Peripheral vascular disease is associated with abnormal arteriolar diameter relationships at bifurcations in the human retina

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ABSTRACT

Arterial diameters at branch points are believed to conform to design principles that optimize circulatory efficiency and maintain constant shear stress across arterial networks. The objective of this study was to examine whether optimality at bifurcations is affected in individuals with atherosclerosis. Retinal images were analysed in normotensive men with abnormal ankle brachial index (n = 13) and healthy controls (n = 8), matched for age and clinic blood pressure. Compared with controls, men with peripheral vascular disease had adverse metabolic profiles (relative insulin resistance and greater total cholesterol levels). In healthy men, retinal arterial diameters at bifurcations conformed to predicted optimal values but in men with peripheral vascular disease, junction exponents deviated significantly from the optimum. Retinal arteriolar bifurcation angles did not differ significantly between the groups. Atherosclerosis is associated with abnormalities in the arteriolar network of the retina. In view of the importance of the endothelium in maintaining network co-ordination of branch diameters this is suggestive of a generalized abnormality of endothelial function in atherosclerosis.

INTRODUCTION

The architecture of an arterial network is a major determinant of circulatory efficiency and has implications for the distribution of shear stress across a network [1]. Two important aspects of arterial network architecture are the relationship of parent and offspring vessel diameters and the angle subtended by branches at a bifurcation [2,3]. A number of theoretical and experimental studies have suggested that arterial diameters at a bifurcation should conform to a power relationship: \( D^n = D^1 + D^2 \), where \( D^n \) is the diameter of parent and offspring vessel and X is a constant termed the junction exponent. Calculations by Murray [2] suggested that when X = 3 network costs are minimized, and it can also be shown that this value predicts constant shear stress throughout the network [1]. Arterial branches in various circulations including the retina in both humans and animals have been shown to conform to this optimal design [4,5].

The retinal arteriolar circulation is readily visualized in vivo and qualitative abnormalities in systemic diseases, such as hypertension, are well recognized. The retinal arterial network is essentially two-dimensional and is amenable to quantitative analysis by image analysis techniques. Retinal arterioles are approx. 50–200 \( \mu \)m in

Key words: atherosclerosis, blood pressure, endothelial function, peripheral vascular disease, retina.
Abbreviations: L-NMMA, \( N^G \)-monomethyl-L-arginine; PVD, peripheral vascular disease; BP, blood pressure; FBF, forearm blood flow; L:D ratio, length to diameter ratio; \( R_{\text{min}} \), minimum forearm vascular resistance.
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diameter [6] and this size of vessel makes an important contribution to systemic vascular resistance [7]. We have shown previously that bifurcation angles are reduced in men and women with hypertension [8] and men with a low birth weight [9], and that junction exponents deviate from optimal values with advancing age [8]. In addition, arteriolar length to diameter ratios, another dimensionless measure of network topography, are increased in hypertensive subjects [10], a finding consistent with that of generalized arteriolar narrowing observed in hypertensive subjects in large-scale epidemiological studies [11,12].

Atherosclerosis is a disorder affecting large arteries. It is commonly associated with metabolic abnormalities such as insulin resistance [13], hyperlipidaemia [13] and impaired vasodilation in response to infused acetylcholine or flow [14]. Recently, the Atherosclerosis Risk in Communities (ARIC) Study has reported a study of retinal changes and atherosclerosis [15]. Generalized arteriolar narrowing was associated with hypertension and with the presence of carotid plaque, but not with any other evidence of clinical or sub-clinical atherosclerosis [15]. We have reported that inhibition of NO synthase by Nω-monomethyl-L-arginine (L-NMMA) induces changes in junction exponents in human retinal arteries [16] and that this may therefore be an indicator of endothelial function in vivo. The objective of the current study was to investigate whether the relationship between arteriolar diameters at retinal bifurcations differs in men with and without symptomatic peripheral vascular disease (PVD). These individuals were matched for blood pressure (BP) and age in order to control for important known confounding factors.

METHODS

The study was carried out in accordance with the Declaration of Helsinki, the protocol was approved by the local research Ethics Committee and all subjects gave written informed consent. Recruitment of subjects and all experimental procedures were performed at the University of Pisa, Italy. Retinal image processing and analysis was performed in London, U.K.

