Abnormal Systemic and Ocular Vascular Response to Temperature Provocation in Primary Open-Angle Glaucoma Patients: A Case for Autonomic Failure?

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PURPOSE. To assess systemic and ocular vascular reactivity in response to warm and cold provocation in untreated patients with primary open-angle glaucoma and normal control subjects.

METHODS. Twenty-four patients with primary open-angle glaucoma and 22 normal control subjects were subjected to a modified cold pressor test involving immersion of the right hand in $40°C$ warm water followed by $4°C$ cold water exposure, and finger and ocular blood flow were assessed by means of peripheral laser Doppler flowmetry and retinal flowmetry, respectively. Finger and body temperature as well as intraocular pressure, systemic blood pressure, systemic pulse pressure, heart rate, and ocular perfusion pressure were also monitored.

RESULTS. The patients with glaucoma demonstrated an increase in diastolic blood pressure ($P = 0.023$), heart rate ($P = 0.010$), and mean ocular perfusion pressure ($P = 0.039$) during immersion of the tested hand in $40°C$ water. During cold provocation, the patients demonstrated a significant decrease in finger ($P = 0.0003$) and ocular blood flow (the parameter velocity measured at the temporal neuroretinal rim area; $P = 0.021$). Normal subjects did not demonstrate any blood flow or finger temperature changes during immersion of the tested hand in $40°C$ water ($P > 0.05$); however, they exhibited increases in systolic blood pressure ($P = 0.034$) and pulse pressure ($P = 0.0009$) and a decrease in finger blood flow ($P = 0.0001$) during cold provocation. In normal subjects, the ocular blood flow was unchanged during high- and low-temperature challenge.

CONCLUSIONS. Cold provocation elicits a different blood pressure, and ocular blood flow response in patients with primary open-angle glaucoma compared with control subjects. These findings suggest a systemic autonomic failure and ocular vascular dysregulation in POAG patients. (*Invest Ophthalmol Vis Sci.* 2004;45:3546–3554) DOI:10.1167/iovs.04-0290

In the search for risk factors involved in the pathogenesis of glaucoma, vascular risk factors are the most extensively studied variables other than increased intraocular pressure (IOP). In particular, a vascular dysregulation leading to local vasospasm or impaired autoregulation has been advocated as a possible contributing factor in the etiology of primary open-angle glaucoma (POAG).[1–5]

Among the factors that may influence blood flow physiology in the human body, variables such as blood pressure (BP) and heart rate (HR) are regulated by the autonomic nervous system (ANS).[6] It follows that any autonomic disturbances could have severe hemodynamic consequences. POAG itself has also been directly linked to ANS dysfunctions. Systemic parasympathetic and sympathetic neuropathies have been reported in patients with POAG and in those with normal tension glaucoma (NTG),[7–10] and it has already been suggested that disturbed autonomic activity may play a role in the pathogenesis of the glaucomatous damage. Nevertheless, further research is needed to prove a direct relationship between glaucoma and ANS failure.

Besides other autonomic function tests, such as the measurement of heart rate variability (HRV), Valsalva maneuver,[11] pressor drug infusion,[12] lower body negative pressure, neck chamber pressure, head-upright tilt,[13,14] and handgrip stress,[15] cold stimulation is also a well-established provocation test used in detecting abnormal vascular reactivity in patients with autonomic failure.[16] The so-called cold pressor test (CPT) generally involves immersion of one hand in cold water with different temperatures (ranging from 0–15°C) and is a well-known stress test that induces a reproducible sympathetic activation in both normal subjects and subjects with arterial hypertension.[17,18] Some of the physiological responses to this test include arteriolar vasoconstriction, increased BP and plasma catecholamines, and reduction in peripheral blood flow.[19] A well-known variation of the CPT involves immersion of the right upper limb in 40°C warm water followed by cooling of the hand.[20–22]

Although cold provocation has a dramatic effect on the systemic vasculature, little is known about its consequences on ocular blood flow. Rojanapongpun and Drance[21] and Nicolela et al.[23] failed to report any blood flow changes as a response to cold stimulation in the ophthalmic artery and retinal circulation of patients with POAG. Nevertheless, in patients with vascular dysregulation manifested by peripheral vasospasm, visual field (VF) defects have been reported after cold provocation[1,24]; it has been suggested that this effect could be the result of an ocular circulatory insufficiency. Because vascular dysregulation has been demonstrated to play a role in the pathogenesis of glaucomatous neuropathy,[1–5] we can hypothesize that cold stimulation could result in an abnormal hemodynamic response in ocular vascular beds. In the present study we sought to assess the systemic vascular reactivity as well as retinal and optic nerve head ocular blood flow (OBF) changes in response to warm and cold provocation in newly diagnosed and untreated POAG patients and normal control subjects.

