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# Morphological Characterization of Systemic Changes in KK-A$^y$ Mice as an Animal Model of Type 2 Diabetes

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**Abstract.** *Background: KK-A$^y$ mice, a relevant model of human type 2 diabetes mellitus, are used worldwide for the assessment of pharmacological effects of new anti-diabetes drugs. Materials and Methods: KK-A$^y$ mice were examined at five weeks of age (non-hyperglycemic condition) and at 10 and 14 weeks of age (hyperglycemic condition). Results: Islet cell hypertrophy was observed in 10- and 14-week-old mice. The area ratio of islet cells to total pancreas significantly increased compared to that of age-matched C57BL/6J mice. Plasma insulin concentration increased in 14-week-old KK-A$^y$ mice. Enlargement of mesangial matrix and increased glomerular area were seen in kidneys of KK-A$^y$ mice. Fatty changes were observed in the liver. Total plasma cholesterol and triglyceride levels increased compared to that of five-week-old KK-A$^y$ mice. Conclusion: The present results on young/adult KK-A$^y$ mice indicate that the hyperglycemic state developing at the early stage of diabetes mellitus is due to related changes in systemic organs.*

It is estimated that 366 million people had diabetes mellitus (DM) in 2011. By 2030, this number is expected to rise to 552 million (1). The World Health Organization classifies DM as type 1 and type 2, of which type 2 DM accounts for more than 90% of patients (2). Type 2 DM is a chronic metabolic disorder and its prevalence has been steadily increasing worldwide. High levels of glucose resulting from insulin resistance and impairment of insulin secretion are considered a major hallmark of type 2 DM. When the diabetic condition is prolonged, complications develop, *e.g.* diabetic nephropathy, retinopathy, and neuropathy. Recently, exercise, diet, and medicinal therapy were recommended for preventing complications of DM.

The KK mouse is an inbred strain established from Japanese native mice (3). This strain is identified as a spontaneously diabetic animal, and several investigators have reported many diabetic traits, such as impaired tolerance to glucose, moderate hyperglycemia, insulin resistance of peripheral tissues, hyperinsulinemia, and renal glomerular changes (4-7). One investigator transferred the yellow obese gene (A$^y$) into KK mice by repeated crossing of yellow obese mice and KK mice (8). A congenic strain of KK mice, thus established, was named KK-A$^y$ mice. KK-A$^y$ mice have also been investigated by many researchers for their diabetic traits because these mice exhibit early onset and prolonged severe hyperinsulinemia (9, 10). Additionally, severe hyperglycemia is developed after two months of existence. With regard to the morphological characterization of the related changes in systemic organs of KK-A$^y$ mice, some investigators have reported on the pancreas, kidney, and liver (11). The islet size significantly increased in the pancreas of 16- and 20-week-old KK-A$^y$ mice (9, 12). Thickening of the glomerular capillary basement membrane and enlargement of mesangial cells were observed in kidneys of 90- to 165-day-old (13- and 24-week-old) KK-A$^y$ mice (11, 13). Triglyceride (TG) and alanine aminotransferase (ALT) levels significantly increased in livers of 14-week-old KK-A$^y$ mice. However, these reports were independent studies of each organ, and almost all of these morphological characterization studies were qualitative, not quantitative. In the present study, we analyzed and compared both biochemical examination of blood and histopathological characteristics in 5-week-old KK-A$^y$ mice without hyperglycemia and in 10- and 14-week-old KK-A$^y$ mice presenting hyperglycemia. We focused on the pancreas, kidney, and liver as organs associated with diabetic conditions. The area ratio of islet cells in the pancreas, glomerular area in the kidney, and area

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*Key Words:* Diabetes, KK-A$^y$ mice, pancreas, kidney, liver, animal model.

Table I. *Body weight, blood glucose level, and plasma insulin concentration in 5-, 10- and 14-week-old KK-A$^y$ and C57BL/6J mice.*

| | Body weight (g) | | | Blood glucose level (mg/dl) | | | Insulin (pg/ml) | | |
|---|---|---|---|---|---|---|---|---|---|
| Age (weeks) | 5 | 10 | 14 | 5 | 10 | 14 | 5 | 10 | 14 |
| C57BL/6J | 14.9±0.2 | 24.2±2.0 | 26.9±1.2 | 156±29 | 160±25 | 110±19 | 13.7±15.1 | 31.6±40.8 | 40.7±24.8 |
| KK-A$^y$ | 16.7±0.2 | 38.5±0.9 | 45.2±5.0 | 209±16 | 517±45* | 504±66* | 105.8±130.1 | 90.7±94.8 | 153.5±24.7* |

*$p$<0.05 *vs.* age-matched control C57BL/6J mice.

