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# Impaired microvascular properties in uncomplicated type I diabetes identified by Doppler ultrasound of the ocular circulation

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## Abstract

**Objective:** Quantification of Doppler flow velocity waveforms has been shown to predict adverse cardiovascular outcomes and identify altered downstream haemodynamics and vascular damage in a number of organ beds. We employed novel techniques to quantify Doppler flow velocity waveforms from the retro bulbar circulation.

**Methods and results:** In total, 39 patients with uncomplicated Type I diabetes mellitus, and no other significant cardiovascular risk factors were compared with 30 control subjects. Flow velocity waveforms were captured from the ophthalmic artery (OA), central retinal artery (CRA) and the common carotid artery. The flow velocity profiles were analysed in the time domain to calculate the resistive index (RI), and time-frequency domain using novel discrete wavelet transform methods for comparison. Analysis of flow waveforms from the OA and CRA identified specific frequency band differences between groups, occurring independently of potential haemodynamic or metabolic confounding influences. No changes were identified in the calculated RI from any arterial site.

**Conclusion:** Novel analysis of the arterial flow velocity waveforms recorded from the retro bulbar circulation identified quantifiable differences in Doppler flow velocity waveform morphology in patients with diabetes prior to the development of overt retinopathy. The technique may be useful as an additional marker of cardiovascular risk.

## Keywords

Endothelium, microcirculation, ocular ultrasound, type I diabetes

## Introduction

Cardiovascular complications represent the major cause of morbidity and mortality in diabetes mellitus (DM). Micro and macro vascular complications lead to target organ damage involving the eye and kidneys, as well as the coronary, lower limb and cerebral circulations. Unfortunately, by the time symptoms develop or events occur as manifestations of target organ damage, the disease process is already at an advanced stage.<sup>1–3</sup>

It is apparent that structural and functional changes in different microvascular beds can provide key predictive information with respect to the development of future cardiovascular risk factors and events.<sup>4–7</sup> Experimental data support the concept that the microvascular beds of the eye and kidney represent preferential targets for systemic disease, where changes in vessel structure and function not only predict local complications but also cardiovascular and cerebrovascular events.<sup>8</sup> Retinopathy, in particular, is a

preclinical marker for microvascular structural abnormalities in the retinal circulation that have been shown to independently predict future macrovascular events including stroke and coronary heart disease.<sup>9–11</sup> Furthermore, it is recognised that diabetes-related retinal vascular structural and functional abnormalities in humans and animal models commences soon after the onset of diabetes.<sup>12–14</sup> Thus it is proposed that

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retinal and cardiovascular diseases share a 'common soil' and that retinopathy may be a marker of underlying subclinical vascular disease that is predictive of the future development of cardiovascular events and mortality.<sup>15</sup>

Microvascular dysfunction profoundly influences Doppler blood flow velocity profiles and the abnormal waveforms are highly predictive of adverse clinical outcomes.<sup>4</sup> Analysis of Doppler flow velocity profiles, recorded over the duration of the cardiac cycle, have focused on flow pulsatility characteristics that are employed to derive the resistive index (RI), an estimate of the resistance to flow imparted by downstream microvascular networks.<sup>16,17</sup> Recent work has focused on developing comprehensive analysis techniques with the aim of extracting further discriminatory information from the flow velocity waveform profiles to identify early structural and functional abnormalities of distal microcirculatory beds.<sup>18</sup> Previous observations from our own and other groups have demonstrated the value of spectral analysis techniques in quantifying arterial waveform structure to identify and track change in downstream microvascular haemodynamics.<sup>18,19</sup>

In this study we employed novel spectral analysis techniques to quantify changes in arterial waveforms from the retrobulbar and carotid circulations to investigate if subclinical microvascular abnormalities can be detected in patients with uncomplicated type 1 DM.

## Methods

### Participants

This parallel group case-control study included 39 patients with uncomplicated Type 1 diabetes, and 30 age and sex-matched controls. All subjects underwent a full history and examination including an electrocardiogram (ECG) prior to entering the trial. Patients were recruited by advertisement through the diabetic clinic at the Belfast City Hospital. Patients were eligible for the study if they were in stable control of their diabetes with glycated haemoglobin (HBA1C) between 6.5 and 10%. Subjects were excluded with any history of overt cardiovascular disease, greater than grade 1 retinopathy, microalbuminuria, or untreated hypertension. Subjects on ACE inhibitors and/aspirin at the time of study stopped the medication for 5 days prior to and including the day of study. Control subjects were recruited from our department, the Belfast City Hospital Trust or the local community using poster or advertisements in the local newsletters or press. All subjects gave fully informed written consent to take part in the study. Written informed consent was obtained from all subjects. The study was performed in accordance with the Declaration of Helsinki (2000) and was approved by the local office for research ethics committee Northern Ireland.

