Retinal Arteriolar Narrowing and Risk of Diabetes Mellitus in Middle-aged Persons

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Type 2 diabetes mellitus affects up to 15 million persons in the United States and is a leading cause of morbidity and mortality in middle-aged persons. Although the main physiological abnormalities are insulin resistance and hyperglycemia, the specific underlying mechanisms determining these changes remain uncertain. Microvascular disease has been hypothesized to contribute to the development of diabetes. This is based on studies that demonstrate microvascular abnormalities (e.g., impaired microvascular reactivity and flow in the skin and skeletal muscles) in persons with type 2 diabetes and in persons at high risk of developing diabetes, such as those with impaired glucose tolerance and first-degree relatives of persons with diabetes.

However, these studies are cross-sectional investigations among highly selected patient groups, often atypical of the general population. Prospective or population-based data are unavailable, largely because changes in the microcirculation are difficult to evaluate outside of experimental settings. The retinal arterioles offer an excellent opportunity to explore, noninvasively, the prognostic importance of microvascular disease. In the Atherosclerosis Risk in Communities (ARIC) study, we developed a technique to quantify narrowing of the retinal arterioles, based on measuring their diameters on digitized photographs.

We have found retinal arteriolar narrowing to be strongly related to concurrent blood pressure and, independently, to past blood pressure, markers of inflammation, and risk of stroke. In this study, we examined the relation of retinal arteriolar narrowing to diabetes, supporting a microvascular role in the development of clinical diabetes.
incident diabetes in middle-aged persons free of this condition in the ARIC study.

METHODS

Study Population

The ARIC study included 15792 women and men 45 to 64 years of age at recruitment in 1987 through 1989. Population samples were selected from 4 US communities: Forsyth County, North Carolina; Jackson, Miss (black participants only); suburbs of Minneapolis, Minn; and Washington County, Maryland. Participants underwent 3 yearly follow-up examinations, in 1990 through 1992 (93% return rate), 1993 through 1995 (86% return rate), and 1996 through 1998 (81% return rate).

Retinal photographs were taken at the third examination (1993-1995). Of the 12887 participants who returned for this examination, we excluded 38 whose race was neither black nor white, 42 nonwhite residents in Minneapolis and Maryland (to permit stratification by race and field center), 2399 with prevalent diabetes, and 19 with retinal vascular occlusions, leaving 10389 eligible for this study. Of these, 1372 had no retinal photographs or ungradable photographs and 1024 did not return for the fourth examination (1996-1998) or had incomplete data to confirm a new diagnosis of diabetes, leaving 7993 who provided data for this study. Comparisons of characteristics between participants included (n=7993) and excluded (n=2396) indicated that those included were younger (n=7993) and excluded (n=2396) individuals free of this condition in the ARIC study.

Diabetes Mellitus

Methods of ascertainment and diagnosis of diabetes in the ARIC study have been previously published. Participants were asked to fast for at least 12 hours before morning blood collection. Glucose was processed via a modified hexokinase/glucose-6-phosphate dehydrogenase procedure.

Diabetes mellitus was defined if any of the following criteria, adapted from the 1997 American Diabetes Association guidelines, were met: fasting (≥8 hours) serum glucose levels of at least 126 mg/dL (7.0 mmol/L), casual (fasting <8 hours) glucose levels of at least 200 mg/dL (11.1 mmol/L), use of diabetic medications, or physician-diagnosed diabetes. Individuals were defined as having incident diabetes if they did not develop diabetes through the third examination but met any of these criteria at the fourth examination.

