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Retinal blood flow and systemic blood pressure in healthy young subjects

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Abstract *Background:* The aim of the present study was to investigate the association between systemic blood pressure and retinal blood flow in healthy young subjects. *Methods:* Three independent study cohorts were included. A cross-sectional study was performed in 420 young male subjects with systolic blood pressure ≤160 mmHg and diastolic blood pressure ≤100 mmHg. Retinal white blood cell flux (n=210) and blood velocity in the central retinal artery (n=210) were measured. In addition, a longitudinal study was performed in 40 young male subjects in whom retinal and systemic haemodynamic parameters were measured thrice within 6 weeks. Retinal white blood cell flux was measured with the blue-field entoptic technique. Blood flow velocity in the central retinal artery was measured by means of colour Doppler imaging. *Results:* Retinal white blood cell flux (r=0.262; P<0.001) and mean flow velocity in the central retinal artery (r=0.174, P=0.010) were significantly associated with mean arterial pressure in the cross-sectional study. In the longitudinal study retinal white blood cell flux and mean flow velocity in the central retinal artery were also correlated with systemic blood pressure. *Conclusions:* Our data indicate a slight but significant increase in retinal blood flow with blood pressure. Whether this is of clinical relevance in eye diseases with altered retinal perfusion, such as diabetic retinopathy, remains to be established.

Introduction

It is well established that the retina has some autoregulatory capacity in response to acute changes in perfusion pressure [2, 11, 14, 15]. However, in its strict sense retinal autoregulation cannot be investigated in human clinical trials, because changes in ocular perfusion pressure cannot be induced without concomitant changes in other vascular beds. In addition, it is currently unknown whether long-term changes in ocular perfusion pressure, as induced for example by systemic hypertension, may induce alterations in retinal blood flow.

Hence, the relation between retinal blood flow and systemic blood pressure is not well established. We therefore investigated the dependence of retinal blood flow on systemic blood pressure in young subjects with normotension or mild untreated hypertension. Two methods were used to assess retinal perfusion in these volunteers. The blue-field entoptic technique was employed to investigate leukocyte movement in perifoveal retinal capillaries. In a separate study population, blood flow velocities in the central retinal artery were measured with colour Doppler imaging.

Materials and methods

Subjects

The study protocols were approved by the local ethics committee and conformed with the ethical standards laid down in the declara-

tion of Helsinki. Three independent study cohorts were included. Four hundred and twenty male non-smoking volunteers between 19 and 40 years of age were studied in protocol A. Retinal white blood cell flux (n=210) and blood velocity in the central retinal artery (n=2 10) were measured. Forty male non-smoking volunteers between 19 and 40 years of age participated in protocol B. All subjects had been drug free for at least 3 weeks at the time of study and did not use any regular medication. The nature of the study was explained, and all subjects gave written consent to participate. Each subject underwent screening that included medical history and physical examination, 12-lead electrocardiography and blood pressure measurement. Inclusion criteria were normal findings in the screening, systolic blood pressure ≤160 mmHg and diastolic blood pressure ≤100 mmHg. Furthermore an ophthalmic examination, including slit-lamp biomicroscopy and indirect funduscopy, was performed. Inclusion criteria were normal ophthalmic findings and a refractive error of less than 3 dioptres in each eye. In all subjects the right eye was studied.

Study design

Protocol A

In 210 subjects retinal white blood cell flux (WBCF) was investigated with the blue-field entoptic technique. In 210 other subjects blood flow velocities in the central retinal artery were measured. All subjects were studied after a resting period of at least 20 min. Stable haemodynamic conditions were ensured by repeated blood pressure measurement. The investigators who performed the ocular haemodynamic measurements were not informed about the blood pressure and pulse rate values obtained in the subjects under study.

Protocol B

In 40 subjects, who did not participate in protocol A, retinal WBCF and blood flow velocities in the central retinal artery were measured on three different days within a period of 6 weeks. All measurements were performed between 8:00 and 12:00.

Study methods

Systolic, diastolic and mean blood pressures (SBP, DBP, MAP) were measured on the upper arm using an automated oscillometric device (HP-CMS patient monitor, Hewlett Packard, Palo Alto, Calif., USA). Pulse rate was automatically recorded from a finger pulse-oximetric device (HP-CMS patient monitor).