### Subject monitoring

Heart rate was monitored continuously throughout each study via three-lead ECG. Blood pressure was measured at the start and end of the study via arterial tonometry (model CR-2000, Hypertension Diagnostics Inc. USA.).

### Procedure

All studies were performed in the early morning in a quiet, temperature-controlled room. The subjects fasted overnight and refrained from consuming alcohol, tobacco or caffeine for 12h prior to testing. Patients with diabetes refrained from taking their morning insulin dose until the study was complete. We employed B-mode and Doppler ultrasound to interrogate the ophthalmic artery using a Philips HDI-3500 ultrasound system. The same operator performed colour Doppler ultrasound examinations, with a 12.5 MHz linear array probe being used to locate and interrogate flow velocity profiles in the central retinal, ophthalmic and carotid arterial circulations.

### Retrobulbar Doppler waveform analysis

The right eye was examined. Colour Doppler ultrasonography examinations were performed by the same operator using an ATL® HDI 3500 ultrasound machine with a 12.5MHz linear array probe. Subject positioning, ultrasound technique and arterial vessel location were identified using a standardised protocol. Subjects were studied supine with their head comfortably supported on a pillow, and maintained fixation with their non-examined eye on a point marked on the ceiling directly above their head. The operator sat behind the subject's head and lightly placed the ultrasound probe, coupled with gel on the closed eyelid of the subject. Ultrasound image quality was optimised and the machine settings were kept constant for the remainder of the entire examination. Colour imaging mode was used to locate the ophthalmic artery (OA) as it coursed along the medial side of the optic nerve, and also the central retinal artery (CRA).

Pulsed Doppler recordings of flow velocity were made using a standard gate size of 1.5 mm with the Doppler angle maintained under 60° as previously described.<sup>19</sup> A 'Cineloop®' recording of the OA and CRA images were saved together with the gate depth and Doppler angle in order to relocate the same part of the vessel for subsequent measurements. An ECG trace was recorded in parallel to the blood flow velocity measurements. A 15-sec train of blood flow velocity signals from each arterial site was recorded at baseline, were digitised at 200 Hz and exported to a personal computer using HDI® lab (ATL, Advanced Technologies Laboratory, Bothell, WA) software. The peak velocity envelopes of 10 consecutive flow velocity waveform signals were stored for off-line analysis using customised software developed in our department.

**Table 1.** Reliability of vascular measures (intra-observer coefficient of variation)

Vascular measure	Mean Value	Standard Deviation	Coefficient of Variation (CV)
Carotid Artery PSV	103.7	10.2	9.9
Carotid Artery EDV	26.27	4.04	15.3
Carotid Artery RI	0.75	0.03	4.0
Ophthalmic Artery PSV	55.9	1.3	2.3
Ophthalmic Artery EDV	13.2	0.84	6.36
Ophthalmic Artery RI	0.76	0.011	1.45
Central Retinal Artery PSV	20.88	1.8	8.6
Central Retinal Artery EDV	5.66	0.89	15.7
Central Retinal Artery RI	0.73	0.053	7.2

PSV, peak systolic velocity; EDV, end diastolic velocity; RI, resistive index

### Carotid artery Doppler waveform analysis

The common carotid artery (CCA) on the side of the eye that was examined was studied in each patient. The patient was in a supine position with their head supported on a single pillow. The head was extended by 10° and rotated by 45° to the opposite side.

Colour Doppler was used to locate the carotid artery and a point 2 cm distal to the carotid bulb was insonated. A 15-sec sample of pulsed Doppler recordings of flow velocity was made using a carrying frequency of 6 MHz and a standard gate size of 1.5 mm with the Doppler angle maintained less than 60°. An ECG trace was recorded in parallel to the blood flow velocity measurements. A 15-s sample of blood flow velocity signals from the carotid artery recorded at baseline was digitised at 200 Hz and exported to a personal computer using HDI® lab (ATL, Advanced Technologies Laboratory, Bothell, WA) software. The peak velocity envelopes of 10 consecutive flow velocity waveform signals were stored for off-line analysis using customised software developed in our department.

