



Associations between macrovascular and renal microvascular dysfunction in type 2 diabetes and non-diabetes: the HELIUS study

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ARTICLE INFO

Keywords:

Microvascular dysfunction
Macrovascular dysfunction
Type 2 diabetes
Cardiovascular disease
Albuminuria
Coronary artery disease
Peripheral artery disease
Stroke
Aortic stiffness
The HELIUS study

ABSTRACT

Background: Although the associations between measures of macrovascular and microvascular dysfunctions are well characterized in diabetes, there is limited data on these associations in individuals without diabetes. We compared the associations between macrovascular dysfunction and renal microvascular dysfunction in individuals with type 2 diabetes (T2D) and without diabetes.

Methods: Cross-sectional analyses of baseline data from the multiethnic Healthy Life in an Urban Setting (HELIUS) study (Amsterdam, the Netherlands), including 986 participants with T2D and 7680 participants without diabetes were done. Logistic regression analyses were used to examine the associations between macrovascular dysfunction [aortic stiffness, coronary artery disease (CAD), peripheral artery disease (PAD), and stroke] and renal microvascular dysfunction [albuminuria] with adjustments for age, sex, ethnicity, waist-to-hip ratio, systolic blood pressure, LDL-cholesterol, and smoking (and HbA1c and diabetes duration for the T2D group).

Results: In the fully adjusted models, aortic stiffness was associated with albuminuria in individuals with T2D [OR 2.55; 95% CI 1.30–4.98], but not without diabetes [0.96; 0.63–1.45]; stroke was associated with albuminuria in T2D [2.40; 1.10–5.25], but not in non-diabetes [1.39; 0.83–2.33]. In age-sex adjusted models, CAD was associated with albuminuria in T2D [1.65; 1.09–2.50] and in non-diabetes [1.56; 1.13–2.15]; the associations were no longer significant in the fully adjusted model. There were no associations between PAD and albuminuria in T2D and non-diabetes.

Conclusions: Our study shows important differences in the associations between measures of macrovascular and renal microvascular dysfunction in T2D and non-diabetes. These findings provide opportunities for future research aimed at prevention and treatment strategies for individuals with vascular dysfunction.

1. Introduction

Globally, vascular dysfunction in individuals with and without diabetes is highly prevalent and remains an important cause of disability, repeated hospitalizations, and early death (Criqui and Victor, 2015; Benjamin et al., 2019; Hayfron-Benjamin et al., 2019). Conventionally, vascular dysfunction is partitioned into diabetes-specific microvascular

disease including retinopathy, nephropathy, and neuropathy, and macrovascular disease including coronary artery disease (CAD), peripheral artery disease (PAD), and cerebrovascular disease (Fowler, 2008).

There is a body of evidence showing that the risk of both macrovascular and microvascular disease in individuals with type 2 diabetes (T2D) may be related, both epidemiologically and mechanistically. For

Abbreviations: ABI, ankle-brachial pressure index; AoPWV, aortic pulse wave velocity; BMI, body mass index; BP, blood pressure; CAD, coronary artery disease; CI, confidence interval; HELIUS, healthy life in an urban setting; OR, odds ratio; PAD, peripheral artery disease; T2D, type 2 diabetes.

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<https://doi.org/10.1016/j.mvr.2021.104162>

Received 30 January 2021; Received in revised form 26 February 2021; Accepted 5 March 2021

Available online 9 March 2021

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example, findings from the UK prospective diabetes study showed that the risk of both T2D related macrovascular and microvascular complications was associated with poor glycemic (Stratton et al., 2000). Mechanistically, an increase in stiffness of the large arteries may impair their buffering capacity, leading to an increase in flow pulsatility and subsequent damage to the microcirculation (Climie et al., 2019). Conversely, microvascular dysfunction may result in increased systemic vascular resistance and an increase in blood pressure (BP), which in turn increases arterial stiffness and influences the risk of macrovascular disease (Climie et al., 2019). Besides, large arteries may be damaged when there is a dysfunction of the small vessels supplying them (Climie et al., 2019). Given the above mechanistic inter-relatedness and the shared risk factors of macrovascular and microvascular dysfunction aside from chronic hyperglycemia (Climie et al., 2019; Krentz et al., 2007), it is biologically plausible that a relationship could exist between dysfunctions of specific microcirculations and macrocirculations, even in individuals without diabetes (Climie et al., 2019). Nevertheless, the relationship between macrovascular and microvascular dysfunction may differ in individuals with T2D and without diabetes, given the evidence that chronic hyperglycemia diminishes the anti-atherogenic role of the vascular endothelium (Climie et al., 2019; Hadi and Suwaidi, 2007). However, epidemiological studies comparing the relationship between macrovascular and microvascular dysfunction in individuals with T2D and without diabetes are lacking. Using a large multiethnic cohort, we tested the hypothesis that the association between measures of macrovascular and microvascular dysfunction differs in individuals with T2D and without diabetes. Understanding these relationships may provide useful leads for novel vascular prevention and treatment strategies, especially in individuals without diabetes.

