PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 151, 231-235 (1976)

A Comparison of the Enzyme Levels and the *In Vitro* Utilization of Various Substrates for Lipogenesis in Pair-Fed Lean and Obese Pigs[1, 2] (39180)

ROY J. MARTIN AND JOE H. HERBEIN

Department of Animal Science, The Pennsylvania State University

Metabolic changes associated with the development of obesity have been under intensive investigation. Laboratory animal models have been effectively utilized to characterize the biochemical events which are influenced by genetically induced obesity. While the obese hyperglycemic mouse (1–6) has been one of the most extensively investigated genetic models of obesity, less attention has been given to the yellow obese mouse (7), diabetic mouse (db/db) (8), Zucker obese rat (9–11), and to the more recently described obese pig (12). A complete description of the metabolic changes associated with the onset of the obese condition is needed to maximize the usefulness of these animal models. For example, those biochemical adaptations common to all types of genetically induced obesities might be considered to be secondary events and not primary to the development of excessive lipid deposition.

The objectives of this report on pair-fed lean and obese pigs are (1) to determine the *in vitro* rates of liver and adipose tissue lipogenesis from various substrates, (2) to compare the *in vitro* utilization of alanine for gluconeogenesis and lipogenesis in liver tissue, and (3) to characterize enzyme profiles in liver and adipose tissue.

Materials and methods. Experimental animals consisted of pigs from a domestic strain (Yorkshire) referred to as lean and a feral strain (Ossabaw) referred to as obese. A description of these animals has been previously reported (12). From weaning (7 weeks of age) to sacrifice (6 months of age), the lean pigs were pair-fed to the obese pigs a corn–soybean meal 14% protein diet. This was done to prevent any differences in food intake from influencing enzyme levels and metabolic rates since the faster-growing lean pig will consume more during this period. At the time of sacrifice liver and subcutaneous adipose tissue was quickly removed and used for *in vitro* tissue slice incubations and homogenized for preparation of enzyme extracts. Both liver and adipose (middle layer of subcutaneous backfat) tissues were sliced with a Sadie–Riggs microtome. Tissue slices (100 mg) were incubated for 2 hr at 37° in 25 ml siliconized flasks placed in a Dubnoff metabolic shaker (90 oscillations per min). Incubation media for adipose slices contained Krebs–Ringer bicarbonate buffer (Ca^{2+} free), 1% albumin, 5 m*M* glucose, 2 m*M* acetate, 5 m*M* glycerol, 2 m*M* leucine, and 1 mU/ml insulin. The labeled substrates were added in separate flasks (in duplicate) in the following amounts per flasks: [U-^{14}C]glucose, 2 μCi; [1-^{14}C]acetate, 1 μCi; [2-^{14}C]glycerol, 2 μCi; [U-^{14}C]leucine, 2 μCi. The reactions were stopped by injection of 0.5 ml 1 *N* H_2SO_4 through the rubber stopper and 0.2 ml of hyamine hydroxide was added to the hanging center wells for trapping carbon dioxide. After 1 hr, center wells were removed and placed in scintillation vials containing scintillation fluid (5 g 2,5-diphenyloxazole and 0.1 g 1,4-bis 2-(5-phenyloxazolyl)benzene per liter of toluene). Adipose slices were extracted for lipid according to the procedure described by de Cingolani (13). Liver slices were handled in a similar manner, except that the labeled substrates used were [U-^{14}C]glucose and [2-^{14}C]pyruvate. In addition, liver slice incorporation of [1-^{14}C]alanine into lipid and glucose was measured in a medium consisting of Krebs–Ringer bicarbonate, 1% albumin, 2 m*M* alanine, and 5 m*M* acetate. Radioactivity of glucose was determined by the procedure of Vernon *et al.* (14).

Liver and adipose tissues were homoge-

[1] Authorized for publication January 22, 1975 as Paper No. 4801 in the journal series of the Pennsylvania Agriculture Experiment Station.

