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The Diabetic Zucker Fatty Rat (41611)

JULIA B. CLARK, CATHY J. PALMER, AND WALTER N. SHAW

Department of Pharmacology, Indiana University School of Medicine and the Lilly Research Laboratory, Eli Lilly and Company, Indianapolis, Indiana 46285

Abstract. A noninsulin-dependent diabetes appeared in fatty rats in our Zucker rat colony. A breeding program yielded a genetic pattern of diabetes consistent with a dominant gene not closely linked to the fatty gene. Fatty males were more frequently affected than fatty females. Since no markers could be identified for heterozygous carriers and since affected fatty rats were 6 months old when diabetes appeared, the diabetic trait could not be sustained in our small colony. Glucose tolerance tests showed that the diabetic fatty rats had little increase in plasma insulin concentration after a glucose load was administered. Plasma insulin concentrations were unchanged relative to control fatty rats. Percentage body fat and plasma triglyceride values were decreased in fatty diabetic rats relative to control fatty rats, however, consistent with insulin resistance in fat and liver. The content of pancreatic insulin was markedly decreased in the diabetic fatty rat relative to either the *ad libitum* or diet-restricted fatty rats. The occurrence of a genetically based diabetes in a normally outbred colony underscores the importance of genetic traits that interact with obesity in determining diabetes in rodent models.

The obesity of the fatty Zucker rat is due to a simple autosomal recessive gene, *fa* (1). The obese (*fa/fa*) rat has been extensively characterized as hyperinsulinemic but euglycemic (2). A colony of Zucker rats was established in the Diabetes Research and Training Center at Indiana University School of Medicine in 1978 with breeding pairs donated by Dr. Walter Shaw of the Eli Lilly Company. In 1980 blood glucose concentrations were determined for two Zucker fatty male breeders, a sire and a male offspring, which were noticed to be urinating excessively. The sire was 11 months old and had a fed blood glucose value of 544 mg/dl which decreased to a fasting value of 184 mg/dl after a 3-week period of diet restriction. The diabetic male offspring was 7 months old and had a fed blood glucose value of 474 mg/dl which decreased to a fasting concentration of 175 mg/dl after a similar period of diet restriction. The entire Zucker colony was then surveyed for high blood glucose concentrations and yielded three more diabetic fatty males, all second generation of the diabetic sire through his diabetic male offspring. This and the Lilly Zucker rat colony are maintained as an outbred colony with breeding pairs having no common grandparents. A modified breeding program was begun with diabetic fatty males as sires and female offspring of diabetic fatty males as dams. This was not a true inbreeding program as circumstances allowed only one successful brother–sister and one father–daughter mating. The results presented here are on the diabetic animals originally found and those produced over the next 12 months, a total of 16 diabetic animals.

Materials and Methods. *Animals.* The Zucker colony is housed in its own room in the Laboratory Animal Resource Center. Animals are individually housed in plastic cages on SaniChips (hardwood chips by L. L. Murphy) in a controlled-environment room, lighted during 0700–1900 hr and kept at 22 ± 3°C. F6 Wayne Lab-Blox were provided as food unless otherwise noted.

Fatty male Zucker rats can be used as breeders if they are diet-restricted (15 g/day) when they reach a weight of 300 g. We also used the periodic schedule of testosterone injections described by Hemmes *et al.* to improve the fertility of the fatty males (3). Fatty females are relatively infertile and do not serve as breeders (4). Heterozygous females (+/*fa*) are used after identification either by virtue of having a fatty father or secondarily after their production of fatty offspring.

The modified breeding program was carried for three generations at which time it was discontinued because of decreasing litter size and the reduced fertility and frequent infertility of the fatty diabetic male breeders.

