Retinal Vascular Changes and 20-Year Incidence of Lower Extremity Amputations in a Cohort With Diabetes

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Objective: To examine the association of retinal vascular changes with the incidence of lower extremity amputations (LEAs) in a cohort with diabetes mellitus.

Methods: Baseline examinations were performed in a cohort of 996 persons with diabetes diagnosed before the age of 30 years and using insulin. History of LEA was obtained at baseline and at 4, 10, 14, and 20 years. The cumulative 20-year incidence of first LEA was calculated by the product-limit method in 906 persons with follow-up information. Retinal arterioles and venules were measured on digitized fundus photographs obtained at baseline by a standard protocol. Generalized arteriolar narrowing was defined as the lowest 25% of the arteriole-venule ratio. Measurements were obtained in at least one eye for 820 persons. Focal arteriolar narrowing and arteriovenous nicking were also determined from fundus photographs by a standard protocol.

Results: The 20-year cumulative incidence of LEAs was 9.9%. The unadjusted risk of undergoing a LEA was higher in persons with generalized arteriolar narrowing than in those without it (15.7% vs 5.7%; odds ratio [OR], 3.08; 95% confidence interval [CI], 1.60-5.68), and in persons with focal narrowing (33.1% vs 6.8%; OR, 5.59; 95% CI, 3.27-9.54). The results for arteriovenous nicking were not significant. With control for age, sex, levels of glycosylated hemoglobin, diastolic blood pressure, and history of ulcers of the feet, the relationships were attenuated but still statistically significant for both generalized narrowing (OR, 1.89; 95% CI, 1.06-3.35) and focal narrowing (OR, 3.56; 95% CI, 1.87-6.78).

Conclusion: In persons with diabetes, generalized as well as focal retinal arteriolar narrowing may reflect damage to the microvasculature, which manifests itself elsewhere in the body as a need for LEAs.

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Although to a large degree preventable, lower extremity amputation (LEA) continues to be an important complication of diabetes mellitus. Approximately one half of LEAs are performed in people with diabetes. Several risk factors for needing an LEA include age, male sex, poor glycemic control, and smoking. It is believed that many LEAs are the result of a vascular etiology, which is demonstrated by associations between LEAs and elevated blood pressure and between LEAs and peripheral vascular disease.

The retina provides a direct view of the vascular system, and changes observed in the retinal arterioles may reflect damage occurring elsewhere. Recent studies indicate that retinal arteriolar narrowing is a marker of elevated blood pressure, inflammation, and compromised endothelial function, and an independent predictor of coronary heart disease. We have previously reported on the incidence of and risk factors for undergoing a LEA through 14 years of follow-up. In the present report, we investigate the association of retinal arteriolar changes with the incidence of LEAs in a cohort with younger-onset diabetes and 20 years of follow-up.

Methods

The population studied for this report comprises the younger-onset cohort of the Wisconsin Epidemiologic Study of Diabetic Retinopathy and the Wisconsin Epidemiologic Study of Cardiovascular Disease in Diabetes. Methods of case identification and descriptions of the population have appeared in previous publications. Briefly, 10,135 patients with diabetes were identified in the practices of primary care physicians in an 11-county area in southern Wisconsin. A 2-part sample of this population was invited to participate in the baseline examination between 1980 and 1982. The first part consisted of the entire group of persons who were diagnosed...
as having diabetes prior to the age of 30 years and who were taking insulin (n=1210), and is referred to as the younger-onset group. The second part, the older-onset group, consisted of a probability sample of persons diagnosed with diabetes at or after the age of 30 years regardless of insulin use (n=1780). Of these selected cohorts, 996 younger-onset and 1370 older-onset persons participated in the baseline examination. Surviving younger-onset persons were invited to participate in 4-year, 10-year, 14-year, and 20-year follow-up examinations. Because of attrition owing to mortality in the older-onset group, examinations were discontinued after the 10-year follow-up examination. Therefore, this cohort is not included in the present analysis.

All examinations followed a similar protocol approved by the institutional Human Subjects Committee. Informed consent was obtained after explanation of the procedures. Pertinent parts of the examination included measuring blood pressure, administering a medical history questionnaire, taking stereoscopic color fundus photographs of 7 standard fields, determining urinary protein level, and obtaining a blood sample to determine the level of glycosylated hemoglobin.

The incidence of LEAs was determined by history, and amputations of toes, feet, or legs were included. Traumatic amputations and amputations related to a condition other than diabetes were excluded. Any question concerning whether an amputation was a result of diabetes was resolved by the subject’s physician. Incidence calculation was based on subjects who had not had an amputation at baseline, and the incidence reported is for the first amputation.

