Retinopathy and RAAS Activation: Results From the Canadian Study of Longevity in Type 1 Diabetes

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OBJECTIVE

The importance of renin-angiotensin-aldosterone system (RAAS) activation in retinopathy for long-standing diabetes is not well understood. We determined retinopathy stage and evaluated associations with other vascular complications before and after physiological RAAS activation in adults with long-standing (≥50 years duration) type 1 diabetes.

RESEARCH DESIGN AND METHODS

Participants underwent retinal examination by digital funduscopic photography and optical coherence tomography and were classified as having nonproliferative diabetic retinopathy (NPDR), proliferative diabetic retinopathy (PDR), or no diabetic retinopathy (NDR) with or without diabetic macular edema (DME). Neuropathy was measured by clinical neuropathy examination scores, electrophysiologically, and by corneal confocal microscopy. Renal function was measured by inulin and para-aminohippurate clearance methods. Arterial stiffness was measured by applanation tonometry. Renal function, blood pressure, and arterial stiffness were measured before and after RAAS activation with angiotensin II (ANGII). Associations were determined using linear regression.

RESULTS

Twelve (16%) of the 75 participants had NDR, 24 (32%) had NPDR, and 39 (52%) had PDR. A low overall prevalence of DME (4%) was observed. Those with PDR had worse nerve function and reduced corneal nerve density, were more likely to have macrovascular disease, and had increased arterial stiffness in response to ANGII compared with those with NPDR or NDR. Prevalence of kidney disease or renal hemodynamic function did not differ by retinopathy status.

CONCLUSIONS

PDR was associated with neuropathy severity and cardiovascular and peripheral vascular disease. In those with PDR, RAAS activation may be linked to vascular stiffening, an effect that persists in long-standing type 1 diabetes.

Diabetic retinopathy is the most common cause of preventable blindness in individuals ages 20–74 years and is the most common vascular complication in type 1 and type 2 diabetes (1–3). On the basis of increasing severity, diabetic retinopathy is classified into nonproliferative diabetic retinopathy (NPDR), defined in early stages by the presence of microaneurysms, retinal vascular closure, and alteration, or proliferative diabetic retinopathy (PDR), defined by the growth of new aberrant blood vessels (neovascularization) susceptible to hemorrhage,

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leakage, and fibrosis (4). Diabetic macular edema (DME) can be present at any stage of retinopathy and is characterized by increased vascular permeability leading to retinal thickening.

Important risk factors for the development of retinopathy continue to be chronic hyperglycemia, hyperlipidemia, hypertension, and diabetes duration (5,6). Given the systemic nature of these risk factors, cooccurrence of retinopathy with other vascular complications is common in patients with diabetes. In the UK Prospective Diabetes Study (UKPDS), long-term hyperglycemia in people with type 2 diabetes, increased arterial stiffness, and hypertension were associated with risk of initiation and progression of diabetic retinopathy (7). Well-established associations also exist between diabetic retinopathy with progressive diabetic kidney disease (DKD) (8). Abnormal blood pressure (BP) patterns, such as nondipping (9), associates with diabetic retinopathy, and comorbid hypertension can accelerate vascular injury to the eye (10,11).

A key pathway implicated in diabetes-related small-vessel disease is overactivation of neurohormones. Activation of the neurohormonal renin-angiotensin-aldosterone system (RAAS) pathway predominates in diabetes in response to hyperglycemia and sodium retention. The RAAS plays a pivotal role in regulating systemic BP through vasoconstriction and fluid-electrolyte homeostasis. At the tissue level, angiotensin II (ANGII), the principal mediator of the RAAS, is implicated in fibrosis, oxidative stress, endothelial damage, thrombosis, inflammation, and vascular remodeling. Of note, systemic RAAS blockers reduce the risk of progression of eye disease but not DKD in adults with type 1 diabetes with normoalbuminuria (12).

