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Retinal vessel reactivity is not attenuated in patients with type 2 diabetes compared with matched controls and is associated with peripheral endothelial function in controls

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ABSTRACT

Background and aims: Attenuated retinal vasoreactivity in patients with type 2 diabetes preceding diabetic retinopathy development has been proposed to reflect local endothelial dysfunction.
Whether retinal vessel reactivity is associated with peripheral endothelial dysfunction and large artery stiffness in patients with type 2 diabetes remains to be elucidated.
Methods: Twenty patients with type 2 diabetes without retinopathy and 20 sex- and age matched controls (diabetes duration: 9.9 years (range 6.0;12.4), 40% male, age: 66.5 ± 7.3 (diabetes) and 65.2 ± 7.6 years (controls)) were included. Endothelial function was assessed using EndoPAT. Arterial stiffness was assessed by carotid-femoral pulse wave velocity using the SphygmoCor. Retinal blood supply regulation was examined by retinal arteriolar diameter change during 1) isometric exercise (hand-weight lifting), 2) exposure to flickering lights, and 3) a combined stimulus of 1) + 2) using the Dynamic Vessel Analyzer.
Results: No significant differences were observed in retinal vessel reactivity in T2DM patients compared to controls. Endothelial function was associated with mean arteriolar diameter change during only the combination intervention, (Beta = 0.033 [0.0013;0.064], $p = 0.042$) in the overall population of patients and controls. When groups were analyzed separately, the associations was statistically significant only in controls. However, formal test for interaction was not statistically significant, $p = 0.40$. No association was observed between pulse wave velocity and retinal arteriolar %-diameter change in patients or controls.
Conclusion: Peripheral endothelial function was associated with retinal arteriolar diameter change in the combined sample. The association seemed to be driven primarily by the controls. Our findings indicate that peripheral endothelial function is reflective of endothelial function in the retina mainly in subjects without T2DM, whereas an association in T2DM without retinopathy was not observed. Further studies are needed in T2DM patients with more advanced retinopathy.

1. Introduction

The prevalence of type 2 diabetes (T2DM) is growing epidemically.[1] Type 2 diabetes is associated with development of late complications in both micro- and macrovascular beds. However, the pathogenic mechanisms underlying the development of complications are complex and it remains unsettled whether common disease mechanisms are involved in both micro- and macrovascular complication development. A number of recent studies found correlations between micro- and macrovascular complications of diabetes, suggesting an interplay between the different vascular beds.[2–5]

Patients with type 2 diabetes and prediabetes have attenuated vasoreactivity of the retinal vessels.[6–10] There is no generally accepted cut-off value for retinal vasoreactivity, however the retinal

Abbreviations: AU, Arbitrary units; cfPWV, Carotid-femoral pulse wave velocity; DR, Diabetic retinopathy; DVA, Dynamic Vessel Analyzer; FMD, Flow-mediated dilation; MAP, Mean arterial pressure; OCT, Optical coherence tomography; PAT, Peripheral arterial tonometry; RH, Reactive hyperaemia; RHI, Reactive hyperaemia index; T2DM, Type 2 diabetes mellitus; UACR, Urinary albumin:creatinine ratio.

Declaration of competing interest: The authors declare that there is no duality of interest associated with this manuscript.

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vasoreactivity was significantly reduced when compared with control subjects without diabetes.

This may reflect dysfunctional autoregulation of the retinal blood flow and has been suggested to play a role in the development of diabetic retinopathy (DR).[11]Moreover, decreasing retinal arteriolar diameter has been associated with improvement of diabetic retinopath.[12]

As the retinal vessel response to diffuse luminance flicker light stimulation is at least in part mediated by nitric oxide,[13] attenuated responses have been proposed to reflect endothelial dysfunction in the retina.[8] Similarly, type 2 diabetes has been associated with impaired peripheral endothelial function,[14] which has in turn been associated with development of macrovascular complications.[15] One study has found that flow-mediated dilation of the brachial artery (FMD) is correlated with retinal arteriolar diameter response to stimulation with flicker light in patients with hypertension and type 1 diabetes mellitus,[16] suggesting that peripheral endothelial dysfunction is associated with retinal vessel dysfunction.

Large artery stiffness is an independent predictor of cardiovascular mortality and increased aortic stiffness has been found to increase cardiovascular mortality in patients with type 2 diabetes.[17,18] Moreover, large artery stiffness has also been associated with microvascular complications in patients with type 2 diabetes, including DR.[2,4,19,20]

Whether retinal vasoreactivity is associated with peripheral endothelial dysfunction and large artery stiffness in patients with type 2 diabetes remains to be elucidated.