Glucose tolerance test. One gram of glucose

0037-9727/83 $1.50

TABLE I. ACTUAL DISTRIBUTION OF FATTY OFFSPRING SIRED BY FATTY-DIABETIC RATS

	Fatty offspring		
	Male	Female	Total
Dam: +/*fa*, no relationship to a fatty-diabetic			
Nondiabetic	3	2	5
Diabetic	*3*	*0*	*3*
Total	6	2	8
Observed frequency for diabetes			37.5
Frequency expected			
Recessive gene			0%
Dominant gene			50%
Dam: +/*fa*, offspring of a fatty-diabetic			
Nondiabetic	1	3	4
Diabetic	*6*	*3*	*9*
Total	7	6	13
Observed frequency for diabetes			69%
Frequency expected			
Diabetic gene linked to *fa* gene			100%
Diabetic gene independent of *fa* gene			50%

is administered by gavage as a 60% solution to animals which have been fasted overnight. Samples of blood are collected from the tail vein into heparinized capillary tubes before administration of the glucose and at 30 and 60 min after glucose administration.

Tissue extraction and biochemical determination. Plasma insulin was determined by a double antibody method (5) in the Immunoassay Core of the Diabetes Research and Training Center or at the Eli Lilly Company (6). For the plasma values listed in Table II, blood was collected after decapitation. Plasma free fatty acid, cholesterol, and triglyceride values were determined by standard techniques (7, 8, 9). At the time of sacrifice, samples of liver, muscle, and pancreas were excised, frozen on Dry Ice, and stored at −20°C until analysis. Liver and muscle glycogen content were quantitated as glucose after extraction with 30% KOH and acid hydrolysis (10). Liver and muscle fatty acid, triglyceride, and cholesterol values were determined after extraction of total lipid (11–15). Frozen pancreas was extracted with acid–ethanol for insulin, which was then quantitated by immunoassay (16). Total carcass fat from all sources was weighed after careful gross dissection.

Results. *Genetics.* Table I presents the anticipated incidence of diabetes among fatty offspring studied. If the incidence of diabetes can be attributed solely to a single gene, then the two types of breeding we studied should show whether or not this is a recessive or dominant gene and whether or not the gene is closely linked to the *fa* gene. Table I lists the two types of breedings studied and their outcomes. The early breedings were of fatty diabetic males to heterozygous females unrelated to diabetic males. The incidence of diabetes was 37.5% among fatty offspring, consistent with a dominant gene (expected frequency 50%) but not a recessive gene (expected frequency 0%). The incidence of diabetes among the offspring of dams who were themselves offspring of diabetic fatties should reveal whether or not the diabetes was linked to the *fa* gene. As seen in Table I, the observed frequency, 69%, was not 100% as would be required for two closely linked genes, whether recessive or dominant. Although the small numbers do not permit a statistically valid analysis, if the diabetes can be attributed to a single gene, then the genetic pattern is most consistent with a dominant gene not closely linked to the *fa* gene. However, diabetes was never found in lean offspring, so the *fa*/*fa*-induced obesity is essential for expression of the diabetes syndrome. Large fasting blood glucose values and abnormal glucose tolerance tests were originally detected only in fatty animals 5–6 months of age. Upon breeding fatty diabetic males to heterozygous daughters of diabetic fatty males, the age of onset of hyperglycemia was decreased and the degree of fasting hyperglycemia increased (data not shown). Two such fatty males were identified at 3 1/2 months of age as diabetic with severe fasting hyperglycemia (220 and 210 mg/dl). We feel that these animals with an early onset of severe hyperglycemia may represent an animal with a double gene dose. Although the diabetic trait appears dominant in the fatty animals, it is not closely linked to the fatty gene. Furthermore, the diabetic trait is not expressed in lean animals, making carriers impossible to identify except after identification of their offspring as diabetic. Since this mode of identification takes so long, we have been unable to establish a breeding colony for diabetic Zuckers.

We attempted to determine if lean Zucker rats could be identified as carriers of a dominant gene for diabetes. Since diabetes was seen only in obese (*fa/fa*) rats, we put lean male Zucker rats who were siblings or offspring of diabetic fatty rats on a cafeteria diet (chow supplemented with peanut butter and chocolate chip cookies) and a 32% sucrose solution replacing drinking water to make lean animals obese (17, 18). One group of rats so treated consisted of 8- to 10-month-old rats and a second group consisted of 6-week-old rats. An initial weight gain of 30% over control was achieved over 6 weeks, but over a longer period net weight was no different than for untreated rats (data not shown). No abnormal glucose tolerance tests were found among any lean rats. Since pregnancy can produce glucose intolerance, the blood glucose values of pregnant siblings or offspring

