Oxygen saturation in retinal vessels and their correlation with endothelial microparticles in diabetes mellitus and/or cardiovascular disease

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Highlight(s):

1. Endothelial microparticles are elevated in patients with cardiovascular disease and diabetes.
2. There is a link between endothelial microparticles and retinal venous oxygen saturation.
3. Retinal vessel oxygenation is linked with disease duration in patients with diabetes but not with geometrical vessel markers.

4. Our study highlights the potential of endothelial microparticles and retinal vessel saturation parameters in their ability to gain further insight into the mechanisms underlying diabetes and diabetic retinopathy.

Abstract

Purpose: Retinal oxygen supply is a critical requirement in ocular function, and when inadequate can lead to retinopathy. Endothelial dysfunction is a leading pathophysiology in diabetes and cardiovascular disease and may be assessed by endothelial microparticles (EMPs). We hypothesised links between retinal vessel oxygenation and EMPs, expecting these indices to be more adverse in those with both DM and CVD.

Methods: Plasma from 34 patients with diabetes mellitus alone (DM), 40 with cardiovascular disease (CVD) alone and 36 with DM plus CVD was probed for EMPs by flow cytometry, but also for vascular markers soluble E-selectin (sEsel) and von Willebrand factor (vWf)(both ELISA) . Retinal vessel fractal dimension, lacunarity, calibres and oxygen saturation were assessed from monochromatic and dual wavelength imaging respectively, intra-ocular pressure by was measured by rebound tonometry (I-CARE).

Results: There was no difference in oxygenation (arterial p=0.725, venous p=0.264, arterio-venous difference 0.375) between the groups, but there were differences in EMPs (p=0.049), vWf (p=0.004) and sEsel (p=0.032) . In the entire cohort, and in diabetes alone, EMPs correlated with venous oxygenation (r=0.24, p=0.009 and r= 0.43, p=0.011 respectively), whilst in DM+CVD, sEsel correlated with venous oxygenation (r=0.55, p=0.002) and with the arterial-venous difference (r= -0.63, p=0.001). In multivariate regression analysis of vascular markers against retinal oximetry indices in the entire group, EMPs were positively linked to venous oxygenation (p=0.037).

Conclusions: Despite differences in systemic markers of vascular function between DM, CVD and DM+CVD, there was no difference in arterial or venous retinal oxygenation, or their difference. However, EMPs were linked to venous oximetry, and may provide further insight into the mechanisms underlying diabetes and diabetic retinopathy.

Introduction

Retinal vessel oxygen saturation is an emerging biomarker with potential in risk stratification of diabetic retinopathy (DR) [1] and linking structural and functional damage in ocular disease [2, 3]. Numerous studies have shown that retinal venous oxygen saturation is increased in DR [4] and is linked with its severity [5]. Endothelial dysfunction and oxidative stress contribute to disease development and progression in diabetes mellitus (DM) and cardiovascular disease (CVD), and can be quantified by plasma levels of von Willebrand factor (vWf) and soluble E selectin [6,7]. Microparticles are an emerging diagnostic marker and possible therapeutic target in early DR and vascular aging [8-10].

Microparticles are small vesicles of around 1 micron in diameter shed from plasma membranes of cells through chemical and physical cell activation, damage, or apoptosis [11]. Endothelial microparticles (EMP) account for between 5-15% of the total circulating microparticles [12, 13]. It is thought that a biological role is to convey and transfer information which can alter cell adhesion, chemotaxis, contribute to angiogenesis, cause cerebral capillary damage, and increased coagulation. A growing body of evidence suggests a potential role of EMPs in the pathogenesis and progression of diabetes, where levels may reflect endothelial damage [8-10,14]. Most research examined EMPs in diabetic patients who also suffer from either DR and or other complications such as diabetic neuropathy [15]. EMPs have also been shown to be linked to macro- and microvascular parameters in patients suffering from cardiovascular disease (CVD), such as reduced flow mediated dilation and poor arterial elasticity [16]. Furthermore, the ability of EMPs to cause damage to cerebral capillaries in a mouse model [17] suggests that changes in retinal vessel indices may be linked to elevated levels of EMPs.

Hence, as both EMPs and retinal oxygen saturation parameters are emerging biomarkers in diabetes we hypothesised links between the two, also assessing two other ~~endothelial markers~~

endothelial markers von Willebrand factor (vWf) and soluble E selectin (sEsel) as comparators [18]. We chose a model where we expected these marker indices to be more adverse in patients with both DM and CVD compared to patients with either disease alone.

