Abstract

Background/Purpose. Recent findings suggest that an objective assessment of retinal vessel caliber from fundus photographs provide information about the association of microvascular characteristics with macrovascular disease. Current methods used to quantify retinal vessel caliber, introduced by Parr1,2 and Hubbard,3 are not independent of scale and are affected by the number of vessels. To improve upon these methods we introduce revised formulas for quantifying vessel caliber.

Methods. Revised formulas were estimated using retinal vessel measurements from 44 young adults free of hypertension and diabetes. Comparisons between the two methods were done using digitized photographs from 4926 participants at the baseline examination of the Beaver Dam Eye Study (BDES), an ongoing population-based cohort study initiated in 1987. Individual arterioles and venules were measured using semi-automated computer software from which summary measures were calculated.

Results. Correlation coefficients between the Parr-Hubbard and revised formulas were high (Pearson correlation coefficients ranging from 0.94 to 0.98). Both arteriolar and venular caliber significantly increased with an increasing number of vessels measured using the Parr-Hubbard formulas (p < 0.001), which in turn affected the relationship to mean arterial blood pressure. To the contrary, the revised formulas were not affected by the number of measured vessels (p > 0.50).

Conclusions. We describe revised formulas for summarizing retinal vessel diameters measured from fundus photographs to be used in future studies and analyses. The revised formulas correlate highly with the previously used Parr-Hubbard formulas, but offer the advantages of being more robust against variability in the number of vessels observed, being independent of image scale, and being easier to implement.

Keywords: retinal vessels; arteriolar narrowing; hypertensive retinopathy; blood pressure; cardiovascular disease

Introduction

The retinal vascular system of the human eye is accessible to direct and repeated observations in vivo. The characteristics of its arterioles and venules in health and disease provide data applicable to the study of systemic vascular disorders.1–13 In 1974 Parr et al1,2 developed a method to quantify retinal arteriolar caliber. In 1999 Hubbard et al3 followed Parr’s approach to quantify retinal venular caliber. Their approaches took into account the relation between parent trunk vessels and their two branches. The Parr-Hubbard formulas have been used to summarize retinal arteriolar and venular diameters measured from digitized photographs in various studies.11–15 While the computation of summary estimates of retinal vessel caliber from these formulas was intuitively reasonable and the resulting associations with blood pressure12 were in keeping with expectations, we noted some features that could lead to spurious variability in our estimates. For example, these calculations allow contributions of a variable number of vessel diameters for each eye to the overall estimate of vessel caliber. This resulted in increasing trends between the number of vessels included in the calculation and the magnitude of summary estimates, potentially decreasing the ability to discern real biological associations of vessel caliber. In addition, the formulas include constant terms in their equations, which in turn cause the result to be depen-
dent upon the units (e.g., microns, pixels) of measurement. We therefore developed revised formulas for summarizing the caliber of arterioles and venules free of such constraints. This paper compares these revised formulas with the previously used Parr-Hubbard formulas.

Materials and methods

Study populations

In this report we use data from two groups. Tenets of the Declaration of Helsinki were followed. First, the revised formulas were estimated from study photographs from 44 young adults free of hypertension and diabetes. This was the same group Hubbard et al. used to develop the formula to quantify venular caliber. Second, data from the Beaver Dam Eye Study (BDES) was used for all other results presented. The BDES is a population-based cohort study of age-related ocular diseases in adults, described in detail elsewhere.

Retinal vessel caliber measurements

Each group had their retinal vessels measured with different technical procedures. In the 44 young normotensive subjects, a 30° color retinal fundus photograph of the right eye centered on the disc (Diabetic Retinopathy Study Standard Field) was projected on white 19 × 24 inch paper at a fixed distance. Edges of vessels that coursed through an area 0.5 to 1 disc diameters from the optic disc margin were marked with a 0.5 mm lead pencil. Measurement at this distance from the disc ensured that arteriolar status had been attained. All photographs were projected at the same magnification. Vessels widths were measured using a dial calipers, accurate to 0.01 inches.

