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Adipose Tissue Glycerokinase Activity in Genetic and Acquired Obesity in Rats and Mice[1] (38667)

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(Introduced by F. J. Stare)

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The glycerophosphate which is required for triglyceride synthesis is normally formed through the glycolytic pathway. Yet, in insulin resistant conditions, such as those associated with obesity, phosphorylation of the glycerol liberated during lipolysis in adipose tissue may play an important role in the increased deposition of fat. In the obese hyperglycemic mouse (Ob/Ob), the activity of the enzyme, glycerokinase, has been demonstrated to be elevated in adipose tissue (1, 2). With the methods available originally, glycerokinase activity was found to be insignificant in adipose tissue of other strains and species tested, but the sensitive assay technique recently developed by Robinson and Newsholme (3) has revealed its presence in adipose tissue of the normal rat as well. The level of glycerokinase activity in adipose tissue from the Ob/Ob mouse is directly related to the serum insulin concentration (4) and to the degree of insulin resistance seen in these animals. In order to test whether adipose tissue glycerokinase is elevated in other forms of genetic and acquired obesity, we have assayed for this enzyme in homogenates of fat cells isolated from various strains of obese rats and mice.

Materials and Methods. The genetically obese animals studied were the Zucker fatty rat (Harriet G. Bird Memorial Laboratories, Stow, MA) and the obese C57BL/6J Ob/Ob and the diabetic C57BL/KsJ Db/Db mice (Jackson Laboratory, Bar Harbor, ME). Acquired obesity was produced in the CD rat (Charles River Breeding Laboratories, Wilmington, MA) by inducing hyperphagia with electrical lesions in the ventromedial nucleus of the hypothalamus (5) at approximately 9 wk of age. These rats are designated VMN obese. Obesity also was produced in the C57BL/6J mouse (Jackson Laboratory) and the CD-1 mouse (Charles River Breeding Laboratories) by intraperitoneal injection of 0.5 mg/g body wt gold thioglucose (GTG) at 7 wk of age. Lean controls for each group were either lean littermates or untreated animals of the same strain. The age at autopsy, the sex and number of animals in each group are shown in Table I. All animals were housed singly in a light and temperature controlled room and fed laboratory chow (Ralston Purina Company, St. Louis, MO) and water *ad libitum*.

On the day of autopsy, the animals were anaesthetized with 50% CO_2–50% O_2 and then killed by decapitation. Blood was collected and serum was separated for insulin and glucose determinations. The epididymal fat tissue and the parametrial fat tissue were removed from the males and females, respectively. The fat cells were isolated from these tissue samples with collagenase using the method of Rodbell (6). The isolated cells from 1 g fat tissue were washed three times with 10 ml Krebs–Ringer bicarbonate buffer, without albumin, as described, resuspended in 2 or 3 ml of buffer, homogenized in a Teflon-pestled tissue grinder and then centrifuged in a cellulose nitrate centrifuge tube for 20 min at 20,000 *g* at 4°. The centrifuge tube was punctured near the bottom to remove the clear middle phase for enzyme assay.

The glycerokinase assay used was derived from the methods of Robinson and Newsholme (3) and Koschinsky *et al.* (4). 100 μl of the clear homogenate were mixed with 50 μl buffer at pH 7.5 (200 mM Tris·Cl, 50 mM mercaptoethanol, 49 mM NaF,

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TABLE I. THE RELATIONSHIP OF GENETIC AND ACQUIRED OBESITY TO SERUM INSULIN AND GLUCOSE IN RATS AND MICE.