### Intra-observer variability of vascular measures

Table 1 demonstrates the coefficient of variation (CV) of the relevant vascular measures from the carotid artery, the OA and the CRA. Measures were performed by a staff member from the department on 10 separate occasions. The CV was then calculated from the results obtained from the 10 visits using the formula:  $(100 \times \text{standard deviation} / \text{mean})$ . CVs were calculated for the peak systolic velocity (PSV), the mean diastolic velocity (EDV), and the RI.

### Spectral analysis of flow velocity waveforms

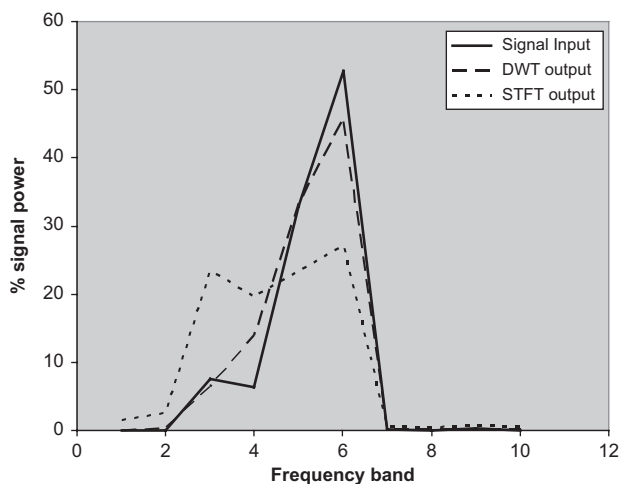
Several discrete parameters (resistive, and pulsatility indices) can be calculated from the Doppler blood flow velocity profiles. The RI is derived from single inflection points describing the excursions of flow (pulsatility) during the cardiac cycle. This index has limited ability to identify

change in the flow waveform structure indicative of functional or structural change in the microvasculature as it is derived from less than 2% of the data contained in the flow velocity envelope. We therefore employed the discrete wavelet transform (DWT), a time–frequency spectral analysis method, in order to quantify changes in the entire flow waveform structure over the duration of the cardiac cycle. The DWT frequency bands describe the blood flow velocity signal bandwidth, 11 bands in this study, the sum of which equals the original signal.<sup>20</sup> The output from each band is summarised as the mean velocity amplitude averaged over the duration of the signal length. The data was log transformed to allow parametric statistical analysis.

With respect to the use of the DWT in our work, a significant amount of time was devoted to the development and assessment of novel algorithms to assess the temporal and spectral content of blood velocity waveforms. This comprised the development of bespoke software applications using Matlab® 7.4.0.287 (R2007a), to enable other group members, without computer programming knowledge, the ability to assess and analyse the spectral content of any blood velocity waveform. In total, 30 different wavelets were investigated for use, through the spectral analysis of synthetically generated blood velocity waveforms of known spectral content. The Daubechies 8 wavelet was chosen based on its accurate representation of the signals' spectral energy content. The mathematical detail employed in the DWT has been described at length elsewhere and is beyond the scope of this paper.<sup>21</sup>

The superiority of the wavelet analysis technique compared with the traditional Fourier approach in resolving the range of frequencies contained in the flow velocity waveforms is well recognised,<sup>22–24</sup> and is shown in Figure 1. Measures of reproducibility of wavelet analysis are summarised in Table 2 demonstrating the % Quartile coefficients of variation in wavelet outputs by frequency band and vascular location. Reproducibility of derived wavelet parameters was investigated by comparison of signals acquired from a control subject on 10 separate occasions.





**Figure 1.** Comparison of Discrete Wavelet Transform (DWT) and Short Time Fourier Transform (STFT).

**Table 2.** Reliability of wavelet analysis of Doppler waveforms (coefficient of variation) (signals acquired from a control subject under study conditions on 10 separate occasions)

Frequency band	Carotid	CRA	Ophthalmic
1	13.2	3.0	5.6
2	11.7	5.9	4.3
3	7.1	4.4	2.8
4	7.7	6.2	3.9
5	4.7	5.5	3.3
6	5.9	6.4	3.0
7	15.1	7.9	1.2
8	1.9	7.3	0.4
9	4.2	8.5	0.1
10	3.6	7.6	2.2

Signals were acquired under study conditions on separate days from the carotid, central retinal, ophthalmic arteries.

### Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences software (SPSS version 15). Descriptive variables are presented as mean  $\pm$  standard deviation and compared using the independent samples *t*-test, when data were normally distributed. The robustness of multivariate normality assumptions was assessed via Hotelling's T-square permutation test. Non-parametric tests were employed as appropriate, and differences tested with the Mann-Whitney *U*-test. Statistical significance was set at  $p < 0.05$ . Co-variate analysis was applied to potential confounding outcome measures, each chosen because of established status as global cardiovascular risk factors (age, sex, smoking status, blood pressure and dyslipidaemia) in turn via multivariate models of covariance in SPSS.

## Results

### Study sample characteristics

We studied 39 patients with uncomplicated Type 1 DM and 30 controls. The study sample characteristics are presented in Table 3. The groups were well matched with respect to age, sex and body mass index. The mean age of the Type 1 diabetic subjects was  $40 \pm 12$  years. The mean duration from diagnosis of diabetes was  $19 \pm 11.8$  years. All patients had yearly retinal photography and no individual has greater than grade 1 retinopathy. All patients were receiving insulin therapy in the form of evening-dose long-acting preparation, and short-acting preparation with meals. The average daily total insulin dose was  $51 \text{ IU} \pm 14$ . There were no significant differences between groups with respect to prior cardiovascular events or family history of overt cardiovascular disease. More patients smoked and had a more favourable lipid profile, probably reflecting the increased prescription of statin therapy. Five patients with Type 1 DM were taking ACE-inhibitor therapy, indicated for optimal blood pressure control, and 11 were taking aspirin. The mean ACR value was significantly higher in patients with diabetes but remained within the defined normal range. Mean HbA1C was  $8.1\% \pm 1.2$  in patients compared with  $5.2 \pm 0.2$  in controls ( $p < 0.01$ ). Patients had a higher systolic blood pressure ( $125 \pm 14.2$  vs.  $118 \pm 14.7$ ;  $p = 0.04$ ) although all values remained within the optimal blood pressure range.

### Doppler waveform analysis

No significant differences were apparent in the RI calculated from discrete points on the OA, central retinal and carotid flow velocity waveforms: 0.71 vs. 0.73  $p = 0.64$  for CCA; 0.71 vs. 0.72  $p = 0.41$  for OA; 0.59 vs. 0.63  $p = 0.07$  for CRA (see Table 4).

Figure 2 represents an illustrative example of typical flow velocity profiles in recordings made from the OA in a patient with Type 1 diabetes and an age and sex-matched control subject. With respect to frequency analysis between groups, there were clear reproducible frequency differences in maximal velocity envelopes recorded at recordings obtained from this vascular site (Bands 3–8  $p < 0.05$ ) (Table 5).

Figure 3 represents an illustrative example of typical flow velocity profiles from the CRA in a patient with Type 1 diabetes and an age and sex-matched control subject. With respect to frequency analysis between groups there was clear reproducible frequency differences in the maximal velocity envelope recorded at this vascular site (Bands 2–8  $p < 0.05$ ) (Table 5).

No visual differences in the maximal flow velocity profiles from recordings made at the carotid artery were seen (Figure 4). However, there were quantifiable differences in frequency bands evident between groups, although to a lesser degree than changes noted in OA and CRA (Bands 3–5  $p < 0.05$ ) (Table 5).

**Table 3.** Subject Characteristics (Values are mean  $\pm$  standard deviation)

	Controls (n=30)		Patients (n=39)		p-value (t-test)
	Mean	S.D.	Mean	S.D.	
Age (yrs)	40.4	12.3	40.0	12.0	0.89
Height (m)	1.7	0.1	1.71	0.1	0.97
Weight (Kg)	76.5	15.2	75.2	11.4	0.69
Body Mass Index	26.1	3.8	25.8	2.7	0.65
Surface Area	1.9	0.2	1.8	0.9	0.79
HBA1C (%)	5.2	0.2	8.1	1.2	<b>0.01</b>
Total Cholesterol (mmol/l)	5.0	0.8	4.3	0.8	<b>0.01</b>
HDL Cholesterol (mmol/l)	1.4	0.3	1.8	0.5	<b>0.01</b>
LDL Cholesterol (mmol/l)	2.9	0.7	2.1	0.6	<b>0.01</b>
Triglycerides (mmol/l)	1.4	0.7	0.9	0.4	<b>0.02</b>
Ratio	3.6	1.3	2.3	0.9	<b>0.01</b>
Creatinine ( $\mu$ mol/l)	78.3	12.9	84.9	5.81	0.53
ACR	0.3	0.3	0.76	0.61	<b>0.01</b>
Homocysteine (mmol/l)	11.1	4.6	10.9	4.2	0.92
Systolic BP (mmHg)	117.4	14.7	125.2	14.1	0.04
Diastolic BP (mmHg)	66.5	11.4	71.5	9.6	0.05
Mean Arterial Pressure	83.6	11.3	89.3	10.3	<b>0.04</b>
Heart Rate baseline	64.1	11.5	71.9	11.6	<b>0.01</b>

