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Fetal Glycerol Metabolism in Experimental Maternal Lipemia (42040)

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Abstract. Maternal lipemia (L), one of the consequences of poorly controlled diabetes in gestation, was induced in pregnant rats by feedings of a diet containing 45% fat. The maternal condition was associated with fetal L and moderate ketonemia. L fetuses had an elevated liver glycerol kinase (EC 2.7.1.30), when assayed 1 day before term (L = 82.5 ± 3.8 nmole/min × mg protein and controls (C) = 67.4 ± 3.9 nmole/min × mg protein; means ± SE, $P < 0.01$). However, neither hepatic cytosolic glycerophosphate ($GcPO_4$) dehydrogenase (EC 1.1.1.94) nor mitochondrial $GcPO_4$ oxidase (EC 1.1.99.5) were altered. $GcPO_4$ oxidase was lower in the striated muscle of L than in that of C fetuses (13.7 ± 1.2 nmole/min × mg protein vs 17.2 ± 0.5 nmole/min × mg protein, $P < 0.05$). The results of the present study suggest that L, *in utero,* may cause an alteration in overall glycerol oxidative capacity in liver and $GcPO_4$ in muscle. These changes appear to be compatible with a shift in the capacity of L fetuses to handle glycerol which may relate to postnatal fuel utilization by L offspring.

In poorly controlled maternal diabetes, the fetus may be subjected to elevated or erratically fluctuating levels of glucose, free fatty acids, and ketone bodies due to shifts in maternal homeostasis. These intracellular fuels are transferred to the fetus which may not metabolize them efficiently, particularly during the latter stages of pregnancy with the offspring of a diabetic mother becoming hyperglycemic and hyperinsulinemic (1). Moreover, the concomitant hyperketonemia and hypertriglyceridemia of diabetes may disturb the normal transplacental exchange of metabolites, influence fetal energy metabolism, and alter the ontogenesis of enzymes involved in carbohydrate and lipid metabolism.

In previous experimental studies, in the rat, where maternal ketosis and hypertriglyceridemia were induced with a high-fat diet, without causing hyperglycemia, fetal triglyceridemia and hyperketonemia resulted (2, 3). Feeding of a high-fat diet has been a widely used dietary manipulation in rats which allows for the investigation of the interactions between lipid and energy metabolism (4–7). This regime could thus mimic one of the metabolic imbalances occurring in poorly controlled diabetes mellitus (8).

Dietary glycerol, once released from fat by lipases, enters the glycolytic pathway after phosphorylation and oxidation, at the triose phosphate level. It has been shown earlier that excess glycerol itself (9) and phosphorylated glycolytic intermediates (10) can interfere with energy metabolism. Hence, an alteration in the ontogenesis of glycerol utilization enzymes and abnormal levels of its intermediary metabolites could further compromise energy production in the perinatal period.

Materials and Methods. *Animals and diets.* Female rats (Crl: (WI) BR, Charles River Labs., Kingston, N.Y.) weighing approximately 240–270 g were mated overnight. A positive sperm smear the following morning was taken as the day of impregnation (Day 1 of gestation). Before and after mating, the rats had free access to a commercial feed (Purina Rodent Chow No. 5001, Ralston Purina Co., St. Louis, Mo.; fat content: 4.5%). The rats were maintained in a temperature controlled room with a 12-hr light cycle. On the 12th day of gestation the rats were assigned to one of two groups. There were 14 dams in each group. One was fed *ad libitum* a high-fat, low-carbohydrate, semipurified diet (L) containing 45% hydrogenated vegetable oil (Crisco), 23% vitamin-free casein (ICN No. 904520), 26% starch, 4% salt mixture (USP XVII, ICN No. 904610), and 2% vitamin diet fortification mixture (ICN No. 904654). The dams serving as controls (C)

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were fed a semipurified diet, similar to the one described above, but containing only 4.5% fat, 66.5% carbohydrate, as starch (45%), and glucose (21.5%). The rats had free access to food and water, including the night prior to their sacrifice. The pregnant rats were killed by cervical dislocation on the 21st day of gestation (1 day prior to delivery) and the fetuses were promptly removed and placed on crushed ice. Blood was immediately drawn from a neck incision and collected in heparinized tubes. The fetuses were rapidly dissected and the tissues from each litter were pooled and maintained on ice. Two pools were made with the blood from the fetuses of the same dam for analysis. Each dam's blood was assayed separately. The plasma obtained by centrifugation was kept in ice before being analyzed the same day.