In the present study, we examined a group of well-regulated patients with type 2 diabetes without retinopathy and a group of sex- and age-matched controls to test the following hypothesis:

Endothelial dysfunction and arterial stiffness are associated with perturbations of the autoregulation of the retinal blood supply in patients with type 2 diabetes.

1.1. Subjects

We included 20 patients with type 2 diabetes and 20 control subjects matched on sex and age. All participants were recruited from an already established cohort that has been described in detail earlier.[19] In brief, 100 patients with type 2 diabetes were recruited from the outpatient clinic at Aarhus University Hospital, Aarhus, Denmark, while 100 sex- and age-matched control subjects were recruited through advertisement in the local press. Enrollment and baseline examinations took place from 2009 to 2011, and 5-year follow up examinations were conducted from 2014 to 2016.

Inclusion criteria for both groups were age ≫ 18 years; for patients diagnosis of type 2 diabetes in accordance with recent consensus criteria[21] and diabetes duration ≪5 years at the time of enrolment 2009–11. Control subjects with previously undiagnosed diabetes were excluded by fasting glucose and oral glucose tolerance tests.

As the cohort was also examined for other research purposes, exclusion criteria included acute or chronic infectious disease, pregnancy or lactation, prior or current cancer and contraindications to MRI. Additional exclusion criteria for this study were ocular disease of any kind (except mild degrees of cataract) and epilepsy (due to flickering light stimulation during the ophthalmological examination), see Supplemental Table 1.

The study protocol was approved by The Central Denmark Region Committees on Health Research Ethics and by the Danish Data Protection Agency. All participants gave their written, informed consent.

2. Materials and methods

2.1. Data collection

Data collection for the present study took place between April 2016 and December 2016. The participants underwent all examinations (assessment of endothelial function, arterial stiffness, ophthalmological examination and dynamic vessel analysis) on the same day. Participants abstained from intake of any caffeinated beverages and tobacco usage on the day of the examinations and were all fasting at least 3 h prior to the examinations. They did, however, take their prescribed medicine as usually.

2.2. Patient characteristics

At the 5-year follow-up, all participants underwent assessment of anthropometrics, HbA_{1C}, plasma cholesterol, urinary albumin:creatinine ratio (UACR), 24-hour blood pressure monitoring, endothelial function, pulse wave velocity and eye examinations. These data were used for baseline characteristics in the present study.

2.3. Endothelial function

Endothelial function was assessed by peripheral arterial tonometry (PAT) during a reactive hyperaemia (RH) protocol using the EndoPAT 2000 device (Itamar Medical Inc., Caesarea, Israel). The system consists of a pneumatic plethysmograph in form of two inflatable digital probes that detect pressure alterations in the finger probes induced by changes in digital pulse wave amplitude. These digital probes are connected to an inflating device through pneumatic tubes and are controlled by a computer. During the RH protocol, the digital probes were placed on both index fingers and a blood pressure cuff (Hokanson SC-12, Bellevue, WA, USA) was placed on the left upper arm; the right arm served as a control. The examination takes 15 min and was conducted with the subject in supine position. After five minutes of baseline measurements arterial blood flow in the left forearm was occluded by inflation of the cuff on the left upper arm to 200 mm Hg or systolic pressure plus 60 mm Hg (whichever was higher) for five minutes after which the measurements proceeded for five minutes. Endothelial function was evaluated via the RH-index (RHI) calculated by the EndoPAT software. The RHI is a ratio of the PAT amplitude post-to-pre occlusion of the tested arm divided by the post-to-pre occlusion ratio of the control arm – a low RHI indicates reduced endothelial function. Data are presented as a natural logarithm of the measured RH-index, LnRHI, as data were skewed.

2.4. Arterial stiffness

Large artery stiffness was evaluated by measuring carotid-femoral pulse wave velocity (cfPWV).[22] All examinations were conducted between 8 a.m. and 12 noon in a quiet room after a minimum of 5 min of rest in the supine position. To assess cfPWV, sequential electrocardiogram-referenced recordings were made at the carotid and femoral artery using an applanation tonometer (SPT—301B; Millar, Houston, TX, USA) and the SphygmoCor equipment and software (AtCor, Medical, Sydney, Australia). The path length was calculated by subtracting the length from the site of the carotid pulse wave measurement to the sternal notch from the length between the sternal notch and the site of the femoral pulse wave measurement. Distance measurements were made using a calliper. Carotid-femoral pulse wave velocity was calculated as a mean of three consecutive measurements.

Office blood pressure measurements were used to calibrate the equipment and was measured on the right arm after a minimum of 5 min' rest in the supine position before the cfPWV measurements (Riester Champion N automatic blood pressure monitor, Riester GmbH, Jungingen, Germany) and calculated as an average of three consecutive measurements.

All examinations were conducted by one trained investigator.

