Adipose tissue cellularity and histochemistry in fetal swine as affected by genetic selection for high or low backfat

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Abstract Adipose tissue development was studied histochemically and histologically in fetuses from lean and obese sows. At 110 days of gestation, fetuses were removed from Ossabaw (obese-feral) sows and from sows selected for high backfat (obese-domestic) and for low backfat (lean) thickness. Body weights were similar for lean (916 ± 225 g) and obese (822 ± 167 g) domestic fetuses, whereas obese feral fetuses were smaller (631 ± 70 g). Histological and histochemical analysis was conducted on subcutaneous tissue from over the shoulder. Staining for lipid-containing fat cells indicated similar concentrations of fat cells throughout the depth of the subcutaneous tissue from obese (domestic and feral) and lean fetuses. Adipocytes from obese fetuses were slightly larger (domestic 23 ± 0.22 μm, feral 21.8 ± 0.26 μm) than cells from lean fetuses (20.7 ± 0.42 μm). The percentage glycogen positive (PAS) adipocytes was low and similar from all three fetal strains. Less than 10% of adipocytes from lean and obese domestic fetuses were esterase-positive, whereas 42% of adipocytes from obese feral fetuses were esterase-positive. All adipocytes from obese fetuses (domestic and feral) were lipoprotein lipase (LPL)-positive whereas all cells from lean fetuses were negative for LPL activity. Therefore, cellular and metabolic differences exist in adipose tissue of lean and obese pigs during the prenatal period of growth and development.—**Hausman, G. J., D. R. Campion, and G. B. Thomas.** Adipose tissue cellularity and histochemistry in fetal swine as affected by genetic selection for high or low backfat. *J. Lipid Res.* 1983. **24:** 223–228.

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An elevated adipose tissue lipoprotein lipase (LPL) activity and larger fat cell size are the earliest indicators of obesity in the Zucker (fa/fa) fatty rat (1, 2). These alterations are observed in the absence of hyperphagia and hypertriglyceridemia and, therefore, may represent a primary defect in the obese Zucker rat (1). These studies (1, 2) did not rule out the possibility of elevated insulin levels in the young obese animal. Insulin stimulates the synthesis of LPL in cultured 3T3-L1 differentiating adipocytes (3–5). Therefore, higher insulin levels in the obese animal could be the cause of elevated LPL activity and increased adipocyte size.

Studies of genetic obesity in rodent models are limited by the lack of adequate tissue and blood sample size in the very young animals. Adequate tissue and blood samples can be obtained from late fetal and young pigs. Therefore, we have studied adipose tissue development in two lines of pigs, one selected for high backfat thickness and another for low backfat thickness (6). The selection for increased backfat resulted in increased total carcass adipose accumulation (6), increased de novo lipogenesis in adipose tissue (7), and increased number and size of adipocytes (8).

Adipose tissue development in the fetal pig is characterized by an increase in adipocyte number with minimal increase in cell size (9, 10). In fetal pigs, the subcutaneous depot can be studied with histological and histochemical techniques (11). For instance, histochemical staining for lipid-containing fat cell clusters in cross sections of subcutaneous tissue indicates the relative number of lipid-containing adipocytes in this depot. Cell diameters can be obtained from these sections and similar sections can be tested for enzyme activities. Therefore, we have studied the development of the subcutaneous adipose depot in late term fetuses from genetically obese and lean sows. Fetuses from another obese (Ossabaw) strain of pig (12) were compared to the two selected lines.

MATERIALS AND METHODS

Obese and lean animals were lines selected over 18 generations from within the Yorkshire and Duroc breed (6). The two breeds were then crossbred and subsequently selection pressure for backfat thickness was removed. Obese and lean sows and Ossabaw sows were housed in individual stalls prior to surgery and fed ad

Abbreviation: LPL, lipoprotein lipase.

TABLE 1. Results of various preincubations on LPL activity of capillaries and adipocytes of porcine subcutaneous adipose tissue

Time/ Minutes	Location of Activity	Preincubation Media[a]			
		A	B	C	D
1	Capillaries	0[b]	+	++	0
	Adipocytes	0	+	++	++
3	C	0	+	++	0
	A	0	+	++	++
6	C	0	+	+	0
	A	0	+	+	+
10	C	0	+	+	0
	A	0	+	+	0
20	C	0	+	0	0
	A	0	+	0	0
30	C	0	+	0	0
	A	0	+	0	0
40	C	0	+	0	0
	A	0	+	0	0

[a] A, 1 M NaCl, pH 6.5; B, control (saline, pH 8.6); C, control (0.1 M Tris base, pH 8.6); D, 10% Na heparin (0.1 M Tris base solution, pH 8.6).

