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Placental Metabolism and Enzyme Activities in Diabetic Pigs (40930)[1]

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Abstract. Placental tissue metabolism and enzyme activities were measured in alloxan diabetic pigs and compared to other tissues. The pig placenta is made up of two placentas, maternal and fetal. These two placentas had different rates of glucose, pyruvate, and alanine utilization. The fetal placenta had higher activities of aspartate aminotransferase when compared to maternal liver and placenta. Diabetes resulted in a decrease in maternal placenta *de novo* fatty acid synthesis and activity of glucose-6-phosphate dehydrogenase. These data support the concept of a metabolic regulatory role for this organ during pregnancy.

The role of the placenta in nutrient transport and endocrinology of pregnancy has been established. However, the metabolic role of this organ remains unclear. Conflicting observations on the effect of insulin on placental metabolism have been reported. Some suggest that the placenta is capable of responding to insulin (1–3); others find no support for insulin responsiveness (4, 5). Glucose transport in the human placenta is a carrier-mediated process (6) but is not an active transport requiring energy expenditure (7). The ovine placenta utilizes large quantities of glucose and metabolizes glutamate of fetal origin to ammonia (8). In order to provide more definitive data on placental metabolism, a series of *in vitro* studies were performed and placental tissue enzyme activities were examined in normal and alloxan diabetic pigs. The data support a metabolic regulatory role for the maternal placenta.

Methods and materials. Animals used in these experiments were pregnant Yorkshire pigs. The methods used for production of diabetes have been described by Ezekwe and Martin (9). Diabetes was induced by intravenous injection of alloxan (40 mg/kg body wt) at 70 days of gestation. Diabetes in pregnant pigs resulted in average blood glucose levels of 410 mg%. Tissues were removed after the pigs were rendered unconscious by inhalation of carbon dioxide on the 112th day of gestation, 2 days prior to the expected parturition date. The dorsal scapular subcutaneous adipose tissue and the uterus were removed. Maternal and fetal placental tissues were separated from the uterine wall by gentle pulling. The liver tissue was removed immediately after the uterus was removed. All tissues were excised and processed within 10 min after the pigs were made unconscious and exsanguinated.

For *in vitro* tracer studies, all tissue slices (100 mg) were preincubated for 45 min and media changed. The second media contained the appropriate labeled substrates (see table footnotes for media details). The second incubation period was for 2 hr at 37° in 25-ml flasks placed in Dubnoff metabolic shakers (90 oscillations per minute). Tissue lipids were extracted for radioactivity by the procedure described by deCingolani (10).

Tissues utilized for enzyme analysis were homogenized in 0.25 *M* sucrose media containing 1 m*M* dithiothreitol and 5m*M* Tris–HCl (pH 7.4). The homogenates (10%) were centrifuged at 37,000*g* for 20 min (4°), and the resulting supernatants were used for enzyme measurement. Enzyme assay procedures for porcine tissues have been previously described by Martin and Herbein (11). Protein concentrations in the extracts were determined by the Folin phenol method (12). The analysis of differences between two means was computed by Student's *t* test as described by Steel and Torrie (13).

Results. An initial experiment to compare relative rates of lipogenesis in mater-

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TABLE I. A COMPARISON OF MATERNAL TISSUE METABOLISM

Tissue	Fatty acid synthesis[a]	Glucose-6-phosphate dehydrogenase[b]
Maternal liver	3.86 ± 1.04[c]	26.2 ± 5.3
Maternal adipose	2.38 ± 1.56	89.7 ± 22.5*
Maternal placenta	3.75 ± 0.73	124.5 ± 10.8*
Fetal placenta	2.11 ± 0.54*	166.3 ± 20.7*

[a] Fatty acid synthesis is expressed as nanomoles of acetate units incorporated per 2 hr/100 mg tissue.

[b] Glucose-6-phosphate dehydrogenase activity is expressed as nanomoles of substrate converted per minute per milligram soluble tissue protein.

[c] Mean ± SEM for eight observations.

* Activities are different when compared to maternal liver ($P < 0.05$).

TABLE II. A COMPARISON OF TOTAL LIPID SYNTHESIS IN MATERNAL TISSUES

Tissue	Treatment	
	Control	Diabetic
Glucose		
Adipose	23.4 ± 4.4[a]	11.7 ± 4.7*
Liver	6.6 ± 0.8	6.8 ± 0.7
Maternal placenta	13.5 ± 2.2	8.8 ± 2.7
Fetal placenta	5.1 ± 1.0	4.1 ± 0.8
Pyruvate		
Liver	28.4 ± 2.5	35.6 ± 11.2
Maternal placenta	6.6 ± 1.6	5.9 ± 1.5
Fetal placenta	3.7 ± 0.6	2.4 ± 0.4*

[a] Mean ± SEM for four observations; activities are expressed as nanomoles of substrate incorporated per 2 hr/100 mg of tissue. Incubations of tissue slices were in Krebs–Ringer bicarbonate buffer with either 10 m*M* glucose (1 μCi [U-^{14}C]glucose/ml) or 10 m*M* pyruvate (1 μCi [2-^{14}C]pyruvate/ml).

