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Retinal vascular function in asymptomatic individuals with a positive family history of cardiovascular disease

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Abstract

Purpose:

To compare retinal microvascular function in healthy individuals with and without a positive FH of CVD.

Methods:

Retinal vessel reactivity was assessed by means of dynamic retinal vessel analysis (DVA) in 38 healthy subjects aged between 30 and 66 years with a positive family history (FH) of cardiovascular disease (CVD), and 37 age- and gender-matched control subjects. Other assessments included blood pressure (BP) profiles, blood glucose and lipid metabolism markers, Framingham risk scores (FRS), carotid intima-media thickness (c-IMT) and brachial flow-mediated dilation (FMD).

Results:

FH positive subjects showed decreased retinal arterial baseline diameter fluctuation, dilation amplitude, percent dilation, and overall constriction response slope ($p = 0.001$; $p = 0.015$; $p = 0.001$; and $p < 0.001$, respectively), and increased percent constriction ($p = 0.008$). On the venous side, baseline corrected flicker response and dilation response slope were decreased in the FH positive group ($p = 0.009$ and $p = 0.010$, respectively). There were no significant differences between groups in c-IMT scores or FMD parameters (all $p > 0.05$). The arterial MC% correlated negatively with decreased HDL-c ($r = -0.52$, $p = 0.002$ in only FH positive group.

Conclusion:

Although macrovascular function is preserved in individuals with FH positive for CVD but with low FRS, there are, however, functional impairments at the retinal microvascular level that correlate with established plasma markers for cardiovascular risk.

Key words: Vascular function, dynamic retinal vessel analysis, family history, cardiovascular risk

INTRODUCTION

It has been previously documented that a positive family history (FH) of cardiovascular disease (CVD) increases the risk for various circulatory pathologies (Myers et al.1990; Shea et al. 1984; Khaleghi et al. 2014; Philips et al. 2007). In addition, as FH encompasses a complex interaction between environmental and genetic variables, it represents a good way of measuring the inherited component of any disease, including CVD (Banerjee 2012). Several risk scores, including the Prospective Cardiovascular Mȕnster (PROCAM), QRISK and Scottish Intercollegiate Guidelines Network (ASSIGN) include various components of FH of CVD but due to lack of standardisation, this information is inconsistently used as a risk predictor in clinical practice. Moreover, these scores have been shown to either over- or underestimate the actual risk in a large number of individuals (Vasan 2006; Cohn 2013; Koenig 2007). Consequently, the need arose for developing other simple tests that are quick and sensitive enough to detect subclinical disease (Wang 2011; Helfand et al.2011; Ge & Wang 2012) For this purpose, one of the most promising avenues is assessing endothelial dysfunction (ED), an entity in which the homeostatic functions of the endothelium are impaired, resulting in abnormal vascular tone, blood flow, immune cell and platelet activity/adhesion (Moncada et al. 1991).

It is well known that ED represents an early predictor of subsequent cardiovascular events or mortality. Moreover, signs of ED may be apparent even in people without an overt disease but with a FH of cardiovascular pathology (Celermajer et al.1994; Vita et al 1990; de Jongh et al.2002). Assessing this parameter is, therefore, important for predicting CVD risk and employing all the necessary preventive and therapeutic measures to decrease morbidity and mortality. Traditionally, the presence of ED is assessed by techniques such as ultrasound flow-mediated dilation (FMD), pulse wave analysis (PWA), plethysmography, and iontophoresis (Ray et al. 2014). These tests however, can be complex, time-consuming, and are performed only in highly specialized services. Dynamic retinal vessel analysis (DVA) represents a non-invasive way of evaluating VED at the retinal microvascular level (Pemp et al. 2009; Dorner et al.2003) and its usefulness in measuring early changes that signal risk for future cardiovascular pathologies in individuals with or without overt disease has already been proven (Pemp et al. 2009; Reimann et al.2009; Kotliar et al. 2011). In addition, it can also detect signs of ED at the retinal microvascular level in the presence of more subtle risk factors for CVD such as ageing

(Seshadri et al. 2015), ethnicity (Patel et al.2011), altered lipid and oxidative stress marker levels (Seshadri et al. 2015), and impaired glucose tolerance (Patel et al.2012).

