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Heidelberg retina flowmeter parameters at the papilla in healthy subjects

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ABSTRACT: Purpose. *To assess the Heidelberg Retina Flowmeter (HRF) parameters "volume", "flow", and "velocity", at the papilla in healthy subjects.*
Methods. *HRF measurements were taken at the papilla (5° x 20°), superficially at level of the retina and at the bottom of the excavation. The effect of increasing frame size (1x1 to 50x50 pixels) on HRF values was assessed in ten subjects. HRF parameters were calculated (50x50 pixels) for 150 eyes of 150 subjects. To assess short-term reliability, measurements were repeated five times in ten subjects.*
Results. *With 50x50 pixels the location of the frame had no influence on HRF values. Reliability was > 90%. Values were significantly higher ($p<0.001$) in the superficial than in the deeper papillary layers. The correlation between HRF parameters was good ($r^2>0.85$).*
Conclusions. *A low magnification (5°x20°) and a 50x50 frame allows a global assessment of HRF parameters at the papilla with high reliability. In healthy eyes, the HRF values are influenced by the level where measurements are made at the papilla. This might be of importance in glaucoma patients with excavated papilla. (Eur J Ophthalmol 1999; 9: 32-6)*

KEY WORDS: Scanning laser, Doppler flowmetry, Glaucoma, Ocular blood flow, Optic nerve head circulation, Eye

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INTRODUCTION

According to the manufacturer (Heidelberg Engineering, Heidelberg, Germany), the Heidelberg Retina Flowmeter (HRF) is an instrument designed to be a non-invasive tool to assess intraocular blood flow in humans (1-8). The HRF combines the principles of confocal laser scanning and Doppler flowmetry measurment (9). After HRF measurements have been taken, parameters supposed to be related to blood flow and termed "volume", "flow", and "velocity", can be calculated in arbitrary units. The present study assessed HRF parameters at the papilla in a healthy population.

SUBJECTS AND METHODS

Subjects

Among visitors to a trade fair, HRF measurements were taken in non-smoking healthy subjects with no history of ophthalmic and vascular diseases and with an inconspicuous eye examination. From the 263 subjects examined, only the data of 72 men (50 ± 14 yrs) and 78 women (49 ± 16 yrs) could be analyzed. Dropouts resulted from bad quality images due to eye movements or blinking during the examination. Often for the same reasons the images of only one eye coud be analyzed, even though both eyes were measured in each subject. When images were of a good quality in both eyes (50 subjects) only data of one randomly selected eye were kept for further analysis. In the end the data of 69 right eyes and 81 left eyes were analyzed.

Heidelberg retina flowmeter

According to the manufacturer, the HRF combines the principles of confocal laser scanning and laser Doppler flowmetry (670 nm, 100µW). After multiple scanning (128 times), the HRF calculates (fast Fourier transformation) a two-dimensional map of the laser

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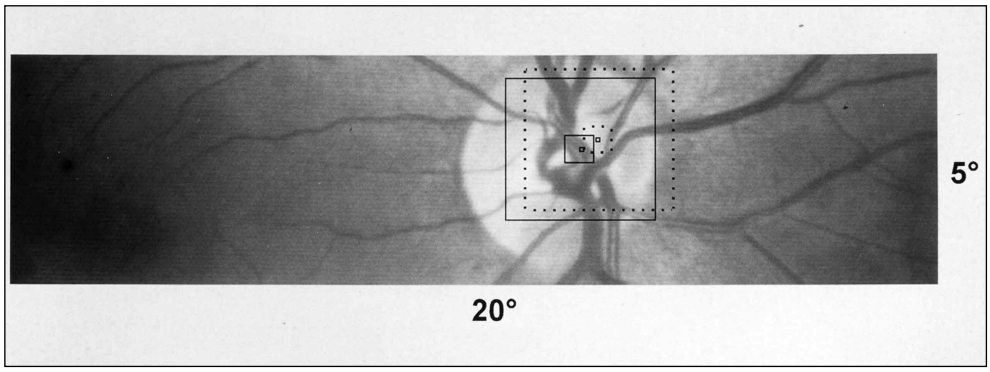