Subjects

The subjects with PVD comprised 13 white males who had previously been recruited from among patients referred for screening and treatment of cardiovascular risk factors. Full eligibility criteria have been published previously [17]. All had normal BP levels (<140/90 mmHg) on repeated clinic measurements while taking no antihypertensive drugs. All had normal glucose tolerance and normal renal function. However, they also all had angiographically demonstrated lower limb atherosclerotic PVD, an ankle/brachial index of less than 0.9 in at least one limb and stable intermittent claudication [17]. The control group consisted of eight healthy white males, group matched for age and clinic BP, who had normal ankle/brachial indices.

Experimental procedures

All subjects underwent a glucose tolerance test [18] and measurements of fasting plasma lipids [17] as described previously.

BP measurement

Clinic systolic and diastolic BP were measured, using a calibrated mercury sphygmomanometer, with patients in the supine position, and were taken as the mean of three readings over 30 min.

Minimum forearm vascular resistance (R_{min})

Forearm blood flow (FBF; ml·100 ml of tissue^{-1}·min^{-1}) was measured by strain-gauge plethysmography (DE Hokanson EC 5R plethysmograph), excluding the hand circulation using a paediatric cuff inflated to suprasystolic pressures [19]. Forearm arterial occlusion was achieved by inflating the plethysmographic cuff to 300 mmHg for 13 min. Dynamic exercise (2–30 hand contractions) was added during the last minute of ischaemia. FBF was measured frequently in basal conditions and at 15-s intervals during the 3 min after ischaemic release. R_{min} was derived as the ratio of pre-ischaemic mean BP (diastolic BP+1/3 pulse pressure) and maximum post-ischaemic FBF.

Retinal photography, digitization and image analysis

Retinal images (35 mm) were taken using a retinal camera (TRC-50, Topcon Optical Company, Tokyo, Japan) with a 50° field of view and a red-free filter to enhance vessel edge definition. Images of the temporal view of the right retina were digitized to a resolution of 3000 × 3000 pixels using a flatbed digital scanner (GS-670, Bio-Rad Laboratories, CA, U.S.A.). A single trained observer performed operator-directed image analysis using a custom-written application package within the MATLAB environment (MATLAB Version 5.2, MathWorks Inc., Natick, Massachusetts, U.S.A.) [20]. For each subject, the five most proximal evaluable arteriolar bifurcations were identified in the upper temporal quadrant of the retina and bifurcation geometry was quantified for each bifurcation. At each bifurcation, the angle
between the parent and each daughter arteriole was measured and the bifurcation angle (\(\omega\)) calculated as:

\[\omega = 360 - (\text{sum of the angles measured between parent and each daughter arteriole}) \]

Internal arteriolar diameters were measured as the width of the red cell column within each vessel. The diameters of the parent (\(D_p\)) and daughter arterioles (\(D_1\) and \(D_2\)) were measured using a validated purpose-written edge-detection program [20]. Vessel width was automatically measured across a total of five parallel cross sections (the central one being operator-selected) and the median was taken as the diameter for that vessel. Arteriolar length was calculated as the actual length from the midpoint of each bifurcation to the midpoint of the preceding bifurcation. The length to diameter (L:D) ratio was estimated on the basis of within-subject standard deviation (\(s_w\)); for measurements of \(\omega\) (mean measured angle, 68.9°), \(s_w = 2.66\), for diameter measurements (mean diameter, 21.1 pixels), \(s_w = 0.92\) pixels, and for length measurements (mean length, 406.9 pixels), \(s_w = 5.09\) pixels.

**Optimality parameter**

The junction exponent, \(X\), is sensitive to small errors in arteriolar diameter measurements, particularly when making measurements from red-free retinal images where a number of sources of error have been identified [20]. For the purposes of this study, we therefore used a novel ‘optimality parameter’, \(\rho\), which measures deviation of junction exponents from the optimal value of 3 [1], and is calculated such that:

\[\rho = \left(D_p^2 - (D_1^2 + D_2^2)\right)^{0.5}/D_p\]

