The Relation of Retinal Vessel Diameter to Changes in Diabetic Nephropathy Structural Parameters in Type 1 Diabetes

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Abstract

Aims/hypothesis—To examine whether retinal vessel diameter in persons with type 1 diabetes mellitus (T1DM) is associated with changes in subclinical anatomic and functional diabetic nephropathy indicators.

Methods—Persons with T1DM had both gradable fundus photographs and renal biopsy data at baseline and 5-year follow-up (n=234). Retinal arteriolar and venular diameters were measured at baseline and follow-up. Central retinal arteriolar (CRAE) and central retinal venule equivalent (CRVE) were computed. Baseline and 5-year follow-up renal structural parameters (e.g., mesangial fractional volume and glomerular basement membrane width (GBMW) and the mesangial matrix fractional volume and GBMW composite glomerulopathy index) were assessed by masked electron microscopic morphometric analyses from percutaneous renal biopsy specimens.

Results—While controlling for other covariates, baseline CRAE was positively associated with change in the glomerulopathy index over the 5 year period. Change in CRAE was inversely related to a change in mesangial matrix fractional volume while change in CRVE was directly related to change in the volume fractions of cortex which was interstitium [Vv(Int/cortex)] over the 5-year period. Baseline CRAE or CRVE or changes in these diameters were not related to changes in other renal anatomic or functional endpoints.

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Conclusions/interpretation—In our study, independent of other factors, baseline CRAE correlated with changes in glomerulopathy index, a composite measure of extracellular matrix accumulation in the mesangium and GBM, while a narrowing of the CRAE was related to mesangial matrix accumulation. Changes in CRVE were related to changes in Vv(Int/cortex), a measure of interstitial expansion in persons with T1DM.

Keywords

Areas 1.02; Research field epidemiology 3.01.01; 3.06.05 nephropathy; 3.06.10 microvascular disease

Despite intensive glycemic and blood pressure (BP) control, diabetic nephropathy (DN) remains an important cause of morbidity and mortality in persons with type 1 diabetes mellitus (T1DM) [1–6]. Detection of persons at risk of DN prior to the development of clinical signs of glomerulopathy is essential in developing preventive approaches at an early reversible stage of the disease [6]. It is, therefore, important to anticipate progression of DN at its preclinical stages. In particular, renal lesions, through much of the natural history of DN, develop in clinical silence (Figure 1). Because carefully measured renal biopsies are not likely to become the standard in the care of persons with T1DM and because renal lesions may already be present before standard tests of renal dysfunction (e.g., microalbuminuria [MA]) reveal it, it is important to have earlier DN risk predictors [7]. Moreover, additional indicators are needed because MA, although useful, is an imprecise predictor of progression to proteinuria (P). We have previously reported significant associations between diabetic retinopathy (DR) and preclinical morphologic changes of DN in baseline assessments of normoalbuminuric (NA), normotensive T1DM persons in the Renin-angiotensin System Study (RASS) [8]. There is suggestive evidence that an additional measurement, assessment of retinal vessel diameter, might also be a useful predictor of chronic kidney disease in persons with T1DM [9,10]. In this report we examine whether retinal vessel diameter (Figure 2) in RASS patients is associated with changes in subclinical anatomic and functional signs of DN.

Research Design and Methods

Description of Cohort

The RASS was a parallel, double-blind, placebo controlled, multicenter, clinical trial of DN primary prevention and retinopathy development and progression conducted at three clinical centers in Minneapolis, Minnesota, United States and Montreal, Quebec and Toronto, Ontario, Canada. The study design and cohort description have been detailed elsewhere [11]. All data were collected with Institutional Review Board approval in conformity with all federal, state, and provincial laws, and the study was in adherence with the tenets of the Declaration of Helsinki as revised in 1983. Informed consent was obtained. Subjects were 15 years of age or older with 2 to 20 years of T1DM and onset before their 45th birthday. All were normotensive (<135/85 mmHg), NA (AER < 20 µg/min on at least 2 of 3 timed overnight urine collections), and had normal or increased GFR (GFR $\geq$ 90 ml/min/1.73 m^2). Two hundred and eighty-five subjects were randomized into one of the following three treatment groups: losartan (an angiotensin-receptor blocker or ARB), enalapril (an angiotensin converting enzyme inhibitor or ACEI), or placebo [11].

Blood Pressure, Weight, Height, and Retinal Measurements

The baseline examination included measurement of BP with the participant seated after resting for 5 minutes, using an automated BP device (DinaMap Vital Signs Monitor #18465X, Critikon Inc., Palatine, IL, USA). Height and weight were measured according to standard anthropometric procedures. Pupils were dilated, and 30° color stereoscopic fundus photographs

were taken of the seven standard fields as defined in the Early Treatment Diabetic Retinopathy Study (ETDRS) protocol [12]. The photographs were graded in a masked fashion by the University of Wisconsin Ocular Epidemiology Reading Center using the modified Airlie House Classification scheme and the ETDRS retinopathy severity scale. Grading protocols and retinopathy severity scales have been described in detail elsewhere [12,13]. DR severity, based on the more severe eye, was grouped as follows: None (Level 10), Mild DR (Levels 20–43) and Moderate to Severe DR (Levels ≥ 47).

