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Supplemental Methods

Skin Capillaroscopy

All participants were asked to refrain from smoking and drinking coffee or tea 3 hours before the measurements.[17] A light meal (breakfast and/or lunch) low in fat content was allowed before the start of the measurements. Skin capillaroscopy measurements were performed in a quiet, temperature–controlled room (T=24°C) with participants in the supine position as previously described.

Briefly, capillaries were visualized in the dorsal skin of the distal phalanges of the third and fourth finger of the right hand by use of a digital video microscope (Capiscope; KK Technology, Honiton, United Kingdom) with a system magnification of 3100. Capillaries were visualized 4.5 mm proximal to the terminal row of capillaries in the middle of the nailfold. The investigator selected a region of interest of 1-mm2 skin area. Capillary density (mean of two fields) was measured under three conditions. First, baseline capillary density was measured. Baseline capillary density was defined as the number of continuously erythrocyte–perfused capillaries per 1 mm2 skin and was counted for 15 seconds. Second, capillary recruitment during post-occlusive peak reactive hyperemia was assessed after 4 minutes of arterial occlusion. Arterial occlusion was applied using a miniature cuff at the base of the investigated finger inflated to suprasystolic pressure (260 mmHg) for 4 minutes. Directly after release of the cuff, all (continuously and intermittently) perfused capillaries were counted for 15 seconds. Third, venous congestion was applied, with the cuff inflated to 60 mmHg for 2 minutes, and all (continuously and intermittently) perfused capillaries were counted for 15 seconds. The number of perfused capillaries was counted in the recorded digital raw data with the use of a semiautomatic procedure (CapiAna) by two

investigators who were blinded to participants' clinical status. The intra- and interobserver coefficients of variation for the counting procedure were 2.5% and 5.6%, respectively, as described previously.

Retinal Vessel Dilation Response

The retinal arteriolar dilation response to flicker light, which is thought to be related to nutritive demands of activated retinal neurons, was measured in a dimly lit room by use of the Dynamic Vessel Analyzer (IMEDOS, Jena, Germany).[18] For safety reasons, participants with an intraocular pressure exceeding 30 mm Hg were excluded from retinal measurements. Per participant, we randomly measured the left or right eye.

During the measurement, the participant was instructed and encouraged to focus on the tip of a fixated needle inside the retinal camera (FF450; Carl Zeiss GmbH, Jena, Germany) while the fundus of the eye was examined under green measuring light (530–600 nm, illumination of fundus approximately 6500 lux). A straight arteriolar segment of approximately 1.5 mm in length located 0.5 to 2.0 disc diameters from the margin of the optic disc in the temporal section was examined. When the specific vessel profile was recognized, vessel diameter was automatically and continuously measured for 150 seconds. A baseline recording of 50 seconds was followed by a 40-second flicker light exposure period (flicker frequency 12.5 Hz, bright-to-dark contrast ratio 25:1) followed by a 60-second recovery period. The Dynamic Vessel Analyzer automatically corrected for alterations in luminance caused by, for example, slight eye movements. During blinks and small eye movements, the registration stopped and restarted once the vessel segments were automatically re-identified.

The integrated Dynamic Vessel Analyzer software (version 4.51; Imedos) automatically calculated baseline diameter and percentage dilation. Baseline diameter was calculated as the average diameter size of the 20- to 50-second recording and was expressed in measurement units, where 1 measurement unit is equal to 1 μm of the Gullstrand eye. Percentage dilation over baseline was based on the average dilation achieved at time points 10 and 40 seconds during the flicker stimulation period. Two regression lines were drawn (at intervals of 0–10 seconds and 10–40 seconds during flicker stimulation) and averaged to assess average percentage dilation. The software successfully assessed 2 regression lines in 95.4% of the curves; only 102 dilation curves (4.6%) were based on one regression line. The purpose of taking the average dilation was to account for interindividual variation in the curve shape during dilation.

Retinal venule dilation was performed in an identical fashion as arteriolar dilation, except the venules were used.

