Asymmetry of Retinal Arteriolar Branch Widths at Junctions Affects Ability of Formulae to Predict Trunk Arteriolar Widths

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Purpose. To describe a revised formula for the estimation of retinal trunk arteriole widths from their respective arteriolar branch widths that improves the summarizing of retinal arteriolar diameters.

Methods. A group of young, healthy individuals underwent retinal photography and arteriolar and venular branching points were identified. Vessel widths of the vessel trunks and their branches were determined. The relationship between the branching coefficient (BC; quotient of the area of the branch and trunk vessels) and the asymmetry index (AI) of the vessel branches was explored. The result was used to formulate a new BC. To test the new BC, a second group of young, healthy individuals also underwent retinal photography. Arteriolar branching points were identified, and the trunk and branch arteriolar widths were recorded. This "revised" BC was compared against the gold standard of the BC as a constant value (1.28), as well as a theoretical formula for the BC that includes the angle between the two vessel branches.

Results. The BC of arterioles (but not venules) related to the AI ($R = 0.275$, $P = 0.0001$; BC arterioles $= 0.78 \pm 0.63 \cdot$ AI). In the second group, the mean arteriolar trunk diameter was 15.56 pixels. The linear regression model for the arteriolar BC was superior to the BC constant of 1.28 (mean difference between estimated and calculated trunk vessel width was 2.16 vs. 2.23 pixels, respectively). The model based on the angle between the branch arterioles was the least accurate (3.43 pixels).

Conclusions. A revised formula has been devised for the arteriolar BC using a linear regression model that incorporates its relationship to the AI. Further studies using this refined formula to calculate the BC are needed to determine whether it improves the ability to detect smaller associations between the retinal vascular network and cardiovascular disease. (*Invest Ophthalmol Vis Sci.* 2006;47:1329–1333) DOI:10.1167/iovs.05-1248

The retinal vascular network is the only human vasculature that can be directly visualized noninvasively in vivo, photographed, and hence subjected to image analysis.[1] Over the past decade, there has been great interest in the use of image-analysis techniques to describe vascular parameters of the retinal vascular branching network that can then be related to various systemic vascular conditions such as hypertension (Hubbard LD, et al. *IOVS* 1992;33:ARVO Abstract 804)[2–4] Derived initially from the work of Parr and Spears[5,6] and Hubbard et al.,[7] summarizing measures of the retinal arteriolar diameters (central retinal arteriolar equivalent [CRAE]) and venular diameters (central retinal venular equivalent [CRVE]) and their quotient (arteriovenous ratio [AVR]) have been of use in large epidemiologic studies.[8–12] The techniques used to combine individual retinal vessel widths into an equivalent central retinal vessel width are based on both theoretical and empiric formulas. Parr and Hubbard derived formulas for the CRAE and CRVE, respectively, by examining a prospective group of retinal images with branching points, calculating the relationship between individual trunk vessels and their respective branch vessels and using a root mean square deviation (RMSD) model that best fit the observed data. The resultant formula for the AVR was of great use for a large number of epidemiologic studies. However, Knudtson et al.[13] pointed out the drawbacks of these formulas including the fact that they were not completely independent of the number of vessels measured and that, because they contained constants within the equations, they were dependent on the units with which the vessels were measured. Hence, Knudtson et al. developed new formulas for calculating the AVR, based on the concept of a BC, where:

$$BC = [(branch\ 1)^2 + (branch\ 2)^2]/Trunk^2$$

In a young, normotensive population, they calculated a mean BC of 1.28 for arterioles and showed that the revised formulas was superior to the previously used Parr-Hubbard formulas.[13] 1.28 compares well with a theoretical BC of 1.26, derived from Murray's law.[14] The derivation of this theoretical value stems from the fact that across any vascular network, Murray calculated that:

$$Trunk\ vessel^3 = (branch\ 1)^3 + (branch\ 2)^3.$$

Hence, if branch 1 and branch 2 are of the same width, then

$$Trunk\ vessel = [2 \cdot (branch\ vessel)]^{1/3}$$

and, hence,

$$Trunk\ vessel = 1.26 \cdot branch\ vessel (1.26 = 2^{1/3}).$$

Thus, the theoretical BC only applies for symmetrical dichotomous vessel branching (where branch 1 = branch 2). We wanted to determine whether variation in the ratio between the widths of the two vessel branches also explain some of the variation in the BC.