Group	Age (weeks)	Sex	No. of animals	Body weight (g)	Weight gain (g/week)	Serum insulin (μunit/ml)	Serum glucose (mg/100 ml)
VMN obese rats	18	F	2	475	31	305	133
Lean controls	18	F	2	338	13	33.0	143
Zucker obese rats	16	F	2	405	12	156	114
Lean controls	16	F	2	226	5	42.5	120
Zucker obese rats	32	M	2	645	8	236	176
Lean controls	32	M	2	479	3	29.0	126
GTG obese mice CD-1	14–16	M	7	63.8 ± 4.7[a]	4.3	158 ± 98	388 ± 321
Lean controls	14–16	M	7	41.1 ± 2.5	1.4	13.6 ± 2.1	200 ± 75
GTG obese mice C57BL/6J	16–18	M	5	33.3 ± 3.5	1.9	28.0 ± 7.3	197 ± 38
Lean controls	16–18	M	3	25.2 ± 2.2	1.0	32.0 ± 10.5	197 ± 11
Ob/Ob obese mice	13–15	M	6	42.8 ± 4.0	2.6	429 ± 62	449 ± 218
Lean controls	13–15	M	4	26.2 ± 0.8	0.7	12.3 ± 1.6	206 ± 66
Db/Db obese mice	13–15	M	5	40.2 ± 1.9	1.8	143 ± 44	1345 ± 566
Lean controls	13–15	M	5	26.0 ± 2.5	1.0	12.1 ± 2.3	160 ± 34

[a] Values represent mean ± SD.

10 m*M* $MgCl_2$, 2 m*M* EDTA, 12 m*M* ATP, 10 m*M* creatine phosphate and 0.3 mg/ml creatine phosphokinase (CalBiochem, La Jolla, CA) and 1 m*M* glycerol-1,3-^{14}C (12 mCi/mmole). The reaction mix was incubated at 37° for 30 min and stopped by the addition of 125 μl of absolute ethanol. Zero time blanks were made by the addition of 125 μl of the ethanol to the incubation buffer before adding the clear homogenate. Twenty-five microliters of each reaction mix was spotted onto a 25 mm diameter Whatman DE81 filter paper disc (Reeve Angel Co., Clifton, NJ). The free glycerol was washed off the filter paper with 250 ml H_2O using a multiple sampling manifold (Millipore Corporation, Bedford, MA) at a rate of 15–30 ml/min, and the discs were counted for radioactivity. Protein concentration in the clear homogenate was determined with biuret reagent. Glycerokinase activity was calculated from the specific radioactivity of the glycerol and expressed as μmoles glycerophosphate formed per min per mg protein.

Serum glucose was determined by the glucose oxidase method (7) and serum insulin by the radioimmunoassay kit (Pharmacia, Piscataway, NJ) using porcine insulin as a standard. Serial dilutions of the serum samples showed no evidence of inhibition of insulin binding to the Sephadex antibody. The standard curve obtained with mouse insulin standard paralleled that using porcine insulin, although the response was less.

Results. The animals were autopsied for isolation of the fat cells and the glycerokinase assayed during the dynamic phase of weight gain, when glycerokinase activity had been shown to be most elevated in the Ob/Ob mouse (4). Weight gain during the week prior to autopsy ranged from 8 to 31 g in the obese rats and 3–13 g in the lean rats and depended on age, sex and strain of the animal (Table I). A similar relationship was observed in the mouse groups. As would be expected, because of their larger size at all ages, the GTG injected CD-1 mice exhibited the greatest weight gain of 4.3 g. The response to the GTG in the C57BL/6J strain was not as pronounced, but the 1.9 g weight gain did fall within the range of the values for the obese C57BL/6J Ob/Ob and the diabetic C57BL/KsJ Db/Db groups and was higher than its lean control group.

Serum insulin concentrations were significantly increased in most forms of acquired and genetic obesity studied, as shown in samples taken at the time of autopsy (Table I). Serum insulin values for the GTG

injected CD-1 mice were elevated in comparison to their lean controls, but no elevation was observed when C57BL/6J mice were injected with GTG. The extent of the obesity was not as pronounced in this latter group. Therefore, it is possible that hyperinsulinemia would have developed later. Serum glucose concentrations were variable, but the usual hyperglycemia observed in the obese Ob/Ob and diabetic Db/Db mouse groups are apparent from the data presented.

