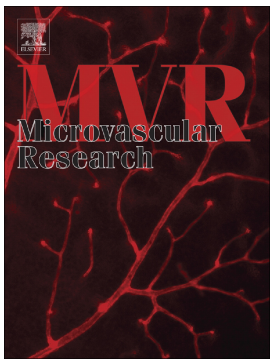

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Retinal and Peripheral Vascular Function in Healthy Individuals with Low Cardiovascular Risk

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Abstract

Objective:

To assess retinal and peripheral microvascular function in individuals with low cardiovascular risk.

Methods and Results:

Retinal microvascular function was assessed using the dynamic vessel analyser (DVA) and peripheral vascular reactivity was measured using the digital thermal monitor (DTM) in 136 healthy participants. In addition, systemic blood pressure (BP) profiles, blood analyses for glucose and lipid metabolism markers (CHOL, HDL-c, LDL-c), as well as the Framingham Risk Score (FRS) were assessed in all participants.

Based on peripheral vascular reactivity scores, participants were separated into 3 groups: high, intermediate and low risk. Participants with high risk showed a significant higher retinal arteriolar time to reach maximum dilation (tMD) than those with intermediate and low risk ($p < 0.001$). In addition, retinal arterial dilation amplitude (DA), and constriction slope ($Slope_{AC}$) were higher in subjects with low risk (p=0.006, p=0.019). Only in high risk participants, peripheral vascular reactivity parameters correlated with retinal arterial functional parameters DA, (r = .3800, p=0.029) and tMD (r = -0.5904, $p < 0.001$).

Conclusion:

We conclude that signs of abnormal vascular function are similarly present and detectable in various microvascular beds, despite existing differences in their anatomical and physiological properties.

Keywords: Endothelial function, cardio-vascular disease, vascular function, vascular imaging, dynamic vessel analysis.

Introduction

In the primary prevention of cardiovascular disease, assessing risk using predictive models such as the *Framingham Risk Score*[1-3] (FRS), the Prospective Cardio- vascular Münster (PROCAM) and the European Society of Cardiology Systematic Coronary Risk Evaluation (SCORE)[4-7] represents common practice. However, such methods either over- or underestimate risks in more than 50% of cases [8-11] Moreover, they are built to predict long-term risk in *populations* and not in *individuals* with specific genetic and environmental influences[12-15]. Indeed, it is now increasingly recognised in medicine that preventive approaches need to be tailored to the risks of individual patients and not only to those measured in general population. Nevertheless, in order to achieve this goal, more research is necessary to identify biomarkers offering a disease-specific individual biological profile in a non-invasive way that is suitable for use in primary care settings.

The assessment of endothelial dysfunction (ED) represents one very important marker for early cardiovascular disease (CVD) risk. Indeed, it is well recognized that ED plays a role in the pathogenesis of coronary heart disease (CHD), and is associated with an increased risk for hypertension, types 1 and 2 diabetes, and cardiovascular complications of obesity [16-18].

Therefore, its quantification it is very important when trying to implement early, successful preventive measures. Assessing ED is usually accomplished using techniques such as ultrasound

flow-mediated dilation (FMD), plethysmography or iontophoresis[19]. These tests are, however, complex and time-consuming, and can only be performed in highly specialized centers. Among the more user-friendly methods developed to measure microvascular function, dynamic retinal vessel analysis (DVA) represents a non-invasive alternative that allows for continuous, live recordings of retinal arterial and venous diameter changes in response to flicker-light stimulation. The main advantage of the DVA assessment is that it provides instant integrated and dynamic data analysis that is specific to each individual. In addition, its output has proven to be modified not only by overt disease but also in the presence of more subtle risk factors for CVD [20-22] including ageing[10], ethnicity [23] and impaired glucose tolerance [24]. Therefore, it is possible to use the assessment of retinal microvascular function as a surrogate marker for ED and early CVD risk.

Another method, called Digital Thermal Monitoring (DTM), has been developed to measures peripheral vascular reactivity using reactive hyperaemia after increasing the blood pressure (BP) to suprasystolic values. The measurements using this instrument have excellent reproducibility and low variability [25], demonstrate strong relationship with flow mediated dilation technique (FMD) [26], coronary calcium score, myocardial perfusion defects, and coronary angiography[10, 27-32] and were validated for detecting ED and CVD risk[1, 10, 26, 30, 33] .In addition, similarly to DVA, DTM is also a non-invasive technique that can be performed in primary care settings, therefore, suitable for early screening. However, these two instruments assess vascular beds with different anatomical properties and exposed to different physiological influences [34,35] . The nature of the provocation stimulus used by each of the instruments to induce vascular dilation and constriction, is also different [13,36,37] .As both methods candidate for

a place in preclinical screening, it would be useful to know that regardless of the provocation stimulus or the properties of the microvascular bed assessed, these existing non-invasive methods are equally sensitive to detecting signs of early CVD risk. Therefore, the aim of the present study was to investigate retinal microvascular function as assessed by the DVA and peripheral vascular reactivity measured using the DTM in apparently healthy, normotensive individuals with no overt cardiovascular disease as well as to identify any relationship between vascular functions in these two different types of microvascular beds.

Materials and Methods

Participants

Community-dwelling volunteers (aged above 18 years) were recruited through local advertisements at the Vascular Research Laboratory and Health Clinics at Aston University (Birmingham, UK). Ethical approval for the study was received from the relevant local and institutional ethics committees. Written informed consent was received from all participants prior to study enrolment and all study procedures were designed and conducted in accordance with the tenets of the Declaration of Helsinki.

