Summary

In 2016, a male rat exhibiting severe hyperglycemia, polydipsia and polyuria and marked enlargement of the kidneys was found in the LEA.PET-*pet* congenic strain, which had been established by introducing the gene (*pet*) responsible for dwarfism (petit) and thymic hypoplasia into the LEA strain. These diabetic phenotypes were confirmed as being genetic traits by inbreeding of this founding diabetic male rat. Rats of the LEA.PET-*pet* congenic strain have never developed diabetes mellitus (DM), and genotyping of the *pet* allele of diabetic rats clearly showed that the diabetic phenotypes were inherited independently of the *pet* allele. Furthermore, all diabetic male rats showed marked enlargement of the kidneys. This genetic trait was therefore called diabetes with enlarged kidneys (DEK). Rats with this trait were repeatedly subjected to brother-sister mating to establish a novel diabetic strain retaining this trait.

Chapter 1 summarizes general information and recent research about DM, as well as animal models of DM. Although most diabetic patients are classified as having type 2 DM, which is usually characterized by hyperglycemia due to insulin resistance and/or insulin deficiency, patients with DM have various lifestyles, genetic backgrounds and

pathological features, limiting the ability of applying a single guideline of medical treatment to all diabetic patients. This problem has been addressed by treating patients with various types of anti-diabetic drugs that differ in their mechanisms of action, as well as by developing various animal models of diabetes with different phenotypes. The recent introduction of sodium glucose cotransporter (SGLT2) inhibitors as anti-diabetic agents has highlighted the important role of the kidneys in glucose metabolism, especially in diabetes. Although the kidneys are impaired by hyperglycemia, these organs are also highly associated with the development of DM. Chapter 1 discusses recent trends in diabetes research and the characteristics of other animal models of diabetes, as well as describing the value of the DEK rat as a novel animal model of diabetes.

Chapter 2 describes the characteristics and mode of inheritance of the DM phenotype in DEK rats. Only some male DEK rats develop non-obesity type 2 DM, suggesting that this diabetic phenotype is a polygenic trait. All rats with hyperglycemia 15 weeks after the onset of diabetes exhibited marked increases in kidney sizes, as well as polydipsia/polyuria. Although DEK rats with DM (DEK-DM) showed age-related deteriorations of enlarged kidneys, dilated renal tubules and tubulointerstitial fibrosis,

their glomerular structure and plasma Cre and electrolyte concentrations were similar to those in DEK rats without DM (DEK-cont), suggesting that renal function was almost normal in DEK-DM rats. These findings suggested that DEK rats have genetic factors associated with renoprotection or tolerance to glomerular lesions induced by diabetes. In addition, DEK-DM showed failure of insulin secretion, with a weak insulin signal detected by immunofluorescent staining of the pancreas. Taken together, these findings indicated that the phenotypes of DEK rats differed from those in other animal models of diabetes and that DEK rats were a novel animal model of diabetes.

Chapter 3 describes the long-term effects of the SGLT2 inhibitor empagliflozin on DEK rats. Treatment of DEK-DM rats with empagliflozin not only normalized blood glucose concentrations, but also improved polyphagia, polydipsia and polyuria. In response to empagliflozin, these rats experience body weight gain and remaining adeps renis, as well as increases in plasma total protein concentrations and decreases in urinary nitrogen excretion and plasma valine and isoleucine concentrations. These results suggested that the enhanced protein catabolism in DEK-DM rats was ameliorated by empagliflozin by improvements in energy balance. Furthermore, although treatment with empagliflozin did

not improve insulin secretion, these rats showed slightly higher insulin positive signals in the pancreas and lower blood glucose concentrations on oral glucose tolerance tests (OGTT) than control rats not administered empagliflozin. These findings indicated that long-term administration of empagliflozin might improve the efficiency of glucose utilization by eliminating chronic hyperglycemia. Although empagliflozin could not prevent the increase of kidney weight and the dilation of renal tubules, it improved the overall function of renal tubules, as shown by reductions in urinary volume and the fractional excretion of electrolytes. Improvements in energy balance and the reabsorption of proteins and amino acids by renal tubules have been found to promote the synthesis of hepatic proteins, suggesting that this mechanism could explain the empagliflozin-associated increases in plasma levels of albumin and total cholesterol. In addition, increased calcium reabsorption associated with the improvement of renal tubule function might inhibit bone resorption and maintain bone mineral density. Taken together, these findings showed that empagliflozin exerted multiple positive effects and ameliorated the symptoms of diabetes in DEK rats, mainly by improving renal tubular function.