2. Materials and methods

2.1. Study design

The rationale, conceptual framework, design, and methodology of the HELIUS study have been described in detail elsewhere (Snijder et al., 2017). For the current analyses, only participants with complete data on renal microvascular function (urinary albumin-creatinine ratio [ACR]) and macrovascular function (ankle-brachial pressure index [ABI], arteriography measurements, CAD (questionnaire and electrocardiography [ECG]), and stroke (questionnaires)) were included. This comprised of 986 participants with T2D and 7680 participants without diabetes; the T2D group excluded individuals who reported the start of their diabetes diagnosis before the age of 30 and/or started using insulin injections right after being diagnosed of diabetes ($n = 21$), as they were more likely to have type 1 diabetes. The study was approved by the Ethics Committee of the Amsterdam Medical Center (MREC 10/100# 17.10.1729) before data collection, and all participants provided written informed consent.

2.2. Assessments

A structured questionnaire was used to record the demographic, socioeconomic, and health-related behaviors of the study participants. Smoking status was classified into nonsmokers and current smokers and the number of pack-years was calculated by multiplying the number of packs (containing 20 cigarettes) smoked a day by the number of years. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). The waist-to-hip ratio (WHR) was determined as the ratio of waist circumference (WC) to hip circumference (HC). BP was measured thrice using the Microlife Watch BP home device, with appropriately sized cuffs after at least 5 min rest while seated. The mean of the last two BP measurements was used for the analyses. Hypertension was defined as systolic BP ≥ 140 mmHg and/or a diastolic BP ≥ 90 mmHg and/or current use of antihypertensive agents.

2.2.1. Biochemical analyses

Fasting blood samples were drawn and plasma samples were used to determine the concentration of glucose by spectrophotometry, using hexokinase as the primary enzyme (Roche Diagnostics, Japan). T2D was defined by fasting plasma glucose concentration of ≥ 7.0 mmol/l and/or HbA1c ≥ 48 mmol/mol and/or the use of glucose-lowering agents. Total cholesterol, triglycerides, and HDL cholesterol were determined by colorimetric spectrophotometry. LDL cholesterol was calculated according to the Friedewald formula. Whole blood samples were used to determine the concentration of HbA1c using high-performance liquid chromatography technology (TOSOH, Tokyo, Japan). Serum creatinine concentration was determined by a kinetic colorimetric spectrophotometric isotope dilution mass spectrometry calibration method (Roche Diagnostics). The eGFR was calculated using the 2009 CKD-EPI (CKD Epidemiology Collaboration) creatinine equation. Participants were asked to bring an early morning urine sample for direct analyses of urinary albumin and creatinine concentration. Urinary albumin concentration (in mg/l) was measured by an immunochemical turbidimetric method (Roche Diagnostics, Japan). Urinary creatinine concentration (in mmol/l) was measured by a kinetic spectrophotometric method (Roche Diagnostics, Japan). Urinary albumin-creatinine ratio (ACR) (expressed in mg/mmol) was calculated by taking the ratio of the urinary albumin and urinary creatinine concentrations.

2.2.2. Physiological measurements

Aortic stiffness measurements were performed in duplicate after 10 min of supine rest using the Arteriograph system (Colson Kft., Hungary) and the mean of these two measurements was used for analyses. The Arteriograph is an operator-independent non-invasive device, which uses oscillometric pressure curves registered by an upper arm BP cuff to determine BP and PWV. The details of the aortic stiffness measurements are described elsewhere (Snijder et al., 2015). In brief, the upper arm cuff was inflated to suprasystolic BP (35–40 mmHg above the conventionally measured systolic BP) for registration of arterial pressure waves. From the pressure waveform, the time difference was calculated between the first pressure wave or 'early systolic peak', which is created by pressure waves traveling from the heart to the periphery, and the second pressure wave of 'late systolic peak', which travels from the periphery to the heart. For the computation of PWV, the Arteriograph system assumes that the late systolic peak is generated by reflected waves from the aortic bifurcation. The wave travel distance, i.e. from the heart to the bifurcation and back, is estimated as the shortest distance between the suprasternal notch and pubic symphysis using a tape measure. PWV (m/s) is calculated from the distance traveled by the pressure wave and the time difference between the early and late systolic peaks. Variability and reproducibility of PWV measurements appear better with the Arteriograph compared to the Complior and Sphygmocor system (Baulmann et al., 2008). PWV measured by the Arteriograph system generates similar PWV values as obtained by Magnetic resonance imaging (Mr et al., 2013).