The fundus photographs were graded for severity of retinopathy following a modified Early Treatment of Diabetic Retinopathy Study (ETDRS) protocol. Retinopathy severity was based on the severity level in the worse eye and defined as: none (level 0), mild (levels 21-37), moderate (levels 43-53), and proliferative diabetic retinopathy (levels ≥60).

Measurements of retinal vessel caliber were obtained from a recent study of cardiovascular disease in the younger-onset cohort. Briefly, photographs of standard field 1 (as defined by the Diabetic Retinopathy Study) were digitized using a CoolScan LS2000 35-mm film scanner (Nikon Corp, Torrance, Calif) and adapted Atherosclerosis Risk in Communities Study protocols. The vessel diameters were measured by a grader on a computer with a high-resolution monitor using custom software for image manipulation, vessel measurement, and data collection. The diameters of all arterioles and venules traversing the measurement zone surrounding the optic disc were measured. Measurements of individual arterioles and venules in that zone were combined in accordance with the formulas of the Atherosclerosis Risk in Communities Study and summarized as the central retinal artery equivalent and the central retinal vein equivalent. Generalized arteriolar narrowing was characterized by the arteriolar-venular (AV) ratio. The AV ratio is approximately normally distributed in the population and compensates for grader variation and magnification differences between photographs. A previous study has indicated that persons with lower AV ratio are at increased risk for cardiovascular disease. In this study, generalized arteriolar narrowing was defined as being in the lowest quartile of AV ratios.

In addition, the fundus photographs were evaluated for focal arteriolar narrowing and arteriovenous (AV) nicking. Focal arteriolar narrowing and AV nicking were recorded in each quadrant of standard field 1. They were considered absent if at least 2 quadrants were gradable for the conditions (for AV nicking, at least 1 quadrant had to be temporal), and the maximum grade was not higher than “questionable.” Focal narrowing was considered present if the width of the constricted area of an arteriole 50 μm or greater in diameter was two thirds or less the width of its proximal and distal segments in at least 1 quadrant. Arteriovenous nicking, a decrease in the diameter of a venule on both sides of an arteriole crossing it, was graded as “less than” or “greater than or equal to” the decrease in standard photograph 9 of the ETDRS. These grades were combined for the present analysis, and AV nicking was considered present if definitely present in any quadrant. Focal narrowing and AV nicking were deemed present in a person if present in either eye.

All risk factor data included in the analysis were obtained at baseline. Age was defined as age at the baseline examination. Duration of diabetes was the time between date of diagnosis and baseline examination. Hypertension was defined as a systolic blood pressure of 140 mm Hg or higher, a diastolic blood pressure of 90 mm Hg or higher, or a history of hypertension with current use of antihypertension medications. Pulse pressure was defined as the difference between systolic and diastolic blood pressures. Proteinuria was defined as a urinary protein level higher than 0.30 g/L. Body mass index was defined as weight in kilograms divided by the square of height in meters. A history of ulcers of the feet or ankles was defined as a breakdown in the skin caused by diabetic peripheral neuropathy or vascular disease. Subjects were classified as nonsmokers if they reported having smoked less than 100 cigarettes in their lifetime; as ex-smokers if they had smoked 100 or more cigarettes but had stopped smoking before the baseline examination; and as current smokers if they had not stopped smoking. Pack-years smoked was defined as the number of packs (of 20 cigarettes) smoked per day multiplied by the number of years smoked. Glycemic control was defined by glycosylated hemoglobin level measured using a microcolumn technique. The normal range of glycosylated hemoglobin obtained in a similar way for a group of subjects without diabetes (n=381) was 4.6% to 7.9%.

Some participants in the baseline and first follow-up examinations who had not had an amputation did not participate in later examinations because they refused, were lost to follow-up, or died. To compute the 20-year cumulative incidence with all the available information contained in the censored observations, the product-limit method was used. To test for univariate trends in incidence, the Mantel-Haenszel test of non-zero correlation, stratified on the follow-up period, was used. Multivariable analyses were based on the discrete linear logistic model. To examine the effect of missing values on the results, additional models were computed containing indicator variables for missing values about generalized narrowing, focal narrowing, and glycosylated hemoglobin.