Several longitudinal epidemiologic studies of diabetic retinopathy have been completed in type 1 diabetes; however, few have studied the relationships between eye, nerve, and renal complications and the influence of RAAS activation after prolonged duration (≥50 years) in adults with type 1 diabetes. As a result, less is known about mechanisms that persist in diabetes-related microvascular complications after long-standing diabetes. Accordingly, in this cross-sectional analysis from the Canadian Study of Longevity in Type 1 Diabetes involving adults with type 1 diabetes for ≥50 years, our aims were to phenotype retinopathy stage and determine associations between the presence of retinopathy and other vascular complications. In addition, we examined the relationship between retinopathy stage and renal and systemic hemodynamic function, including arterial stiffness, at baseline and dynamically after RAAS activation with an infusion of exogenous ANGII.

RESEARCH DESIGN AND METHODS

Study Design

This study represents a secondary analysis of the second phase of the Canadian Study of Longevity in Type 1 Diabetes, which was a cross-sectional cohort study of 75 participants with type 1 diabetes for ≥50 years and 75 age- and sex-matched control subjects. The primary research objective was to determine mechanisms of nephropathy resistance. In this report, we have included only results from participants with type 1 diabetes because age- and sex-matched comparators did not undergo retinal examinations. Participants completed 2 days of vascular measurement procedures (~2–4 weeks apart) in Toronto, Ontario, Canada, during the period from February 2015 to September 2016. Complete details of these vascular measurements have been outlined elsewhere (13). Participants with type 1 diabetes were recruited from the nationwide registry of ~450 Canadians with long-standing type 1 diabetes (≥50 years) established during the first phase of the Canadian Study of Longevity in Type 1 Diabetes and were invited to participate in the second phase on the basis of geography and proximity to the greater Toronto area, as previously described (14,15). All participants provided written informed consent before inclusion in this study, and the study was approved by the institutional research ethics boards of the University Health Network and Mount Sinai Hospital in Toronto, Ontario, Canada.

Experimental Procedures

Retinopathy Phenotyping

Participants with type 1 diabetes underwent retinal examination at the Toronto Western Hospital in the Donald K. Johnson Eye Centre on study day 1. The examination involved Optos 200Tx Ultra-widefield digital retinal imaging (Optos, Dunfermline, U.K.) and optical coherence tomography (OCT) (CIRRUS HD-OCT; Zeiss). On the basis of funduscopy results and Early Treatment Diabetic Retinopathy Study (ETDRS) classification, participants were divided into three categories: no diabetic retinopathy (NDR), NPDR, and PDR. The presence or absence of DME on OCT was determined.

Renal Hemodynamic Function Testing

For the purposes of these tests, all participants underwent RAAS inhibitor washout 30 days before the renal and vascular function testing, which occurred on study day 2. Renal hemodynamic function was measured at *1*) baseline, *2*) after a 0.5 ng/kg/min (low-dose) infusion of intravenous ANGII (51.2 μg/vial prepared in a 400 ng/mL solution) (Clinalfa; Bachem, Läufelfingen, Switzerland), *3*) after a 1 ng/kg/min (high-dose) infusion of intravenous ANGII, and *4*) during a 90-min recovery period. ANGII was administered to stimulate RAAS, which was a key component of the experimental design for the study's primary outcome. Initiation of ANGII was withheld if a participant's BP increased >150/80 mmHg, and ANGII was stopped if a participant's BP increased to >160/100 mmHg. For 7 days before study day 2, participants were instructed to maintain a minimum sodium intake of 150 mmol/day and a protein diet of 1.5 g/kg/day. For participants who could not tolerate RAAS withdrawal (consistent home BP readings >140/80 mmHg), calcium channel blockade with amlodipine was used (16,17).

After an overnight fast, participants arrived for study day 2, and peripheral intravenous catheters were placed for blood sampling, infusion of inulin and para-aminohippurate (PAH), and infusion of dextrose (5% dilution) or insulin (0.2 IU/mL dilution) (for euglycemic clamp). After a rest period of ~15 min, baseline blood and urine were collected. Ad libitum water consumption was allowed up to a maximum of 500 mL. Participants remained supine throughout the study but were allowed to ambulate for subjective voiding. All participants underwent the same experimental procedures, except that participants with type 1 diabetes underwent a minimum 2-h euglycemic clamp to achieve a constant blood

glucose range between 4 and 6 mmol/L before and during measurement of renal hemodynamic function and arterial stiffness. Blood was drawn for hematocrit, total protein, inulin, and PAH measurements.