**Table 4.** Comparison of resistive index between groups

Location	Patient group	Mean	Mann Whitney U	Significance
CCA	Control	0.7173	-0.466	0.641
	Diabetic	0.7250		
OA	Control	0.7050	-0.828	0.408
	Diabetic	0.7216		
CRA	Control	0.5868	-1.828	0.068
	Diabetic	0.6362		

CCA, common carotid artery; CRA, central retinal artery; OA, ophthalmic artery

### Adjustment for confounders

Table 6 shows the results of diabetic vs. control comparisons, for only those power bands significant ( $p < 0.01$ ) before adjustment and then after adjustment for age, sex, body mass index, smoking, hypertension and dyslipidaemia. Observed differences were preserved between groups, suggesting that the changes observed reflect the disease state as opposed to other established cardiovascular risk factors.

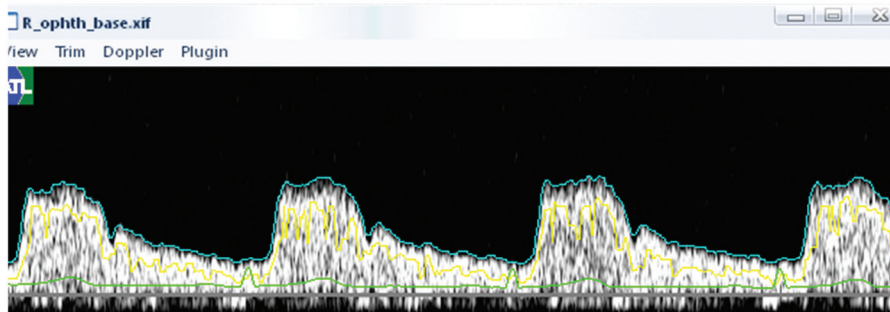
### Discussion

Doppler flow velocity waveforms between patients with uncomplicated type 1 DM and control group patients were visually different. In addition, novel spectral analysis of the Doppler flow velocity waveform envelope recorded from the retrobulbar circulation identified quantifiable differences in the waveform morphology. No visual differences in flow velocity profiles were evident between groups from

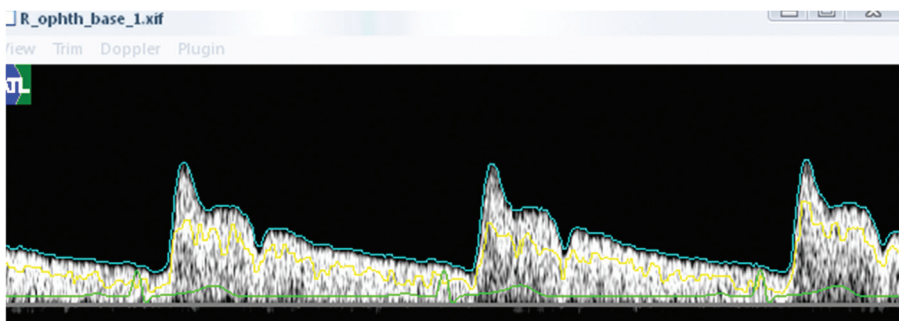
recordings made at the carotid artery, but subtle differences in frequency analysis were noted. No differences were found in the RI from flow velocity waveforms captured from any arterial site. Of note, following adjustment for various potential confounding influences, the observed differences were preserved between groups, thus suggesting that the differences reflect the disease state as opposed to other established cardiovascular risk factors.

