

# Glomerular Vascular Changes in KK-A<sup>y</sup> Mice with Early Diabetes: Scanning Electron Microscopy of Vascular Resin Casts

Hiroyoshi NINOMIYA<sup>1,3</sup>, Tomo INOMATA<sup>1,3</sup> and Kikumi OGIHARA<sup>2</sup>

Departments of <sup>1</sup>Laboratory Animal Science, <sup>2</sup>Environmental Pathology and <sup>3</sup>Research Institute of Biosciences, Azabu University, 1-17-71 Fuchinobe, Sagami-hara, Kanagawa 229, Japan

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**ABSTRACT.** Vascular changes, the angioarchitecture of renal glomerular fine vessels in early diabetic KK-A<sup>y</sup> mice of 4 months of age were examined by scanning electron microscopy of corrosion casts. Histologically, enlargement of glomeruli and dilation of glomerular capillaries were found. In resin cast specimens, glomeruli with diabetes were larger than normal, and an increase in diameter of the glomerular vessels was found in diabetic mice. No capillary proliferation, distortion of glomerular vessels and destruction of glomerular capillary loops were found in diabetic mice. The increased vascular diameter may explain the increase in blood flow in the glomeruli, affecting kidney function in early diabetes. — **KEY WORDS:** diabetes, glomerulus, KK-A<sup>y</sup> mouse.

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Congenital diabetes mellitus has been reported in NOD, C57BL/ob, C57BL/db, KK, NZO and NON mice, BB, GK, Zucker fatty rats, hamsters and other animals [3]. Glomerular lesions similar to those found in human diabetes have been studied with these animals [7–10]. Swelling of the glomerulus, expansion of glomerular capillary lumina and enhanced glomerular perfusion are reported to be major symptoms during the initial stage of the disease [1, 4, 5, 10].

Glomerulosclerosis, diffuse sclerosis of mesangium and thickening of basal laminae and proliferation of glomerular cells can be observed in young KK mice (2 to 4 months old) [9, 10]. Abnormal glucose tolerance begins at 4 months of age in the animals [7]. Few detailed studies, however, have been directed to the initial alteration in capillaries in diabetic glomerular change. The capillary changes at the initial stage may show an angioarchitecture clearly different from those of late stage and/or very severe glomerular change.

The present study focused on an early stage in the glomerular capillary changes of diabetes mellitus. In this study, these changes were examined using scanning electron microscopy (SEM) of resin casts of capillaries of diabetic KK-A<sup>y</sup> mice. A comparison was made with those of nondiabetic C57BL/6 mice.

Fourteen diabetic KK-A<sup>y</sup> mice (male, Nippon Clea Co., Ltd.) with blood sugar levels above 400 mg/dl were used for this study. Since early diabetic changes such as a diffuse sclerosis of the mesangium were already found in young animals [9, 10], the age of the diabetic animals was 4 months. The mice were killed by an overdose of anesthetic ether. Blood sugar levels were measured at autopsy by means of the enzymatic ultraviolet test with hexokinase (Nippon Roche Co., Ltd.). The Ames paper strip (Bayer Diagnostics Co., Ltd.) was used for determination of glucose and proteins in the urine. Four additional normal C57BL/6 mice (male, 4 months of age, Nippon Clea Co., Ltd.) were sacrificed to serve as controls. Blood sugar levels of C57BL/6 mice ranged 194 to 220 mg/dl.

**Histological preparation:** The left kidney was taken out and prepared for light and transmission electron microscopy (TEM) with routine techniques. Kidneys of the four nondiabetic C57BL/6 mice were treated the same way.

**Corrosion cast preparation:** The aorta was isolated and a catheter inserted at a position immediately cranial to the renal artery. The kidneys were perfused with Ringer's solution at 37°C. The renal arterial vasculature was filled with acrylic resin. After polymerization, the kidney was immersed in a 20% NaOH solution, then samples were rinsed in distilled water. Under a dissecting microscope, each glomerulus was isolated, mounted on an aluminum stub and spattered with gold for scanning electron microscopy (SEM). Caliber estimation of the individual vascular casts was performed on scanning electron micrographs (magnification  $\times 500$ ). The mean glomerular diameter was evaluated for 30 glomeruli per animal. The dimension of capillaries was determined by measuring 10 capillaries on the equator line of each glomerulus.

**Light microscopy:** In all the diabetic mice studied, enlargement of glomeruli and expansion of glomerular capillaries were found in the kidneys of KK-A<sup>y</sup> mice but not in C57BL/6 controls (Figs. 1-A & B).

**TEM:** No characteristic diabetic lesions such as thickening of basement membrane and/or mesangial cell proliferation were found in any diabetic mice except for the dilation of glomerular capillaries (Fig. 2).

