Characterization of an Obese Syndrome in the Pig[1] (37285)

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(Introduced by E. W. Hartsook)

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A number of genetic obese models have been described in the rodent family. These obese animals have been shown to possess certain metabolic abnormalities ranging from specific changes in tissue enzyme levels to general endocrine imbalances. Abnormalities found in rodents possessing the obese syndrome have been recently reviewed by Bray and York (1). Until now there have been no reports on the obese pig. The objectives of this initial study of obese pigs were to characterize the gross changes in adipose and muscle tissue development and to determine enzyme patterns in liver and adipose tissue. In addition, the adaptations of adipose tissue enzymes were examined in pigs subjected to a fasting–refeeding schedule.

Materials and Methods. In the first experiment lean pigs from the domestic strain (Yorkshire) were compared to obese pigs from a feral strain of pigs (Ossabaw). The feral strain normally inhabits Ossabaw Island off the coast of Georgia. On this island the pigs have no natural predators. Survival during the winter months when food supply is scarce, is probably dependent on the ability of the pig to store large quantities of fat during the late summer and fall months when food supply is abundant. Both lean and obese strains were maintained at The Pennsylvania State University Swine Center and fed *ad libitum* a corn and soybean meal diet containing 14% protein. The pigs were approximately 5 mo of age at the time of sacrifice. Carcass characteristics and organ weights were measured at The Pennsylvania State University Meats Lab. Backfat thickness was measured at the first rib, last rib and last lumbar vertebra and the average was calculated. The cross-sectional area of the longissimus dorsi muscle was determined be-

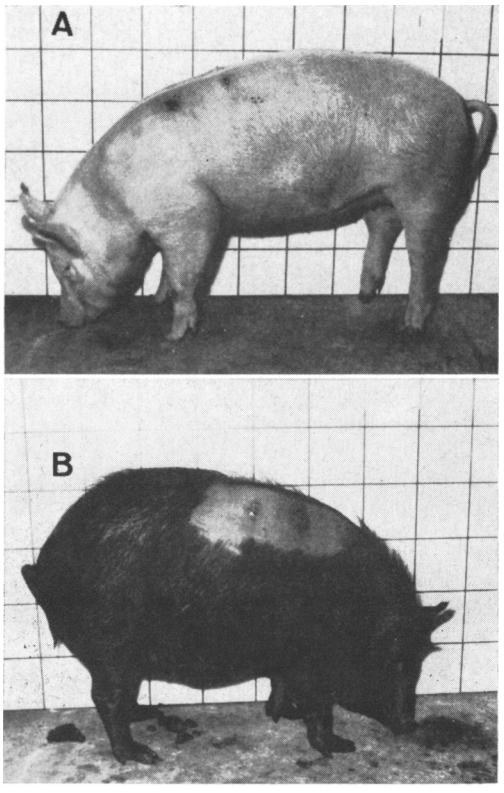

FIG. 1. Comparison of lean and obese type pigs. (A) Domestic breed Yorkshire (lean) and (B) feral breed Ossabaw (obese).

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tween the 10th and 11th ribs. Adipose tissue samples were excised from the subcutaneous and perirenal sites and kept in thermos jars containing warm Krebs Ringer buffer (37°) for transportation to the laboratory. Keeping the tissue at this temperature facilitated tissue homogenization and extraction. The livers were removed, weighed and samples were placed in plastic bags on ice for preparation of tissue extracts.

In a second study the effects of fasting and refeeding on adipose tissue enzyme adaptation were measured in lean and obese pigs. Samples were obtained by biopsy of the subcutaneous adipose before the start of fasting, on the third and seventh day of fasting and on the third and seventh days of refeeding. These samples were also placed in thermos bottles containing warm buffer before transporting to the laboratory.