Fig. 1 - *Once HRF measurements have been taken at the back of the eye (5°x20°), the parameters are calculated after selecting a certain number of pixels on the monitor (frame size). This schematic representaton shows the proportions of three different frame sizes (2x2, 10x10, 50x50 pixels) to the papilla. In this example two locations have been chosen: one within a vessel (full line), one outside a vessel (dotted line).*

Doppler shift within a thin (300 µm) slice of tissue, over a rectangular area (5° x 20°) of the posterior pole of the eye. The laser Doppler shifts values (frequency shifts) recorded at different locations on the posterior pole of the eye are displayed on a monitor in a color-coded image (256 x 64 pixels). For each pixel, a frequency shift is calculated. Different sized frames can then be selected on the monitor (1x1 to 50x50 pixels) and three parameters (HRF parameters), termed “volume”, “flow”, and “velocity”, are calculated and displayed, in arbitrary units.

HRF measurements

In the present sutdy, measurements were taken (5° x 20°) at the papilla, superficially, at the retinal surface, and deeper in the papilla, approximately at the bottom of the excavation. To make measurements deeper in the papilla, a sharp image was first obtained at the retina surface, then the image was defocused posteriorly by about 0.48 ± 0.16 mm. The amount of defocusing necessary to theoretically reach the bottom of the excavation was determined using depth profiles obtained using a Heidelberg Retina Tomograph (Heidelberg Engineering, Heidelberg, Germany). HRF measurements were made by two investigators (SMG, AL).

In ten subjects (randomly selected from the 150 person pool) we first assessed how HRF parameters were influenced by the frame size selected on the monitor or by the fact that the center of this frame was within or outside a major vessel at the papilla (Fig. 1). At these two locations, the frame size was gradually increased (1x1, 2x2, 4x4, 10x10, 20x20, 30x30, 40x40, and 50x50 pixels), and HRF parameters were calculated for each frame (Fig. 2).

To assess short-term reproducibility, measurements were repeated five times in ten additional volunteers within ten minutes. Measurements were taken in the superficial papillary layer, and HRF parameters were calculated for a 50x50 pixel frame. In 150 eyes of 150 subjects measured in both a superficial and a deep layer of the papilla, HRF parameters were calculated with a 50x50 pixel frame.

Statistical analysis

Results are given as absolute values (arbitrary units) and expressed as means ± standard deviation. Results were compared by analysis of variance (ANOVA). P values less than 0.05 were considered significant.

RESULTS

When a frame of less than 20x20 pixels was selected to calculate HRF parameters at the papilla, the fact whether the center of this selection was within or outside a vessel greatly influenced the values. These values were significantly higher for all three HRF parameters within the vessels (as opposed those outside the vessels: $p < 0.001$) (Fig. 2).

When a frame of 20x20 pixels or larger was selected, there was no statistical difference in HRF values whether the center was within or outside a major vessel of the papilla. There was also no statistical difference between values calculated using 30x30, 40x40, or 50x50 pixel frames. Furthermore, for a frame centered outside the vessel, the HRF values were independent of the frame size between 10x10 and 50x50. Because when more than 20x20 pixels were select-

ed, the location of the center of the frame at the papilla had no statistical influence on HRF values, we always calculated HRF parameters on the basis of the 50x50 pixel frame.

Short-term reliability (coefficient of reliability = variance between frame sizes/total variance) for the HRF parameters at the retina surface and using a 50x50 pixel frame was calculated from five measurements taken in ten additional volunteers. HRF parameters showed high reliability "volume" 89.95%, "flow" 91.36%,

TABLE I - HRF PARAMETER VALUES IN 150 EYES OF 150 HEALTHY SUBJECTS (mean ± SD in arbitrary units)

HRF parameter	Superficial layer of the papilla	Deep layer of the papilla
"volume"	40.6 ± 13.1	24.9 ± 8.0
"flow"	1144 ± 374	716 ± 214
"velocity"	3.43 ± 0.90	2.36 ± 0.61