Vascular pathology underlies most of the complications associated with DM, and may have its origins in endothelial dysfunction that has a profound effect on microvascular beds, where structural and functional abnormalities often pre-date or accompany the earliest stages of the cardiovascular disease process.<sup>25,26</sup> Maladaptive remodelling of the microvasculature is a characteristic feature and primary driver promoting target organ damage in DM.<sup>27,28</sup> Microvascular dysfunction identified in target organs not only predicts further organ dysfunction but identifies an increased risk for future macrovascular events.<sup>29</sup> As

*Representative ophthalmic artery waveform from a 49-year-old patient with Type 1 diabetes mellitus (note loss of morphology of initial diastolic decay pattern):*

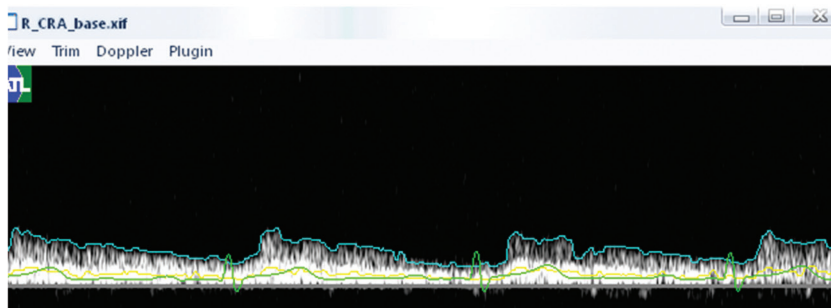


*Representative ophthalmic artery waveform from a 49-year-old healthy matched control:*

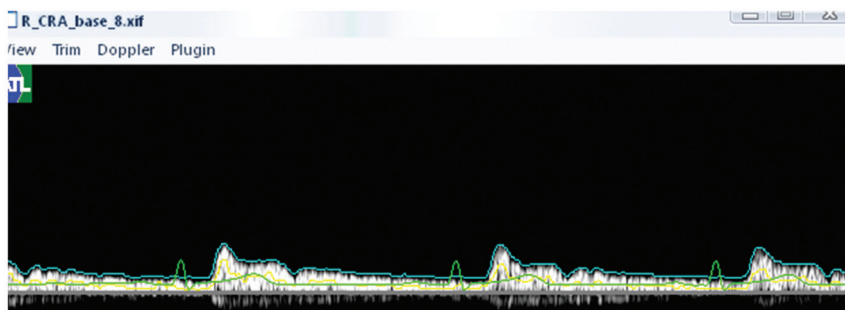


**Figure 2.** Visual differences in waveform morphology from the ophthalmic artery in a patient with Type I diabetes and an age and sex-matched control.

*Representative central retinal artery waveform from a 29-year-old patient with Type 1 diabetes mellitus (note loss of morphology of diastolic decay pattern):*



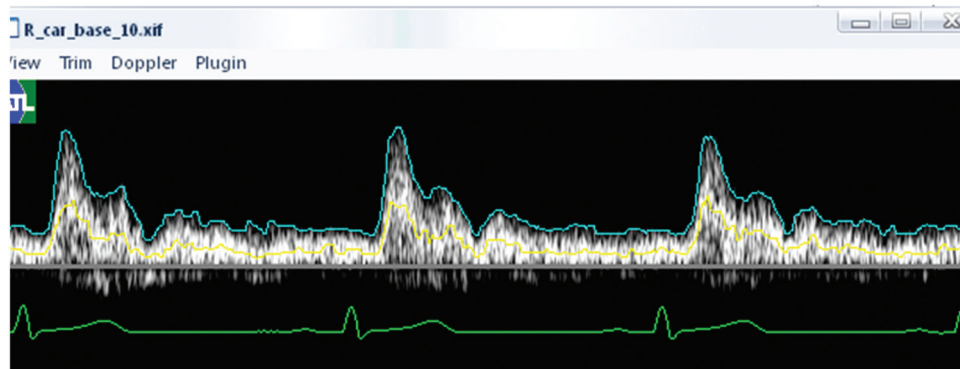
*Representative central retinal artery waveform from a 29-year-old healthy matched control:*