Renal hemodynamic function was measured using inulin and PAH clearance techniques standardized per 1.73 m^2 body surface area, which measures the glomerular filtration rate (GFR_{INULIN}) and effective renal plasma flow ($ERPF_{PAH}$), respectively (18–20). Filtration fraction (FF) was determined by dividing the GFR_{INULIN} by the $ERPF_{PAH}$. Renal blood flow (RBF) was calculated by dividing the $ERPF_{PAH}$ by (1 − hematocrit). Renal vascular resistance (RVR) was derived according to mean arterial pressure divided by RBF, as described elsewhere (21,22).

Systemic Hemodynamic Function Testing

Arterial stiffness was measured using right-side radial artery waveforms by high-fidelity micromanometer (SPC-301; Millar Instruments) and central aortic pressure waveforms (SphygmoCor; AtCor Medical Systems, Sydney, New South Wales, Australia) before and after each dose of ANGII. Systemic arterial stiffness was determined by the augmentation index (AIx), calculated as the difference between the second systolic peak and inflection point, expressed as a percentage of the central pulse pressure and corrected to an average heart rate of 75 beats/min. The aortic pulse wave velocity (PWV) was measured by sequentially recording electrocardiogram-gated right-side carotid and radial artery waveforms. Our group has published and validated the use of the SphygmoCor device previously (23).

Large and Small Nerve Fiber Structure and Function Testing

During study day 1, all participants underwent neurological evaluations of signs and symptoms of neuropathy; the Toronto Clinical Neuropathy Score (TCNS) and Michigan Neuropathy Screening Instrument (MNSI) examination scores were calculated on the basis of these neurological evaluations (14,24). On study day 2, participants underwent nerve conduction studies using the Counterpoint device (Alpine Biomed, Fountain Valley, CA) and performed in accordance with the standards of the American Association for Neuromuscular and Electrodiagnostic Medicine; cooling detection threshold (CDT) testing using the TSA-II NeuroSensory Analyzer (Medoc, Ramat-Yishai, Israel); vibration perception threshold (VPT) testing using a neurothesiometer (Bailey Instruments Ltd, Trafford Park, U.K.); and in vivo corneal confocal microscopy using the Rostock Cornea Module of the Heidelberg Retinal Tomograph III (Heidelberg Engineering, Smithfield, RI) for determination of the corneal nerve morphological parameters corneal nerve fiber length (CNFL), corneal nerve fiber density (CNFD), and corneal nerve branch density (CNBD). CDT and VPT were determined using the limits algorithms method. CNFL, CNBD, and CNFD were determined using fully automated software (ACCMetrics 2.0 provided by X. Chen and M.A. Dabbah, University of Manchester, Manchester, U.K.).

Autonomic Function Testing

Heart rate variability (HRV) measurements were performed at baseline in all study participants with SphygmoCor using methods described previously (23). In brief, the device obtains an electrocardiographic recording using three electrodes placed on the patient's chest while lying supine. Two 10-min segments were recorded and averaged to measure vagal tone (root mean square successive difference), sympathetic activity (SD of normal-to-normal interval), and the ratio of low-power to high-power frequencies (LF/HF) of R-R intervals.

Definitions of Other Complications

Coronary artery disease was defined by self-reported diagnosis or by angina, angioplasty, or coronary artery bypass, and peripheral vascular disease was defined by self-reported diagnosis or leg angioplasty or leg bypass. Coronary artery calcification scoring also was used as described elsewhere (25). Nephropathy was defined by the presence of MDRD estimated GFR ($eGFR_{MDRD}$) $<$60 mL/min/1.73 m^2 or 24-h urine albumin excretion $\geq$30 mg/day. Neuropathy was defined by the presence of signs and/or symptoms corroborated by abnormal nerve conduction, according to consensus criteria (26).