Retinal vessel diameters were measured at baseline and at the 5-year follow-up RASS examinations using a computer-assisted technique based on a standard protocol which entailed the following: retinal photographs of field 1 (centered at the optic nerve head), were converted to digital images by a high-resolution scanner using identical settings for all photographs [14]. Retinal vessel measurements were done independently for each examination and each eye. Trained graders masked to participant characteristics measured the diameters of all arterioles and venules coursing through a specified area one-half to one disc diameter surrounding the optic disc using a computer software program. On average, between 7 and 14 arterioles and an equal number of venules were measured per eye. Individual arteriolar and venular measurements were combined into summary indices that reflect the average retinal arteriolar (central retinal arteriole equivalent [CRAE]) and venular (central retinal venule equivalent [CRVE]) diameter of an eye based on the Parr-Hubbard-Knudtson formula [15]. Graders regularly participated in quality control exercises; the inter-and intra-grader variability was small (interclass and intraclass correlations > 0.90 for CRAE and CRVE.

Measures of Renal Function and Glycemia

GFR was estimated at baseline and follow-up by plasma disappearance of nonradioactive iohexol (Omnipaque 300, Shearing Co. as detailed elsewhere and expressed in ml/min adjusted up to $1.73m^2$) [16,17]. AER was measured in timed overnight urine collections using a sensitive fluorescence immunoassay [18] and expressed in μg/minute. NA was defined as having at least two of three AER readings < 20 μg/min. Glycosylated hemoglobin was measured by HPLC (DIAMAT glycosylated hemoglobin analyzer, BioRad, Hercules, CA, USA) until April 2002 when the TOSOH method was introduced (Tosoh Medics Inc., San Francisco, CA, USA) [19].

Electron Microscopy (EM)

Baseline percutaneous renal biopsies were obtained within six months prior to and follow-up biopsies five years after randomization [20]. Tissue processing was as described elsewhere [8]. The center-most intact glomeruli in the EM blocks were selected for study and an image calibration grid was obtained at the beginning of each microscopy session [21]. EM digital images (X3900) stored in tagged image file format (TIFF) format (Adobe Photoshop 6.0 software, San Jose, CA, USA) were used to build montages of entire glomerular cross-sections on which counting grids were superimposed. These montages were used for the measurement of the fraction of the glomerulus occupied by the mesangium or mesangial fractional volume [Vv(Mes/glom)], mesangial matrix fractional volume [Vv(MM/glom)], mesangial cell fractional volume (Vv(MC/glom)), and surface density (surface/volume) of peripheral glomerular capillary GBM [Sv(PGBM)/glom] [8,21–26]. After the center-most glomerulus in a block was randomly entered, 10 to 20 evenly-spaced digital images representing an unbiased systematic sample of approximately 30% of the glomerular profile was obtained at x11,000 for measurement of GBM width [21,22,24,25] and mesangial composition [8,21,26].

A glomerulopathy index (GI = GBM width/10 + [Vv(MM/glom) × 100]), a composite expression of glomerular extracellular matrix accumulation in diabetes, was computed using

the formula as described by Rudberg et al. [27]. All EM determinations were by a single masked observer.

Light microscopy

Zenker's fixed periodic acid-Schiff stained light microscopy slides were randomly selected for determining the index of arteriolar hyalinosis (IAH) in all cortical arterioles ≤ 1 average tubular diameter in size based on the fraction of each arteriolar wall replaced by hyaline as simultaneously estimated by two masked observers. IAH was calculated as published [22]. Volume fractions of cortex which was interstitium [Vv(Int/cortex)] and of cortical tubules per total tubules which were atrophic [Vv(AT/TT)] were estimated by a masked observer at X300 by point counting [28].

Statistics

Statistical analyses were conducted in SAS version 9 (SAS Institute Inc., Cary, NC, USA). Means were compared for statistically significant differences by the t-test and analysis of variance when two or more than two groups, respectively, were involved. Pearson correlation coefficients were computed for pair-wise associations of variables. Multivariable associations between renal anatomic and functional endpoints (and changes in these measures) with CRAE and CRVE and other independent variables such as age, sex, duration of diabetes, glycosylated hemoglobin, BP, and body mass were explored by multiple linear regression. Similarly, multivariable relationships between renal anatomic and functional characteristics defined as normal or abnormal with CRAE or CRVE and other independent variables were evaluated by logistic regression and selected covariates in the model were chosen because they were statistically significantly associated with the specific outcome.

Results

Characteristics of the cohort baseline examination

Of the 285 participants, one had insufficient glomeruli for renal morphometric measurements, 28 had renal biopsy data without gradable fundus photographs for retinal vessel measurement, and 257 had both gradable fundus photographs and biopsy data at baseline. Persons without gradable fundus photographs and biopsy data were older than those without gradable fundus photographs and biopsy data (p=0.002); there were no statistically significant differences between groups for other characteristics [8].

Baseline renal and retinal characteristics of the cohort are presented in Table 1 for 257 of the 285 persons with data available for both vessel measurement and at least one of the anatomic or functional DN measures. The cohort had a mean age of 30.2 ± 9.6 years, a mean duration of T1DM of 11.3 ± 4.7 years, and a mean glycosylated hemoglobin level of $8.5 \pm 1.6\%$. Ninety-two per cent of the cohort had no (36.6%) or mild (55.3%) DR (Table 1).

Relationships of CRAE and CRVE to Change in Renal Endpoints

Of the 257 participants with both gradable retinal vessel measurements and biopsy data at baseline, 234 had both gradable fundus photographs and biopsy data at follow-up. Persons with biopsy data at follow-up were younger than those without (p=0.03); additionally, persons without both follow-up biopsy data and gradable fundus photographs had larger baseline Vv (Mes/glom) (p=0.04) and Vv(MM/glom) (p=0.002) than those with biopsy data, otherwise, there were no statistically significant differences between groups for other characteristics [8].

