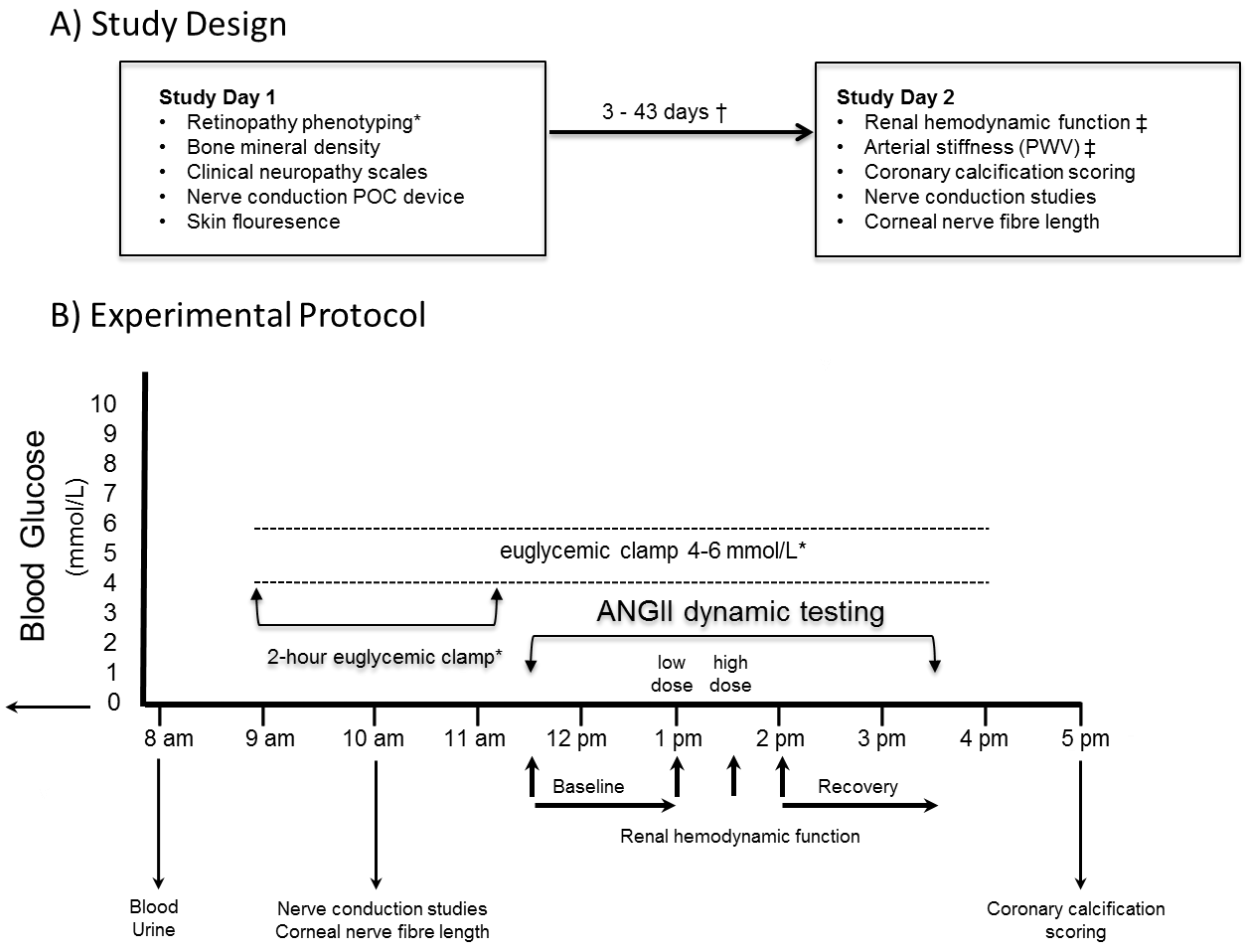
SUPPLEMENTARY DATA

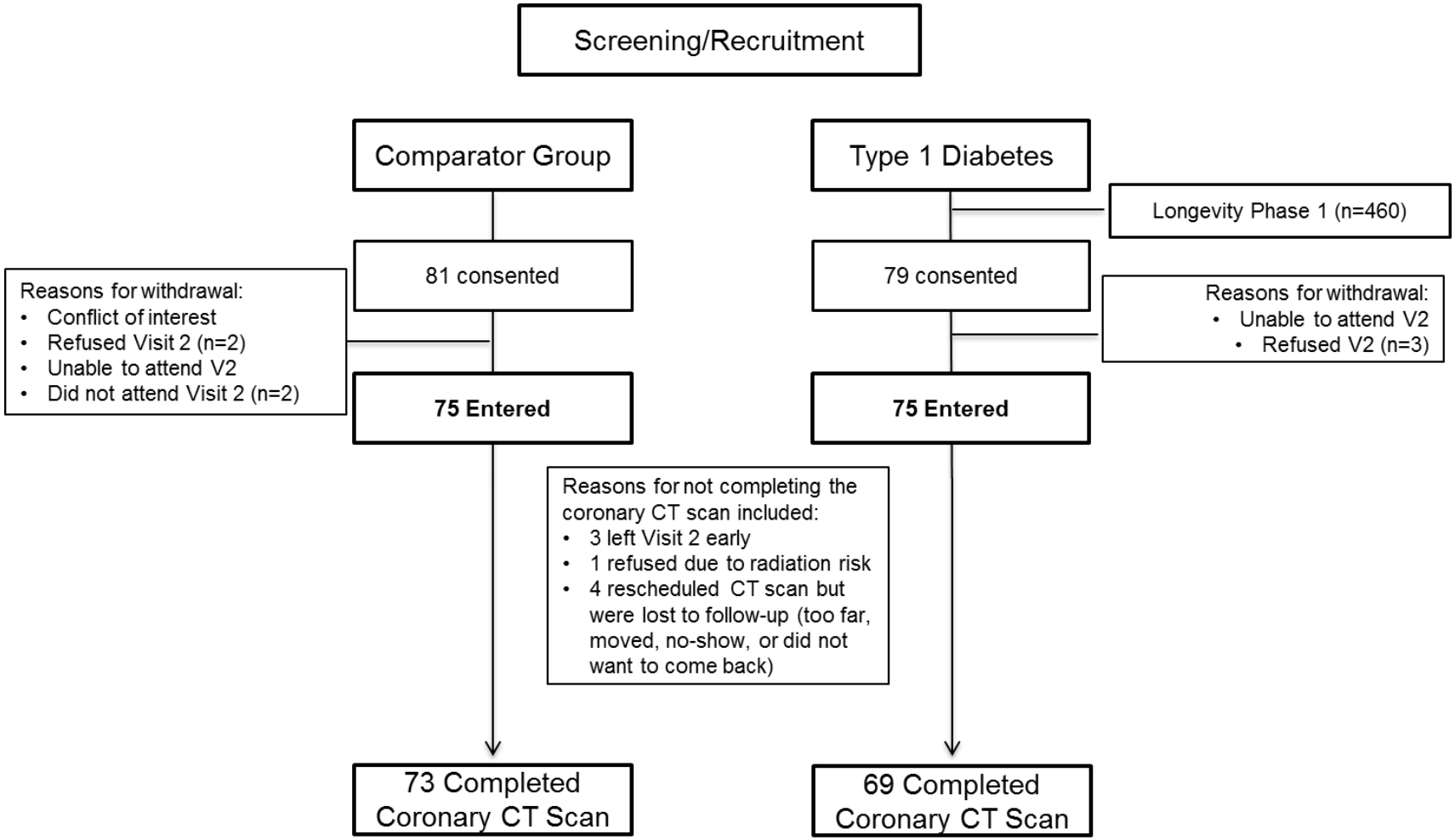
Supplementary Figure S1. Study design and experimental protocol



Legend: *Type 1 diabetes only. † RAASi withdrawal. ‡ At baseline and during ANGII
ANGII, angiotensin II; POC, point of care; PWV, pulse wave velocity; RAASi, renin-angiotensin-aldosterone system inhibitor.