2.5. Ophthalmological examination

Participants underwent a standard ophthalmological examination including assessment of intraocular pressure (IOP) (Nidek, Tonoref II, Japan), measurement of best-corrected visual acuity using Snellen charts and a slit-lamp examination. Subsequently, best corrected visual acuity was measured using ETDRS charts, and mydriasis was induced using phenylephrine 10% (SAD, Denmark) and tropicamide 1% (Alcon, Denmark) eye drops followed by a 60° fundus photography centred on the fovea (Canon CF 60Z, Amstelveen, Holland). Further examinations involved assessment of central retinal thickness by optical coherence tomography (OCT) scanning (Cirrus HD OCT, Carl Zeiss Meditec, Dublin, CA, USA) and video recordings of the retinal vessels using the Dynamic Vessel Analyzer (DVA, Imedos, Jena, Germany).

2.6. Dynamic vessel analysis

The DVA consists of a fundus camera and a video unit connected to a computer. This allows continuous recordings of the retina throughout the examination protocol and software measures changes in retinal vessel diameter real-time. Thus, the diameter response of the retinal arterioles is determined during an increase in systemic blood pressure induced by isometric exercise, stimulation of the retinal metabolism with flickering light and during a combination of these two experimental conditions to examine a potential interaction. In healthy individuals an increase in systemic blood pressure leads to vasoconstriction, keeping the retinal blood flow constant. Conversely, stimulation of the retinal metabolism leads to vasodilatation to accommodate the retinal oxygen demand.

The setup has been described in detail previously,[23] as has the examination protocol used in the present study.[24] To summarize, the subject was positioned in front of the camera in seated position during the entire examination, and gaze was fixed at the tip of a small bar with an adjustable position within the viewing system of the DVA. The investigator marked a linear vessel segment without branches on the upper or lower temporal arteriole and the adjoining venule located between a half and two disk diameters from the margin of the optic disk. The DVA software then measured the diameter 25 times per second for every 10 μm along the marked segments throughout the examination. The examination lasted for 14 min and was divided into 7 uninterrupted phases of 2 min each (see Fig. 1). Phase 1 consisted of an initial resting period followed by phase 2 where the arterial pressure was raised by isometric exercise (lift of a 2-kg hand weight in the right arm). Subsequently, 2 min' rest followed in phase 3. In phase 4 the participant was subject to stimulation of the retina by flickering lights at 12.5 Hz before yet another resting period in phase 5. Phase 6 consisted of a combination of the two interventions (isometric exercise and flickering light stimulation). The examination was concluded by a final resting period in phase 7. Blood pressure was monitored during each of the phases, except for the last.

Data presented here were sampled from the last 45 s of each phase.

2.7. Statistical analyses

Distribution of data was assessed by histograms and QQ plots. Normally distributed data are presented as mean ± standard deviation (SD), whereas skewed data (diabetes duration, HbA_{1C}, blood glucose,

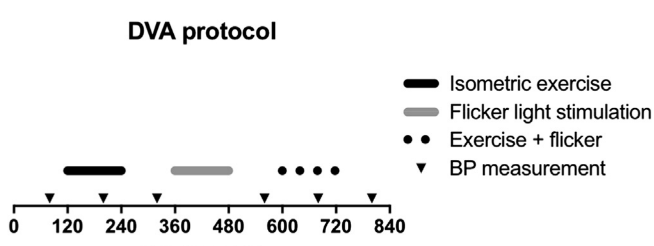

Fig. 1. Timeline over the protocol for the examination of the dynamic characteristics of the retinal vasculature using the DVA.

triglycerides, RH-index, UACR) are presented as median (range). Categorical values are presented as numbers (%).

Retinal arteriolar diameters are reported in arbitrary units (AU) approximately corresponding to μm in the Gullstrand eye.[25] Mean arterial pressure (MAP) was calculated as diastolic blood pressure + (⅓ x pulse pressure).

Mixed model analyses were initially conducted to analyse the repeated measurement data in the retinal vessel analysis. However, as patients returned to baseline levels in the resting period between examinations, retinal arteriolar diameter changes in the two groups could be assessed with paired *t*-tests for each intervention and the results of these analyses are reported. We tested for interaction between group (diabetes/control) and intervention response for each intervention in subsequent linear regression analyses.

Linear regression analyses were conducted to test for association between PWV and LnRHI with retinal arteriolar diameter change during each intervention. Both unadjusted results and results adjusted for sex, age and blood pressure are presented.

We tested for interaction between endothelial function and group (type 2 diabetes/control) and between PWV and group (type 2 diabetes/control) in the association with arteriolar function. When the interaction term was non-significant, a common coefficient for patients with type 2 diabetes and controls was calculated. As the power to detect a significant interaction term could be relatively low given the study sample size, we also report the associations for patients with type 2 diabetes and controls separately.