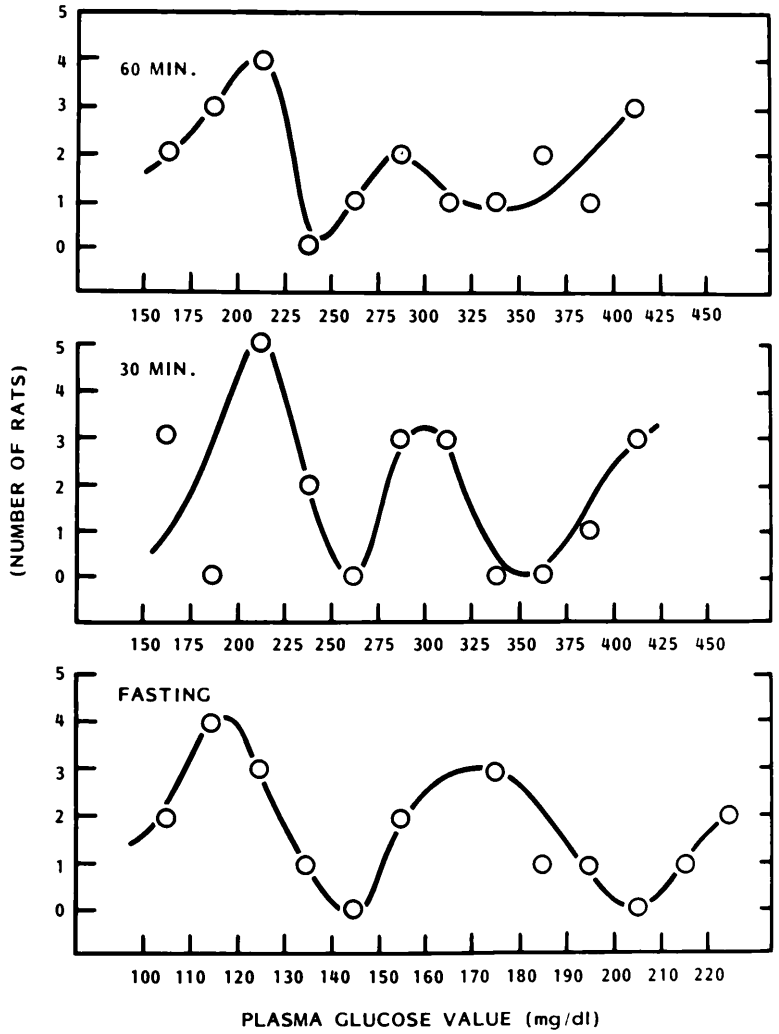

FIG. 1. Graph of glucose tolerance test-derived plasma glucose values which reveals diabetic rat populations. Plasma glucose values were determined before and 30 and 60 min after administration of 1 g glucose to the rats after an overnight fast. The number of rats with plasma glucose values in a given 10 mg/dl range are plotted against the plasma glucose ranges found. Diabetic animals had fasting blood glucose values above 140 mg/dl, severe diabetic animals had fasting blood glucose values above 200 mg/dl.

of diabetic rats were monitored. No abnormal values were found. We were therefore unsuccessful in identifying lean carriers for diabetes.

Characterization of the diabetes. Glucose tolerance tests were administered to 36 fatty and lean Zucker rats ages 5 1/2 to 16 months old. Of the 12 diabetic rats identified, all were fatty rats. Two were female, 10 were male. Since the number of females was small, only the data for males were analyzed statistically, although the data from the females did not appear different. A graph of the glucose values obtained with only the glucose tolerance test of the male rats is shown in Fig. 1. Based on the three populations in this graph, animals were classified as normal, diabetic or severely diabetic. The average values for glucose and insulin resulting from this classification are shown in Figs. 2 and 3. Plasma glucose concentrations rose in all groups 30 min after glucose administration but at 60 min had fallen only in the lean animals (Fig. 2). Fasting glucose values were the same in lean and nondiabetic fatty rats at zero time but the values for fatty rats did not fall at 60 min. Insulin values increased in all groups following administration of glucose (Fig. 3). Fasting concentrations of insulin were higher in the fatty rats, nondiabetic and diabetic, than in the lean rats. As with the plasma glucose values, the major differences among the groups appeared at 30 min. Fatty nondiabetic obese rats had