Statistical Methods

The AVR was categorized into quartiles (with the first quartile indicating the most severe arteriolar narrowing and the fourth the least) and also analyzed as a continuous variable (per 1-SD difference in AVR). We used analysis of covariance to compare the AVR and its components (summary measures of retinal arteriolar narrowing and diabetes mellitus).
nal arteriolar and venular diameters) between persons who did and did not develop diabetes. We used logistic regression models to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) of incident diabetes, comparing a given quartile of AVR vs the fourth quartile or a 1-SD difference in AVR. We initially adjusted for age (years), sex, race, and field center. In multivariable models, we additionally adjusted for 6-year mean arterial blood pressure, fasting glucose levels, fasting insulin levels, family history of diabetes, BMI, waist-hip ratio, sports and leisure activity indexes, high school education, pack-years of cigarette smoking, alcohol consumption status (ever, never), total and high-density lipoprotein cholesterol levels, and triglyceride levels. In a separate model, we also adjusted for WBC (1000 cells/mm³), plasma fibrinogen, factor VIII, vWF, and carotid IMT, since these are possible confounders.14

We performed the following supplementary analyses. First, we used a higher cutoff (≥141 mg/dL [7.8 mmol/L]) for the fasting glucose value required for the diagnosis of diabetes (alternate diabetes definition). Second, we repeated analyses excluding persons with signs typical of diabetic retinopathy (eg, microaneurysms), since these persons may have preexisting diabetes. Third, we evaluated associations separately in persons with and without impaired fasting glucose levels (110-124 mg/dL [6.1-6.9 mmol/L]) at the third examination and tested interactions with other diabetes risk factors by stratification and by inclusion of cross-product terms in the logistic regression.

**RESULTS**

The baseline mean AVR in the population was 0.843 (SD, 0.08; median, 0.844; range, 0.57-1.22). TABLE 1 compares baseline characteristics of persons with retinal AVR falling in the first quartile (0.57-0.79) with those in the fourth (0.91-1.22). In general, persons in the first compared with the fourth AVR quartile were older, more likely to be men and to be black, and, after adjusting for age, sex, race, and field center, more likely to have a poorer diabetes risk profile.

Over a median follow-up of 3.5 years (range, 0.7-5.5 years), 291 (3.6%) persons developed diabetes. The incidence of diabetes increased from 2.4% to 5.2% with decreasing quartiles of AVR (TABLE 2). After adjustment for age, sex, race, and field center, persons in the lowest compared with the highest AVR quartile were twice as likely to develop diabetes (OR, 2.09; 95% CI, 1.47-2.98). This association was attenuated but still significant after additional adjustment for fasting glucose and insulin levels, family history of diabetes, and other risk factors (OR, 1.71; 95% CI, 1.13-2.57). These associations were somewhat stronger when we used an alternate definition of incident diabetes (fasting glucose ≥141 mg/dL [7.8 mmol/L] used as the cutoff) (Table 2).

When persons with signs of diabetic retinopathy (n=382) were excluded, the association was similar (OR, 1.75; 95% CI, 1.15-2.66, comparing the first with the fourth AVR quartile). Further adjustment for WBC, plasma fibrinogen, factor VIII, vWF, and carotid IMT in the multivariable model resulted in a slightly stronger association (OR, 1.98; 95% CI, 1.15-3.41, comparing the first with the fourth AVR quartile).

The results of AVR analyzed as a continuous variable are presented in TABLE 3. Each 1-SD decrease in the AVR (a decrease of 0.08) in the total sample