[b] ++, high activity; +, low activity; 0, no activity.

libitum a diet of 67.7% ground shell corn, 10% oats, 5% wheat bran, 8% soybean meal (50% protein), 4% poultry by-product meal, 2.5% alfalfa meal (17% protein), and 2.85% vitamin and mineral mix.

At 110 days of gestation, sows were laparotomized under halothane anesthesia and the fetuses were removed and exsanguinated. Five obese, three lean, and four Ossabaw sows were used. Tissue samples were obtained from each of three fetuses per sow from the lean and Ossabaw strains, whereas two fetuses per sow were sampled from the obese strain.

Tissue handling

Neck and shoulder dorsal-most subcutaneous tissue, including some underlying muscle was removed from the fetuses. This tissue was removed in a manner that resulted in a rectangular-shaped piece of tissue, the length of which was equal to the length extending from a point just caudal to the head to the fifth and sixth rib. The tissue was divided into 2- to 4-mm sections cut perpendicular to the long axis of the body. One-half of the sections were fixed in Bouin's fixative for 1–2 days and routinely processed into paraffin blocks. The remaining sections were frozen in isopentane cooled in liquid nitrogen and stored in an upright ultracold freezer (−60°C) until analyzed.

Section preparation and staining

Mounting of sections in paraffin blocks or for the cryostat was done to assure that histological sections would be perpendicular to the skin surface. Specific procedures for handling and staining fresh frozen cryostat sections are reported elsewhere (11). Sections (5–10 μm) from paraffin blocks were stained with PAS reagents (13), Picro Ponceau reagents (13), and Harris Hematoxylin (HH). Air-dried, fresh frozen cryostat (−20°C) sections (10–30 μm) were reacted for esterase activity (14), LPL activity (15), or were stained with oil red O (11), the Picro Ponceau reagents (13), toluidine blue (13), and HH.

Characterization of the histochemical test for lipoprotein lipase

In this study we applied the method of Moskowitz and Moskowitz (15) to air-dried, unfixed 30-μm sections cut from rapidly frozen tissue. Lipoprotein lipase liberates free fatty acids from the lipoproteins. Calcium forms insoluble soaps with the fatty acids at the site of enzyme activity. Calcium soaps are then converted into a visible black deposit of lead sulfide by exposure to lead nitrate and finally an ammonium sulfide solution. This technique used no added substrate but utilizes the endogenous blood chylomicrons and cellular stores of triglycerides as substrates. Therefore, a series of experiments was conducted to characterize the reaction that was being detected. Sections of adipose tissue from 1-day-old pigs were used in the following experiments. The pH of the incubating media was varied in one set of experiments. No reaction product was evident at pH 6 whereas most adipocytes were stained at pH 7. Capillaries were lightly stained and all adipocytes were positive at pH 8. At pH 9 all capillaries and adipocytes were clearly delineated with reaction product. These results indicate an alkaline pH optima for capillary LPL staining. Purified pig adipose tissue LPL exhibited a pH optimum of 8.8 (16).

When incubation times were varied (1–8 hr), the amount of reaction product was proportional to the duration of incubation time up to 5 hr, at which time the amount of reaction product plateaued. When sections were preincubated in Lugdol's Iodine solution for 2 min and then incubated in a normal medium for 8 hr, there was no reaction product present. This indicated an enzymatic nature to the reaction since Lugdol's Iodine inactivates lipase enzymes by blocking sulfhydryl groups on the enzyme. Results of various preincubations (**Table 1**) showed that preincubating at 1.0 M NaCl for as little as 1 min gave complete inhibition of lipase activity of adipocytes and capillaries. When late fetal and early postnatal sections were incubated in media containing 0.5 M NaCl, there was complete inhibition, while incubating in 0.25 M NaCl gave partial inhibition. These results indicated an enzymatic nature to the reaction since Bensadoun et al. (16) reported that purified pig adipose tissue lipoprotein lipase was 80% inhibited in the presence of 0.6 M and higher NaCl concentra-