Table II. *Absolute and comparative weight of kidney in 5-, 10- and 14-week-old KK-A$^y$ and C57BL/6J mice.*

| | Absolute weight of kidney (mg) | | | Comparative weight of kidney (mg) | | |
|---|---|---|---|---|---|---|
| Age (weeks) | 5 | 10 | 14 | 5 | 10 | 14 |
| C57BL/6J | 183.1±6.8 | 280.1±23.3 | 284.3±33.1 | 1233.2±55.7 | 1284.9±94.5 | 1130.8±59.9 |
| KK-A$^y$ | 196.9±2.0 | 467.3±57.8* | 491.6±28.1* | 1179.0±10.4 | 1178.4±154.9 | 1185.6±86.4 |

*$p$<0.05 *vs.* age-matched control C57BL/6J mice.

Table III. *Plasma concentration of alanine aminotransferase (ALT), total cholesterol (T-CHO), and triglyceride (TG) in 5-, 10- and 14-week-old KK-A$^y$ and C57BL/6J mice.*

| | ALT (U/l) | | | T-CHO (mg/dl) | | | TG (mg/dl) | | |
|---|---|---|---|---|---|---|---|---|---|
| Age (weeks) | 5 | 10 | 14 | 5 | 10 | 14 | 5 | 10 | 14 |
| C57BL/6J | 33±5 | 39±9 | 27±17 | 99±7 | 117±11 | 97±13 | 62±5 | 60±6 | 71±28 |
| KK-A$^y$ | 26±5 | 43±8 | 24±2 | 116±7 | 120±20 | 175±30* | 137±28* | 171±27* | 200±66* |

*$p$<0.05 *vs.* age-matched control C57BL/6J mice.

ratio of fat deposition in the liver were morphometrically and quantitatively analyzed to determine the degree of damage in relation to the diabetic condition.

## Materials and Methods

*Reagents*. The following materials were obtained from Wako Pure Chemical Co. (Osaka, Japan): Hematoxylin, eosin, formalin, disodium hydrogen phosphate dodehydrate, sodium dihydrogen phosphate dihydrate, ethanol, acetic acid, isopropanol, glycerin jelly, periodic acid, and sodium bisulfite. The following material was obtained from MERCK Co. (Tokyo, Japan): eosin G. The following materials were obtained from Waldeck GmbH & Co KG (Münster, Germany): oil red O and phloxine B. All reagents were of analytical or reagent grade and were used without purification.

*Animals*. C57BL/6J mice, the most widely used inbred strain as non-diabetic mouse model (14), and KK-A$^y$ mice were obtained from CLEA Japan Inc (Tokyo, Japan) and allowed free access to solid food (MT, Oriental Yeast Co., ltd. Tokyo, Japan) and tap water. They were housed in an air-conditioned room at a temperature of 23˚C±1˚C and a humidity of 60%±10%, with lights on from 8:00 to 20:00. The animal studies were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University (KPU, 2012-083) and were performed according to the Guidelines for Animal Experimentation at KPU.

*Observation of clinical signs, and measurement of body weight, and food consumption*. Throughout the study, all animals were observed once a day for clinical signs, general remarks, and behavior. Body weight and food consumption were measured once a week at 7:00.

*Blood chemical analyses*. At the time of sacrifice, a blood sample for glucose level measurement was obtained from the tail vein of each mouse and blood glucose levels were determined with a Glucocard (Arkray, Kyoto). Mice were subjected to a 12-h fast, and blood samples were collected from the cavernous sinus under anesthesia by using heparinized tools. Collected samples were then centrifuged at 650 ×*g* for 10 min at 4˚C, and the resultant plasma samples were used to analyze various biochemical parameters. Plasma concentrations of ALT, TG, and total cholesterol (T-CHO) were determined by using Fuji Dry Chem 4000 (Fuji Medical Co., Tokyo, Japan).

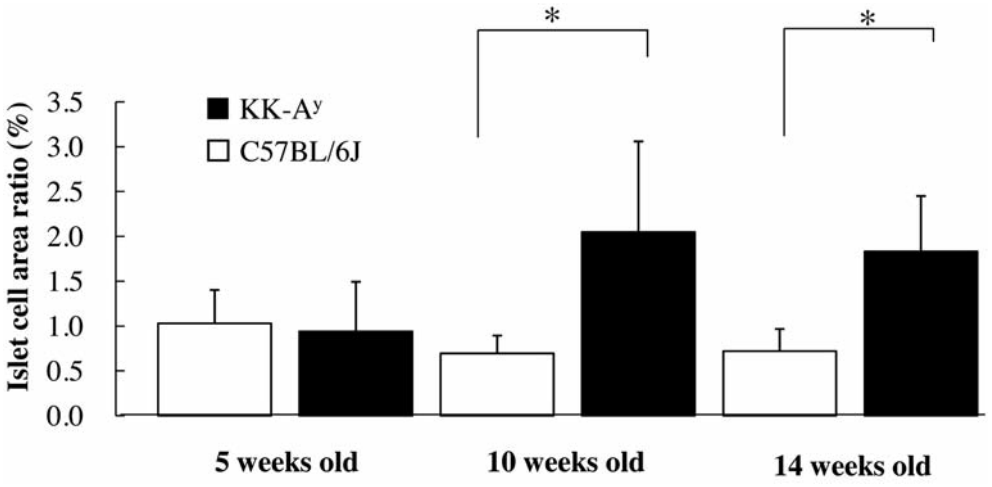


Figure 1. *Islet cell area ratio in pancreas of 5-, 10-, and 14-week-old C57BL/6J and KK-A$^y$ mice. Islet cell hypertrophy was observed and the islet cell area ratio in the pancreas significantly increased in comparison to that of C57BL/6J mice. *p<0.05.*