Study exclusion criteria were defined as a history or current diagnosis of cardiovascular or cerebrovascular disease including hypertension, coronary artery disease, heart failure, arrhythmia, stroke, transient ischemic attacks and peripheral vascular disease. In addition, patients suffering from any inflammatory or autoimmune diseases, history of cold hands and/or Raynaud's syndrome, as well as, smokers, diabetics (self-reported), or those with severe dyslipidaemia (defined as plasma triglyceride levels above 6 mmol/L, cholesterol levels above 7

mmol/L) have also been excluded. The use of vasoactive or antidyslipidemic medications, including dietary supplements containing vitamins or antioxidants and bronchodilators also served as exclusion criteria. Participants with elevated intraocular pressures (IOP > 21 mmHg), retinal disease, intraocular surgery, neuro-ophthalmic disease, cataract or other media opacities that may affect the ocular vascular system or prevent retinal vascular examination were also excluded from the study.

General Assessments

All measurements were performed between 8 and 11am following a 12-h overnight fast, which included refraining from alcohol and caffeine. General preliminary assessments were conducted and included general health history questionnaires, measures of height and weight, BMI= (weight in KG/Height $(m)^2$), Heart rate (HR), Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP ≈ (⅔ X DBP) + (⅓ X SBP)), IOP and ocular perfusion pressure (OPP)=2/3*((2/3*DBP+1/3*SBP))-IOP. Additionally, blood samples were assessed for total cholesterol (CHOL), high-density lipoprotein (HDL) and low-density lipoprotein (LDL).

The Framingham Risk Score (FRS)

The FRS is a widely used gender-specific algorithm originally developed to estimate CVD risk [38]. In the present study FRS was calculated using the current versio[39] based on risk factors such as age, gender, CHOL, HDL-c, SBP, treatment for hypertension, smoking status, and diabetes. Risk factors such as age, treatment for hypertension, smoking status and diabetes were identified from self-report questionnaires and CHOL, HDL-c, and SBP values were as those determined on

the day of study assessment. The scoring algorithm is based on gender-specific points assigned for each risk factor variable that can be determined using FRS tables i.e. point scores by age group; age group and total CHOL; age group and smoking status; HDL-c level; SBP and treatment status. Ten-year risk percentage is then calculated by total points (1 point, 6%; 2 points, 8%; 3 points, 10%; 4 points, 12%; 5 points, 16%; 6 points, 20%; 7 points, 25%; 10 points or more, > 30%).

For each participant, an absolute CVD risk percentage over 10 years was calculated and only subjects with a CVD risk <10% were included in the study [37].

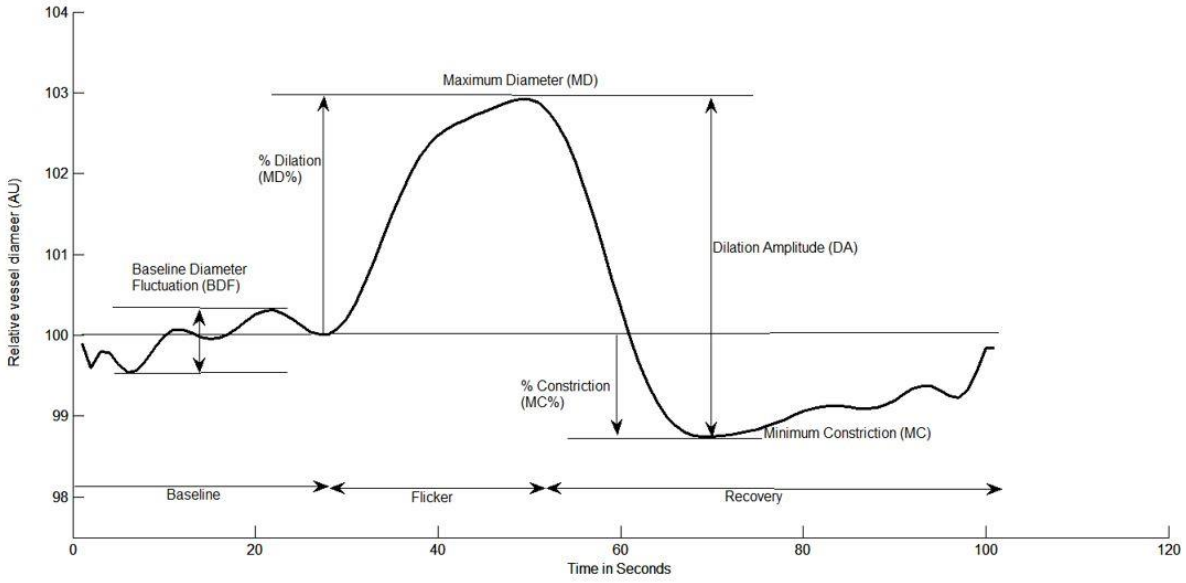
Vascular assessments

1. **DVA**

Retinal vessel reactivity was measured using the dynamic vessel analyser (DVA, IMEDOS GmbH, Jena, Germany) according to an already established protocol[40-42]. All measurements were performed in a temperature-controlled room (22c°) following dilatation with 1% tropicamide (Chauvin Pharmaceuticals Ltd, UK). Measurements were conducted in one unselected eye for each subject. Subjects were also asked to fixate on a target which controlled their eye movements and to stabilize the fundus image. Two segments of the inferior temporal artery and vein of approximately 0.5-1mm and 1.5-2 Disc diameters from the optic nerve head were selected for continuous diameter recording. The automated protocol included a 50 second baseline diameter measurement under still illumination at (25Hz) followed by a 20 second flicker stimulation (opto-electronically generated at 12.5Hz) and an 80 second recovery period which was repeated 3 times for a total evaluation of 350 seconds. The vessel reactivity