We performed a prospective study to ascertain the relationship between the degree of asymmetry at retinal vascular junctions (asymmetry index [AI] = branch 1/branch 2) and the BC.

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Because calculations of the AVR, CRAE, and CRVE measure vessel branches 0.5 to 1.0 disc diameters from the optic disc margin, we further explored whether any relationship existed between how far from the optic disc the vessel branch occurred and the BC, as there may be a rationale for lower-order venules and arterioles being more predictive as risk factors for cardiovascular disease than larger vessels as they exhibit greater regulation via local factors, rather than autonomic control of the central retinal artery.[15] In addition, we examined the influence of the angle between the branch vessels on the BC. Finally, we compared different formulas based on the BC in their predictive ability to calculate the trunk vessel diameters from the two branch vessel widths, including a technique described by Zamir[16] that incorporates the angle between the vessel branches.

Methods

Ethical approval for the study was obtained from the local institutional review board, and the study was performed according to the tenets of the Declaration of Helsinki. The initial part of the study involved recruiting 13 individuals who were within the age group 18 to 40 years. All individuals recruited were healthy, normotensive persons, with no prior history of hypertension or any other cardiovascular diseases. After full informed consent was obtained, 1% tropicamide was instilled into the eye. After approximately 20 minutes, the pupils were inspected to ensure full dilation. A 60° color photograph of the fundus of each of the individuals, centered on the optic disc, was taken with a fundus camera, (model 60UVi; Canon, Tokyo, Japan), with a digital camera (model 20D; Canon), set at maximum resolution (8 megapixels). The image was taken at the same resolution for all photographs (3504 × 2336 pixels), and all images were stored as TIFF files.

All images were analyzed with a custom-written image-analysis program (written in the MatLab environment; The MathWorks, Natick, MA). Images were converted from color to grayscale and underwent contrast enhancement (contrast-limited, adaptive histogram equalization). Using the original color image, we identified arteriolar and venular branching points in each image. A point either side of the vessel was chosen to create a line perpendicular to the vessel. With microdensitometry, the profile of this created line was fitted to a single Gaussian curve. In addition to the selected line's intensity profile, four other intensity profiles (2 and 4 pixels either side of the selected line) were automatically created by the computer image analysis program. Each intensity profile created was either rejected or accepted by the operator as being a good fit of the selected vessel's profile. At least three of the five profiles had to be deemed a good fit before the vessel's width could be calculated. In addition, the SD of the acceptable vessel intensity profiles had to be equal to or less than 10% of the mean width before the vessel width would be recorded. If it was greater than 10%, then the measurements would be rejected, and the vessel would be remeasured. The vessel width was calculated as the mean width of the intensity profiles at half the height of the intensity profile peak.[17] Widths were calculated for both branches and for the trunk vessel of each evaluable branching point in each image. A portion of vessel segment that was fairly uniform in thickness and not obscured by other crossing vessels was chosen. In addition, the distance from the branching point of all vessels measured for each individual vascular junction was made as uniform as possible. Only vessel junctions that were of good photographic image quality were measured.

To determine the degree of eccentricity of each vessel junction from the optic disc, we measured the distance (in pixels) from the branching point to the nearest circumferential edge of the optic disc. In addition, we measured the vertical optic disc (also in pixels), and expressed the distance of the branching point from the edge of the optic disc in units of disc diameters.

To calculate the bifurcation angle (ω), a region of interest within the grayscale enhanced digital image was magnified in a separate window, and the angle between the two daughter arterioles was calculated according to the cosine rule.[18]

This dataset was then used to formulate a new calculation of the BC, based on a step-wise, least-squares linear regression model. Variables that were considered for inclusion in this model were the AI of the retinal vessel widths (AI), the eccentricity (in disc diameters) of the branching point, and the angle (ω) between the branch vessels.