Statistics

Statistical analyses were performed using SAS 9.4 for Windows software (SAS Institute, Cary, NC). We compared general clinical and biochemical characteristics between retinopathy classes using ANOVA, Kruskal-Wallis test, or χ^2 test, depending on variable distribution. Renal and systemic hemodynamic function at baseline and in response to ANGII infusion, large nerve fiber function, small nerve fiber structure and function, and autonomic assessments were compared using multivariable linear regression models; crude unadjusted *P* values for trend were calculated as well as those adjusted for age, sex, and HbA_{1c}. An α-level of 0.05 (two-sided) was used for tests of statistical significance. The planned sample size was based on previous studies in type 1 diabetes longevity cohorts of nephropathy resistance (27) and is explained elsewhere (28). We decided a priori not to perform corrections for multiple testing, and therefore these results should be considered hypothesis generating.

RESULTS

Baseline Parameters

Of the 75 participants, 12 (16%) had NDR, 24 (32%) had NPDR, and 39 (52%) had PDR (Table 1). At baseline, those with NDR had lower mean HbA_{1c} compared with those with NPDR and PDR (7.4 ± 0.7% and 7.5 ± 0.9%, respectively; *P* for trend = 0.019). Of note, those with more severe eye disease (PDR) had lower systolic and diastolic BP values but a significantly higher urine albumin-to-creatine ratio (UACR) (2.21 [interquartile range (IQR) 1.27, 6.00] vs. 1.29 [0.72, 2.60] vs. 1.14 [0.82, 1.97] mg/g; *P* for trend = 0.016) compared with those with less severe eye disease (NPDR) or with NDR despite higher use of RAAS inhibitors among those with PDR compared with NPDR or NDR. History of cardiovascular and peripheral vascular disease history was significantly higher in participants with PDR (33.3%) than in those with NPDR (8.3%) or NDR (0%). Diabetic sensory polyneuropathy was prevalent across all groups irrespective of retinopathy status but was numerically higher in the PDR group (95%) than in the NPDR (86%) or NDR (75%) groups. No significant differences were observed in retinal thickness across the three groups.

Measures of Neuropathy in Patients According to Retinopathy Status

Clinical measures of neuropathy, including the MNSI examination score,

Table 1—Clinical characteristics of study participants stratified by retinopathy status

Characteristic	NDR (*n* = 12)	NPDR (*n* = 24)	PDR (*n* = 39)	*P* value
Clinical				
Female sex	5 (41.7)	13 (54.2)	23 (59)	0.57
Age (years)	67.2 ± 9.1	65.6 ± 8.1	65.5 ± 7.4	0.81
Duration of type 1 diabetes (years)	53 (50, 55)	53 (50, 57)	55 (52, 59)	0.11
Onset of type 1 diabetes (age)	14.5 (7, 21.5)	12 (8, 16)	9 (6, 15)	0.30
Total daily insulin (units)	32.4 ± 9.3	37.9 ± 15.5	35.3 ± 12.9	0.53
Weight (kg)	67.7 ± 10.7	74.4 ± 12.8	73.0 ± 12.0	0.28
BMI (kg/m^2)	24.6 ± 2.8	27.1 ± 3.7	26.9 ± 4.0	0.14
Systolic BP baseline (mmHg)	135 ± 13	139 ± 15	128 ± 15	**0.03**
Diastolic BP baseline (mmHg)	77 ± 10	71 ± 9	67 ± 7	**<0.001**
Heart rate baseline (beats/min)	71 ± 12	68 ± 11	71 ± 12	0.66
RAAS inhibitor medication use	7 (58)	19 (79)	36 (92)	**0.02**
Statin use	7 (58)	21 (88)	32 (84)	0.09
Aspirin use	4 (33)	16 (67)	25 (66)	0.10
Biochemical				
HbA_{1c} (%)	6.7 ± 0.6	7.4 ± 0.7	7.5 ± 0.9	**0.02**
Glucose (mmol/L)	7.3 ± 2.9	8.1 ± 3.5	9.2 ± 3.8	0.22
Total cholesterol (mmol/L)	4.11 ± 1.21	3.80 ± 0.76	3.84 ± 0.60	0.49
HDL (mmol/L)	1.75 ± 0.45	1.58 ± 0.39	1.66 ± 0.48	0.57
LDL (mmol/L)	2.02 ± 0.90	1.86 ± 0.56	1.81 ± 0.37	0.50
Triglycerides (mmol/L)	0.75 ± 0.53	0.78 ± 0.38	0.80 ± 0.37	0.93
$eGFR_{MDRD}$ (mL/min/1.73 m^2)	80 ± 17	75 ± 15	68 ± 18	0.08
$eGFR_{MDRD}$ <60 mL/min/1.73 m^2	3 (25)	3 (12.5)	11 (28.2)	0.34
UACR (mg/mmol)	1.14 (0.82, 1.97)	1.29 (0.72, 2.60)	2.21 (1.27, 6.00)	**0.02**
UACR >2 mg/mmol	1 (8.3)	5 (20.8)	15 (38.5)	0.08
24-h urinary albumin (mg/day)	13 (9, 15)	11 (8, 15)	14 (10, 45)	0.052
24-h urinary albumin >30 mg/day	1 (8.3)	3 (12.5)	11 (28.2)	0.17
Renin concentration (ng/L)	10.57 ± 6.34	14.41 ± 29.56	20.85 ± 20.26	**0.044**
Aldosterone (pmol/L)	180 ± 87	159 ± 68	206 ± 121	0.60
Uric acid (μmol/L)	291 ± 66	270 ± 82	291 ± 98	0.62
Complications				
Coronary artery/peripheral vascular disease	0 (0)	2 (8.3)	13 (33.3)	**0.009**
DKD	3 (25)	6 (25)	16 (41)	0.34
Neuropathy	9 (75)	19 (86.4)	37 (94.9)	0.14
DME	0 (0)	2 (8.3)	1 (2.6)	0.40
OCT				
Central subfield thickness OD	262.2 ± 25.3	250.2 ± 43.2	263.6 ± 45.7	0.46
Central subfield thickness OS	275.8 ± 61.7	254.6 ± 39.6	252.8 ± 52.7	0.38
Cube volume OD	9.4 ± 0.7	9.6 ± 0.8	9.8 ± 1.0	0.38
Cube volume OS	9.7 ± 1.1	9.7 ± 0.8	9.8 ± 0.9	0.87
Cube average thickness OD	263.3 ± 19.3	268.0 ± 22.9	274.2 ± 28.7	0.38
Cube average thickness OS	270.5 ± 31.1	269.5 ± 21.6	273.2 ± 24.5	0.84