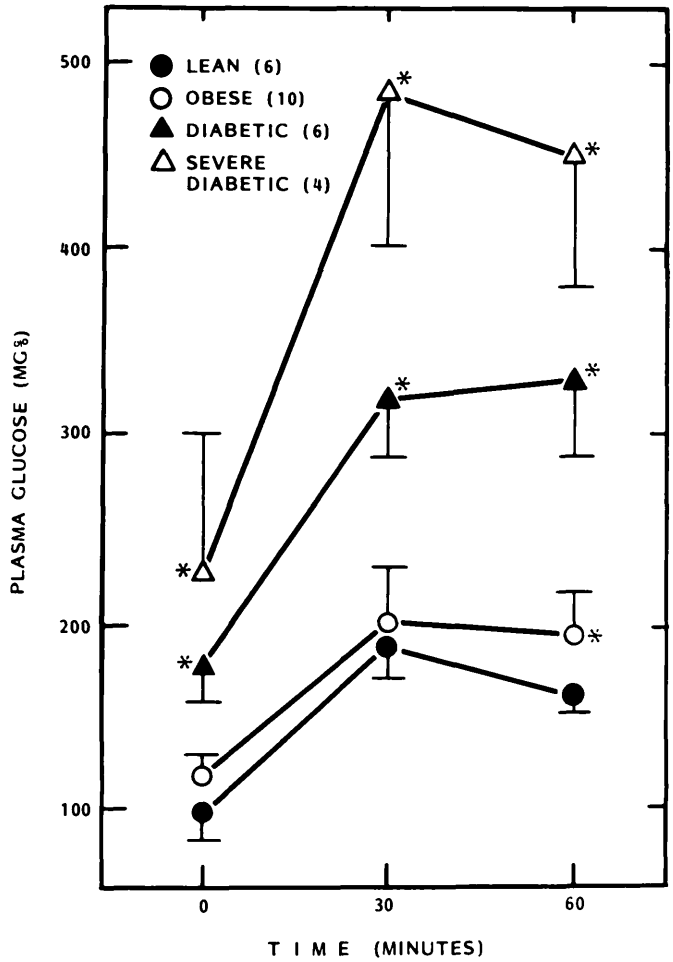

FIG. 2. Glucose tolerance test. Animals were grouped according to body type (lean or obese) or as diabetic (diabetic or severe diabetic) based on the graph in Fig. 1. All diabetic animals were obese. Asterisks (also in Fig. 3) indicate a significant change from lean animals.

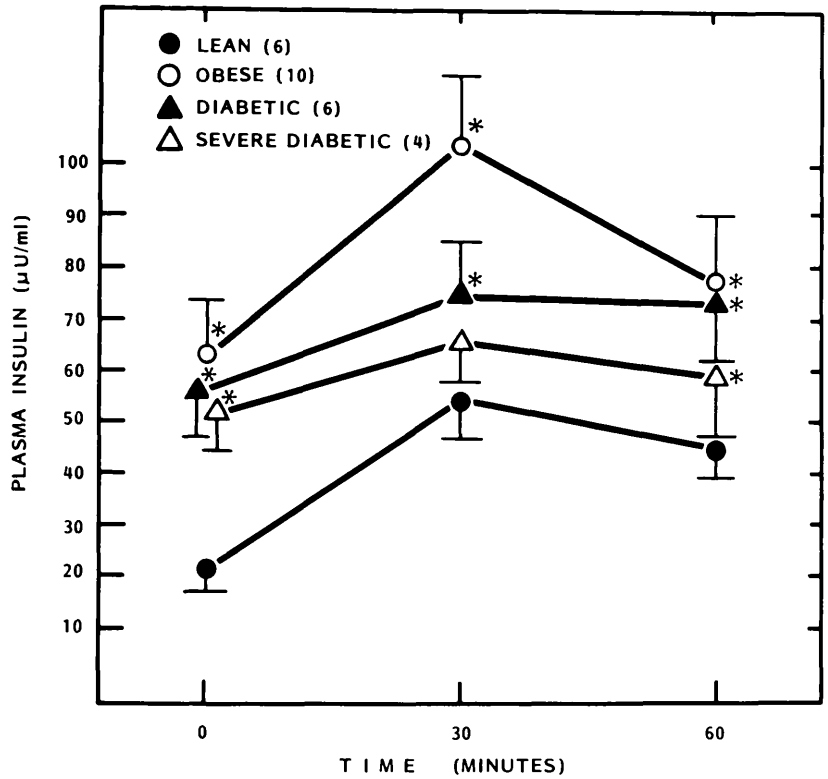

FIG. 3. Glucose tolerance test. Animals were grouped according to body type (lean or obese) or as diabetic (diabetic or severe diabetic) based on the graph in Fig. 1. All diabetic animals were obese.

