 0.05$; **, $p < 0.01$ and ***, $p < 0.001$. Dotted line corresponds to the amino acid ratio value of 1.

the only parameter used to calculate the transfer that differed between the two groups was the uterine blood flow, with no difference in either maternal alanine specific radioactivity or the radioactivity present in the left minus the right fetuses (Table 1).

To determine whether an altered intrinsic placental metabolism together with the reduced uterine blood flow could be contributing to this alanine decreased placental transfer, we studied the *in vitro* utilization of ($U-^{14}C$)-alanine by placental slices from diabetic and control animals. Placental slices were incubated for 50 min in Krebs Ringer bicarbonate supplemented with 0.4 mM of ($U-^{14}C$)-L-alanine. It was observed that neither alanine uptake or its conversion into CO_2 or lactate differed between the two groups. The same experiment was carried out but using different L-

alanine concentrations in the media to obtain the kinetic constant of these parameters. The inverse plots for alanine uptake and its conversion into lactate and CO₂ show that both V_{max} and K_m values were similar for placentas from diabetic and control animals. In order to determine whether this lack of difference in the placenta metabolic handling of alanine between the two groups could be also the case of other amino acids, it was decided to extend the experiment to a non-metabolizable amino acid such as α-aminoisobutyric acid. Results of this experiment showed again no difference between the two groups in the placental uptake of this amino acid at any of the studied concentrations. Used kinetic constants derived from the inverse plots were similar to those found for alanine and did not differ in placentas from diabetic and control animals.

DISCUSSION

The present study shows that streptozotocin diabetic pregnant rats have reduced maternal and fetal body weights, fetal plasma amino acids levels and blood flow to the uterus. Reduction in fetal body weight in the diabetic pregnant rat is in agreement with previous findings (7). This change may be related to reductions in fetal plasma insulin levels present in animals under similar conditions (3), although the decreased uterine

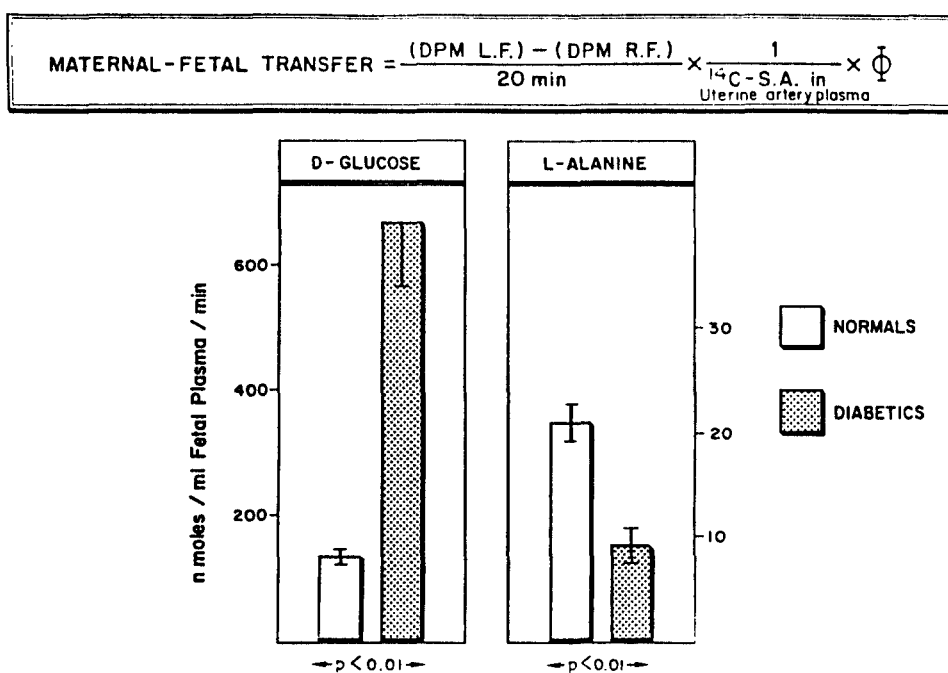


Fig. 2. Placental transfer of glucose and alanine in normal (open bars) and diabetic (shaded bars) 20 day pregnant rats. Means \pm S.E.M. of 5-7 rats/group. Placental transfer was estimated as the difference in total plasma radioactivity between the left (L.F) and right fetuses (R.F) divided by the time of infusion with (U-¹⁴C)-D-glucose or (U-¹⁴C)-L-alanine through the maternal left uterine artery (20 min) and by the ¹⁴C specific radioactivity (SA) in the maternal artery, and the blood flow (Φ) to the left uterine horn (equation shown in the upper part of the Figure).

Table 1. Parameters used to calculate placental glucose and alanine transfer in STZ-diabetic 20 day pregnant rats

| | CONTROLS | DIABETICS | p |
|--|-----------|------------|-------|
| Left uterine blood flow (ml/min) | 5.31±0.8 | 2.44±0.23 | * |
| (U- ¹⁴ C)-D-Glucose Maternal glucose (mM) | 5.1±0.3 | 24.40±2.30 | x |
| Maternal glucose S. A (dpm/μmol) | 1242±236 | 310±52 | * |
| (DPM L.F. - DPM R.F) (dpm/ml) | 2868±529 | 7497±1092 | * |
| (U- ¹⁴ C)-L-Alanine Maternal alanine (μmoles/L) | 390±35 | 402±29 | N. S. |
| Maternal ALA S. A. (dpm/μmol) | 2215±206 | 2336±601 | N. S. |
| (DPM L.F. - DPM R.F.) (dpm/ml) | 11173±887 | 8916±1557 | N. S. |

Means ± S.E.M. of 7-9 rats/group. Statistical comparisons vs. controls are shown: *, p < 0.01 and x, p < 0.001.

blood flow and plasma amino acid levels, could be also 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 (8).

Placental D-glucose transfer values were previously found to be linearly correlated to maternal glycemia in spite of reduced uterine blood flow and augmented placental glucose transfer is a direct consequence of maternal hyperglycemia (3). Reduced placental transfer of L-alanine in the diabetic rat may be however related to the decreased uterine blood flow. This parameter was significantly reduced in diabetic rats whereas neither of the other parameters used to calculate materno-fetal L-alanine transfer differ between the two experimental groups. Variations in uterine blood flow have been reported to affect the transfer to the fetus of molecules with high placental extraction coefficients in other experimental conditions (9). Thus, the reduced uterine blood flow in the diabetic rat may participate in the decreased placental L-alanine transfer observed in these animals.

Placental slices produce a considerable amount of lactate and CO₂ from alanine. These findings demonstrate that rat placenta actively synthesizes lactate from alanine supporting the hypothesis that placenta modifies the quality of maternal metabolites reaching the fetus, lactate being the major product of this effect. Lactate production from other metabolites besides alanine crossing the placenta was previously shown by us (6) and these findings are of special interest under the current belief that lactate is an important energy fuel for the fetus (10). The present results show that

neither alanine uptake or its conversion into lactate by placental slices differ between diabetic and control rats. These findings demonstrate that intrinsic placental metabolism in diabetic rat was not altered.

If L-alanine may be considered representative of most amino acids, we may therefore conclude from present results that reduced circulating amino acids in fetuses from streptozotocin diabetic rats is due to an impairment in mother to fetus amino acid transfer secondary to reduced uterine blood flow and not to an altered placental metabolic capacity to handle amino acids coming from maternal circulation.

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