Data are mean ± SD, median (IQR), or *n* (%). Boldface indicates significance at $P < 0.05$. OD, right eye; OS, left eye.

indicated more severe impairment in the PDR group than in the NPDR or NDR groups (Table 2). In all studies of nerve conduction (including peroneal amplitude potential, conduction velocity, and f-wave latency as well as sural amplitude potential and conduction velocity), participants with PDR had more evidence of impaired nerve function than those with NPDR or NDR. In addition, in measures that reflect the most significant neurological involvement, including CDT at the toe, lower-limb VPT, and CNFD, participants with PDR had the most severe impairment compared with those with NPDR or NDR. For assessment of autonomic dysfunction measured by HRV, the LF/HF ratio was the lowest in the PDR group, reflecting a decrease in sympathetic tone relative to parasympathetic tone.

Measures of Renal Function, BP, and Arterial Stiffness Before and After RAAS Stimulation

At baseline, before stimulation with ANGII, no differences in renal hemodynamic function (GFR, ERPF, RVR, RBF, FF), BP, or arterial stiffness were observed across the groups irrespective of retinopathy status (Supplementary Table 1). Similarly, after stimulation with low-dose ANGII, renal hemodynamic function and systemic hemodynamics (reflected by BP) were comparable across the groups, and responses in peripheral vascular stiffening also did not change (Supplementary Table 2). In contrast, an exaggerated response in the PDR group was observed after high-dose ANGII, with significant increases seen in carotid-radial PWV compared with the NPDR and NDR groups, even after adjustment (Table 3).