TABLE 2. Effect of fasting on LPL activity in capillaries and adipocytes of porcine subcutaneous adipose tissue

Period of Fasting	pH of Incubating Media	Adipocytes	Capillaries
hr			
0	6	0[a]	0
	7	++	0
	7.4	++	0
	8.6	++	++
	9.0	++	++
6	6	0	0
	7	+	0
	7.4	+	0
	8.6	0	0
	9.0	0	0
30	6	+	0
	7	+	0
	7.4	0	0
	8.6	0	0
	9.0	0	0

[a] ++, high activity; +, low activity; 0, no activity.

tions. Preincubating in solutions containing sodium heparin for as little as 1 min (Table 1) resulted in no reaction product in capillaries. Since postheparinized plasma contains considerable lipoprotein lipase activity, our results indicated that the enzyme was removed from the capillaries by the heparinized solutions. Longer preincubations with heparin (Table 1) resulted in partial or total loss of reaction product from the adipocytes as well. Since the control sections (Table 1) showed low activity throughout the various preincubation times, the loss of adipocyte reaction product was probably due to extraction of the enzyme by heparin. The effect of starvation on lipoprotein lipase activity of capillaries and adipocytes is summarized in **Table 2**. Starvation for 30 hr resulted in complete inhibition of enzyme activity in capillaries and, at some pH levels, in adipocytes. This is totally expected since the animals were mobilizing free fatty acids from adipose tissue as evidenced by actual decreases in adipose tissue lipid (oil red O stained sections) when compared to nonstarved pigs.

Fat cell diameters

Fat cell diameters were determined on cryostat sections according to the method of Sjöström Björntorp, and Vrána (17). One-hundred fat cells from each of six fetuses per strain were measured. The mean ± S. E. of these (six) means was used to indicate fat cell size for a given strain.

Statistics

Comparison of means was accomplished by the Student *t*-method for comparison of two means (18).

RESULTS

Adipose tissue from fetuses of all three strains was morphologically similar (**Fig. 1**). Fat cell clusters were similar in concentration and distribution in outer and inner layers of subcutaneous adipose tissue (Fig. 1). Regardless of strain, the thickness of subcutaneous tissue was positively related to body size (**Table 3**). There-

