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Early Development of Adipose Cell Lipogenesis and Glycerol Utilization in Zucker Obese Rats[1, 2] (38738)

R. J. MARTIN AND P. M. LAMPREY

Animal Science Department, The Pennsylvania State University, University Park, Pennsylvania 16802

Metabolic adaptations to genetically induced obesities have been previously described in the mouse (1) and pig (2). This is a report on the metabolic changes that occur in adipose tissue during early development of the Zucker obese rat (3). Changes in adipose cell numbers (4), adipose tissue lipolysis (5, 6), and adipose lipogenesis (7) have been previously reported in the obese rat. However, very little data are available on the pattern of development of lipogenic activity and enzyme profiles in the genetically obese rat. The objective of this study was to determine if there was a qualitative change in adipose cell metabolism during early development which would explain the excessive accumulation of fat in the Zucker obese rat.

Methods. The experimental animals were male obese (fa/fa) Zucker rats and their lean (Fa/?) controls. Adipose sampling was done at 5, 7, 10, and 14 wk of age with an equal number (6) of lean and obese done at each age period.

At 5 wk of age, the obese rats were started on a pairfeeding schedule with their lean littermate controls. Purina Lab Rat Chow was fed during a short feeding period (8:00 AM–11:00 AM) daily. As discussed previously (8), this procedure was used to eliminate the influence of intake level and feeding pattern on tissue metabolism. The following procedure was used to obtain adipose samples. Sodium pentobarbital (10 mg/180 g body wt) was injected intraperitoneally. Recovery time varied but averaged about three hours from injection to return to feeding. In the preparation the animals were shaved, swabbed with alcohol, and stretched out on a sterile drop cloth over a styrofoam board. A ventral incision (1–1.5 cm) was made in the skin just to the right or left of midline in the area midway between pelvis and rib cage as this location facilitates exposure of the distal end of the epididymal fat pad, which is the area from which the sample was made. A second incision (approx. 5 mm) was made in the body wall immediately below the outer incision. The fat pad was grasped with Graefe forceps, partially exposed, a small portion (100–200 mg) removed, and the remainder allowed to slip back into the body cavity. A single gut suture was made in the muscle to close the body wall incision. The outer incision was repaired with 6–0 silk suturing material. After sampling, the animals were placed in clean cages, away from drafts until full recovery, at which time they were returned to their original cages. Sutures were removed a week later. No animals were sampled more than twice.

Adipose tissue was homogenized in 0.25 *M* sucrose media containing 1 m*M* dithiothreitol, 1 mM EDTA, and 5 mM Tris-HCl (pH 7.4). The 10% homogenates were centrifuged at 27,000 *g* for 20 min at 0° and the resulting supernatants were used for enzyme assays. All assays were determined on a Gilford 2400 recording spectrophotometer at 25° under the conditions of substrate saturation of enzyme. The soluble protein content of tissue extracts was determined by the method of Lowry *et al.* (9). Malic enzyme (ME) (EC 1.1.1.40) was determined by the method of Ochoa (10); citrate cleavage enzyme (CCE) (EC 4.1.3.8) was determined by the method of Cottam and Srere (11); and glucose-6-PO_4 dehydrogenase (EC 1.1.1.49) was assayed by the method of Glock and McLean (12). Glycerokinase (EC 2.7.1.30) was determined by a radiochemical assay previously described (13). Plasma glycerol was determined by the method of Wieland (14).

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Adipose cells were isolated by an adaption of the method of Rodbell (15). Small pieces of adipose tissue were incubated in siliconized 25 ml flasks containing 3 ml of Krebs–Ringer bicarbonate buffer medium (40 mg albumin, 6 mg collagenase and 3 mg glucose) at 37° for about 45 min. After dispersion, cells were washed four times with Krebs–Ringer bicarbonate buffer (2% albumin). Adipose cell suspensions were added to 1 ml of the appropriate media (Krebs–Ringer bicarbonate and 2% albumin with 5 m*M* glucose-U-^{14}C or 10 m*M* glycerol-2-^{14}C) and incubated in a Dubnoff shaker at 90 oscillations/minute at 37° for 2 hr under an atmosphere of O_2:CO_2 (95:5). At the end of the incubation, CO_2 was released by the addition of 0.5 ml 1 *N* H_2SO_4. The flasks were incubated for an additional hour to collect the CO_2 in plastic wells that contained 0.2 ml hydroxide of Hyamine. The plastic wells were added directly to the scintillation vials for counting in 10 ml of scintillation fluid (4 g PPO and 100 mg POPOP per liter of toluene). Lipids were extracted from the incubation media and cells by a modification (16) of the method of Dole and Meinertz (17).