SUBJECTS AND METHODS

Subjects

Successive newly diagnosed and previously untreated POAG patients attending the Fast Track Glaucoma Clinic at the Heartlands and Solihull NHS Trust (Birmingham, UK) were recruited for the study. Patients underwent diurnal IOP phasing and POAG was diagnosed in the

presence of IOP measurements greater than 21 mm Hg by applanation tonometry (with at least two of the measurements being higher than 24 mm Hg), glaucomatous cupping of the optic disc on funduscopic examination, normal open anterior chamber angles by gonioscopy, and repeatable VF defects consistent with a diagnosis of glaucoma, using program 24-2 of the Humphrey Field Analyzer (HFA; Carl Zeiss Meditec, Dublin, CA). Patients with closed iridocorneal angles; evidence of secondary glaucoma; pseudoexfoliation; pigmentary dispersion; a history of intraocular surgery; any form of retinal or neuro-ophthalmologic disease that could result in visual field defects; or a history of chronic systemic disease, including diabetes mellitus, or occlusive vascular disorders were not included in the study. The normal control group was recruited from patients' spouses and included only those subjects who had never had any significant systemic or ocular disease, including chronic cardiovascular diseases and glaucoma. Further exclusion criteria for both groups were refractive errors higher than $+2$ or -3 D, a medical history of Raynaud's phenomenon, neurologic or metabolic diseases, and chronic intake of vasoactive drugs. Before the study, ethical approval was obtained from the local medical ethics committee, and written informed consent was received from all subjects. The study was designed and conducted in accordance with the tenets of Declaration of Helsinki.

Experimental Setup

All subjects were tested in the morning, between 8 and 10 AM, in a room with a constant temperature, ranging between 21°C and 23°C (mean $\pm$ SD: 21.31 $\pm$ 1.15°C). Subjects were instructed to avoid ingesting any stimulants, including coffee, tea, or cigarette smoke on the morning of the study. In preparation for the test, each subject rested in a sitting position for 15 to 20 minutes in a quiet room to achieve sufficient mental and physical calm. The experiment comprised five steps: (1) a 10-minute baseline period; (2) a 5-minute immersion of the right hand up to the wrist in 40°C water; (3) a 5- to 10-minute recovery period with the hand wrapped in a towel to avoid a cooling evaporation effect on hand temperature; (4) immersion of the right hand in ice water (4°C) for 1 minute; and (5) a 15-minute final recovery period, with the hand wrapped in a towel. During cold provocation, subjects were instructed to signal verbally when the water "begins to produce pain." The cold provocation was interrupted at the moment when pain occurred.

For each subject, peripheral finger blood flow (FBF) by means of peripheral laser Doppler flowmetry and finger temperature (FT) in the left hand were measured continuously during the experiment. In addition, IOP, tympanic temperature (TT), BP, heart rate (HR), and OBF parameters (volume, flow, and velocity) in the temporal areas of the neuroretinal rim (NRR) and peripapillary retina were assessed with the Heidelberg Retina Flowmeter (HRF; Heidelberg Engineering, GmbH, Heidelberg, Germany) at the end of each step of the experiment. The techniques are described later.

Peripheral Blood Flow and Peripheral and Central Temperature

Skin perfusion was assessed by means of a blood perfusion monitor (Periflux System 5001; Perimed, Stockholm, Sweden) that uses a low-power solid-state laser diode as a coherent source of light. The optical output power at the probe end is less than 2 mW and the wavelength is typically 780 nm. The apparatus and the technique have been described in detail by Nilsson et al.[25,26] In summary, this device measures the microvascular perfusion using a laser beam that, when it strikes an object in motion (such as red blood cells), undergoes a change in light frequency known as the Doppler shift. All measurements were performed with an upper frequency limit of 32 kHz, an output circuit time constant of 0.2 second, and a gain factor of $\times 3$. For measurements of FBF, the probe was attached with an adhesive tape to the pulp of the second finger of the left hand. The hand of the seated subjects rested comfortably on a table, at heart level. The probe of the

perfusion monitor was placed to avoid any undue pressure and kept in this position, so that the signal level indicator remained green. The FBF was then recorded continuously on a computer.

The FT was measured using a copper-constantan sensor (ML309 Thermistor Pod) connected to a signal acquisition unit (PowerLab/4SP unit; A&D Instruments Ltd., Oxford, UK). This type of sensor is isolated from the environment with neoprene and is suitable for skin temperature measurements in the range of 5° to 45°C. The sensor was fixed with a thin layer of adhesive tape on the dorsal aspect of the second finger of the left hand near the area of peripheral blood flow measurement. The signal acquisition unit was connected to a computer, permitting continuous recording of peripheral temperature during the experiment.

FBF and FT were measured continuously during the experiment, and average levels were calculated for each step of the experiment.

A noncontact infrared ear thermometer (ThermoTek One Second Tympanic Thermometer, P.M.S. Instruments Ltd., Maidenhead, UK) was used to measure body temperature. Within 1 second, the noncontact infrared ear thermometer measures tympanic temperature, which represents core body temperature. This device can evaluate temperatures between 20°C and 42.2°C and works by detecting infrared energy emitted from the deep auditory canal and tympanic membrane. Infrared ear thermometry findings are reproducible and provide a relatively close estimate of pulmonary artery core temperature.[27,28]

Systemic BP and HR

Clinical BP and HR were obtained with an automatic BP monitor (UA-779; A&D Instruments Ltd.). This device measures BP automatically, using the same principle as a conventional mercury sphygmomanometer, with a cuff and microphone. During the BP measurements, the cuff was placed around the upper right arm approximately at heart level. The systolic BP (SBP) and diastolic BP (DBP) were measured three times (1 minute apart), and the average readings for SBP and DBP were then used to calculate the mean BP (MBP) using the formula: MBP = $\frac{2}{3}$DBP + $\frac{1}{3}$SBP. Pulse pressure (PP) was calculated as: PP = SBP − DBP.