To investigate the consequences of errors in measurements of vessel diameter on the estimation of \(X\) and \(\rho\), a Monte Carlo simulation was carried out. A normally distributed error with a S.D. of 1 pixel was imposed on a theoretically optimal bifurcation with a parent diameter of 20 pixels. The size of vessel and magnitude of error were chosen to imitate typical measurements and errors in retinal images [20]. One thousand sets of data were generated with the error present in all three elements of the bifurcation using Prism 3.02 (GraphPad Software Inc., San Diego, CA, U.S.A.) and analysis was conducted using Excel 2000 (Microsoft Corporation, Redmond, WA, U.S.A.). From these data \(X\) was estimated to be 3.51 ± 2.8 pixels (mean ± S.D.) and \(\rho = -0.01 ± 0.54\). The simulation therefore indicated that errors in the measurement of vessel diameter result in an overestimation of \(X\) compared with the true value, while the estimated value of \(\rho\) was close to the theoretical value. Moreover the degree of scatter in estimates of \(\rho\) was considerably less than in estimates of \(X\).

**Statistical analysis**

Anthropomorphic, biochemical and haemodynamic data are presented as mean (± standard deviation) and stratified by group. Normally distributed continuous variables were compared between groups using two-tailed \(t\) tests. For those variables where data were not normally distributed (triacylglycerols and areas under glucose and insulin curves following a glucose tolerance test) groups were compared using a non-parametric Mann–Whitney \(U\) test. As a categorical variable, the frequency of smoking in each of the groups was compared using the \(\chi^2\) statistic. For each subject, the median values of each retinal parameter (\(\omega\), L:D ratio and \(\rho\)) were calculated. Mean values and S.D. were calculated for each retinal parameter in each group, and compared between groups using two-tailed \(t\) tests. All statistical analyses were performed using SPSS software (SPSS Version 10, SPSS Inc., Chicago, IL, U.S.A.).

**RESULTS**

**Subject characteristics**

Table 1 shows characteristics of subjects, stratified by the presence or absence of PVD. No subjects were currently taking antihypertensive or lipid-lowering medication, but all those with PVD were taking anti-platelet medication (aspirin or ticlopidine). There were equal numbers of current smokers in each group (\(\chi^2 = 0.387\)).

**Differences in biochemical and haemodynamic parameters**

Compared with healthy subjects, subjects with PVD had significantly greater levels of total cholesterol and higher stimulated insulin levels following a glucose tolerance test (greater area under the insulin curve and peak insulin levels, \(P = 0.036\) and 0.030 respectively; Table 1). There was a non-significant trend towards a greater area under the glucose curve following a glucose tolerance test (\(P = 0.057\)). There were no significant differences between controls and PVD subjects with respect to systolic BP (mean ± S.D.; 126 ± 9 vs. 123 ± 11 mmHg respectively, \(P = 0.434\)) or diastolic BP (77 ± 9 vs. 75 ± 5 mmHg respectively, \(P = 0.610\)). In keeping with the similar BPs, \(R_{\text{min}}\) was similar in the control and PVD groups (1.6 ± 0.4 vs. 1.8 ± 0.5 mmHg · ml⁻¹ · 100 ml tissue⁻¹ · min⁻¹, \(P = 0.259\)).

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Table 1  Subject characteristics and biochemical data

Comparisons between groups were made using two-tailed t tests for normally-distributed data and Mann–Whitney U tests for non-normally-distributed data (triacylglycerols, AUC glucose and AUC insulin; where AUC is the area under the curve following an oral glucose tolerance test). BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Data are means ± S.D. (range).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensives</th>
<th>Normotensives with PVD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>58 ± 12 (43–78)</td>
<td>56 ± 8 (46–73)</td>
<td>0.588</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.3 ± 2.2 (22.8–29.0)</td>
<td>25.3 ± 2.5 (20.9–29.4)</td>
<td>0.968</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.35 ± 1.23 (4.03–6.98)</td>
<td>6.28 ± 0.67 (4.89–7.37)</td>
<td>0.036</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.14 ± 0.28 (0.85–1.81)</td>
<td>1.07 ± 0.29 (0.62–1.58)</td>
<td>0.588</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.58 ± 1.18 (1.62–5.38)</td>
<td>4.25 ± 0.62 (3.33–5.52)</td>
<td>0.095</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/l)</td>
<td>1.38 ± 0.86 (0.60–3.50)</td>
<td>2.08 ± 1.11 (0.95–4.09)</td>
<td>0.124</td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>4.66 ± 0.69 (3.28–5.77)</td>
<td>5.10 ± 0.51 (4.39–6.33)</td>
<td>0.103</td>
</tr>
<tr>
<td>AUC glucose (mmol · l⁻¹ · 2 h⁻¹)</td>
<td>688 ± 257 (543–889)</td>
<td>923 ± 206 (602–1294)</td>
<td>0.057</td>
</tr>
<tr>
<td>AUC insulin (nmol · l⁻¹ · 2 h⁻¹)</td>
<td>35.3 ± 14.7 (20.9–62.9)</td>
<td>60.0 ± 29.5 (30.9–119.6)</td>
<td>0.030</td>
</tr>
<tr>
<td>Peak insulin (pmol/l)</td>
<td>484 ± 216 (207–855)</td>
<td>763 ± 309 (355–1306)</td>
<td>0.038</td>
</tr>
</tbody>
</table>

