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Placental Development and Fatty Acid Metabolism in Pigs Fed *ad Libitum* or Restricted during Gestation[1] (41093)

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Abstract. Placental metabolism and histochemistry were studied during three periods of gestation in pigs fed either *ad lib* or restricted. Placental tissue fatty acid synthesis and esterification were depressed in those pigs which were restricted. Palmitate oxidation to carbon dioxide was not influenced by dietary manipulation. Between the gestational age of 45 and 112 days, placental villi increased in length and maternal and chorionic epithelial cells decreased in height. A comparison of maternal and fetal placental cells revealed differences in lipid content and fatty acid synthesis. It is proposed that both maternal and fetal placentas are capable of responding to maternal nutritional state and may be important in altering metabolites available to the fetus.

The role of the placenta in regulating metabolites available to the fetus is poorly understood. In addition to acting as a selective transport barrier, the placenta is capable of altering metabolites as they are transported to the fetal circulation (1, 2).

The metabolism of placenta-derived nutrients may also be modified by the physiological state of the mother. Maternal pig placenta oxidation of alanine and tissue activity glucose-6-PO_4 dehydrogenase are depressed in alloxan diabetic pigs (2). Treatment of the pregnant rat with estrogen resulted in an increase in placental pyruvate kinase and acetyl CoA carboxylase (3). PEP carboxykinase in the placental tissue of the rat is depressed by treatment with triamcinolone (4).

In the present study, the role of nutritional status on placental tissue development and metabolism was investigated. Since the pig placenta was made up of maternal and fetal epithelial cell layers which had different rates of glucose, pyruvate, and alanine utilization (2), a comparison of these cell layers was made. Food intake restriction during pregnancy resulted in decreased rates of placental tissue fatty acid synthesis and esterification. When compared to the fetal placenta, the maternal placenta had higher rates of fatty acid synthesis, esterification, and glycogen levels.

Materials and Methods. Animals used in these experiments were pregnant Yorkshire pigs of known breeding dates. At 35 days of pregnancy, pigs were alloted to two treatment groups, *ad lib* fed and restricted (1.8 kg per day). The diet consisted of a balanced corn-soybean based gestation ration (16% protein). Average feed intake for the *ad lib* fed pigs was 2.8 kg per day. At 65, 85, and 112 days of gestation, three pigs from each group were rendered unconscious by inhalation of carbon dioxide and hysterectomy was performed. Maternal and fetal placental tissues were separated from the uterine wall by gentle pulling and cut into small slices (50–100 mg) for *in vitro* incubation.

Placental tissue obtained for histology were either fixed in Bouin's fixative for 3 days and processed into paraffin blocks or frozen in isopentate cooled in liquid nitrogen and stored (−60°) until analysis. Paraffin sections were stained with Mayer's hematoxylin and eosin. Air-dried, fresh-frozen cryostat (−20°) sections were reacted for esterase (lipase) 15 min in cold formal calcium fixative (5); for glycogen (6); and for lipid with Oil Red O (5).

For *in vitro* tracer studies, tissues were preincubated for 45 min in Krebs Ringer bicarbonate buffer (one-half normal Ca) with 10 m*M* glucose, 2% fatty acid poor al-

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bumin and 1.0 m*M* Na-palmitate, and 10 m*M* lactate and media changed. The second media consisted of the above components as well as tracers (1 mCi ^3H_2O and 0.5 Ci [1-^{14}C]palmitate/ml of media). All *in vitro* studies were done in 25-ml Erlenmeyer flasks incubated in 2 ml of media at 37° in a Dubnoff metabolic shaker (90 oscillations per minute) under continuous gassing with a mixture of 95% O_2–5% CO_2. The incubations were stopped by the injection of 0.5 ml of 1 *N* H_2SO_4. Carbon dioxide was captured in 0.2 ml of hyamine hydroxide contained in suspended plastic wells. The ^{14}C label recovered in hyamine hydroxide was counted in 10 ml of scintillation fluid (4 g PPO and 100 mg POPOP per liter of toluene). Placental tissues were removed from the media, rinsed, and placed in glass-stoppered tubes for lipid extraction (7). Palmitate metabolism may be influenced by the concentration of palmitate in the media. The concentration utilized in this experiment (1 m*M* palmitate) is near the upper limit for serum palmitate concentrations in the pregnant pig. A channels ratio method was used to separate counts from ^{14}C and tritium. Statistical analysis was done by analysis of variance. Differences between means were compared to Duncan's new multiple-range test (8).