The univariate relationships of baseline retinal characteristics to changes in renal endpoints are presented in Table 2. CRAE was associated with change in glomerulopathy index (GI), while

CRVE was associated with change in Vv(MM/glom), Sv(PGBM/glom), and GI. Relationships of CRAE to changes in renal endpoints, controlling for other factors are presented in Tables 3 and 4. While controlling only for duration of diabetes, CRAE was associated with change in GBM width, Vv(MM/glom), GI, and Sv(PGBM/glom). The relationship of CRAE with change in GI remained statistically significant after adding other covariates to the model. These relationships did not change when DR severity was added to the models (data not shown). Other relationships of CRAE and changes in renal structural endpoints were not statistically significant.

While controlling for duration of diabetes, CRVE was positively associated with change in Vv (MM/Glom), GI, Sv(PGBM), and with the incidence of abnormal GBM, and abnormal GI (Tables 3 and 4). None of these relationships remained statistically significant while controlling for other covariates in the model.

Relationships of Change in CRAE and CRVE to Changes in Renal Endpoints

Changes in CRAE over the five year period were related to changes in Vv(MM/glom) (r=−0.14, p=0.04) and incident abnormal Vv(MM/glom) (r=−0.17, p=0.03) while changes in CRVE were related to changes in Vv(Int/cortex) (r=0.20, p<0.01). The relation of changes in CRAE remained associated with changes in Vv(MM/glom) and incident abnormal Vv(MM/glom) and that between changes in CRVE and changes in Vv(Int/cortex) while controlling for other risk factors (data not shown). Changes in CRAE or CRVE were not statistically significantly associated with changes in other renal parameters (data not shown).

Discussion

Changes in retinal vessel diameters occur early in the course of T1DM, possibly reflecting structural and/or functional alterations resulting from changes in retinal blood flow and the effects of chronic hyperglycemia, inflammation, and endothelial dysfunction [29,30]. Retinal arteriolar narrowing (a small CRAE), for example, is thought to be related to cumulative small vessel damage from the effects of hypertension while retinal venular widening (a large CRVE) has been thought to reflect retinal hyperperfusion and/or lactic acidosis associated with hyperglycemia and hypoxia (Figure 2) [14,29,30]. In persons with T1DM, independent of DR severity, narrower retinal arteriolar and wider retinal venular diameters are thought to reflect microvascular changes from processes described above [31–38]. These may be markers of similar changes in the microvascular diameters in the cerebral, coronary, peripheral, and renal circulations in persons with or without T1DM or indicators of pathogenetic processes damaging to other targets of diabetic microvascular injury [32,38,39].

The RASS is the first study to examine the relationship of CRAE and CRVE with changes in diabetic renal structural parameters in sequential research biopsies in T1DM persons. CRAE and CRVE were associated with abnormalities and increases in renal structural parameters which reflect extracellular matrix accumulation, the central pathologic processes leading to renal dysfunction in DN [7,40]. These included increases in GBM width and mesangial matrix fractional volume that, as the composite GI, are closely associated with albuminuria in T1DM and decrease in glomerular filtration surface density [Sv(PGBM] which results from mesangial expansion [40] and are ultimately associated with GFR loss in T1DM (Figure 1) [23].

In the main, these relationships were independent of diabetes duration or DR severity. Speculation on these associations could take several directions. Increased CRVE and CRAE could be related to increased retinal blood flow which was perhaps mirrored by increases in renal blood flow, long posited as a candidate mechanism for DN pathogenesis through hemodynamic injury [41]. Alternately, both increased retinal and renal blood flow could reflect

compensatory responses to metabolic disturbances which could be the drivers of tissue injury in diabetes, such as relative tissue hypoxia [42] or oxidative stress [43].

Regardless of the mechanism, the RASS, which directly compares structural changes at relatively early stages of DN and DR, adds information indicating important relationships between these two microvascular complications which could not be derived from renal functional studies, since these were all normal at RASS baseline. It is not clear from these data whether the associations between the baseline retinal diameters parameters and the change in important DN structural parameters in T1DM patients without renal functional abnormalities will provide added precision to early DN risk predictions [44]. In contrast to the relationship of baseline CRAE and renal structural change as measured by GI, an increase in the CRAE over the 5 years of RASS was inversely related to Vv(MM/glom). Clearly, longer-term studies involving larger numbers of type 1 patients with hypertension and albuminuria would be necessary to further describe and unravel these complex dynamic relationships.

In the RASS, an increase in the CRVE was related to an increase in Vv(Int/cortex). The latter is measure of interstitial expansion which at these earlier stages of DN would mainly result from an increase in the cellular component of the interstitium [28]. Wider retinal venules are associated with systemic inflammation, consistent with the finding that the latter may be involved in the early increase in Vv(Int/cortex) [45].

In the RASS, there were no relationships found between retinal vessel diameters and renal functional endpoints. This was in contrast to the findings in persons with T1DM in the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) [9]. In that study, there was an increased risk of incident proteinuria (RR comparing 4th to 1–3rd quartiles, 1.53, 95% CI 1.19, 1.97], and renal insufficiency (RR 1.41, 95% CI 1.05, 2.17) in persons with wider retinal venular diameters independent of age, sex, duration of diabetes, glycosylated hemoglobin levels, baseline DR severity, and other factors. No relation was found with retinal arteriolar diameter at baseline with these renal endpoints in that study. Comparisons of RASS findings with those from the WESDR are limited due to differences in the two cohorts. The findings from the WESDR are with functional outcomes in a diabetic cohort that included persons with hypertension and microalbuminuria at baseline, while those in the RASS cohort were all normotensive and NA at baseline. Moreover, WESDR participants were studied in a period of poorer glycemic control. Perhaps if the RASS had included hypertensive albuminuric persons with longer duration of type 1 diabetes, as in the WESDR, stronger associations of retinal vessel diameters with renal anatomic abnormalities might have been found.