Finally, another group of normal, nonhypertensive individuals with no prior history of cardiovascular disease and aged between 18 and 40 years were recruited. This "testing" group also had mydriatic retinal photography performed. A 50° color photograph of the fundus centered on the optic disc was taken, using a fundus camera (Topcon, Tokyo, Japan) with a digital camera (Nikon, Tokyo, Japan), set at a different resolution (3008 × 1960 pixels). Again, all images were stored in TIFF format. This "testing" group underwent the same image analysis measurements as the first group. The ability of the BC calculated using the linear regression model from the first group to predict trunk vessel arteriolar measurements from the two branch arteriolar vessels in this "testing" group was compared with a constant BC of 1.28 calculated by Knudtson et al.[13] In addition, we compared the two predictive formulas given earlier in the article for summarizing retinal vessel widths with a theoretical formula based on work by Zamir,[16] which incorporates the angle (ω) between retinal vessel branches to calculate the BC. Zamir calculates that the BC for a symmetrical dichotomous junction should be $[2 \cdot (\cos \omega + 1)]^{1/2}$.

Statistical Analysis

Inspection of the four variables (BC, AI, angle and degree of eccentricity) revealed normal distributions and hence were subject to parametric analysis. To justify using multiple branching point measurements from individual fundi, we compared the within-group (each fundus) variation of the BC with the between-group variation, by using the intraclass correlation coefficient (ICC). Student's t-test was used to compare means of the variables between the "training" and "testing" groups. Pearson's correlation coefficient was used for all bivariate correlations. Step-wise linear regression was used to model a formula for the BC as the dependent variable, with the AI, the angle between the branch vessels (ω), and the degree of eccentricity as the independent variables. The plot of the residuals of the linear regression model were inspected to confirm random scattering of the residual Y values about $X = 0$ and reasonable homoscedasticity. Nonlinear regression models were evaluated but were an inferior fit to a linear model. Statistical significance was set at $P < 0.05$.

Results

For the "training" group, 16 fundi of 13 individuals (8 women; mean age, 27 years; range, 19–38) were photographed and analyzed. These 16 fundi provided 125 individual arteriolar branching points and 90 venular branching points. The ICC for the arteriolar BC of the 16 fundi was −0.16, (95% confidence interval [CI], 0.16–0.19). This indicates that each fundus had as much variation as between the different fundi, and therefore we justify our use of individual fundi to provide multiple numbers of branching points.

For the second "testing" group, 22 fundus images of 11 individuals (7 women; mean age, 26 years; range, 21–32) were analyzed. These 22 fundal images provided 86 evaluable arteriolar branching points.

Table 1 shows the mean and 95% CI for both the arterioles and venules in the "training" group of the BC, AI, angle at vessel bifurcation, and degree of eccentricity. In addition, it shows the mean and 95% CI for the arterioles of the "testing" group.

Training Group

Arteriolar Branching Points. The AI was the only parameter in this group that was significantly associated with the BC

TABLE 1. Study Group Data

	Training Group		**Testing Group**	
	Arteriolar (n = 125)	**Venular (n = 90)**	**Arteriolar Only (n = 86)**	***t*-Test (For Arteriolar Branches)**
Branching coefficient	1.25 (1.18–1.32)	1.22 (1.14–1.3)	1.26 (1.17–1.34)	P = 0.86
Asymmetry index	0.74 (0.72–0.78)	0.75 (0.71–0.78)	0.78 (0.75–0.81)	P = 0.18
Angle (deg)	79 (76.2–82.5)	75.8 (72.5–79.1)	81.2 (77.9–84.5)	P = 0.46
Degree of eccentricity (disc diameters)	2.04 (1.9–2.19)	2.4 (2.18–2.62)	1.58 (1.42–1.75)	P = 0.0001*

Data show the mean branching coefficient, asymmetry index, angle between the two branches, and degree of eccentricity for the arteriolar branching points of both the "training"and "testing"groups, as well as the venular branching points of the "testing"group. Data in parentheses are ranges. n = Number of branching points.

* $P < 0.05$.

(R = 0.275, P = 0.002). A stepwise linear regression model was constructed with the BC as the dependent variable and the AI as the independent variable (Fig. 1: BC = 0.78 + 0.63 · AI; P = 0.002, N = 125.

Neither the degree of eccentricity (R = 0.022, P = 0.81) nor the angle (ω) between the two arteriolar vessel branches (R = 0.03, P = 0.75) was associated with the arteriolar BC.

Venular Branching Points. Neither the AI, the degree of eccentricity, nor the angle (ω) was associated with the BC for the venular junctions (R = 0.03, P = 0.76; R = 0.05, P = 0.63; R = −0.59, P = 0.58, respectively).