Chapter 4 describes the characterization of enlarged kidneys and their association with

hyperglycemia in DEK-DM rats. The gradual increase in kidney weight of DEK-DM rats was found to be dependent on the duration and degree of diabetes. The number of nephrons was higher in DEK-DM than in DEK-cont rats, whereas blood glucose concentrations were positively correlated with kidney weight and nephron number in DEK-DM but not DEK-cont rats. The similar glomerular sizes and single glomerular Ccre in DEK-cont and DEK-DM rats indicated that glomerular hypertrophy and hyperfiltration were not involved in renal enlargement in DEK-DM rats. Therefore, enlarged kidneys in DEK-DM rats were apparently associated with increased nephron number rather than individual nephron hypertrophy. Because nephrogenesis ends soon after birth in rats and nephron precursor stem cells are absent from adult kidneys, it is unlikely that nephron number increased as blood glucose levels increased in DEK-DM rats. Rather, rats with an increased number of nephrons might develop diabetes. In addition, the density of glomeruli was found to be reduced in expanded cortical sections of DEK-DM rats, suggesting that an increase in renal tubules corresponded to 90% of the parenchyma in the kidneys. Taken together, these findings suggested that the increase in renal parenchyma involved in increases in the numbers of nephrons and renal tubules was a

prerequisite for the development of diabetes. In contrast, because DEK-DM rats showed increases in nephron number and homogeneous glomerular size but not pathological hypertrophy, the renal phenotype of DEK-DM rats is highly renoprotective against the progression of diabetic nephropathy (DN).

To obtain direct evidence of the effects of increased nephron mass on the development of diabetes, DEK-DM rats were subjected to uninephrectomy (1/2Nx) immediately after the onset of diabetes. Blood glucose levels remained normal for 28 days after 1/2Nx, with this level remaining lower than in Sham-operated rats for >84 days after the operation. In addition, 1/2Nx improved systemic symptoms, including body weight gain suppression, polyuria, polydipsia, and hyperphagia, similar to the ability of the SGLT2 inhibitor empagliflozin to suppress renal glucose reabsorption (Chapter 3). Because overall improvements induced by the SGLT2 inhibitor or 1/2Nx were not accompanied by improvements in insulin secretion, the former improvements are thought to be due to partial or generation suppression of renal function associated with elevated blood glucose levels. In addition, 1/2Nx increased plasma concentrations of total cholesterol, sodium, albumin, and total protein and reduced urinary excretion of glucose, urinary nitrogen, and

protein in DEK-DM rats. Similar results were observed in response to SGLT2 inhibitor, suggesting that 1/2Nx and SGLT2 inhibitor have a common mechanism of action, with improvements in negative energy balance and osmotic diuresis due to the attenuation of hyperglycemia. The increases in the number of PCNA-positive epithelial cells and the kidney weight of DEK-DM rats were further enhanced by 1/2Nx, suggesting that renal function in these rats could be increased by further enlargement. Taken together, these findings indicate that the kidneys of DEK-DM rats were associated with their development of diabetes and were resistant to DN.

The final chapter discusses the mechanisms of diabetes development involving the pancreas and kidneys. First, the dysfunction of islet endocrine cells was caused not only by external factors that affect the islets but also by internal factors that cause islet endocrine cells to lose their identity. Because structural defects in the islets of DEK-DM rats were unaccompanied by pathological alterations that secondarily disrupt pancreatic islets, such as inflammation or fibrosis, DEK rats seem to have genetic defects in factors required to maintain normal islet structure and function. Second, the structural and functional alterations in renal tubules may also be involved in the onset and progression

of diabetes in DEK-DM rats. The increase in kidney weight and the severity of tubular dilation apparently correlated with the duration and severity of diabetes in DEK-DM rats. These renal alterations persisted despite reductions in hyperglycemia by empagliflozin. Therefore, we hypothesized that diabetes does not cause renal changes, but that these changes are involved in the development of diabetes in DEK rats. This hypothesis was supported by ability of uninephrectomy to suppress the onset of diabetes. Although tubular hyperplasia and renal hypertrophy are considered among the earliest renal pathological changes in diabetes, the renal parenchyma in DEK-DM rats continued to grow without leading to end stage renal failure, even when diabetes had progressed or functional load was increased by nephrectomy. Because the size of the renal parenchyma reflects renal reserve capacity, renal growth in DEK-DM rats can delay the progression of DN. In contrast, because kidneys have the second highest glycemic control potential, following the liver, renal hyperfunction accompanied by increased parenchyma was found to be strongly associated with systemic glucose control and may worsen hyperglycemia. Diabetes in DEK rats is thought to be caused by the combined involvement of pancreatic and renal factors, but this mechanism has not been analyzed.

Diabetes research using DEK rats may provide useful information on the intrapancreatic mechanism leading to insulin deficiency and may validate the hypothesis that the kidneys are involved in the development and deterioration of diabetes.