Ankle-brachial pressure index (ABI) measurements were performed in the supine position using a validated oscillometric device (Microlife WatchBP Office ABI, Switzerland) with appropriate sized cuffs, after at least 10 min of supine rest (Saladini et al., 2011). Systolic BP was measured twice in the right and left brachial arteries and twice in the right and left posterior tibial arteries. ABI was calculated by taking the highest arm systolic BP as the denominator, and the lowest ankle systolic BP as the numerator. The lowest of the left and right ABI measurements was used for analyses. ABI obtained by the oscillometric method using the Microlife WatchBP Office ABI obtained by the oscillometric method has been shown to correlate well with ABI acquired by Doppler ultrasound with a 95% agreement between the two methods in diagnosing PAD (Kollias et al., 2011).

Standard 12-lead supine digital resting electrocardiography (ECG) was recorded (GE MAC5500, 500 samples/s) and processed with the Modular ECG Analysis System (MEANS) program, (van Bommel et al.,

1990) which determines common P-wave, QRS, and T-wave onsets and offsets for all 12 leads together on 1 representative averaged beat. All onsets and offsets were manually checked and adjusted when necessary. The QRS offset/J-point was positioned after a potential end-QRS notch/slur. Three methods were used to evaluate each ECG, namely the Minnesota coding, the GE Marquette 12SL report, and assessment by a cardiologist.

2.2.3. Definition of macrovascular and microvascular dysfunction

Macrovascular dysfunction was defined as the presence of aortic stiffness, PAD, CAD, and stroke. Aortic stiffness was defined as AoPWV greater than 12 m/s (Rajzer et al., 2008). PAD was defined as ABI $\leq$ 0.90 (Gerhard-Herman et al., 2017). In defining normal ABI in the regression analyses models, an ABI $>$ 1.4 was excluded as it could be suggestive of non-compressible vessels (Gerhard-Herman et al., 2017). CAD was based on the Rose Angina Questionnaire (Rose Chest Pain Questionnaire, 2002) and/or the presence of pathological Q waves in at least 2 contiguous leads on ECG. ST-segment abnormalities were not included in the definition of CAD due to the highly variable ethnicity-dependent prevalence of ECGs with ST-segment elevations exceeding STEMI thresholds in apparently healthy individuals (ter Haar et al., 2020). Stroke was self-reported.

Microvascular dysfunction was based on albuminuria. Albuminuria was defined as ACR $\geq$ 3 mg/mmol [category $\geq$ A2] according to the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines (Lamb et al., 2013). In a sensitivity analysis, we assessed the associations of measures of macrovascular dysfunction with nephropathy, defined as the presence of albuminuria and/or low eGFR according to the 2012 KDIGO guidelines (Lamb et al., 2013). Low eGFR was defined as eGFR $<$ 60 ml/min/1.73 m² (category $\geq$ G3) (Haneda et al., 2015). Low eGFR alone was not used as a measure of renal microvascular dysfunction as it could result from macrovascular disease such as renal artery stenosis.

2.3. Statistical analysis

Differences in demographic, socioeconomic (level of education), behavioral, clinical, and biological measures were compared between participants with T2D and without diabetes. Data with normal distributions were presented as mean ($\pm$ standard deviation), whereas those not normally distributed were presented as median (interquartile range). For data not normally distributed, the Kruskal-Wallis ANOVA test was used to compare medians among study groups. Categorical data were presented as frequencies (percentages). Multivariable logistic regression analyses were used to examine the associations between measures of macrovascular and microvascular dysfunction, with adjustment for potential covariates. Odds ratios (ORs) and their corresponding 95% CI were estimated. The minimal sufficient adjustment sets for estimating the direct effect of macrovascular dysfunction on microvascular dysfunction was determined by a directed acyclic graph (DAG) (DAG available at <http://dagitty.net/mrPADou>). DAG was chosen because the traditional methods of adjusting for potential confounders may introduce conditional associations and bias rather than minimize it (Shrier and Platt, 2008). Based on the DAG, the minimal sufficient adjustment sets were sex, age, ethnicity, diabetes, dyslipidemia, obesity, and smoking. We included systolic BP as a covariate due to its contribution to microvascular and macrovascular dysfunction (Climie et al., 2019). Three models were used to examine the data. Model 1 was unadjusted; Model 2 was adjusted for age and sex; Model 3 was further adjusted for ethnicity, WHR, systolic BP, LDL-cholesterol concentration, and smoking pack-years (and HbA1c and diabetes duration in participants with T2D). A statistical test of significance was set at a p-value $<$ 0.05. Data were analyzed using the IBM SPSS version 23 for Windows.

Table 1

Characteristics of the study population.