Results. Placental *de novo* fatty acid synthesis is shown in Table I. The stage of gestation did not have any apparent influence on placental synthesis of fatty acids. However, food restriction during pregnancy resulted in a significant decrease in fatty acid synthesis. Maternal placenta had higher rates of de novo fatty acid synthesis than fetal placenta.

Fatty acid oxidation as measured by palmitate oxidation to carbon dioxide showed no significant influence of age, treatment, or origin of placental tissue (Table II).

Esterification activities were estimated by measuring the rates of [^{14}C]palmitate incorporated into triglyceride-fatty acid (Table III). The gestational age had no apparent influence on esterification of fatty acids. Restricted feeding resulted in a significant decrease in esterification activities. Maternal placentas had higher rates of esterification than fetal placentas.

Morphological changes which occur

TABLE I. *In Vitro* Placental Fatty Acid Synthesis[a]

Tissue	Treatment	Days of gestation		
		65	85	112
Maternal placenta	*Ad lib*	1025 ± 563[b]	1883 ± 563	1098 ± 292
	Restricted	1098 ± 274	870 ± 62	885 ± 105
Fetal placenta	*Ad lib*	431 ± 31	1526 ± 37	680 ± 148
	Restricted	452 ± 83	378 ± 28	557 ± 22
	df	*F*	*P*[a]	
		Analysis of variance		
Source				
Day	2	1.60	0.25	
Treatment	1	6.27	0.02	
Tissue	1	14.09	0.003	
		Significant mean separation		
Treatment				
Ad lib	16	1031	0.05[c]	
Restricted	16	711	0.05[c]	
Tissue				
Maternal	18	1025	0.05[c]	
Fetal	14	671	0.05[c]	

[a] Fatty acid synthesis is expressed as nanomoles of acetate units incorporated/g tissue per 2 hr of incubation.
[b] Mean ± SEM.
[c] Mean separation *P* value based on Duncan's new multiple-range test ($P < 0.05$).

TABLE II. *In Vitro* Placental Fatty Acid Oxidation[a]

Tissue	Treatment	Days of gestation 65	85	112
Maternal placenta	*Ad lib*	802 ± 227[b]	1027 ± 43	529 ± 116
	Restricted	633 ± 280	582 ± 239	815 ± 201
Fetal placenta	*Ad lib*	720 ± 256	1319 ± 480	489 ± 168
	Restricted	516 ± 211	314 ± 255	665 ± 184
		Analysis of variance		
	df	*F*	*P*	
Source				
Day	2	14.2	NS	
Treatment	1	7.2	NS	
Tissue	1	0.1	NS	

[a] Fatty acid oxidation is expressed as nanomoles of palmitate oxidized/g tissue/2 hr of incubation.
[b] Mean ± SEM.

during placental development are illustrated in Fig. 1. Between 45 and 110 days, the increased length of villi results in an increased surface area for exchange between maternal and fetal tissues. In addition, it has been shown that by 110 days of gestation the effective placental barrier separating fetal and maternal blood stream measures 2 μm or less as a result of capillary network development around the chorioallantois villi (9). The height of maternal and chorionic epithelial cells decreases as gestation proceeds which also reduces the transplacental intervascular distance. No

TABLE III. *In Vitro* Placental Fatty Acid Esterification[a]

Tissue	Treatment	Days of gestation 65	85	112
Maternal placenta	*Ad lib*	375 ± 57[b]	669 ± 250	387 ± 110
	Restricted	505 ± 196	304 ± 29	311 ± 45
Fetal placenta	*Ad lib*	156 ± 18	540 ± 11	242 ± 54
	Restricted	150 ± 42	139 ± 13	219 ± 14
	df	*F*	*P*[c]	
		Analysis of variance		
Day	2	1.53	NS	
Treatment	1	5.98	0.035	
Tissue	1	15.11	0.0025	
	N	Mean	*P*	
		Significant mean separation		
Treatment				
Ad lib	16	368.4	0.05	
Restricted	16	255.9	0.05	
Tissue				
Maternal	18	367.4	0.05	
Fetal	14	241.1	0.05	

[a] Fatty acid esterification is expressed as nanomoles of palmitate esterified/g tissue/2 hr incubation.
[b] Mean ± SEM.
[c] Mean separation *P* value based on Duncan's new multiple-range test.