There are a number of strengths to our study, including the objective determination of retinal vessel caliber using standardized protocols and the use of careful morphometric techniques in 2 research renal biopsies spaced 5 years apart. However, caution should be observed in interpreting the findings herein. It is possible that the ARB or ACEI treatment used in the RASS masked a possible relationship of retinal vessel measurements to renal endpoints. Although, neither treatment arm in our study was shown to have a protective affect on renal structural or functional endpoints [46], both ARB and ACEI treatment significantly reduced retinopathy progression in RASS. This may have limited detection of associations of changes in CRAE or CRVE and renal structural or functional endpoints that might have been present in a natural history study. However, when the analyses were limited to the 85 persons in the placebo group the associations of CRAE or CRVE to the renal endpoints were essentially unchanged (Klein R. Unpublished data, 2010). Also, our study included estimates of CRAE and CRVE taken at a single and random point in the pulse cycle. However, we have previously shown that the effect of variability from the pulse cycle was not large and was unlikely to affect any of the associations under study [47]. It is possible that the measure chosen as a marker of narrowing of the retinal arterioles, CRAE, may not have been as sensitive as other measures of structural

and physiological changes in the microvasculature, e.g., retinal arteriole wall-to-lumen ratio, resulting in diminishing our ability to demonstrate stronger relationships with renal structural abnormalities [48].

In summary, retinal vessel diameters were related to the early stages of DN lesion development in matrix accumulation and interstitial expansion but were unrelated to changes in renal function in normotensive NA persons with T1DM. At this point in time we can only speculate that widened retinal venular diameter reflects the cumulative effects of various diabetic processes on the systemic microvasculature, including the kidney. However, it is too early to use this measure clinically to determine risk for renal anatomic damage prior to the appearance of functional changes, e.g., albuminuria and decreasing glomerular filtration in people with type 1 diabetes

Acknowledgments

Abbreviations used

ACEI	Angiotensin-receptor-converting enzyme inhibitor
ARB	Angiotensin-receptor blocker
BP	Blood pressure
CRAE	Central retinal arteriole equivalent
CRVE	Central retinal venule equivalent
DN	Diabetic nephropathy
DR	Diabetic retinopathy
ETDRS	Early Treatment Diabetic Retinopathy Study
GBMW	Glomerular basement membrane width
GI	Glomerulopathy index
H	Retinal hemorrhages
IAH	Index of arteriolar hyalinosis
IRMA	Intraretinal microvascular abnormalities
MA	Microalbuminuria
NA	Normoalbuminuric
P	Proteinuria
RASS	Renin-angiotensin System Study
SCr	Serum creatinine
SP	Standard photo

| Sv(PGBM)/glom | Surface density (surface/volume) of peripheral glomerular capillary glomerular basement membrane |
T1DM	Type 1 diabetes mellitus
TIFF	Tagged image file format
Vv(AT/TT)	Cortical tubules per total tubules which were atrophic
Vv(Int/cortex)	Volume fraction of cortex which was interstitium
Vv(Mes/glom)	Mesangial fractional volume per glomerulus
Vv(MM/glom)	Mesangial matrix fractional volume per glomerulus
Vv(MC/glom)	Mesangial cell fractional volume per glomerulus
WESDR	Wisconsin Epidemiologic Study of Diabetic Retinopathy