Materials and Methods

The study was approved by the NRES Committee East Midlands-Leicester, UK (Ref: 12/EM/0062) and the Aston University Ethics Committee, and adhered to the Declaration of Helsinki. Patients were recruited from the cardiovascular rehabilitation unit, hypertensive, and diabetic outpatient clinics at City Hospital (Birmingham, UK). All patients gave written informed consent. Three groups were defined prior to recruitment: patients suffering from DM but free from CVD: patients suffering from CVD but free from DM, and patients with both DM and CVD. DM was defined by attendance at a diabetes clinic and HbA_{1c} >50 mmol/mol (6.7%). CVD was defined as history of myocardial infarction, coronary artery stenosis/occlusion, coronary artery bypass grafting, stroke, iliac/femoral artery stenosis or bypass or lower limb/foot/toe amputation. Exclusion criteria included recent (<3 month) myocardial infarction, stroke, or surgery, cancer, autoimmune disease (such as rheumatoid arthritis) or ophthalmological disease such as age-related macular degeneration. Clinical, laboratory, medication and demographic data were collected and are shown in table 1.

Patients were advised to abstain from caffeinated drinks for a minimum of 12 hours prior to ocular examination. All were seated in a temperature-controlled room (21^{o} C +/-2^{o}) for a minimum of 15-20 minutes to achieve a stable blood pressure (systolic blood pressure [SBP] and diastolic blood pressure [DBP] were measured using a digital sphygmomanometer (UA767, PMS Instruments, UK)). Intraocular pressure (IOP) was measured using a non-contact tonometer (I-Care, Mainline Instruments Ltd., UK) after which pupils were dilated with

1% tropicamide (Chauvin Pharmaceuticals Ltd., Kingston-Upon-Thames, UK). Diabetic retinopathy (DR) was assessed by grading two full colour 50 degree retinal photographs (one macula and one ONH centred image) according to the grading system used by the National Diabetic Eye Screening programme used in England (UK).

Retinal vessel oximetry

Once pupils were fully dilated, three images were obtained per patient with the camera angle set at 30° and the optic nerve head (ONH) centred [19]. Oxygen saturation measurements were performed using the "oxygen tool" and VesselMap software (Version 2, Imedos Systems, Imedos GmbH, Jena, Germany) as described elsewhere [20]. In brief, retinal images were taken with a customized dual wavelength filter (transmission bands at 548 and 610 nm; bandwidth 10 nm each) inserted in the illumination pathway of the fundus camera (Zeiss FF450+). Optical densities of retinal vessels were measured as the logarithmic ratio of the fundus reflection at the vessel centre and its surrounding tissue. The optical density ratio (ODR) at 610 and 548 nm has been found to be inversely proportional to the vessel haemoglobin oxygen saturation when compensating for the vessel diameter and fundus pigmentation [20]. The measurement area consisted of a concentric annulus around the optic nerve head (ONH) which was half a disc diameter (DD) distant from the ONH and of one DD in width (ring area A see Figure 1). This distance and length was chosen in order to obtain results which could be used for comparison to earlier publications using the same device. Oxygen saturations were obtained of all major retinal arteries and veins crossing the measurement annulus. Following this process average arterial and venous vessel saturation for the entire measurement annulus were calculated using the software's "multi-measurement tool" (Visualis software,Imedos Systems, Jena, Germany) [19]. The multi-measurement tool allows for automatic image registration and analysis of several images. This allowed us to measure the same retinal arteries and veins of three images to calculate averages across images and vessel (separately for arteries and veins) [19]. Finally,

we calculated arterial minus venous retinal vessel saturation (A-V SO_2) which is thought to be an indirect marker of relative retinal oxygen metabolism and is independent of retinal blood flow velocity [4, 21].

Retinal vessel calibres

All retinal vessel diameters were measured using a semi-automated piece of software (VesselMap 2, Imedos, Germany) by a single observer (RH). In brief, following image selection of one monochromatic (where the monochromatic image has been obtained using the inbuilt red-free filter of the retinal camera) optic nerve head (ONH) centred retinal image, two concentric rings with a radius of 1 disc diameter (DD) and 1.5 DD radius were fitted around the ONH to demarcate the ½ DD wide measurement annulus (measurement zone B see figure 2). The six largest retinal arteries and veins passing through the created measurement annulus were included in the calculation of the central retinal artery equivalent (CRAE) and the central retinal vein equivalent (CRVE) [22].