Adipose and liver tissue samples were homogenized in 0.25 *M* sucrose media (containing 1 m*M* dithiothreitol) with a Vertis 45 homogenizer for 15 sec. This procedure permitted greater recovery of enzyme activity than the glass Teflon homogenizer. The homogenates were centrifuged at 27,000*g* for 20 min (4°) and the resulting supernatants were used for enzyme measurement.

Enzyme assays. Malic enzyme (EC 1.1.1.40) (ME) was measured by the procedure described by Ochoa (2). Glucose-6-P dehydrogenase (EC 1.1.1.49) (G6PD) and 6-P gluconate dehydrogenase (EC 1.1.1.44) (6PGD) were assayed by the method of Glock and McLean (3). NADP-isocitrate dehydrogenase (EC 1.1.1.42) (ICDH) was assayed by the procedure of Plaut (4). Assay of alanine aminotransferase (EC 2.6.1.2) (GPT) was performed on liver extracts by the procedure of Segal and Matsuzawa (5). Malate dehydrogenase (EC 1.1.1.37) (MDH) and aspartate aminotransferase (EC 2.6.1.10) (GOT) were assayed according to the procedure of Baldwin and Milligan (6). Fructose diphosphatase (EC 3.1.3.11) (FDPase) was assayed by the procedure of Taketa and Powell (7). Levels of α-glycerol PO_4 dehydrogenase (αGPD) were measured by the procedure of Fitch and Chaikoff (8). Citrate cleavage enzyme (CCE) was assayed by the method described by Cottam and Srere (9).

Protein concentration in adipose tissue extracts was determined by the method of Low-

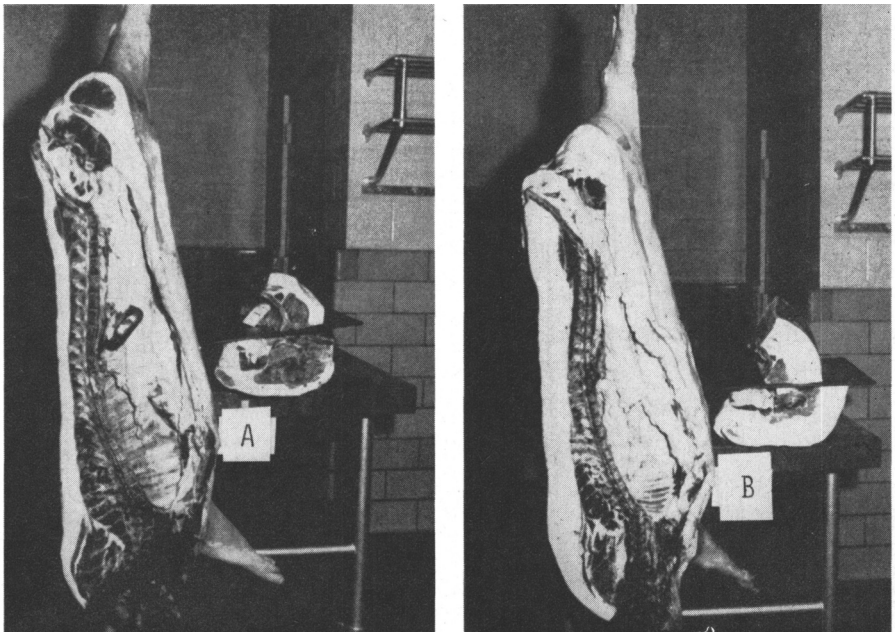

FIG. 2. Comparison of body carcass characteristics from (A) lean and (B) obese pigs. Note the greater lipid deposition of the obese strain.

TABLE I. General Characteristics of Experimental Animals.

Exptl groups[a]	Body Weight (kg)	Body Length (cm)	Backfat thickness (cm)	Muscle area (cm^2)	Feed intake[b] (kg/wk)	Plasma glucose (mg/100 ml)
Lean	92.6 ± 2.6	73.9 ± 0.6	2.8 ± 0.2	35.5 ± 2.2	14.2 ± 1.2	126 ± 9
Obese	58.0 ± 4.3	58.2 ± 1.1	8.1 ± 0.2	13.9 ± 1.1	15.1 ± 1.5	103 ± 12

[a] Values represent the mean of five animals ± SEM. All animals were approximately 8 wk of age at the time of sacrifice.