* Significantly different from control ($P < 0.05$).

nal and fetal placental tissue with maternal liver and adipose tissues revealed some similarities and differences among these tissues (Table I). Fatty acid synthesis was comparable in maternal liver, adipose, and placenta. Fetal placenta fatty acid synthesis was significantly lower than maternal liver or placenta. On the other hand, fetal placenta had the highest activity of glucose-6-phosphate dehydrogenase (G6PD) and maternal liver the lowest activity of G6PD.

A comparison of total lipid synthesis from glucose and pyruvate was made between control and diabetic tissues (Table II). Diabetes in the 112-day pregnant sow resulted in a depression of glucose incorporation into total lipid of adipose tissue. There were no significant treatment differences in glucose utilization for lipid synthesis in maternal liver and placenta, and fetal placenta. Fetal placenta utilization of pyruvate for total lipid synthesis was lowest of all tissues measured and was significantly depressed in diabetic pigs.

The effect of maternal diabetes was determined on *de novo* fatty acid synthesis as measured with tritiated water incorporation into lipids and fatty acids by maternal and fetal placentas (Table III). In both maternal and fetal placentas *de novo* fatty acid synthesis and total lipid synthesis was depressed by diabetes. The majority of tritium label was found in the total lipids fraction

TABLE III. MATERNAL AND FETAL PLACENTAL TISSUE *de Novo* FATTY ACID SYNTHESIS IN DIABETIC PIGS

Tissue	Tritiated water incorporation	Treatment	
		Control	Diabetic
Maternal placenta	Total lipids	80.7 ± 2.5[a]	20.1 ± 6.2*
	Fatty acids	4.6 ± 0.7	2.2 ± 0.7*
Fetal placenta	Total lipids	61.8 ± 7.5	6.1 ± 1.5*
	Fatty acids	2.0 ± 0.9	0.25 ± .15*
P value maternal vs fetal		<0.05	<0.01

[a] Mean ± SEM for four observations; activities are expressed as nanomoles of acetate units incorporated per 2 hr/100 mg tissue. Incubations of tissues were in Krebs–Ringer bicarbonate buffer with 10 m*M* glucose and 2 mCi of tritiated water per flask.

* Treatment differences are significant ($P < 0.05$).

TABLE IV. MATERNAL AND FETAL PLACENTAL TISSUE METABOLISM OF ALANINE IN CONTROL AND DIABETIC PIGS

Tissue	[^{14}C]Alanine incorporation into	Treatment	
		Control	Diabetic
Maternal placenta	Total lipid	77.6 ± 24.0[a]	11.7 ± 7.0*
	Fatty acid	1.6 ± 0.5	1.7 ± 1.0
	Carbon dioxide	4324.0 ± 1078.0	1799.0 ± 150.0*
Fetal placenta	Total lipid	22.6 ± 6.9	1.6 ± 0.6*
	Fatty acid	0.2 ± 0.1	NM[b]
	Carbon dioxide	722.0 ± 289.0	370.0 ± 91

[a] Mean ± SEM for four observations; activities are expressed as nanomoles of alanine incorporated per 2 hr/100 mg tissue. Incubations of tissues were in Krebs–Ringer bicarbonate buffer with 10 m*M* alanine (1 μCi [2-^{14}C]alanine/ml).

[b] Nonmeasurable within the sensitivity of this assay system.

* Significantly different from control ($P < .05$).

and not in the fatty acids of the triglycerides.

Maternal and fetal placental alanine metabolism was studied in diabetic pigs (Table IV). [2-^{14}C]Alanine was primarily oxidized to carbon dioxide in both tissues and the oxidation rate was decreased by diabetes. Alanine incorporation into total lipids was decreased by diabetes in both tissues. Very little of the [2-^{14}C]alanine was found in the triglyceride fatty acids.

Enzyme activities were measured in maternal and fetal placentas of control and diabetic pigs (Tables V and VI). Diabetes resulted in a depression of glucose-6-PO_4 dehydrogenase (G6PD) activity in the maternal placenta only. Other placental enzymes such as 6-phosphogluconate dehydrogenase (6PGD), NADP malate dehydrogenase (ME), NAD malate dehydrogenase (MDH), and pyruvate kinase (PK) were not influenced by diabetes. The following enzyme activities were lower in the fetal placenta when compared to the maternal placenta: G6PD, 6PGD, MDH, and PK. The fetal placenta had higher activities of the amino acid transaminases, alanine aminotransferase (GPT), and aspartate