There is still a debate if ED can be considered an independent risk factor for CVD in low-risk individuals (Cardona et al. 2015) Therefore, it is possible that ED's capabilities to predict risk for CVD can be increased by association with other traditional risk factors, such as FH. Therefore, this study investigates if individuals with low personal risk (FRS <= 10%) but with FH of CVD exhibit early signs of vascular dysfunction at microvascular levels when compared to those without FH. In addition, the relationship between these changes and the circulatory markers for CVD risk is also analysed.

METHODS

Study 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 this study was received from Aston University's ethics committee and written informed consent was received from all participants prior to study enrolment. All study procedures were designed and conducted in accordance with the tenets of the Declaration of Helsinki.

Study participants were considered for inclusion on the basis of self-declared FH of CVD (to include coronary artery disease, heart failure, arrhythmia, and vascular disease) in a first-degree relative, identified by way of self-report questionnaire.

Study exclusion criteria were defined as subject history or current diagnosis of cardiovascular or cerebrovascular disease including coronary artery disease, heart failure, arrhythmia, stroke, transient ischemic attacks, peripheral vascular disease, as well as, hypertension, diabetes, and or severe dyslipidemia (defined as plasma triglyceride levels > 6 mmol/L or cholesterol levels > 7 mmol/L). Smoking and the use of vasoactive medications, such as dietary supplements containing vitamins or antioxidants and bronchodilators, also served as exclusion criteria for the study participants. In addition, potential subjects were screened for ocular diseases and were excluded from if they had a refractive error of more than ±3DS and more than ±1DC equivalent, elevated intraocular pressures (> 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.

General assessments

All study-related measurements were performed between 8 and 11 AM following a 12-hour overnight fast, which included refraining from alcohol or caffeine. Standard anthropometric measures of height and weight were recorded to determine body mass index (BMI = weight/height^2). Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were measured using an automatic BP monitor (UA-767; A&D Instruments Ltd, UK) to determine mean arterial pressure (MAP = 2/3 DBP + 1/3 SBP). Intraocular pressure (IOP) was measured using non-contact tonometry (Pulsair; Keeler Ltd, UK) to determine ocular perfusion pressure (OPP = 2/3 MAP – IOP). In addition, blood and plasma samples drawn from the antecubital fossa vein were assessed immediately for fasting glucose (GLUC), triglycerides (TG), total cholesterol (CHOL), and high-density lipoprotein cholesterol (HDL-c) using the Reflotron Desktop Analyzer (Roche Diagnostics, UK). Low-density lipoprotein cholesterol (LDL-c) values were calculated as per the Friedewald equation (Friedewald et al. 1972).

Cardiovascular risk scores for each subject were also calculated using the current version of the Framingham risk score (FRS) published by an expert panel of the National Heart, Lung and Blood Institute (NHLBI 2002), which includes the following risk factors: age, sex, CHOL, HDL-c, SBP, treatment for hypertension, smoking status, and diabetes. Age, sex, treatment for hypertension, smoking, and the presence of diabetes were identified from self-report questionnaires, whereas CHOL, HDL-c, and SBP values were as those determined on the day of study. The risk scoring algorithm is based on gender-specific points assigned for each risk factor variable. Ten-year risk percentage was then calculated based on 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%). Finally, the absolute CVD risk percentage was classified as low risk (< 10%), intermediate risk (10-20%), or high risk (> 20%) (Ford et al. 2004). Only those individuals classified in the low-risk category were considered for this study.