All Beaver Dam participants had stereoscopic 30° color retinal photographs taken of both eyes, centered on the disc (Diabetic Retinopathy Study Standard Field). Measurement of retinal vessel diameter has been described previously. In brief, these photographs were converted to digital images by a high-resolution scanner (Fig. 1). Calibration of the image was performed on the basis of the standard disc diameter (1850 μm), as established by the Early Treatment Diabetic Retinopathy Study, as a defined unit of measurement. The diameters of all arterioles and venules coursing through a standard area 0.5 to 1 disc diameters from the optic disc margin (Zone B in Fig. 1) were measured in microns (3.8 μm per pixel) using a custom computer program (Retinal Analysis, University of Wisconsin-Madison), according to a protocol described elsewhere.

Parr-Hubbard formulas

The Parr-Hubbard method summarized the individual retinal vessel measurements into the central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE). It has been suggested that smaller arterioles (i.e. those farther away from the optic nerve) are more affected by hypertension. For this reason the branches of arterioles were measured, where possible, if the measurement of the trunk diameter was ≥ 85 μm. Arteriolar caliber measurements were thus combined into the CRAE as the branch variant (substituting branch measurements for any trunk arteriole ≥ 85 μm) or the trunk variant (using trunk measurements regardless of their diameter). To correct for possible magnification errors, that affect arterioles and venules similarly, the ratio between CRAE (branch or trunk) to CRVE was calculated. For this report we use CRAET (trunk variant), CRAEB (branch variant), CRVE, AVRT (ratio using CRAET), and AVR (ratio using CRAEB) to abbreviate the Parr-Hubbard summary measures.

Revised formulas

We have developed revised formulas to summarize the retinal vessel diameters restricted to the six largest retinal arterioles and venules measured from the photographs. To compensate for the considerable variation in the number of bifurcations in an eye, the relationship between trunks and branches was expressed in terms of an empirically derived branching coefficient:

\[ \text{Branching Coefficient } = \frac{w^2}{w^2 + w^2} \]  

where \( w \) is the width of the parent vessel and \( w \) is the width of its daughter vessel.
Revised formulas for summarizing retinal vessel diameters

where \( w_1 \), \( w_2 \), and \( W \) are, respectively, the widths of the narrower branch, the wider branch, and the parent trunk.

We estimated the branching coefficient for arterioles and venules separately using data from the 44 young normotensive adults that Hubbard et al. used to develop the venular formula.\(^3\) One hundred eighty-seven branch-trunk arteriole relationships were measured, yielding a branching coefficient of \( 1.28, 95\% \text{ CI} = (1.25, 1.32) \), comparable to the cited theoretical branching coefficient of \( 1.26.\)\(^{22}\) One hundred fifty-one branch-trunk venule relationships were measured, yielding a branching coefficient of \( 1.11, 95\% \text{ CI} = (1.08, 1.14) \). We then inserted our estimates of the branching coefficient into equation 1 and solved for \( W \) to yield the following two formulas to approximate vessel equivalents:

\[
\text{Arterioles: } \hat{W} = 0.88 \ast (w_1^2 + w_2^2)^{1/2} \tag{2}
\]

\[
\text{Venules: } \hat{W} = 0.95 \ast (w_1^2 + w_2^2)^{1/2} \tag{3}
\]

where \( w_1 \) and \( w_2 \) are the same as described above, and \( \hat{W} \) is the estimate of parent trunk arteriole or venule.

Using these formulas, including only the six largest arterioles and the six largest venules, we used an iterative procedure of pairing up the largest vessels with the smallest and repeating until we reached a single number that we will still call a central vessel equivalent. As an example, assume in a retinal photograph the six largest arterioles are 100, 90, 80, 70, 60, and 50 \( \mu m \). First put 100 and 50 into equation 4 to yield 98.4 \( \mu m \). Similarly pair up 90 and 60 to yield 95.2 \( \mu m \) and 80 and 70 to yield 93.5 \( \mu m \). After the first iteration there are three values: 98.4, 95.2, and 93.5. Continue with the next iteration by pairing up the largest and the smallest (98.4 and 93.5 to yield 119.4 \( \mu m \)). The middle number (95.2) carries over to the final iteration. Finally, pairing up 119.4 and 95.2 yields 134.4 \( \mu m \) for CRAET-6.