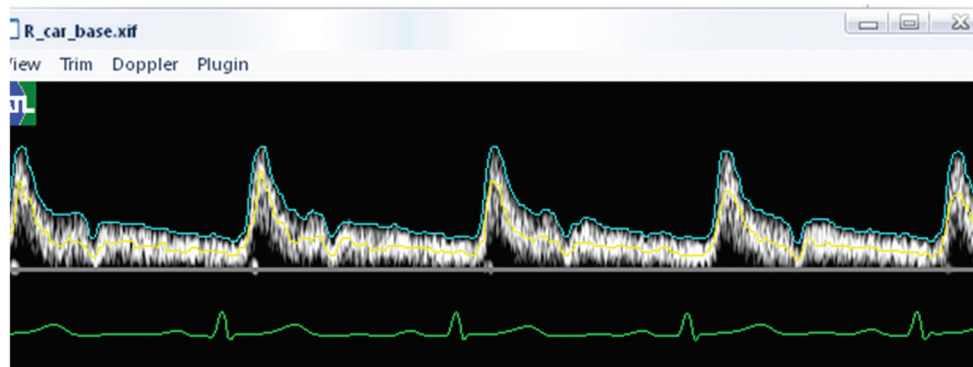
**Figure 3.** Visual differences in waveform morphology from the central retinal artery in a patient with Type I diabetes and an age and sex-matched control.



*Representative carotid artery waveform from a 29-year-old patient with Type 1 diabetes mellitus:*



*Representative carotid artery waveform from a 29-year-old healthy matched control:*



**Figure 4.** Minimal visual differences in waveform morphology from the carotid artery, which supplies the retrobulbar circulation, in a patient with Type 1 diabetes and an age and sex-matched control.

remodelling of the microvasculature represents one of the first manifestations of target organ damage and is a dynamic process that is reversible, assessment and monitoring of this section of the vasculature may hold therapeutic and prognostic significance.<sup>4,30</sup> In the Diabetes Control and Complications Trial, HbA1C and duration of diabetes explained only about 11% of the variation in retinopathy risk, suggesting a need for additional markers of risk for diabetic microvascular complications.<sup>31</sup> Crucially, changes in the retrobulbar circulation in patients in this study are apparent before the patient has developed significant retinopathy.

Microvascular dysfunction profoundly influences Doppler blood velocity flow patterns, and the abnormal waveforms are highly predictive of adverse clinical outcomes.<sup>4</sup> The RI has traditionally been employed to mark the presence of a change in flow waveshape of the Doppler waveform with disease, and an elevated RI may not only predict future organ damage but is linked to other markers of vascular damage and adverse clinical outcome.<sup>32-34</sup> However, as a measure of downstream resistance in a vascular bed we, and others, have shown the RI has significant

limitations in identifying the effects of altered microvascular haemodynamics in influencing the pattern of wave reflection and change in flow velocity waveshape.<sup>18,19,35</sup> This is because the index is influenced not only by downstream resistance but also compliance that alters the pulsatile and steady-state characteristics (impedance properties) of distal microvascular networks and the patterns of wave reflection.<sup>17,36</sup> In addition, this index has limited ability to identify change in the flow waveform structure indicative of functional or structural change in the microvasculature, as it is derived from less than 2% of the data contained in the flow velocity envelope. We therefore employed the DWT, a time–frequency spectral analysis method, in order to quantify changes in the entire flow waveform structure over the duration of the cardiac cycle. Pathological change in the microvasculature in diabetes, including basement membrane thickening, may have little effect on altering flow resistance,<sup>37</sup> but may influence the pattern of wave reflection and thus structure of the flow velocity profile.<sup>18</sup> The ultimate shape of flow and pressure waveforms reflects the interaction between the output generated by the left ventricle and the magnitude and timing of wave reflection

**Table 5.** Differences in frequency band content (log transformed mean velocity amplitude) of waveform data measured at the OA and CRA between groups

Ophthalmic Artery (OA)			
FrqBnd2	-2.249	-2.149	<b>0.098</b>
FrqBnd3	-1.034	-0.783	<b>0.001</b>
FrqBnd4	0.061	0.390	<b>0.001</b>
FrqBnd5	0.527	0.758	<b>0.007</b>
FrqBnd6	0.813	1.026	<b>0.015</b>
FrqBnd7	1.151	1.460	<b>0.003</b>
FrqBnd8	-0.595	-1.040	<b>0.027</b>
FrqBnd9	-1.115	-1.046	0.668
FrqBnd10	-1.174	-1.096	0.652
FrqBnd11	-0.969	-0.725	0.234
Central Retinal Artery (CRA)			
FrqBnd2	-2.707	-2.614	<b>0.027</b>
FrqBnd3	-1.772	-1.651	<b>0.007</b>
FrqBnd4	-1.168	-0.992	<b>0.002</b>
FrqBnd5	-0.716	-0.559	<b>0.022</b>
FrqBnd6	-0.197	-0.062	<b>0.049</b>
FrqBnd7	0.315	0.528	<b>0.015</b>
FrqBnd8	-0.896	-1.474	<b>0.003</b>
FrqBnd9	-1.226	-1.386	0.319
FrqBnd10	-1.370	-1.396	0.869
FrqBnd11	-1.073	-0.862	0.273

CRA, central retinal artery; OA, ophthalmic artery

from impedance mismatches arising predominantly from reflection sites from microcirculatory beds.