Vascular assessments

Carotid intima-media thickness measurements of the common carotid arteries (c-IMT) were obtained for all participants, as described previously (Seshadri et al. 2015), and in accordance with an already published protocol (Salonen et al. 1991). Briefly, with the subject in supine position, a high-resolution B-mode ultrasound system (Siemens, Acuson-Sequoia, UK) was used to capture an image of the right then left carotid artery at the level of the carotid bifurcation. Measurements of the thickness of the inner two layers of the artery were then taken from the central region of the inferior arterial wall at a site proximal to the artery bifurcation using the instrument's in-built software caliper system.

Flow-mediated dilation (FMD) of the brachial artery was determined using a high-resolution ultrasound imaging system with a 7 mm 8MHz linear-array (Siemens; Acuson-Sequoia, UK). The brachial artery was imaged above the antecubital fossa in a longitudinal plane. A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall was selected for continuous 2D grey-scale imaging. The vessel diameter, from the anterior to the posterior interface between the media and adventitia, was then continually measured using a specialized, artificial neural networking, wall-detection software (VIA® Software, UK). According to a published protocol (Corretti et al. 2002), a baseline image of the artery was first acquired. The brachial artery was then temporarily occluded by BP cuff inflated 50 mmHg above each individual's SBP for a standardized five minutes. Image recording was then continued for an additional two minutes following cuff deflation. Endothelium-dependent FMD (FMD_{ED}) was expressed as a percentage of the maximal artery dilation during hyperemia ($MD_{hyperemia}$) relative to the average vessel diameter recorded during the 2-minute baseline image acquisition ($AD_{baseline}$).

Dynamic retinal vessel analysis (DVA) was carried out using the retinal vessel analyzer system (IMEDOS; GmbH, Jena, Germany). All measurements were performed in one unselected eye following full pupil dilation with Tropicamide 1% (Chauvin Pharmaceuticals Ltd, UK), and in a quiet, temperature-controlled room (22˚C). A visual fixation target was used to control eye movements and to position the region of interest at the center of the fundus image. Within this region, a segment approximately 0.5 to 1 mm and 1 to 2 disc diameters from the optic nerve head

was selected for continual diameter recording, for both the inferior temporal retinal artery and retinal vein. Arterial and venous vessel diameters were then assessed simultaneously and continuously over 350 seconds, according to an accepted and widely used protocol (Nagel et al. 2006). In short, the whole vessel diameter measurement duration (350 seconds) consists of a 50-second baseline measurement followed by continual diameter recordings during three consecutive 100-second flicker cycles, each comprised of 20 seconds of flickering light (stimulus) interrupted by 80 seconds of still illumination (recovery). Visualization plots to evaluate the dynamic nature of the vessels' response profiles were created by extracting the raw response data from the device software and applying a statistical polynomial regression algorithm using MATLAB (Mathworks, USA) according to a method perfected in-house (Mroczkowska et al. 2012) The following retinal vessel reactivity and time course parameters were then calculated for both the artery and the vein (Heitmar et al. 2010). The differences between maximum and minimum baseline vessel diameter was termed as baseline-diameter fluctuation (BDF), the maximum diameter (MD) was used to describe the maximal vessel dilation in response to flicker-light stimulation expressed as a percentage relative to baseline, the time taken (seconds) to reach the maximum vessel diameter during the twenty-second flicker exposure was termed as MD reaction time (tMD), the minimal vessel diameter within thirty seconds of the recovery period was calculated as a percentage relative to baseline and expressed as the maximum constriction (MC) whilst the time taken (seconds) to reach maximal vessel constriction was termed maximum constriction reaction time (tMC), and finally, the difference between maximal dilation and constriction responses was termed as the dilation amplitude (DA). More recently, an additional parameter was introduced by our research group that can be used describe the change in vessel diameter as a function of time (slope) and can be calculated for the dilation ($Slope_D$ = (MD-baseline)/tMD) and constriction components ($Slope_C$ = (MC-baseline)/tMC) of the vessel response profile (Mroczkowska et al. 2012).