Testing Group

Because there was no association between the venular BC and any of the variables from the "training" group, only arteriolar vascular junctions were analyzed in the "testing" group. As in the "training" group, the only parameter associated with the BC was the AI (R = 0.22, P = 0.041).

The predictive ability of the regression model for the BC formed from the "training" group to determine the trunk diameter from the two branch vessel diameters was compared with the constant BC of Knudtson et al.[13] (1.28), as well as the theoretical formula of the BC by Zamir.[16] This predictive ability of the three models for the BC was compared by using a least-squares technique.

The mean difference between the calculated and measured arteriolar widths for the three different formulas to predict the trunk diameter from the two branch diameters is summarized in Table 2 below (mean arteriolar trunk width = 15.56 pixels).

A subanalysis was performed on only those trunk blood vessels that were greater than 15 pixels in diameter, as these may more accurately reflect the larger vessels that are measured to calculate the CRAE, CRVE, and AVR in epidemiologic studies. In total, 49 trunk vessels measured greater than 15 pixels in diameter. The mean difference (in pixels) between the calculated and measured vessel widths using the least-squares technique was 2.57 for the linear regression model, 2.67 for the constant BC, and 4.83 for the Zamir formula.

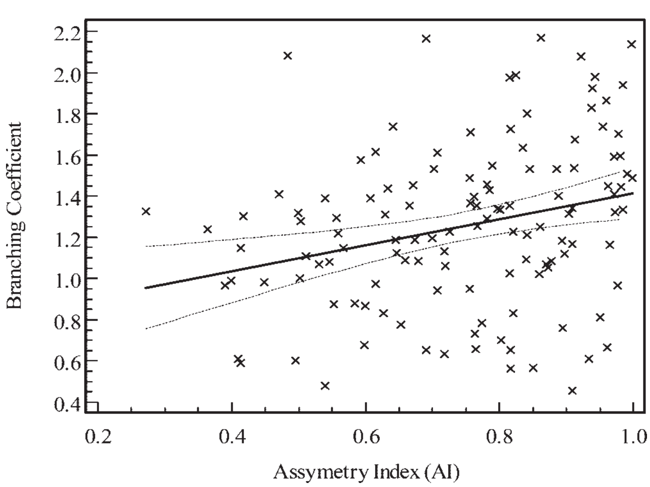

FIGURE 1. A linear regression model of BC versus AI for arteriolar junctions. BC = 0.78 + 0.63 · AI; P = 0.002, N = 125.

DISCUSSION

This study shows that the BC of both retinal arterioles and venules varies greatly, even in a young, healthy, normotensive population. Although our mean BC for retinal arterioles closely matches both the calculated value by Knudtson et al.[13] and the theoretical optimum value for a symmetrical dichotomous vessel junction, a better model of fit for the observed values of the BC can be achieved by relating it to the degree of asymmetry of the two vessel branches. This linear regression model equation was then tested on a different, independent dataset that was not used to derive the calculated formula, and we found the formula to be slightly superior, both to the formula using a fixed BC of 1.28 and to the theoretical formula devised by Zamir,[16] which relates the BC to the angle between the two vessel branches.

This relationship of the BC to the AI was also observed by Parr and Spears,[5] when they developed their empiric formula to calculate the trunk arteriole from retinal branch arterioles. However, rather than try to alter the BC with this observation, they developed a new formula that required a conversion of measured units into micrometers (necessary because of the presence of a constant within their formulated equation). Knudtson et al.[13] pointed out the limitations of this approach, and the distinct advantages of using a dimensionless parameter such as the BC. However, we believe that rather than describe the BC as a constant value, relating it in terms of the AI may improve its accuracy in predicting arteriolar trunk vessel widths from the respective branches. This approach retains the advantages of having a dimensionless BC, as the AI is a ratio and therefore remains dimensionless. In addition we deliberately used a different camera set-up, degree of field of view, and resolution of retinal photographs between both the "training" group that was used to derive the linear model and the "testing" group used to test the formulated model. The calculation of the BC should be independent of all these factors. A relationship between the AI and the BC should also be expected

TABLE 2. Differences in Widths

	Mean Difference between Calculated and Measured Widths	Range of Difference between Calculated and Measured Widths	Standard Deviation of Differences
Linear regression model (BC = 0.78 + 0.63 × AI)	± 2.16	± 0.01–10.58	± 1.83
Constant BC = 1 1.28	± 2.23	± 0.06–11.76	± 1.90
BC = $(2\times(\cos\omega + 1)^{1/2}$	3.43	± 0.08–14.34	± 2.79

Data demonstrate the mean difference, range of difference, and standard deviation of the differences between the calculated and measured arteriolar trunk vessel widths using the three different summarizing formulae. Data are pixel units.

n (branching points) = 86.

from theoretical formulas, as the optimum BC of 1.26 derives from the assumption of a symmetrical, dichotomous branching.