Intraocular Pressure

Because the design of the experiment did not allow the patient to be seated at a slit lamp, IOP was measured by a handheld tonometer (Tono-Pen XL; Mentor Ophthalmics Inc., Norwell, MA) after application of 1 drop of 0.4% benoxinate hydrochloride. The IOP and MBP measurements were used to calculate the mean ocular perfusion pressure (MOPP): MOPP = $\frac{2}{3}$(MBP − IOP).

Ocular Blood Flow

In the glaucoma patients, measurements were performed in the eye with the most advanced disease (as determined by ophthalmoscopy and VF analysis). In normal subjects, the test eye was randomly selected. Perfusion parameters in the superior temporal regions of the neuroretinal rim and peripapillary retina were measured for each step of the experiment, using the HRF system. The HRF principle has been described in detail.[29,30] In short, this noninvasive technique combines laser Doppler flowmetry using an infrared laser diode with wavelength of 780 nm and a power of 200 μW, with the confocal scanning laser principle. The HRF scanning system enables the measurement of back-scattered intensity for a retinal area of approximately 2.7 $\times$ 0.7 mm (10° $\times$ 2.5° scan), mapped with a resolution of 256 points $\times$ 64 lines.[29] The total time for scanning and data acquisition is 2.048 seconds. Each of the 64 horizontal lines is scanned 128 times with a line repetition rate of 4000 Hz. For each retinal point in the scanned area, the back-scattered intensity can be measured as a function of time to produce an intensity–time curve. The signal undergoes a fast-Fourier transformation, which results in a power spectrum for each retinal point. This spectrum is used to calculate the variables volume, flow, and velocity for each retinal point, expressed as the mean and SD of

the measurements and displayed in arbitrary units. In this study, capillary volume, flow, and velocity were recorded for all images with a 10 × 10-pixel measurement frame, avoiding areas with large vessels.

To collect HRF data, we needed to define the border between the cup and the rim as well as the disc margin. For this purpose, we used a confocal laser retinal tomograph, the Heidelberg Retina Tomograph (HRT; Heidelberg Engineering). The method used by this device has been described in detail.[31–37] In short, this instrument is a confocal scanning laser (670 nm) ophthalmoscope that obtains topographic images as a series of 32 optical sections perpendicular to the optical axis of the eye at consecutive focal planes. The image consists of 256 × 256 pixels, and at each pixel the retinal height is determined. Based on Gullstrand's model eye, magnification error is corrected using the patient's keratometric readings. In eligible eyes, after keratometric values were measured, one topographic image was obtained through a dilated pupil (1% tropicamide; Alcon, Fort Worth, TX). This is in contrast to the standard protocol of three images.[38] However, because of the principal of regression to the mean, limiting the number of acquired topographic images to one image per eye is not expected to alter the results in a screening procedure such as the one used in this study.[39]

During the imaging procedure, the subjects fixated on a distant target with the fellow (nontest) eye. For the topographic images, the optic disc margin was outlined along the inner margin of the scleral ring by an experienced investigator (DG). With a transparency overlaid on the monitor's screen, anatomic landmarks (blood vessels, optic nerve contour, and contour of the cup) were drawn manually.[40]

Three to five HRF measurements were recorded by the same experienced investigator (DG) at the end of each step of the experiment. Only the best-quality images were selected for statistical purposes. Quality inclusion criteria were lack of movement during the recording and good illumination of the image. Images were included if the direct current (DC) values were between 70 and 200.[41,42] Measurements were performed, first on one location on the temporal NRR (TNRR) by a central alignment technique,[43] and then on one location

on the temporal retina (TR) while avoiding the large vessels. The transparency obtained on HRT measurements was overlaid on the HRF images of the same subject, ensuring a near-perfect adjustment. A 10° frame was then drawn on the area of interest. The same transparency was used for all subsequent images obtained from the same subject during the experiment, to ensure that the exact location of the measurement frames was reproduced.

Statistical Analysis

Data are expressed as the mean ± SD. Differences between the groups at baseline were calculated using Student's *t*-test for unpaired variables. Warm- and cold-induced changes were calculated using repeated-measurements ANOVA (re-ANOVA) followed by post hoc analysis using the Tukey honest significant difference (HSD) test. Peripheral blood flow and temperature were corrected for the influence of the ambiance and central temperatures. OBF was corrected for changes in MOPP in an analysis of covariance (ANCOVA). This approach was performed in a multivariate analysis, to ensure independence of the change at each step from the other changes in re-ANOVA. Pearson's linear correlation factor was used to study the relationship between changes in peripheral and ocular blood flow parameters. Holm's sequentially rejective method was used to correct for multiple comparisons.[44] Statistical analyses were performed on computer (Statistica, ver. 6.0 for Windows; StatSoft, Tulsa, OK). Statistical significance was defined as $P < 0.05$.

