Retinal Vascular Geometry Predicts Incident Retinopathy in Young People With Type 1 Diabetes

A prospective study from adolescence

OBJECTIVE—To examine the association between retinal vascular geometry and subsequent development of incident retinopathy in young patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS—A prospective cohort study was conducted of 736 people with type 1 diabetes aged 12 to 20 years, retinopathy-free at baseline, attending an Australian tertiary care hospital. Retinopathy was determined from seven-field retinal photographs according to the modified Airlie House Classification. Retinal vascular geometry, including length/diameter ratio (LDR) and simple tortuosity (ST), was quantified in baseline retinal photographs. Generalized estimating equations were used to determine risk of retinopathy associated with baseline LDR and ST, adjusting for other factors.

RESULTS—After a median 3.8 (interquartile range 2.4–6.1) years of follow-up, incident retinopathy developed in 287 of 736 (39%). In multivariate analysis, lower LDR (odds ratio 1.8 [95% CI 1.2–2.6]; 1st vs. 4th quartile) and greater ST (1.5 [1.0–2.2]; 4th vs. 1st quartile) predicted incident retinopathy after adjusting for diabetes duration, sex, A1C, blood pressure, total cholesterol, and BMI. In subgroup analysis by sex, LDR predicted incident retinopathy in male and female participants (2.1 [1.1–4.0] and 1.7 [1.1–2.7]; 1st vs. 4th quartiles, respectively) and greater arteriolar ST predicted incident retinopathy in male participants (2.4 [1.1–4.4]; 4th vs. 1st quartile) only.

CONCLUSIONS—Lower arteriolar LDR and greater ST were independently associated with incident retinopathy in young people with type 1 diabetes. These vascular geometric parameters may serve as risk markers for diabetic retinopathy and provide insights into the early structural changes in diabetic microvascular complications.

The retina offers a unique opportunity to noninvasively and repeatedly examine the microvasculature in vivo. Improved imaging techniques and advances in computer-based retinal image analysis have allowed a better understanding of retinal vascular parameters and their relationship with pathophysiologic processes in and beyond the retina (1,2). Diabetic retinopathy (DR) is the most common microvascular complication in individuals with diabetes and is a risk indicator of other complications, with mild retinopathy being associated with several-fold increases in the risk of stroke, coronary heart disease, and heart failure independent of other risk factors (3). A better understanding of DR may allow prevention and early treatment of those at risk for vision loss and life-threatening cardiovascular disease.

Hypothetically, optimal retinal microvascular architecture will deliver the most efficient blood flow (4). Alterations in retinal architecture may reflect early vascular dysfunction, concurrent compensatory mechanisms, and predict later disease (5). Indeed, subtle variations in retinal vessel caliber have been associated with cardiovascular risk factors (6) and, more recently, diabetic retinopathy and nephropathy (7,8). Some diabetes studies, however, have highlighted the variable nature of retinal caliber with ambient glycemia (9). Thus, geometric parameters, such as vessel simple tortuosity (ST), which reflects the undulation of a vessel along its course, and length/diameter ratio (LDR), which uses the axial length between two defined branching points divided by the vessel’s diameter, may be more robust predictors of diabetic microvascular and macrovascular complications.

We previously demonstrated in a cross-sectional analysis that an adverse diabetes risk profile (higher A1C and systolic blood pressure) is associated with lower retinal arteriolar LDR and greater tortuosity in young people with type 1 diabetes (10). We therefore hypothesized that changes in LDR and ST might predict incident retinopathy and conducted this study to prospectively assess the association between retinal vascular geometric parameters (LDR and ST) and incident retinopathy in young patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS

Study population and assessment

A total of 951 adolescents aged between 12 and 20 years with type 1 diabetes of at least 2 years’ duration, who were...
retinopathy-free at baseline and had a minimum of two assessments through the Diabetes Complications Assessment Service at the Children’s Hospital at Westmead between 1990 and 2007, agreed to participate in the study. All participants had at least a 2-year study follow-up from the baseline visit. Of these, 736 (77%) had baseline photographs suitable for retinal vascular geometry grading and were included in this longitudinal study. The hospital ethics committee approved the study, and written informed consent was obtained from all participants.