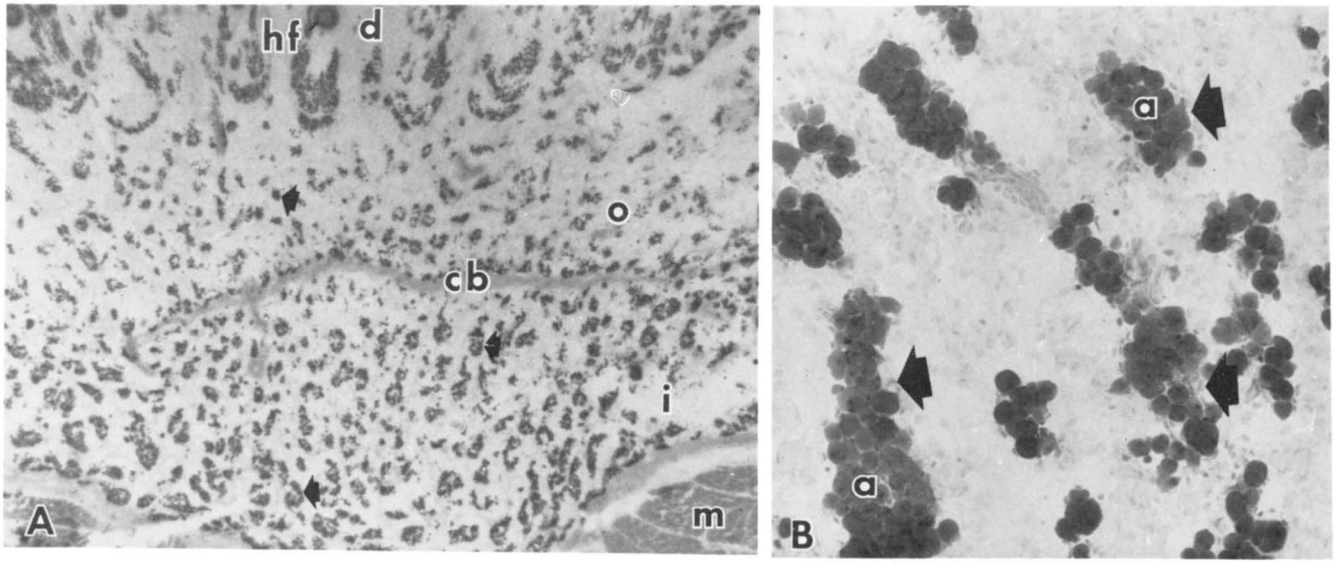

Fig. 1. Morphology of subcutaneous adipose tissue from a domestic-lean fetus (A). Numerous fat cell clusters (arrows) are distributed throughout the outer (o) and inner (i) layers of subcutaneous adipose tissue. Fat cell cluster distribution is similar for fetuses from all three maternal strains. Fat cell clusters are tightly arranged groups of adipocytes (B, a). The morphology of fat cell clusters from a domestic-lean fetus is shown in B. Fat cell cluster morphology was similar for fetuses from all three strains. Indicated are dermis (d), hair follicle (hf), muscle (m), and connective tissue border (cb) between outer and inner layers of adipose tissue. Frozen sections (24 μm) stained for lipid with oil red O and counterstained with Harris Hematoxylin. A, ×15 and B, ×320. (Reduced in reproduction).

TABLE 3. Body weights, fat cell size, and esterase and LPL histochemistry of fat cells from lean and obese pig fetuses

Maternal Strain	n	Body[a] weight	Outer Layer[a] Thickness	Fat Cell[a] Size	LPL-positive Cells	Esterase-positive Cells	PAS-positive Cells
		g	*mm*	*μm*	%	%	%
Lean	9	916 ± 130	2.65 ± 0.1	20.7 ± 0.42[c]	0[b]	<10[b]	6[b]
Domestic-obese	10	882 ± 68	2.6 ± 0.1	23.0 ± 0.22[d]	100	<10	6.5
Feral-obese	12	631 ± 26	2.0 ± 0.1	21.8 ± 0.26[e]	100	42	10

[a] Mean ± SEM of six animals.
[b] One hundred cells from each fetus in this study were counted and the percentage positive cells were recorded; the average percentage of positive cells for each strain was calculated and reported here.
[c,d,e] Vertical values with different subscripts differ ($P < 0.01$).

fore, the tissue was thicker from the selected lines relative to the Ossabaw strain, since Ossabaw fetuses were smaller (Table 3).

Adipocytes from obese fetuses were larger ($P < 0.01$) than cells from lean fetuses (Table 3). Frequency distributions of adipocyte diameters indicated considerable overlap of cell sizes between the strains (**Fig. 2**). The percentage PAS positive cells was low and similar for all three fetal strains (Table 3). Forty-two percent of adipocytes from Ossabaw fetuses were esterase-positive whereas less than 10% of cells from fetuses of the selected lines were positive for esterase activity (Table 3). All cells (100%) from obese fetuses were LPL-positive whereas all cells (100%) from lean fetuses were negative for LPL activity (Table 3 and **Fig. 3**).