RESULTS

Thirty-two glaucoma patients (12 men and 20 women) and 34 control subjects (20 men and 14 women) participated in the experiment. In one case (a normal subject, male) the test had to be stopped after 30 seconds of cold provocation due to pain sensation, and the subject was excluded from the experiment. Careful image analysis and subsequent rejection of those sub-

TABLE 1. Systemic BP, HR, IOP, and Central Temperature in the Study Groups

Parameter	Glaucoma (24 Patients)	Normal (22 Subjects)	*P*	*P**
At baseline				
IOP (mm Hg)	23.63 ± 4.89	17.95 ± 3.74	0.00005	0.0004
SBP (mm Hg)	134.46 ± 18.90	127.95 ± 21.95	0.28600	NS
DBP (mm Hg)	76.04 ± 11.80	77.73 ± 9.83	0.60300	NS
MBP (mm Hg)	95.51 ± 13.29	94.47 ± 13.01	0.78900	NS
PP (mm Hg)	58.42 ± 12.61	50.23 ± 15.84	0.05800	NS
MOPP (mm Hg)	39.63 ± 8.56	44.30 ± 9.92	0.09400	NS
HR (beats/min)	66.35 ± 12.06	68.86 ± 9.09	0.43500	NS
TT (°C)	35.59 ± 0.62	35.65 ± 0.48	0.70700	NS
After warm stimulation				
IOP (mm Hg)	23.42 ± 4.99	17.68 ± 3.27	0.00004	0.0003
SBP (mm Hg)	135.67 ± 24.40	126.77 ± 21.80	0.20000	NS
DBP (mm Hg)	81.08 ± 16.50	79.68 ± 12.69	0.75000	NS
MBP (mm Hg)	99.28 ± 18.55	95.38 ± 15.32	0.44400	NS
MOPP (mm Hg)	42.77 ± 11.22	45.90 ± 11.27	0.34500	NS
PP (mm Hg)	54.58 ± 12.68	47.09 ± 11.79	0.04400	NS
HR (beats/min)	69.29 ± 12.53	69.59 ± 8.82	0.92600	NS
TT (°C)	35.57 ± 0.60	35.67 ± 0.49	0.71000	NS
After cold stimulation				
IOP (mm Hg)	23.96 ± 4.63	18.27 ± 3.72	0.00004	0.0003
SBP (mm Hg)	134.21 ± 18.55	133.04 ± 17.26	0.82700	NS
DBP (mm Hg)	78.33 ± 13.02	78.77 ± 10.40	0.90100	NS
MBP (mm Hg)	96.96 ± 13.54	96.86 ± 11.89	0.98000	NS
MOPP (mm Hg)	40.68 ± 8.58	46.30 ± 9.25	0.03800	NS
PP (mm Hg)	55.88 ± 14.16	54.27 ± 11.62	0.67800	NS
HR (beats/min)	66.52 ± 13.63	67.54 ± 7.63	0.76200	NS
TT (°C)	35.53 ± 0.70	35.64 ± 0.40	0.85800	NS

* Corrected by means of Holm's sequentially rejective method.

TABLE 2. Peripheral Temperature and Peripheral and Ocular Blood Flow Parameters

Parameter	Glaucoma (24 patients)	Normal (22 subjects)	*P*	*P**
At baseline				
FT (°C)	32.26 ± 3.35	34.27 ± 2.66	0.030	NS
FBF (AU)	218.70 ± 129.42	297.90 ± 144.16	0.056	NS
Volume TNRR (AU)	13.44 ± 4.08	12.81 ± 2.69	0.565	NS
Flow TNRR (AU)	236.43 ± 86.97	212.83 ± 52.18	0.317	NS
Velocity TNRR (AU)	0.76 ± 0.38	0.76 ± 0.18	0.983	NS
Volume TR (AU)	16.55 ± 3.74	16.90 ± 2.66	0.730	NS
Flow TR (AU)	292.58 ± 88.72	317.97 ± 79.52	0.339	NS
Velocity TR (AU)	1.04 ± 0.30	1.14 ± 0.27	0.312	NS
After warm stimulation				
FT (°C)	32.81 ± 3.41	34.48 ± 2.59	0.070	NS
FBF (AU)	211.46 ± 124.60	263.72 ± 142.78	0.192	NS
Volume TNRR (AU)	13.04 ± 4.37	11.90 ± 2.98	0.368	NS
Flow TNRR (AU)	226.97 ± 88.74	205.46 ± 42.12	0.367	NS
Velocity TNRR (AU)	0.80 ± 0.30	0.74 ± 0.15	0.480	NS
Volume TR (AU)	17.00 ± 3.68	16.87 ± 3.69	0.912	NS
Flow TR (AU)	294.43 ± 83.60	318.56 ± 67.83	0.330	NS
Velocity TR (AU)	1.04 ± 0.29	1.14 ± 0.23	0.265	NS
After cold stimulation				
FT (°C)	32.77 ± 3.48	34.25 ± 2.60	0.112	NS
FBF (AU)	131.15 ± 96.10	151.44.90 ± 119.31	0.527	NS
Volume TNRR (AU)	13.29 ± 4.71	12.05 ± 3.35	0.383	NS
Flow TNRR (AU)	209.11 ± 78.09	223.64 ± 57.36	0.533	NS
Velocity TNRR (AU)	0.73 ± 0.24	0.81 ± 0.22	0.349	NS
Volume TR (AU)	14.82 ± 4.16	16.76 ± 3.07	0.125	NS
Flow TR (AU)	280.71 ± 90.71	300.01 ± 79.60	0.504	NS
Velocity TR (AU)	1.00 ± 0.32	1.08 ± 0.27	0.547	NS

* Corrected by means of Holm's sequentially rejective method.

jects who exhibited poor HRF image quality resulted in further exclusion of eight glaucoma patients (3 men and 5 women) and 11 control subjects (6 men and 5 women). Finally, 24 glaucoma patients (9 men and 15 women, mean age ± SD: 68.38 ± 11.92 years) and 22 normal subjects (13 men and 9 women, mean age ± SD: 62.59 ± 9.43 years) were included in the statistical analysis.