CONCLUSIONS

Hyperglycemia contributes to the pathogenesis of diabetic retinopathy through multiple interactive pathways, including increased production of advanced glycation end products, IGF-I, vascular

Table 2—Neuropathy grading stratified by retinopathy status

	NDR (n = 12)	NPDR (n = 24)	PDR (n = 39)	P value	
				Crude	Adjusted
DSPN (by Toronto consensus)	9 (75)	19 (86.4)	37 (94.9)	0.14	0.14
Clinical neuropathy examination scores					
TCNS total (out of 19)	5.7 ± 4.1	6.0 ± 3.2	7.7 ± 4.1	0.12	0.14
TCNS symptoms (out of 6)	1.1 ± 1.5	1.0 ± 1.1	1.6 ± 1.4	0.22	0.26
TCNS sensory (out of 5)	1.4 ± 1.2	1.4 ± 1.2	1.9 ± 1.6	0.31	0.31
MNSI examination score (out of 10)	2.0 ± 1.6	2.8 ± 1.8	3.6 ± 2.2	**0.048**	**0.048**
Neuropathy symptom questionnaire score					
MNSI questionnaire score	1.7 ± 1.9	1.8 ± 1.9	2.5 ± 2.0	0.24	0.36
Nerve conduction studies					
Peroneal amplitude potential (μV)	2.7 ± 1.7	2.2 ± 1.6	1.3 ± 1.2	**0.003**	**0.003**
Peroneal conduction velocity (m/s)	39.0 ± 7.3	37.9 ± 7.1	34.0 ± 8.1	0.06	**0.047**
Peroneal f-wave latency (ms)	59.9 ± 9.3	60.9 ± 8.7	65.1 ± 5.9	0.06	**0.02**
Sural amplitude potential (μV)	4.1 ± 2.2	4.2 ± 3.6	1.9 ± 2.2	**0.003**	**0.003**
Sural conduction velocity (m/s)	39.7 ± 6.0	38.0 ± 6.4	34.6 ± 5.7	**0.02**	**0.03**
CDT					
At the toe (°C)	23.8 ± 5.1	23.7 ± 5.9	18.8 ± 7.1	**0.005**	**0.008**
VPTs					
Upper limb (V)	5.3 ± 1.6	6.0 ± 1.9	6.7 ± 2.0	0.12	0.23
Lower limb (V)	18.7 ± 7.6	20.1 ± 9.8	26.8 ± 10.6	**0.04**	**0.02**
Corneal nerve morphology					
CNFL (mm/mm^2)	8.5 ± 5.2	9.7 ± 4.8	7.3 ± 3.9	0.14	0.14
CNBD (branches/mm^2)	15.9 ± 22.1	17.0 ± 21.5	11.0 ± 12.8	0.77	0.46
CNFD (fibers/mm^2)	10.6 ± 11.4	13.49 ± 10.0	7.2 ± 6.7	**0.046**	**0.03**
HRV					
SDNN	36.5 ± 15.5	42.4 ± 24.4	39.4 ± 43.4	0.09	0.76
RMSSD	26.1 ± 17.2	31.0 ± 26.4	28.5 ± 32.7	0.46	0.80
LF/HF ratio	2.3 ± 2.2	3.3 ± 2.67	2.0 ± 1.5	0.13	**0.04**

Data are mean ± SD or n (%). Boldface indicates significance at $P < 0.05$. Adjusted P values have age, sex, and HbA_{1c} as covariates. DSPN, distal symmetric polyneuropathy; RMSSD, root mean square successive difference; SDNN, SD of normal-to-normal interval.

endothelial growth factor, endothelin, nitric oxide, oxidative damage, and proinflammatory cytokines (29–33). Overactivation of the RAAS in response to hyperglycemia also is implicated in the pathogenesis of diabetes-related complications in the retina, nerves, and kidney and is an important therapeutic target in type 1 diabetes. Despite what is known about these underlying pathogenic mechanisms in the early development of diabetes-related complications, whether the same mechanisms are active in the setting of longstanding type 1 diabetes is not known. Accordingly, the Canadian Study for Longevity in Diabetes gave a unique opportunity to study these important questions, providing insight into the prevalence of retinopathy and associations with other vascular complications to determine the influence of RAAS activation on these associations in adults with type 1 diabetes duration ≥50 years.