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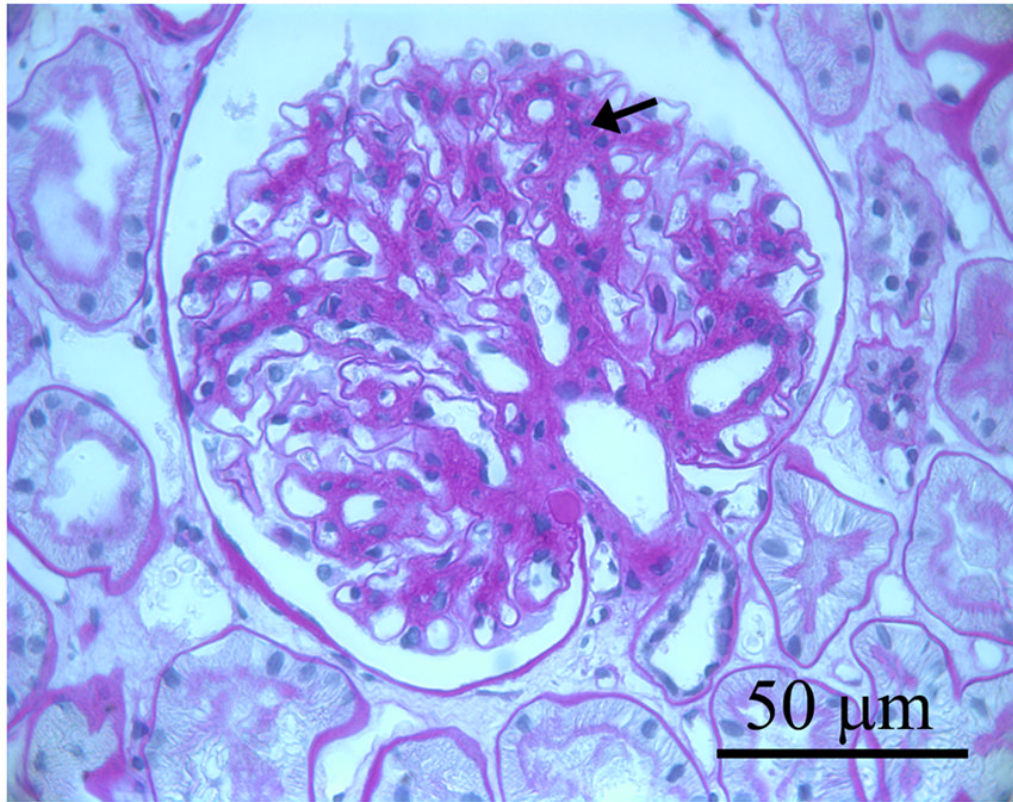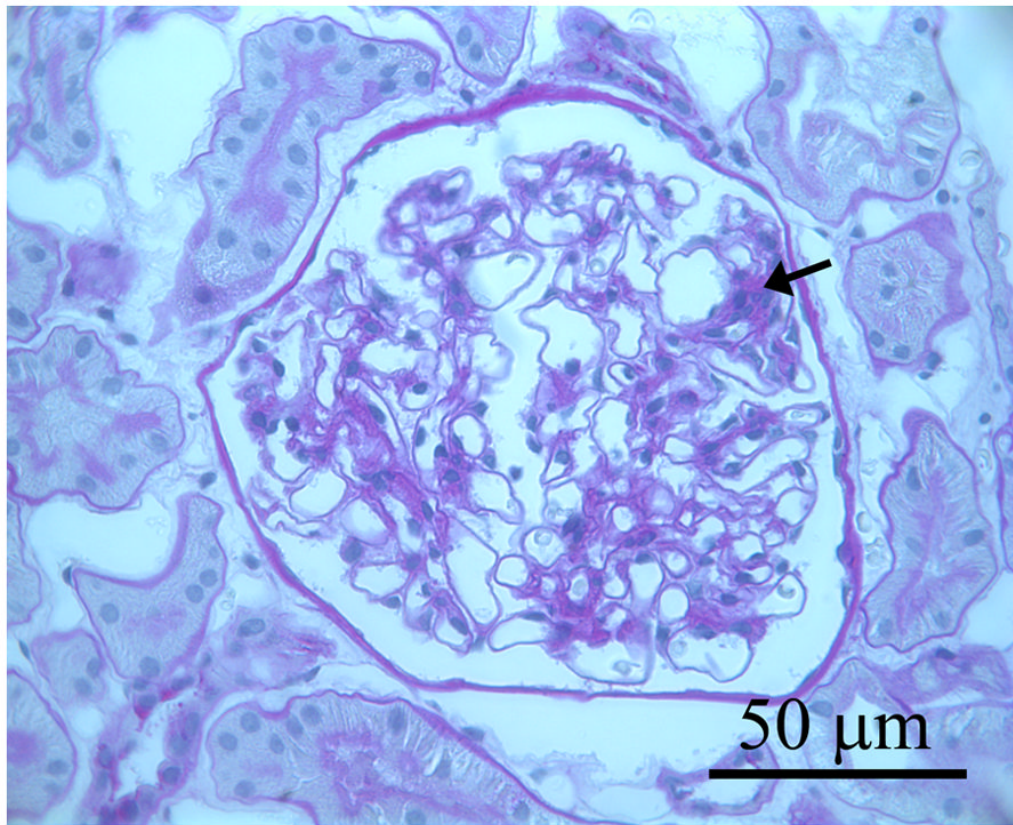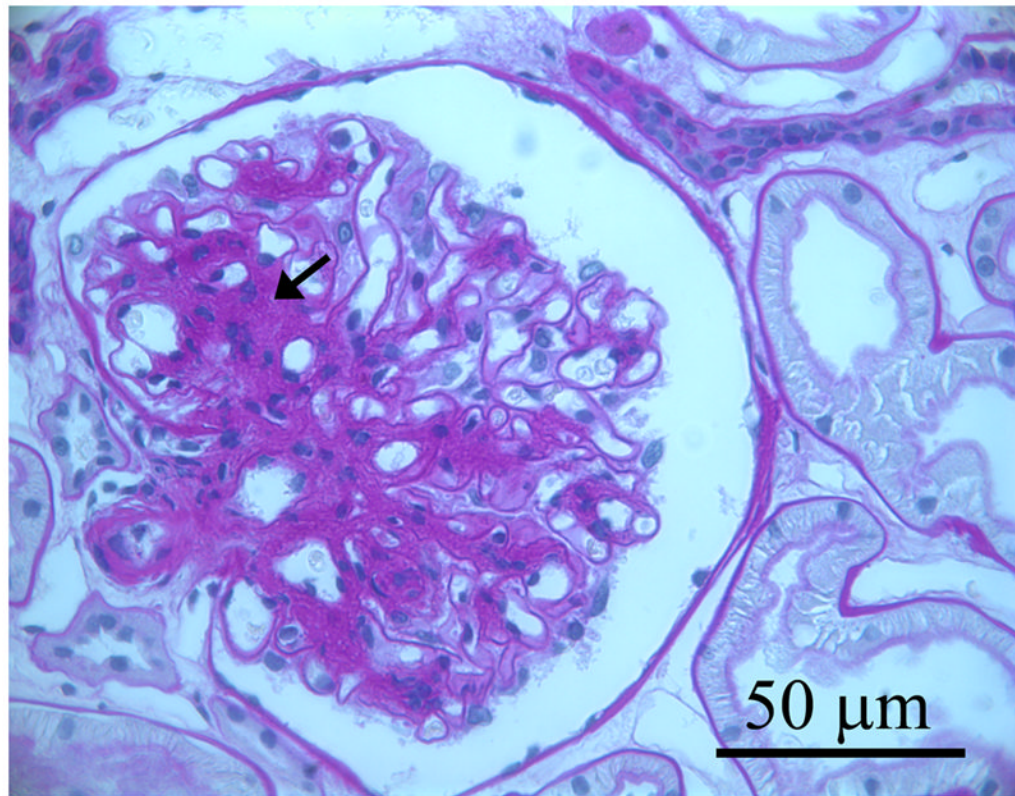
Figure 1.
Three photomicrographs from three separate baseline biopsies of glomerular tissues for light microscopy that were fixed in Zenker's solution and embedded in paraffin and stained with periodic acid Schiff (PAS): a. glomerulus in which the mesangium appears normal; b. shows a glomerulus with mild mesangial expansion with increased PAS positive matrix material between the capillary loops and branching from the