Statistical analyses were conducted using Stata 14 (StataCorp LP, College Station, TX, USA).

3. Results

3.1. Subject characteristics

Clinical and biochemical data for patients and controls are presented in Table 1. Patients with type 2 diabetes had good glycemic control and had comparable office and 24 h blood pressure, and higher BMI compared to controls, Table 1. No significant difference between the two groups with regard to smoking status or previous cardiovascular disease was observed.

Patients had significantly lower levels of total cholesterol, HDL and LDL cholesterol. Conversely, triglyceride levels were significantly higher in patients with type 2 diabetes. Moreover, patients with type 2 diabetes had significantly higher urinary albumin:creatinine ratios.

A significantly larger proportion of patients with type 2 diabetes were treated with antihypertensive agents, acetylsalicylic acid and statins.

No statistically significant differences were observed regarding the ophthalmological parameters intraocular pressure and visual acuity. Controls did, however, have higher measures of central retinal thickness compared to patients. In addition, none of the patients with diabetes had any degree of DR.

3.2. Endothelial function and arterial stiffness

No difference was observed between the two groups regarding endothelial function as assessed by LnRHI (0.71 ± 0.30 vs. 0.81 ± 0.30, $p = 0.32$) (Fig. 2), but patients with type 2 diabetes had significantly higher cfPWV compared to controls (9.3 ± 1.8 m/s vs. 8.3 ± 2.2 m/s, $p = 0.049$) (Fig. 3).

3.3. Retinal arteriolar diameter response

There was no significant difference in retinal arteriolar baseline diameter between the two groups (116.2 ± 14.4 AU vs. 107.0 ± 10.6 AU, $p = 0.065$). The retinal arteriolar diameter responses to each intervention during dynamic retinal vessel analysis are

Table 1
Clinical and biochemical characteristics of the study population.

Characteristic	T2DM ($n = 20$)	Controls ($n = 20$)	p-Value
Clinical			
Age, years	66.5 ± 7.3	65.2 ± 7.6	
Male sex, n (%)	8 (40)	8 (40)	
Diabetes duration, years	9.9 (6.0;12.4)	n/a	
24 h blood pressure, mmHg			
Systolic	121 ± 12[a]	122 ± 10[b]	0.90
Diastolic	71 ± 7[a]	73 ± 8[b]	0.98
Office blood pressure, mmHg			
Systolic	127 ± 13	130 ± 14	0.56
Diastolic	75 ± 7	78 ± 10	0.27
Heart rate (beats/min)	67 ± 11[c]	58 ± 12	0.02
BMI, kg/m^2	31.1 ± 6.2	26.1 ± 3.4	0.009
Smoking status			0.28
Current, n (%)	6 (30)	2 (10)	
Former n (%)	6 (30)	7 (35)	
Never, n (%)	8 (40)	11 (55)	
Previous CVD, n (%)	2 (10)	0 (0)	0.15
Biochemical			
HbA_{1C}, mmol/mol	49 (39;86)	38 (33;46)	0.0001
HbA_{1C}, (%)	6.6 (6.3;7.3)	5.6 (5.5;6.0)	0.0001
Blood glucose, mmol/L	7.6 (4.5;19.1)	5.7 (3.9;7.1)[d]	0.002
Total cholesterol, mmol/L	4.0 ± 1.31	5.2 ± 0.9	0.004
HDL-C, mmol/L	1.4 ± 0.5	1.7 ± 0.5	0.01
LDL-C, mmol/L	1.8 ± 0.9	3.0 ± 0.9	0.002
Triglycerides, mmol/L	1.8 (0.5;11.4)	1.0 (0.6;2.4)	0.03
Urinary albumin:creatinine ratio, mg/g	3 (0;159)[e]	0 (0;85)	0.06
Medication			
Antihypertensive treatment, n (%)	16 (80)	7 (35)	0.004
Diabetes treatment			
Metformin, n (%)	16 (80)	0 (0)	
Sulfonylureas, n (%)	3 (15)	0 (0)	
GLP-1 agonist, n (%)	2 (10)	0 (0)	
DPP4 inhibitor, n (%)	4 (20)	0 (0)	
Insulin, n (%)	5 (25)	0 (0)	
Acetylsalicylic acid, n (%)	18 (90)	4 (20)	≪0.001
Statin, n (%)	18 (90)	6 (30)	≪0.001
Ophthalmological			
Intraocular pressure, mmHg	13.1 ± 2.2	12.6 ± 2.9	0.41
Best-corrected visual acuity, ETDRS-letters[f]	90 ± 6	92 ± 5	0.55
Central retinal thickness, μm	252 ± 23	275 ± 25	0.0045