Retinal WBCF was assessed with the blue-field entoptic technique. This non-invasive method is described in detail by Riva and Petrig [12]. The blue-field entoptic phenomenon can be seen best by looking into a blue light with a narrow optical spectrum at a wavelength of approximately 430 nm. Under these conditions tiny corpuscles can be seen flying around swiftly in an area with a radius of 10–15 degrees of arc centred at the fovea. Most likely this phenomenon is caused by the fact that red but not white blood cells absorb short-wavelength light. Thus, the passage of a white blood cell is perceived as a flying corpuscle. For determination of retinal haemodynamic parameters, a simulated particle field is shown to the subjects under study. By comparison with their own entoptic observation subjects can adjust the number of white blood cells (WBCD) and the mean flow velocity (WBCV). Retinal WBCF is then calculated as the product of WBCD and WBCV. All subjects had a training session which consisted of at least five matching tests. Only subjects who were able to provide reproducible values with a SD of less than 20% were included. For study purposes each measurement consisted of at least five matching tests, and the means of velocity and density were calculated. Again, only values with a SD of less than 20% were accepted as accurate. Subjects who did not reach a SD lower than 20% at any matching trial during the study (protocol B) were excluded from the trial.

Mean blood flow velocity (MFV), peak systolic flow velocity (PSV), and end-diastolic flow velocity (EDV) were determined in the right central retinal artery (CRA) with colour Doppler imaging as described in detail elsewhere [7]. MFV was measured manually as time mean of the spectral outline. Measurements were performed with a 7.5-MHz probe (CFM 750, Vingmed Sound, Horten, Norway). The resistive index (RI) in the CRA was calculated as RI=(PSV-EDV)/(PSV). All parameters were determined as mean values over at least three cardiac cycles.

Data analysis

All statistical analyses were done using the Statistica software package (Release 4.5, StatSoft, Tulsa, Okla., USA). The association between ocular and systemic haemodynamic parameters as obtained in protocol A was investigated using linear correlation analysis. The three values obtained for each subject in protocol B were averaged. A deviation from this mean was then calculated for each value. For example the individual deviation in mean arterial pressure $DMAP_i$ was calculated as $MAP_i–MAP_{mean}$. The association between these deviations from the mean were investigated using linear correlation analysis. Data are presented as means ± SD. A two-tailed P <0.05 was considered the level of significance.

Results

Protocol A

Subjects' characteristics are shown in Table 1. Data are presented separately for the subjects participating in the blue-field measurements and for the subjects participating in the colour Doppler experiments. The age of the subjects was comparable between the two groups.

Table 1 Subjects' characteristics (protocol A): 210 subjects underwent measurement of retinal white blood cell flux with the blue-field entoptic technique; 210 other subjects underwent colour Doppler imaging of the central retinal artery. Data are presented as means ± SD

Blue-field entoptic technique (n=210)	
Age (years)	27.7±4.8
Systolic blood pressure (mmHg)	131.9±14.1
Diastolic blood pressure (mmHg)	70.1±14.1
Mean arterial pressure (mmHg)	90.8±13.1
Pulse rate (beats/min)	64.5±8.1
White blood cell velocity (relative units)	1.24±0.29
White blood cell density (relative units)	112.7±29.5
White blood cell flux (relative units)	136.7±39.3
Colour Doppler imaging (n=210)	
Age (years)	27.8±5.1
Systolic blood pressure (mmHg)	130.9±13.7
Diastolic blood pressure (mmHg)	68.1±12.7
Mean arterial pressure (mmHg)	89.0±12.6
Pulse rate (beats/min)	63.2±7.8
Mean flow velocity (cm/s)	6.4±0.6
Resistive index	0.72±0.04

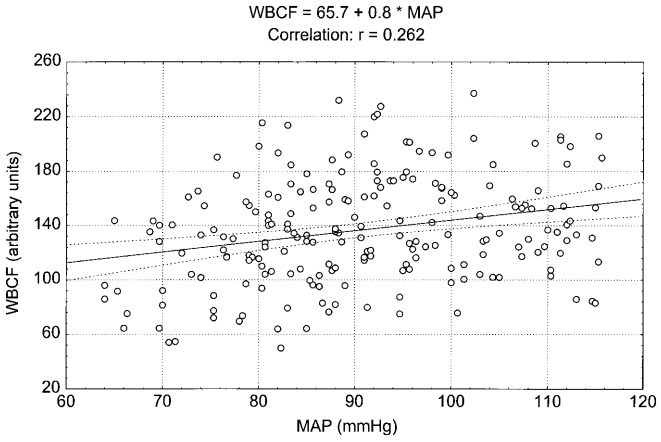

Fig. 1 Association between retinal white blood cell flux (*WBCF*) and mean arterial pressure (*MAP*). The regression line and the 95% confidence interval are shown (*n*=210)