*Tissue fixation and processing*. Pancreata, kidneys, and livers from 3–4 KK-A$^y$ and C57BL/6J mice were removed at five, 10, and 14 weeks of age fixed in 10% buffered formalin, sectioned at a thickness of 3 μm, and stained with hematoxylin and eosin (HE). In addition, sequential sections of kidneys and livers were stained with periodic acid-schiff reagent and oil red O, respectively.

*Morphometric analyses of pancreas, kidney, and liver.* HE-stained sections of each tissue were scanned with a high-resolution digital slide scanner (NanoZoomer 2.2 Digital Pathology, Hamamatsu Photonics, Hamamatsu, Japan) to prepare digital images. The ndpi image files were opened in color mode with NDP.view software (Hamamatsu Photonics, Hamamatsu, Japan). The islet cell ratio was calculated as: (area of islet cells/total area of pancreas specimen)×100, and the area ratio of fat deposition was calculated as: (area of fat deposition/total area of liver specimen)×100. The glomerular area was measured in HE sections and average values were calculated. Histopathological and morphometric evaluations were conducted by T.M. and reviewed by a toxicologic pathologist certificated by the International Academy of Toxicologic Pathology (K.Y.), according to the previously defined histopathological terminology and diagnostic criteria (15, 16).

*Statistical analysis*. All discrete values are expressed as the mean±standard deviation (SD) and statistically analyzed with the two-tailed independent Student's *t*-test for unpaired samples after confirming the homogeneity of variance. The statistical analysis was used to examine the significance of differences in the area ratio of pancreatic islet cells in the pancreas, fat deposition in the liver, and glomerular area in the kidney. *p*-Values of <0.05 were considered to indicate statistical significance.

## Results

*General remarks*. During the course of the present study, abnormal clinical signs were not observed. Body weight and food consumption were significantly increased in 10- and 14-week-old KK-A$^y$ mice compared with age-matched C57BL/6J mice (Table I).

*Pancreatic and related changes*. At five weeks of age, no abnormal changes were detected in KK-A$^y$ mice. The islet cell area ratios were similar to those of C57BL/6J mice: 0.94% and 1.03% in KK-A$^y$ and C57BL/6J mice, respectively (Figure 1). At the age of 10 and 14 weeks, islet cell hypertrophy was observed in KK-A$^y$ mice (Figure 2). Islet cell hypertrophy was characterized by the cell shape, *i.e.* cells retained a normal round to oval outline or became lobular and irregular. The regional distribution of cell types was retained in hyperplastic islets. The enlarged islet cells showed no atypia in mice at the age of 10 and 14. Islet cell area ratios significantly increased in KK-A$^y$ mice compared with age-matched C57BL/6J mice, or 2.05% and 1.83% in 10- and 14-week-old KK-A$^y$ mice, and 0.70% and 0.72% in C57BL/6J mice, respectively (Figure 1).

At the age of five weeks, the mice of each strain had similar blood glucose levels. At the age of 10 and 14 weeks, KK-A$^y$ mice had significantly higher blood glucose levels than age-matched C57BL/6J mice (Table I).

In addition, plasma insulin concentrations of KK-A$^y$ mice increased with age. The levels were significantly increased in 14-week-old KK-A$^y$ mice compared with age-matched C57BL/6J mice (Table I).

*Renal and related changes*. The absolute weights of the kidneys of KK-A$^y$ mice were significantly increased compared with age-matched C57BL/6J mice. However, the comparative weights of the kidneys of KK-A$^y$ mice were not different from those of C57BL/6J mice of the same age (Table II).

