GENETICS

Mutant C57Bl/KsLepr$^{db/+}$ Mice as a Genetic Model of Type 2 Diabetes Mellitus

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The genetic model of diabetes mellitus was studied on mutant C57Bl/KsLepr$^{db/+}$ mice. These mice were characterized by high concentrations of glucose and glycosylated hemoglobin in the blood, polyuria, polyphagia, polydipsia, progressive obesity, biphasic morphological changes in insular islets of the pancreas (hyperplasia and atrophy), fatty degeneration of the liver, and hypoplasia of the spleen tissue and lymph nodes. Our results indicate that C57Bl/KsLepr$^{db/+}$ mice serve as an adequate model of type 2 diabetes mellitus. This model is suitable for testing of therapeutic methods for type 2 diabetes mellitus.

Key Words: *type 2 diabetes mellitus; db/db mice; experimental model*

Type 2 diabetes mellitus (DM) is one of the most common chronic diseases. The development of DM is associated with a decrease in receptor-mediated cell sensitivity to endogenous insulin [1,2,4]. Type 2 DM develops against the background of progressive immune dysfunction and is followed by autoimmune damage to the pancreatic islet tissue and vascular wall [3,5,6].

Experimental studies of this disorder involve toxic model of DM, which is induced by administration of alloxan or streptozotocin to animals. However, this treatment results in the development of disturbances that are typical of type 1 DM. The model of autoimmune DM also simulates type 1 DM. There is no experimental model for type 2 DM.

Here we evaluated whether mutant C57Bl/KsLepr$^{db/+}$ mice can be used as an experimental model for type 2 DM.

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MATERIALS AND METHODS

Experiments were performed on 90 C57Bl/KsLepr$^{db/+}$ mice (experimental group) carrying recessive gene for leptin receptor Lepr^{db} (*db*, linkage group 8, chromosome IV). The homozygous state of *db* gene (db/db) induces a variety of changes that are typical of DM, including β-cell degranulation in pancreatic islets, but without insulin deficiency. Male and female animals homozygous for the *db* gene are sterile.

Two groups of control specimens consisted of phenotypically healthy C57Bl/KsLepr$^{db/+}$ heterozygous mice (n=30) and C57Bl/10 mice (n=30). The control and experimental groups had free access to water and food. The animals were examined over 12 months (starting from the 4th-8th week of life).

Body weight, daily amount of food and water consumption, and lifetime of animals were analyzed. The concentrations of glucose, glycosylated hemoglobin, and lipids (total cholesterol, triglycerides, high-density lipoproteins [HDL], and low-

TABLE 1. Concentrations of Glucose and Glycosylated Hemoglobin in the Blood and Weight Indexes in Mice of Various Strains and Ages

Parameter	Age, months	Mouse strain		
		C57Bl/KsLepr$^{db/+}$ (type 2 DM)	C57Bl/KsLepr$^{db/+}$ (control)	C57Bl/10 (control)
Glucose, mmol/liter	2	15.10±3.83* (*n*=90)	5.80±0.42 (*n*=30)	5.90±0.03 (*n*=30)
Glycosylated hemoglobin, %		5.30±1.11*	3.60±0.01	3.20±0.13
Weight, g		33.00±2.37*	11.00±2.69	16.00±2.49
Glucose, mmol/liter	4	21.20±1.49* (*n*=78)	4.60±0.39 (*n*=30)	4.90±0.69 (*n*=30)
Glycosylated hemoglobin, %		6.3±1.06*	3.70±0.21	3.70±0.02
Weight, g		39.00±2.68*	18.00±2.26	21.00±2.27
Glucose, mmol/liter	6	24.2±0.9* (*n*=37)	5.70±0.62 (*n*=30)	5.40±0.38 (*n*=30)
Glycosylated hemoglobin, %		7.10±1.25*	3.50±0.02	3.50±0.01
Weight, g		21.00±2.35*	24.00±1.80	27.00±1.64

Note. Here and in Tables 2 and 3: *$p<0.05$ compared to the control.

density lipoproteins [LDL]) were measured in the whole blood. Biochemical studies were performed with 45 μl blood. Blood samples were taken from the saphenous vein after 16-18-h starvation [4].