[b] Feed intake is expressed as weekly feed intake per 100 kg of body weight.

ry *et al.* (10). Blood glucose was determined on samples obtained during sacrifice by the glucostat method (Worthington).

Results. Lean and obese pigs are shown in Figs. 1 and 2. The differences in fat deposition are more obvious in Fig. 2. The most striking differences were found in the subcutaneous and perirenal fat sites. Also noticeable is the inferior muscle development found in obese pigs. General characteristics of the experimental animals are given in Table I. When examined at a similar age, the obese pig was considerably lighter in weight and shorter than the lean pig. Backfat thickness and perirenal fat weight are greater in the obese pig indicating extensive fat deposition. The cross-sectional area of the longissimus dorsi muscle was larger in the lean pig then the obese pig. Organ weights of the two groups of pigs are given in Table II. In general, most organs were not grossly different in the two groups of pigs. The liver weight was greater in the obese pig.

Liver tissue enzyme levels are given in Table III. Enzymes normally associated with elevated rates of lipogenesis (G6PD, 6PGD, ME and αGPD) were not significantly different in the lean and obese pig. However, those enzymes involved in gluconeogenesis and amino acid metabolism were elevated in the obese pig. Activities of selected adipose tissues enzymes are given in Table IV. Levels of perirenal adipose tissue enzymes associated with lipogenesis were markedly increased in the obese pig indicating a greater capacity for fat synthesis in this tissue. Differences were also observed in the enzyme profiles of subcutaneous fat from the two groups (Table V).

The influence of fasting and refeeding on adipose tissue enzyme adaptation is shown in Figs. 3 and 4. Both CCE and ME level are depressed during fasting in the lean and obese groups. However, the response to refeeding was different. The lean pig responded in the classical fashion by elevated levels of both enzymes. The obese pig adipose tissue enzymes did not return to normal levels until the seventh day of refeeding. These results indicated that the dynamic adaptation of fasting and refeeding was not operative in the obese pig.

Discussion. The pigs used in this study demonstrated extremes in body tissue devel-

TABLE II. Organ Weights of Lean and Obese Pigs.

Exptl group[a]	Weight Liver (kg)	Perirenal fat[b] (g)	Adrenal (g)	Pituitary (g)	Full gastrointestinal tract (kg)
Lean	1.38 ± 0.05	299 ± 34	37.6 ± 0.7	2.8 ± 0.1	6.4 ± 0.5
Obese	1.98 ± 0.13	738 ± 135	30.9 ± 1.1	2.2 ± 0.1	5.8 ± 0.4

[a] Values represent the mean of five determinations on different animals ± SEM. All animals were approximately 8 wk of age at the time of sacrifice.

[b] Perirenal fat weight was determined on the left half of the carcass only.

TABLE III. Levels of Liver Tissue Enzymes from Lean and Obese Pigs.

Enzymes	Exptl groups[a]; (μmoles/min/kg body wt)		
	Lean	Obese	*p* Value
Glucose-6-P dehydrogenase	12.8 ± 1.8	13.0 ± 1.7	NS
6-P gluconate dehydrogenase	16.3 ± 0.8	23.8 ± 2.5	<.05
Malic enzyme	15.2 ± 0.7	12.8 ± 2.1	NS
α-Glycerol-P dehydrogenase	61.0 ± 6.5	91.5 ± 11.6	NS
Fructose diphosphatase	75.6 ± 4.6	124.2 ± 13.0	<.01
Aspartate aminotransferase	94.4 ± 8.8	153.8 ± 19.6	<.05
Alanine aminotransferase	20.3 ± 0.8	39.0 ± 2.5	<.01