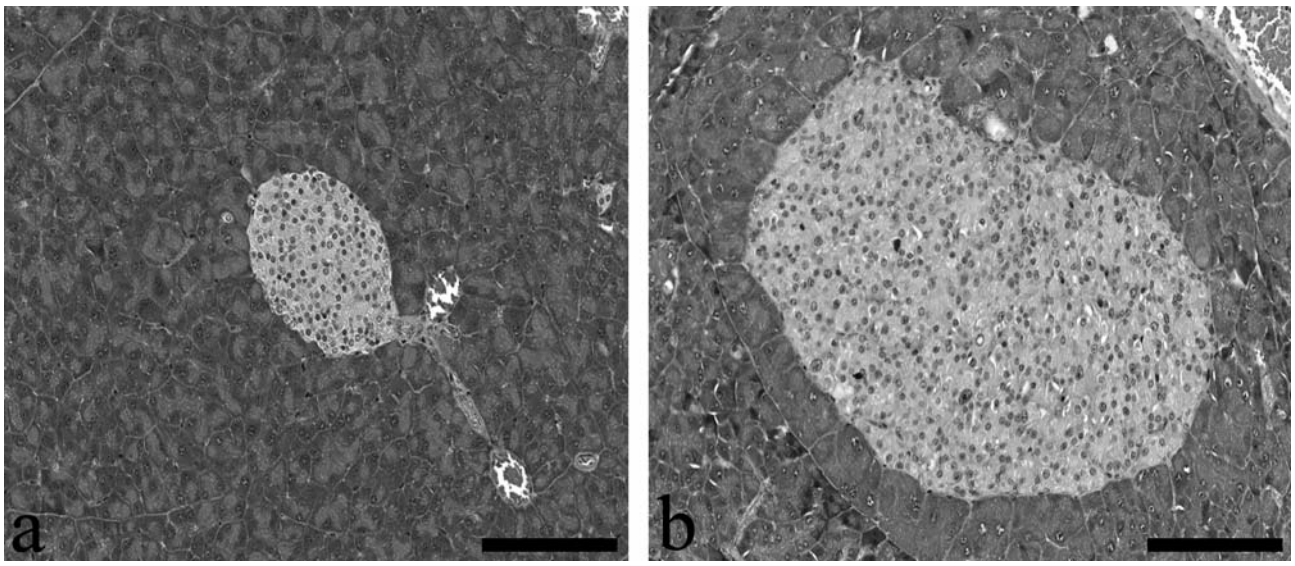


Figure 2. *Islet cells in 14-week-old C57BL/6J (a) and KK-A$^y$ mice (b). Islet size and number of islet cells in C57BL/6J mice were within normal ranges. However, in KK-A$^y$ mice, islet cells were enlarged and the number of islet cells was higher than those in C57BL/6J mice. Hematoxylin-eosin staining (scale bar=200 μm).*

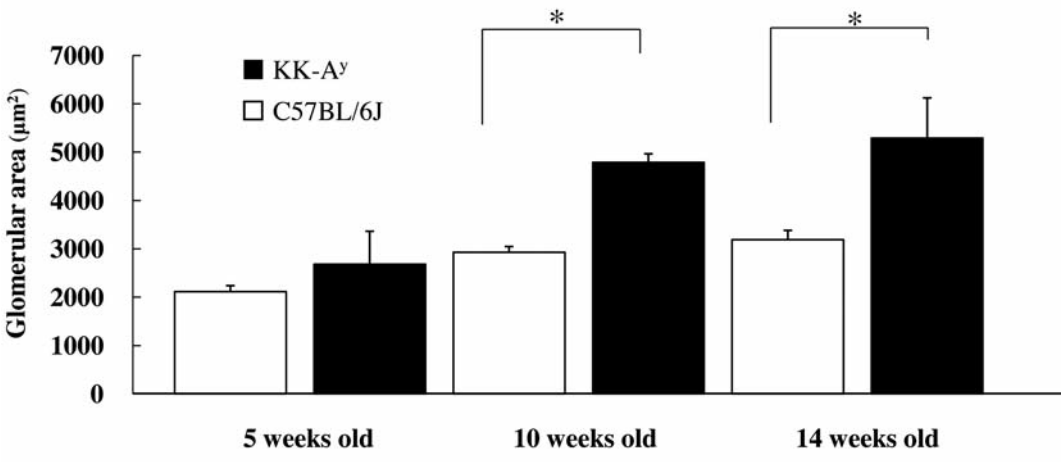


Figure 3. *Glomerular area in kidneys of 5-, 10-, and 14-week-old C57BL/6J and KK-A$^y$ mice. The index significantly increased in comparison to age-matched control C57BL/6J mice. *p<0.05.*

The kidneys of 5-week-old KK-A$^y$ mice revealed normal histopathological characteristics, and the glomerular areas were similar to those in C57BL/6J mice. The glomerular areas were 2,683 and 2,116 μm$^2$ in KK-A$^y$ and C57BL/6J mice, respectively (Figure 3).

At 10 and 14 weeks of age, glomerular hypertrophy due to enlargement of the mesangial matrix was observed in KK-A$^y$ mice (Figure 4). The glomerular areas significantly increased in 10- and 14-week-old KK-A$^y$ mice compared with age-matched C57BL/6J mice; these were 4,790 and 5,292 μm$^2$ in KK-A$^y$ mice and 2,928 and 3,191 μm$^2$ in C57BL/6J mice at the age of 10 and 14 weeks, respectively (Figure 3).

*Hepatic and related changes.* The livers of 5-week-old KK-A$^y$ mice revealed normal histopathological characteristics. The area ratios of fat deposition were similar to that of C57BL/6J mice: 0.61 and 0.60 μm$^2$ in KK-A$^y$ and C57BL/6J mice, respectively (Figure 5).