Participants were prospectively followed up using standardized interviews, clinical examinations, and laboratory investigations at baseline and at subsequent visits, as previously described (10). Briefly, BMI (kg/m²) was assessed and blood pressure (BP) was measured with a sphygmomanometer using an appropriately sized cuff in seated patients after 5 min of rest. Venous blood samples were obtained for measurement of A1C and total cholesterol levels.

Definition of retinopathy
Mydriatic seven-field stereoscopic fundal photography of both eyes was performed using a TRC 50-VT Topcon Fundus photography of both eyes was performed initially with Mydriatic seven-minute cuff in seated patients after 5 min of rest. A manometer using an appropriately sized sure (BP) was measured with a sphygmomanometer. Blood pressure was calculated as the ratio between the central retinal arteriolar equivalent (CRAE) and the central retinal venular equivalent (CRVE), as previously described. Measurements were based on the biggest six arterioles and six venules within zone B and C. The grader ensured that the vessel type selected by the program was correct. The software then combined the individual measurements into summary indices:

- LDR was calculated as the length from the midpoint of the first branch to the midpoint of the second branch divided by the diameter of the parent vessel at the first branch.
- ST was calculated as the ratio between the actual path length of the vessel segment (measured by tracking) and the straight-line length of the same segment.

Statistical analysis
All statistical procedures were performed using PASW 18.0 software (SPSS Inc., Chicago, IL). Descriptive statistics are presented as mean and SD for normally distributed data or median and interquartile range (IQR) for skewed distributions. Differences between independent samples were evaluated using the Student t test for normally distributed data, or the Mann-Whitney U test or the Kruskal-Wallis test for skewed data. ANOVA was used to determine differences in LDR quartile groups with the Tukey test for post hoc analysis. Differences between groups of categoric variables were compared using the χ² test.

Longitudinal analysis was performed using generalized estimating equations (GEE), with presence or absence of retinopathy at the respective visit as the outcome variable. The use of GEE allowed all visits for each individual to be included in the analysis and accounted for correlations between repeated observations for a given patient. Age; sex; diabetes duration; A1C; systolic BP (SBP); diastolic BP (DBP); BMI SD scores (SDS); total cholesterol; and albumin excretion rate (AER), expressed as log AER, were included in the models as covariates. Baseline arterial and venular ST and LDR values were log-transformed for analysis as continuous variables. ST and LDR were categorized into quartiles to examine for threshold effects. Clinically relevant interaction terms were included in the models. Analysis was stratified by sex in view of sex differences in vessel calibers. Results of significant GEE models are reported as odds ratio (OR) and 95% CI.

RESULTS
Baseline characteristics are reported in Table 1. Median age was 13.7 (IQR 12.9–15.1) years and diabetes duration was 5.0 (3.3–7.6) years. After a median 3.8 (2.4–6.1) years of follow-up with 3.0 (2.0–4.0) visits per participant and 2,745 clinical assessments, diabetic retinopathy developed in 287 of 736 participants (39.0%). Those who developed incident retinopathy were generally younger, had a longer diabetes duration, had higher A1C levels, had higher BP, and were less likely to be following a regimen of multiple daily insulin injections (Table 1). A higher proportion of female subjects (42.1%) developed retinopathy than male subjects (35.8%, P = 0.08).

The lowest quartile for arteriolar LDR (LDRa) had a greater proportion of individuals with incident retinopathy, higher A1C, higher SBP and DBP, and fewer patients receiving intensive insulin therapy (Table 1). Arteriolar caliber did not differ among LDRa quartiles in male patients (F(3,374) = 0.67, P = 0.6). Arteriolar caliber differed among LDRa quartiles in female patients (F(3,374) = 4.68, P = 0.003). Post hoc comparisons among female subjects indicated those in the first and fourth quartile groups had wider arterioles (161.6 and 158.5 µm, respectively) than those in the second and third LDRa quartiles (154.1 and 156.1 µm, respectively; P = 0.003).

Retinal arteriolar and venular calibers were greater in those with longer (>5 years) diabetes duration (158 vs. 154 µm [P = 0.001] and 220 vs. 215 µm [P = 0.004]).
respectively) and in female versus male patients (arterioles: 158 vs. 154 μm [P < 0.001]; venules: 220 vs. 215 μm [P < 0.001]).

