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PLACENTAL MODIFICATION OF TRANSFERRED MATERNAL SUBSTRATES IN NORMAL AND DIABETIC RATS

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Altered placental composition and intrinsic metabolism has been reported to be present in the diabetic mother both in humans (1-3) and rats (3-6) but functional significance of these abnormalities in what maternofetal metabolite transfer is concerned has not been established. The problem is of major importance as maternal diabetes affects fetal growth (7-10) which directly depends on the quality and quantity of metabolic fuels crossing the placenta.

Following our recently reported method to study the "in vivo" placental metabolite transfer in the rat (11) we found that in the late pregnant diabetic rat placental glucose transfer was enhanced and that a certain proportion of the transferred glucose appeared as lactate in fetal plasma (12). In normal rats we also found that maternofetal transfer of L-alanine involves its partial conversion into lactate by the placenta (13). In the present work the comparative maternofetal transfer of D-glucose and L-alanine was determined in the streptozotocin diabetic late pregnant rats, and the study was extended to establish the placental synthesis of lactate from both substrates.

EXPERIMENTAL ANIMALS AND "IN VIVO" PLACENTAL TRANSFER QUANTIFICATION

Pregnant rats were made diabetics by the intravenous injection of 45 mg/Kg streptozotocin (or buffer in case of the normal controls) at day 7 of gestation and they were studied at day 20th. Animals were anesthesized with sodium pentobarbital (33 mg/Kg, intravenously) and subjected to the surgical procedure already described (11). They were infused for 20 min with medium containing the 14C-labelled tracer through a cannula placed counter-current into the left uterine artery with colateral 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. Other experimental details were as already described (11-13). Results of radioactivity were always corrected by considering 1x10 dpm as the total infused radioactivity per rat. Comparison of radioactivity present in fetal plasma from the left and right uterine horns, specific activity of the tracer in the left uterine artery and uterine blood flow were used to calculate the actual placental transfer (11,12). Left uterine blood flow was determined by infusing other experimental rats with 99mTc-

labelled albumin microspheres, as previously described (14). As shown in Table 1, maternofetal trasfer of D-glucose appeared significantly greater whereas that of L-alanine was lower in diabetic than in normal control rats. Placental D-glucose transfer values were previously found to be linearly correlated to maternal glycemia in spite of reduced uterine blood flow in case of the diabetic rat (14) indicating that in this condition augmented placental glucose transfer is a direct consequence of maternal hyperglycemia. Reduced placental transfer of L-alanine in the diabetic rat may be however related to the decreased uterine blood flow. As also shown in Table 1, this parameter was significantly reduced in diabetic rats whereas neither of the other parameters used to calculate maternofetal L-alanine transfer differed between the two experimental groups (data not shown). Variations in uterine blood flow have been reported to affect the transfer to the fetus of molecules with low placental extraction coefficients in other experimental conditions (15-17). Thus, the reduced uterine blood flow in the diabetic rat may participate in the decreased placental L-alanine transfer observed in these animals.

Table 1. Effect of STZ diabetes on maternofetal D-glucose and L-alanine transfer and uterine blood flow in 20-day pregnant rats

	Normal	Diabetic	p
Transfer of D-glucose (nmoles/ml/min)	154±30	635±100	<0.01
Transfer of L-alanine (nmoles/ml/min)	21±2	10±3	<0.01
Uterine blood flow (ml/min)	4.8±0.3	2.4±0.3	<0.01

Mean±SEM, p= Diabetics vs. Normals, n=5-7 rats/group

QUALITY OF TRANSFERRED METABOLITES

After the infusion of either (U-14C)-D-glucose or (U-14C)-L-alanine through the maternal left uterine artery distribution of radioactivity in plasma of fetuses from the left uterine horn appeared different to that found in mother's plasma. As shown in Table 2, in animals infused with (U-14C)-D-glucose practically all radioactivity in mother's plasma remained as 14C-glucose whereas in fetal plasma a considerable proportion of label appeared as 14C-lactate. Maternal diabetes intenselly reduced the proportional appearance of 14C-lactate in fetal plasma, indicating a reduced capacity to metabolyze the glucose load reaching the fetus. After maternal infusions with (U14C)-L-alanine radioactivity in morher's plasma appeared distributed in almost equal parts for alanine,lactate and glucose whereas in left fetus plasma there appeared more 14C-lactate than 14C-alanine and only negligible amounts of 14C-glucose, in agreement with the known lack of gluconeogenesis in the rat fetus. Contrary to what it occurs with labelled D-glucose infusion, the diabetic condition of the mother does not modify the distribution of radioactivity in left fetal plasma after maternal infusion with labelled L-alanine.