parameters were determined for each flicker cycle and averaged over three cycles. Further analysis of the vessel response profile was explored through extraction of the raw data and applying a statistical polynomial regression algorithm (MATLAB; Mathowrks v12, MA, USA)[43, 44] .The following vessel reactivity and time-course parameters were determined for each flicker cycle and then averaged over the three cycles, with the artery and vein regarded separately as follows: the average baseline diameter and range of maximum and minimum baseline vessel diameters (baseline diameter fluctuation, BDF); the maximum vessel dilation diameter during flicker stimulation expressed as a percentage change relative to baseline diameter (MD%) and the time taken in seconds to reach the maximum diameter (tMD); the maximum vessel constriction diameter during the postflicker recovery period expressed as a percentage change relative to baseline diameter (MC%) and the time taken in seconds to reach the maximum vessel constriction diameter (tMC); the overall dilation amplitude (DA) calculated as the difference between MD and MC; and the baseline-corrected flicker response (BCFR) used to describe the overall dilation amplitude after normalizing for fluctuations in baseline diameters (DA-BDF). In addition, the arterial (A) and venous (V) dilation slopes ($Slope_{AD/VD}$ = (MD-baseline diameter)/tMD) and constriction slopes ($Slope_{AC/VC}$ = (MC-MD)/tMC) were also calculated (Figure 1).

Figure 1: Graphical presentation of the dynamic vessel response profile displaying the parameters calculated and used in analysis. (DA) calculated as (MD-MC). (MD%) calculated as the percent increase from baseline to MD. (MC%) calculated as the percent constriction below baseline following MD.

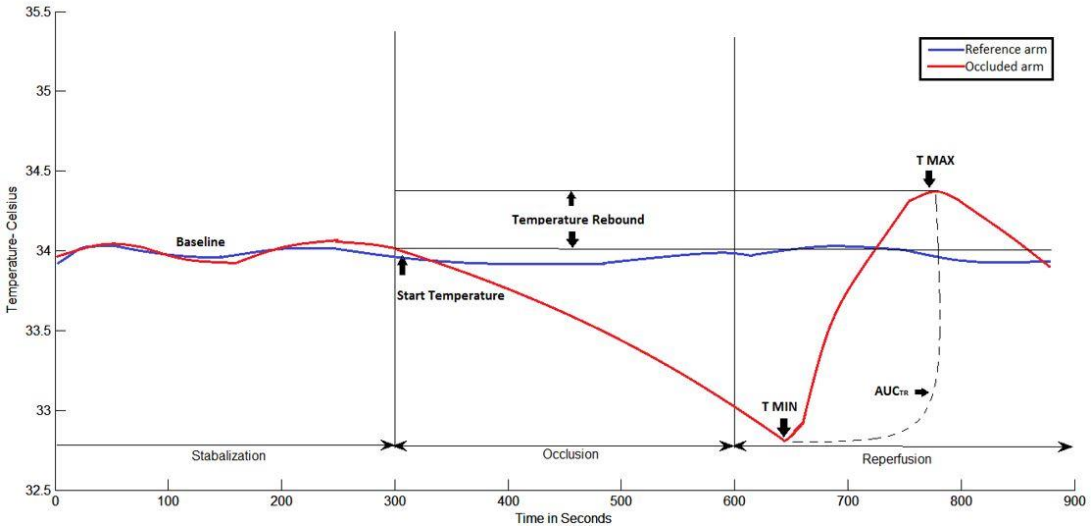


2. Digital Thermal Monitoring (DTM)

The peripheral microvascular reactivity at the level of the fingertips was assessed using VENDYS 5000BC DTM system (Endothelix, Inc., Houston, TX, USA). This FDA approved device consists of a computer-based thermometry system (0.006°C thermal resolution), with two special thermocouple fingertip probes designed to minimize the area of skin-probe contact and fingertip pressure. A standard sphygmomanometer cuff and a compressor unit to control cuff inflation and deflation is included to facilitate the occlusion-hyperaemia protocol[45] . The test is conducted with the patient at rest for 30 minutes in the supine position, in a quiet, dimmed room with ambient temperature of 22°C to 26°C. VENDYS DTM probes are affixed to the index finger of each hand and after a period of stabilization of basal skin temperature (defined as stabilization within a 0.05°C threshold) the temperature is measured in the index fingers of both hands (of which the right arm only is subjected to occlusion-hyperaemia) with an automated, operator-independent protocol. The right upper arm cuff is rapidly inflated to ≥50 mmHg above systolic pressure for 5 minutes and then rapidly deflated to invoke reactive hyperaemia distally. Thermal tracings are measured continuously and digitized automatically using a computer-based thermometry system with 0.006°C thermal resolution. Dual channel temperature data is simultaneously acquired at a 1 Hz sample rate. Figure 2 shows a representative example of a temperature-time trace and the primary DTM-derived measures, related to thermal debt and recovery that were recorded and calculated. *Temperature rebound* (TR): maximum temperature- start temperature (just before cuff inflation); *adjusted temperature rebound* (aTR): temperature rebound/ start temperature; *area under the curve temperature rebound* (AUCTR): area under the curve between maximum temperature and minimum temperature. The post-occlusive adjusted temperature rebound aTR determined by

the software algorithm is directly associated with the extent of the subjects vascular reactivity[46]. An aTR below 1 is considered poor cardiovascular reactivity, whereas an aTR between 1 and 2 is considered intermediate vascular reactivity and an aTR of greater than 2 is considered healthy vascular reactivity [46].

Figure 2: Graphical representation of the Digital Thermal Monitor software analysis; T MAX, maximum temperature; TMIN, minimum temperature; AUCTR, Area under the curve temperature rebound.



Statistical Analysis

All analyses were performed using Statistica® software (version 13, StatSoft Inc., Tusla, OK, USA). Distributions of continuous variables were determined by the Shapiro-Wilks test. In cases where normality of the data could not be confirmed appropriate data transformations were made or non-parametric statistical alternatives were used. Univariate associations were determined using Pearson's (normally distributed data) or Spearman's method (non-normally distributed data), and forward stepwise regression analyses were performed to test the influence of clinical parameters and circulating markers on the measured vascular reactivity variables. Differences between groups were subsequently assessed using one-way ANOVA or ANCOVA, as appropriate, followed by Tukeys post hoc analysis. P-values of less than 0.05 were considered significant.