Statistical analysis

All statistical analyses were performed using Statistica® software (Version 9, StatSoft Inc, USA). The Shapiro-Wilk test was used to determine the distribution of the data. Multivariate analysis was used to test the influence of age, BMI, BP, circulating markers, and systemic parameters on the measured variables. Differences between groups were subsequently assessed using

independent sample *t*-tests or ANCOVA, as appropriate. Within-group correlations between retinal vascular reactivity and systemic parameters were explored using Pearson's or Spearman's rank method, as appropriate. A *p*-value of less than 0.05 was considered significant, except in certain cases where a stricter *p*-value of less than 0.01 was adopted in order to correct for multiple comparisons.

What is described in the results section is RESULTS

In total, 102 participants were screened for eligibility of which 27 were excluded on the basis of having moderate or high FRS scores (>10%) or if the quality of the DVA recording was below the acceptable quality control. The remaining 75 healthy subjects were then classified into one of two groups based on the self-declared presence or absence of CVD in a first-degree relative (FH positive: $n = 38$, controls: $n = 37$, respectively). The number of participants in each group was similar (Chi-square $p = 0.909$) as was the within-group distributions of male (M) and female (F) participants (FH positive: M = 20; F = 18, Control: M = 22, F = 15, Chi-square $p = 0.551$).

Clinical characteristics

A summary of the clinical and systemic vascular characteristics of the FH positive and control subjects is presented in Table 1. There were no significant group differences in age, BMI, SBP, DBP, HR, MAP, IOP, OPP, GLUC, TG, CHOL, LDL-c, TG: HDL-c, and FRS (all $p > 0.05$). However, in comparison to controls, FH positive subjects exhibited lower levels of HDL-c ($p = 0.030$) and higher CHOL: HDL-C ratios ($p = 0.021$).

Systemic vascular parameters

After correcting for all the possible influences identified through the multivariate analyses, there were no significant group differences in c-IMT scores brachial $AD_{baseline}$, $MD_{hyperaemia}$, or FMD_{ED} (all $p > 0.05$ ANCOVA, Table 1)

Retinal vascular parameters

Arterial response. After controlling for influential covariates in ANCOVA models, statistically significant differences in arterial BDF ($p = 0.001$), MD% ($p = 0.001$), MC% ($p = 0.008$), and $Slope_{AC}$ ($p < 0.001$) (Fig. 1 A) were identified between the two study groups (Table 2). No significant group differences in any of the other measured arterial DVA parameters were identified (all $p > 0.05$).

Venous response. After controlling for influential covariates in ANCOVA models as appropriate, group comparisons showed statistically lower venous BCFR ($p = 0.009$) and dilation

response $Slope_{VD}$ ($p = 0.010$) in the FH positive group in comparison to controls (Table 2; Fig. 1B). No significant group differences in any of the other measured venous DVA parameters were identified (all $p > 0.05$).

Correlations between microvascular retinal function and systemic parameters

In the FH positive group, increased arterial MC% was associated with decreased HDL-c ($r = -0.52$, $p = 0.002$, Fig. 2). A similar trend could not be observed in the control group ($p > 0.05$). No other significant correlations were identified in either of the study groups ($p > 0.05$).

DISCUSSION

This study has demonstrated that independent of systemic influences, early signs of microvascular dysfunction are detectable at the retinal arterial and venous levels of otherwise healthy subjects with a positive FH for CVD, even when the macrovascular structure and function (as assessed by c-IMT and FMD measurements) are within normal limits. These changes correlated with established plasma markers for cardiovascular risk.