SUPPLEMENTARY DATA

Supplementary Figure S2. Patient flow diagram



SUPPLEMENTARY DATA

Supplementary Table S1. Microvascular endpoints in the comparator group without diabetes stratified by the presence or absence of high CAC

	Comparator Group			
	<300 AU n=63	≥300 AU n=10	Crude p-value	Adjusted p-value*
Neuropathy Endpoints				
Clinical Neuropathy Exam Scores				
TCNS total	2.2 ± 2.3	3.8 ± 2.8	0.067	0.064
TCNS symptoms subscale	0.4 ± 0.8	0.5 ± 0.7	0.53	0.24
TCNS sensory subscale	0.6 ± 0.9	1.2 ± 0.5	0.23	0.38
Large Nerve Fibre Function				
Peroneal nerve AMP (µV)	4.6 ± 1.8	4.6 ± 2.5	0.95	0.87
Peroneal nerve CV (m/s)	46.7 ± 4.2	44.4 ± 3.5	0.10	0.79
Peroneal nerve f-wave latency (ms)	51.0 ± 7.9	53.8 ± 3.8	**0.029**	0.80
Sural nerve AMP (µV)	11.6 ± 6.1	7.9 ± 3.1	0.076	0.63
Sural nerve CV (m/s)	50.1 ± 4.1	47.0 ± 3.1	**0.044**	0.79
CDT at the toe (°C)	27.9 ± 2.6	28.2 ± 1.6	0.82	0.062
VPT Upper limb (volts)	4.1 ± 1.1	4.4 ± 1.2	0.52	0.47
VPT Lower limb (volts)	10.7 ± 4.3	11.6 ± 5.0	0.61	0.26
Corneal Nerve Morphology				
$CNFL_{Auto}$ (mm/mm^2)	13.4 ± 4.5	13.3 ± 4.6	0.94	0.88
$CNBD_{Auto}$ ($branches/mm^2$)	29.3 ± 20.2	40.6 ± 29.1	0.14	0.12
$CNFD_{Auto}$ ($fibres/mm^2$)	19.9 ± 9.7	19.1 ± 6.9	0.86	0.94
Heart Rate Variability				
SDNN	59.5 ± 33.6	43.8 ± 15.0	0.15	0.22
RMSSD	50.2 ± 42.4	42.9 ± 21.6	0.91	0.85
LF/HF Ratio	1.79 ± 1.48	1.36 ± 0.82	0.57	0.18
Renal Hemodynamic Endpoints				
CKD criteria met	2 (3.2%)	3 (30.0%)	**0.002**	**0.021**
GFR_{INULIN} ($mL/min/1.73m^2$)	105 ± 19	111 ± 21	0.37	0.54
$ERPF_{PAH}$ ($mL/min/1.73m^2$)	494 ± 136	527 ± 108	0.48	0.26
RBF ($mL/min/1.73m^2$)	793 ± 217	886 ± 223	0.21	0.23
FF (%)	22.0 ± 3.9	21.4 ± 3.9	0.65	0.21
RVR (mmHg/L/min•100)	11.5 ± 3.9	10.8 ± 3.7	0.59	0.85
R_A ($dyne•s•cm^{-5}$)	4441 ± 2085	4268 ± 2056	0.64	0.86
R_E ($dyne•s•cm^{-5}$)	1229 ± 256	1163 ± 354	0.31	0.40

Data expressed as mean±SD or n(%). Retinal imaging was not completed in in the comparator group.

*Adjusted for age and sex.

TCNS, Toronto clinical neuropathy score; AMP, amplitude potential; CV, conduction velocity; CDT, cooling detection threshold; VPT, vibration perception threshold; CNFL, corneal nerve fibre length; CNBD corneal nerve branch density; CNFD, corneal nerve fibre density; SDNN, standard deviation of the NN (R-R) interval; RMSSD; root mean square successive differences LF, low frequency; HF, high frequency; CKD, chronic kidney disease [GFR/albuminuria criteria for CKD met]; GFR_{INULIN}, glomerular filtration rate; $ERPF_{PAH}$, effective renal plasma flow; RBF, renal blood flow; FF, filtration fraction; RVR, renal vascular resistance; R_A, renal afferent arteriolar resistance; R_E, renal efferent arteriolar resistance.

SUPPLEMENTARY DATA

Supplementary Table S2. Change in renal and systemic hemodynamic function in response to exogenous ANGII in the in the comparator group without diabetes stratified by the presence or absence of high CAC

	Comparator Group		Crude p-value	Adjusted p-value*
	<300 AU n=63	≥300 AU n=10		
ΔGFR_{INULIN} (%)	-2.5±12.6	-6.3±11.1	0.42	0.59
$\Delta ERPF_{PAH}$ (%)	-14.8±8.2	-14.4±5.7	0.89	0.60
ΔRBF (%)	-16.0±8.6	-15.2±6.1	0.81	0.74
ΔFF (%)	15.2±15.9	9.6±12.2	0.34	0.80
ΔRVR (%)	30.8±18.5	29.5±16.6	0.85	0.79
ΔR_A (%)	48.0±36.7	50.0±33.9	0.88	0.58
ΔR_E (%)	20.9±22.0	12.3±15.0	0.29	0.59
ΔMAP (%)	8.6±8.6	9.1±8.1	0.88	0.98
ΔHeart Rate (%)	-0.4±7.1	1.2±4.0	0.53	0.76

Data expressed as mean±SD.