Results

A total of 150 participants were initially screened for study inclusion of which 14 individuals were excluded based on having a moderate to high FRS (>10%). The remaining 136 participants with low FRS (<10%) were included in the final analysis and classified into one of three groups based on the aTR vascular reactivity index cut-off [22, 35] : group 1: aTR= 0-1 (high risk; 45 subjects: 22 males, 23 females, p>0.05); group 2: aTR >1<2 (intermediate risk; 46 subjects: 25 males, 21 females, p>0.05), and group 3: aTR ≥ 2 (low risk; 45 subjects: 22 males, 23 females, p>0.05). The aTR values for each group are presented in table 1.

Abbreviations: TR, temperature rebound; aTR, adjusted temperature rebound; AUCTR, Area under the curve temperature rebound

Table 1. Digital Thermal monitoring parameters

	Mean (SD)			**P value**
Parameter	**Group 1 (aTR<1)**	**Group 2 (aTR>1<2)**	**Group 3 (aTR>2)**	<0.001
TR	-0.17 (0.60)	0.03 (0.54)	0.84 (1.26)	<0.001
aTR	0.73 (0.35)	1.73 (0.19)	2.62 (0.78)	<0.001
AUC_{TR}	121 (68.52)	267 (72.64)	420 (144.90)	<0.001

General characteristics of the study population

Table 2 provides a summary of the anthropometric and other measured variables, showing no statistically significant differences between our study groups (all $p > 0.05$).

Table 2. Characteristics of the study groups

	Mean (SD)			
Variable	**Group 1 (aTR>0)**	**Group 2 (aTR>1<2)**	**Group 3 (aTR>2)**	**P value**
N	45	46	45	
Age (years)	34(11.51)	29(9.56)	32(10.57)	0.098
BMI (Kg/m^2)	25(4.36)	24(4.50)	24(4.69)	0.428
SBP (mmHg)	122(11.98)	126(12.77)	122(11.22)	0.233
DBP (mmHg)	72(7.86)	73(9.01)	71(8.40)	0.462
HR (bpm)	64(11.45)	65(11.2)	67(9.60)	0.395
MAP	89(8.43)	91(9.19)	88(8.68)	0.306
IOP (mmHg)	14(2.25)	14(2.76)	13(2.85)	0.234
OPP	45(5.78)	46(7.03)	45(6.05)	0.740

CHOL mmol/L	4.38(0.82)	4.20(0.94)	4.76(1.09)	0.479
HDL-C mmol/L	1.48(0.56)	1.55(0.43)	1.67(0.59)	0.749
LDL-C mmol/L	2.48(0.90)	2.06(1.03)	2.46(1.07)	0.673
FRS %	3.73(3.65)	2.17(1.82)	2.71(2.01)	0.593

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; IOP, intraocular pressure; OPP, ocular perfusion pressure; CHOL, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FRS: Framingham Risk Score in %. * Significant *p*-values are indicated in bold where $p < 0.05$ was considered significant.

Retinal vascular function measurements

The results of retinal arteries and veins function assessments are displayed in Table 3. After controlling for all influential covariates identified by multivariate regression analysis such as age, BMI, BP, and CHOL there was a clinically significant difference in the arterial tMD between the 3 study groups (Table 3, p<0.001). Post hoc comparisons using the Tukey test indicated that the mean score for group 1 (M = 25.27, SD = 7.79) was significantly different than group 2 (M = 21.48, SD = 5.44) and group 3 (M = 17.28, SD = 4.17). In addition, there was also a significant difference between groups 2 and 3. Group 3 (low risk) showed a significantly higher DA (Table 3 , p=0.006). Post hoc comparisons using the Tukey test indicated that the mean score for group 3 (M= 29.54, SD 7.80) was significantly different than groups 2 (M=24.97, SD 7.54) and group 1 (M= 23.80, SD 10.76). However, Groups 1 and 2 did not significantly differ from each other. Group 3 (low risk) also showed a significantly higher $Slope_{AC}$ (p=0.019). Post hoc comparisons using the Tukey HSD test indicated that the mean score for group 3 (M=0.59, SD 0.63) was significantly different than groups 2 (M=-0.50, SD 0.29) and group 1 (M= -0.34, SD 0.18). However, Groups 1 and 2 did not significantly differ from each other. There were no other significant differences at the retinal microvascular level in either the measured arteries or veins (all p>0.05).

Figure 3: Comparison of retinal arterial response profile across groups based on the temperature rebound parameters. AU, arbitrary units; MD, maximum diameter during flicker; MC, maximum constriction post flicker; tMD, reaction time in seconds to MD; DA, difference between MD&MC during flicker; SlopeAC, calculated as (MC-MD)/(mCRT). Group 1, aTR= 0-1; group 2, aTR >1<2; group 3, aTR ≥ 2.