[2] Supported in part by NIH Grant HD 0709-03.

nized in 0.25 *M* sucrose media containing 1 m*M* dithiothreitol and 5 m*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. Liver homogenates used for glucose-6-phosphatase (G6Pase) were prepared in 0.1 *M* citrate buffer (pH 6.5). Enzyme assay procedures used have been previously cited (4, 15, 16). Protein concentration in liver extracts was determined by the Folin phenol method (17). Adipose tissue cellularity was determined by the method of Hirsch and Gallian (18) and used for expression of enzyme data. Statistical calculations were performed using an analysis of variance for each parameter in either the obese or lean group. All results are expressed as mean ± SEM.

Results. A comparison of *in vitro* liver slice utilization of pyruvate and glucose for oxidation and lipogenesis showed no significant differences between lean and obese pigs (Table I). The rate of pyruvate utilization was higher than that of glucose in pig liver tissues. The rate of lipogenesis in pig liver tissue is considerably lower than that of pig adipose tissue.

Adipose tissue utilization of various substrates for lipid synthesis is shown in Table II. Glucose, acetate, and glycerol are more rapidly incorporated into triglyceride and fatty acid in adipose cells from obese pigs. Leucine incorporation into lipid fractions by lean and obese pigs did not differ significantly. Of the substrates utilized, glucose supplied 86 and 94% of the glyceride-glycerol synthesized in lean and obese pigs, respectively. Glycerol was not a major contributor to glyceride-glycerol synthesis (only 3.5 to 5.5%) in spite of the presence of adipose cell glycerokinase (see Table V). Leucine and glycerol were not as important a source of carbon for fatty acid synthesis as either acetate or glucose, contributing only 3.7 and 2.8%, respectively, to fatty acid carbon in adipose tissue from obese pigs.

In order to test for a shift in the metabolism of amino acids, alanine incorporation into glucose and lipids was measured in liver slices *in vitro* (Table III). A significant increase ($P < 0.05$) in alanine incorporation into glucose was observed in obese pigs. As in Table I, there was no significant difference in liver lipogenesis.

Liver tissue enzymes of pair-fed lean and obese pigs are shown in Table IV. Of the enzymes normally associated with lipogenesis, only malic enzyme and citrate cleavage enzyme were elevated in the obese pig. Pentose phosphate pathway enzymes (G6PD and 6PGD) and fatty acid synthetase were not significantly different in the lean and obese pig. Pyruvate kinase levels were significantly higher in the lean pig. Of the enzymes associated with gluconeogenesis and amino acid catabolism, G6Pase and SDH were significantly higher ($P < 0.05$) in the obese pig. While other enzymes in this category were slightly elevated in the obese pig, there were no significant differences detected in this study. Lowry protein values were highest in the livers of lean pigs while liver weight was similar in both groups.

TABLE I. CONVERSION OF GLUCOSE AND PYRUVATE INTO CARBON DIOXIDE AND LIPID BY LIVER TISSUE FROM LEAN AND OBESE PAIR-FED PIGS.

Substrate	Fraction	Experimental groups		*P* values[+]
		Lean	Obese	
Glucose	Carbon dioxide	259 ± 32*	389 ± 102	NS
	Triglyceride	67 ± 3	79 ± 7	NS
	Fatty acid	18 ± 1	20 ± 1	NS
Pyruvate	Carbon dioxide	8274 ± 1651	7088 ± 1310	NS
	Triglyceride	469 ± 86	422 ± 66	NS
	Fatty acid	146 ± 39	154 ± 51	NS

* Mean ± SEM for six animals. Activity is expressed as nanomoles of substrate incorporated per hour per gram tissue.

[+] Probability of nonsignificance; NS = not significant.

TABLE II. CONVERSION OF GLUCOSE, ACETATE, GLYCEROL, AND LEUCINE INTO LIPID BY ADIPOSE TISSUE FROM LEAN AND OBESE PAIR-FED PIGS.