Baseline Data

There was no significant difference in age between study groups ($P > 0.05$). Moreover, the number of men and women included in the study groups were similar ($P > 0.05$, χ^2 test). Table 1 shows the systemic hemodynamic parameters (BP, PP, MOPP, HR), as well as IOP, and TT for both study groups. Peripheral and ocular blood flow parameters and FT are summarized in Table 2. The IOP was significantly higher ($P = 0.0004$) in the untreated glaucoma patients than in normal subjects. There were no significant differences between study groups with regard to systemic BP, PP, MOPP, HR, and central temperature ($P > 0.05$, Table 1). Moreover, the study groups were also comparable with regard to FT, FBF, and OBF parameters (flow, velocity, volume; $P > 0.05$, Table 2).

Warm Stimulation

Normal Subjects. In normal subjects, warm stimulation did not have any effect on any systemic or ocular measured parameters when compared with baseline ($P > 0.05$).

Glaucoma Patients. Warm stimulation resulted in a significant increase in DBP (mean change ± SD: 6.56 ± 13.40, $P = 0.023$), HR, (mean change ± SD: 5.40 ± 8.06, $P = 0.010$), and MOPP (mean change ± SD: 6.68 ± 19.72, $P = 0.039$) compared with baseline (Fig. 1). OBF parameters, however, remained unchanged after immersion of the right hand in 40°C

warm water ($P > 0.05$, after correcting for the influence of MOPP).

Intergroup Differences. Intergroup differences in the measured systemic, peripheral, and ocular parameters after warm stimulation are listed in Tables 1 and 2. The only statistically significant difference between groups was in the level of IOP ($P = 0.0005$). The changes between baseline and warm stimulation are shown in Tables 3 and 4. There were no statistically significant differences between the two study groups ($P > 0.05$).

Cold Stimulation

Normal Subjects. In normal subjects, immersion of the right hand in 4°C cold water for 1 minute resulted in a statistically significant increase in SBP (mean change ± SD: 4.65 ± 2.53, $P = 0.034$) and PP (mean change ± SD: 11.58 ± 16.58, $P = 0.0009$) compared with baseline (Fig. 1). The cold provocation also resulted in a significant decrease in FBF compared with baseline (mean change ± SD: −50.86 ± 26.01, $P = 0.0001$, Fig. 2). In normal subjects, however, cold stimulation did not influence the OBF parameters.

Glaucoma Patients. IOP, systemic BP, PP, MOPP, and HR were not significantly modified by cold stimulation; however, after correcting for the influence of room and body temperature, there was a significant decrease in FBF (mean change ± SD: −29.95 ± 44.67, $P = 0.0003$, Fig. 2) compared with baseline. In the glaucoma group, cold stimulation determined a significant decrease in the parameter velocity (mean change ± SD: 15.55 ± 22.67, $P = 0.021$, Fig. 3) measured at the TNRR level. These changes were significant after correction for the influence of MOPP on these parameters.

Intergroup Differences. Intergroup differences in the measured systemic, peripheral, and ocular parameters after immersion of the right hand in 4°C cold water for 1 minute are
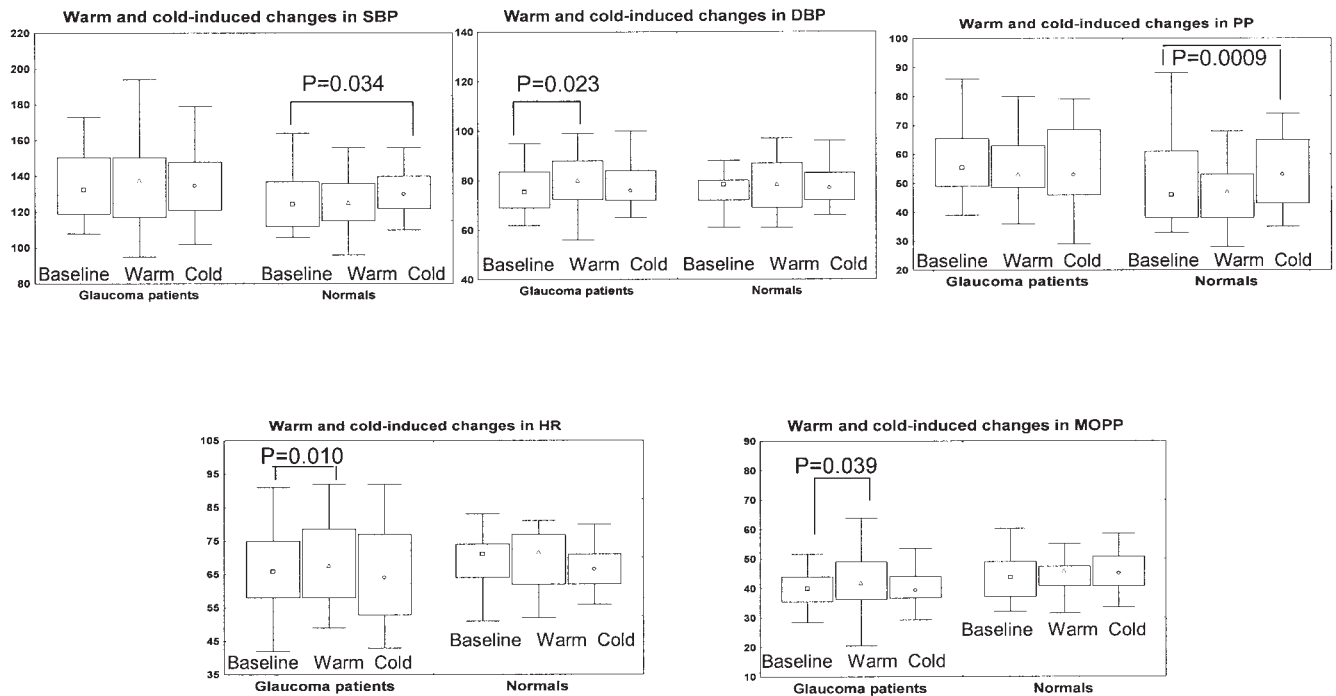
FIGURE 1.   Warm- and cold-induced changes in systemic blood pressure, heart rate, and mean ocular perfusion pressure in glaucoma patients and normal control subjects.