Biochemical determinations. Fetal livers and striated muscles of a hind leg of each fetus were immediately dissected and homogenized in 4 vol of ice-cold 0.25 *M* sucrose containing 5 m*M* Tris, 1 m*M* EDTA, and 1 m*M* mercaptoethanol, adjusted to pH 7.4, and surrounded by ice. The homogenate was centrifuged at 600*g* for 15 min to spin down debris. The supernatant was removed and further centrifuged at 7500*g* for 10 min. Both operations were done at 4°C. These last supernatants were used for glycerol kinase (EC 2.7.1.30) (11) and glycerophosphate ($GcPO_4$) dehydrogenase assays (EC 1.1.1.94) (12). The pellet from the second centrifugation was resuspended in an equal volume of the buffer described above and used for $GcPO_4$ oxidase (EC 1.1.99.5) assay (13). An aliquot of the original homogenate was promptly deproteinized with an equal volume of 2 *M* $HClO_4$. The supernatant obtained after centrifugation was neutralized with $KHCO_3$ and centrifuged again. All these operations were performed at 0–4°C. Protein was determined in the homogenates and resuspended pellet by a colorimetric method (14). Plasma triglycerides were assayed with a commercial kit (Sigma Chem. Co., St. Louis, Mo., No. 335-UV). Free fatty acids were determined spectrophotometrically after extraction (15) and ketone bodies (β-hydroxybutyrate and acetoacetate) were assayed by an enzymatic fluorometric method (16). A colorimetric procedure was applied for the determination of cholesterol (17). Nutritional supplies and reagents were purchased from ICN Nutritional Biochemicals, Cleveland, Ohio, Fisher Scientific Company, Fair Lawn, New Jersey, and Sigma Chemical Company.

Results are expressed as means ± SE. Significant differences between means were assessed by a two-tailed Student's *t* test (18).

Results and Discussion. The dams fed the high-fat diet from the 12th day of gestation on (L) gained more weight than their regularly fed controls (C) (Table I). Since the litter weights of the two groups were similar and individual fetal weights only marginally different, the high-fat diet probably caused mostly fat deposition in the dams. The pregnant rats of the L group were characterized by a high concentration of plasma triglycerides. The same was true for the respective fetuses, but they only had about one-third the concentration measured on the maternal side. Free fatty acids were indistinguishable between L and C dams. However, a gradient developed across the placenta, with the fetal concentration slightly over one-half the maternal free fatty acid levels in both groups of fetuses. Plasma cholesterol did not differ between the dams or the fetuses in the L and C groups.

The L dams did not exhibit ketonemia, in terms both of β-hydroxybutyrate or acetoacetate. Nevertheless, plasma β-hydroxybutyrate was elevated in L fetuses, without alteration of plasma acetoacetate. No significant correlation could be established between the plasma β-hydroxybutyrate and acetoacetate of either dams or fetuses. No concentration gradient was observed between dams and fetuses, regardless of the dietary treatment.

Fetal liver glycerol kinase was significantly increased in L fetuses over C values (Fig. 1). However, neither the hepatic cytosolic $GcPO_4$ dehydrogenase nor the mitochondrial $GcPO_4$ oxidase differed in the fetuses obtained from either group of dams.

Muscle glycerol kinase and $GcPO_4$ dehydrogenase were similar in the two groups of fetuses (Fig. 2). Nevertheless, mitochondrial $GcPO_4$ oxidase was lower in striated muscle of L fetuses than in the tissue of the respective C animals.

A comparative elevation of fetal plasma triglycerides secondary to maternal ingestion

TABLE I. WEIGHT CHANGES AND PLASMA METABOLITES IN LIPEMIC AND CONTROL DAMS AND FETUSES (1 DAY BEFORE DELIVERY)

	Initial weight (g)	Weight gain (g)	Plasma triglycerides (mg/dl)	Plasma free fatty acids (mg/dl)	Plasma cholesterol (mg/dl)	Plasma β-hydroxybutyrate (mg/dl)	Plasma acetoacetate (mg/dl)	Plasma β-hydroxybutyrate/ acetoacetate ratio
Dams								
Lipemic	264.5 ± 14.0	114.5 ± 4.8	280 ± 25	46.7 ± 5.1	93 ± 11	3.11 ± 0.55	1.29 ± 0.31	3.48 ± 0.88
	(14)	(14)	(13)	(14)	(14)	(12)	(11)	(10)
		**	***					
Controls	249.2 ± 8.8	96.9 ± 4.5	142 ± 21	44.1 ± 4.1	99 ± 6	2.65 ± 0.64	1.33 ± 0.20	3.00 ± 0.86
	(14)	(14)	(13)	(13)	(14)	(13)	(9)	(8)
	Mean litter weight (g)	Individual weight (g)						
Fetuses								
Lipemic	39.5 ± 2.2	3.86 ± 0.04	77 ± 4†††	29.5 ± 2.1††	95 ± 8	3.82 ± 0.57	0.92 ± 0.14	7.04 ± 2.50
	(14)	(143)	(27)	(25)	(26)	(27)	(19)	(19)
		*	**			*		
Controls	40.8 ± 3.8	4.10 ± 0.07	50 ± 4†††	24.6 ± 3.3††	96 ± 9	2.26 ± 0.28	1.01 ± 0.12	3.53 ± 0.93
	(14)	(139)	(26)	(27)	(24)	(24)	(16)	(15)