In the livers of 10- and 14-week-old KK-A$^y$ mice, centrilobular vacuolation of hepatocytes was observed, compared with C57BL/6J mice. The vacuoles were strongly

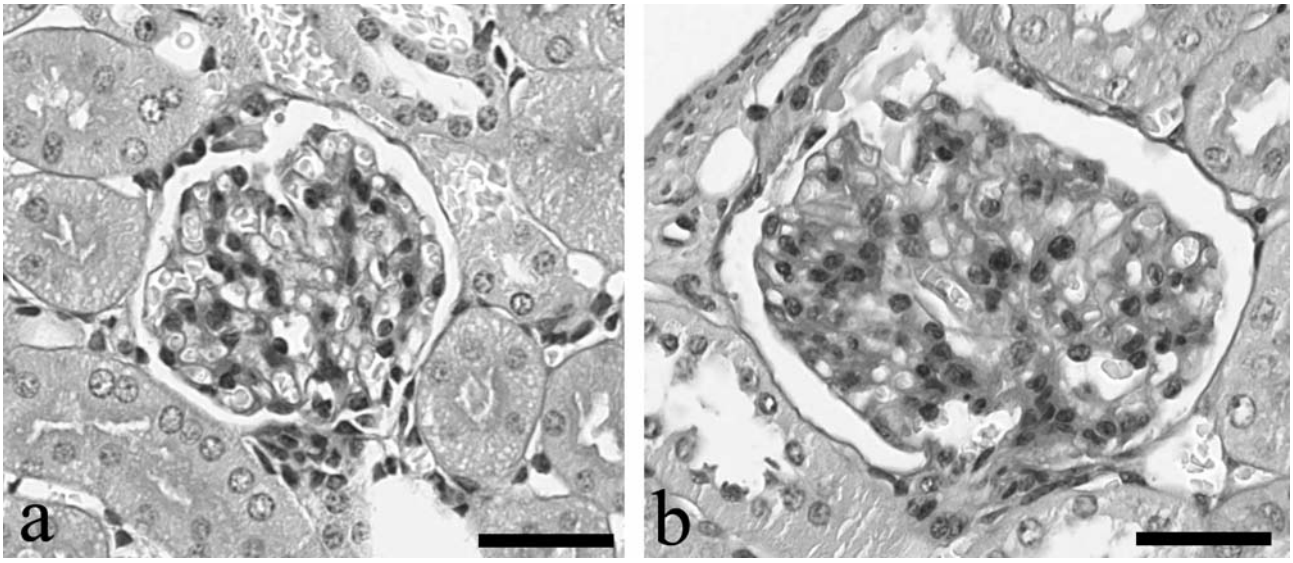


Figure 4. *Glomerular morphology in 10-week-old C57BL/6J (a) and KK-A$^y$ mice (b). The size of glomerular and mesangial matrices was in the normal ranges. However, in KK-A$^y$ mice, glomerular hypertrophy due to enlargement of the mesangial matrix was observed. Periodic acid-Schiff staining (scale bar=50 μm).*

stained by oil red O, showing fat deposition (Figure 6). The area ratios of fat deposition were significantly increased in 10- and 14-week-old KK-A$^y$ mice compared with age-matched C57BL/6J mice; these were 0.96% and 1.46% in KK-A$^y$ mice, and 0.48% and 0.72% in C57BL/6J mice at the age of 10 and 14 weeks, respectively (Figure 5).

In KK-A$^y$ mice, irrespective of the age, plasma concentrations of ALT were similar to those of age-matched C57BL/6J mice (Table III). T-CHO levels were 175 mg/dl in 14-week-old KK-A$^y$ mice, and significantly increased at 14 weeks of age compared with age-matched C57BL/6J mice (97 mg/dl). Plasma TG levels significantly increased in KK-A$^y$ mice at the age of five, 10, and 14 weeks compared with age-matched C57BL/6J mice (Table III).

*Other organs*. The related changes in other main systemic organs, spleen, eye, and gastrointestinal tract, were also examined histopathologically; however, no abnormal changes related to DM were detected in these organs.

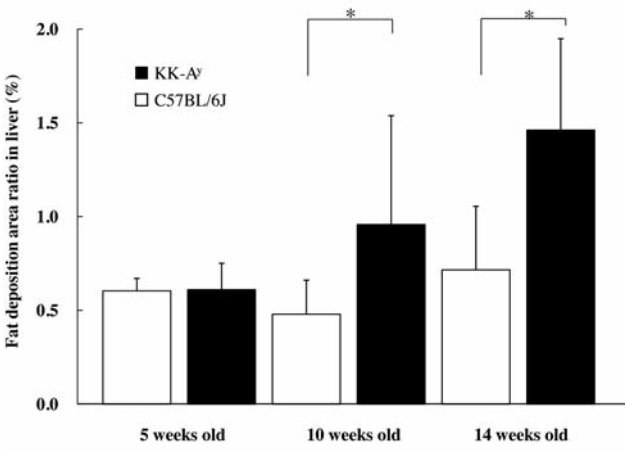


Figure 5. *Area ratio of fat deposition in liver of 5-, 10-, and 14-week-old KK-A$^y$ and C57BL/6J mice. The index significantly increased in comparison to that of age-matched control C57BL/6J mice. *p<0.05.*

## Discussion

The present study provides information on the state of the pancreas, kidney, and liver in 10- and 14-week-old KK-A$^y$ mice. Our quantitative data confirm the morphometric changes in each organ. The islet cell ratio in the pancreas, area ratio of fat deposition in the liver, and glomerular area in the kidney were significantly higher in KK-A$^y$ mice compared with age-matched C57BL/6J mice. In previous reports, thickening of the glomerular capillary basement membrane and pancreatic islet cell hypertrophy were seen in 10- to 13-week-old KK-A$^y$ mice; however, there was little detailed information about morphometric analyses (9, 17).