Although the improvement in predictability of the linear regression model in determining trunk arteriolar widths compared with the technique by Knudtson et al.[13] was modest, even a small improvement may have significant advantages in the ability to detect cardiovascular associations with retinal vascular geometrical measurements such as the AVR in larger epidemiologic studies. Although the difference between the linear regression model and the technique by Knudtson et al. was not statistically significant ($P = 0.287$), power calculations ($\alpha = 0.05$, $1 - \beta = 0.8$) estimate a sample size of approximately 11,000 to be able to detect this difference as significant. The linear regression model developed in this study improves on the predictability of trunk arteriolar widths by approximately 3% when compared with the method described by Knudtson et al., who showed that their revised calculations for the AVR led to tighter CIs for cardiovascular associations, when compared with the original Parr-Hubbard formula. Further large epidemiologic studies are needed to determine whether this revised BC model is able to reveal smaller associations between retinal vascular changes and cardiovascular disease.

We chose a much larger field of view than either Knudtson et al.[13] or Parr and Spears,[5,6] because we also wished to explore whether the degree of eccentricity of the retinal junctions influenced the calculated BC. Although there was a significant difference between the "training" group and the "testing" group in degree of eccentricity of the arteriolar junctions, no relationship between the degree of eccentricity and BC was found. Thus, we can conclude that the calculated BC from peripheral arteriolar junctions is no different from more central arteriolar junctions. In addition, we performed a subanalysis on only the larger arterioles (>15 pixels) to confirm that the appropriateness of the linear regression model over a constant BC of 1.28 persists in this group. Thus, this model appears valid in calculating the AVR using only the six largest arterioles and venules within 0.5 to 1.0 disc diameters of the edge of the optic disc, as recommended by Knudtson et al.

Of the three BC-based formulas tested in this study, the principle based on the theoretical formula by Zamir,[16] using the angle between the two branch vessels, was the least accurate. We found no association between the angle between the two branches and the BC for either arterioles or venules. Zamir's formula has been proposed as an optimal system of vessel branching designed to minimize drag in a vascular network. Our findings suggest that the optimal principle of minimum work (minimum energy requirements) across the retinal vascular network as devised by Murray plays a more prominent role in a healthy, young, normotensive population.

It is interesting that there was no relationship between the BC of venules and the AI. This finding suggests that venules may exhibit different optimal principles than arterioles, possibly due to their different physiological function, although this is not entirely clear. Our mean venular BC (1.22) was also different from the mean BC calculated by Knudtson et al. (1.11).[13] The lack of association between the BC and AI for venules also emphasizes that there is no intrinsic mathematical relationship between these entities that makes association inevitable, thus providing further evidence of the significance of the arteriolar association.

Based on our findings, we would recommend calculating the CRAE using the same methodology as Knudtson et al.,[13] except incorporating our linear regression model for the BC, rather than a constant. For the CRVE, as no relationship exists between the AI and the BC, we recommend using a constant BC as before.

In determining the degree of eccentricity of the retinal vascular junctions, this study makes the assumption that the average optic disc size between individuals approximates to the same to measure in terms of optic disc diameters. It also assumes that in peripheral vascular junctions, the greater degree of ocular curvature does not unduly influence the relative widths between the vessel trunks and branches. In addition, a limitation of the study is the assumption that all the individuals photographed were normotensive, as no blood pressure measurements were taken. It is possible that some of the subjects had undiagnosed hypertension or other cardiovascular disease.

In conclusion, we describe a new equation to calculate the BC of retinal arterioles in terms of the asymmetry of the two vessel branches, based on an empirically derived linear regression model. In a dataset of retinal photographs from a young, healthy, normotensive population that was not used to derive the new formula, the model appeared superior to using a constant value for the BC. This further revision to summarizing retinal arteriolar widths may improve our ability to detect smaller associations between cardiovascular disease and retinal vascular network geometry.

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