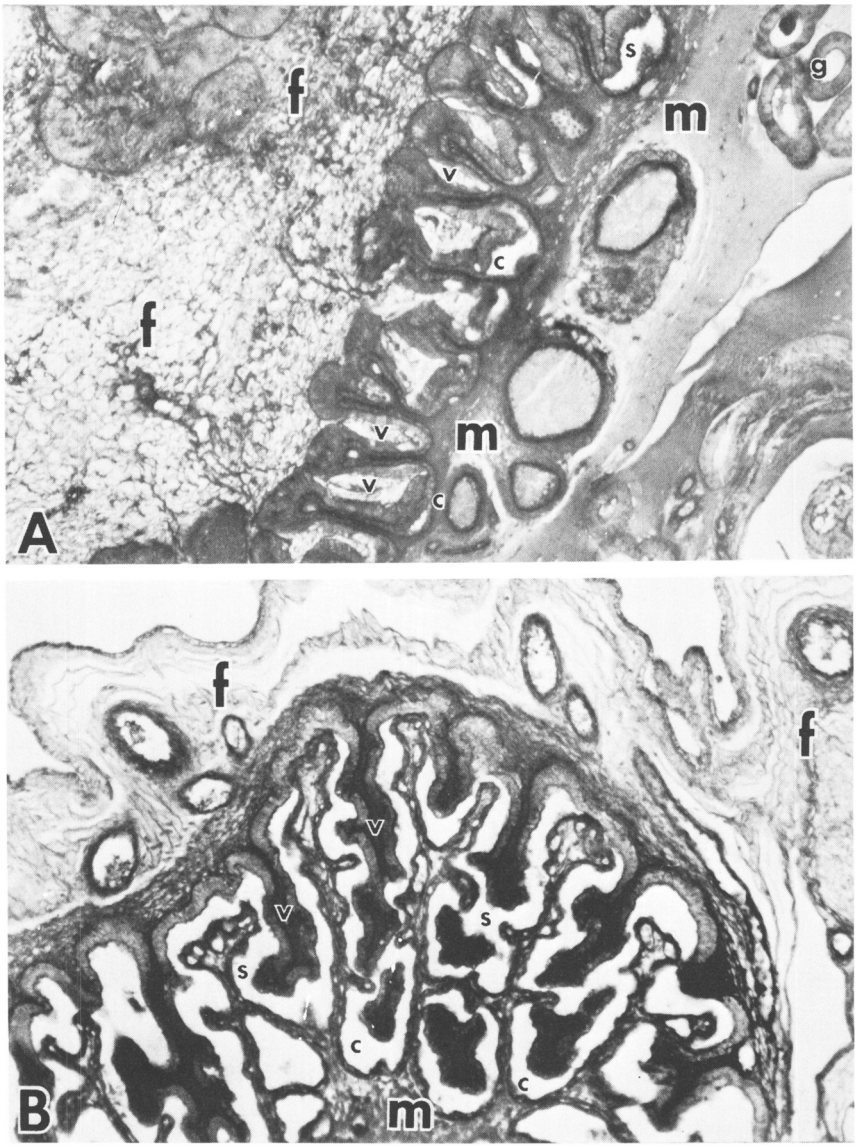

FIG. 1. Alterations in placenta morphology between 45 days (A) and 112 days (B) of gestation in the pig. Paraffin sections (10 μm) stained with the PAS reagents and Harris hematoxylin, ×90. Chorioallantois villi (v) have increased in length and are more deeply embedded into the maternal uterine epithelial crypts (c) in the 112-day placenta. Between 45 and 112 days the height of both the maternal epithelial and chorionic cells has decreased. Fresh-frozen cryostat sections of placenta from all four gestational stages indicated the maternal epithelium and chorion to lie in apposition with no apparent gap between these two cell layers. The space (s) between the maternal epithelium and chorion in these sections is therefore artefactual and due to the fixation and/or embedding process. Also indicated on the micrographs are fetal (f) and maternal (m) placenta and uterine glands (g).

influence of diet restriction was observed on these morphological changes. No changes in histochemical parameters or markers were induced by diet.