Retinal vessel fractal dimension and lacunarity

Retinal vessel fractal dimension and lacunarity were measured from monochromatic retinal images using MONA® software (VITO Health, Mol, Belgium) assessing 50-degree monochromatic ONH centred retinal images [23]. Vessel density of the superficial retinal vessels (arteries and veins) was analysed in a concentric annulus around the ONH which was 1/2 DD distant from the ONH and 1.5 DD in width (measurement zone C see Figure 2). Following vessel segmentation, a box counting method was applied to derive a numerical value for the following two markers: vessel density as defined by fractal dimension (Df) and lacunarity. Lacunarity is often described as the ‘gapiness’ or ‘gaps between the vasculature’. Geometrical retinal vessel markers, such as calibres, fractal dimension and lacunarity were calculated for descriptive purposes of our sample and to establish if vessel geometry is

comparable across groups.

Endothelial markers

Venous blood was collected into citric acid, and plasma obtained after centrifugation at 1000g for 20 minutes. Von Willebrand factor (vWf) and soluble E selectin (sEsel) were measured by commercial enzyme linked immune-sorbent assay (ELISA) (Dako-Cytomation, Ely, Cambs UK and R&D Systems, Abingdon, UK). Endothelial microparticles (EMPs) were determined by flow cytometry (Apogee Flow Systems, Hemel Hempstead, UK) using fluorochrome-linked monoclonal antibodies to CD144 (R&D Systems, Abingdon, UK). Briefly, citrated plasma was centrifuged at 1500g for 15 min, an aliquot of the supernatant taken for further centrifugation at 13,000g for 2 minutes in accordance with the ISTH protocol [24]. A 25 μL aliquot of the second supernatant was mixed with 25 μL of the monoclonal antibody at room temperature, the volume made up to 1 mL with PBS, and the mixture applied to the flow cytometer. A minimum of 10^6 events were collected for analysis. The number of EMPs/μL was calculated without reference to the viscosity of the plasma. The size of the particle was confirmed with polystyrene beads of 110, 200, 500 nm and 1μm diameter together with 300 and 880 nm silica beads (Apogee Flow Systems, Hemel Hempstead, UK).

Statistical Analysis

We hypothesised that any of the three systemic markers of vascular function (EMPs, vWf, sEsel) would be linked to any of three markers of retinal vessel oxygenation (in arteries, in veins, and the difference between arteries and veins), calling for a sample size of 90 [25, 26]. However, in view of the likelihood that some indices would themselves be linked, for additional confidence we over-recruited by at least 15% in any one group, 25% overall. We tested the hypothesis in a three-by-three general linear model with multivariate linear regression analysis of the three retinal indices as dependent variables versus each of the three vascular indices as independent variables. Cross-sectional differences between the three groups

were assessed using analysis of variance or the Kruskal-Wallis test, depending on distribution. Between group differences were sought using Tukey's post-hoc test. Categorical data was analysed by the chi-squared test. Associations between continuously variable factors were sought using Pearson's or Spearman's correlation, dependent on distribution. Statistical significance was set at $p<0.05$, and results are reported as either mean (standard deviation) or as median (upper quartile-lower quartile). All data were analysed using Minitab version 17 (Minitab, Coventry, UK).

Results

Table 1 shows clinical, demographic, medication, and co-morbidity details. Patients with DM+CVD had lower DBP than the other two groups, whilst heart rate, HbA1c and body mass index were lower in those with CVD alone. Coronary artery disease was present in 31 (72%), peripheral artery disease in 5 (12%), and cerebrovascular disease in 8 (19%) of the patients with CVD alone. Similarly, coronary artery disease was present in 30 (81%), peripheral artery disease in 4 (11%) and cerebrovascular disease in 3 (8%) of the patients with both DM and CVD. There was no difference in the distribution of the type of CVD between the groups ($p=0.405$) and none of the patients with DM presented with diabetic retinopathy.

Table 2 shows ocular and endothelial indices, and intra-ocular pressure. Intra-ocular pressure was higher in DM compared to CVD. Retinal vessel oxygen saturations parameters and structural indices were comparable between the three groups, but EMPs were higher in DM and DM+CVD compared to CVD alone, vWf was higher in DM+CVD compared to the two other groups, and sEsel was higher in both CVD groups compared to DM alone.