Differences in retinal parameters

Bifurcation angles did not differ between controls (79 ± 11°) and those with PVD (81 ± 6°, P = 0.725; Figure 1A). Arteriolar L:D ratios in healthy men (18.5 ± 7.4) tended to be greater than in men with PVD (14.3 ± 3.5, P = 0.077), although this difference was not statistically significant (Figure 1B). In control subjects, junction exponents did not differ from the optimum value (ρ = 0.11 ± 0.63; Figure 1C). In contrast, in men with PVD, junction exponents deviated significantly from optimum (ρ = −0.36 ± 0.23). The optimality parameter differed significantly between the two groups (P = 0.013).

DISCUSSION

Arteriolar bifurcations are abnormal in the retina of normotensive men with PVD, in that junction exponents deviate significantly from optimal values. In contrast, internal arteriolar diameters at bifurcations in matched healthy controls are related so that power losses are minimized and shear stress is uniform. Since both groups had similar BPs and R<sub>min</sub> was similar in both groups it is unlikely that structural changes secondary to hypertension can account for these observations. Indeed retinal arteriolar bifurcation angles and L:D ratios, parameters that are reported to be altered in hypertension [8,10], did not differ significantly between the control and PVD groups in the current study.

There is extensive evidence to suggest that normal arterial vascular networks are optimally designed to minimize power losses and maintain uniform levels of shear stress [1]. Evidence from animal models indicates that the endothelium plays a key role in co-ordinating
such vascular behaviour, largely via production of NO in response to shear stress. In isolated perfused rabbit ear preparations with unimpaired endothelial function, junction exponents remained close to optimum values even when preparations were vasoconstricted by 5-hydroxtryptamine. In contrast, when NO was inhibited, vasoconstriction was associated with marked deviation of junction exponents from optimum values [21]. The concept that endothelial function, and NO in particular, is important in the maintenance of network topography is further supported by two recent in vivo studies of the human retinal circulation. Despite inducing changes in arteriolar diameter, neither hyperoxia nor hypercapnia resulted in an alteration of junction exponents [22]. However, vasoconstriction induced by inhibition of NO synthesis with L-NMMA was associated with an alteration in junction exponents away from the optimum value [16].

Our data are consistent with the presence of abnormal endothelial function in the retina in subjects with PVD and extend previous reports showing evidence of systemic endothelial dysfunction in atherosclerosis [23,24]. Endothelial dysfunction in large arteries appears to be an early finding in atherosclerosis [25], and may be related to the presence of risk factors for vascular disease [26]. Subjects with PVD are known to have a variety of metabolic and hemodynamic abnormalities [13,27]. In the current study, subjects with PVD, despite having normal glucose tolerance, were relatively insulin resistant (demonstrated by higher stimulated insulin concentrations) and had higher total cholesterol levels than the control group. It is therefore possible that the retinal arteriolar defect is a consequence of the metabolic abnormalities observed in subjects with PVD, either via an effect on endothelial function or via structural effects such as changes in vascular smooth muscle cell morphology at bifurcations. It is equally possible, however, that some metabolic differences may be a consequence of abnormalities in microvascular structure and altered blood flow in some territories, e.g. skeletal muscle [28].

In conclusion, we have demonstrated differences in the retinal arteriolar network in a group of men with symptomatic PVD compared with healthy men matched for known confounding factors. This suggests that PVD is associated with abnormal endothelial function in the retinal microvasculature, and suggests the presence of a systemic abnormality of endothelial function in atherosclerosis.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the Medical Research Council (U.K.).

REFERENCES


Received 20 September 2001/13 December 2001; accepted 12 April 2002