TABLE V. PLACENTAL TISSUE ENZYME ACTIVITY IN CONTROL AND DIABETIC PIGS

Enzyme	Tissue	Control	Diabetic
Glucose-6-phosphate dehydrogenase	Maternal placenta	53.4 ± 9.1[a]	33.2 ± 2.3**
	Fetal placenta	16.8 ± 3.9*	12.6 ± 2.6*
6-Phosphogluconate dehydrogenase	Maternal placenta	67.1 ± 7.3	70.4 ± 12.8
	Fetal placenta	37.9 ± 6.5*	45.0 ± 16.7*
NADP–malate dehydrogenase	Maternal placenta	200.0 ± 23.0	137.0 ± 25.0
	Fetal placenta	153.0 ± 10.0	153.0 ± 39.0
NAD–malate dehydrogenase	Maternal placenta	2453.0 ± 299.0	2735.0 ± 714.0
	Fetal placenta	1374.0 ± 308.0*	1234.0 ± 265.0*
Pyruvate kinase	Maternal placenta	632.0 ± 197.0	812.0 ± 230.0
	Fetal placenta	102.0 ± 31.0*	235.0 ± 103.0*

[a] Mean ± SEM for four observations; enzyme activities are expressed as nmole/min/mg protein.

* Tissue differences are significant ($P < 0.05$).

** Treatment differences are significant ($P < 0.05$).

TABLE VI. PLACENTAL TISSUE ENZYME ACTIVITIES IN CONTROL AND DIABETIC PIGS

Enzyme	Tissue	Control	Diabetic
Alanine amino transferase	Maternal placenta	12.1 ± 3.0[a]	8.4 ± 1.6[a]
	Fetal placenta	21.7 ± 2.1[b]	18.5 ± 1.9[b]
	Maternal liver	62.0 ± 12.1[c]	54.9 ± 4.8[c]
Aspartate amino transferase	Maternal placenta	576.0 ± 196.0[a]	535.0 ± 153.0[a]
	Fetal placenta	4171.0 ± 815.0[c]	4083.0 ± 1107.0[c]
	Maternal liver	1282.0 ± 101.0[b]	2073.0 ± 210.0[b]*

[a,b,c] Mean ± SEM for four observations; vertical means with different superscripts are significantly different ($P < 0.05$). Activities are expressed as nmole/min/mg protein.
* Treatment differences are significant ($P < 0.05$).

aminotransferase (GOT) (Table VI). Fetal placental GOT activity was even higher than that of maternal liver. While maternal liver GOT activity responded to diabetes none of the placental tissues GPT or GOT activities were influenced by diabetes.

Discussion. The role of the placenta in altering the metabolites available to the fetus has been previously proposed (3, 5, 8). The present studies indicate that the placenta does respond to maternal alloxan diabetes by decreasing the rates of *de novo* fatty acid synthesis, alanine oxidation, and the activity of glucose-6-phosphate dehydrogenase. That the placenta is an organ responsive to the insulin status of the mother is also supported by the following observations: (i) specific insulin receptors have been found in human placental tissue (14); (ii) specific binding of insulin to microsomal membranes from the placenta of insulin-dependent diabetic was significantly decreased when compared to normals (1); (iii) insulin increases placental glycogen synthesis and *in vitro* glucose transport (2, 3, 15).

Other observations do not support the concept of insulin sensitivity of the placenta. Battaglia *et al.* (4) using ovine cotyledons could not demonstrate insulin stimulation of glucose uptake. Since tissues from ruminant animals do not readily respond to insulin *in vitro* (16–18), a lack of insulin response in ovine cotyledons probably reflects a species difference. Szabo and Grimaldi (19) could not demonstrate an insulin response in human placental slices collected after delivery. It may be difficult to obtain an insulin response in tissues which have been subjected to degeneration and stress of delivery.

Diabetes induced by streptozotocin did not induce adaptive changes in the activities of placental enzymes of glycolysis, NADPH generation, and lipogenesis in the rat (5). There are no apparent differences in the approach and methodology of the present study to explain this discrepancy, other than specie and placental type. In the present study it was possible to separate maternal and fetal placenta. In the rat there is no maternal placenta only fetal placenta. Therefore, the lack of response to diabetes of the pig fetal placenta is similar to that found in the rat placenta (5).

Throughout this study differences in maternal and fetal placental metabolism were noted. When compared to the fetal placenta, the maternal placenta had higher rates of glucose and pyruvate conversion to triglyceride, fatty acid synthesis, alanine oxidation to carbon dioxide, and increased enzyme activities (G6PD, 6PGD, ME, and PK) associated with NADPH production and glycolysis. On the other hand, the fetal placenta had higher activities of enzymes associated with amino acid transaminase (GPT, GOT). In fact, the fetal placenta had two- to threefold higher activities of GOT when compared to maternal liver. Since glutamate primarily enters the placenta from the fetal circulation, the fetal placenta may be important in the removal of ammonia produced by amino acid catabolism by the fetal placenta (8).

Based on these observations, it is pro-

posed that the fetal placenta of the pig may be important in amino acid catabolism and that the maternal placenta may be important in metabolic adjustments of carbohydrate and lipid metabolism as influenced by maternal metabolic status.

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