the highest insulin values, higher ($P < 0.01$) than either the fatty diabetic or the lean rats ($P < 0.001$).

Glucose tolerance tests were repeated twice more over a 4-month period in the same animals. There was no age-related deterioration of glucose tolerance tests (data not shown).

Plasma and tissue values. The results of studies relating to plasma and tissues of age-matched fatty groups (nondiabetic, diet-restricted, diabetic) and lean rats are shown in Tables II and III. Only male obese rats are included because only males were diet-restricted for breeding and diet restriction does affect the values. An analysis of variance (ANOVA) was performed on the data in Tables II and III (19). When ANOVA indicated the inequality of the means at a probability level of 0.05, the individual means were tested against each other for equality by the Newman–Keuls test at the 0.05 confidence level (19).

The plasma values in Table II show that both diabetes and diet restriction attenuate the hyperinsulinemia, hypertriglyceridemia, and hypercholesterolemia that is characteristic of the fatty rat. The plasma free fatty acid concentration is higher in fatty diabetic and fatty nondiabetic rats than in diet-restricted fatty and lean rats.

The tissue values are shown in Table III. The major unique characteristics of the fatty diabetic rat are the decreased pancreatic insulin and body fat relative to that in the nondiabetic fatty and diet-restricted fatty rats and the greater liver glycogen content relative to all other groups. No difference among the groups were found for muscle glycogen, liver fatty acids, and liver cholesterol. Liver triglycerides of the nondiabetic fatty rats were greater than that of all other groups.

Discussion. The fatty Zucker rat has become a classic model of hyperinsulinemic, euglycemic obesity. We have demonstrated here that a marked fasting hyperglycemia appeared in selected animals and that this trait ap-

TABLE II. COMPARISON OF PLASMA VALUES: YEAR OLD ZUCKER RATS

Plasma concentration	Body type: Lean	Fatty	Fatty (diet restricted)	Fatty-diabetic (diet restricted)
Glucose: mg/dl	132 ± 8 (21)	125 ± 9 (13)	139 ± 9 (8)	207 ± 28 (7)*
Insulin: μU/ml	58 ± 7 (20)	326 ± 66 (12)*	147 ± 29 (8)	180 ± 38 (7)
Triglycerides: mg/dl	120 ± 11 (20)	427 ± 49 (10)*	269 ± 69 (9)*	180 ± 21 (6)
Cholesterol: mg/dl	94 ± 10 (21)	222 ± 13 (13)*	161 ± 15 (10)	152 ± 20 (7)
Free fatty acids: μEq/L	315 ± 31 (20)	590 ± 69 (12)*	348 ± 91 (10)	621 ± 98 (7)*

Note. The values are the mean ± SEM of number of animals shown in parentheses.
* Values significantly ($P \leq 0.05$) greater than those found for the lean animals.

peared to be genetically transmitted. Diabetic animals were always obese and were typically 6 months old before the appearance of fasting hyperglycemia. No ketone bodies were detected in the urine (data not shown). Glucose tolerance tests showed that the diabetic obese animals did not have a marked increase in plasma insulin in response to an oral glucose load. However, the plasma insulin concentration of the diabetic animals was as elevated as that of the euglycemic obese rats indicating a more marked insulin resistance in the diabetic rats. On sacrifice, the content of pancreatic insulin in the diabetic fatty rat was similar to that of the lean rats. The values of pancreatic insulin were five times higher in nondiabetic fatty or fatty diet-restricted rats than in lean rats. Although the pancreatic insulin content was much lower in the diet-restricted diabetic fatty than in the diet-restricted fatty rats, the plasma insulin concentrations in the fed state (determined at sacrifice) were similar. Percentage body fat and the plasma triglyceride concentrations were decreased in the diet-restricted fatty diabetic rat compared to the diet-restricted fatty rats. These two parameters are indicators of the insulin sensitivity of fat and liver respectively. Although no hard data are given, despite smaller plasma insulin concentrations, the fatty diabetic Zucker rat shows insulin resistance as expressed in its hyperglycemia, reduced body fat, and reduced plasma triglyceride concentration. The Zucker diabetic fatty rat exhibits both prime characteristics of non-insulin-dependent diabetes described in man as reduced insulin secretory capacity and decreased tissue insulin sensitivity (20).