	Diabetes	No diabetes	p-value
N	986	7680	
Male sex	505 (51.2%)	3568 (46.5%)	0.005
Age, years	55.58 ($\pm$ 8.27)	44.21 ($\pm$ 12.96)	$<$ 0.001
Higher education	135 (13.8%)	2508 (32.9%)	$<$ 0.001
Ethnicity			$<$ 0.001
Dutch	80 (8.1%)	2245 (29.2%)	
South-Asian Surinamese	234 (23.7%)	907 (11.8%)	
African Surinamese	325 (33.0%)	2038 (26.5%)	
Ghanaian	160 (16.2%)	879 (11.4%)	
Turkish	86 (8.7%)	785 (10.2%)	
Moroccan	69 (7.0%)	564 (7.3%)	
Others	32 (3.2%)	262 (3.4%)	
Smoking	230 (23.5%)	2014 (26.3%)	0.177
Smoking (pack-years) ^a	0.00 (11.03)	0.00 (6.30)	0.178
Duration of diabetes, years	8.21 ($\pm$ 6.72)	–	
Anti-lipid medications	446 (45.2%)	434 (5.7%)	$<$ 0.001
BMI, kg/m ²	29.8 ($\pm$ 5.1)	26.1 ($\pm$ 4.7)	$<$ 0.001
Waist to hip ratio	0.98 ($\pm$ 0.07)	0.89 ($\pm$ 0.08)	$<$ 0.001
Heart rate beats per minute	71.9 ($\pm$ 11.0)	67.9 ($\pm$ 9.8)	$<$ 0.001
Systolic BP, mmHg	139.7 ($\pm$ 17.2)	127.5 ($\pm$ 17.2)	$<$ 0.001
Diastolic BP, mmHg	83.1 ($\pm$ 9.8)	79.2 ($\pm$ 10.8)	$<$ 0.001
Hypertension	715 (72.6%)	2428 (31.7%)	$<$ 0.001
Biochemical			
HbA1c, mmol/mol	56.2 ($\pm$ 15.1)	37.1 ($\pm$ 4.4)	$<$ 0.001
Total cholesterol, mmol/l	4.6 ($\pm$ 1.1)	5.0 ($\pm$ 1.0)	$<$ 0.001
LDL-cholesterol, mmol/l	2.8 ($\pm$ 1.0)	3.1 ($\pm$ 0.9)	$<$ 0.001
Micro- and macrovascular Function			
AoPWV (m/s)	9.8 ($\pm$ 2.2)	8.2 ($\pm$ 2.2)	$<$ 0.001
Aortic stiffness (%)	157 (15.9%)	568 (7.4%)	$<$ 0.001
ABI	1.15 (0.11)	1.13 (0.12)	$<$ 0.001
PAD	30 (3.0%)	324 (4.2%)	0.087
CAD	196 (19.9%)	890 (11.6%)	$<$ 0.001
Stroke (%)	85 (8.6%)	322 (4.2%)	$<$ 0.001
ACR, mg/mmol ^a	0.39 (1.12)	0.24 (0.30)	$<$ 0.001
Albuminuria (%)	137 (13.9%)	275 (3.6%)	$<$ 0.001
eGFR, ml/min/1.73m ²	94.1 ($\pm$ 19.4)	100.6 ($\pm$ 17.2)	$<$ 0.001
eGFR $<$ 60 ml/min/1.73m ² (%)	47 (4.8%)	75 (1.0%)	$<$ 0.001
Nephropathy	162 (16.4%)	330 (4.3%)	$<$ 0.001

Data are mean ($\pm$ standard deviation), median (IQR), or n (%).

Abbreviations: ABI = ankle-brachial pressure index; AoPWV = aortic pulse wave velocity; BMI = body mass index; CAD = coronary artery disease; eGFR = estimated glomerular filtration rate; LDL = low density lipoprotein; PAD = peripheral artery disease.

^a Data presented as median (interquartile range).

3. Results

3.1. General characteristics

The baseline characteristics of the study population are shown in Table 1. Compared to individuals without diabetes participants with T2D were older, more frequently male, less educated, and had higher mean BMI, WHR, systolic BP, and diastolic BP. The mean concentration of total and LDL-cholesterol were lower in the T2D group; the T2D group had a higher proportion of participants on anti-lipid medications.

3.2. Rates of macrovascular and microvascular dysfunction

Participants with T2D had significantly higher mean AoPWV, higher median ACR, and lower mean eGFR than those without diabetes (Table 1). The mean ABI was higher in T2D than in non-diabetes. The rates of prevalent aortic stiffness, stroke, and CAD were approximately twice as high in individuals with T2D compared with individuals without diabetes. However, the rates of PAD were similar in the two groups. The renal microvascular dysfunction rate (assessed by either albuminuria alone or nephropathy) was nearly four times higher in individuals with T2D than in non-diabetes individuals.