Patients with DM develop hyperglycemia by intermediary uptake of glucose due to insulin resistance, hyperinsulinemia (18). The patient's body has to produce much more insulin to reduce the blood glucose level. As a result, β-cell islets

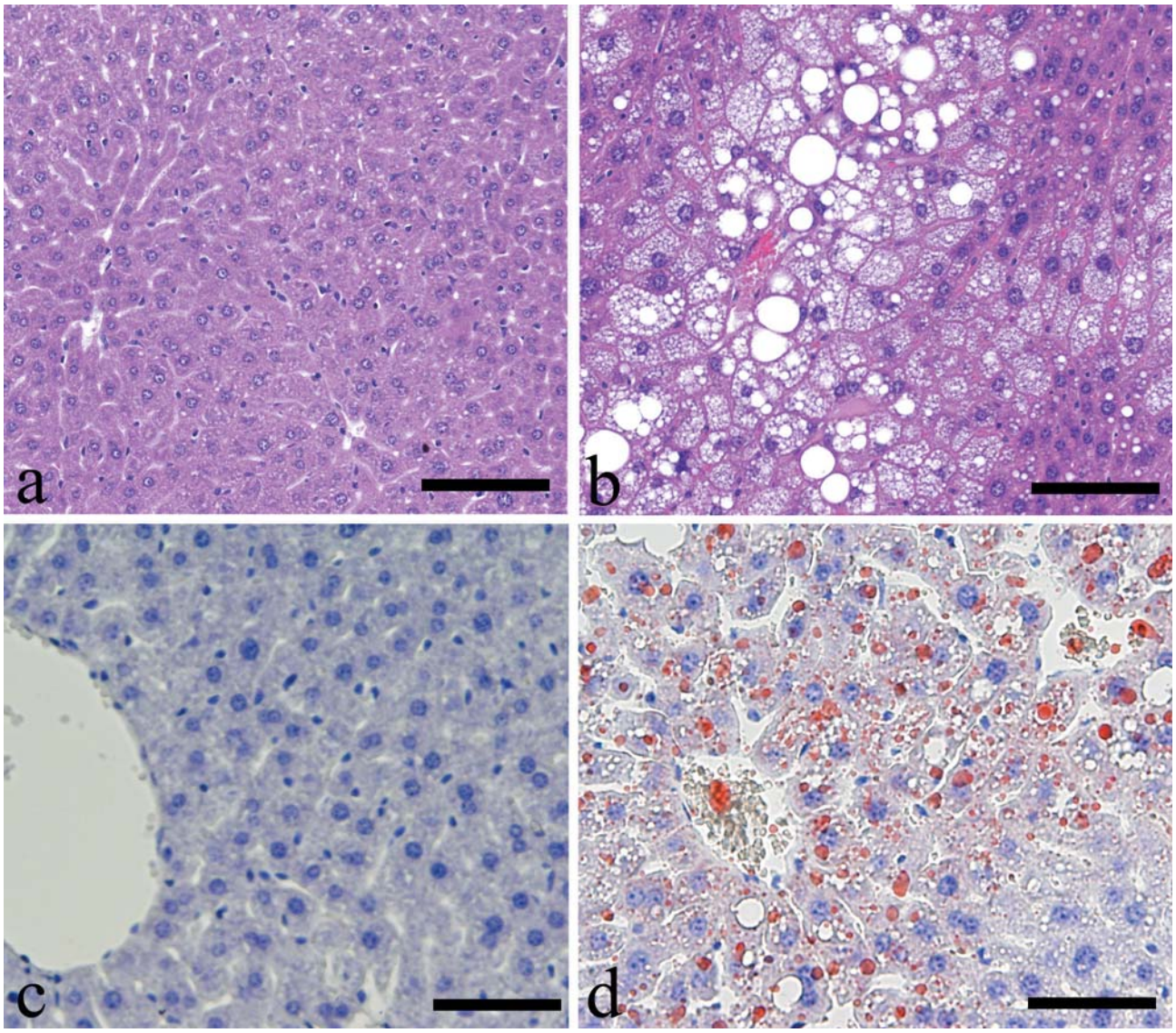


Figure 6. *Hepatic morphology in 14-week-old KK-A$^y$ and C57BL/6J mice. Normal morphology in C57BL/6J mice (a) and centrilobular vacillation of hepatocytes in KK-A$^y$ mice (b) were seen. Hematoxylin-eosin staining. No staining in C57BL/6J mice (c) and oil red O-positive cells in KK-A$^y$ mice (d) were seen. Oil red O staining (scale bar=100 μm).*