listed in Tables 1 and 2. The only statistically significant difference between groups was in IOP ($P = 0.0003$).

Intergroup differences in cold-induced changes (percentage of baseline values) are listed in Tables 3 and 4. These changes were comparable between study groups for all the measured parameters ($P > 0.05$) with the exception of PP. Normal subjects exhibited higher PP changes from baseline values than glaucoma during cold provocation ($P = 0.021$).

The percent change in FBF did not correlate with changes in the measured ocular blood flow parameters for the same stage of the experiment in either of experimental groups ($P > 0.05$).

DISCUSSION

The present study assessed the response of BP as well as peripheral and ocular blood flow to warm and cold provocation. Our results disclosed a difference in the systemic and ocular vascular response to these stimuli between untreated POAG patients and otherwise healthy age-matched control

subjects. Whereas DBP, HR, and MOPP increased during immersion of the test hand in 40°C water in POAG patients, in control subjects, SBP and PP increased markedly during cold provocation. Moreover, in glaucoma patients, cold stimulation did not affect systemic BP; however, it resulted in a significant reduction in blood velocity at the TNRR level. No OBF changes were observed in normal subjects after cold provocation. Cold provocation resulted in a similar reduction in FBF in both study groups.

The CPT is a well-known and widely used provocation test able to determine the integrity of the ANS function. Similar tests include the Valsalva maneuver,[11] pressor drug infusion,[12] lower body negative pressure, neck chamber pressure, head-upright tilt,[13,14] and handgrip stress.[15] The initial normal response to CPT consists of cutaneous vasoconstriction and an increase in systemic BP. A blunt BP response to CPT may indicate autonomic dysregulation.[19,45] In our study, glaucoma patients exhibited high DBP and HR during warm but not cold provocation. This observation is of extreme interest. It is thought that the vascular responses to warm immersion are the

TABLE 3.   Intergroup Differences in Warm- and Cold-Induced Changes

Parameter	Warm-Induced Changes			Cold-Induced Changes			
	Glaucoma	Normal	*P*	Glaucoma	Normal	*P*	*P**
IOP	-2.52 ± 4.02	-1.32 ± 3.56	0.294	1.80 ± 4.57	1.63 ± 3.52	0.886	NS
SBP	0.87 ± 11.36	-0.75 ± 7.13	0.570	0.04 ± 6.28	4.65 ± 2.53	0.015	NS
DBP	6.56 ± 13.40	2.37 ± 7.82	0.208	3.05 ± 5.39	1.39 ± 6.51	0.273	NS
MBP	3.80 ± 11.85	0.81 ± 6.27	0.297	1.61 ± 5.67	2.78 ± 4.32	0.458	NS
PP	-5.93 ± 15.94	-3.34 ± 18.43	0.611	-3.82 ± 16.66	11.58 ± 16.58	0.003	0.021
MOPP	6.78 ± 19.72	1.84 ± 10.49	0.302	1.62 ± 5.97	3.34 ± 6.47	0.354	NS
HR	5.40 ± 8.06	1.35 ± 6.92	0.077	0.56 ± 7.13	-1.27 ± 8.78	0.458	NS

Data are expressed as a percentage of baseline.
* Corrected by means of Holm's sequentially rejective method.