**Prospective analysis**

In univariate analysis, lower LDRa (OR 1.9 [95% CI 1.3–2.7]; 1st vs. 4th quartile) and higher arteriolar ST (STa 1.6 [1.1–2.3]; 4th vs. 1st quartile) predicted incident retinopathy. Log LDRa (0.71 [0.51–0.98]) and log STa (×102; 1.01 [1.00–1.02]) predicted incident retinopathy as continuous variables. Other significant risk factors for incident retinopathy included longer diabetes duration and higher BP.

In multivariate analysis, lower LDRa (OR 1.8 [95% CI 1.2–2.7]; 1st vs. 4th quartile) and higher STa (1.6 [1.1–2.4]; 4th vs. 1st quartile) predicted incident retinopathy after accounting for age, sex, diabetes duration, A1C, diastolic blood pressure, BMI, and total cholesterol (Table 2). Log LDRa and log STa did not reach statistical significance in multivariate analysis for the group as a whole.

In subgroup univariate analysis by sex, lower LDRa predicted incident retinopathy in both male and female participants (OR 1.8 [95% CI 1.1–2.9] and 2.0 [1.1–3.7], respectively; 1st vs. 4th quartile). Higher STa predicted incident retinopathy in male subjects (2.1 [1.2–3.6]; 4th vs. 1st quartile) with a similar trend observed in female patients (1.3 [0.8–2.2]; 4th vs. 1st quartile). Log LDRa (0.58 [0.40–0.83]) and log STa (1.02 [1.00–1.04]), as continuous variables, predicted incident retinopathy in male patients. A similar trend in female patients did not reach statistical significance (log LDRa: 0.86 [0.48–1.5]; log STa: 1.01 [0.99–1.02]). Higher A1C, cholesterol, and DBP were significant risk factors for retinopathy in male patients.

**Table 2—LDR and ST predict incident retinopathy in prospective GEE univariate and multivariate models**

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Pts (n)</th>
<th>Univariate</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>Multivariate 1*</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>Multivariate 2+</th>
<th>OR (95% CI)</th>
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<td>736</td>
<td>287</td>
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<tr>
<td>1 (&lt;19.8)</td>
<td>184</td>
<td>92</td>
<td>1.9 (1.3–2.7)</td>
<td>0.001</td>
<td>1.8 (1.2–2.7)</td>
<td>0.003</td>
<td>1.8 (1.2–2.6)</td>
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<td>2 (19.8–65.6)</td>
<td>184</td>
<td>68</td>
<td>1.3 (0.9–1.9)</td>
<td>0.2</td>
<td>1.3 (0.9–2.0)</td>
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<td>3 (65.6–114.2)</td>
<td>184</td>
<td>71</td>
<td>1.2 (0.8–1.8)</td>
<td>0.3</td>
<td>1.3 (0.9–2.0)</td>
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<td>4 (&gt;114.2)</td>
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<td>STa Quartile</td>
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<td>1 (&lt;1,091)</td>
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<tr>
<td>2 (1,091–1,107)</td>
<td>184</td>
<td>76</td>
<td>1.5 (1.0–2.2)</td>
<td>0.03</td>
<td>1.6 (1.1–2.4)</td>
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<td>3 (1,108–1,127)</td>
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<td>69</td>
<td>1.5 (1.1–2.2)</td>
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<tr>
<td>4 (&gt;1,127)</td>
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<td>76</td>
<td>1.6 (1.1–2.3)</td>
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Pts, patients. *Model 1 adjusted for age, sex, and diabetes duration. †Model 2 adjusted for model 1 covariates plus A1C, DBP, BMI, total cholesterol, and relevant interactions (sex*A1C and sex*cholesterol).
CONCLUSIONS—This longitudinal study of initially retinopathy-free young people with type 1 diabetes found that greater ST predicted incident retinopathy in both sexes, independent of established risk factors, including diabetes duration and total cholesterol. Furthermore, there appeared to be sex-specific trends and total cholesterol, BP, and diabetes duration, A1C, total cholesterol, and DBP as significant covariates. Female multivariate model included diabetes duration and BMI as significant covariates.
Higher arteriolar tortuosity found among those with subsequent retinopathy may result from compensatory changes in vessel path due to increased vessel density associated with earlier branching and lower LDR. Furthermore, abnormal vessel wall function with loss of tensile forces and increased intramural pressure may alter vessel integrity increasing tortuosity. These findings contrast with an earlier study in which lower arteriolar tortuosity was associated with ischemic heart disease in nondiabetic subjects (13). However, the proposed mechanism of decreased angiogenesis and impaired oxygenation is not consistent with our hypothesis. That is, increased tortuosity is, at least in part, an adaptation to increased vessel density (increased angiogenesis) to overcome neuroretinal hypoxia. Thus, increased vessel tortuosity may be a marker of greater angiogenesis in the development of retinopathy.