The genetics of diabetes in the fatty Zucker rat is not clear. Only fatty (*fa/fa*) and not lean (*fa/?*) animals became diabetic, and diabetic animals appeared in each generation. Our data are consistent with a dominant gene not closely linked to the *fa* gene. It is of in-

TABLE III. COMPARISON OF TISSUE VALUES: YEAR OLD ZUCKER RATS

Tissue Values	Body type: Lean	Fatty	Fatty (diet restricted)	Fatty-diabetic (diet restricted)
Pancreatic insulin: mU/Gm	112 ± 26 (4)	550 ± 94 (4)*	463 ± 58 (4)*	96 ± 20 (6)
% Body weight as carcass fat	4.2 ± 0.7 (6)	40 ± 1 (4)*	30 ± 3 (4)*	24 ± 2 (7)*
Muscle glycogen: mg/Gm	0.6 ± 0.2 (5)	1.0 ± 0.2 (4)	0.7 ± 0.2 (4)	1.3 ± 0.2 (7)
Liver glycogen: mg/Gm	21.4 ± 1.4 (6)	27.1 ± 1.4 (4)	23.8 ± 0.6 (4)	50.0 ± 4.7 (7)*
Liver cholesterol: mg/Gm	1.65 ± 0.15 (6)	1.67 ± 0.2 (7)	1.53 ± 0.09 (3)	1.80 ± 0.15 (11)
Liver triglycerides: mg/Gm	26.8 ± 8.0 (6)	75.1 ± 8.6 (4)	35.1 ± 5.3 (3)	45.1 ± 12.3 (8)
Liver fatty acids: mg/Gm	11.4 ± 0.8 (6)	12.0 ± 1.2 (4)	11.7 ± 2.0 (3)	13.9 ± 1.6 (8)

Note. The values are the mean ± SEM of the number of animals shown in parentheses.
* Values significantly ($P \leq 0.05$) greater than those found for the lean animals.

terest that most (13 of 19) of our diabetic rats were fatty males and not females. We wondered if the testosterone injections used to improve fertility of some fatty males might influence the occurrence of diabetes, but diabetes did subsequently occur in fatty males not included in the testosterone regimen. Ikeda and co-workers have reported that when the *fa* gene is transferred to the Wistar–Kyoto rat, fatty males, but not fatty females, develop diabetes at 6 weeks of age (21). This result points to the importance of the strain as well as the sex of the animal. The background strains are Okamoto–Aoki and Wistar–Kyoto for the obese Koletsky rat (22, 23). The background strains in the Zucker are Sherman and Merck stock M (1). Leiter and co-workers have reported that the expression of the *db* gene in mice is dependent on the strain of mouse studied (24, 25). Of eight strains tested, *db/db* always produced obesity, produced hyperglycemia in both sexes in three strains, and in males only in three other strains. An association could be made with certain H-2 haplotypes and the susceptibility to diabetes.

In summary, we have demonstrated the occurrence of a genetically transmitted diabetes of the noninsulin-dependent type in our Zucker rat colony. This trait was not readily propagated in our colony. Work by others suggests that obesity in appropriate strains of rats and mice may be associated with an noninsulin-dependent diabetes. The occurrence of a genetically based diabetes in an outbred colony underscores the importance of genetic traits other than obesity in determining diabetes in rodent models. It is of interest to note that in rodent models, obese males show a higher or exclusive incidence of diabetes relative to females (21, 24, 25). Since obese men have a higher incidence of diabetes than obese women (26) the rodent models may be additionally suitable for the study of this sex-linked influence in man.

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