[a] Values represent the mean of five animals ± SEM. Activity is expressed as a function of body size since it has been our experience that enzyme activity is more consistent with the physiological function when expressed in this manner. This technique eliminates complicating phenomena such as changes in liver labile proteins and daily fluctuations in liver lipid, glycogen and water contents.

opment. The obese pigs possess a greater capacity for lipid synthesis and storage and a marked impairment in muscle development when compared to the domestic pig. Unlike the Zucker fatty rat (11) and the obob mouse (12) the obese pig can reproduce large litters (unpublished data). Because of their size, blood sampling and tissue biopsies are accomplished without significantly altering body tissue functions. The genetic obese rodents such as the Zucker fatty rat (11) and the obese hyperglycemic mouse (12) will consume more calories than their lean littermates. This is an additional variable which, if not regulated, can complicate interpretation of data comparing the genetic lean and obese animal. The obese pig does not develop hyperphagia even during the stages of rapid lipid deposition. Apparently, the shift in metabolite utilization from muscle to adipose tissue development is sufficient to result in excess lipid deposition.

The enzyme patterns in liver and adipose tissue of lean and obese rodents have been reported by several researchers (13–16). This is the first report of enzyme levels in genetically obese pigs. It has been established that there is considerable species variation in regard to the principal tissue(s) involved in the production of body fat. O'Hea and Leveille (17) have shown that in the pig the major site of fat synthesis is the adipose tissue. The present studies indicate that the lipogenic enzyme adaptation to the obese state occurs in adipose tissue and not in liver tissue of the obese pig. Levels of G6PD and 6PGD were found to be elevated in both liver and adipose tissue of obese mice when

TABLE IV. Levels of Perirenal Adipose Tissue Enzymes from Lean and Obese Pigs.

Enzymes	Exptl groups[a]; (nmoles/min/mg protein)		
	Lean	Obese	*p* Value
Glucose-6-P dehydrogenase	90 ± 6	213 ± 16	<.01
6-P gluconate dehydrogenase	43 ± 2	77 ± 9	<.01
Malic enzyme	112 ± 18	384 ± 34	<.01
Citrate cleavage enzyme	18 ± 2	41 ± 8	<.01
α-Glycerol-P dehydrogenase	72 ± 9	93 ± 6	<.10
Malic dehydrogenase	158 ± 11	169 ± 24	NS
		(mg/g tissue)	
Soluble protein	9.6 ± 1.3	8.6 ± 0.3	NS

[a] Values represent the mean of five animals ± SEM.

TABLE V. Levels of Subcutaneous Adipose Tissue Enzymes from Lean and Obese Pigs.

	Exptl group[a]; (nmoles/min/mg protein)		
Enzymes	Lean	Obese	*p* Value
Glucose-6-P dehydrogenase	95 ± 8	145 ± 20	<.05
6-P gluconate dehydrogenase	47 ± 7	70 ± 6	<.025
Malic enzyme	143 ± 18	344 ± 59	<.025
		(mg/g tissue)	
Soluble protein	9.8 ± 1.8	8.7 ± 1.0	NS

[a] Values represent the mean of five animals ± SEM.

compared to lean controls (18). However, hyperphagia in the obese mice was not prevented and could have caused these changes in enzyme pattern. We have shown that if the obese mouse is subjected to dietary restriction the liver lipogenic enzyme levels are essentially the same as the lean control, whereas the adipose tissue lipogenic enzymes remain elevated (19). Hyperphagia was not a factor in this study of obese pigs.

Additional studies of glucose conversion to fatty acids by adipose cells are required to determine the significance of these shifts in enzyme levels. Further analysis of enzymes more directly related to the synthesis of lipids such as acyl-CoA synthetase and fatty acid synthetase would also permit more positive interpretation of these data.