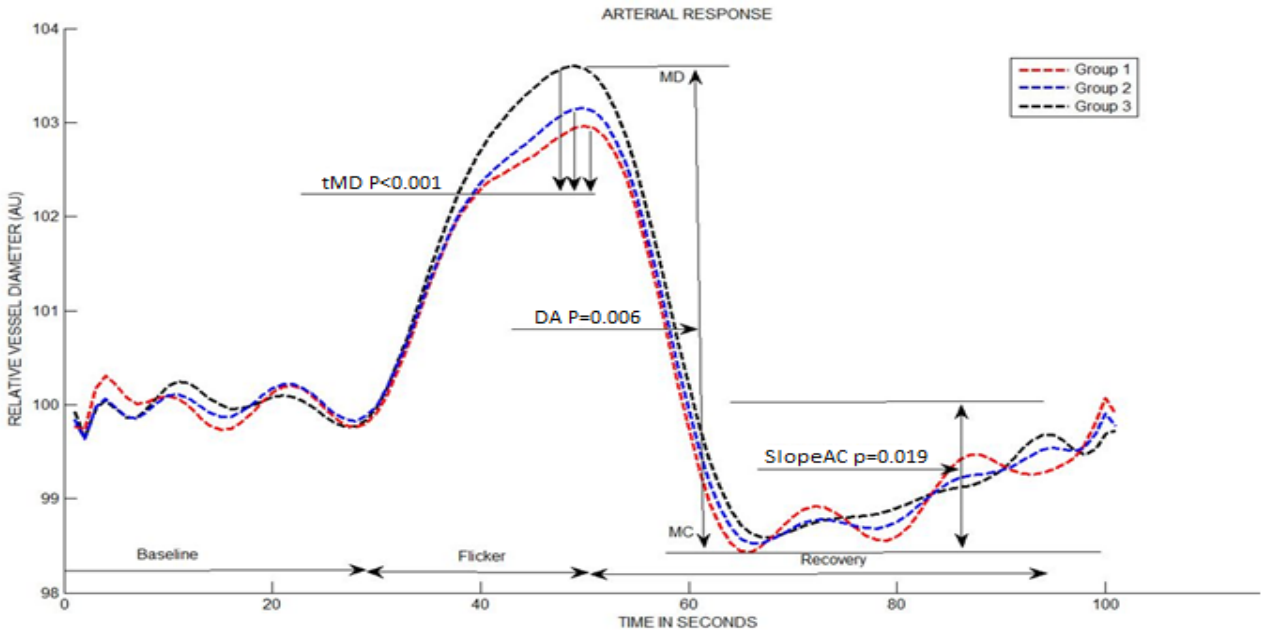


Table 3. Summary of Retinal Arteries and Veins Vascular Function Parameters

		Mean (SD)			
Arteries	**Group 1**	**Group 2**	**Group 3**	**P Value**	
	(aTR>0)	**(aTR>1<2)**	**(aTR>2)**	Anova/Ancova	Tukey post-hoc
Baseline	100(0.02)	100(0.03)	99.96(0.25)	0.398	-
BDF	5.43(2.42)	6.35(3.57)	6.24(2.66)	0.269	-
BCFR	3.02(2.52)	3.33(2.78)	2.86(2.63)	0.695	-
MD	105.05(2.75)	105.50(2.47)	104.95(1.96)	0.537	-
tMD	25.27(7.79)	21.48(5.44)	17.28(4.17)	<0.001*	1>2>3
Dilation %	5.05(2.74)	5.50(2.73)	4.98(1.96)	0.57	-
MC	96.59(1.80)	95.81(3.01)	95.83(2.39)	0.234	-
tMC	29.07(9.09)	25.67(6.23)	26.42(6.57)	0.871	-
Constriction %	-3.41(1.78)	-4.20(2.98)	-4.13(2.43)	0.247	-
DA	23.80(10.76)	24.97(7.54)	29.54(7.80)	0.006*	3>2,1
$Slope_{AD}$	0.31(0.23)	0.33(0.22)	0.38(0.23)	0.415	-
$Slope_{AC}$	-0.34(0.18)	-0.50(0.29)	0.59(0.63)	0.019*	3>2,1

		Mean (SD)		
Veins	**Group 1**	**Group 2**	**Group 3**	**P Value**
	(aTR>0)	**(aTR>1<2)**	**(aTR>2)**	Anova/Ancova
Baseline	100(0.03)	99(0.03)	99(0.16)	0.254
BDF	4.69(2.82)	4.93(2.43)	5.27(2.74)	0.587
BCFR	3.12(2.13)	3.11(2.58)	3.35(3.28)	0.889
MD	106(3.51)	105(2.25)	106(3.43)	0.453
tMD	21.42(4.87)	22.23(5.70)	22.19(6.44)	0.75
Dilation %	6.11(3.49)	5.81(2.25)	6.65(3.42)	0.427
MC	98.31(6.41)	97.75(2.03)	98.01(1.55)	0.422
tMC	33.78(6.41)	33.42(6.88)	33.25(7.92)	0.906
Constriction %	-1.83(1.93)	-2.26(1.99)	-1.98(1.55)	0.611
DA	31.50(7.08)	31.18(8.62)	31.05(8.71)	0.964
$Slope_{VD}$	0.31(0.16)	0.29(0.14)	0.37(0.21)	0.101
$Slope_{VC}$	-0.28(0.16)	-0.28(0.16)	-0.34(-0.24)	0.300

ANOVA, analysis of variance; ANCOVA, analysis of covariance; Baseline, baseline diameter; BDF, baseline diameter fluctuation; BCFR, Baseline corrected flicker response; mDRT, reaction time to MD; MD (%), percent dilation; mCRT, reaction time to MC; MC (%), percent constriction; DA, dilation amplitude (difference between MD and MC during flicker) $Slope_{VD}$, slope of venous dilation; $Slope_{VC}$, slope of venous constriction. * Significant *p*-values are indicated in bold where $p < 0.05$ was considered significant.

Within group correlations between retinal and peripheral vascular function measures.

In group 1, retinal arterial DA, correlated positively ($r = .3800$, p=0.029); and tMD negatively ($r = -0.5904$, $p < 0.001$, Figure 4) with aTR. No significant correlations between aTR and any of the other measured retinal arterial and venous reactivity parameters were identified for the groups 2 and 3 (all p>0.05).