[a] $n = 19$.
[b] $n = 17$.
[c] $n = 19$.
[d] $n = 19$.
[e] $n = 19$.
[f] 90 ETDRS-letters corresponds to a 20/15 Snellen equivalent.

featured in Fig. 4 and Table 2. Mean arterial pressure increased in both patients and controls during isometric exercise (20 mm Hg vs. 21 mm Hg) alone and in combination with flicker (19 mm Hg vs. 19 mm Hg). The increase was not statistically significantly different in the two groups, (see Supplemental Table 2). Isometric exercise alone did not lead to a significant change in mean retinal arteriolar diameter in any of the two groups (type 2 diabetes: $p = 0.97$; controls: $p = 0.34$) and no group difference in the response to the intervention was observed, Table 2. Stimulation with flickering light elicited a significant arteriolar vasodilatation in both groups (type 2 diabetes: $p = 0.01$, controls: $p = 0.004$), but no significant group difference in the response to flicker light stimulation in the two groups was observed, Table 2. Similarly, a combination of these two stimuli entailed a significant vasodilatation of the retinal arterioles in both groups (type 2 diabetes: $p = 0.001$; controls: $p = 0.0001$); however, no significant group difference in the response to the intervention was observed, Table 2.

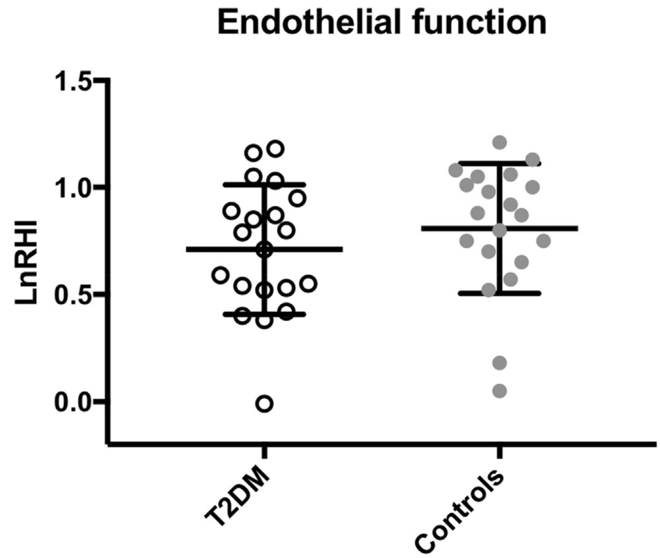

Fig. 2. Scatter dot-plot over endothelial function assessed by PAT for the two groups.

3.4. Association between endothelial function, arterial stiffness and retinal arteriolar function

No significant interaction was observed between endothelial function and type 2 diabetes/control group or between PWV and type 2 diabetes/control group in the tests for association with retinal arteriolar function (all p-values were $\gg 0.05$). Therefore, a common beta-coefficient for both groups was calculated Table 3. Baseline heart rate was not associated with the DVA parameters in multivariate analyses, data not shown.

3.4.1. Endothelial function

There was no association between endothelial function and retinal arteriolar function during the first two interventions (Table 3). Endothelial function was, however, associated with retinal arteriolar function during the combination intervention with both isometric exercise and flicker light stimulation. This association remained significant also when adjusting for age, sex and change in MAP during the intervention (Beta = 0.045 [0.010;0.079], $p = 0.014$).

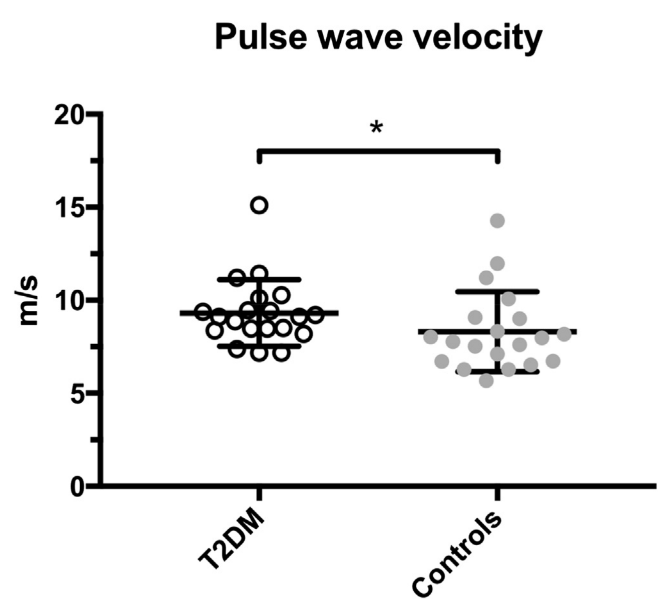

Fig. 3. Scatter dot-plot showing arterial stiffness assessed as cfPWV for the two groups.