The mean values for glycerokinase activity, expressed as μmoles glycerophosphate formed/min/mg protein, were 0.72 $\pm$ 0.09 (SEM) for the Ob/Ob mouse group in comparison to 0.12 $\pm$ 0.03 for the lean control group (Fig. 1). Surprisingly, a similar relationship was observed in the diabetic Db/Db mouse group, in which glycerokinase activity was 0.61 $\pm$ 0.19 in comparison to 0.18 $\pm$ 0.07 for its lean control group. Elevated fat cell glycerokinase activities were not observed in the other forms of obesity studied when compared to the appropriate control animals. Of interest were the strain and specie differences in glycerokinase activity. Both the lean and the obese CD-1 mouse groups exhibited fat cell glycerokinase activities approaching the high levels found in the obese Ob/Ob and the diabetic Db/Db mouse groups. The C57BL/6J mouse group injected with GTG did not respond with an elevation in its fat cell glycerokinase activity. Glycerokinase activity in fat cells of the Zucker and the CD strains of rats were similar and unaffected by obesity.

Because the weight of the adipose tissue sample removed as well as the total body adipose tissue was much larger in the obese animals in comparison to the lean controls, glycerokinase activity expressed as total enzyme activity of the fat cells of the epididymal or parametrial fat tissue removed would more realistically represent the total capacity of adipose tissue for reutilization of liberated glycerol. Mean values of total glycerokinase activity of the fat cells from the tissue excised are shown in Fig. 2. The Ob/Ob and the Db/Db groups had 17 and five times greater total glycerokinase activity than their lean controls, respectively The GTG injected CD-1 mouse group and the VMN obese and the Zucker obese rat groups all had more total glycerokinase activity than their lean groups when expressed in this manner.

Discussion. Koschinsky *et al.* (4) have shown the direct relationship of glycero-

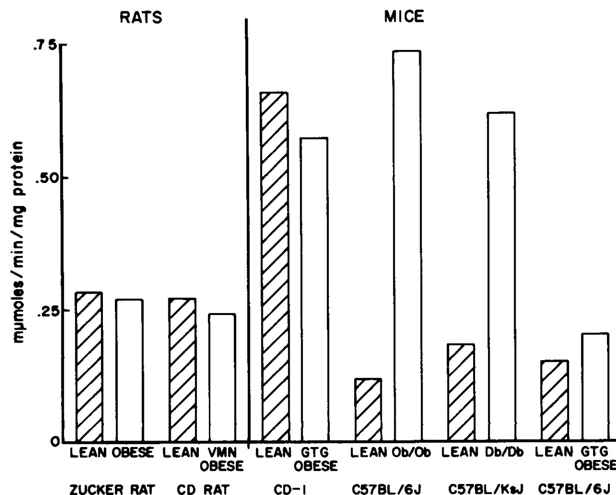

FIG. 1. Glycerokinase activity in isolated fat cells from rats and mice with genetic or acquired obesity and their lean controls. Bars represent mean values for glycerokinase activity expressed as μmoles glycerophosphate formed/min/mg protein for the groups listed in Table I. Genetically obese groups are the Zucker obese rat, the Ob/Ob obese mouse and the Db/Db diabetic mouse. VMN obese rats were lesioned in the ventromedial nucleus of the hypothalamus. GTG obese mice were injected with gold thioglucose.