Firstly, spontaneous variations in baseline diameters before the onset of flicker (BDF) were found to be reduced in the FH positive subjects in comparison to controls. We can hypothesize that this observation is the result of an imbalance in the adaptive mechanism that enables a healthy vessel to adjust its baseline diameters to an increased metabolic demand after flickering stimulation (Kotliar et al. 2004; Vilser et al. 2002; Kotliar et al. 2011). In addition, a reduction in arterial dilation and over-constrictions post-flicker were also observed in individuals with positive FH as compared to controls. It is possible that the participants with a positive FH for CVD, although otherwise healthy, displayed some limitations in the functional vascular reserves that may only be evident after provocative stressors showing that increased challenges cannot be met. In addition, we also observed that FH positive subjects demonstrated a decreased retinal venous dilation response slope, a composite parameter that could reflect a decrease in retinal venous dilation diameter and/or a delay in reaching maximal dilation diameter. Although retinal veins are thought to play a more passive role in autoregulatory function (Kotliar et al. 2004) we could speculate that the functional changes observed at both arterial and venous levels could signal key generalised microvascular alterations associated with the presence of non-modifiable risk factors, such as FH. It is possible that such limitation in the retinal vascular function are inherited and will predispose individuals with a positive FH to CVD later in life.

Our observations of abnormal retinal microvascular responses in this category of individuals is in line with previous studies reporting reduced coronary blood flow(Schachinger et al. 1999) and skin reactive hyperemia in otherwise healthy subjects with FH of hypertension (Maver et al. 2004) or diabetes (Lee et al. 2011) Moreover, previous studies have also demonstrated that an impaired microvascular endothelial function may be evident as early as adolescence in those with a FH of cardiovascular disease (de Jongh et al. 2002) . Indeed, poor microvessel function

represents an inherited phenotype for CVD risk in patients with familial risk factors (Noon et al. 1997; Barrett-Connor et al. 1984; Colditz et al. 1986; Marenberg et al. 1994; Friedlander et al. 1985; Grech et al.1992).

However, beside genetic factors, other mechanisms could also be contributing to the two above-mentioned pathological entities (Martino et al. 2015) of circulatory function. Longitudinal, more complex studies are, therefore, required to understand the relative importance of our observations in FH positive individuals. From our experience, in individuals with low CVD risk, retinal microvascular function can be affected by various factors such as ethnicity (Patel et al. 2011) and ageing (Seshadri et al. 2015a). In addition, we have also published that body's antioxidant capacity is also linked to the vascular function as measured at the microvascular retinal level (Seshadri et al. 2015b). This later observation is very important and could offer us a possible explanation for the present findings. Indeed, it has been independently shown that individuals with a positive FH for CVD display higher levels of oxidative stress than the normal population (Kelishadi et al. 2009). Although the levels of antioxidant molecules were not determined and compared in this study, it can be hypothesized that this factor could have contributed to our findings.

Our study also found a favourable relationship between high HDL-C level and microvascular function at the retinal arterial level. Indeed, subjects without a positive family history of CVD had higher HDL-C levels than those with positive FH and in the latter category, the abnormal HDL-C level was associated with an abnormal vascular constriction response. It has been previously shown that high levels of HDL-C do not only offer protection against atherosclerosis but also predicts a favourable course of symptomatic vascular disease (Kim et al. 2014). Therefore, our study comes to confirm the necessity of treating low HDL-C levels in individuals without overt CVD but with risk factors such as history of premature CHD (<50 years old in a first-degree relative) (Link et al. 2007).

In conclusion, the results of the present study show that familial risk for CVD is associated with alterations in retinal vascular function that correlate with circulating markers for CVD risk. With traditional cardiovascular risk scoring systems such as the FRS known to severely underestimate relative risk (Vasan 2006; Cohn 2013) the findings of the present study suggest that functional

retinal assessments can provide an improved diagnostic capability for the detection of subclinical microvascular dysfunction. This approach could be promising in furthering the concept of early vascular screening and prevention strategies, an important step towards the development of individualized and targeted endothelial therapies that can improve the life-time risk in individuals that were traditionally considered low-risk (Chia 2016).