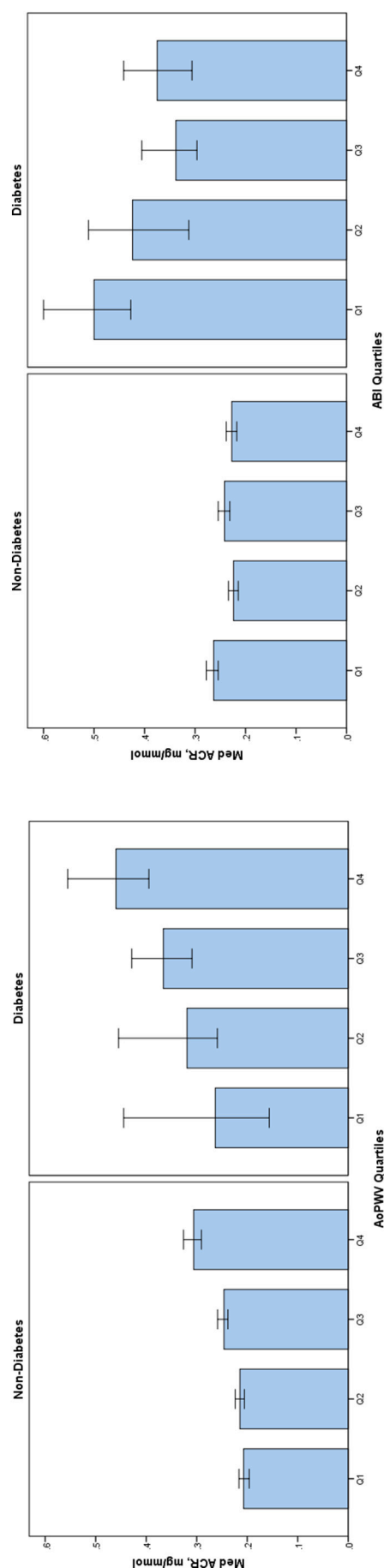


Fig. 1. Median ACR in A) AoPWV Quartiles and B) ABI Quartiles in Individuals with T2D and without Diabetes. Error bars are 95% confidence intervals.

Abbreviations: ABI = ankle-brachial pressure index; ACR = albumin-creatinine ratio; AoPWV = aortic pulse wave velocity.

3.3. Associations of AoPWV and ABI with ACR

The median ACR in the various AoPWV and ABI quartiles stratified by diabetes status are shown in Fig. 1. In both individuals with T2D and without diabetes, there was a significant increase in the median ACR across the AoPWV quartiles ($p < 0.001$ for trend across AoPWV quartiles). Individuals without diabetes in the fourth ($p < 0.001$), and third ($p < 0.001$), but not the second ($p = 0.173$) AoPWV quartile had higher median ACR compared with the first AoPWV quartile. In the T2D group, individuals in the fourth ($p = 0.045$), but not the third ($p = 0.245$) or second ($p = 0.694$) AoPWV quartile had higher median ACR compared with the first AoPWV quartile.

The median ACR decreased across the ABI quartiles in individuals with T2D ($p = 0.013$) and without diabetes ($p < 0.001$). Individuals without diabetes in the first ABI quartile had significantly higher median ACR compared with individuals in the other quartiles ($p < 0.001$). In the T2D group, individuals in the first ABI quartile had significantly higher median ACR compared with individuals in the third ($p = 0.002$) and fourth ($p = 0.008$) quartiles.

3.4. Association between macrovascular dysfunction and albuminuria

The associations between macrovascular dysfunction and albuminuria are shown in Table 2. In models adjusted for age and sex, aortic stiffness, CAD, and stroke were significantly associated with albuminuria in T2D. After further adjustment for ethnicity, systolic BP, WHR, LDL-cholesterol concentration, smoking pack-years, diabetes duration, and HbA1c, aortic stiffness [adjusted odds ratio 2.55; 95% CI, 1.30–4.98, $p = 0.006$] and stroke [2.40, 1.10–5.25, $p = 0.028$] but not CAD [1.47; 0.80–2.72, $p = 0.219$] were significantly associated with albuminuria in individuals with T2D. In individuals without diabetes, aortic stiffness [1.67; 1.15–2.43, $p = 0.007$] and CAD [1.56 (1.13–2.15), $p = 0.007$] was associated with albuminuria in the age and sex-adjusted model; these associations were no longer significant in the fully adjusted models. There were no significant associations between PAD and albuminuria in individuals with T2D and without diabetes.

3.5. Association between macrovascular dysfunction and nephropathy

The associations of aortic stiffness, CAD, stroke, and PAD with nephropathy (based on albuminuria and/or low eGFR) were similar to those based on albuminuria alone (Table 3). In the fully adjusted models, aortic stiffness was associated with nephropathy in individuals with T2D [OR 2.22; 1.17–4.20, $p = 0.014$], but not without diabetes [OR 1.01; 0.69–1.47, $p = 0.961$]. In models adjusted for age, sex, ethnicity, WHR, systolic BP, LDL-cholesterol concentration, and smoking pack-years, stroke was associated with nephropathy in T2D [OR 1.83; 1.06–3.17, $p = 0.030$], but not in individuals without diabetes [1.18; 0.72–1.95, $p = 0.514$]; in T2D, the association was no longer significant after further adjustment for diabetes duration and HbA1c.