**SEM:** The glomeruli described were limited to those just beneath the capsule of the kidney. An increase in diameters of the glomerulus, afferent arterioles, glomerular capillaries and efferent arterioles were found in diabetic mice (Figs. 3-A & B). The average diameters of the diabetic glomeruli, afferent arterioles, capillaries and efferent arterioles measured  $123.3 \pm 13.3$ ,  $20.0 \pm 3.4$ ,  $9.8 \pm 2.3$  and  $15.4 \pm 3.2$   $\mu\text{m}$ , respectively. In contrast, in nondiabetic C57BL/6 mice, the average diameters of those vessels were less and measured  $85.2 \pm 8.1$ ,  $16.7 \pm 1.2$ ,  $7.4 \pm 1.2$  and  $13.0 \pm 3.3$   $\mu\text{m}$ . There was neither capillary proliferation, distortion of fine glomerular vessels nor destruction of the glomerular

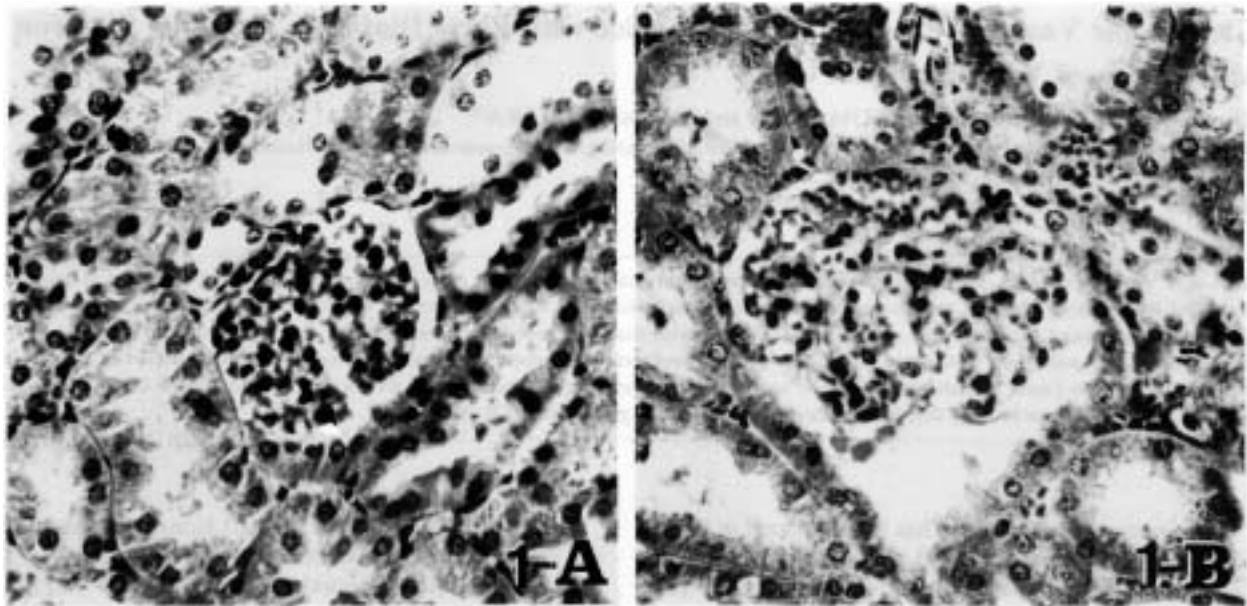


Fig. 1. Light micrograph of glomerulus. H.E. stain.  $\times 200$ . 1-A. Nondiabetic glomerulus of C57BL/6 (4 months of age). 1-B. Early diabetic glomerulus of KK-A<sup>y</sup> mouse (4 months of age) showing enlargement of glomerulus and dilated capillaries.



Fig. 2. Electron micrograph of early diabetic glomerulus (4 months of age). No severe diabetic glomerular lesion are seen. The basal laminae appear to be normal.  $\times 3,000$ .

capillary network in diabetic mice.

SEM study of corrosion casts allowed easy detection and detailed observation of even slight vascular changes. Use of this technique provided a clear demonstration of the increase in diameter of glomerular fine vessels in early diabetes. These findings confirmed previous histological studies [5, 10]. Diameters of the afferent arteriole, glomerular capillary and efferent arteriole were greater in

the early diabetic animals than in the normal controls, viz.  $20.0 \pm 3.4$  versus  $16.7 \pm 1.2 \mu\text{m}$  (16.5% increase in diameter),  $9.8 \pm 2.3$  versus  $7.4 \pm 1.2 \mu\text{m}$  (24.5% increase in diameter) and  $15.4 \pm 3.2$  versus  $13.0 \pm 3.3 \mu\text{m}$  (15.6% increase in diameter) respectively.

An elevated GFR (glomerular filtration rate) is a well established feature in early diabetes in humans [1] as well as rats [2]. The mechanism of this elevated GFR is activated