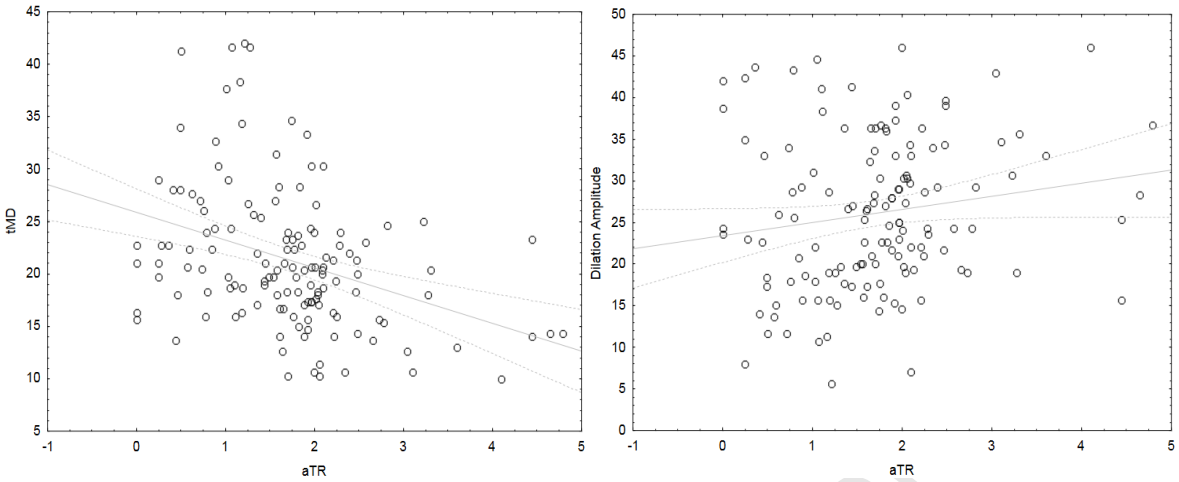


Figure 4: Correlations between retinal and peripheral vascular function measures
tMD: Time to maximum dilation; DA, Dilation Amplitude; aTR, adjusted temperature rebound**.**

Discussion

This study demonstrates for the first time that in individuals without over CVD but classified as high risk using the DTM technique, there are signs of abnormal retinal function, with higher tMD when comparing to the intermediate and low-risk groups. In addition, only in the high-risk group there was a correlation between vascular reactivity at the peripheral and retinal level. Although the precise mechanisms behind these changes need further elucidation, it has been previously hypotesized that an increased tMD could be the result of either subclinical atherosclerotic vessel wall changes, increased arterial stiffness, or reduced NO bio-availability to peripheral tissues[23], therefore, the observed vascular functional abnormalities in our high-risk individuals may represent a combination of either one or all these factors[47, 48]. Moreover, we were also able to demonstrate that low-risk individuals have a higher dilatatory capacity of the retinal arteries than the other two groups, with the intermediate and high risk groups demonstrating a more reduced response in dilation after flickering stimulus. This parallelism in response in two completely different vascular beds is surprising, considering that in

asymptomatic individuals, vascular functional changes are only evident after applying stressors that are very different for various diagnostic instruments. Indeed, the dynamics of dilation and constriction of the retinal microvessels as assessed by the DVA are the result of an increased metabolic demand in response to flickering light and subsequent nitric oxide (NO) release. In addition, neurovascular coupling, as well as the basal tone of the retinal vasculature, which is determined by the summation of both metabolic and myogenic regulatory mechanisms, also play a role[37,49, 50]. The DTM, on the other hand, uses reactive hyperaemia after a period of ischemia and, therefore, its output is strongly dependent on the balance between endothelial NO synthase (eNOS) and cyclooxygenase (COX) inhibition which attenuates the acetylcholine (Ach)-induced, endothelium- dependent, cutaneous vasodilatation[51] . Other responsible factors also include a non-NO, non-prostanoid-dependent pathway, potentially attributable to the endothelium-derived hyperpolarizing factor[52] (EDHF). Despite all the above differences, both instruments were able to detect, in those individuals classified as “high risk”, early functional abnormalities that seem to have co-existed in both their peripheral and retinal microvessels beds. In addition, the functional abnormalities seem to have a relationship to each other, demonstrating that a diseases vascular bed defies the local physiological rules and behaves in a functional manner that is general across all microvessel of the affected individual. This represents a sign of individual pathological vascular response which proves that endothelial dysfunction, when present, is a general process and is detectable with accuracy regardless of the method used or location of the vascular bed.

Previously, relationships between structural abnormalities at the retinal and peripheral circulation, have been demonstrated in various diseases[53-56]. Nevertheless, this is for the first time when similar vascular dysfunctions are reported using functional parameters and in individuals without overt disease. As molecular and imaging biomarkers drive the shift towards personalized medicine, it seems that assessing retinal or peripheral vessel reactivity can be used with the same benefit for profiling individualized vascular risk. Nevertheless, further studies, to include individuals with various pathologies, would be needed to confirm our assumptions.

Conclusion

The findings of this study showed associations between retinal and peripheral vascular measurements. The identification of abnormalities in vascular function in accessible peripheral arteries provides a means for the early detection in pre-symptomatic groups. The clinical information gathered with retinal and peripheral vascular function is essential given that they can provide prognostic information to previously un-identified 'at-risk' individuals.

DISCLOSURE

All authors read and approved the final version of the manuscript. The authors have no financial or personal conflicts of interest to declare.

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Figure Legends:

Figure 1: Graphical presentation of the dynamic vessel response profile displaying the parameters calculated and used in analysis. (DA) calculated as (MD-MC). (MD%) calculated as the percent increase from baseline to MD. (MC%) calculated as the percent constriction below baseline following MD.

Figure 2: Graphical representation of the Digital Thermal Monitor software analysis; T MAX, maximum temperature; TMIN, minimum temperature; AUCTR, Area under the curve temperature rebound.