Table 3 shows correlations between the retinal vessel saturation parameters, HbA1c, and endothelial markers. The only significant relationship was between EMPs and retinal venous oxygenation. In the entire cohort, their correlation coefficient (r) was 0.24 ($p=0.009$). The

duration of the diabetic disease failed to correlate with any of the endothelial markers. However, the correlation between disease duration and arterial oxygenation was significant in the DM+CVD group (r=0.44, p=0.009) but not in the DM only group (r=0.21, p=0.24). Similarly, venous oxygenation and disease duration correlated significantly in the DM+CVD group (r=0.43, p=0.009) but not in the DM only group (r=0.33, p=0.064). The correlation between disease duration and the arterial/venous difference was r= - 0.21 (p=0.228) in the DM+CVD group and r= -0.29 (p=0.101) in the DM only group. Table 4 shows the multivariate regression analysis of the entire cohort: the only significant relationship was between EMPs and venous oxygenation.

Discussion

Diabetes is a major risk factor for CVD, with endothelial damage being a leading pathophysiology. We broadly hypothesised that ocular/retinal oxygenation indices would be worse in those with both conditions. The increased vWf in DM+CVD supports this hypothesis, other vascular markers do not. EMPs were raised in both DM groups whilst soluble E selectin was raised in both CVD groups. However, there were no differences in retinal vessel oxygen saturation and vessel diameters between the three groups, and we find it notably that HbA1c failed to correlate with any oxygenation index in any group. Velocity in retinal blood vessels has recently been shown to negatively correlate with oxygen saturation measured in the same vessels [21]. As velocity can change depending on systemic disease and local factors it can also be altered due to vascular dilation (or narrowing) all of which can impact on the measurement of retinal oxygen saturation measurements. However, while we did not measure velocity in our sample, we used a standardized protocol to measure retinal vessel diameters [27] which showed no difference between groups. Notably, IOP was higher in DM than in CVD but this finding has no clinical or methodological relevance as all groups were within

what is considered a normal IOP range [28]. Disease duration correlated with arterial and venous oxygenation (but not the difference) in the combined DM+CVD group – correlations in the DM only group were not significant. This finding shows that despite a shift in arterial and venous oxygen saturation, there was no change in oxygen metabolism in patients with DM and CVD, although this shift in both arterial and venous saturation may be an indication of an altered oxygen metabolism. Previous research examining DM patients with and without diabetic retinopathy (DR) showed no difference in retinal oxygen saturation levels between healthy controls and patients with DM and no DR or DR but not requiring treatment and agrees with our findings as is the increase in saturation parameters with increasing disease duration [5,29, 30].

EMPs are vesicular structures shed from damaged/activated/apoptotic endothelial cells and are reputed to have a role in coagulation, inflammation, endothelial function, and angiogenesis and can disturb vascular homeostasis, contributing to diseases progression [15]. Elevated EMP levels have been found in plasma from patients with vascular diseases and are thought to be surrogate markers of progression of endothelial dysfunction [15,31]. Hypoxia related vascular diseases such as acute coronary syndromes, stroke, and organ transplantation, attract increased attention owing to their high morbidity and mortality. Evidence from previous studies shows that hypoxia-induced oxidative stress, coagulation, inflammation, and angiogenesis play a major role in the pathological process of hypoxia related vascular endothelial injury. Hypoxia itself, hypoxia-induced oxidative stress, coagulation, and inflammation can induce the release of EMPs [15]. In addition, EMPs play an important role in cell-to-cell communication and function, have pro-coagulant, proinflammatory, angiogenic and other functions, all of which affect pathological processes therefore suggesting that EMPs may help to identify hypoxia related vascular disease mechanisms and improve risk stratification [31].

We found that numbers of EMPs in the peripheral circulation were only related to retinal venous oxygen saturation. In common with all other studies taking peripheral blood, we cannot state levels therein are an accurate reflection of the state of the retinal vessel endothelium, but may be a marker of any pathology, if, indeed, any is present. Increased retinal venous oxygen saturation has previously been linked with more advanced disease stages of diabetic retinopathy and in patients with severe loss of visual function due to glaucoma [3, 5]. EMPs were highest in both diabetic groups and while our patients were free from clinically-defined DR, retinal venous oxygen saturation was linked with disease duration in DM+CVD, and with EMPs in diabetes alone, which may point towards a potential role in diabetic eye disease progression. Hence, we speculate that increased levels of EMPs in diabetes could potentially be a marker for increased risk of DR. However, this requires further evaluation by observing patients with diabetes but free of DR longitudinally until they develop DR in order to verify and validate.