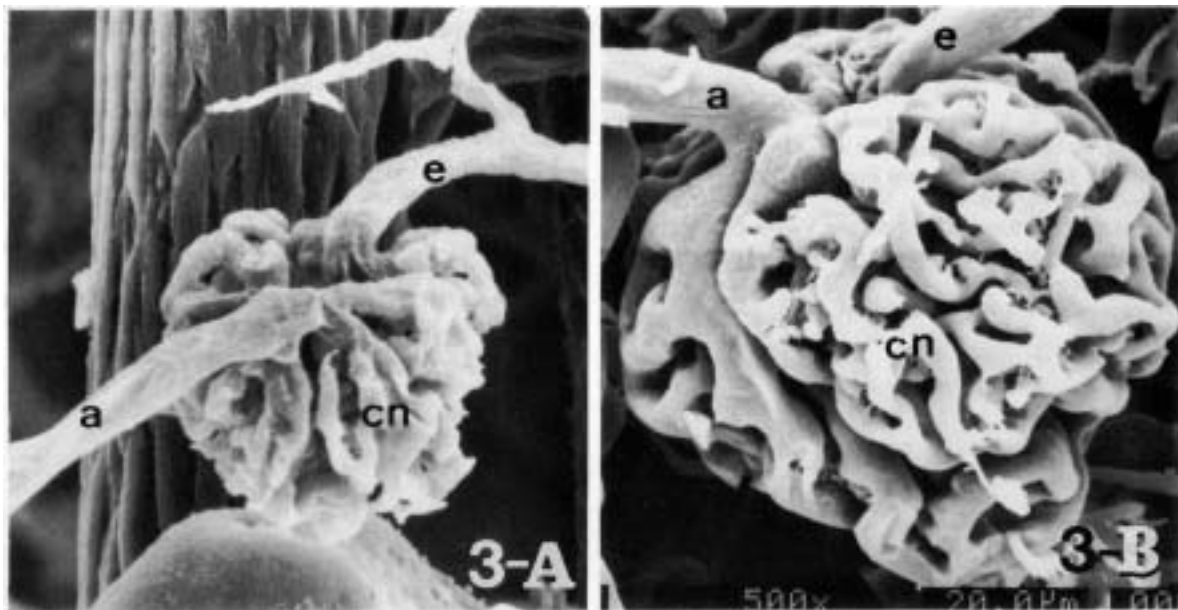


Fig. 3. Scanning electron micrographs of resin cast of glomerulus (4 months of age).  $\times 500$ . 3-A. Nondiabetic glomerulus. 3-B. Early diabetic glomerulus showing glomerular enlargement and thick afferent arteriole (a), capillaries (cn) and efferent arteriole (e) in diameter as compared with those of nondiabetic glomerulus.

by an enhanced RPF (renal plasma flow), an increased transglomerular hydrostatic pressure gradient and an increased glomerular ultrafiltration coefficient [4]. The GFR determinants are closely related to the hemodynamics in the glomeruli.

Although we cannot directly determine the functional significance of the GFR determinants, it is reasonable to assume that an increase in the diameter of glomerular vessels may affect the kidney function in early diabetes. In support of this concept, Poiseuille's law [6] states that when the diameter of a vessel increases 10 to 20%, blood flow in the vessel increases 20 to 40%. Interestingly, this 20 to 40% blood flow increase in the kidney has been reported in human early diabetes compared with non-diabetic controls [5]. The increased renal blood flow may be the factor behind the enhanced RPF and increased glomerular ultrafiltration coefficient affect GFR. It is also well known that parallel reductions in afferent and efferent resistances induce blood flow increase in early diabetes. The present study demonstrates an increase in diameters of the afferent and efferent arterioles in KK- $A^y$  diabetes mice. This increase in diameter of arterioles may allow increased blood flow into the glomerulus, resulting in increasing RPF.

The present study has clearly shown enlargement of the capillary lumina, which represents an expansion of the surface area available for filtration. Since the ultrafiltration coefficient is determined by the fluid permeability of the glomerular capillary and the area of its surface available for filtration [1], the enhanced ultrafiltration characteristic of early diabetes may well be caused by the enlargement of glomerular capillaries. Furthermore, an increase in the caliber of the afferent arterioles has been shown in the

present study. Dilation of the afferent arteriole increase the glomerular pressure, with a corresponding increase in glomerular filtration rate [1].

Although segmental thickening of the basement membrane and proliferation of cells have been observed in diabetic KK mice from 2 to 4 months old [7, 9, 10], we could not find these glomerular changes in the present study. However, our findings regarding the dilation of capillaries in the glomeruli of young diabetic mice are consistent with the findings of one other author [10]. These glomerular changes in KK- $A^y$  mice increase in severity with age and are similar to those observed in human diabetes. The typical nodular glomerular sclerosis seen in humans does not develop in mice but severe sclerosis of arterioles develops in old diabetic mice more severely than in humans [9, 10]. The striking glomerular vascular changes we observed in young diabetic KK- $A^y$  mice bear a certain similarity to those described in human early diabetes. The enlargement of glomerular fine vessels may be considered as a common feature. In the late and/or severe diabetes, an increase of mesangium and thickening of basement membranes encapsulating glomerular capillaries has been reported [8]. These mesangial and basement membrane lesions cause distortion and narrowing of glomerular capillaries that leads to a decrease in GFR [1]. The fine vascular configuration of the glomerulus at the late stage appears to be quite different from that seen at an early stage of diabetes. Detailed study of glomerular vascular changes in diabetes ranging from an early stage to advanced stages with various diabetic animal models such as NON, NOD, C57BL/6 mice and BB Wistar rats remains to be carried out and may be helpful in elucidating kidney functional aspects

of diabetes.

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