*Adjusted for age and sex.

GFR_{INULIN}, glomerular filtration rate; $ERPF_{PAH}$, effective renal plasma flow; RBF, renal blood flow; FF, filtration fraction; RVR, renal vascular resistance; R_A, renal afferent arteriolar resistance; R_E, renal efferent arteriolar resistance; MAP, mean arterial pressure.

SUPPLEMENTARY DATA

Supplementary Table S3. Associations between quartiles of Agatston score values and microvascular endpoints in the group with type 1 diabetes

	Type 1 Diabetes					
	Score ≤222 AU n=18	222< Score ≤1000 AU n=17	1000< Score ≤2373 n=17	Score >2373 AU n=17	Crude p-value	Adjusted p-value
Neuropathy Endpoints						
DSP by Toronto consensus	13(72%)	15(88%)	16(94%)	17(100%)	**0.013**	0.063
Clinical Exam Scores						
TCNS total	4.8 ± 2.8	6.1 ± 3.9	7.5 ± 4.2	8.8 ± 3.9	**0.003**	**0.009**
TCNS symptoms subscale	0.7 ± 1.1	1.5 ± 1.7	1.2 ± 1.2	1.9 ± 1.2	**0.029**	**0.035**
TCNS sensory subscale	0.9 ± 1.0	1.7 ± 1.5	2.0 ± 1.5	1.9 ± 1.6	**0.040**	0.17
Large Nerve Fibre Function						
Peroneal nerve AMP (µV)	2.75 ± 1.55	1.94 ± 1.70	1.42 ± 1.26	1.08 ± 1.04	**0.001**	**0.010**
Peroneal nerve CV (m/s)	41.57 ± 3.50	35.32 ± 9.19	34.79 ± 7.40	32.76 ± 8.32	**0.002**	**0.017**
Peroneal nerve f-wave latency (ms)	60.84 ± 9.65	62.91 ± 7.02	62.00 ± 8.11	65.01 ± 6.16	0.15	0.60
Sural nerve AMP (µV)	4.69 ± 3.46	2.96 ± 2.49	2.21 ± 2.72	1.61 ± 2.10	**0.002**	**0.022**
Sural nerve CV (m/s)	41.16 ± 3.83	36.18 ± 6.15	34.65 ± 6.44	33.85 ± 6.29	**<0.001**	**0.008**
CDT at the toe (°C)	25.40 ± 4.21	21.30 ± 6.20	20.63 ± 7.06	17.50 ± 7.45	**0.001**	**0.006**
VPT Upper limb (volts)	4.72 ± 1.55	6.27 ± 1.53	7.17 ± 2.41	6.93 ± 1.65	**0.005**	**0.031**
VPT Lower limb (volts)	14.48 ± 7.36	22.13 ± 9.01	27.26 ± 8.91	28.48 ± 10.32	**<0.001**	**0.004**
Corneal Nerve Morphology						
$CNFL_{Auto}$ (mm/mm^2)	8.95 ± 5.47	8.18 ± 4.18	8.11 ± 3.88	7.05 ± 3.14	0.19	0.38
$CNBD_{Auto}$ (branches/mm^2)	15.26 ± 18.48	9.37 ± 15.06	12.11 ± 11.35	11.76 ± 14.34	0.58	0.83
$CNFD_{Auto}$ (fibres/mm^2)	12.87 ± 11.37	7.37 ± 6.44	7.62 ± 7.39	8.09 ± 6.35	0.093	0.30
Heart Rate Variability						
SDNN	38.26 ± 27.35	37.35 ± 17.85	24.28 ± 13.06	55.24 ± 55.28	0.21	0.28
RMSSD	28.84 ± 26.04	28.02 ± 17.43	12.29 ± 7.92	39.51 ± 32.84	0.43	0.67
LF/HF Ratio	2.15 ± 1.61	2.75 ± 2.35	2.86 ± 2.87	1.94 ± 1.24	0.87	0.88
Retinopathy Endpoints						
None	6(33%)	2(12%)	1(6%)	1(6%)		
NPDR	5(28%)	6(35%)	5(29%)	5(29%)	**0.037**	**0.033**
PDR	1(39%)	9(53%)	11(65%)	11(65%)		
Renal Hemodynamic Endpoints						
DKD	7(39%)	6(35%)	4(24%)	7(41%)	0.15	0.27
GFR_{INULIN} (mL/min/1.73m^2)	98.86 ± 17.89	110.64 ± 17.42	103.61 ± 18.44	100.43 ± 13.43	0.93	0.20
$ERPF_{PAH}$ (mL/min/1.73m^2)	453.61 ± 89.04	451.96 ± 106.34	460.32 ± 128.76	418.27 ± 80.60	0.44	0.47
RBF (mL/min/1.73m^2)	697.54 ± 147.83	697.31 ± 166.53	698.29 ± 214.91	645.08 ± 136.95	0.45	0.43