The concentration of glycosylated hemoglobin was measured on a NycoCard Reader II device. Glucose concentration was measured on an ACCU-CHEK device. The concentrations of total cholesterol, triglycerides, HDL, and LDL were measured on a Cholestech LDX analyzer. LDL concentration was calculated automatically as follows: LDL=total cholesterol-(triglycerides/2.19+HDL). The endocrine and exocrine parts of the pancreas, liver, kidneys, and spleen were examined by histological methods. Paraffin sections were obtained on the 2nd, 4th, and 6th months of life and stained with hematoxylin and eosin. Gomori staining of the pancreatic tissue with aldehyde fuchsin allowed us to visualize α-cells and β-cells in Langerhans islets (LI). Liver glycogen and kidney lipohyaline were assayed by periodic acid—Schiff reaction. Fatty degeneration of hepatocytes was studied by staining cryostat sections of the liver with Sudan III and IV.

Morphometry was performed to evaluate the average area of LI, average number of cells in LI, and average area of lymphoid follicles in the spleen. The calculation was conducted on a personal computer using T-Morfometriya software (telemedical system).

The results were analyzed on a personal computer with Biostat software. Intergroup differences were evaluated by Student's *t* test with Bonferroni correction. The differences were significant at $p<0.05$.

RESULTS

Study of carbohydrate metabolism in mice of the experimental group showed that blood glucose concentration increases to 9-15 mmol/liter on the 3rd-4th week of life. The concentration of glycosylated hemoglobin exceeded 5.0-5.8% by the 6th-7th week. These results suggest that db/db mice have genetic signs of impaired carbohydrate metabolism. The dynamics of changes in carbohydrate metabolism in these mice was compared with the control (normal carbohydrate metabolism). Progressive changes were revealed in blood concentration of glucose and glycosylated hemoglobin in db/db mice on the 2nd, 4th, and 6th months of life, which significantly differed from that in heterozygous C57Bl/KsLepr$^{db/+}$ mice and C57Bl/10 mice of the control groups (Table 1).

TABLE 2. Lipid Metabolism in 4-Month-Old Mice of Various Strains

Mouse strain	Total cholesterol, mmol/liter	HDL, mmol/liter	Triglycerides, mmol/liter	LDL, mmol/liter
C57Bl/KsLepr$^{db/+}$ (type 2 DM)	3.23±0.50*	1.74±0.26*	0.69±0.15*	1.00±0.45*
C57Bl/KsLepr$^{db/+}$ (control)	3.02±0.32	1.28±0.39	0.85±0.33	1.42±0.48