become hypertrophic, which causes more insulin production (19). Insulin resistance continues for prolonged periods, β-cells become overloaded, and finally become necrotic. In fact, islet cell hypertrophy is indicated at the early stage of the clinical state of DM. When estimating the pharmacological effect of compounds on DM, it is very important to know the clinical state of the model animal. In our study, hypertrophy of islet cells was seen in 10- and 14-week-old KK-A$^y$ mice, along with a significant increase in the plasma insulin concentration. The islet cell area ratio of 10- and 14-week-old KK-A$^y$ mice was significantly higher compared with age-matched C57BL/6J mice. In the pancreas, necrotic areas were not detected. In 20-week-old KK-A$^y$ mice, the following characteristics were reported: increased number of islets, enlarged area of islets, abundance of large vacuolations, lipid droplets, and fat proliferation (12). In our study, enlarged areas of islets and increased numbersf islets were observed in 10- and 14-week-old KK-A$^y$ mice, but lipid droplets and fat proliferation in islets were not observed. These differences were possibly due to the different ages studied. These results suggest that 10- and 14-week-old KK-A$^y$ mice present morphologically the early stage of clinical DM.

Diabetic nephropathy is the most frequent cause of chronic kidney disease. Early alteration in diabetic nephropathy includes the development of glomerular hyperfiltration and glomerular hypertrophy, followed by increased urinary albumin excretion, eventually leading to glomerular sclerosis (20). The overgrowth of mesangial matrix causes glomerular degeneration, sclerosis, and development of nephropathy (13). In 90- and 165-day-old (13- and 24-week-old) KK-A$^y$ mice, thickening of the glomerular basement membrane was reported (21). In addition, 16-week-old KK-A$^y$ mice exhibited enlargement of the mesangial matrix; this continuous pathological state causes diabetic nephropathy (2, 11). These results suggest that KK-A$^y$ mice developed early-stage diabetic nephropathy between 90 to 165 days of age (13- and 24-week-old). In our study, we observed that the glomerular areas of 10- and 14-week-old KK-A$^y$ mice were significantly higher compared with age-matched C57BL/6J mice (Figures 3 and 4). KK-A$^y$ mice exhibited enlargement of the glomerular mesangial matrix. Thus, we consider that most of the increase in the glomerular area is due to enlargement of the mesangial matrix. Enlargement of glomerular matrix presented in 10-week-old KK-A$^y$ mice clinically corresponds to the early stage of diabetic nephropathy in humans.

Excessive fat accumulation in the liver characterizes insulin-resistant individuals with either too much or too little subcutaneous fat (22). Obesity and insulin resistance are the most common associates of non-alcoholic fatty liver disease (23). KK-A$^y$ mice developed impaired glucose tolerance, moderate hyperglycemia, insulin resistance of peripheral tissues, hyperinsulinemia, and renal glomerular changes. In addition, aged KK-A$^y$ mice presented fatty liver, very similar to humans (9). Moreover, KK-A$^y$ mice exhibited high blood levels of TG, T-CHO, AST, and ALT at 28 weeks of age (24). In our study, high plasma TG concentrations were noted in 5-week-old KK-A$^y$ mice, but remarkable changes in the liver were not observed. We observed centrilobular vacuolation of hepatocytes in 10- and 14-week-old KK-A$^y$ mice, and the result of oil red O staining indicates that vacuolation derived from fat deposition, suggesting fatty liver. In addition, blood T-CHO levels in 14-week-old KK-A$^y$ mice were higher than those in age-matched C57BL/6J mice. These results suggest that 5-week-old KK-A$^y$ mice developed hypertriglyceridemia and 10-week-old KK-A$^y$ mice developed fatty liver, similar to DM in humans.

In conclusion, young KK-A$^y$ mice show related changes in systemic organs, *e.g.* enlargement of islet cells, nephropathy, and fatty liver, which are similar to the characteristics of DM in human. This mouse as a diabetic model has been used for studies on the benefits of drug treatments. On the basis of our study results, we conclude that for the appropriate assessment of therapeutic drugs, administration should start at 10 weeks of age. After administration, analyses of the islet cell area ratio in the pancreas, glomerular area, and fat deposition in the liver are critical. In addition, we conclude that evaluation of the morphological assessment is very important because biochemical parameters, such as plasma insulin levels and TG levels, were not always correlated with islet size and fat deposition.