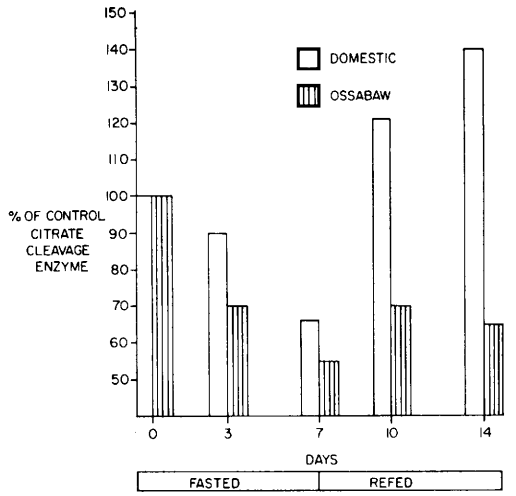

FIG. 3. Effects of fasting and refeeding on subcutaneous adipose tissue citrate cleavage enzyme of domestic (lean) and Ossabaw (obese) pigs. Enzyme activities were expressed per milligram of protein first then calculated as a percentage of control value.

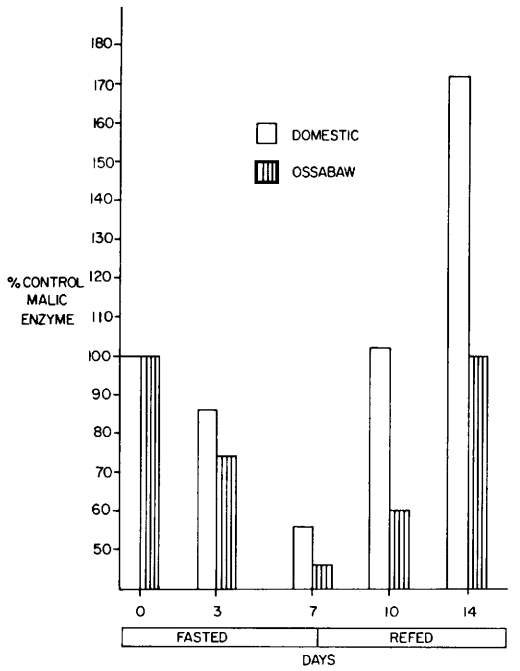

FIG. 4. Effects of fasting and refeeding on subcutaneous adipose tissue malic enzyme of domestic and Ossabaw pigs. Enzyme activities were expressed per milligram of protein first then calculated as a percentage of control value.

The effects of fasting and refeeding on adipose tissue enzyme levels indicate that adaptive changes in the obese pig are impaired. Similarly, when the obese hyperglycemic mouse was fasted and refed the classical hyperlipogenic response in adipose tissue was not observed (20). This lack of adaptive response may be related to the decreased adipose tissue sensitivity to hormones observed in other obese animals (21, 22) and man

(23).

Another phenomena observed in genetic obese animals is the increased level of liver gluconeogenic enzymes (13, 16, 19). Seidman, Harland and Teeber (24) have shown that liver FDPase and glucose-6-phosphatase are elevated in obese hyperglycemic mice. The differences in liver FDPase and amino acid transaminases found in obese pigs appear to reflect a similar pattern of adaptation. These enzyme patterns may be caused by elevated levels of glucocorticoids found in obese animals (1) and obese patients (25). Furthermore, the shift in utilization of amino acids from protein synthesis to glucose synthesis and fat synthesis would be expected in the obese pig with inferior muscle development and extensive lipid deposition. Whether this shift in amino acid utilization is really the cause of, or the result of the obese syndrome, has yet to be determined.

Summary. Metabolic abnormalities associated with obesity were studied with two strains of pigs possessing varying propensities for lipid and protein deposition. The lean strain has a subcutaneous fat thickness of 2.8 cm and the obese strain, 8.0 cm. Adipose tissue enzymes associated with lipogenesis were elevated severalfold in the obese pig. The same enzymes in the liver were not altered. Gluconeogenic enzymes were elevated in the obese pig indicating a shift in the metabolism of amino acids. Enzymatic response to fasting and refeeding appears to be more dynamic in the lean type pig.

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