Histochemical locations of esterase activity, lipid, and glycogen are shown in Figs. 2, 3, and 4. Esterase activity and lipid are primarily found in maternal epithelial

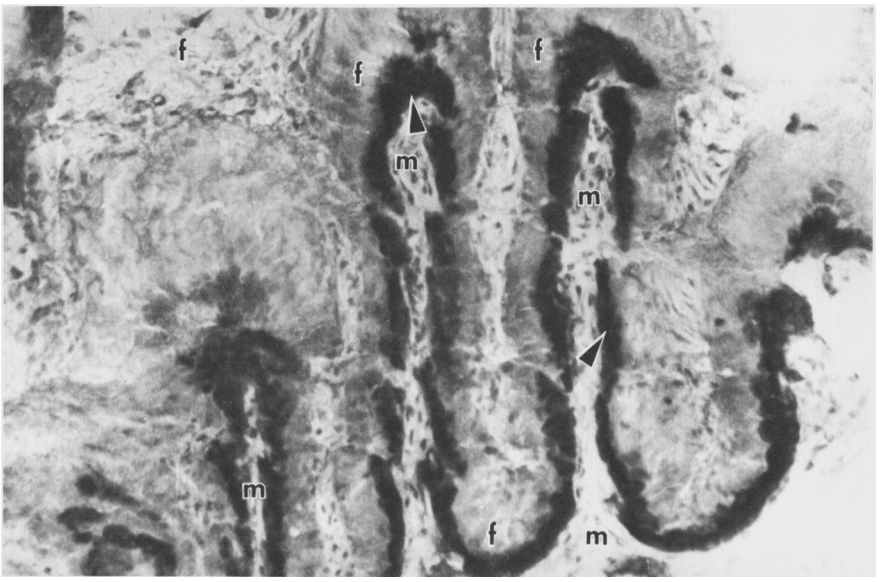

FIG. 2. Fresh frozen cryostat (24-μm) sections of placenta from a sow of 65 days of gestation, $\times$180. This section has been reacted for esterase activity. Note the dark reaction product (arrows) is only located in the maternal epithelial cells. Indicated on the micrograph is fetal (f) and maternal (m) placenta.

cells. The higher level of lipid found in these cells is supported by the increased rates of *in vitro* lipogenesis of maternal placental tissue (see Table I).

The elevated levels of esterase activity may reflect the presence of lipoprotein lipase which would be essential for removal of circulating maternal blood triglycerides.

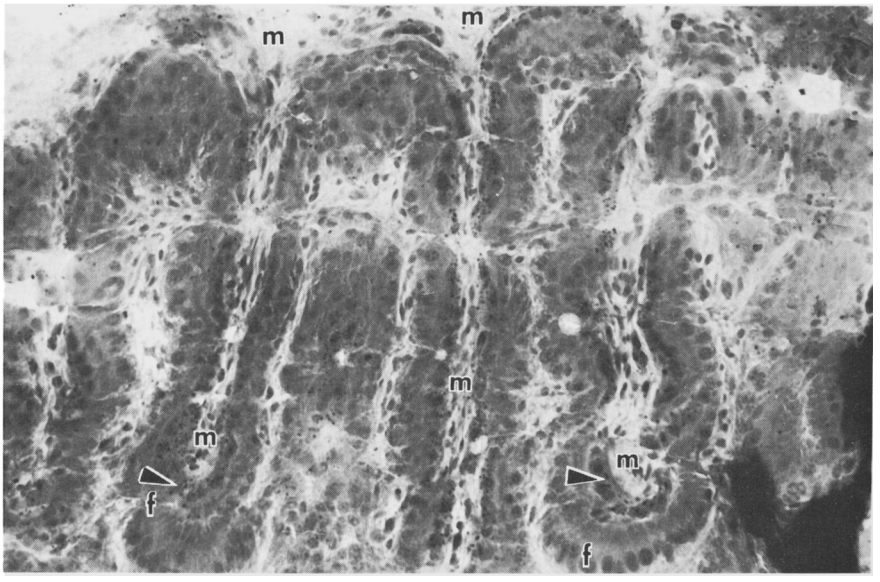

FIG. 3. Fresh frozen cryostat (24-μm) section of placenta from a sow of 85 days of gestation, $\times$180. This section has been stained for lipid (Oil Red O) and nuclei (Harris hematoxylin). Note the lipid droplets (arrows) are only located in the maternal epithelial cells. Indicated on the micrograph is fetal (f) and maternal (m) placenta.