Changes in the vascular system of the eye such as vessel narrowing, vascular remodelling and rarefaction, can be assessed by retinal vessel geometrical parameters such as vessel calibres, branching pattern, vessel density and areas of vessel dropout or gaps (lacunarity) and have been suggested to be a consequence of hypertension [32], predictive of diabetic complications [33] and a surrogate marker for poor perfusion [34]. Having a less dense vascular network and/ or narrower vessels could therefore contribute deficient of supply in oxygen. While we have measured vessel oxygen saturation and found no association between geometrical vessel markers and saturation parameters, this does not allow the conclusion of a comparable supply between disease groups nor does it rule out an insufficient supply as this will also depend on other parameters such as blood flow [35, 36].

An explanation of the link between EMPs and venous saturation may be found in the fact that both markers have previously been reported to be elevated in diabetes mellitus [15] and

implicated in disease progression. Increased venous saturation has previously suggested to be a result of less oxygen demand from the retinal tissue due to a lack of "functional" tissue [3, 4] in individuals with glaucoma and whereas in DM it has been attributed to vascular remodelling and shunt vessels [4]. We cannot rule out either of these two mechanisms in our cohort, owing to the fact that glaucoma is a long-term complication of DM and age is a risk factor both glaucoma and DM/DR. EMPs are highest in the two diabetic groups, possibly reflecting disease activity as EMPs have shown to mediate several inflammatory processes [15]. The lack of patients with DR in our sample limit our findings and would benefit from further studies assessing patients with various stages of DR. We acknowledge the limitation of the lack of a healthy control group, the assumption that without further differentiation/assessment of the severity of the diseases that those with both CVD and DM have more endothelial disease severity, relatively small numbers, and that these real-world patients were taking a variety of medications that may influence the measured indices. Indeed, our data offer a snap-shot of local pharmaceutical practice and its consequences. Nevertheless, our pilot data point to a potential role for EMPs in the assessment of retinal vessel pathophysiology.

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Declarations

Ethics approval and consent to participate

The study was approved by the NRES Committee East Midlands-Leicester (Ref: 12/EM/0062) and the Aston University Ethics Committee and adhered to the Declaration of Helsinki. Written informed consent was obtained from each patient.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

ADB recruited patients and conducted all blood analyses. ADB and RH jointly conducted the statistical analyses. RH obtained all ocular data and drafted the manuscript. Both authors revised and reviewed the final version of the manuscript.

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Table 1: Clinical data, medications, and demographics

	DM [n=34]	CVD [n=40]	DM & CVD [n=36]
Age [years]	64 (10)	64 (11)	65 (9)
Gender [m/f]	24/12	30/13	32/5
SBP [mm Hg]	130 (13)	125 (19)	125 (16)
DBP [mm Hg]	75 (9)	75 (13)	67 (10)*
HR [beats per minute]	76 (14)	67 (11)**	72 (16)
BMI [kg/m^2]	31 (6)	27 (4)*	31 (6)
HbA1c [mmol/mol][%]	58 (15), 7.5 (3.5)	42 (3), 6.0 (2.4)*	61 (20), 7.7 (4.0)
DM duration [years]	11 (4.5-16)	-	10 (4-17)
eGFR (ml/min/1.73)	66 (20)	74 (12)	67 (20)
Calcium channel blocker	20, 58%	13, 32%	14, 38%
ACEI/ARB	23, 68%	31, 78%	30, 83%
Metformin	26, 76%	-	21, 58%
Sulphonylurea	7, 20%	-	7, 19%
DPP-4 inhibitor	8, 24%	-	6, 16%
Insulin	15, 44%	-	14, 38%
GLP-1 agonist	4, 11%	-	4, 11%
Lipid-lowering	26, 76%	39, 98%	35, 97%
Anti-platelet	11, 32%	39, 98%	36, 100%
Nitrate	-	11, 26%	11, 30%
Oral anticoagulant	6, 17%	5, 12%	5, 14%
Beta-blocker	10, 28%	19, 44%	22, 59%
Diuretic	18, 50%	9, 21%	18, 49%

M: male; f: female; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; HR: Heart Rate; IOP: Intraocular Pressure; BMI: Body mass index; HbA1C: glycated haemoglobin; DM: Diabetes Mellitus; ACEI: angiotensin-converting-enzyme inhibitor; ARB: Angiotensin receptor blockers; DPP-4 = dipeptidyl peptidase-4 ('gliptins'); GLP-1 = glucagon-like peptide-1 (exenatide, liraglutide). Data presented as mean with standard deviation, median and interquartile range, or as number of subjects and percentage. *different compared to the two other groups ($p<0.01$); **different to the CVD group ($p<0.01$).