Of the 996 younger-onset subjects who were participants at baseline, 891, 765, 634, and 570, respectively, had their 4-, 10-, 14-, and 20-year follow-up examinations. An additional 27, 50, 65, and 82 subjects, respectively, did not have these examinations but provided information concerning LEA by interview at the time of the missed examinations. Among the total population of younger-onset subjects who participated in the 4-year follow-up examination, 906 were at risk for a first LEA and 820 (90.5% of these) had at least 1 eye gradable for vessel caliber measurements. Also, among these 906 subjects, 857 (94.6%) were gradable for focal narrowing and 833 (94.1%) were gradable for AV nicking. Table 1 compares baseline characteristics of these subjects with those of persons who either did not participate in the follow-up or who did not have photographs gradable for vessel caliber measurements. Compared with the study...
subjects, those who were excluded were older, had a longer history of diabetes, higher blood pressure, and a greater body mass, and they were more likely to be men, to have proteinuria, and to have more severe retinopathy at baseline.

In the total group at risk, there were 79 LEAs, for a cumulative 20-year incidence of 9.9% (95% confidence interval [CI], 7.8%-12.0%). The incidence of LEAs was higher in subjects without photographs gradable for vessel measurements (21 LEAs [32.0%]) than in subjects with such photographs (58 LEAs [8.0%]) (P<.001).

Table 2 presents the cumulative 20-year incidence of LEAs by quartile of arteriolar diameter, venular diameter, A/V ratio, severity of diabetic retinopathy, and absence or presence of focal narrowing and AV nicking. The trends in incidence are not statistically significant for the arteriolar or venular diameters, or for AV nicking. However, there was a statistically significant trend for the A/V ratio, with the lowest quartile showing the highest incidence of LEAs. The odds ratio (OR) for generalized arteriolar narrowing (first quartile vs second through fourth quartiles) was 3.08 (95% CI, 1.82-5.21). These results were substantially unchanged after adjusting for age and sex (OR, 2.73; 95% CI, 1.60-4.68) or when analyzing men and women separately (data not shown).

There is also a statistically significant relationship between increasing severity of retinopathy and higher incidence of LEAs (Table 2). This relationship also re-
mains after controlling for age and sex (data not shown). In addition, participants with focal narrowing are at significantly increased risk for a LEA (OR, 3.59; 95% CI, 3.27-9.54). These odds are reduced but remain significant when controlling for age and sex (OR, 3.48; 95% CI, 3.27-9.54). These odds are reduced but remain significant when controlling for age and sex (OR, 3.48; 95% CI, 3.27-9.54). These odds are reduced but remain significant when controlling for age and sex (OR, 3.48; 95% CI, 3.27-9.54). These odds are reduced but remain significant when controlling for age and sex (OR, 3.48; 95% CI, 3.27-9.54).

In univariate analyses, older age, male sex, longer duration of diabetes, higher systolic and diastolic blood pressure, presence of hypertension, higher pulse pressure, higher levels of glycosylated hemoglobin, presence of proteinuria, a history of ulcers of the feet or ankles, and, in subjects 18 years or older, more pack-years smoked were associated with greater incidence of LEAs. Because many of these variables are correlated, a multivariable model was developed to determine which were mutually and independently associated with incidence of LEAs. The resulting set of variables included age, sex, levels of glycosylated hemoglobin, history of ulcers of the feet or ankles, diastolic blood pressure, and pack-years smoked. Controlling for these additional risk factors resulted in the models in Table 3 displaying the associations of generalized narrowing, diabetic retinopathy, and focal narrowing with cumulative 20-year incidence of LEAs. The ORs are reduced compared with the unadjusted ratios or with the ratios simultaneously adjusted for age and sex, but are still statistically significant. When including indicator variables for missing values about generalized arteriolar narrowing and levels of glycosylated hemoglobin, the OR for generalized narrowing became 1.80 (95% CI, 1.05-3.09). Similarly, for focal narrowing the OR became 2.81 (95% CI, 1.59-4.98). The ORs for diabetic retinopathy were nearly unchanged by this procedure (data not shown). Additional adjustment for pack-years smoked among subjects older than 18 years did not further reduce the ORs (data not shown). In model 4 of Table 3, all of the ocular variables were included in addition to the systemic risk factors. In this instance, only diabetic retinopathy remained statistically significant. Generalized narrowing and focal narrowing were no longer statistically significant.