Table 3—Percent change in renal and systemic hemodynamic function in response to high-dose ANGII stratified by retinopathy status

Percent change	NDR (n = 12)	NPDR (n = 24)	PDR (n = 39)	P value	
				Crude	Adjusted
Measured					
GFR_{INULIN}	−5.2 ± 7.2	−4.1 ± 10.6	−7.0 ± 9.2	0.57	0.65
$ERPF_{PAH}$	−12.7 ± 8.1	−13.4 ± 5.9	−13.0 ± 7.3	0.96	0.95
RVR	24.7 ± 16.4	23.3 ± 12.4	27.8 ± 12.9	0.51	0.56
RBF	−13.2 ± 8.7	−14.3 ± 6.0	−13.3 ± 7.3	0.88	0.87
FF	8.9 ± 6.4	10.9 ± 10.7	7.4 ± 12.4	0.58	0.55
MAP	8.3 ± 7.1	5.2 ± 6.8	9.3 ± 6.1	0.09	0.08
Heart rate	2.3 ± 5.9	0.8 ± 6.0	4.8 ± 8.0	0.13	0.16
Arterial stiffness					
AIx					
Aortic AIx	5.0 ± 4.5	4.0 ± 6.5	3.2 ± 4.6	0.63	0.51
Carotid AIx	7.0 ± 4.7	3.8 ± 6.9	5.3 ± 5.8	0.35	0.37
PWV					
Carotid radial	−4.9 ± 11.43	8.2 ± 18.5	12.9 ± 20.2	**0.03**	**0.04**
Carotid femoral	7.9 ± 25.16	8.0 ± 24.5	9.2 ± 31.1	0.99	0.86

Data are mean ± SD. Boldface indicates significance at $P < 0.05$. ANGII dose 1 ng/kg/min. Adjusted P values have age, sex, and HbA_{1c} as covariates. MAP, mean arterial pressure.

In this study, we observed that participants with PDR were more likely to be taking RAAS inhibitors, to have a higher frequency of cardiovascular or peripheral vascular disease, and to have higher UACR levels, likely reflecting the higher overall risk profile of this group. Although it is not possible to determine why some patients in this cohort developed PDR while others did not after similar

durations of type 1 diabetes, it seems unlikely that glycemic control alone is sufficient to fully explain the observed between-group differences and differing vascular risk profiles. Whereas the NDR group had significantly lower mean HbA_{1c} levels than the NPDR and PDR groups, differences between participants with NPDR and those with PDR were modest. Accordingly, other factors, such as differences in vascular function, neurohormones, growth factors, genetics, and lifestyle, may play a role in determining retinopathy severity at the individual level.

The association between retinopathy and risk for DKD is well established in diabetes (34). In the setting of type 2 diabetes, patients with high levels of UACR have twice the risk of developing diabetic retinopathy than those with normal UACR levels. For example, Rodríguez-Poncelas et al. (35) demonstrated that impaired renal function is linked with increased diabetic retinopathy risk. Consistent with these studies and others, the PDR group in this Canadian Study of Longevity in Type 1 Diabetes demonstrated significantly higher UACR, which is associated with an increased risk of DKD progression, illustrating that the interaction between eye and kidney disease progression also may exist in patients with long-standing type 1 diabetes. Significant differences in measured renal hemodynamic function, however, were not observed among the retinopathy groups studied. This observation is perhaps not surprising, considering that the majority of participants in this study had preserved renal function ($eGFR_{MDRD}$ >60 mL/min/1.73 m^2). As such, as observed in other longevity cohorts, the study population is likely enriched with protective factors against DKD, which precludes us from making broader conclusions about retinopathy risk in patients with significant renal functional impairment. Moreover, the observation that PDR can be present without DKD, even after long-standing type 1 diabetes, highlights that divergent tissue-specific mechanisms that differentially affect the retinal versus renal vasculature may play an important role in the group studied. As a final comment, renal hemodynamic responses to an exogenous infusion of ANGII did not differ according to retinopathy status, suggesting that at least at the microvascular level, the degree of RAAS activation may not be helpful to ascertain retinopathy risk in this study group.