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## References

1 Olokoba AB, Obateru OA and Olokoba LB: Type 2 diabetes mellitus: A review of current trends. Oman Med J *27*: 269-273, 2012.
2 Amos AF, McCarty DJ and Zimmet P: The rising global burden of diabetes and its complications: Estimates and projections to the year 2010. Diabet Med *14*: S1-S85, 1997.
3 Kondo K, Nozawa K, Tomita T and Ezak K: Inbred strains resulting from Japanese mice. Bull Exp Animal *6*: 107-112, 1957 in Japanese.
4 Nakamura M: A diabetic strain of the mouse. Proc Jpn Acad *38*: 348-352, 1962.
5 Tsuchida I: Pathogenesis of hyperglycemia and adiposity in KK-$A_y$ mice. J Jpn Diabetic Soc *9*: 67-84, 1966 in Japanese.
6 Dulin WE and Gerristen GC: Proc. 6th Congr. Int. Diabetes Fed. Stockholm, 1967.
7 Treser G, Oppermann W, Ehrenreich T, Lange K, Levine R and Camerini-Davalos RA: Glomerular lesions in a strain of genetically diabetic mice. Proc Soc Exp Biol Med *129*: 820-823, 1968.
8 Fujita T, Sugiyama Y, Yaketomi S, Sohda T, Kawamatsu Y, Iwatsuka H and Suzuoki Z: Reduction of insulin resistance in obese and/or diabetic animals by 5-[4-(1-methycyclohexyl-methoxy)benzyl]-thiazolidine-2,4-dione (ADD-3878,U-63,287, ciglitazone), a new antidiabetic agent. Diabetes *32*: 804-810, 1983.
9 Iwatsuka H, Shino A and Suzuoki Z: General survey of diabetic features of yellow KK mice. Endocrinol Jpn *17*: 23-35,1970.
10 Chang AY, Wyse BM, Copeland EJ, Peterson T and Ledbetter SR: The Upjohn colony of KK-$A_y$ mice. A model for obese Type II diabetes. *In*: Diabetes 1985. Serrano-Rios M and Lefebvre PJ (eds.), Amsterdam, Elsevier, pp. 466-470, 1986.
11 Okazaki M, Saito Y, Udaka Y, Maruyama M, Murakami H, Ota S, Kikuchi T and Oguchi K: Diabetic nephropathy in KK and KK-$A_y$ mice. Exp Anim *51*: 191-196, 2002.
12 Chen L, Zhang XL, Li J, Han J. Fu JY, Tian JY, Pan L and Ye F: The semi-quantitative method for evaluating lipid accumulation in pancreas of diabetic mice. Acta Pharmaceut Sin *46*: 664-668, 2011 in Chinese, English abstract.

13 Haschek WM, Rousseaux CG and Walling MA: Handbook of Toxicologic Pathology (Second Edition). Volume 2., Elsevier Inc, San diego, Calif., Academic press: pp. 282-285, 2001.
14 Mao XQ, Wu Y, Wu K, Liu M, Zhang JF, Zou F and Ou-Yang JP: Astragalus polysaccharide reduces hepatic endoplasmic reticulum stress and restores glucose homeostasis in a diabetic KK-$A_y$ mouse model. Acta Pharmacol Sin *28*: 1947-1956, 2007.
15 Greaves P: Histopathology of preclinical toxicity studies. Interpretation and Relevance in Drug Safety Evaluation. Fourth Edition. Amsterdam, the Netherlands, Academic Press: pp. 433-614, 2012.
16 Boorman GA, Eustis SL, Elwell MR, Montgomery CA Jr. and Mackenzie WF: Pathology of the Fischer Rat. Reference and Atlas. San Diego, Academic Press: pp. 548-549, 1990.
17 Diani AR, Sawada GA, Hannah BA, Jodelis KS, Connell MA, Connell CL, Vidmar TJ and Wyse BM: Analysis of pancreatic islet cells and hormone content in the spontaneously diabetic KK-$A_y$ mouse by morphometry, immunocytochemistry and radioimmuno-asssay. Virch Arch A Pathol Anat Histopathol *412*: 53-61, 1987.
18 Lillioja S, Mott DM, Howard BV, Bennettt PH, Yki-Javinen H, Freymond D, Nyomba BL, Zurlo F, Swinburn B and Bogardus C: Impaired glucose tolerance as a disorder of insulin action. Longitudinal and cross-sectional studies in Pima Indians. N Engl J Med *318*: 1217-1225, 1988.
19 Kloppel G, Mattias L, Habich K, Oberholzer M and Heitz PU: Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. Surv Synth Pathol Res *4*: 110-125, 1985.
20 Chiarelli F, Gaspari S and Marcovecchio ML: Role of growth factors in diabetic kidney disease. Horm Metab Res *41*: 585-593, 2009.
21 Diani R, Sawada GA, Zhang NY, Wyse BM, Connell CL, Vidmar TJ and Connell MA: The KKAy mouse: A model for the rapid development of glomerular capillary basement membrane thickening. Blood Vessels *24*: 297-303, 1987.
22 Yki-Jarvinen H and Westerbacka J: The fatty liver and insulin resistance. Curr Mol Med *5*: 287-295, 2005.
23 Neuschwander-Tetri BA and Caldwell SH: Nonalcoholic steatohepatitis: Summary of an AASLD Single Topic Conference. Hepatology *37*: 1202-1219, 2003.
24 Yang S, Lou JL and Wang Q: Effect of puerarin on liver injury in KK-$A_y$ mice with type 2 diabetes mellitus. Zhongguo Zhong Xi Yi Jie He Za Zhi 29: 707-710, 2009 in Chinese, English abstract.

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