Despite differences noted in frequency analysis, the visual similarity of the flow velocity amplitudes and waveform structure recorded from the CCA strongly suggests that the flow input into the OA was similar between groups. This indicates that changes in the magnitude and timing of wave reflection from distal microvascular beds, beyond the OA and CRA recording sites, alter waveform morphology. Our data suggest that to detect early damage in these susceptible organs by identifying the altered signature of wave reflection, recording of the flow velocity signal must take place in close proximity to the microvascular bed.

The pathophysiological explanation may reside in the fact that the magnitude of wave reflection from the low-impedance ocular circulation will be small, and would therefore be masked by contributions from other circulations in recordings made at sites in more proximal conduit arteries. Furthermore, reflected flow waves emanating from the ocular microcirculation would be expected to undergo a degree of attenuation and dissipation before reaching the carotid artery.<sup>38-40</sup> Techniques providing a global assessment of the circulation may not capture, or cannot localise, findings to a specific site or target organ of interest in the arterial system.<sup>29</sup>

These results support previous findings<sup>18,41</sup> indicating that comprehensive spectral analysis of retrobulbar flow velocity waveforms can identify altered structure or tone in the ocular microcirculatory bed at an early preclinical stage.

**Table 6.** Differences in frequency band content between groups following adjustment for confounding influences-the frequency band changes in the ophthalmic and central retinal artery are preserved following adjustment

Power band	Numbers		Before adjustment					After adjustment <sup>†</sup>					
	(Diabetics, Controls)	b	SE(b)	GM ratio	(95% CI)	p	b	SE(b)	GM ratio	(95% CI)	p		
CRA													
3	67 (37,30)	0.1180	0.0421	1.13	1.03	1.22	<b>0.007</b>	0.1154	0.0479	1.12	1.02	1.24	<b>0.019</b>
4	67 (37,30)	0.1764	0.0554	1.19	1.07	1.33	<b>0.002</b>	0.1676	0.0602	1.18	1.05	1.33	<b>0.007</b>
8	67 (37,30)	-0.5187	0.1668	0.60	0.43	0.83	<b>0.003</b>	-0.3714	0.1849	0.69	0.48	1.00	<b>0.049</b>
OA													
3	66 (36,30)	0.2560	0.0755	1.29	1.11	1.50	<b>0.001</b>	0.2440	0.0810	1.28	1.09	1.50	<b>0.004</b>
4	66 (36,30)	0.3258	0.0871	1.39	1.16	1.65	<b>0.001</b>	0.2879	0.0950	1.33	1.10	1.61	<b>0.004</b>
5	66 (36,30)	0.2294	0.0848	1.26	1.06	1.49	<b>0.007</b>	0.1881	0.0936	1.21	1.00	1.46	<b>0.049</b>
7	66 (36,30)	0.3098	0.1040	1.36	1.11	1.68	<b>0.003</b>	0.2818	0.1089	1.33	1.07	1.65	<b>0.012</b>

<sup>†</sup>Adjustment for: Age, Sex, BMI, Smoking (yes/no), hypertension (yes/no) and dyslipidaemia (yes/no)

b – coefficient representing difference in means between Diabetics and Controls on log<sub>e</sub> scale

SE, standard error; GM, geometric mean

The technique represents a sensitive, non-invasive assessment tool with potential to track and monitor the effect of therapeutic interventions in relation to the development of complications with risk factors for cardiovascular disease.

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We have no potential conflicts of interest to disclose.

### Author contributions

CJL performed data collection and wrote manuscript; AMcC analysed data; CEA analysed data; PKH researched data and reviewed manuscript; CEQ researched data; CCP provided statistical input; VMcC researched data; CMcG edited/reviewed manuscript; MTH edited/reviewed manuscript; GEMcV conceived research and edited/reviewed manuscript.

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