<table>
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<tr>
<th>Characteristic</th>
<th>Grade or Increment</th>
<th>OR (95% CI)</th>
<th>P Value</th>
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<tr>
<td>Model 1</td>
<td></td>
<td></td>
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<tr>
<td>Generalized narrowing</td>
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<td>1.89 (1.06-3.35)</td>
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<td>Diabetic retinopathy</td>
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<td>0.95 (0.39-2.45)</td>
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<td></td>
<td>Moderate</td>
<td>1.59 (0.56-4.53)</td>
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<td>Focal narrowing</td>
<td>Present</td>
<td>3.56 (1.87-6.78)</td>
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<td>Model 4</td>
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<tr>
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<td>2.14 (0.93-4.89)</td>
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Abbreviations: CI, confidence interval; OR, odds ratio; PDR, proliferative diabetic retinopathy.
*Controlling for baseline age, sex, glycosylated hemoglobin, diastolic blood pressure, and history of sores and ulcers on the feet and ankles.

The present study shows that in people with diabetes diagnosed before the age of 30 years, generalized and focal retinal arteriolar narrowing and the presence of diabetic retinopathy are associated with an increased 20-year cumulative risk of LEAs independent of their level of glycosylated hemoglobin and blood pressure, and a history of ulcers of the feet. This suggests that microvascular disease, as reflected in the retina, may be an important predictor of the need for a LEA.

Despite a growing knowledge of the pathogenesis and treatment of diabetes, complications that lead to LEA continue to be a major problem for people with the disease. A substantial proportion of LEAs may be attributed to a vascular, possibly microvascular, etiology. In people with diabetes, those with peripheral vascular disease have a substantially higher risk of undergoing a LEA than those without it.21-23,26,27 High blood pressure has also been implicated as a risk factor for LEA.21,24,25 In the general population, generalized arteriolar narrowing and focal arteriolar changes have recently been shown to be associated with the risk of cardiovascular disease29,31-33 and stroke,44,46 suggesting that changes in the retinal vasculature may reflect adverse microvascular changes occurring elsewhere. Thus, the association of retinal arteriolar narrowing and LEA in people with diabetes supports the hypothesis that systemic microvascular disease may contribute toward risk of LEA. If supported by further research, this finding may have important clinical implications as it suggests that medical therapy targeting the microvasculature may reduce the risk of foot ulcers and LEAs.

However, we found that when also controlling for the level of severity of diabetic retinopathy, the associations of generalized and focal arteriolar narrowing with LEA were no longer statistically significant. This suggests that these parameters provide no more information about the risk of having a LEA than that provided by the retinopathy level—a factor that is routinely assessed in people with diabetes. If there is a small residual effect of generalized or focal arteriolar narrowing, this study does not have the power to detect it.

In addition to the association of LEA with retinal microvascular disease, we confirmed several modifiable risk factors that have been identified from past examinations of this cohort.25,35,36 These include glucose control, blood pressure, and smoking. Earlier publications have extensively discussed these relationships.25,35,36

Serum levels for total and high-density lipoprotein cholesterol were not obtained until the second examination, between 1984 and 1986, and only for approximately one third of the participants. Thus, it is not possible to control for these variables at baseline, and an insufficient number of LEAs were performed following that examination to conduct a reliable analysis. However, results from the Atherosclerosis Risk in
Communities Study indicate that there is no relationship between blood lipid levels and retinal vascular measurements.\textsuperscript{32} Thus, blood lipids are unlikely to be confounders of the association of retinal vascular factors and LEAs.

There are several limitations to this study. First, there were losses to follow-up in the cohort, owing largely to deaths.\textsuperscript{25,35,36} A differential incidence of LEAs in these persons would result in underestimation of the incidence rate. In an earlier publication using a probable-case scenario, we estimated that the cumulative incidence of LEAs could be underreported by as much as 27%.\textsuperscript{23}

Losses to follow-up may also affect the relationships between risk factors and LEAs. However, the risk factors for LEAs are also risk factors for death.\textsuperscript{40-43} Thus, the most likely effect of losses to follow-up is to move the results toward the null hypothesis or to weaken the associations between the risk factors and LEAs.

Most importantly, there is possible bias because some photographs were ungradable for vessel measurements. If such ungradable was related to both the risk factor of interest and LEAs, then exclusion of cases with missing data could influence the magnitude and significance of the relationship between the risk factor and LEAs. We addressed this concern by including these cases in the models, with an indicator variable for the missing data. This allowed us to recover much of the missing data and to examine how this affected the relationship between risk factor and LEAs. That this procedure had little effect on the relationships suggests that the results are not unduly influenced by the fact that data are missing.

The results of this study suggest a link between microvascular changes in the retinal vessels and risk of undergoing a LEA in people with younger-onset diabetes. These results provide evidence that microvascular processes, as reflected in the retina, may contribute to the risk of LEA.

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