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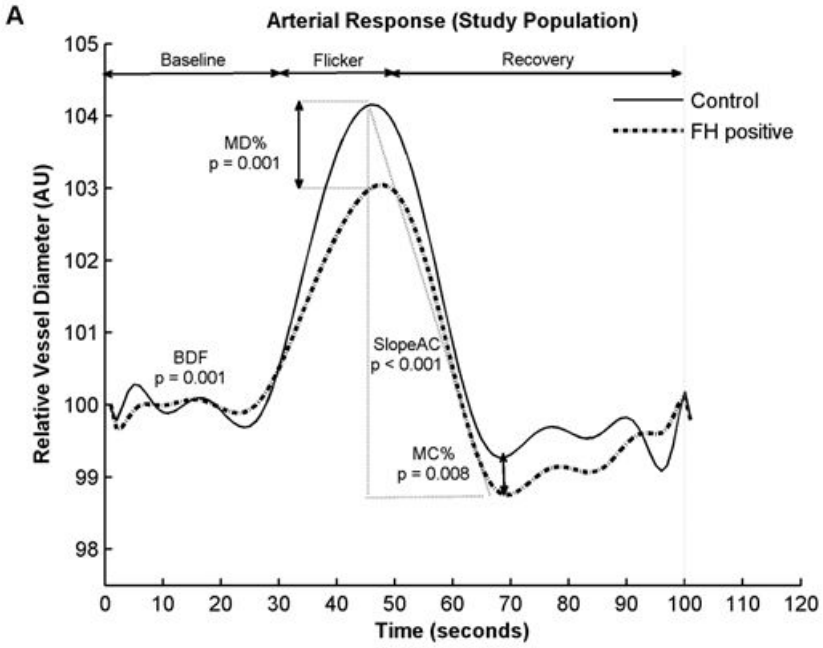
Figure Legends

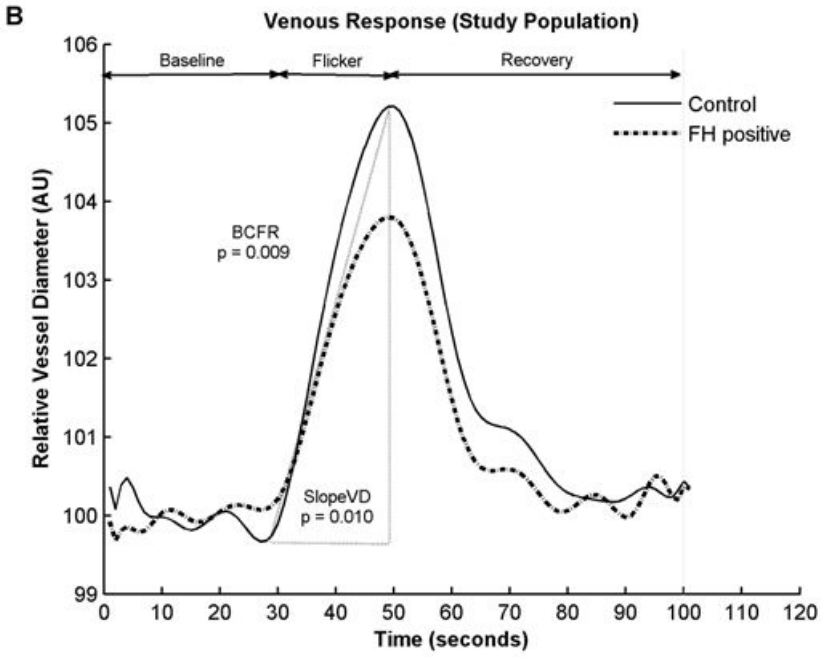
Figure 1. Group comparisons of retinal vascular response profiles.

(A) Arterial response, (B) Venous response. Abbreviations: AU, arbitrary units; BDF, baseline diameter fluctuation; BCFR, baseline corrected flicker response; FH, family history; MD%, percent dilation; MC%, percent constriction; $Slope_{AC}$, arterial constriction slope; $Slope_{VD}$, venous dilation slope.

Figure 2. Correlation between HDL-c and retinal arterial percent constriction in the FH positive group.

Abbreviations: FH, Family history; HDL-c, high-density lipoprotein cholesterol; MC%, percent constriction.