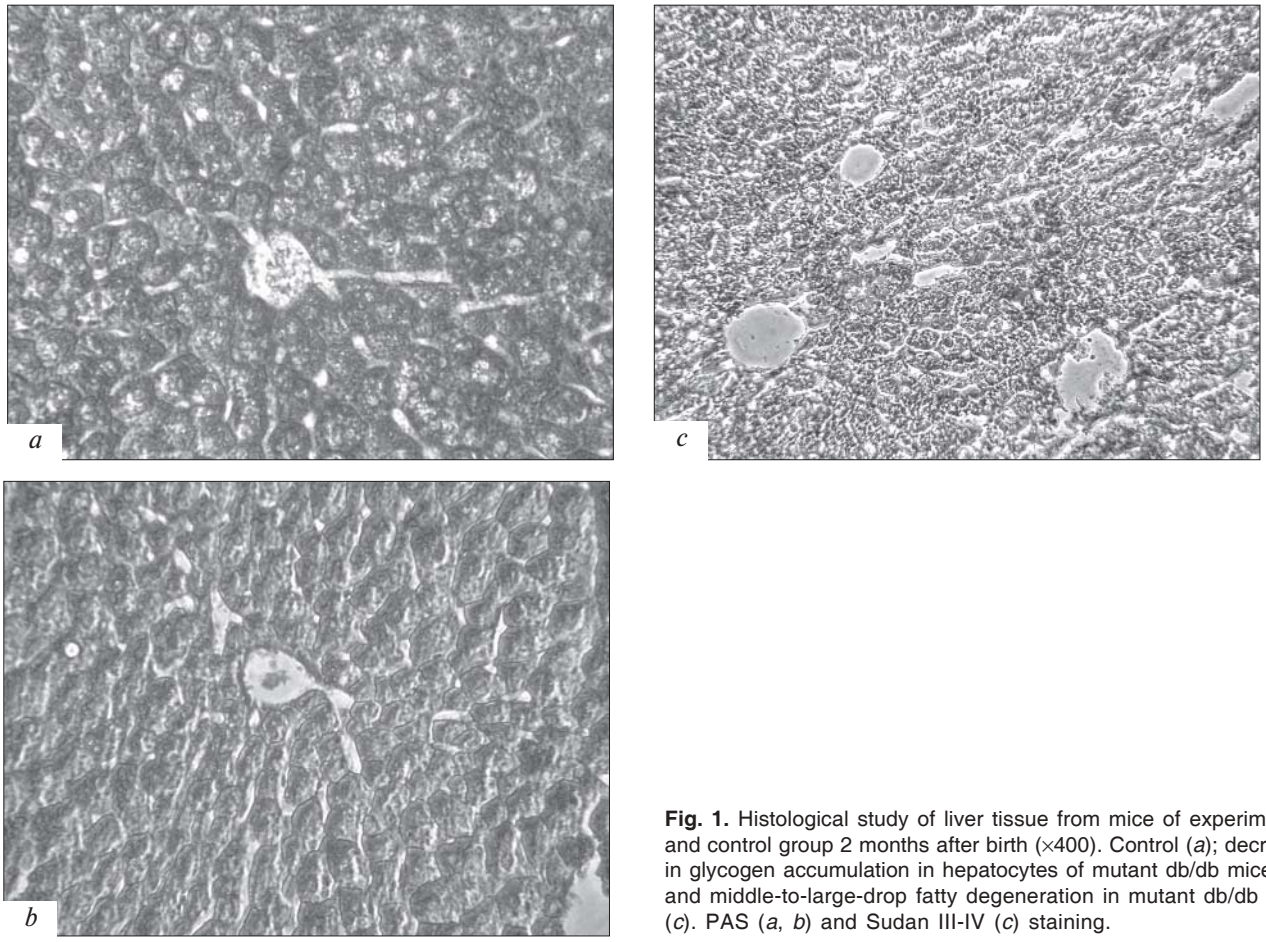

Fig. 1. Histological study of liver tissue from mice of experimental and control group 2 months after birth (×400). Control (*a*); decrease in glycogen accumulation in hepatocytes of mutant db/db mice (*b*); and middle-to-large-drop fatty degeneration in mutant db/db mice (*c*). PAS (*a*, *b*) and Sudan III-IV (*c*) staining.

All parameters of lipid metabolism in db/db mice and heterozygous animals significantly differed 4 months after birth (Table 2).