base of the glomerulus (hilus) to the periphery (black arrows); c. shows a glomerulus with moderate mesangial expansion (black arrows).
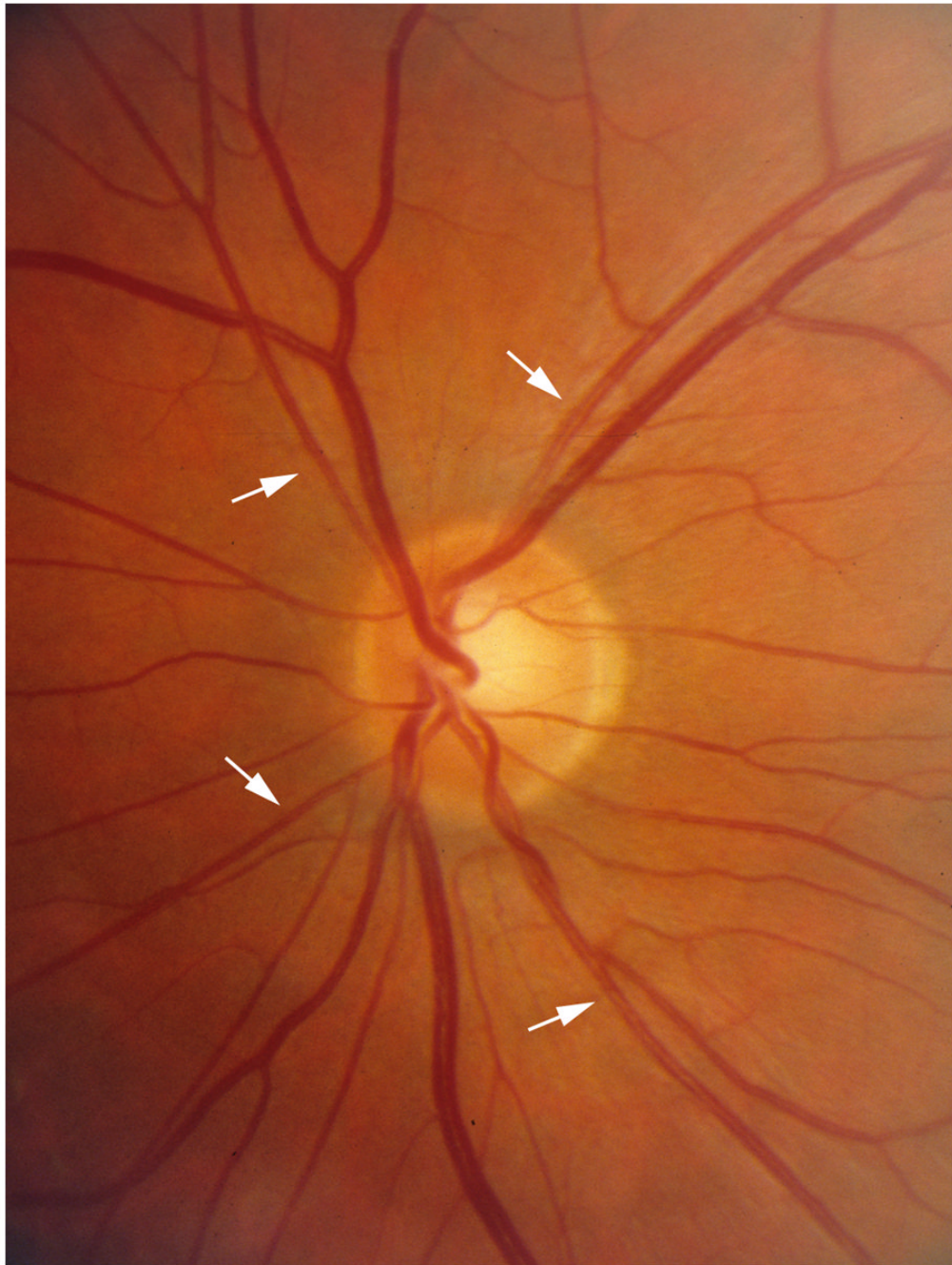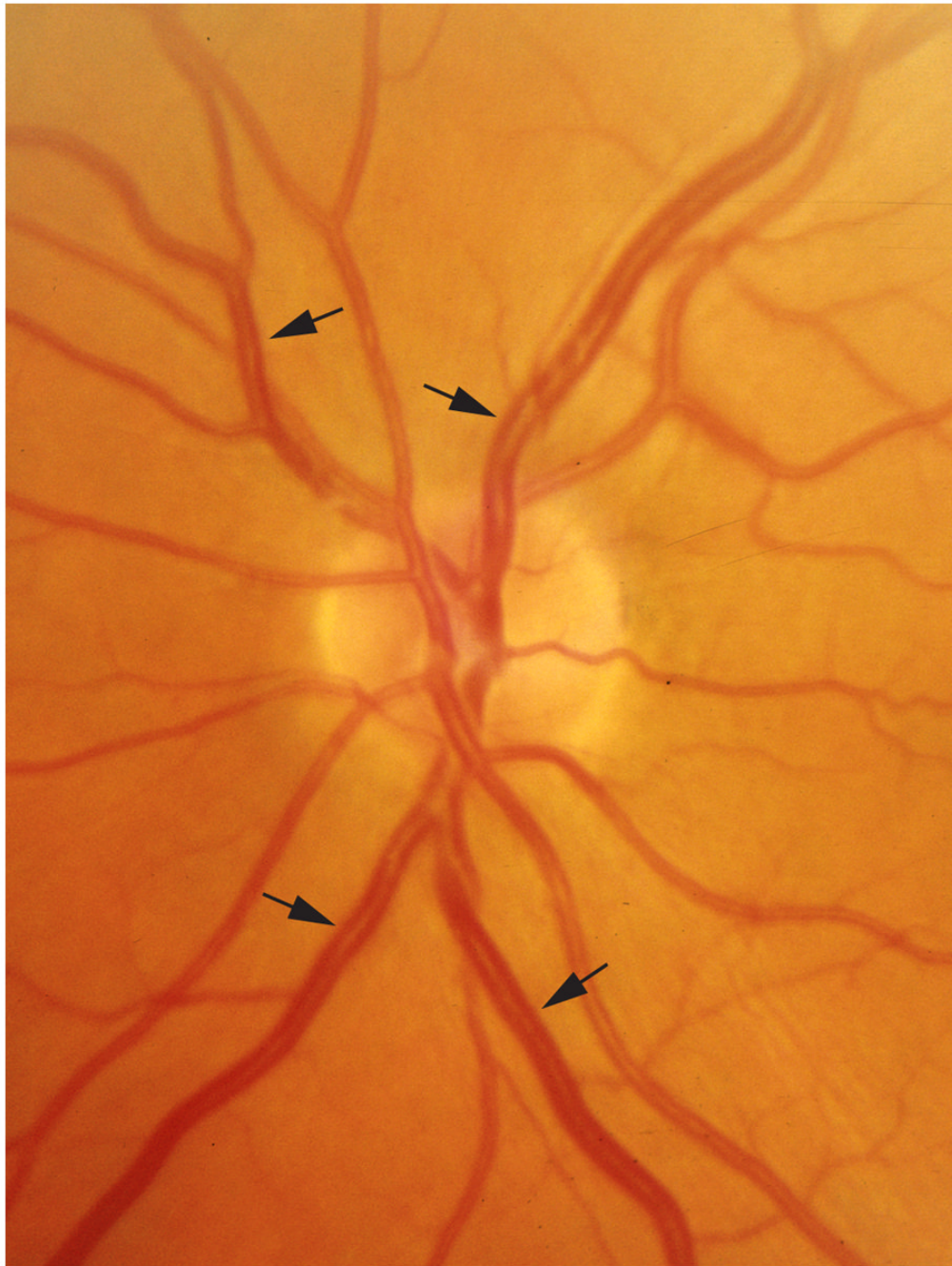
Figure 2.
a. Retinal image of left eye showing narrow retinal arterioles (white arrows, CRAE 99:m, mean and standard deviation in the RASS 159±13:m) and normal retinal venules (CRVE 222:m, mean and standard deviation in the RASS 228±22:m) and b. Retinal image of a left eye showing wide retinal venules (black arrows, CRAE 177 :m, CRVE 304 :m) and normal retinal arterioles.