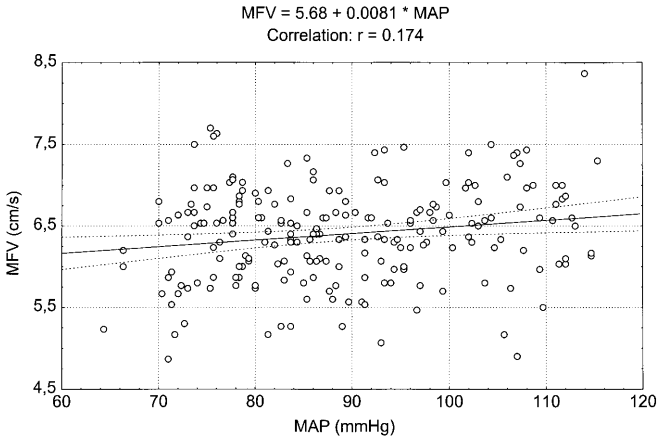

Fig. 2 Association between mean flow velocity (*MFV*) and mean arterial pressure (*MAP*). The regression line and the 95% confidence interval are shown (*n*=210)

We observed a significant direct correlation between WBCF and MAP (Fig. 1) and between WBCV and MAP (each $P<0.001$). The correlation coefficient between WBCD and MAP was considerably lower ($P=0.033$). The correlation coefficients between retinal WBCF ($r=0.34$; $P<0.001$), retinal WBCV ($r=0.37$; $P<0.001$), retinal WBCD ($r=0.16$; $P=0.023$) and SBP were in the same range. Correlation coefficients between parameters of retinal leukocyte movement and DBP were only slightly smaller (WBCF: $r=0.31$; $P<0.001$; WBCV: $r=0.34$; $P<0.001$; WBCD: $r=0.14$; $P=0.047$). By contrast, none of the parameters assessed with the blue-field technique was dependent on pulse rate.

Mean flow velocity in the central retinal artery was also dependent on MAP ($P=0.010$; Fig. 2), SBP ($r=0.16$; $P=0.018$) and DBP ($r=0.17$; $P=0.012$), but correlation coefficients were smaller than those observed for retinal white blood cell flux and blood pressure. RI and blood pressure were negatively correlated (MAP: $r=–0.19$; $P=0.006$; SBP: $r=–0.21$; $P=0.002$; and DBP $r=–0.21$; $P=0.002$), but again correlation coefficients were small. Neither MFV nor RI was associated with pulse rate.

Protocol B

Subjects' characteristics are shown in Table 2. We observed a significant direct correlation between DWBCF and DMAP ($r=0.256$; $P=0.005$; Fig. 3) and DWBCV and DMAP ($r=0.336$; $P<0.001$). The correlation between retinal DWBCD and DMAP was not significant. DMFV in the central retinal artery was dependent on DMAP ($r=0.204$; $P=0.026$; Fig. 4), whereas DRI and DMAP were not correlated. None of the retinal haemodynamic variables were dependent on pulse rate. There was a significant positive correlation between DMFV and DWBCV ($r=0.23$; $P=0.01$).

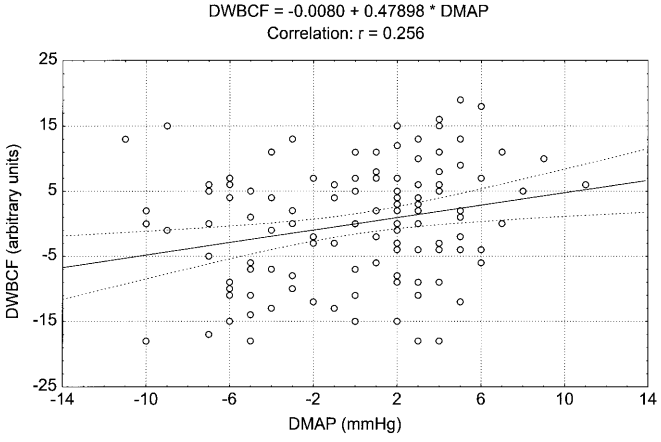

Fig. 3 Association between individual deviations in retinal white blood cell flux (*DWBCF*) and mean arterial pressure (*DMAP*). The regression line and the 95% confidence interval are shown (*n*=40)