Figure 3: Comparison of retinal arterial response profile across groups based on the temperature rebound parameters. AU, arbitrary units; MD, maximum diameter during flicker; MC, maximum constriction post flicker; mDRT, reaction time in seconds to MD; DA, difference between MD&MC during flicker; $Slope_{AC,}$ calculated as (MC-MD)/(mCRT).

Figure 4: Correlations between retinal and peripheral vascular function measures, graphs group 1-2

tMD, Reaction time in seconds; BDF, Baseline diameter fluctuation; MC, minimum constriction; DA, Dilation Amplitude; Slope vc, Vein constriction slope; %constriction, % constriction from baseline; aTR, adjusted temperature rebound**.**

Table Legends:

Table 1: TR, temperature rebound; aTR, adjusted temperature rebound; AUCTR, Area under the curve temperature rebound

Table 2: Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; IOP, intraocular pressure; OPP, ocular perfusion pressure; CHOL, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FRS: Framingham Risk Score in %. * Significant *p*-values are indicated in bold where $p < 0.05$ was considered significant.

Table 3: ANOVA, analysis of variance; ANCOVA, analysis of covariance; Baseline, baseline diameter; BDF, baseline diameter fluctuation; BCFR, Baseline corrected flicker response; tMD, reaction time to MD; MD (%), percent dilation; mCRT, reaction time to MC; MC (%), percent constriction; DA, dilation amplitude (difference between MD and MC during flicker) $Slope_{AD}$, slope of arterial dilation; $Slope_{AC}$, slope of arterial constriction. * Significant *p*-values are indicated in bold where $p < 0.05$ was considered significant.

Highlights

- Early functional abnormalities co-exist in both peripheral and retinal microvessels beds
- Peripheral & retinal vascular dysfunction are surrogate markers for early CVD risk
- Retinal vascular analyser can be used for assessing individual vascular risk

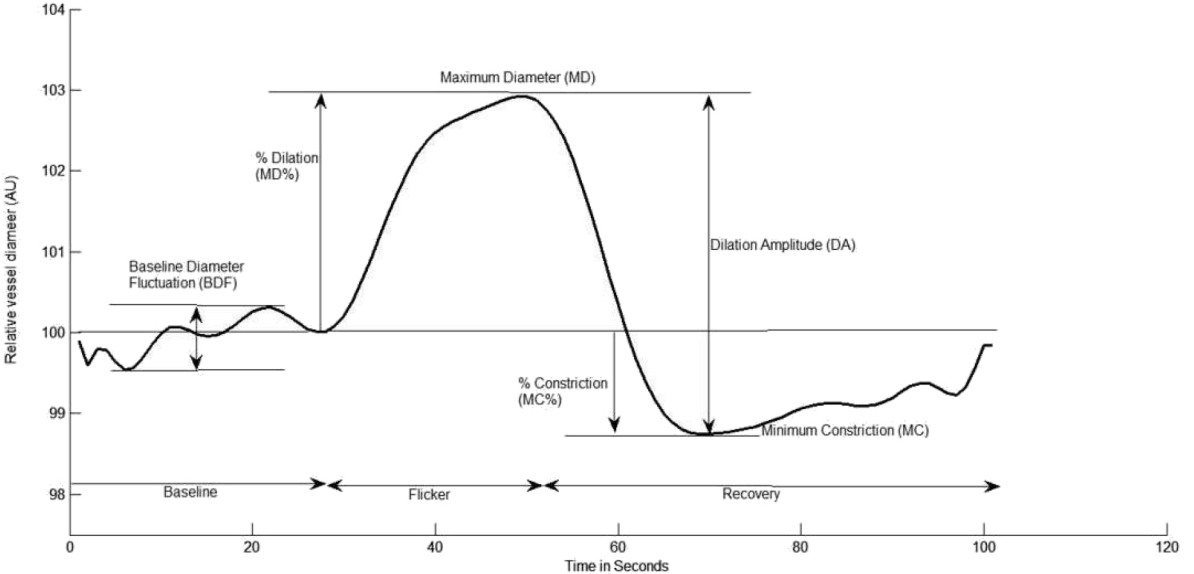
Maximum Diameter (MD)
% Dilation
(MD%)
Dilation Amplitude (DA)
Baseline Diameter
Fluctuation (BDF)
% Constriction
(MC%)
Minimum Constriction (MC)
Baseline
Flicker
Recovery
Relative vessel diameer (AU)
Time in Seconds
104
103
102
101
100
99
98
0
20
40
60
80
100
120

Figure 1

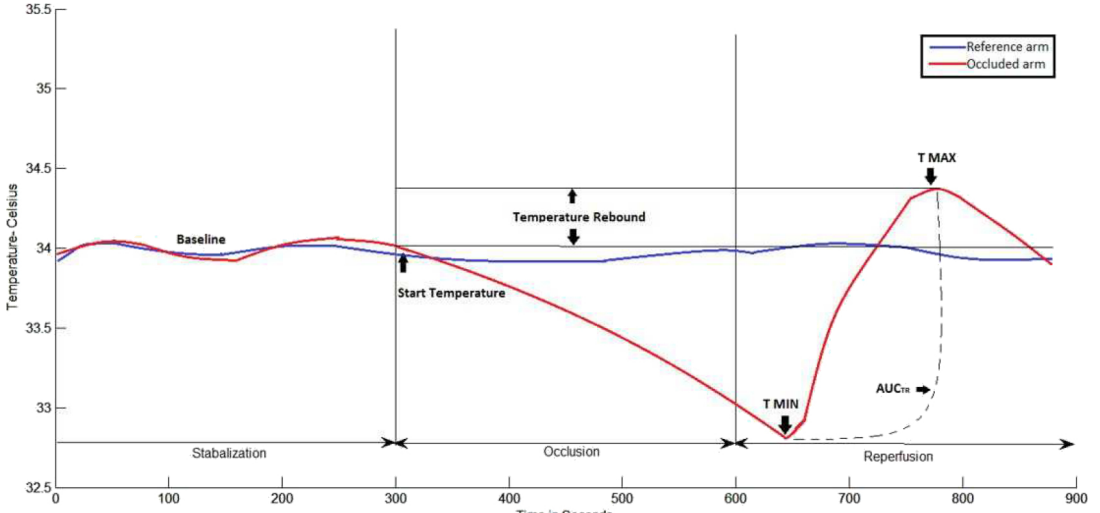
Reference arm
Occluded arm
T MAX
Temperature Rebound
Baseline
Start Temperature
T MIN
AUCTR
Stabalization
Occlusion
Reperfusion
Temperature- Celsius
Time in Seconds
35.5
35
34.5
34
33.5
33
32.5
0
100
200
300
400
500
600
700
800
900