4. Discussion

4.1. Key findings

In the present study, we observed differential associations between the specific measures of macrovascular dysfunction and renal microvascular dysfunction in individuals with T2D and without diabetes. In individuals with T2D, aortic stiffness, stroke, and CAD were associated with albuminuria in the unadjusted, and age-sex adjusted models; the associations were significant in fully adjusted models for aortic stiffness and stroke. In non-diabetes, aortic stiffness and CAD were associated with albuminuria in only the unadjusted, and age-sex adjusted models. There were no associations between PAD and albuminuria in individuals with T2D and without diabetes. We observed similar associations when renal microvascular dysfunction was based on combined albuminuria

Table 2

Association between macrovascular dysfunction and albuminuria in diabetes and non-diabetes.

	Albuminuria [OR (95% CI), p-value]			
	Model 1	Model 2	Model 3	Model 4
Diabetes				
Aortic Stiffness	2.58 (1.70–3.91), <0.001	2.55 (1.66–3.91), <0.001	1.69 (1.04–2.75), 0.034	2.55 (1.30–4.98), 0.006
PAD	1.98 (0.83–4.72), 0.122	2.00 (0.84–4.78), 0.117	2.16 (0.56–8.38), 0.266	1.63 (0.28–9.38), 0.582
CAD	1.68 (1.11–2.53), 0.014	1.65 (1.09–2.50), 0.017	1.47 (0.94–2.32), 0.093	1.47 (0.80–2.72), 0.219
Stroke	2.74 (1.64–4.57), <0.001	2.66 (1.59–4.45), <0.001	2.46 (1.39–4.33), 0.002	2.40 (1.10–5.25), 0.028
Non-diabetes				
Aortic Stiffness	2.22 (1.57–3.14), <0.001	1.67 (1.15–2.43), 0.007	0.96 (0.63–1.45), 0.837	
PAD	0.67 (0.33–1.37), 0.277	0.71 (0.35–1.45), 0.348	0.77 (0.34–1.74), 0.527	
CAD	1.65 (1.20–2.27), 0.002	1.56 (1.13–2.15), 0.007	1.35 (0.96–1.88), 0.083	
Stroke	1.54 (0.93–2.55), 0.094	1.44 (0.87–2.38), 0.161	1.39 (0.83–2.33), 0.212	

Abbreviations: CAD = coronary artery disease; CI = confidence interval, OR = odds ratio; PAD = peripheral artery disease.

Model 1: unadjusted; Model 2: adjusted for age and sex; Model 3: additionally adjusted for ethnicity, WHR, systolic blood pressure, LDL-cholesterol concentration, and smoking pack-years; Model 4: additionally adjusted HbA1c and duration of diabetes.

Table 3

Association between macrovascular dysfunction and nephropathy in diabetes and non-diabetes.

	Nephropathy [OR (95% CI), p-value]			
	Model 1	Model 2	Model 3	Model 4
Diabetes				
Aortic Stiffness	2.45 (1.64–3.64), <0.001	2.28 (1.51–3.42), <0.001	1.55 (0.99–2.45), 0.058	2.22 (1.17–4.20), 0.014
PAD	1.60 (0.68–3.81), 0.283	1.65 (0.69–3.94), 0.257	1.70 (0.47–6.16), 0.420	1.42 (0.27–7.57), 0.682
CAD	1.58 (1.07–2.34), 0.021	1.55 (1.05–2.30), 0.028	1.35 (0.88–2.07), 0.165	1.23 (0.68–2.22), 0.488
Stroke	2.32 (1.40–3.82), 0.001	2.18 (1.32–3.61), 0.002	1.83 (1.06–3.17), 0.030	1.95 (0.91–4.16), 0.085
Non-diabetes				
Aortic Stiffness	2.48 (1.82–3.37), <0.001	1.61 (1.15–2.26), 0.005	1.01 (0.69–1.47), 0.961	
PAD	0.55 (0.27–1.13), 0.103	0.61 (0.30–1.24), 0.169	0.66 (0.30–1.46), 0.304	
CAD	1.60 (1.19–2.15), 0.002	1.49 (1.10–2.00), 0.009	1.32 (0.96–1.80), 0.086	
Stroke	1.34 (0.82–2.18), 0.241	1.21 (0.74–1.98), 0.454	1.18 (0.72–1.95), 0.514	

Abbreviations: CAD = coronary artery disease; CI = confidence interval, OR = odds ratio; PAD = peripheral artery disease.

Model 1: unadjusted; Model 2: adjusted for age and sex; Model 3: additionally adjusted for ethnicity, WHR, systolic blood pressure, LDL-cholesterol concentration, and smoking pack-years; Model 4: additionally adjusted HbA1c and duration of diabetes.

and low eGFR.