Substrate	Fraction	Lean	Obese	*P* values[+]
Glucose	Triglyceride	12.66 ± 1.98*	32.22 ± 2.16	0.01
	Fatty acid	10.32 ± 1.74	27.36 ± 1.56	0.01
	Glyceride-glycerol	2.34 ± 0.30	4.80 ± 0.60	0.02
Acetate	Triglyceride	4.56 ± 0.60	14.80 ± 2.08	0.01
	Fatty acid	4.34 ± 0.29	14.70 ± 1.96	0.01
	Glyceride-glycerol	0.24 ± 0.12	0.10 ± 0.06	NS
Glycerol	Triglyceride	0.66 ± 0.06	1.44 ± 0.15	0.01
	Fatty acid	0.51 ± 0.06	1.26 ± 0.12	0.01
	Glyceride-glycerol	0.15 ± 0.03	0.18 ± 0.03	NS
Leucine	Triglyceride	1.32 ± 0.18	1.62 ± 0.24	NS
	Fatty acid	1.31 ± 0.24	1.68 ± 0.25	NS
	Glyceride-glycerol	—	—	
Total	Triglyceride	19.20 ± 2.41	50.08 ± 3.76	0.01
	Fatty acid	16.48 ± 2.20	45.00 ± 3.49	0.01
	Glyceride-glycerol	2.72 ± 1.01	5.08 ± 1.28	NS

* Results are expressed as microgramcarbon atoms of substrate converted per hour/10^5 adipose cells.
[+] Probability of nonsignificance; NS = not significant.

Levels of adipose tissue enzymes in pair-fed lean and obese pigs are shown in Table V. Levels of glucose-6-phosphate dehydrogenase, 6 phosphogluconate dehydrogenase, malic enzyme, citrate cleavage enzyme, malate dehydrogenase, isocitrate dehydrogenase, and glycerophoshate dehydrogenase were higher in adipose tissue of the obese pig. Adipose tissue pyruvate kinase, glycerokinase, and fatty acid synthetase were similar in both groups of pigs.

Discussion. This study supports an earlier observation that the elevated rate of lipid deposition in obese pigs is achieved by increases in the rate of adipose cell metabolism (12). While glucose and acetate are effectively utilized for adipose cell fatty acid synthesis, glycerol and leucine were of minor importance. It is significant to point out that glucose supplied approximately 90% and glycerol supplied 5% of the carbon for glyceride-glycerol synthesis. The suggested role of adipose tissue glycerokinase as a major factor in decreasing fatty acid release in obese animals (2) is not supported in this animal model. Others have reported an increase in adipose tissue enzymes (19) and *in vitro* lipogenesis (20) in pig with high genetic potential for fat deposition.

In this study, both groups of pigs were receiving a diet which exceeded their nitrogen requirement for growth. The obese pig deposited less nitrogen due to inferior muscle development (21). This means that a greater portion of the dietary amino acids must have been catabolized by the liver in obese pigs. The excess nitrogen was probably being excreted as urea. Amino acid catabolizing enzymes were generally elevated in liver tissue from obese pigs suggesting greater liver tissue metabolism of amino acids. Alanine incorporation into glucose was also elevated, suggesting that a portion of the excess amino acids could have been converted to glucose, and then the glucose could have been utilized for fatty acid synthesis by adipose tissue. While adipose tissue has the capacity to utilize amino acids directly for fatty acid synthesis, no evidence for an increase in this capacity was found in obese pigs. An elevation of gluconeogenic capacity has been reported for other animal models of obesity which have

TABLE III. CONVERSION OF ALANINE INTO GLUCOSE AND LIPID BY LIVER TISSUE FROM LEAN AND OBESE PAIR-FED PIGS.

Fraction	Experimental groups		*P* values[+]
	Lean	Obese	
Glucose	79 ± 3*	93 ± 4	0.05
Lipid	2.4 ± 0.5	2.9 ± 0.3	NS

* Mean ± SEM for six animals. Activity expressed as nanomoles of alanine incorporated into glucose per hour per 100 mg tissue.
[+] Probability of nonsignificance; NS = not significant.

TABLE IV. LIVER TISSUE ENZYME LEVELS IN LEAN AND OBESE PAIR-FED PIGS.