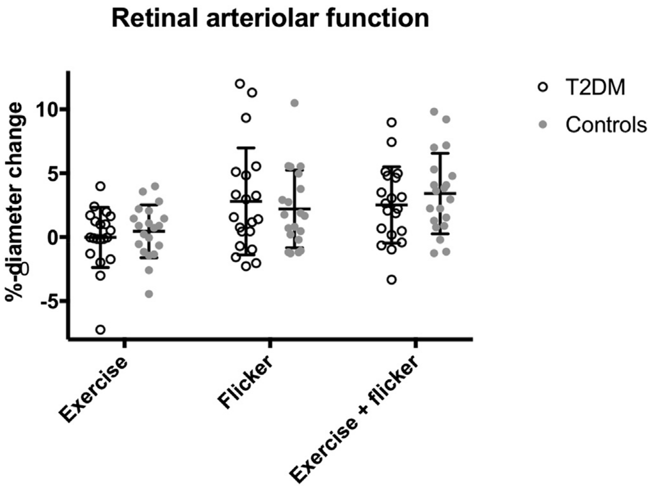

Fig. 4. Retinal arteriolar %-diameter change in response to isometric exercise, flicker and a combination of exercise and flicker for both groups.

3.4.2. Arterial stiffness

No association was observed between pulse wave velocity and retinal arteriolar %-diameter change for any of the three interventions. This was the case for both crude and adjusted estimates.

The association between endothelial function, arterial stiffness and retinal arteriolar function is also reported separately for patients with type 2 diabetes and controls in Table 4.

Whereas both crude and adjusted estimates of association between retinal arteriolar %-diameter change in response to both exercise and flicker light and endothelial function were statistically significant in the control group, the association did not reach the level of statistical significance in the diabetic subjects. As for the common linear regression analysis, no association was found between retinal arteriolar function and arterial stiffness in the separate estimates.

4. Discussion

The first main finding of this study was that retinal vessel reactivity was not significantly different in patients with type 2 diabetes and sex- and age matched control. This observation contrasts with findings in previous studies.[6–10] Differences in diabetes duration, glycemic regulation, medications or other patient characteristics may contribute to these differences. The second main finding was that peripheral endothelial function was associated with retinal arteriolar function during a combination stimulus of isometric exercise and flickering light stimulation in patients with type 2 diabetes and controls. The association was statistically significant only in the control group when the two groups were analyzed separately, indicating that the observed association in the combined diabetes and control sample was primarily driven by the controls. However, in formal statistical analysis for effect modification by group, the interaction term was not significant. Thus, from a statistical point of view, we cannot conclude that the observed association is significantly different in controls and patients with type 2 diabetes. This could reflect lack of power in the statistical analyses, and further conclusions in this regard must await studies with more participants. Our finding suggests that peripheral endothelial function might reflect microvascular endothelial function in the in subjects without T2DM, whereas our data did not indicate an association in T2DM patients without retinopathy. The same association was observed between endothelial function and flicker-induced vasodilatation, but this association was not statistically significant. The observed association between peripheral and retinal endothelial function in the overall sample is in line with recent studies, where attenuated flicker-induced vasodilatation was associated with impaired FMD in diabetic subjects with and without DR.[16,26] However, as noted above, our findings contrasts with previous studies as the association was not statistically significant in the diabetes patients when they were analyzed separately. The third main finding was that cfPWV as a measure of large artery stiffness was not associated with retinal vascular reactivity. The findings imply that the two parameters are determined by different pathogenic mechanisms.

In addition, control subjects had increased central retinal thickness compared to patients with type 2 diabetes, both within the normal range. Thinning of the central retina has recently been suggested to be an early feature of DR.[27]

Endothelial dysfunction has been suggested to be implicated in the early development of diabetic angiopathy, including DR.[28,29] Several studies have reported attenuated flicker-induced vasodilatation in patients with diabetes.[6–8,11] Furthermore, these changes in retinal autoregulation have been reported in subjects with prediabetes[9,11] and appear to deteriorate with increasing degrees of retinopathy,[30] underlining the role of dysregulation of retinal blood flow in the development of DR.

In the present study, we did not observe attenuated flicker-induced vasodilatation in diabetic subjects. In addition, contrary to previous studies,[14,31] no difference in peripheral endothelial function was observed between controls and diabetic subjects in the present study. This discrepancy could reflect that the patients in this study population were well-controlled regarding conventional cardiovascular risk factors (blood pressure, glucose and cholesterol). However, little is known about risk factors associated with attenuated vasoreactivity, as the previous studies investigating retinal vasoreactivity have matched controls with regard to known risk factors for cardiovascular disease.