TABLE 4. Intergroup Differences in Warm and Cold-Induced Changes

Parameter	Warm-Induced Changes			Cold-Induced Changes		
	Glaucoma	Normal	*P*	Glaucoma	Normal	*P*
TT	0.01 ± 0.01	−0.02 ± 0.01	0.897	0.05 ± 0.04	0.02 ± 0.03	0.833
FT	1.75 ± 3.28	0.65 ± 1.19	0.144	1.17 ± 4.08	−0.01 ± 2.32	0.236
FBF	7.21 ± 50.31	−9.44 ± 28.60	0.180	−29.95 ± 44.67	−50.86 ± 26.01	0.062
Volume TNRR	2.68 ± 17.87	−4.32 ± 16.40	0.243	3.67 ± 29.38	−9.43 ± 12.47	0.485
Flow TNRR	−0.06 ± 25.49	0.57 ± 27.27	0.944	−12.84 ± 27.10	−6.22 ± 23.61	0.470
Velocity TNRR	−1.40 ± 24.41	2.04 ± 25.99	0.691	−15.55 ± 22.67	−4.52 ± 15.72	0.135
Volume TR	0.54 ± 16.20	1.25 ± 8.89	0.874	−10.13 ± 28.31	4.18 ± 19.09	0.103
Flow TR	−1.02 ± 13.98	3.00 ± 19.18	0.459	−3.45 ± 28.32	−4.75 ± 22.10	0.885
Velocity TR	−1.96 ± 13.43	2.61 ± 18.44	0.381	−3.72 ± 26.58	−5.06 ± 21.20	0.875

Data are expressed as a percentage of baseline.

result of stimulation of the preoptico-anterior hypothalamic area in the brain.[46] Severe whole-body heating results in an increased HR without changes in systemic BP,[47] whereas mild heating has no influence on either HR or systemic BP.[48] Changing the stimulated skin area further decreases the temperature effect on systemic BP.[48] Therefore, the immersion of one hand in warm (40°C) water for 5 minutes was unlikely to provoke any HR or BP changes in our subjects. Indeed, the control group did not exhibit any systemic circulatory changes during warm stimulation. The most obvious explanation for the high BP and HR during warm provocation in our glaucoma patients could be an anticipatory reaction to the physical stress. This reaction occurred even though the patients were allowed 15 to 20 minutes in a quiet room to achieve a sufficient mental and physical calm before the experiment. In the glaucoma patients, the hemodynamic response due to the anticipation of the test may have been stronger than the effect of the warm stimulation itself, and the result was an increase in BP and HR. The type of vascular hyperactivity exhibited by our patients is similar to that demonstrated in subjects at risk for cardiovascular diseases, in whom the anticipatory and recovery vascular responses are better predictors for subsequent circulatory disturbances than the direct stress-induced reactivity.[49] Theoretically, we can hypothesize that in their normal daily life, glaucoma patients respond to challenge by cardiovascular reactions that last longer than in normal subjects. This type of

reaction could in fact represent a sign of autonomic dysfunction. This conclusion is further supported by the observation that in our glaucoma patients, immersion of the tested hand in cold (4°C) water did not affect the systemic BP. Similar results were reported by Nicolela et al.[23] in POAG patients and control subjects after 30 minutes of body surface cooling. They observed that despite the increase in plasma endothelin (ET)-1, glaucoma patients did not exhibit any change in their systemic BP, whereas control subjects demonstrated a significant increase in DBP and MOPP with cooling. The authors did not offer any explanation for this finding. We suggest that a blunted BP response to cold provocation in POAG patients could signal an abnormal neural pathway resulting from a lack of sympathetic participation. Whether this type of reaction has a role in glaucoma pathogenesis is still to be clarified.

Our study demonstrated an abnormal decrease of the blood velocity in the temporal region of the NRR after a 1-minute cold provocation in patients with POAG. To our knowledge, this is the first observation of an abnormal ocular hemodynamic response to cold provocation in POAG patients. Previous studies failed to report any OBF changes to CPT in either retrobulbar[21] or retinal circulations.[23] Our study also failed to demonstrate any hemodynamic changes in temporal peripapillary retinal circulation; however, cooling significantly decreased the blood velocity measured at the temporal NRR level. Because the vessels in the retina and in the prelaminar portion of the optic nerve have no neural innervation,[50,51] it is reasonable to as-
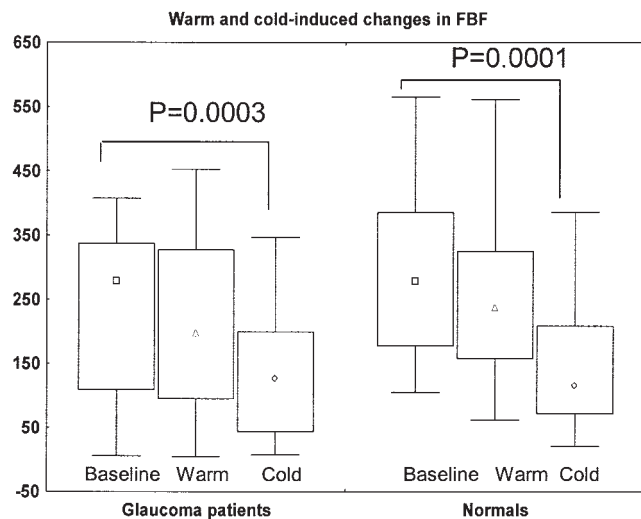

FIGURE 2. Warm and cold-induced changes in FBF in glaucoma patients and normal control subjects.
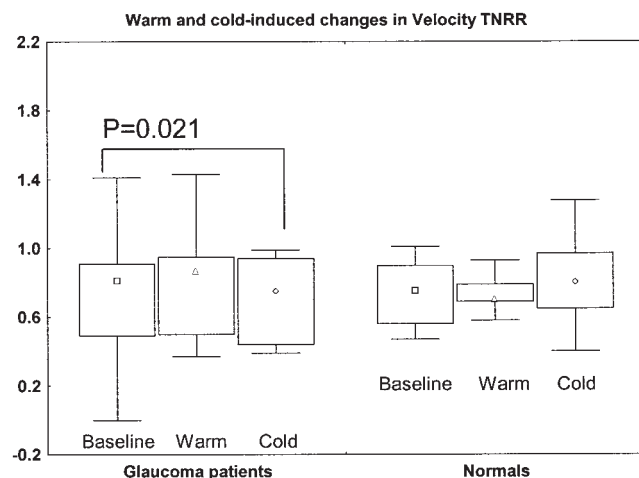