Table 1

Baseline Characteristics of the Renin-angiotensin System Study Cohort.

Variable	*N*=257	
	Mean or %	SD
Age (years)	30.2	9.6
Sex (% male)	48	
Diabetes duration (years)	11.3	4.7
Glycosylated hemoglobin(%)	8.5	1.6
Body mass (kg/m^2)	25.8	3.7
Systolic blood pressure (mmHg)	120	12
Diastolic blood pressure (mmHg)	70	8
Mean arterial blood pressure (mmHg)	87	9
Serum creatinine (mg/dl)	0.81	0.14
Glomerular filtration rate (ml • min • 1.73 m^2)	129	20
Albumin excretion rate (μg/min)	6.4	5.9
Mesangial fractional volume/glomerulus[a]	0.21	0.04
Mesangial matrix fractional volume/glomerulus[a]	0.11	0.03
Mesangial cell fractional volume/glomerulus[a]	0.072	0.020
Glomerular basement membrane width[a] (nm)	476	92
Peripheral GBM[b] surface density/glomerulus[a]	0.13	0.02
Volume fractions of cortex which was interstitium[b]	0.110	0.036
Volume fraction cortical tubules per total tubules which were atrophic[c]	0.004	0.019
Index of arteriolar hyalinosis[d]	1.1	0.2
Glomerulopathy index[a]	58.1	10.6
Diabetic Retinopathy Severity		
None, %	34.6	
Mild, %	55.3	
Moderate to Severe, %	10.2	
Central retinal arteriole equivalent (μm)	159	13
Central retinal venule equivalent (μm)	228	22

GBM: glomerular basement membrane.

[a] *N* = 256

[b] *N* = 223

[c] *N* = 210

[d] *N* = 231

Table 2

Correlations of Central Retinal Arteriole Equivalent (CRAE) and Central Retinal Venule Equivalent (CRVE) at Baseline with Change in Diabetic Nephropathy Measures and Incident Abnormal Values over 5-year of Follow-up.