Furthermore, none of the patients had any degree of retinopathy, despite a mean diabetes duration of 9.9 years. Thus, the sample of patients included in this study may have a lower propensity for retinopathy development than the general population of type 2 diabetics. Moreover, the age of the study population could be of importance as increasing age previously has been associated with a decline in retinal autoregulation.[32] The mean age of the present study population was in the mid-60s, whereas previous studies referred to in the present work have had study populations with a mean age in the 50s.

Large artery stiffness has previously been associated with different measures of damage to the microvasculature in the brain, kidney as well as in the retina.[3,4,19,20,33] One theory is that increased large artery stiffness leads to transmission of excess pulsatile energy into the microvasculature due to the altered biomechanical properties of the vasculature. This theory has been supported by recent work by Mitchell et al., who demonstrated a correlation between higher flow pulsatility in the carotid artery, cfPWV and microvascular lesions in the brain.[33]

The findings of the present study may infer that the detrimental effects of transmission of excess pulsatile energy into the microvasculature is of greatest importance in high-flow organs such as the brain and kidneys.

Strengths of the study include the matched design on sex and age, and the involvement of interventions to evaluate retinal vessel function. Additionally, the two groups were comparable on other parameters as well, e.g. blood pressure and previous cardiovascular disease. None of the patients with type 2 diabetes had significant signs of retinopathy, making them a homogenous group of participants. However, our

Table 2
Retinal arteriolar %-diameter change in response to isometric exercise, flicker and a combination of exercise and flicker for both groups.

Intervention	Retinal arteriolar %-diameter change		p-Value
	T2DM ($n = 20$)	Controls ($n = 20$)	
Exercise, M ± SD	−0.020 ± 2.35	0.45 ± 2.07	0.44
Flicker, M ± SD	2.80 ± 4.19	2.20 ± 3.04	0.59
Exercise + flicker, M ± SD	2.52 ± 2.98	3.41 ± 3.15	0.32

M: Mean, SD: Standard deviation.

Table 3
Linear regression models used to test for association between LnRHI and retinal arteriolar %-diameter change and PWV and retinal arteriolar %-diameter change for each stimulus.

Intervention/regression model	Exercise		Flicker		Exercise + flicker	
	β-Coefficient (95% CI)	*p*-Value	β-Coefficient (95% CI)	*p*-Value	β-Coefficient (95% CI)	*p*-Value
LnRHI						
Crude	−0.0027 (−0.021;0.015)	0.76	0.033 (−0.0052;0.071)	0.09	0.033 (0.0013;0.064)	0.042
Model 2	−0.0025 (−0.021;0.016)	0.78	0.034 (−0.0017;0.069)	0.06	0.033 (0.0022;0.065)	0.037
Model 3	−0.0076 (−0.031;0.016)	0.50	0.040 (−0.0041;0.083)	0.07	0.043 (0.0077;0.078)	0.02
cfPWV						
Crude	−0.00061 (−0.0033;0.0020)	0.63	−0.000061 (−0.0050;0.0049)	0.98	−0.0013 (−0.0052;0.0027)	0.51
Model 2	−0.00071 (−0.0035;0.0020)	0.59	0.00013 (−0.0049;0.0051)	0.96	−0.0014 (−0.0057;0.0029)	0.51
Model 3	−0.0031 (−0.0068;0.00064)	0.10	0.00043 (−0.0051;0.0060)	0.87	0.00016 (−0.0048;0.0052)	0.95

Model 2: Adjusted for age, sex and group (type 2 diabetes/control).
Model 3: Adjusted for age, sex, group (type 2 diabetes/control) and baseline MAP.

observations may thus not apply to patients with type 2 diabetes with other characteristics, including patients with retinopathy.

A limitation of the study is its cross-sectional design with regard to the associations between LnRHI/cfPWV and retinal vasoreactivity, making it difficult to determine a potential direction of causality. A larger proportion of patients was treated with antihypertensives, statins and acetylsalicylic acid. The possible implications of this pharmacological disparity are difficult to assess, but particularly antihypertensive treatment has been demonstrated to lower PWV.[34,35] In addition, control subjects with hypertension and dyslipidemia were included in the study, which could have influenced the results. The sample size limited the number of covariates to be included in the multivariate analysis, and potential residual confounding from the abovementioned variables could affect the results. Another possible biasing factor is that we measured reactivity of large retinal arterioles, whereas the pathological lesions that lead to diabetic retinopathy often occur in smaller vessels harbouring the microcirculation.[36] Moreover, as the site of the measurements was limited to either the upper or lower temporal arteriole, the results may not reflect regional pathology elsewhere in the retina. Indeed, regional differences in retinal vasoreactivity has been suggested to contribute to the regional distribution of diabetic retinopathy lesions.[37] We found no significant differences in retinal vessel reactivity and in endothelial function in patients with type 2 diabetes compared to sex- and age matched control. Our study sample size was comparable to previous studies that observed significant differences in retinal vessel reactivity[6,7] and reduced endothelial function.[16] Accordingly, lack of power seems less likely as an explanation for our findings.