Figure 2

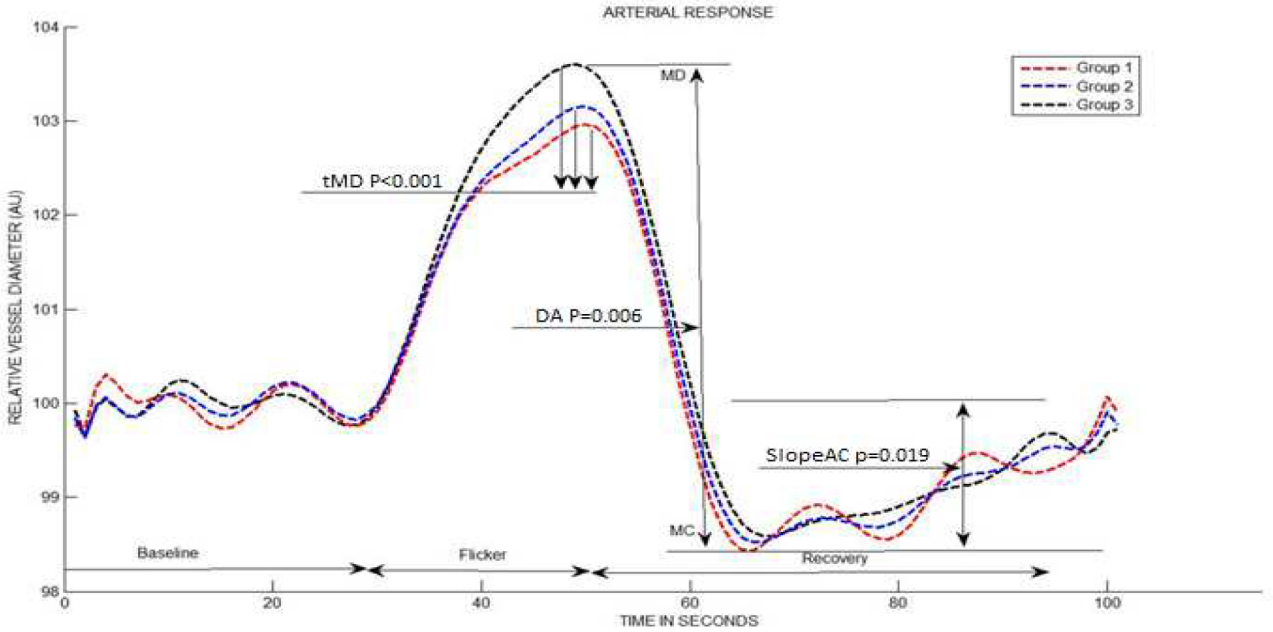
ARTERIAL RESPONSE
Group 1
Group 2
Group 3
MD
tMD P<0.001
DA P=0.006
SlopeAC p=0.019
MC
Baseline
Flicker
Recovery
RELATIVE VESSEL DIAMETER (AU)
TIME IN SECONDS
104
103
102
101
100
99
98
0
20
40
60
80
100

Figure 3

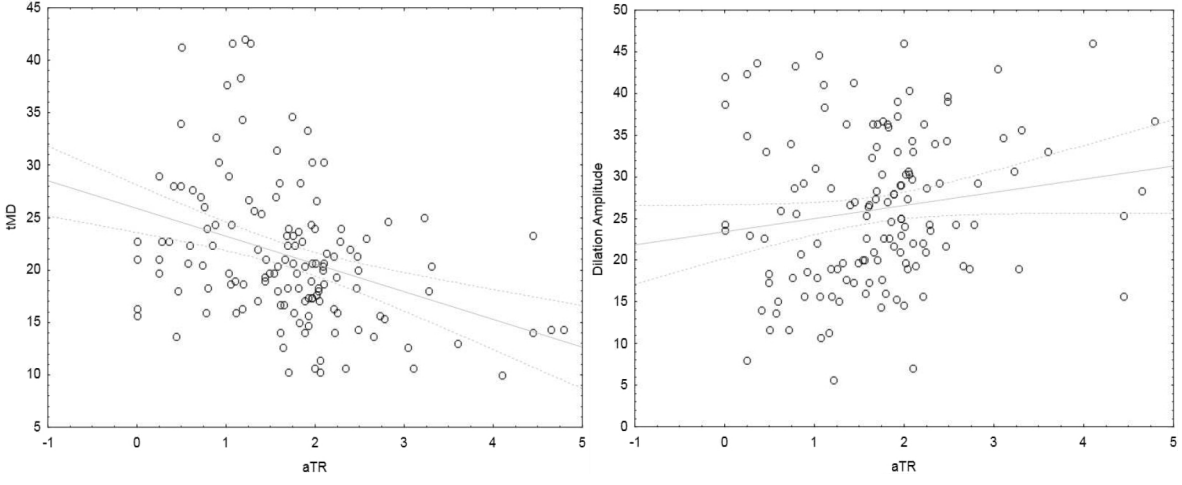

Figure 4