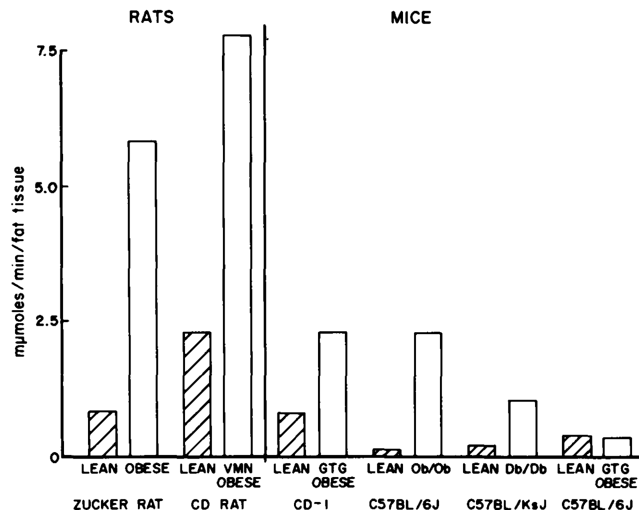

FIG. 2. Total glycerokinase activity in isolated fat cells from adipose tissue in genetic and acquired obesity. Bars represent mean values for total glycerokinase activity expressed as μmoles glycerophosphate formed/min/fat tissue for the groups listed in Table I. Fat tissues were the epididymal in the male mouse groups and the parametrial in the female rat groups.

kinase activity to circulating insulin in the C57BL/6J Ob/Ob mouse. Our results confirm these data for the Ob/Ob mouse. In addition, we have reported a similar elevation of glycerokinase activity in the C57BL/KsJ Db/Db mouse also associated with high circulating insulin levels. The Db and Ob mutations are separate mutations and are both associated with early hyperglycemia, hyperinsulinemia and obesity. Metabolic differences between the Ob and the Db mutations which have been previously observed have been influenced by the background strain genes, the C57BL/6J strain normally carrying the Ob mutation and C57BL/KsJ, the Db mutation (8). Transfer of the Db gene to the C57BL/6J strain and vice versa results in the metabolic differences associated with the background strain and not differences unique to the specific gene. Similarly, our observation of increased glycerokinase activity in both mutations indicates that this response is not unique to the Ob gene as previously concluded, but a response of genes present in both the C57BL/KsJ and the C57BL/6J strains.

We did not observe a similar elevation of glycerokinase activity in gonadal adipose tissue associated with the hyperinsulinemia in other forms of genetic and acquired obesity studied. Therefore, it appears that circulating insulin concentration does not universally control glycerokinase activity. Persico *et al.* (9) suggested that insulin controls glycerokinase only in mammalian subcutaneous and brown adipose tissue, but not in epididymal or perirenal fat. Both Persico *et al.* (9) and Koschinsky *et al.* (4) have suggested that the control by insulin may be due to enzyme induction since they have demonstrated that actinomycin D inhibits the increased glycerokinase activity resulting from exogenous insulin administration. It is not possible to conclude that this is a direct enzyme induction by insulin, but may be secondary to other metabolic changes during hyperinsulinemia.

The total glycerokinase activity in epididymal or parametrial adipose tissue increases relative to the amount of tissue hypertrophy or hyperplasia present in the various forms of obesity studied as well as being dependent upon the species and strain of animal studied. Such an increase in total activity could contribute to the maintenance of the obese condition by providing additional glycerophosphate for esterification of fatty acids. But this is not unique for glycerokinase, since other lipogenic enzymes show similar increases. Theoretically, the

in vitro glycerokinase activity has ample capacity to provide the 1–2.5 nanomoles α-glycerophosphate per mg protein in adipose tissue. Yet, adipose tissue α-glycerophosphate dehydrogenase is approximately 100 times more active in the Ob/Ob mouse (10, 11) suggesting that α-glycerophosphate could be formed by either glycolysis or glyceroneogenesis from gluconeogenic precursors. Considering the available evidence, it is unlikely that glycerokinase is limiting for triglyceride synthesis even in the Db/Db and the Ob/Ob obese mutant mice.

Summary. Glycerokinase activity in isolated fat cells was elevated in both Ob/Ob and Db/Db mice in comparison to their lean controls and this elevation was associated with obesity, hyperinsulinemia and hyperglycemia. In the other forms of acquired and genetic obesity in the rats and mice studied (also associated with hyperinsulinemia), adipose tissue glycerokinase activity was not elevated in comparison to lean control groups when expressed on a mg protein basis. It is concluded that the elevated glycerokinase activity is not due to the specific Db or Ob mutation, but is secondary to the obesity and hyperinsulinemia interacting with the similar genetic background in the C57BL/KsJ and the C57BL/6J mouse strains.

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