	Type 1 Diabetes					
	Score ≤222 AU n=18	222< Score ≤1000 AU n=17	1000< Score ≤2373 n=17	Score >2373 AU n=17	Crude p-value	Adjusted p-value
FF (%)	0.22 ± 0.03	0.25 ± 0.05	0.23 ± 0.03	0.25 ± 0.04	0.18	0.56
RVR (mmHg/L/min•100)	0.13 ± 0.03	0.14 ± 0.04	0.14 ± 0.04	0.14 ± 0.04	0.28	0.51
R_A (dyne•s•cm^{-5})	4486.33 ± 1462.90	4888.43 ± 2067.29	4853.03 ± 1583.05	5072.60 ± 1759.22	0.35	0.42
R_E (dyne•s•cm^{-5})	2226.44 ± 462.25	2608.85 ± 652.18	2399.42 ± 449.06	2529.20 ± 586.68	0.19	0.66

Data expressed as mean±SD or n(%). Bold p-values indicate values <0.05. P-values from ordinal logistic regression model with quartile of Agatston score used as the dependent variable, and indicated microvascular endpoint as the independent variable. The adjusted p-value includes age, sex, and HbA1c as covariates. The p-value for the test of proportional odds was >0.05 in all crude models except for SDNN and RMSSD. The p-value for the test of proportional odds was >0.05 in all adjusted models except for nephropathy status (by study definition), SDNN, RMSSD, GFR_{INULIN}, $ERPF_{PAH}$, and RBF.
TCNS, Toronto clinical neuropathy score; AMP, amplitude potential; CV, conduction velocity; CDT, cooling detection threshold; VPT, vibration perception threshold; CNFL, corneal nerve fibre length; CNBD corneal nerve branch density; CNFD, corneal nerve fibre density; SDNN, standard deviation of the NN (R-R) interval; RMSSD; root mean square successive differences LF, low frequency; HF, high frequency; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; DKD, diabetic kidney disease; GFR_{INULIN}, glomerular filtration rate; $ERPF_{PAH}$, effective renal plasma flow; RBF, renal blood flow; FF, filtration fraction; RVR, renal vascular resistance; R_A, renal afferent arteriolar resistance; R_E, renal efferent arteriolar resistance.

SUPPLEMENTARY DATA

Supplementary Table S4. Change in renal and systemic hemodynamics in response to exogenous ANGII stimulation in the group with type 1 diabetes stratified by the presence or absence of high Agatston score values

	Type 1 Diabetes					
	Score ≤222 AU n=18	222< Score ≤1000 AU n=17	1000< Score ≤2373 n=17	Score >2373 AU n=17	Crude p-value	Adjusted p-value*
ΔGFR_{INULIN} (%)	-2.97 ± 6.00	-5.63 ± 13.70	-8.72 ± 7.88	-6.92 ± 10.06	0.21	0.53
$\Delta ERPF_{PAH}$ (%)	-13.05 ± 5.70	-9.92 ± 6.68	-17.06 ± 8.89	-12.80 ± 5.09	0.77	0.56
ΔRBF (%)	-14.27 ± 6.30	-10.10 ± 6.21	-17.52 ± 8.72	-12.98 ± 5.09	0.99	0.68
ΔFF (%)	11.88 ± 8.15	4.61 ± 10.94	10.93 ± 12.43	7.14 ± 13.42	0.44	0.99
ΔRVR (%)	27.08 ± 13.12	20.04 ± 12.58	33.12 ± 13.84	23.23 ± 11.34	0.62	0.79
ΔR_A (%)	44.45 ± 27.85	39.85 ± 30.40	51.09 ± 19.41	38.76 ± 21.04	0.56	0.61
ΔR_E (%)	16.31 ± 11.22	5.67 ± 13.78	14.11 ± 14.96	8.86 ± 15.96	0.37	0.92
ΔMAP (%)	8.40 ± 7.75	7.67 ± 7.04	8.37 ± 4.93	6.85 ± 6.77	0.69	0.28
ΔHeart Rate (%)	0.66 ± 6.02	4.89 ± 7.69	5.86 ± 8.00	2.33 ± 6.96	0.34	0.33