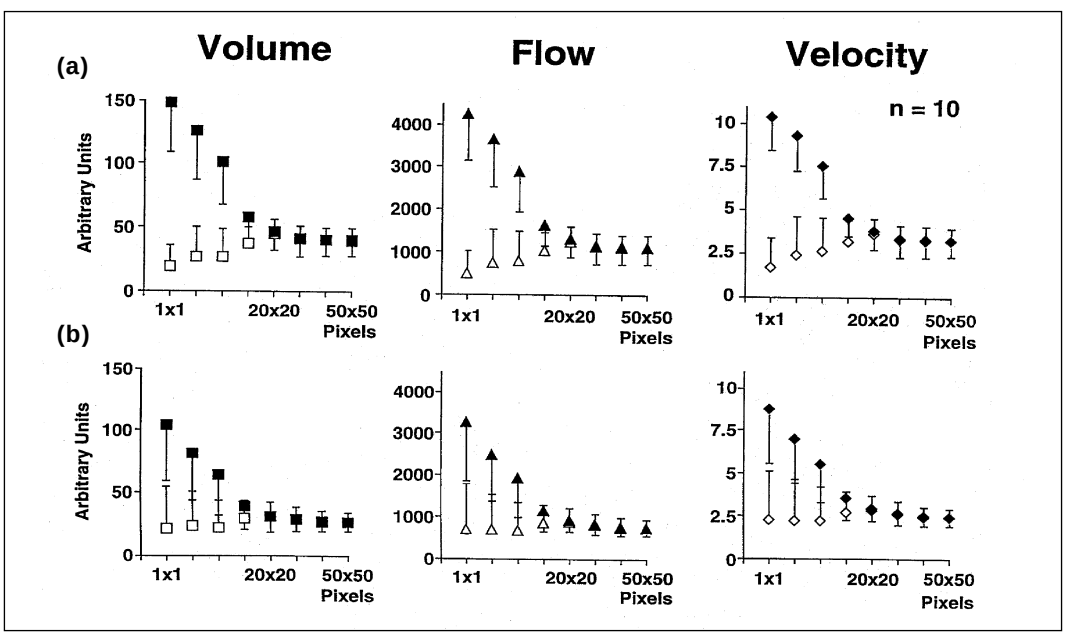

Fig. 2 - *Distribution of HRF parameters volume, flow, and velocity (mean ± SD) calculated after selecting increasing frame sizes (1x1, 2x2, 4x4, 10x10, 20x20, 30x30, 40x40, 50x50). Two locations have been chosen as the center of the different frame sizes, one within a vessel (filled symbols), one outside a vessel (open symbols). HRF measurements at the papilla were taken on a superficial layer (**a**), and (**b**) around the bottom of the excavation.*