Results. The pattern of development of ME in adipose tissue of obese rats is shown in Fig. 1. The highest activity was observed at 5 and 7 wk for the obese and lean rats, respectively. Thereafter, activity declined to its lowest level at 14 wk. The obese had

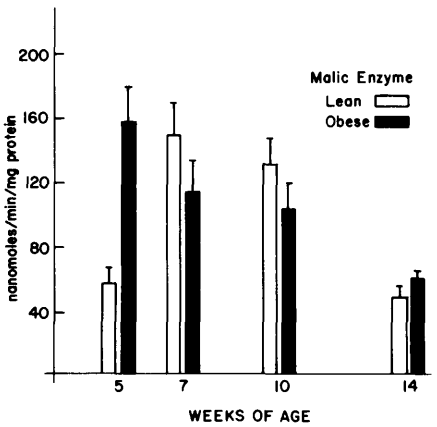

FIG. 1. Malic enzyme levels of adipose tissue from lean and obese Zucker rats at different ages. Bars indicate the mean ± SEM for six animals.

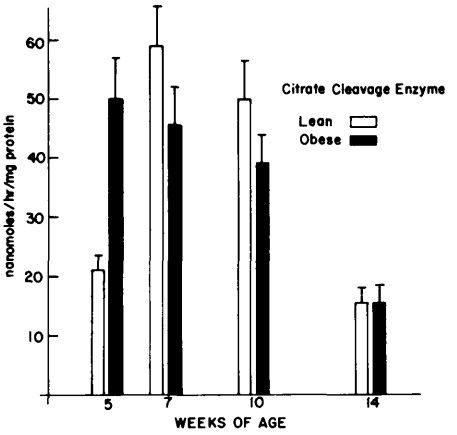

FIG. 2. Citrate cleavage enzyme levels of adipose tissue from lean and obese Zucker rats at different ages. Bars indicate the mean ± SEM for six animals.

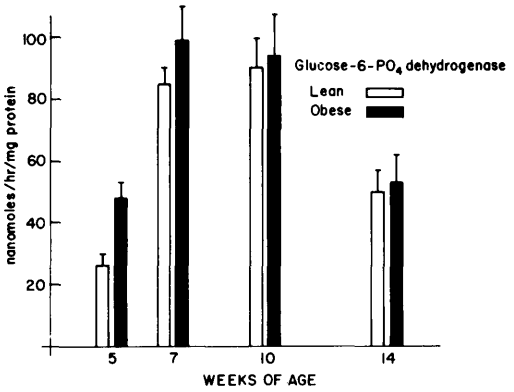

FIG. 3. Glucose-6-PO_4 dehydrogenase enzyme levels of adipose tissue from lean and obese Zucker rats at different ages. Bars indicate the mean ± SEM for six animals.

higher levels of ME at 5 wk of age. The same trend was observed for CCE (Fig. 2). For adipose G6PD the highest levels were observed at 7 and 10 wk for both the lean and obese rats (Fig. 3). Only at 5 wk were adipose tissue G6PD levels higher in the obese than in the lean rats.

Development of adipose tissue metabolism is shown in Figs. 4 and 5. Glucose oxidation was highest at 5 wk of age and declined very rapidly to the lowest levels by 14 wk in the obese rat. In the lean rat the peak of glucose oxidation was reached at an older age. Glucose conversion to fatty acid showed a similar pattern. At 5 wk of age, lipogenesis was four times higher in the obese

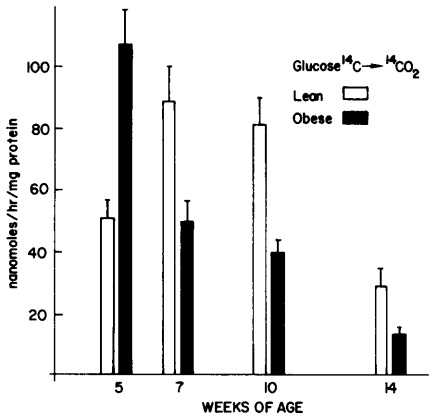

FIG. 4. *In vitro* conversion of U-^{14}C-glucose into carbon dioxide by isolated adipose cells from lean and obese Zucker rats. Bars indicate the mean ± SEM for six animals.