5. Conclusions

Endothelial function assessed by peripheral arterial tonometry was associated with retinal vascular reactivity in response to a combination of isometric exercise and flickering light stimulation. This finding suggests that peripheral endothelial function is reflective of endothelial dysfunction in the retina. Our findings indicate that peripheral endothelial function is reflective of endothelial function in the retina mainly in subjects without T2DM, whereas no firm conclusions can be drawn in T2DM without retinopathy. Further studies are needed in T2DM patients with more advanced retinopathy.

Future perspectives

The findings of the present study contribute to the understanding of the complex pathophysiology of microvascular complication development in patients with type 2 diabetes. A more comprehensive understanding of these mechanisms could prove useful in the clinic in relation to early identification of late complications and risk stratification of patients with type 2 diabetes.

Table 4
Linear regression models used to test for association between LnRHI and retinal arteriolar %-diameter change and PWV and retinal arteriolar %-diameter change for each stimulus in both groups.

Intervention/regression model	Exercise		Flicker		Exercise + flicker	
	β-Coefficient (95% CI)	*p*-Value	β-Coefficient (95% CI)	*p*-Value	β-Coefficient (95% CI)	*p*-Value
T2DM						
LnRHI						
Crude	−0.0054 (−0.044;0.033)	0.77	0.047 (−0.017;0.11)	0.14	0.013 (−0.035;0.062)	0.57
Model 2	−0.0066 (−0.052;0.039)	0.77	0.037 (−0.040;0.11)	0.32	0.0076 (−0.049;0.064)	0.78
Model 3	−0.018 (−0.074;0.038)	0.50	0.052 (−0.046;0.15)	0.27	0.016 (−0.056;0.088)	0.65
Controls						
LnRHI						
Crude	0.000026 (−0.034;0.034)	0.99	0.018 (−0.031;0.067)	0.44	0.052 (0.0071;0.096)	0.03
Model 2	−0.0034 (−0.043;0.036)	0.86	0.033 (−0.021;0.086)	0.22	0.066 (0.017;0.12)	0.01
Model 3	−0.0063 (−0.047;0.034)	0.74	0.040 (−0.011;0.092)	0.11	0.074 (0.029;0.12)	0.003
T2DM						
cfPWV						
Crude	−0.00054 (−0.0070;0.0060)	0.86	0.0062 (−0.0050;0.017)	0.26	0.0015 (−0.0067;0.0097)	0.71
Model 2	−0.00071 (−0.0077;0.0062)	0.83	0.0075 (−0.0039;0.019)	0.18	0.0018 (−0.0067;0.010)	0.66
Model 3	−0.0041 (−0.013;0.0050)	0.35	0.0085 (−0.0076;0.025)	0.28	0.037 (−0.0081;0.015)	0.51
Controls						
cfPWV						
Crude	−0.0066 (−0.0054;0.0041)	0.77	−0.0044 (−0.011;0.0023)	0.19	−0.0032 (−0.010;0.0039)	0.36
Model 2	−0.00052 (−0.0056;0.0046)	0.83	−0.0048 (−0.012;0.0020)	0.15	−0.0033 (−0.011;0.0043)	0.37
Model 3	−0.0011 (−0.0093;0.0070)	0.76	−0.0047 (−0.016;0.0063)	0.37	−0.00083 (−0.013;0.012)	0.89

Model 2: Adjusted for age and sex.
Model 3: Adjusted for age, sex and baseline MAP.

The study population of the present study were very well regulated with regard to glycaemia and traditional cardiovascular risk factors. Future studies including patients with dysregulated glycaemia and more advanced retinpathy are needed to further investigate the association between peripheral endothelial function and retinal vessel reactivity.

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Data availability

All data generated and/or analyzed in the present study are available from the corresponding author on request.

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Contribution statement

J.M.B. developed study hypothesis; developed the study design; acquired, analyzed and interpreted data; and drafted and critically revised the manuscript. K.L.F. provided technical support, and critically revised the manuscript. L.P. provided technical support, interpreted data and critically revised the manuscript. L.V. acquired and interpreted data; and critically revised the manuscript. S.T.K. generated the study hypothesis; and critically revised and edited the manuscript. T.B. developed the study hypothesis; developed the study design; interpreted data; and critically revised the manuscript. P.L.P. generated the study hypothesis; developed the study design; handled supervision; interpreted data; and critically revised and edited the manuscript. E.L. generated the study hypothesis; developed the study design; analyzed and interpreted data; provided technical support; handled supervision; and critically revised and edited the manuscript. J.M.B is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jdiacomp.2019.05.024.

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