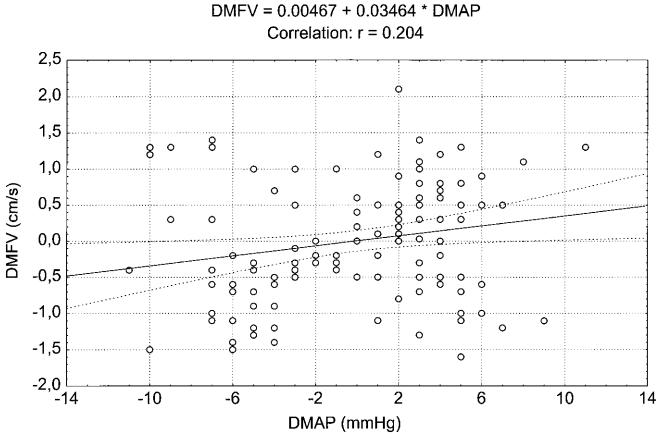

Fig. 4 Association between individual deviations in mean flow velocity (*DMFV*) and mean arterial pressure (*DMAP*). The regression line and the 95% confidence interval is shown (*n*=40)

Table 2 Subjects' characteristics (protocol B). Data are presented as means ± SD (*n*=40)

Age (years)	24.2±3.9
Systolic blood pressure (mmHg)	124.6±10.0
Diastolic blood pressure (mmHg)	61.7±9.4
Mean arterial pressure (mmHg)	82.4±9.8
Pulse rate (beats/min)	59.9±8.3
White blood cell velocity (relative units)	1.22±0.41
White blood cell density (relative units)	116.3±30.7
White blood cell flux (relative units)	138.9±42.6
Mean flow velocity (cm/s)	6.1±0.7
Resistive index	0.73±0.03

Discussion

In the present study in subjects with normal or slightly elevated blood pressure we observed an association between retinal WBCF and MAP. Our data also indicate that perifoveal leukocyte velocity is dependent on MAP, whereas retinal leukocyte density shows only little blood pressure dependence. The hypothesis that retinal blood flow is dependent on blood pressure is further supported by our observation that MFV in the central retinal artery is correlated with MAP.

Generally the dependence of retinal haemodynamic variables on blood pressure in the present study was small. Our data indicate that WBCF increases between 3.6% (protocol B) and 5.6% (protocol A**)** per 10-mmHg increase in MAP. Accordingly, MFV increases between 1.3% (protocol A) and 5.8% (protocol B) per 10-mmHg increase in MAP. Whether this is of clinical relevance remains to be shown. However, even a small hypertension-induced elevation of retinal blood flow may increase the risk of onset or progression of ocular vascular disease [6].

In the present study we focused on young male subjects because retinal WBCF decreases with age [4]. Patients with any systemic or ocular pathology likely to influence retinal blood flow were excluded from the present study. In addition, we included only subjects who did not receive any regular medication. We considered this important because any type of systemic vasoactive medication may influence retinal blood flow [5, 8].

Measurement of retinal blood flow with the blue-field entoptic technique is a subjective method. Therefore, only subjects whose results displayed little variability were included in the present study. There is evidence from previous trials that reproducible data can be obtained with this system if subjects are carefully selected [3, 9]. However, this technique assesses leukocyte movement, and it is not entirely clear whether results can be directly applied to volumetric blood flow in the retina. There is some evidence that leukocytes move slower in retinal capillaries than erythrocytes [1]. Whether erythrocyte movement is differentially affected by hypertension in the retina remains to be established. This may, in principle, be investigated using combined laser Doppler velocimetry and vessel size determination [13]. For calculation of total blood flow with this technique it is necessary to measure all veins or arteries entering the optic nerve. This procedure is much more time-consuming than measurements with the blue-field entoptic technique and was therefore not employed in the present study.

In addition, it is widely accepted that leukocyte velocity can be adequately assessed with the blue-field technique, but it is unclear whether the number of leukocytes as perceived in the blue light is an adequate measure of vascular volume. To overcome the problem of inter-individual variability we performed a study in which subjects were measured thrice within 6 weeks (protocol B). The results of this longitudinal study are, however, comparable to those with the cross-sectional approach.

Colour Doppler imaging can be applied to measure blood flow velocities in retrobulbar vessels, but no quantitative information on vessel diameters can be obtained. Hence, this method does not allow for quantification of blood flow through the central retinal artery. Nevertheless, we observed a significant association between SBP and MFV in the central retinal artery, which may indicate increased blood flow through this artery with increasing blood pressure. Again, the results in protocol B are in good agreement with those observed in the cross-sectional study.

The present study's findings regarding RI are difficult to interpret. We have previously shown that RI is not an adequate measure of vascular resistance in the retina [10]. Hence, it seems very unlikely that the correlation between RI and blood pressure observed in the present study contains any information on the dependence of retinal perfusion on ocular perfusion pressure.

Concluding, our data indicate a slight but significant increase in retinal blood flow with increasing blood pressure. Whether this is of clinical relevance in eye diseases with altered retinal perfusion, such as diabetic retinopathy, remains to be established.

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