Data expressed as mean±SD. P-values from ordinal logistic regression model with quartile of Agatston score used as the dependent variable, and indicated change in hemodynamic variable as the independent variable. The adjusted p-value includes age, sex, and HbA1c as covariates. The p-value for the test of proportional odds was >0.05 in all crude models except for ΔRVR. The p-value for the test of proportional odds was >0.05 in all adjusted models except for $\Delta ERPF_{PAH}$, ΔRVR, and ΔR_A.

GFR_{INULIN}, glomerular filtration rate; $ERPF_{PAH}$, effective renal plasma flow; RBF, renal blood flow; FF, filtration fraction; RVR, renal vascular resistance; R_A, renal afferent arteriolar resistance; R_E, renal efferent arteriolar resistance; MAP, mean arterial pressure.

SUPPLEMENTARY DATA

Supplementary Table S5. Microvascular endpoints in the comparator group without diabetes stratified by category of Agatston score

	Comparator Group			Crude p-value	Adjusted p-value
	Score=0 AU n=34	0< Score <300 AU n=29	Score ≥300 AU n=10		
Neuropathy Endpoints					
Clinical Neuropathy Exam Scores					
TCNS total	1.9 ± 2.3	2.6 ± 2.3	3.8 ± 2.8	**0.030**	**0.043**
TCNS symptoms subscale	0.4 ± 0.7	0.5 ± 1.0	0.5 ± 0.7	0.62	0.15
TCNS sensory subscale	0.4 ± 0.8	0.8 ± 1.0	1.0 ± 1.2	0.037	0.18
Large Nerve Fibre Function					
Peroneal nerve AMP (µV)	4.5 ± 1.5	4.7 ± 2.2	4.6 ± 2.5	0.83	0.81
Peroneal nerve CV (m/s)	47.0 ± 4.2	46.3 ± 4.2	44.4 ± 3.5	0.12	0.74
Peroneal nerve f-wave latency (ms)	49.5 ± 7.5	52.7 ± 8.1	53.8 ± 3.8	0.075	0.91
Sural nerve AMP (µV)	13.4 ± 6.0	9.5 ± 5.5	7.9 ± 3.1	**0.004**	0.31
Sural nerve CV (m/s)	50.8 ± 3.4	49.4 ± 4.7	47.0 ± 3.2	**0.027**	0.38
CDT at the toe (°C)	28.5 ± 2.2	27.1 ± 2.9	28.2 ± 1.6	0.21	0.44
VPT Upper limb (volts)	3.9 ± 1.2	4.4 ± 1.0	4.4 ± 1.2	0.14	0.70
VPT Lower limb (volts)	9.9 ± 4.2	11.7 ± 4.2	11.6 ± 5.0	0.19	0.50
Corneal Nerve Morphology					
$CNFL_{Auto}$ (mm/mm^2)	14.2 ± 3.7	12.5 ± 5.1	13.3 ± 4.6	0.27	0.24
$CNBD_{Auto}$ (branches/mm^2)	32.3 ± 19.1	26.0 ± 21.2	40.6 ± 29.1	0.92	0.44
$CNFD_{Auto}$ (fibres/mm^2)	21.4 ± 8.9	18.2 ± 10.4	19.1 ± 6.9	0.28	0.64
Heart Rate Variability					
SDNN	61.5 ± 31.7	57.1 ± 36.1	43.8 ± 15.0	0.26	0.43
RMSSD	51.8 ± 36.6	48.4 ± 48.8	42.9 ± 21.6	0.61	0.89
LF/HF Ratio	1.7 ± 1.7	1.9 ± 1.2	1.4 ± 0.8	0.86	0.74
Renal Hemodynamic Endpoints					
CKD criteria met	1 (3%)	1 (3%)	3 (30%)	**0.016**	**0.015**
GFR_{INULIN} (mL/min/1.73m^2)	102.8 ± 19.7	108.1 ± 17.1	111.0 ± 20.6	0.14	0.22
$ERPF_{PAH}$ (mL/min/1.73m^2)	497.6 ± 142.9	490.5 ± 130.1	526.8 ± 108.0	0.75	0.19
RBF (mL/min/1.73m^2)	785.0 ± 213.3	801.8 ± 224.3	886.3 ± 222.9	0.29	0.14
FF (%)	0.21 ± 0.03	0.23 ± 0.04	0.21 ± 0.04	0.38	0.50
RVR (mmHg/L/min•100)	0.12 ± 0.04	0.11 ± 0.03	0.11 ± 0.04	0.65	0.58
R_A (dyne•s•cm^{-5})	4465.3 ± 2439.3	4412.8 ± 1614.3	4268.2 ± 2056.0	0.80	0.80
R_E (dyne•s•cm^{-5})	1197.0 ± 220.2	1266.9 ± 292.3	1162.7 ± 353.9	0.77	0.72