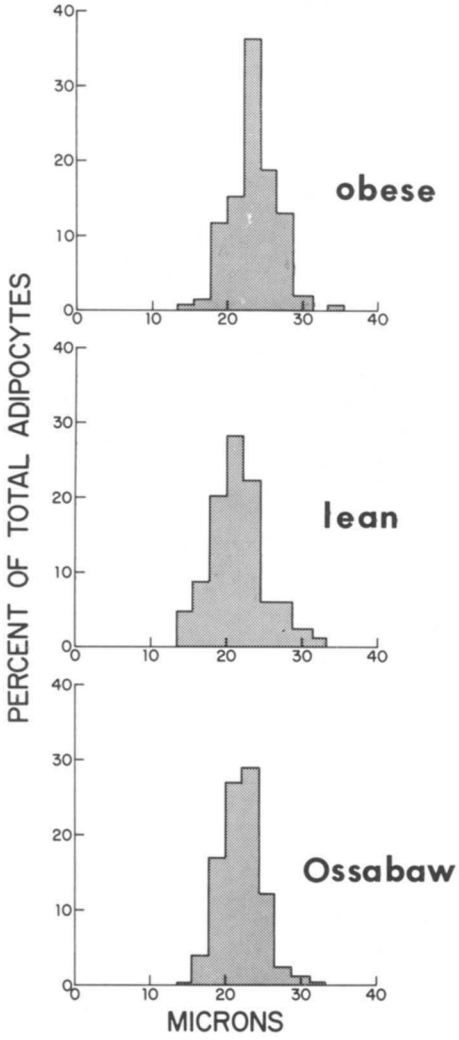

Fig. 2. Adipocyte diameter distributions from obese, lean, and Ossabaw fetuses. Note that there is considerable overlap of cell sizes from all three fetal strains.

DISCUSSION

These results demonstrate that either forced or natural selection for obesity in adult swine results in alterations in fetal adipocyte cellularity. Fetuses from obese mothers had fat cells that were larger and apparently more capable of LPL synthesis than cells from fetuses of nonobese mothers. These results are completely compatible with data obtained from young, obese Zucker rats (1, 2). In the rat studies (1, 2), however, it can not be ascertained if the elevated LPL activity and increased cell size are asynchronous or synchronous events.

A biochemical analysis for adipose tissue LPL from the fetuses used in this study indicated significantly higher levels of enzyme in obese and Ossabaw fetuses than in lean fetuses (19). Our histochemical evidence indicates that all fat cells from fetuses of obese mothers are LPL-positive even though some of these cells are smaller or similar in size to cells from fetuses of lean mothers (Fig. 2). Therefore, the distinction between lean and obese fetuses based on LPL histochemistry was independent of cell size differences. Our results indicate that the increased capacity for LPL synthesis may precede the increased cell size seen in obesity.

Body composition analysis of pig fetuses from obese and lean mothers indicates similar percentages of body protein (14–16%) and body lipid (1–2%) (19). Therefore, unlike previously reported rat studies (1, 2), the present study represents a comparison of lean and obese animals in the pre-obese state. Hormone and metabolite levels were measured in blood samples obtained from