4.2. Discussion of key findings

Macrovascular and microvascular complications have similar etiologic characteristics, with chronic hyperglycemia driving several metabolic and structural derangements including the formation of advanced glycation end products, activation of inflammatory pathways, and increased oxidative stress (Orasanu and Plutzky, 2009; Dal Canto et al., 2019). Expectedly, we observed higher rates of nephropathy, aortic stiffness, CAD, and strokes in individuals with T2D, compared with non-diabetes, which is consistent with previous reports (Dal Canto et al., 2019). Our observation of similar rates of PAD in T2D and non-diabetes is inconsistent with the existing mechanistic model establishing diabetes as a major driver of atherosclerotic occlusive disease of the lower extremities, also described in previous epidemiological reports (Association, A. D., 2003; Shu and Santulli, 2018; Hayfron-Benjamin et al., 2020). Given the existing evidence that the manifestation of atherosclerosis in one vascular territory generally indicates an increased likelihood that it exists in other vascular territories (Climie et al., 2019; Dal Canto et al., 2019), it remains unclear why the rates of PAD are similar while the rates of other measures of macrovascular dysfunction vary in T2D and non-diabetes. However, there is evidence suggesting that T2D alone is not an independent risk factor for PAD (Shu and Santulli, 2018; Wilcox et al., 2018) and that other risk factors may play equally important roles (Maahs et al., 2010). Therefore, apart from T2D, other risk factors may increase PAD rates to values comparable to T2D. These risk factors may not necessarily be significant drivers of atherosclerotic cardiovascular disease in other vascular territories. Indeed, previous reports have

shown that the prognostic potency of the individual risk factors for atherogenesis differs in the various arterial beds (Gregorio et al., 2010).

This study shows that aortic stiffness was associated with albuminuria in individuals with T2D, even after full adjustment. Our results confirm previous findings (Kim et al., 2017), and further shows that this association persists after adjustment for a wide range of cardiometabolic risk factors. The association between aortic stiffness and renal microvascular dysfunction in the setting of T2D may thus be independent of the conventional cardiometabolic risk factors. This lends credence to the theory that the association between aortic stiffness and end-organ dysfunction is largely mechanical, with the impaired aortic buffering capacity driving higher flow pulsatility and subsequent damage to the microcirculation (Climie et al., 2019). Our study has also provided data on the association between aortic stiffness and albuminuria in individuals without diabetes. The absence of association between aortic stiffness and albuminuria in non-diabetes after full adjustments suggests that the presence of T2D may play a critical role in the increased susceptibility of the renal microvascular system to mechanical injury following surges in flow pulsatility due to aortic stiffness (Climie et al., 2019; Dronavalli et al., 2008). This further highlights diabetes as a central mechanism in microvascular dysfunction (Fowler, 2008). It is worth noting that the non-diabetes group was younger than the diabetes group; at a younger age, the microvasculature may be more resistant to injury from surges in flow pulsatility due to aortic stiffness (Jin, 2019). Age differences could therefore explain the difference in the association between aortic stiffness and albuminuria in T2D and non-diabetes groups in this study.

Similar to aortic stiffness, we observed significant associations between stroke and albuminuria in T2D, after full adjustment. This is

consistent with prior studies linking stroke and albuminuria in diabetes (Ninomiya et al., 2009). One mechanistic model associating stroke and albuminuria is diabetes is based on the evidence associating albuminuria with increased carotid artery intima-media thickness (Mykkanen et al., 1997). Therefore albuminuria may complicate carotid artery atherosclerosis, increasing the risk of atherothrombotic stroke. In this study, albuminuria was not associated with stroke in non-diabetes individuals. Although the biological basis is unclear, this finding may indicate that unlike in diabetes, albuminuria in non-diabetes may not necessarily indicate a generalized systemic vasculopathy independent of cardiovascular risk factors (Dronavalli et al., 2008). Further studies may be required to confirm or refute this assumption.

In this study, CAD was associated with albuminuria in T2D in the unadjusted and age-sex adjusted models. Further adjustments for cardiovascular risk factors attenuated this association, suggesting that the relationship between CAD and albuminuria in T2D may be partly dependent on the conventional cardiovascular risk factors, which is consistent with previous reports (Stehouwer and Smulders, 2006; Perkovic et al., 2008). This finding seems to support the hypothesis that albuminuria is likely a marker of longer diabetes duration or more severe diabetes and not necessarily an independent risk factor for diabetes-related vascular damage including CAD (Wattanakit et al., 2008). In non-diabetes, our findings of an association between CAD and albuminuria supports a limited hospital-based study that showed an association between echocardiographic signs of ischemia and microalbuminuria in individuals without diabetes (Al-Saffar et al., 2015). Further, our study shows that the association persists after age-sex adjustment, but not after further adjustment for the conventional cardiovascular risk factors.