Data expressed as mean±SD or n(%). Retinopathy was not phenotyping in the comparator group. P-values from ordinal logistic regression model with quartile of Agatston score used as the dependent variable, and indicated microvascular endpoint as the independent variable. The adjusted p-value includes age and sex as covariates. The p-value for the test of proportional odds was >0.05 in all crude and adjusted models..

TCNS, Toronto clinical neuropathy score; AMP, amplitude potential; CV, conduction velocity; CDT, cooling detection threshold; VPT, vibration perception threshold; CNFL, corneal nerve fibre length; CNBD corneal nerve branch density; CNFD, corneal nerve fibre density; SDNN, standard deviation of the NN (R-R) interval; RMSSD; root mean square successive differences LF, low frequency; HF, high frequency; CKD, chronic kidney disease [GFR/albuminuria criteria for CKD met]; GFR_{INULIN}, glomerular filtration rate; $ERPF_{PAH}$, effective renal plasma flow; RBF, renal blood flow; FF, filtration fraction; RVR, renal vascular resistance; R_A, renal afferent arteriolar resistance; R_E, renal efferent arteriolar resistance.

Supplementary Table S6. Change in renal and systemic hemodynamic function in response to exogenous ANGII in the comparator group without diabetes stratified by category of Agatston score

	Comparator Group				
	AU=0 n=34	0< Score <300 AU n=29	Score ≥300 AU n=10	Crude p-value	Adjusted p-value
ΔGFR_{INULIN} (%)	-3.7 ± 8.3	-1.1 ± 16.3	-6.3 ± 11.1	>0.99	0.61
$\Delta ERPF_{PAH}$ (%)	-15.8 ± 9.3	-13.7 ± 6.7	-14.4 ± 5.7	0.36	0.56
ΔRBF (%)	-16.9 ± 9.6	-14.8 ± 7.1	-15.2 ± 6.1	0.59	0.47
ΔFF (%)	15.4 ± 13.5	14.9 ± 18.5	9.6 ± 12.2	0.67	0.46
ΔRVR (%)	32.0 ± 23.0	29.3 ± 10.9	29.5 ± 16.6	0.83	0.53
ΔR_A (%)	51.7 ± 45.9	43.4 ± 20.6	50.0 ± 33.9	0.88	0.75
ΔR_E (%)	20.3 ± 16.7	21.5 ± 27.4	12.3 ± 15.0	0.71	0.45
ΔMAP (%)	8.0 ± 10.1	9.2 ± 6.4	9.1 ± 8.1	0.41	0.40
ΔHeart Rate (%)	-1.3 ± 7.4	0.5 ± 6.8	1.2 ± 4.0	0.22	0.56

Data expressed as mean±SD. P-values from ordinal logistic regression model with quartile of Agatston score used as the dependent variable, and indicated change in hemodynamic variable as the independent variable. The adjusted p-value includes age and sex as covariates. The p-value for the test of proportional odds was >0.05 in all crude and adjusted models.

GFR_{INULIN}, glomerular filtration rate; $ERPF_{PAH}$, effective renal plasma flow; RBF, renal blood flow; FF, filtration fraction; RVR, renal vascular resistance; R_A, renal afferent arteriolar resistance; R_E, renal efferent arteriolar resistance; MAP, mean arterial pressure.