Note. Means ± SE. (N) = number of determinations. * $P < 0.05$; ** $P < 0.02$; *** $P < 0.001$ vs Controls. †† $P < 0.02$; ††† $P < 0.001$ vs corresponding dams. See text for preparation of specimens.

of a high-fat diet did occur with this animal model of maternal L, in spite of evidence of active lipolysis when triglycerides circulate through the placenta (19). The L fetuses still had higher levels of triglycerides than the C, suggesting a dissimilar permeability to triglycerides in the two types of fetuses, or a greater rate of resynthesis of triglycerides in the L fetuses. Neonatal triglyceridemia and ketonemia have also been demonstrated in lactating pups born to dams treated in a similar fashion (2, 5, 7).

The increased levels of β-hydroxybutyrate found in the offspring of L dams have been attributed by other investigators to lower rates of free fatty acid oxidation and an altered redox potential in the hepatocyte (5, 6). The pattern resulting from this dietary manipulation thus differs from the maternal ketosis occurring in gestation after fasting, since in this case ketone levels are higher in the mother than in the fetus (20, 21).

Fetal triglyceridemia and ketonemia in the rat were concomitant with an enhanced hepatic glycerol kinase. This finding agrees with the characterization of this enzyme as a mechanism activated in an energetic "affluent state" (22), which could well be representative of the L fetuses' condition. These metabolic changes occurred simultaneously with a reduction of $GcPO_4$ oxidase in muscle, suggesting immaturity in tissue utilization of an ancillary fuel.

Biochemical changes in $GcPO_4$ metabolism, comparable to those induced in the fetuses of L dams, have been reported earlier

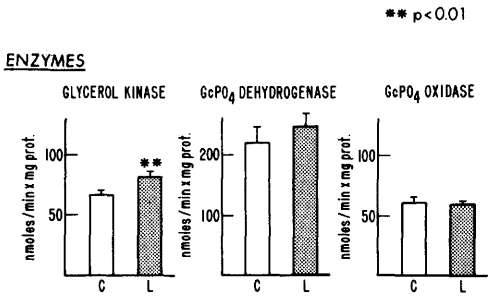

FIG. 1. Liver glycerol kinase, glycerophosphate ($GcPO_4$) dehydrogenase, and $GcPO_4$ oxidase in C and L fetuses obtained 1 day prior to scheduled delivery. The bar heights represent the means and the SE range. $N = 14$ per group.

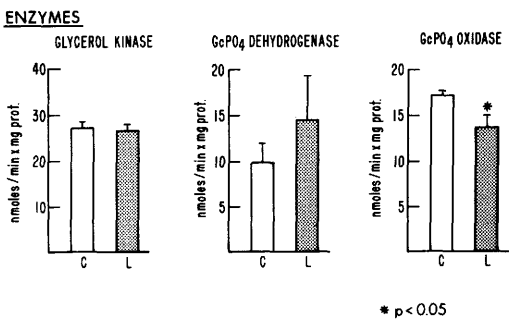

FIG. 2. Glycerol metabolism enzymes in striated muscle of C and L fetuses 1 day before term. $N = 10$ for each group. Abbreviations and statistical significance as in Fig. 1.

in fully developed animals subjected to dietary manipulations. Liver glycerol kinase is particularly sensitive to nutritional imbalance, as reported in adult rats fed excessive amounts of fat for 1 week (11)—another version of energy surplus (22). There is also linkage between hepatic glycerol kinase, $GcPO_4$, and $GcPO_4$ oxidase. Since $GcPO_4$ has been demonstrated to be a competitive inhibitor of glycerol as a substrate for glycerol kinase (23), a negative feedback mechanism could be part of the regulatory process.

Therefore, the present study indicates that dietary-induced L and ketonemia appear to be also responsible for an additional metabolic abnormality affecting overall glycerol utilization. An increase of glycerol kinase activity without a concomitant capacity for $GcPO_4$ disposal could lead to abnormal levels of phosphorylated intermediates of glycerol which have the potential of inhibiting gluconeogenesis and glycogenolysis (10). The findings of this investigation may contribute to the understanding of the causes of one of the multiple derangements known to occur in the offspring of poorly controlled diabetic mothers.

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