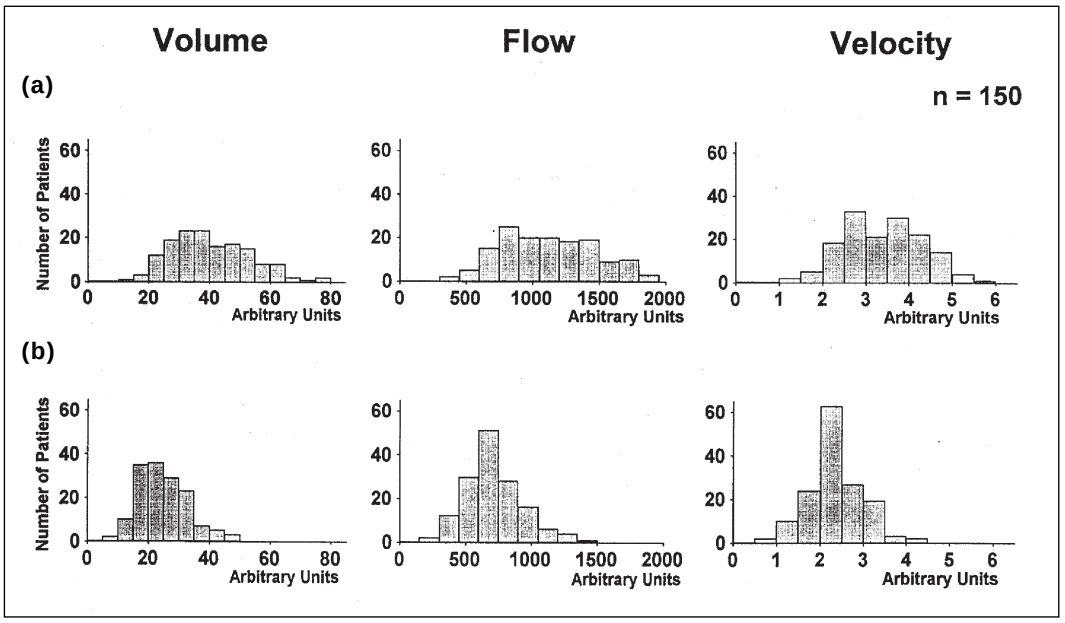

Fig. 3 - *Histogram of the distribution of the HRF-parameters volume, flow, and velocity in 150 eyes of 150 healthy subjects. Measurements in a superficial layer (**a**) showed a wider distribu-tion of values and gave a higher mean value than those taken in a deeper layer (**b**) of the papilla.*

"velocity" 91.90%).

In the 150 eyes of the 150 subjects means ± SD of the HRF parameters were calculated. Values of the deeper layer were lower (39% for "volume", 37% for "flow", and 31% for "velocity") than those from the superficial layer (Tab. I). This difference was highly significant (ANOVA for repeated measurements: $p<0.001$). The values were normally distributed when expressed logarithmically, although the interindividual distribution was very wide (Fig. 3). An intergroup comparison by ANOVA did not show any real influence of sex or investigator on HRF values. In addition, analysis of covariance disclosed no real influence of age or IOP on HRF values.

Correlations between the three HRF parameters, at the retinal level, were close ("flow-volume".$r^2=0.92$; "flow-velocity".$r^2=0.98$; "volume-velocity".$r^2=0.86$).

DISCUSSION

This study investigated HRF parameters at the papilla after measurements made with an angle of field of 5° x 20° in healthy eyes. When a frame of 20x20 pixels or more was used to calculate those parameters, their values were independent of frame location and frame size within the papilla. For a frame centered outside the vessel, HRF values were also independent of the frame size between 10x10 and 50x50 pixels. The short-term reliability of HRF parameters after repeated measurements was high for a 50x50 pixel frame, although the interindividual distribution was very wide. In addition, values measured in the superficial layer of the papilla were much higher than at the bottom of the excavation. All three HRF parameters appeared to be closely correlated.

When a frame of more than 20x20 pixels was selected for calculation, HRF values were not statistically different regardless of whether the center of the frame was within or outside a vessel. This suggests that a larger frame gives a better overall assessment of HRF parameters at the papilla. Thus, it was decided to calculate HRF parameters at the papilla based on a 50x50 pixel frame. Reliability in this condition was high, with values comparable to other reports (2, 3), even when they were calculated with a higher magnification (2.5°x10°) and a smaller frame (10x10 pixels).

The HRF values termed "volume", "flow", and "velocity", calculated in arbitrary units, were highly correlated. That the three parameters are closely related therefore suggests it might be sufficient to assess only one of them.

In view of the large differences between healthy individuals, it appears that to detect a difference in HRF values between two populations, either this difference or the population studied has to be very large. Statistical analysis suggests that this large distribution is not influenced by such factors as age, IOP, sex, or investigator.

Interestingly, there was a significant difference between measurements taken superficially and in deeper layers of the papilla. Though one can only speculate on the reason this difference might be important when measuring glaucomatous eyes with an excavated papilla.

In summary, it appears that with the HRF instrument, a 50x50 pixel frame allows a global assessment of HRF parameters at the papilla. Under these conditions measurements are reliable although the interindividual distribution is wide. As the three parameters appear to be closely correlated, it seems sufficient to calculate only one HRF parameter. However, HRF values are lower at the bottom of the excavation than at the retina surface.

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