FIGURE 3. Warm- and cold-induced changes in the retinal flowmetry parameter Velocity, measured at the TNRR in glaucoma patients and normal control subjects.

sume that a local neural-mediated vasoconstriction cannot explain our observation. We cannot exclude, however, a possible sympathetic influence on the blood flow downstream from the measurement point, at the level of posterior ciliary and central retinal arteries. These vessels have a rich supply of autonomic nerves[50,52,53] and could, in theory, react to a powerful vasoconstrictor stress such as CPT. Nevertheless, our glaucoma patients did not demonstrate a systemic sympathetic activation during cold provocation, as measured by an increase in systemic BP. Although activation of thermoreceptors in the skin during CPT could result in different activation of the arteries situated closer to the body's surface than of those situated away (e.g., ocular arteries),[19] it is difficult to presume that in our patients, in that the sympathetic response to cooling manifested only at the retrobulbar vessels level. A more probable explanation would be the involvement of endothelial factors. Indeed, in a recently published study Nicolela et al.[23] demonstrated a significant increase in ET-1 levels after body cooling in POAG patients. Although they failed to demonstrate any hemodynamic changes in the retinal circulation, they speculated that a high ET-1 concentration after CPT could influence the optic nerve blood flow. This conclusion does not come as a surprise. It has already been demonstrated that in humans, systemic injection of ET-1 results in a reduction of both pulsatile and optic nerve blood flow.[54] Altered ocular blood flow during sympathetic provocation was also reported by other investigators. In a recent study, Gugleta et al.[55] demonstrated that a handgrip stress is associated with a decrease in choroidal blood flow (CBF) in subjects with vasospasm and in POAG patients with progressive damage. They also hypothesized that an increased level of plasma ET-1 or a deficient release of endothelium-derived vasodilators could be responsible for the observed alteration in CBF during a handgrip test. Although we did not measure plasma ET-1 levels in our patients, in the light of previous research it is possible that high plasma levels of this extremely potent vasoconstrictor peptide resulted in a decreased blood flow at the NRR level in our glaucoma patients.

It can be argued that the observed vascular effects could be, at least partially, the result of a possible pain sensation associated with cold provocation.[56] Indeed, CPT is not only a stressor commonly used to test sympathetic reaction but is also a widely used technique in pain research. When testing pain, however, CPT is rather unreliable. The pain triggered by this test is influenced by a large variety of factors, such as the individual ability to cope, ambient temperature, and mental preparation.[57,58] In our study the room temperature was controlled and maintained between 21°C and 23°C. In addition, our patients were instructed to signal verbally when the water "begins to produce pain." However, only one subject had to remove the hand from the cold water after 30 seconds due to pain sensation.

The mechanism by which the water's temperature relates to the response of nociceptors and pain perception is still incompletely understood. Other provocation tests, such as handgrip can also be painful.[59] However, no significant relationship has been found between cardiovascular responses and perception of pain during handgrip test.[60] Both handgrip stress and CPT have similar cardiovascular effects[61]; therefore, we can hypothesize that a possible pain sensation induced by cold provocation has limited value in determining the final cardiovascular response to this type of test.

In our study, optic nerve blood flow was assessed by means of HRF technology. This is a noninvasive, high-resolution mapping technique estimating capillary blood flow of the optic nerve head and retina with acceptable reliability[62-64] and adequate ability to show blood flow alterations.[65] Moreover, in our study we used a central alignment technique described by

Sehi and Flanagan,[43] which allows an optimal focal plane for the flowmetry of the NRR and more repeatable measurements of the blood flow at this level. Using this technique, we observed that blood flow in the anterior optic nerve of glaucoma patients was normal under baseline conditions but became disturbed when challenged. This observation suggests that in glaucoma patients the blood flow may not be reduced continuously. If chronically reduced blood flow were able to induce glaucomatous damage, vascular diseases with chronic reduction in blood flow such as arteriosclerosis would have to be related to the prevalence of glaucoma. However, neither risk factors for arteriosclerosis nor arteriosclerotic alterations in the carotid vessels have been demonstrated to be related to the prevalence of glaucomatous optic neuropathy.[66-70] An alternative explanation could be that these patients have ocular vascular dysregulation, which could predispose the optic nerve head to ischemia and reperfusion damage. Indeed, vascular dysregulation has been advocated as possible contributing factor in the etiology of POAG.[5,71-74] The abnormal reduction of blood flow at the NRR in response to a stress factor such as cold could increase the susceptibility of the optic nerve to high IOP and low perfusion pressure in these individuals.

In summary, patients with POAG may show a blunted BP response and a reduction in optic nerve head blood flow in response to cold provocation. This reaction could be a manifestation of an autonomic dysfunction. The observed systemic and ocular hemodynamic alterations may result in the onset or progression of glaucomatous optic neuropathy.

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