A
Arterial Response (Study Population)
Baseline
Flicker
Recovery
Control
FH positive
MD%
p = 0.001
BDF
p = 0.001
SlopeAC
p < 0.001
MC%
p = 0.008
Relative Vessel Diameter (AU)
Time (seconds)
105
104
103
102
101
100
99
98
0
10
20
30
40
50
60
70
80
90
100
110
120


B
Venous Response (Study Population)
Baseline
Flicker
Recovery
Control
FH positive
BCFR
p = 0.009
SlopeVD
p = 0.010
Relative Vessel Diameter (AU)
Time (seconds)
106
105
104
103
102
101
100
99
0
10
20
30
40
50
60
70
80
90
100
110
120

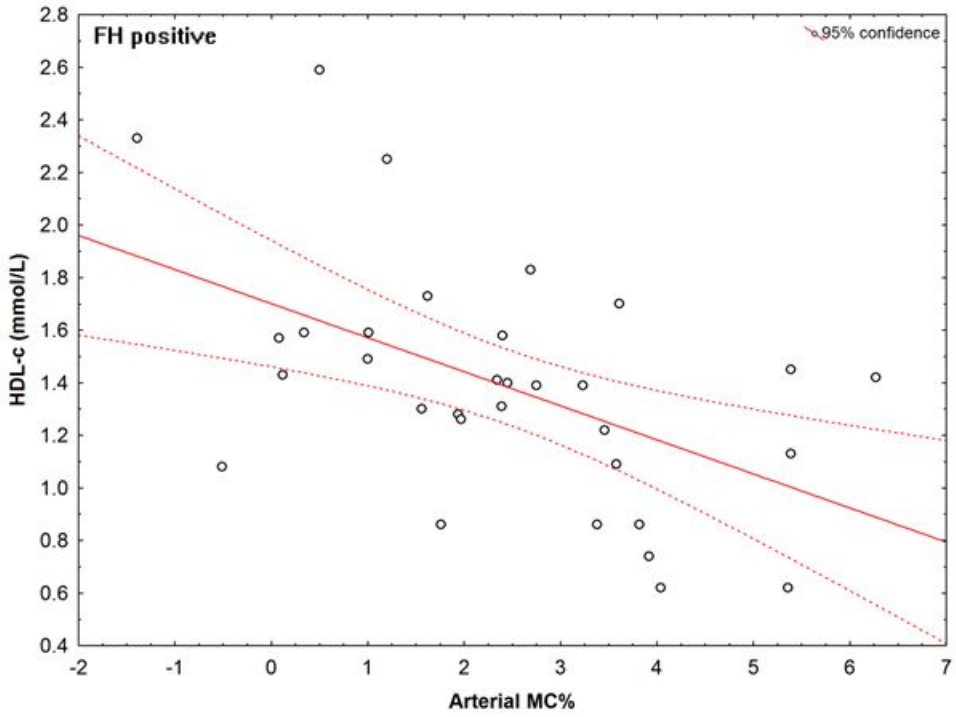

FH positive
95% confidence
HDL-c (mmol/L)
Arterial MC%
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
-2
-1
0
1
2
3
4
5
6
7