Table 2: Ocular and endothelial parameters

	DM [n=34]	CVD [n=40]	DM & CVD [n=36]	P value
IOP (mm Hg)	15.2 (3.0)**	13.2 (1.8)	14.1 (2.3)	0.002
Structural retinal vessel indices				
CRAE [au]	178 (21)	175 (18)	175 (11)	0.662
CRVE [au]	212 (20)	210 (19)	216 (18)	0.379
AVR	0.84 (0.09)	0.83 (0.08)	0.82 (0.08)	0.456
Df	1.38 (0.05)	1.39 (0.05)	1.38 (0.05)	0.590
Lacunarity	1.10 (0.04)	1.11 (0.04)	1.10 (0.034)	0.476
Oxygenation indices				
Arterial oxygen saturation [%]	97.7 (4.5)	96.7 (5.8)	96.7 (7.8)	0.725
Venous oxygen saturation [%]	63.7 (10.5)	59.4 (10.5)	60.8 (12.7)	0.264
Arterial - venous saturation [%]	34.00 (9.00)	37.3 (9.2)	35.8 (11.4)	0.375
Systemic endothelial indices				
Endothelial microparticles (n/µL)	4.36 (2.12-8.85)	2.86 (3.00-8.51)*	4.14 (1.21-8.13)	0.049
Von Willebrand factor (IU/dL)	108 (21)	111 (19)	124 (22)**	0.004
Soluble E selectin (ng/mL)	22 (9)**	26 (8)	27 (12)	0.032

Data presented as mean with standard deviation, media with interquartile range, or as number of subjects and percentage. Tukey's post-hoc test, *lower than the other two groups and **higher than the two other groups. CRAE: Central Retinal Artery Equivalent; CRVE: Central Retinal Vein Equivalent; AVR: Arterio-Venous Ratio; D_f: fractal dimension.

Table 3: Correlations

	Group	Arterial O_2	Venous O_2	Arterial-venous difference
HbA1c	Diabetes	0.10: 0.561	0.02: 0.901	0.114: 0.521
	CVD	0.16: 0.329	-0.03: 0.836	0.10: 0.531
	Both	0.01: 0.995	0.24: 0.144	-0.17: 0.311
Soluble E selectin	Diabetes	-0.01: 0.981	-0.20: 0.260	0.14: 0.426
	CVD	0.05: 0.744	-0.10: 0.528	-0.03: 0.836
	Both	-0.35: 0.068	**0.55: 0.002**	**-0.63: 0.001**
Von Willebrand factor	Diabetes	0.01: 0.995	-0.24: 0.177	0.18: 0.303
	CVD	0.15: 0.327	0.26: 0.092	-0.06: 0.691
	Both	0.01: 0.973	0.113: 0.448	-0.07: 0.641
Endothelial microparticles	Diabetes	-0.27: 0.125	**0.43: 0.011**	0.06: 0.755
	CVD	0.03: 0.825	0.24: 0.125	-0.18: 0.250
	Both	0.01: 0.941	-0.07: 0.680	0.09: 0.563

Data are Spearman correlation coefficient and p value. HbA1c = Glycated haemoglobin, CVD = cardiovascular disease. Sample sizes: Diabetes n=35, CVD n= 42, Both n=37.

Table 4: Multivariate regression analysis

Dependent retinal index	**Independent endothelial index**		
	Endothelial microparticles	Von Willebrand factor	Soluble E selectin
Arterial oxygenation	0.251	0.578	0.466
Venous oxygenation	**0.037**	0.843	0.388
Arterial-venous difference	0.105	0.895	0.151

Data are p values.

Figure legends:

Figure 1: Retinal vessel oxygen parameters were assessed in a concentric measurement annulus of ½ disc diameter width (denoted by A), ½ disc diameter distant to the optic disc margin.

Figure 2: Retinal vessel calibers were assessed in a concentric measurement annulus of ½ disc diameter width (denoted by A), ½ disc diameter distant to the optic disc margin. Retinal vessel fractal dimension and lacunarity were assessed in a concentric measurement annulus of 1.5 disc diameter width (denoted by B), ½ disc diameter distant to the optic disc margin.