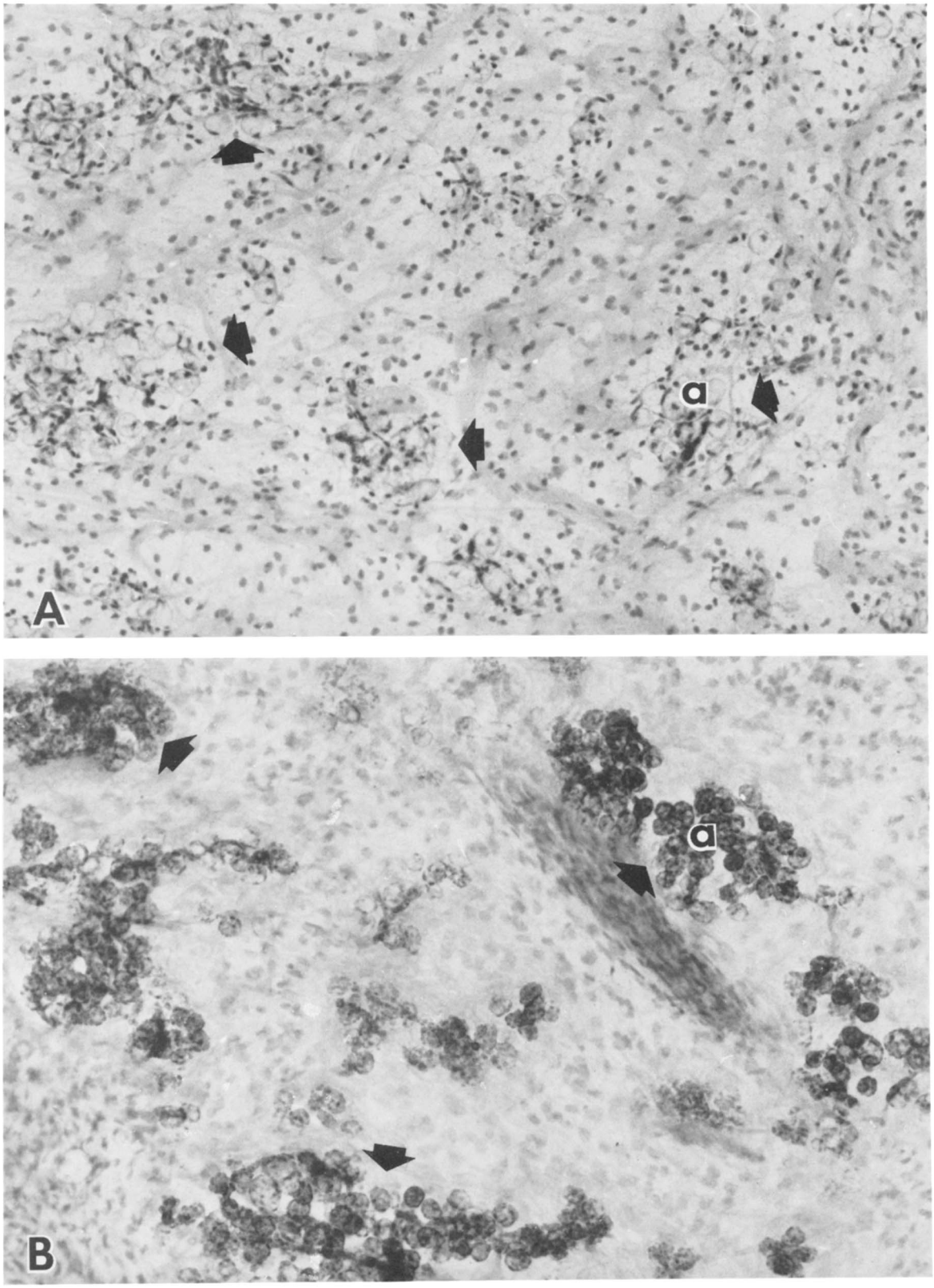

Fig. 3. Domestic-lean (A) and obese (B) adipose tissue sections reacted for lipoprotein lipase activity (LPL). All adipocytes (a) reactive positively for LPL activity in sections from domestic-obese fetuses (B), whereas no reaction product is evident in adipocytes from domestic-lean fetuses (A, a). Indicated are fat cell clusters (arrows) and adipocytes (a). Frozen sections (24 μm) incubated for LPL activity at a pH of 8.6 for 5 hr at 37°C, A and B, ×320.

fetuses from obese and lean mothers (20). Plasma growth hormone levels were lower and cortisol and glucagon levels were higher in fetuses from obese mothers relative to fetuses from lean mothers (20). Glucose, triglyceride, and fatty acid levels in plasma were not different between the two strains of pigs (20). The differences in cellularity and histochemistry noted between the fetal strains may be the result of an altered endocrine status. An ontogeny study examining fetuses at several earlier ages could elucidate which characteristic of the fetal obese strain developed first (altered fat cells or altered endocrine status).

Adipose tissue from pig fetuses of diabetic (alloxan-induced) mothers contained adipocytes that were larger and histochemically distinct from control adipocytes (21). All adipocytes from fetuses of diabetic mothers contained glycogen (PAS-positive) (21). The similar and low level of glycogen-containing (PAS-positive) cells in all three fetal strains (present study) indicates that fetuses in obese mothers and fetuses in diabetic mothers are exposed to dissimilar metabolic and endocrine profiles.

Domestic pigs had larger adipocytes (30 vs. 24 μm) than did obese feral pigs in a previous comparison of 1–2-day-old animals (22). Therefore, during the immediate perinatal period, adipocyte hypertrophy in domestic pigs is significant while little hypertrophy is noted for cells in feral pigs. The greater esterase response of fetal feral adipocytes relative to cells from domestic fetuses (present study) may explain the relative lack of cell hypertrophy in the feral animals. The esterase response is probably indicative of activity similar to hormone-sensitive lipase enzyme. A greater lipase response in feral pigs would be compatible with a greater adaptation to stress that allows the feral pigs to survive in the wild.

This study has demonstrated that the early development of fetal adipose tissue LPL is possibly a key initial event in the development of genetic obesity. Subsequent studies are needed to determine if the enhanced LPL activity represents a response to an endocrine signal or if it is due to a genetic alteration in the tissue. The size of pig fetuses and the long gestation period of swine allows continued and expanded investigation into the etiology of genetic obesity.

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