Table 2. Percentual distribution of 14C-components in plasma 20 min after maternal infusion with labelled substrates through the left uterine artery in the 20 day pregnant rat

	Normal		Diabetic	
	Mother	Left fetus	Mother	Left fetus
Infusion with (U-14C)-D-glucose				
14C-glucose	91±1	50±3	96±1**	85±2***
14C-lactate	9+1	51±2	4±1**	14±2***
Infusion with (U14C)-L-alanine				
14C-alanine	35±2	39±2	34±6	42±3
14C-lactate	34+2	58±3	31±4	57±3
14C-glucose	31±3	1±0	34±6	1±0

Mean+SEM of % total plasma radioactivity.*=statistical significance of Diabetics vs. Normals(**=p<0.01,***=p<0.001)

ROLE OF THE PLACENTA ON LACTATE PRODUCTION FROM TRANSFERRED D-GLUCOSE AND L-ALANINE

By using the in situ placental infusion technique we demonstrated for the first time that most of lactate appeared in the rat fetus from transferred L-alanine is synthesyzed by the placenta (13). To further support this finding and to determine whether this is also the case for transferred glucose, placental slices from 20 day pregnant normal rats were incubated "in vitro" in the presence of physiological concentrations of either (U-14C)-D-glucose or (U-14C)-L-alanine. Methodological details were as described (13). As shown in Table 3, placental slices produced a considerable amount of lactate from both offered substrates. Although not necessarily related, lactate production from glucose appeared even greater than tissue uptake. These findings demonstrate that as it occurred for L-alanine rat placenta actively synthesizes lactate from glucose supporting the hypothesis that placenta modifies the quality of maternal metabolites reaching the fetus, lactate being the major product of such effect. This is of special interest under the current believe that lactate is an important energy fuel for the fetus (18,19) and indicates that changes in the placental lactate production from transferred metabolites in the diabetic mother may contribute to the altered development of her fetuses.

Table 3 Lactate production from (U-14C)-D-glucose and (U-14C)-L-alanine by 20 day pregnant rat placenta slices incubated for 50 min

	(nmoles/100 mg fresh tissue)			
	Tissue uptake		Medium lactate	
Incubations with:	Normal	Diabetic	Normal	Diabetic
D-glucose(5mM)	256±20	259±18	335±52	479±81
L-alanine(0.4mM)	58±7	40±2*	31±3	30±2

Mean+SEM; *=Significance Diabetics vs Normals (p<0.05),n=4-6 rats

SUMMARY AND CONCLUSIONS

Main results of the present study expressed in a quantitative manner are summaryzed in Figure 1. Placental glucose transfer is greatly enhanced in the diabetic late pregnant rat but proportional lactate formation from transferred glucose is

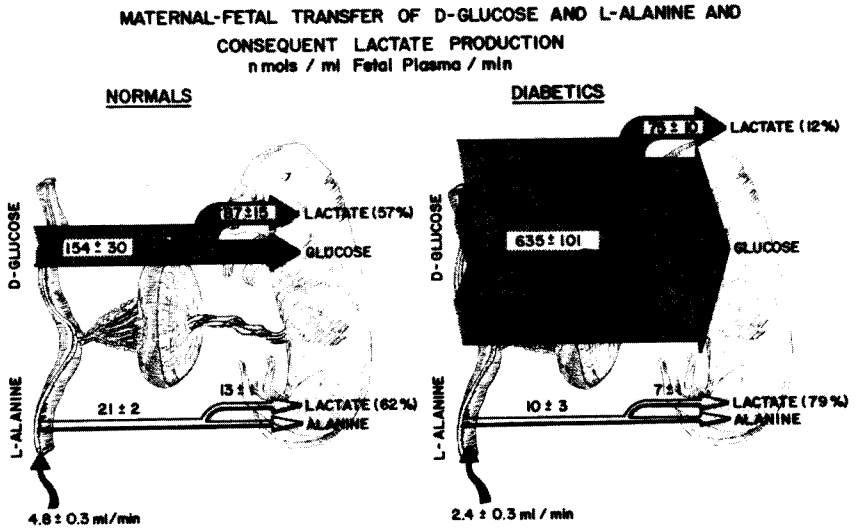

Figure 1

reduced in the diabetic mother indicating a limited capacity to utilyze the enormeous load of glucose reaching the fetoplacental unit. Placental transfer of L-alanine is impaired in the diabetic pregnant rat, and this effect seems to be related to the reduced uterine blood flow of these animals. Rat placenta also synthesyzes lactate from transferred L-alanine, the process being unmodified in the diabetic mother.

Extrapolation of these findings to human beings should be done with caution because besides obvious differences, maternal diabetes in women is normally milder than that present in streptozotocin treated rats since some control of glucose homeostasis is always attempted. Present findings demonstrate that maternofetal transfer of both D-glucose and L-alanine are greatly altered in the diabetic rat mother and that the placenta itself converts to lactate a certain proportion of the transferred maternal substrates. It is proposed that these changes may play a major role in the metabolic economy of the fetus from a diabetic mother, being partially responsible of its abnormal development.

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