Skin Hyperemic Response

Skin blood flow was measured as described previously by means of a laser-Doppler system (Periflux 5000; Perimed, Järfalla, Sweden) equipped with a thermostatic laser-Doppler probe (PF457; Perimed) at the dorsal side of the wrist of the left hand.[18] The laser-Doppler output was recorded for 25 minutes with a sample rate of 32 Hz, which gives semi-quantitative assessment of skin blood flow expressed in arbitrary perfusion units. Skin blood flow was first recorded unheated for 2 minutes to serve as a baseline. After the 2 minutes of baseline, the temperature of the probe was rapidly and locally increased to 44°C and was then kept constant until the end of the registration. The heat-induced skin hyperemic response was expressed as the percentage increase in average

perfusion units during the 23-minute heating phase over the average baseline perfusion units). The response is thought to be related to skin metabolic and thermoregulatory function.

Supplemental Table 1: Baseline Characteristics of Participants by Subcohort

Subcohort	Capillaroscopy	Laser-Doppler	Flicker-Light
Age (years) ± SD	60 (9)	60 (8)	60 (8)
Male, n (%)	421 (54)	818 (52)	1094 (51)
BMI (kg/m^2) ± SD	27 (5)	27 (5)	27 (5)
Smoking, n (%)			
Never	223 (29)	485 (32)	735 (35)
Former	416 (55)	837 (55)	1104 (53)
Current	122 (16)	197 (13)	264 (13)
Diabetes Status*, n (%)			
Normal	413 (53)	823 (53)	1204 (56)
Prediabetes	125 (16)	237 (15)	315 (15)
Type 2 Diabetes	236 (30)	504 (32)	619 (29)
HbA1C (%) ± SD	6.0 (0.8)	6.0 (1.0)	6.0 (1.0)
Retinopathy, n (%)	15 (3)	26 (2)	34 (2)
CVD, n (%)	135 (18)	262 (17)	319 (15)
Office Systolic BP (mm Hg) ± SD	137 (19)	136 (18)	135 (18)
Office Diastolic BP (mm Hg) ± SD	77 (10)	76 (10)	74 (7)
24 hr Systolic BP (mm Hg) ± SD	120 (12)	120 (12)	119 (11)
24 hr Diastolic BP (mm Hg) ± SD	75 (7)	74 (7)	74 (7)
On BP Meds, n (%)	308 (40)	663 (42)	830 (39)
On HLD Med n (%)	292 (38)	616 (40)	765 (36)
eGFR (ml/min/1.73m^2) ± SD	81 (17)	81 (16)	81 (17)

CKD Stage n (%)			
None (eGFR>60ml/min/1.73m^2)	705 (91)	1451 (93)	1989 (93)
CKD 3a	61 (8)	102 (7)	131 (6)
CKD3b	8 (1)	11 (1)	17 (1)
CKD4	0 (0)	0 (0)	1 (0.1)
Urine Albumin/Creatinine Ratio (mg/g) ± IQR	5 [4-9]	5 (3-9)	4 (2-8)
Calcium (mg/dl) ± SD	9.3(0.3)	9.3 (0.3)	9.3 (0.3)

Supplemental Table 2: Association of Phosphate with Microvascular Function Stratified by Sex*

Women		Men		
Per 1 mg/dl increase	P Value	Per 1 mg/dl increase	P Value	P Interaction
%Post-Occlusive Reactive Hyperemia				
-5.3 (-13.5, 2.8)	0.20	-4.9 (-11.2, 1.4)	0.13	0.61
% Capillary Recruitment Increase in Response to Venous Congestion				
-6.0 (-14.6, 2.5)	0.16	-3.9 (-10.7, 2.9)	0.26	0.76
% Retinal Arteriolar Dilation Increase in Response to Flicker Light				
-0.07 (-0.32, 0.46)	0.72	-0.32 (-0.72, 0.07)	0.11	0.22
% Retinal Venule Dilation Increase in Response to Flicker Light				
-0.40 (-0.71, -0.10)	0.01	-0.12 (-0.42, 0.18)	0.42	0.32

* Adjusted for age, sex, BMI, smoking status, 24-hour ambulatory systolic blood pressure, use of anti-hypertensives, use of lipid modifying agents, diabetes status, eGFR and serum calcium.