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Lack of Nitric Oxide Results in Increased Glomerular Capillary Basement Membrane Thickness

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Capillary basement membrane thickening (CBM) is an ultrastructural hallmark of diabetic retinopathy and nephropathy. Elevated levels of nitric oxide (NO) and superoxide can uncouple constitutive endothelial nitric oxide synthase (eNOS) and reduce availability of nitric oxide (NO) [1]. Inhibition of inducible NOS (iNOS) results in coronary microvascular remodeling in rats [2] and interstitial fibrosis is exacerbated in kidneys of mice lacking the gene for iNOS [3]. Numerous studies have investigated pharmacological inhibition of eNOS and iNOS; however, there are questions as to the specificity of the drugs and the issue of side effects. Mice with gene deletions of specific NOS isoforms provide a better experimental design for investigating the role of NO in vascular biology. Previous studies of CBM thickening in galactose induced diabetic retinopathy demonstrated increased CBM thickness in the retinal capillaries of nondiabetic eNOS knockout (eNOS KO) mice as well as in diabetic WT, eNOS KO and iNOS KO mice [4]. In order to establish a baseline for studies of glomerular CBM thickening in this galactose induced model of diabetes we investigated the role of NO from eNOS and iNOS in glomeralular CBM by studying wild type (WT), eNOS knockout (eNOS KO) and iNOS knockout (iNOS KO) mice fed a normal rodent diet.

Breeder pairs of WT C57Bl/6, eNOS KO and iNOS KO mice on a C57Bl/6 line were obtained from Jackson Labs, Bar Harbor, ME). Age matched male mice of each strain were fed a normal rodent diet (Purina, St. Louis, MO) and sacrificed after 3 months. Kidneys were fixed, processed for NADH oxidase localization, embedded, and sectioned for examination by transmission electron microscopy (TEM). CBM thickness was measured by the orthogonal intercept method [5] and TEM magnification was calibrated with a 2,160 ln/mm calibration grating (Electron Microscopy Sciences, Hatfield, PA).

Morphology of the eNOS KO and iNOS KO kidneys was comparable to that of WT (Fig. 1-3) and there was localization of NADH oxidase in the vasculature of all three strains of mice (Figs. 2, 3). The foot processes of podocytes appeared to be broader in eNOS KO mice (Fig. 3). Results of the glomerular CBM thickness are summarized in Table 1. The CBM thickness of age matched WT and iNOS KO on a normal diet were comparable; however the CBM thickness of eNOS KO mice was 1.3 times thicker than the CBM of age matched WT or iNOS KO mice.

Increased CBM thickness in the eNOS KO mice as compared to WT and iNOS KO mice suggests that endothelial derived NO plays a role in vascular homeostasis and that lack of availability of NO in diabetic and galactosemic models of diabetes plays an important role in CBM thickening in diabetes.

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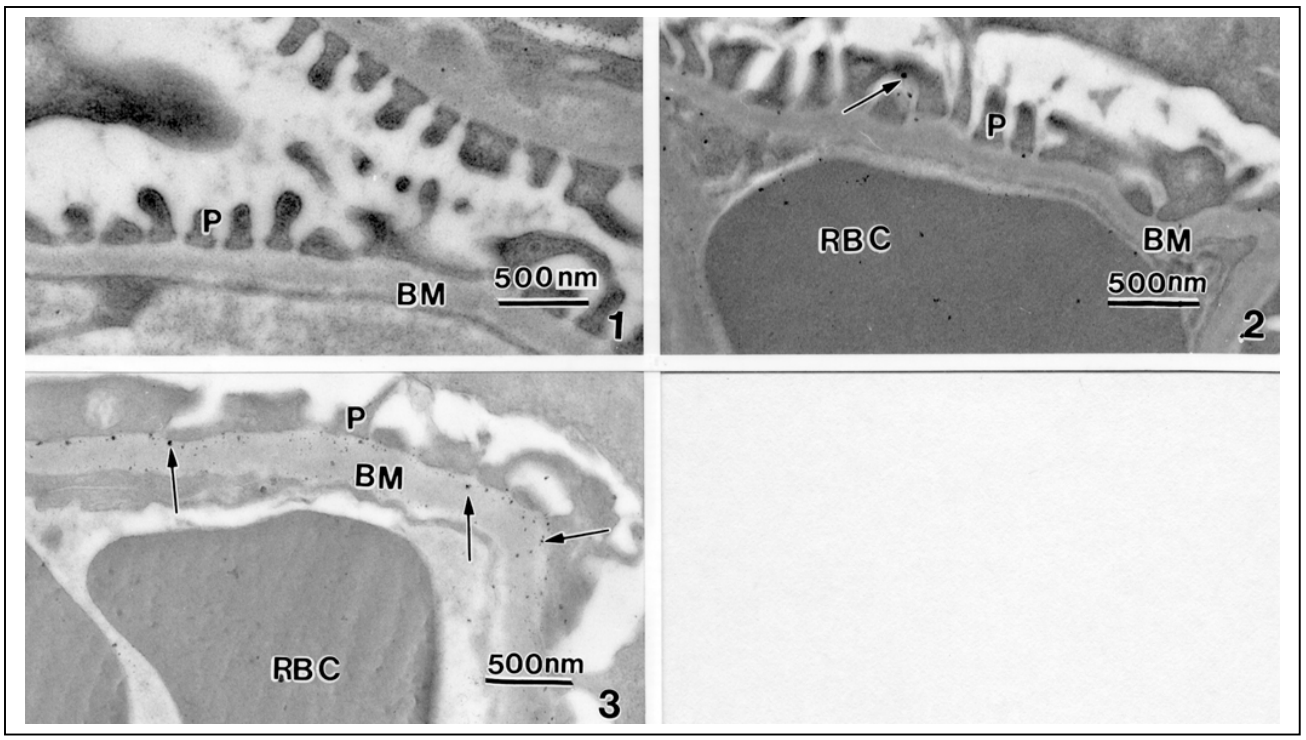

Fig. 1 Glomerular CBM in WT mouse. Basement membrane, BM; podocyte foot process, P.
Fig. 2 Glomerular CBM in iNOS KO mouse. Arrows indicate electron dense precipitate formed by localization of NADH oxidase. Basement membrane, BM; podocyte foot process, P; red blood cell, RBC.
Fig. 3 Glomerular CBM in eNOS KO mouse. Arrows indicate electron dense precipitate formed by localization of NADH oxidase. Basement membrane, BM; podocyte foot process, P; red blood cell, RBC.

Table 1. Glomerular CBM thickness in wild type and NOS knockout mice on a normal diet.

	CBM THICKNESS (nm)
WT Normal Diet	155.4 ± 18.6
iNOS KO Normal Diet	148.1 ± 16.6
eNOS KO Normal Diet	**195.6 ± 22.7**

Numbers in bold are significantly different, $p = 0.05$.

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