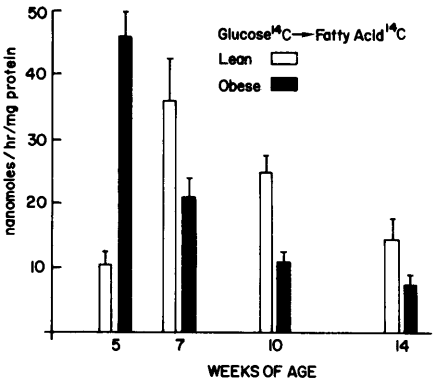

FIG. 5. *In vitro* conversion of U-^{14}C-glucose into fatty acids by isolated adipose cells from lean and obese Zucker rats. Bars indicate the mean ± SEM for six animals.

rat adipose tissue than in the lean rat. At all other ages the lean rat had a greater rate of lipogenesis from glucose.

Glycerol utilization by adipose cells from lean and obese rats at various ages is presented in Table I. There appeared to be very little change in absolute level of glycerol utilization with age. The peak of glycerol conversion to CO_2 and triglyceride appeared at 10 wk of age. However, the shift in the utilization of this substrate was not nearly as dramatic as that observed for glucose utilization or for enzyme levels. The obese rat had significantly ($P < 0.05$) higher levels of glycerol utilization at five weeks. Thereafter, the difference between the lean and obese was not statistically significant. Glycerol conversion to fatty acids was very low at all ages investigated. It would have been interesting to determine the rate of glycerol utilization during maximum lipogenesis from some other substrate such as glucose or acetate. When glycerokinase activity was determined at 14 wk of age, there was a three-fold higher level of this enzyme in adipose tissue of genetically obese rats (Table II). Plasma levels of glycerol were also shown to be elevated in the obese rat at this age.

Discussion. In this study elevated levels of lipogenic and oxidative capacities of adipose cells from obese rats were observed only at 5 wk of age. Thereafter, the capacity of lipogenesis appeared to be either the same as or slightly lower than the pair-fed lean littermates. The same trend was observed

TABLE I. GLYCEROL-^{14}C INCORPORATION INTO $^{14}CO_2$, TRIGLYCERIDE-^{14}C AND FATTY ACID-^{14}C OF ISOLATED FAT CELLS FROM LEAN AND OBESE RATS.

Fraction[a]	Animal	Age (wks) 5	7	10	14
Carbon dioxide	Lean	1.51 ± 0.11	1.50 ± 0.10	2.15 ± 0.33	1.51 ± 0.23
	Obese	2.16 ± 0.13[b]	1.82 ± 0.19	2.05 ± 0.24	1.96 ± 0.11
Triglyceride	Lean	5.52 ± 0.45	4.60 ± 0.51	7.01 ± 0.66	6.00 ± 0.96
	Obese	7.06 ± 0.56[b]	5.38 ± 0.72	8.39 ± 0.84	4.74 ± 0.84
Fatty acid	Lean	0.26 ± 0.03	0.33 ± 0.04	0.16 ± 0.03	0.14 ± 0.02
	Obese	0.24 ± 0.41	0.54 ± 0.04	0.17 ± 0.01	0.16 ± 0.04

[a] Values represent the mean ± SEM. Activity is reported as nanomoles of glycerol incorporated into product/hr/mg protein, six animals per mean.

[b] Significantly different from lean animals ($P < 0.05$).

TABLE II. A COMPARISON OF ADIPOSE TISSUE GLYCEROKINASE ACTIVITY AND PLASMA GLYCEROL IN LEAN AND OBESE RATS AT 14 WEEKS.

Variable compared	Lean (7)	Obese (4)	P^b
Glycerokinase (nanomoles/hr/mg protein)	34 ± 9[a]	107 ± 43	0.10
Plasma glycerol (nanomoles/ml)	140 ± 20	290 ± 60	0.01

[a] Values represent the mean ± SEM of seven lean and four obese rats.