We previously demonstrated that retinal arteriolar dilatation was associated with retinopathy (7). Our present results, which suggest that vessel caliber is greater in those with longer diabetes duration, are consistent with previous studies suggesting a progression in adults with type 2 diabetes from early vasoconstriction to subsequent vasodilatation with increasing diabetes duration (14,15). Retinal vascular smooth muscle cells are crucial in the regulation of retinal blood flow through the action of large-conductance Ca2+-activated K+ channels. These channels show reduced Ca2+ sensitivity during early diabetes, resulting in early sustained vasoconstriction compared with nondiabetic control individuals (16). Subsequent smooth muscle cell and pericyte loss lead to further vasomotor dysregulation, loss of structural support, capillary dropout, and sustained vasodilatation (17). This loss in structural integrity of vessels may also contribute to the observed increase in tortuosity.

We previously described the sex dimorphism of greater retinal vascular calibers in girls (7); however, the mechanisms underlying these differences are not clear. In this cohort, a higher proportion of girls developed retinopathy. Girls generally progress through puberty earlier than boys, and the differential observed may represent accelerated retinal vascular changes in girls. There is increasing evidence that puberty and sex hormones may accelerate microvascular complications (18–20), and some have postulated that younger women with diabetes may be more susceptible to macrovascular and microvascular disease than men (21). Our finding that retinopathy developed in those who had an earlier diagnosis of diabetes is consistent with previous findings that a longer prepubertal diabetes duration increased the risk of retinopathy (18).

The importance of higher DBP, albeit in the normal range, is highlighted in this study because it remained a significant independent predictor of retinopathy, consistent with our previous findings (22). This suggests that early microvascular changes have organ-specific and systemic hemodynamic effects. The greatest resistance in systemic circulation is offered by capillary beds. Early microvascular changes and dysregulation may increase systemic BP, which in turn may lead to further retinal vascular changes in a self-perpetuating cycle. These findings are consistent with previous studies associating greater arteriolar tortuosity with hypertension in young (23) and older (24) age-groups.

The strengths of this study include the longitudinal design, a large patient cohort with multiple visits per individual, standardized quantitative evaluation of retinal vascular measures by a single grader masked to the clinical status, and the use of GEEs to include every available patient visit in the analysis. Repeated LDR and ST measures at each visit would strengthen this study.

Potential limitations include the differences in length of follow-up and number of visits per individual: our median was 3.8 years, and some patients had only 2 years of follow-up; however, the GEE model accounts for uneven follow-up and missing data. The cohort was from a tertiary referral center and may be biased toward closer monitoring, tighter metabolic control, and a potential bias toward under-representation of retinopathy. However, this attests to the robust nature of the measures studied.

Manual intervention by the grader to ensure correct identification of vessels and their precise location was sometimes required by the semiautomated grading program. This introduced the potential for measurement errors to occur. However, because these measurement errors would occur randomly, the positive associations found in this study are unlikely to be significantly affected.

In summary, retinal vascular geometry, specifically retinal arteriolar LDR and tortuosity, may predict incident retinopathy in young people with type 1 diabetes. These noninvasive retinal measures may aid in elucidating early mechanistic pathways for microvascular complications, assist in risk stratification, and offer a potential to monitor the benefit of therapy early in the course of disease. Further studies replicating these results in other populations are necessary to support these novel findings.

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P.B.-A. digitized photos for grading, analyzed data, and wrote the manuscript. M.E.C. advised on statistical analysis, contributed to the introduction and discussion, and reviewed the manuscript. M.B.S. graded photographs, advised on statistical analysis, and reviewed the manuscript. A.J.J. reviewed the manuscript and contributed to discussion. T.Y.W. contributed to study design and methods and reviewed the manuscript. J.J.W. reviewed the manuscript and contributed to discussion. N.C. contributed to methods and reviewed the manuscript. K.C.D. contributed to the study design, introduction, data analysis, and discussion, and reviewed the manuscript.

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Retinal vascular geometry and DR