The branches of arterioles \( \geq 85 \mu m \) were still taken so that we have a branch and trunk variant. For this report we use CRAET-6, CRAEB-6, CRVE-6, AVRT-6, and AVRBlog-6 to distinguish between the Parr-Hubbard and the revised method. Definitions of other variables appear in other reports.\(^{16,23,24}\)

**Statistical procedures**

Age and summary indices of retinal vessel caliber were analyzed as continuous variables. Mean arterial blood pressure (MABP) was analyzed both continuously and as a 6-level ordered factor (<75, 75–84, 85–94, 95–104, 105–114, and >114 mm Hg). The number of measured vessels (regardless of the summary formula) was analyzed both continuously and as a three-level ordered factor (arterioles: <9, 9–11, >11; venules: <8, 8–10, >10). In most instances, ordinary linear regression was used to evaluate significance. The correlation between methods was assessed by the Pearson correlation coefficient. Confidence intervals for correlations were calculated using methods presented by Snedecor and Cochran.\(^{25}\) We performed all analyses with SAS Version 8.0 (SAS Institute Inc, Cary, NC).

**Results**

Table 1 shows the summary statistics for the two methods for right eyes in the Beaver Dam population. The central retinal vessel equivalents produced by the two methods correlate quite highly, with Pearson correlation coefficients ranging from 0.94 to 0.98. It should be noted that the mean ratio of the arteriolar to the venular measure obtained from the revised formulas is substantially smaller than that from the Parr-Hubbard formulas. Coefficients of variation were similar between the two methods.

<table>
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<tr>
<th>Caliber</th>
<th>Method</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Coef. var</th>
<th>Corr.</th>
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\( N = \) Number of participants.

\( SD = \) Standard deviation.

\( \text{Coef. var} = \) Coefficient of variation \(( 100 \ast (SD/\text{Mean}) )\).

\( \text{Corr.} = \) Pearson correlation coefficient.

Table 1. Summary statistics for right eye vessel caliber.
Figure 2 shows the relationship between the number of measurable arterioles and venules and the central retinal equivalents in right eyes, both from the entire Beaver Dam population ($n = 4157$) and a subgroup of younger, “normal” subjects (aged 43–50 years, free of hypertension, cardiovascular disease, and diabetes ($n = 690$)). Using the revised formulas, in the younger subgroup there was no association between the number of vessels and corresponding central retinal equivalents (CRAET-6: $p = 0.51$; CRVE-6: $p = 0.79$). In contrast, using the Parr-Hubbard formulas, in the younger subgroup there was a strong increasing trend between the number of vessels and corresponding central retinal equivalents (for CRAET: $p < 0.001$; for CRVE: $p < 0.001$). In the whole population there was a significant increasing trend with both methods, but the magnitude of change was much more pronounced using the Parr-Hubbard method.

Arteriolar caliber is known to decrease with an increase in blood pressure. The Pearson correlation coefficient between MABP and arteriolar caliber were similar for the two methods (CRAET: $-0.26$, 95% CI = $(-0.29, -0.22)$; CRAET-6: $-0.24$, 95% CI = $(-0.28, -0.20)$). Venular caliber was not significantly associated with MABP with either method. We examined if stratifying by the number of measurable vessels has an effect on this relationship (Fig. 3). After adjusting for age, for each category increase in the number of arterioles CRAET increased by 3.3 $\mu$m, 95% CI = (2.8, 3.7), whereas for each category increase in the number of arterioles CRAET-6 did not significantly increase ($p > 0.05$). Results were similar using the branch variants instead of trunks (results not shown). Further, results were similar for venules, with the Parr-Hubbard method more affected by the number of venules than the revised method (2.8 $\mu$m versus 1.0 $\mu$m increase for CRVE and CRVE-6, respectively).

We further investigated the relationship between the number of measured vessels and MABP. There was an inverse relationship with MABP and the number of arterioles (Pearson correlation = $-0.09$, 95% CI = $(-0.13, -0.05)$) and there was no relationship between MABP and the number of venules (Pearson correlation = $-0.03$, 95% CI = $(-0.07, 0.01)$).