Although renal hemodynamic function did not differ significantly by retinopathy status, several measures of neuropathy were associated with retinopathy status. Clinical TCNS and MNSI examination scores and measures of nerve conduction were much slower in patients with PDR than in those with NPDR. Overall, the PDR group exhibited the most significant neurological dysfunction, again likely reflecting the higher risk profile of this group. Because neuropathy score responses to RAAS activation cannot be measured in humans, we are not able to comment on the potential contributory influence of RAAS activation on neuropathy severity in adults with long-standing type 1 diabetes. We did observe that participants with PDR exhibited lower LF/HF ratios when measuring HRV, thereby suggesting that the PDR group had less sympathetic tone compared with parasympathetic tone, possibly reflecting the autonomic dysfunction characteristic of diabetes. Therefore, future work should examine the relationship between factors associated with autonomic nervous system disease and the development of retinopathy, including BP regulation and diurnal patterns, factors implicated in the development of microvascular complications in younger cohorts (26,36–38).

Increased arterial stiffness is a marker of atherosclerotic disease and is linked to inflammation. In this study, we used arterial PWV as a measure of arterial stiffness and did not observe significant differences categorized by retinopathy severity either at baseline or after low-dose exogenous ANGII. Of note, in response to high-dose ANGII, the PDR group exhibited exaggerated carotid radial PWV and lower systolic and diastolic BP responses than the NPDR or NDR groups. These results are similar to those obtained by Tanaka et al. (33) that showed that PDR is associated with aortic PWV in Japanese patients with type 2 diabetes. Others have suggested that the stage of diabetic retinopathy may be associated with PWV through the accumulation of fluorescent advanced glycation end products, which also is associated with increased arterial stiffness (39). Although the mechanisms responsible for mediating these effects on vascular stiffening cannot be inferred from this study, we speculate that the PDR group's exaggerated response to RAAS in the peripheral vasculature might reflect increased ANGII responsivity at the ANGII receptor level as a result of heightened ANGII receptor expression or receptor affinity (16,23,31,40) or for reasons that remain to be determined. Consequently, cardiovascular and peripheral vascular diseases were more frequent among the PDR group, suggesting shared risk factors between those with retinal small-vessel disease and macrovascular disease in this cohort. Of note, Gordin et al. (41) recently studied the prevalence of cardiovascular disease, stratified by chronic kidney disease (CKD) and PDR status, in a type 1 diabetes longevity cohort in the U.S. They observed a lower prevalence of cardiovascular disease in those with CKD but without PDR than in those with CKD with PDR. They suggested that the higher prevalence of cardiovascular disease in those with PDR is reflective of systemic vascular dysfunction or that tissue-specific factors may be responsible.

The current study has several limitations worth highlighting, including the small sample size and cross-sectional design. As a consequence, we cannot imply causality of the observations in this study. Assumptions around glycemic and metabolic control were made on the basis of recent laboratory parameters, which may not reflect the average degree of glycemic control over an individual's diabetes duration. The results from this study cannot be applied to youth with type 1 diabetes, individuals who have only had type 1 diabetes for a short period, or individuals with type 2 diabetes. There is also the potential for survivorship bias in studying longevity cohorts. To be eligible for inclusion in this study, participants had to live with type 1 diabetes for ≥50 years. As such, those with progressive or advanced nephropathy or macrovascular disease may not have been captured in this longevity study because of related mortality, which limits the overall generalizability of these findings. Furthermore, we do not have detailed ophthalmic records or retinal examinations over time and recognize that some patients were being treated actively for retinopathy; thus, we cannot make inferences about progression of disease. Tissue-specific levels of RAAS in

the retina and kidney were not measured because of the invasive nature of capturing these data. As such, our conclusions around the influence of RAAS activation reflect systemic activation but do not necessarily reflect tissue-specific levels of local RAAS expression in the retina, nerves, or kidney. Finally, adjustments for multiple comparisons were generated only for age and sex, but residual confounding on these variables and other unmeasured or unknown confounders may still exist. Despite these limitations, the strengths of this study include gold standard measures of microvascular complications at baseline and after dynamic RAAS activation at two different physiological doses.

In conclusion, retinopathy was prevalent after prolonged type 1 diabetes duration, and retinopathy severity associated with several measures of neuropathy and with higher UACR. Differential exaggerated responses to RAAS activation in the peripheral vasculature of the PDR group highlights that even in the absence of DKD, neurohormonal abnormalities are likely still operant, and perhaps accentuated, in patients with PDR even after long-standing type 1 diabetes duration.

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