Enzymes	Experimental groups		P values+
	Lean	Obese	
Glucose-6-PO_4 dehydrogenase	0.473 ± 0.077*	0.500 ± 0.062	NS
6 P-gluconate dehydrogenase	1.860 ± 0.20	1.880 ± 0.04	NS
Malic enzyme	0.356 ± 0.44	0.580 ± 0.066	0.05
Citrate cleavage enzyme	0.070 ± 0.024	0.118 ± 0.018	0.05
Fatty acid synthetase	0.016 ± 0.003	0.021 ± 0.094	NS
Pyruvate kinase	2.36 ± 0.23	1.88 ± 0.12	0.05
Glucose-6-phosphatase	13.19 ± 0.75	15.68 ± 0.58	0.05
Fructose 1,6-diphosphatase	3.25 ± 0.12	3.61 ± 0.23	NS
Alanine aminotransferase	1.44 ± 0.22	1.74 ± 0.22	NS
Aspartate aminotransferase	23.96 ± 6.09	29.76 ± 3.90	NS
Serine dehydratase	0.006 ± 0.002	0.014 ± 0.005	0.05
Glycerokinase	0.054 ± 0.021	0.074 ± 0.017	NS
Lowry protein (mg/g)	250 ± 18	22 ± 9	0.05
g liver/kg body wt	12 ± 1	13 ± 1	NS

* Mean ± SEM for six animals. Activity is expressed as μmoles/min/g wet tissue.
+ Probability of nonsignificance; NS = not significant.

TABLE V. ADIPOSE TISSUE ENZYME LEVELS IN LEAN AND OBESE PAIR-FED PIGS.

Enzymes	Experimental groups		P values+
	Lean	Obese	
Glucose-6-PO_4 dehydrogenase	106 ± 11*	342 ± 42	0.01
6 P-gluconate dehydrogenase	58 ± 6	138 ± 12	0.01
Malic enzyme	101 ± 11	401 ± 30	0.01
Citrate cleavage enzyme	12 ± 1	34 ± 4	0.01
Fatty acid synthetase	22 ± 5	24 ± 9	NS
Pyruvate kinase	15 ± 3	17 ± 4	NS
Glycerokinase	2.5 ± 0.4	1.9 ± 0.4	NS
Malate dehydrogenase	858 ± 329	2285 ± 308	0.01
Isocitrate dehydrogenase	100 ± 36	339 ± 157	0.05
Glycerol PO_4 dehydrogenase	64 ± 3	134 ± 10	0.01

* Mean ± SEM for six animals. Activity is expressed as nanomoles/min/10^5 adipose cells.
+ Probability of nonsignificance; NS = not significant.

inferior muscle growth (4, 8, 11). The amino acid requirement for leaner, more muscular pigs is higher than for the fatter, less muscular pig (22). It would appear that if the dietary intake of amino acids were matched to the actual requirement of both lean and obese animals, a shift in liver amino acid metabolism may not be observed. An alternate explanation of elevated rates of liver catabolism of amino acids could be the overproduction of glucocorticoids in obese pigs. More data are required to distinguish between these two possibilities.

Adipose cell lipogenic capacity was elevated several fold in the obese pig whereas liver tissue enzyme activities and *in vitro* lipogenesis were similar in both groups. We found a similar pattern in obese hyperglycemic mice when pair-fed to their lean littermates (4). Insulin may be responsible for elevated rates of adipose lipogenesis. However, the elevated levels of serum insulin found in the obese hyperglycemic mouse (23) are not observed in the obese pig at this age (24). At present we are testing for differences in serum factors which may potentially lead to greater anabolic activity in adipose tissue in the obese pig.

Summary. In this study of spontaneous obesity of pigs, specific metabolic shifts were observed, which explain an increase in fat deposition. Liver tissue utilization of pyruvate and glucose for oxidation and lipogenesis showed no significant difference between lean and obese pigs. Adipose tissue utilization of glucose, acetate and glycerol

for triglyceride and fatty acid synthesis was greater in obese pigs than lean pigs ($P < 0.01$). No significant difference in leucine incorporation into lipid fractions was found. Of the substrates utilized, glucose supplied 86 and 94% of the glyceride-glycerol synthesized in lean and obese pigs, respectively. Glycerol was not a major contributor to glyceride-glycerol synthesis (3.5 to 5.5%), in spite of the presence of adipose tissue glycerokinase. An increase ($P < 0.05$) in alanine incorporation into glucose was observed in liver tissue from obese pigs. In general, the levels of enzyme activities associated with gluconeogenesis, glycolysis, and lipogenesis supported the findings of *in vitro* utilization of these substrates.

The authors wish to express their gratitude to V. Hazlett, J. Watkins, P. Lamprey, and P. Hartman for their assistance in conduct of this study and in the preparation of this manuscript.

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Received September 24, 1975. P.S.E.B.M. 1976, Vol. 151.