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**Figure 2.** Relationship between the summary measurements and the number of vessels measured (regardless of formula used) for the entire Beaver Dam population and a younger sub-population. *young sub-population: ages 43–50, free of hypertension, diabetes, and cardiovascular disease.*
Discussion

We have discovered several methodological issues with the standard Parr-Hubbard formulas used in previous studies.\(^{11–15}\) First, the Parr-Hubbard formulas allow a variable number of vessel diameters for each eye to affect the overall estimate of vessel caliber. Second, the Parr-Hubbard formulas include constant terms in their equations, making them sensitive to scale. To address these issues, we developed revised formulas that summarize the retinal vessel diameters using the six largest arterioles and venules measured from photographs. The analyses presented comparing the methods showed that the revised formulas were not affected by the number of vessels measured and do not include constant terms in the equation. We therefore believe that the revised formulas may provide more precise and consistent estimates of retinal vessel caliber in an eye than the previously used Parr-Hubbard formulas.

Compared to average vessel caliber using the Parr-Hubbard formulas, we note that venular caliber increased, while arteriolar caliber decreased using the revised formulas (Table 1). Although this was unexpected, we believe a mean AVR of 0.69 calculated using the revised method was more in keeping with biological expectations than a mean AVR of 0.85 calculated using the Parr-Hubbard method. Consistent with this, Kagan \textit{et al.} using a different measurement approach reported mean arteriole to venule ratios of upper temporal vessels ranging from 0.68 to 0.73.\(^7\)

Parr \textit{et al.} claimed that because the volume of a vascular bed is related to the cross-sectional area of the feeding vessels, the width of the central retinal artery should be similar for normal eyes.\(^1\) Thus, the method for summarizing retinal vessel diameters in normal eyes should theoretically be independent of the number of vessels measured. By considering vessel caliber in a younger group free of known disease to have “normal” eyes, we demonstrated that the
number of vessels affected the summary values for the Parr-Hubbard method, but not for the revised method (Fig. 2). This provides further evidence that the revised formula may be more robust compared to the Parr-Hubbard formulas.

An inverse relationship between the number of vessels to blood pressure has been reported by Kagan et al. We found a similar inverse relationship with MAP in the Beaver Dam population. Because results from the Parr-Hubbard formulas were clearly affected by the number of measurable vessels (Fig. 2), the confounding effect of using different numbers of vessels may decrease the ability to discern biological associations by not considering the number of measurable arterioles when assessing relationships between blood pressure and CRAET (Fig. 3). The revised formulas did not have this problem because the relationship between blood pressure and CRAET-6 was independent of the number of arterioles measured. The results were analogous for venules, although we did detect a slight effect of the number of venules on the relationship between blood pressure and CRVE-6.

The revised formulas (equations 2 and 3) do not contain constant values as do the Parr-Hubbard formulas. The latter were developed assuming that the vessels would be measured in microns. Using different digital photographic approaches, images may be measured in different units (e.g. pixels vs. microns), in which case the same constant values would not apply. Our revised formulas can be used on images of any magnification and measured with any scale.

A technical advantage of using larger vessels is that they are easier to measure. The color of the blood column is more obvious, making it easier to type a vessel as an arteriole or venule, and the vessel walls are better defined, making it easier to gauge the diameter accurately. We are currently in the process of developing automated imaging software that easily measures and identifies arteriole/venule status on the larger vessels, but is less consistent for the smaller vessels. Therefore using only large vessels in the summary formulas greatly facilitates development of an automated retinal vessel measurement system.

Earlier reports have used the Parr-Hubbard method, some of which are included in the list of references. Reanalysis of some of those previously published analyses using the revised formulas showed that overall associations did not change and led to tighter confidence intervals (Knudtson et al. unpublished data).

In conclusion we describe revised formulas for summarizing retinal vessel diameters measured from fundus photographs to be used in future studies and analyses. The revised formulas correlate highly with the previously used Parr-Hubbard formulas and provide a comparable representation of the vascular bed, but offer the advantages of being robust to variability in the number of vessels observed, being independent of image scale, and being easier to implement.

Acknowledgements

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