Total cholesterol concentration in the blood from experimental animals was much higher compared to the control, which is consistent with published data on lipid metabolism disorders during type 2 DM. Other parameters (triglycerides, HDL, and LDL) were not interpreted due to the impossibility of measuring the concentration of very-low-density lipoproteins (VLDL), the major dyslipidemic substances [8], on the specified device. By the 2nd-4th month of life, rapid increase in body weight of db/db mice and a rise in total cholesterol

TABLE 3. Morphometric Study of the Pancreas and Spleen and Weight of the Spleen in Mice of Various Strains and Ages

Parameter	Age, months	Mouse strain		
		C57Bl/KsLepr$^{db/+}$ (type 2 DM)	C57Bl/KsLepr$^{db/+}$ (control)	C57Bl/10 (control)
Area of LI, arb. units	2	87 516.5±1250.0*	41 954.7±1017.0	43 038.7±1352.0
Cell number in LI		250±27*	186±34	214±22
Area of LI, arb. units	4	28 569.8±1017.0*	41 954.7±1051.0	43 038.7±1150.0
Cell number in LI		98.41*	186±38	214±47
Area of LI, arb. units	6	9910.6±634.0*	41 954.7±690.0	43 038.7±512.0
Cell number in LI		75±32*	186±40	214±57
Area of splenic follicles, arb. units	6	26 123.9±320.0*	65 404.2±405.0	54 669.6±398.0
Weight of the spleen, g		0.045±0.01*	0.089±0.016	0.099±0.013

concentration were observed (Table 1) and interpreted as signs of obesity typical of type 2 DM.

db/db mice had various clinical signs of type 2 DM, including polydipsia and polyuria (30 ml of daily water consumption *vs*. 3-4 ml in control animals), polyphagia (2-fold greater amount of daily food intake compared to control animals), and skin maceration on withers (3-35% mice).

db/db mice were also characterized by rapid body weight loss from the 4th to the 6th month of life. The concentrations of glucose and glycosylated hemoglobin in the blood remained high. Starting from this period, the course of DM was similar to clinical signs of type 1 DM. Body weight of some mice decreased to 10-13 g (cachexia) 7-14 days before death. During this period, the animals were characterized by hypoglycemia (blood glucose concentration 3.0-3.8 mmol/liter), loss of appetite (by 5 times), and reduction of water consumption (by 10 times). The average lifetime of db/db mice was 174±30 days (*vs.* 1.5-2.0 years in control animals).

Histological changes in the pancreas, liver, and spleen of db/db mice were typical of type 2 DM. By the 2nd month of life, the area and cellularity of LI in the pancreas of db/db mice were higher compared to healthy animals of the control groups (Table 3). Basophilic β-cells prevailed in LI of db/db mice, which reflected high production of insulin. However, the number and size of LI significantly decreased by the 4th month of life. LI had loosened shape. The area of LI and number of cells (including basophilic cells) decreased. The number, area, and cellularity of LI significantly decreased by the 6th month of life (Table 3). Very small LI with insignificant amounts of basophilic cells (9-15 cells per group) were found between layers of the connective and adipose tissue.

Macroscopic and microscopic changes in the liver of db/db mice were well pronounced by the 4th-6th month of life. Progressive decrease in glycogen accumulation in hepatocytes and signs of middle-to-large-drop fatty degeneration were found even in 2-month-old animals (Fig. 1). Pronounced changes in immunocompetent organs (spleen and splenic lymph node) were also revealed in db/db mice. The weight of the spleen in 6-month-old db/db mice was 2-fold lower than in control animals (Table 3). Hypoplasia and atrophy of lymphoid follicles were observed in the spleen. Typical pattern of structure and germinal centers were absent in most animals. The area of lymphoid follicles in the spleen and regional lymph node of db/db mice was 2-fold lower compared to control animals. The weight of the spleen was compared with morphometric data on the area of follicles in the spleen and lymph node. db/db mice had signs for immunodeficiency, which always accompanies type 2 DM.

Our results show that DM in C57Bl/KsLepr$^{db/+}$ mice is an adequate model, which reproduces carbohydrate metabolic disorders, dysfunction of LI in the spleen, impairment of lipid metabolism (increased concentrations of total cholesterol and fatty degeneration of the liver), and immune dysfunction. The genetic model of DM can be considered as a model of type 2 DM suitable for the search and evaluation of new therapeutic methods for this disorder under experimental conditions.

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