[b] Indicates the probability that means in the same row are not significantly different.

for the enzymes normally associated with enhanced lipogenic capacity (ME, CCE, and G6PD). If enzyme or lipogenic activities were expressed as total activity per rat, the obese rat would have had greater lipogenic activity at all ages. This would have been primarily due to the greater numbers of adipose cells observed in the obese rat (4). In this study we were primarily interested in determining if there was a qualitative change in the adipose cells from obese rats which would explain the large accumulation of fat in the adipose depots. The only indication of an increased specific lipogenic capacity was observed at 5 wk of age.

It appeared that the normal elevation in adipose lipogenesis following weaning (18) occurred at an earlier age in the genetically obese rat. The explanation given for this type of developmental response in the rat is that the shift from a high fat diet (rat milk) to a relatively high carbohydrate diet (dry diet) produces a shift in glucose metabolism toward greater *de novo* fatty acid synthesis. Therefore, it might be expected that the obese rat pups were consuming larger quantities of dry diet before the lean pups. At 5 wk of age the obese rat pups are already showing physical signs of excessive fat accumulation. At this age the maximum level of lipogenesis, CCE, and ME were already observed in the obese rat.

Our studies were not designed to regulate food consumption prior to the initial sampling period. There are two questions that should be resolved before the observed increase in specific lipogenic capacity at five weeks of age can be adequately interpreted. The first is what is the timing of initiation of dry diet consumption in the Zucker obese rat. The second is what is the quantitative intake pattern (expressed per metabolic body size) in the Zucker rat 3–4 wk postweaning.

Mayer (19) proposed that adipose tissue glycerokinase was the key enzyme responsible for the metabolic abnormalities associated with the obese hyperglycemic mouse. Since then a number of studies have shown that glycerokinase levels in adipose tissue may be a secondary consequence of elevated levels of plasma insulin (20, 21). At 14 wk of age, we observed a slightly higher level of this enzyme in adipose tissue of pair-fed obese rats (Table II). In the Zucker rat the maximum levels of plasma insulin are observed at this age (20). Therefore, it is likely that the elevated levels of plasma insulin are responsible for the increased glycerokinase levels observed in the Zucker rat. An alternate explanation for elevated levels of glycerokinase are the higher levels of plasma glycerol. The significance of glycerol utilization by adipose tissue as a causal agent in obesity is questionable since the rate of utilization was very similar in lean and obese rats (Table I). As indicated in an earlier study of obese hyperglycemic mice (22), it is likely that increased glycerokinase levels observed in obese animals represents a secondary shift in metabolism due to the onset of obesity and does not appear to cause the obesity.

The similarity in adipose cell lipogenic capacity between lean and obese pair-fed rats is in good agreement with previous studies (7, 8). In addition to the increased number of adipose cells (4), the elevated levels of liver lipogenesis (8), of liver production of very low density lipoproteins (23) and of liver output of triglycerides (23) may be considered prominant factors in the development of obesity in the Zucker obese rat. From the data presented, there is no indication of a qualitative change in adipose cell lipogenesis in the pair-fed obese rat after five weeks of age that would explain excessive fat deposition.

Summary. A study of adipose cell metabolism was made at ages 5, 7, 10, and 14 wk of age in genetically obese Zucker rats. Adipose samples were surgically removed and used for *in vitro* adipose cell incubations and for characterization of enzyme patterns. Lipogenic capacity from glucose and enzymes normally associated with lipogenesis (malic enzyme, citrate cleavage enzyme and glucose-6-PO_4 dehydrogenase) followed the same pattern of development. At 5 wk of age, the adipose cells of obese animals had a greater capacity for fat synthesis than the lean rats. At all other ages lipogenic activity and enzyme levels were either similar or less than the pair-fed lean littermates. Glycerol utilization by isolated fat cells was similar; however, adipose tissue glycerokinase was elevated in obese rats at 14 wk of age. It was concluded that there was no apparent change in specific lipogenic capacity of fat cells from the obese rat when compared to its lean littermate. It was also concluded that increased adipose glycerokinase activity in obese rats represented a secondary shift in metabolism.

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