Table 1. Clinical and systemic vascular characteristics

Variable	Mean (SD) FH positive (1)	Mean (SD) Control (2)	*p*-value	Significance
N	38	37	0.909	-
Gender	20M : 18F	22M : 15F	0.551	-
Age (years)	47 (11)	45 (11)	0.468	-
BMI (kg/m^2)	27.13 (4.45)	26.75 (4.72)	0.727	-
SBP (mmHg)	121 (11)	121 (12)	0.752	-
DBP (mmHg)	74 (9)	76 (9)	0.227	-
HR (bpm)	65 (10)	68 (8)	0.096	-
MAP	89.36 (8.90)	91.29 (9.05)	0.356	-
IOP (mmHg)	14 (2)	14 (3)	0.439	-
OPP	45.15 (6.36)	46.91 (5.54)	0.206	-
GLUC (mmol/L)	4.95 (1.07)	5.08 (0.58)	0.539	-
TG (mmol/L)	1.14 (0.44)	1.15 (0.62)	0.958	-
CHOL (mmol/L)	4.68 (1.02)	4.55 (0.98)	0.578	-
HDL-C (mmol/L)	1.16 (0.41)*	1.39 (0.45)	**0.030***	1 < 2
LDL-C (mmol/L)	3.00 (0.97)	2.67 (0.92)	0.156	-
TG/HDL-c	1.11 (0.56)	1.00 (0.84)	0.515	-
CHOL/HDL-c	4.39 (1.36)*	3.61 (1.36)	**0.021***	1 > 2
FRS %	5.91 (4.16)	4.85 (3.78)	0.105	-
c-IMT	0.55 (0.11)	0.58 (0.11)	0.318	-
$AD_{baseline}$ (mm)	4.16 (0.93)	4.44 (0.88)	0.174	-
$MD_{hyperaemia}$ (mm)	4.61 (1.12)	5.14 (1.26)	0.060	-
FMD_{ED}	10.88 (9.42)	12.13 (8.63)	0.520	-

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; GLUC, glucose; TG, triglycerides; CHOL, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; FRS%, Framingham risk score; c-IMT, carotid intima media thickness; $AD_{baseline}$, brachial baseline diameter; $MD_{hyperaemia}$, maximum brachial diameter during hyperaemia; FMD_{ED}, endothelium-dependent flow-mediated dilation. * Significant *p*-values are indicated in bold where $p < 0.05$ was considered

Table 1. Group differences in retinal vascular reactivity parameters

DVA parameter	Mean (SD) FH positive (1)	Mean (SD) Control (2)	*p*-value (*t*-test/ANCOVA)	Significance
Arteries:				
Baseline	100.02 (0.05)	100.01 (0.06)	0.552	-
BDF	5.13 (2.10)	6.50 (2.88)	**0.001***	1 < 2
BCFR	1.04 (2.00)	1.12 (3.07)	0.882	-
DA	6.16 (2.67)	7.51 (2.76)	0.015	-
MD%	3.74 (2.25)	5.22 (2.50)	**0.001***	1 < 2
MC%	-2.42 (1.87)	-2.29 (2.31)	**0.008***	1 > 2
tMD (seconds)	19 (6)	22 (8)	0.048	-
tMC(seconds)	29 (6)	26 (8)	0.064	-
$Slope_{AD}$	0.24 (0.12)	0.30 (0.15)	0.070	-
$Slope_{AC}$	-0.25 (0.13)	-0.38 (0.25)	**<0.001***	1 < 2
Veins:				
Baseline	100.00 (0.04)	100.00 (0.03)	0.775	-
BDF	3.94 (1.78)	3.79 (1.68)	0.011	-
BCFR	1.40 (2.42)	2.35 (1.83)	**0.009***	1 < 2
DA	5.33 (2.91)	6.09 (2.60)	0.242	-
MD%	4.44 (2.74)	5.43 (2.38)	0.045	-
MC%	-0.90 (1.59)	-0.66 (1.04)	0.461	-
tMD (seconds)	21 (6)	20 (4)	0.200	-
tMC (seconds)	28 (7)	31 (8)	0.058	-
$Slope_{VD}$	0.23 (0.13)	0.29 (0.12)	**0.010***	1 < 2
$Slope_{VC}$	-0.23 (0.16)	-0.23 (0.14)	0.960	-

Abbreviations: Baseline, average baseline diameter: BDF, baseline diameter fluctuation; DA, dilation amplitude; BCFR, baseline corrected flicker response; MD%, percent dilation (during flicker); MC%, percent constriction (post-flicker); tMD, time taken to reach maximal dilation (MD) diameter; tMC, time taken to reach maximal constriction (MC) diameter; $Slope_{AD/VD}$, slope of arterial/venous dilation; $Slope_{AC/VC}$, slope of arterial/venous constriction. *Significant *p*-values are indicated in bold where $p < 0.01$ was considered significant