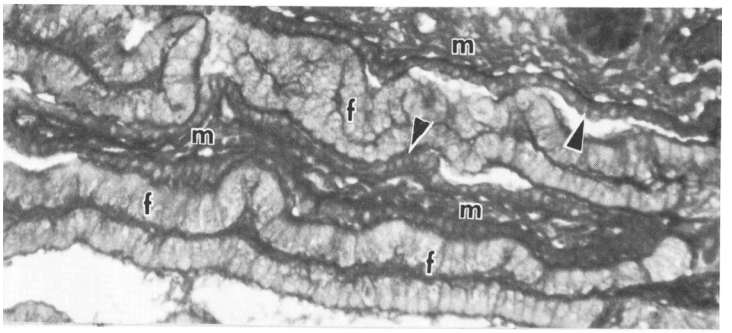

FIG. 4. Paraffin (10-μm) section of placenta from a sow of 45 days of gestation. This is a PAS-stained section, ×180. Note the dark reaction product (arrows) is located primarily in the maternal epithelial cells. The reaction product is usually indicative of glycogen. Indicated on the micrograph are fetal (f) and maternal (m) placenta.

Glycogen deposition in placental tissue has been previously reported (10). The PAS-stained section (Fig. 4) shows that glycogen-like material is primarily located in maternal epithelial cells.

Discussion. Other studies have demonstrated that the placenta has the necessary enzyme profile and the capacity for *de novo* fatty acid synthesis (2–4). In addition, it has been estimated that only 15% of fetal palmitic acid was of maternal serum origin (11). The ability of material and fetal placental tissue to alter fatty acid synthesis in response to a change in maternal nutritional status has been demonstrated here. The mechanism of reduced fatty acid synthesis may involve a decrease in maternal insulin and/or an increase in serum fatty acids which would inhibit placental acetyl CoA carboxylase (12). That the pig placenta is capable of responding to insulin status has been previously demonstrated in this laboratory (2). In addition, it has been shown that insulin increases placental glycogen synthesis and *in vitro* glucose transport (10, 13).

Placental fatty acid esterification was also influenced by maternal nutrition. The rate of esterification, which is dependent on an adequate supply of α-glycerol phosphate, was probably caused by the decreased availability of glucose to the placenta in the pigs on the restricted intake.

The sheep placenta utilized glucose as a major source of energy (1). In the feed-restricted pigs it was anticipated that fatty acid oxidation to carbon dioxide would be elevated because of a decreased availability of glucose. However, this was not observed. In these studies, no attempt was made to measure ketone body release from labelled palmitate. Therefore, incomplete oxidation of fatty acids to acetoacetate and β-hydroxybutyrate could have been altered without influencing the rate of palmitate oxidation to carbon dioxide. Further studies will be required to clarify this aspect of placental fatty acid metabolism.

Comparisons of maternal and fetal placental cells revealed several metabolic and histochemical differences. *In vitro* rates of *de novo* fatty acid synthesis and esterification were higher in maternal placenta. In addition, histochemical staining for lipid revealed higher levels of lipid in maternal placenta. The accumulation of lipid in this tissue suggests that transport through this tissue may be rate limiting for transport of maternally derived lipids to the fetus. The fact that esterase activity was also higher in maternal placental cells suggests that lipoprotein lipase may also be available in these cells to facilitate the uptake of maternally derived lipids.

Fruther comparisons of maternal and fetal placental histochemistry revealed highest levels of glycogen in maternal placental cells. Several factors may be responsible for this observation. The higher levels of maternal serum glucose and insulin could have stimulated glycogen synthesis in maternal placental cells (14). Demers *et al.* (10)

have demonstrated that insulin increases the activity of placental glycogen synthesis. Conversely, fetal serum glucagon levels are higher than maternal serum levels and would tend to deplete any glycogen stored in fetal placental cells (15). Alternately, it has been found that receptor and transport mechanisms differ for maternal and fetal placental membranes (16).

In summary, this report illustrates unique differences in maternal and fetal placental cells. In addition, the effect of maternal nutritional status on placental fatty acid metabolism is documented. It is proposed that the placentas have the necessary metabolic capabilities to alter metabolite availability and to respond to maternal dietary restriction.

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