	N at risk[a]	CRAE		CRVE	
		r[b]	P	r[b]	P
Change in:					
Glomerular filtration rate	236	−0.03	0.61	0.00	0.98
Albumin excretion rate	234	0.00	0.96	0.01	0.93
Mesangial fractional volume/glomerulus	234	0.08	0.20	0.07	0.28
Mesangial matrix fractional volume/glomerulus	234	0.12	0.07	0.13	0.05
Mesangial cell fractional volume/glomerulus	234	−0.02	0.71	−0.00	0.99
Glomerular basement membrane (GBM) width (nm)	234	0.11	0.08	0.10	0.12
Peripheral GBM surface density/glomerulus	234	−0.11	0.09	−0.16	0.02
Volume fraction of cortex which was interstitium	183	−0.03	0.64	0.08	0.26
Volume fraction cortical tubules per total tubules which were atrophic	165	−0.09	0.23	−0.011	0.16
Glomerulopathy index	234	0.14	0.03	0.13	0.04
Incident abnormal:					
Mesangial fractional volume/glomerulus (n = 30)	206	−0.05	0.50	−0.04	0.61
Mesangial matrix fractional volume/glomerulus (n = 43)	165	0.11	0.14	0.12	0.13
Mesangial cell fractional volume/glomerulus (n = 20)	216	0.02	0.74	−0.00	0.95
GBM width (n = 27)	93	0.09	0.41	0.10	0.35
Peripheral GBM surface density/glomerulus (n = 61)	206	0.08	0.25	0.14	0.05
Glomerulopathy index (n = 27)	86	0.23	0.04	0.22	0.04

[a] Numbers at risk vary due to gradable measurement of nephropathy measures and those with prevalent abnormal values at baseline.

[b] r = Pearson correlation for continuous measures (top 9 rows) and Spearman correlation for categorical measures (bottom 6 rows).

Table 3

Relation of Central Retinal Arteriole Equivalent and Central Retinal Venule Equivalent at Baseline to Change in Renal Endpoint Models.

	Independent Variable							
	Central retinal arteriole equivalent per SD				Central retinal venule equivalent per SD			
	Duration adjusted		Multivariate[a] adjusted		Duration adjusted		Multivariate[a] adjusted	
Dependent Variable	Est (SE)	P	Est (SE)	P	Est (SE)	P	Est (SE)	P
Change in glomerular filtration rate	−0.52 (1.15)	0.65			0.08 (1.13)	0.94		
Change in albumin excretion rate	0.04 (1.39)	0.97			0.09 (1.37)	0.95		
Change in mesangial fractional volume/glomerulus	0.0051 (0.0033)	0.13			0.0042 (0.0033)	0.20		
Change in mesangial matrix fractional volume/glomerulus	0.0045 (0.0022)	0.04	0.0032 (0.0022)	0.14	0.0046 (0.0021)	0.03	0.0033 (0.0021)	0.13
Change in mesangial cell fractional volume/glomerulus	−0.0003 (0.0015)	0.86			0.0002 (0.0015)	0.89		
Change in volume fraction of cortex which was interstitium	−0.0032 (0.0044)	0.46			0.0040 (0.0043)	0.36		
Change in volume fraction cortical tubules per total tubules which were atrophic	−0.0012 (0.0036)	0.73			−0.0019 (0.0035)	0.58		
Change in glomerular basement membrane width (nm)	9.30 (4.47)	0.04	7.29 (4.43)	0.10	8.06 (4.38)	0.07	5.03 (4.36)	0.25
Change in glomerulopathy index	1.38 (0.55)	0.01	1.06 (0.53)	0.05	1.27 (0.54)	0.02	0.81 (0.52)	0.12

[a] From linear regression model.

[b] Multivariate adjustment:

Change in glomerular basement membrane (GBM) width (nm): diabetes duration, glycosylated hemoglobin, sex.

Change in glomerulopathy index: diabetes duration, glycosylated hemoglobin, sex, drug trial treatment.

Change in peripheral GBM surface density/glomerulus: diabetes duration, glycosylated hemoglobin, study site.

Change in mesangial matrix fractional volume/glomerulus: diabetes duration, glycosylated hemoglobin.

Table 4

Relation of Central Retinal Arteriole Equivalent and Central Retinal Venule Equivalent at Baseline to 5-year Incidence of Renal Abnormality Models.

	Independent Variable							
	Central retinal arteriole equivalent per SD				Central retinal venule equivalent per SD			
	Duration adjusted		Multivariate adjusted[a]		Duration adjusted		Multivariate adjusted[a]	
Incident Variable	OR (95% CI)[b]	P	OR (95% CI)[b]	P	OR (95% CI)[b]	P	OR (95% CI)[b]	P
Abnormal mesangial fractional volume/glomerulus	0.86 (0.58, 1.27)	0.45			0.88 (0.59, 1.31)	0.53		
Abnormal mesangial matrix fractional volume/glomerulus	1.38 (0.96, 1.98)	0.08			1.30 (0.92, 1.85)	0.14		
Abnormal mesangial cell fractional volume	1.03 (0.64, 1.64)	0.91			1.00 (0.63, 1.58)	0.99		
Abnormal glomerular basement membrane (GBM) width	1.36 (0.85, 2.19)	0.20			1.38 (0.85, 2.23)	0.19		
Abnormal peripheral GBM surface density/glomerulus	1.23 (0.90, 1.67)	0.19			1.37 (1.01, 1.85)	0.04	1.07 (0.76, 1.50)	0.70
Abnormal glomerulopathy index	1.93 (1.16, 3.20)	0.01	1.48 (0.76, 2.86)	0.25	1.81 (1.07, 3.05)	0.03	1.25 (0.64, 2.45)	0.51

[a]Multivariate adjustment:

Abnormal peripheral GBM surface density/glomerulus: diabetes duration, glycosylated hemoglobin, study site.

Abnormal glomerulopathy index: diabetes duration, glycosylated hemoglobin, age.

[b]OR (95% CI) from logistic regression model.