In this study, there were no significant associations between PAD and markers of renal microvascular injury in T2D and non-diabetes. Existing reports on the association between PAD and albuminuria in diabetes and non-diabetes have reported conflicting findings (Wattanakit et al., 2008; Wu et al., 2010; Yap et al., 2014). Given the evidence that PAD is an indicator of widespread atherosclerosis in other vascular territories, such as the cerebral and coronary circulations (Bennett et al., 2009), it remains unclear why PAD was not associated with albuminuria. It is plausible that the lack of association between PAD and albuminuria, especially in the setting of diabetes could be due to a misclassification of PAD based on the recommended diagnostic criteria ($ABI \leq 0.9$). In diabetes, hardening or non-compressibility of the distal arteries could lead to an elevation of ABI (Gerhard-Herman et al., 2017). Therefore, individuals with PAD with non-compressible distal arteries may have $ABI > 0.9$, thereby masking PAD diagnosis based on ABI measurement. This is less of an issue in non-diabetes as this vascular phenotype affects the more proximal arteries (He et al., 2014).

4.3. Strengths and limitations

Our study provides new important information on the association between measures of macrovascular and renal microvascular dysfunction in non-diabetes and has simultaneously compared these findings with the associations in T2D. Another strength of this study is that we used well-standardized study protocols and multiple measures of macrovascular dysfunction, and adjusted for a wide range of covariates in our logistic regression models. Our study is limited by its cross-sectional design. Additionally, we were not able to stratify the analysis for ethnicity due to the relatively lower sample sizes per ethnic group. Further, coronary arteriography was not performed in the evaluation of CAD, due to feasibility. Assessment of renal microvascular dysfunction based on albuminuria may be limited, especially in non-diabetes individuals where albuminuria may result from causes other than microvascular injury (Haider and Aslam, 2020). We also did not adjust for inflammation, a potential mediator between albuminuria and macrovascular disease (Festa et al., 2000). Finally, CAD and stroke were self-reported, which could have led to reporting bias.

4.4. Conclusion

Our study shows important differences in the associations between measures of macrovascular and renal microvascular dysfunction in T2D and non-diabetes. Measures of macrovascular dysfunction were more frequently associated with renal microvascular dysfunction in individuals with T2D compared with non-diabetes. The above observations indicate that the mechanisms linking macrovascular and renal microvascular dysfunction in diabetes and non-diabetes may differ. These findings provide opportunities for future research aimed at integrated preventive and treatment strategies for individuals with vascular dysfunction.

Data availability

The HELIUS data are owned by the Amsterdam University Medical Center, location AMC in Amsterdam, The Netherlands. Any researcher can request the data by submitting a proposal to the HELIUS Executive Board as outlined at <http://www.heliusstudy.nl/en/researchers/collaboration>

CRediT authorship contribution statement

1. Charles F. Hayfron-Benjamin: Conceptualization, Methodology, Formal analysis, Data Interpretation, Writing - Original Draft, Writing - Review & Editing, and approval of final manuscript
2. Albert G. B. Amoah: Conceptualization, Methodology, Data Interpretation, Writing - Original Draft, Writing - Review & Editing, and approval of final manuscript
3. Anke H. Maitland - van der Zee: Conceptualization, Methodology, Data Interpretation, Writing - Original Draft, Writing - Review & Editing, and approval of final manuscript
4. Eric Peter Moll van Charante: Methodology, Data Interpretation, Writing - Original Draft, Writing - Review & Editing, and approval of final manuscript
5. Henrike Galenkamp: Methodology, Data Interpretation, Writing - Original Draft, Writing - Review & Editing, and approval of final manuscript
6. Bert-Jan van den Born: Conceptualization, Methodology, Data Interpretation, Writing - Original Draft, Writing - Review & Editing, and approval of final manuscript
7. Charles Agyemang: Conceptualization, Methodology, Formal analysis, Data Interpretation, Writing - Original Draft, Writing - Review & Editing, and approval of final manuscript

Approval statement

This statement is to certify that all authors have seen and approved the manuscript being submitted, have contributed significantly to the work, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission to the *Microvascular Research Journal*. We attest that the article is the Authors' original work, has not received prior publication and is not under consideration for publication elsewhere.

Funding

The HELIUS study is conducted by the Amsterdam University Medical Center, location AMC and the Public Health Service of Amsterdam. Both organizations provided core support for HELIUS. The HELIUS study is also funded by the Dutch Heart Foundation (Hartstichting; grant no. 2010T084), the Netherlands Organization for Health Research and Development (ZonMw; grant no. 200500003), the European Union (Seventh Framework Programme, FP-7; grant no. 278901), and the European Fund for the Integration of non-EU immigrants (EIF; grant no. 2013EIF013).

Declaration of competing interest

The authors report no relationships that could be construed as a conflict of interest.

Acknowledgments

We are most grateful to the participants of the HELIUS study and the management team, research nurses, interviewers, research assistants, and other staff who have taken part in gathering the data of this study.

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