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**Table 1. Baseline Characteristics, by Quartile Extremes of AVR**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Crude</th>
<th>Adjusted†</th>
<th>Adjusted‡</th>
<th>Adjusted§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Population (n = 7993)</td>
<td>First AVR Quartile</td>
<td>Second AVR Quartile (Range: 0.57-0.79)</td>
<td>Third AVR Quartile (Range: 0.80-0.84)</td>
<td>Fourth AVR Quartile (Range: 0.85-0.90)</td>
</tr>
<tr>
<td>Age, mean (SE), y</td>
<td>59.4</td>
<td>59.7 (0.12)</td>
<td>59.5 (0.12)</td>
<td>59.4 (0.12)</td>
</tr>
<tr>
<td>Men, %</td>
<td>43.3</td>
<td>51.8</td>
<td>44.8</td>
<td>41.6</td>
</tr>
<tr>
<td>Black, %</td>
<td>17.1</td>
<td>21.2</td>
<td>19.4</td>
<td>15.2</td>
</tr>
<tr>
<td>High school graduate, %</td>
<td>84.3</td>
<td>84.7</td>
<td>83.6</td>
<td>85.4</td>
</tr>
<tr>
<td>Family history of diabetes, %</td>
<td>21.6</td>
<td>20.1</td>
<td>21.2</td>
<td>22.1</td>
</tr>
<tr>
<td>Fasting glucose, mean (SE), mg/dL§</td>
<td>98.1</td>
<td>99.1 (0.22)</td>
<td>98.2 (0.22)</td>
<td>98.0 (0.22)</td>
</tr>
<tr>
<td>Fasting insulin, mean (SE), µU/mL</td>
<td>9.9</td>
<td>10.5 (0.16)</td>
<td>10.3 (0.16)</td>
<td>9.6 (0.16)</td>
</tr>
<tr>
<td>Blood pressure, mean (SE), mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>122.2</td>
<td>128.5 (0.37)</td>
<td>123.9 (0.37)</td>
<td>120.4 (0.37)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>71.6</td>
<td>75.2 (0.21)</td>
<td>72.6 (0.21)</td>
<td>70.7 (0.21)</td>
</tr>
<tr>
<td>Body mass index, mean (SE), kg/m²</td>
<td>27.9</td>
<td>28.7 (0.11)</td>
<td>28.1 (0.11)</td>
<td>27.6 (0.11)</td>
</tr>
<tr>
<td>Waist-hip ratio, mean (SE)</td>
<td>0.93</td>
<td>0.94 (0.001)</td>
<td>0.94 (0.001)</td>
<td>0.93 (0.001)</td>
</tr>
<tr>
<td>Sports activity index, mean (SE)</td>
<td>2.57</td>
<td>2.53 (0.02)</td>
<td>2.53 (0.02)</td>
<td>2.63 (0.02)</td>
</tr>
<tr>
<td>Cigarette smoking, current, %</td>
<td>16.6</td>
<td>18.2</td>
<td>17.4</td>
<td>15.5</td>
</tr>
<tr>
<td>Alcohol use, current, %</td>
<td>57.2</td>
<td>60.1</td>
<td>57.6</td>
<td>56.2</td>
</tr>
</tbody>
</table>

**Notes:**

*AVR indicates arteriole-to-venule ratio. See “Methods” section for definitions of characteristics.

†Values are adjusted for age, sex, race, and field center (except for age, men, and black, which are unadjusted for age, sex, and race, respectively) using analysis of covariance.

‡Represents overall difference among AVR quartiles.

§To convert values for fasting glucose to mmol/L, multiply values by 0.0555.
was independently associated with a 26% increase in risk of diabetes (OR, 1.26; 95% CI, 1.09-1.46). This association was generally similar in people stratified into “high-risk” and “low-risk” baseline characteristics, being possibly stronger in black than in white participants and in persons with lower BMIs and waist-hip ratios. Analysis repeated using the logarithmic transformation of AVR did not improve the fit of the models presented (data not shown).

Focal signs of hypertensive retinopathy, such as arteriovenous nicking and focal arteriolar narrowing, were not related to incident diabetes (adjusted OR, 0.94; 95% CI, 0.65-1.35, and adjusted OR, 1.12; 95% CI, 0.78-1.59, respectively).

**COMMENT**

In this prospective cohort study of middle-aged persons without diabetes, retinal arteriolar were significantly narrower in persons who subsequently developed diabetes during the ensuing 3.5 years compared with those who did not. After controlling for fasting glucose level and insulin level 6 years prior, family history of diabetes, blood pressure, adiposity, physical activity, and other known risk factors, retinal arteriolar narrowing (as reflected by a lower AVR) was independently associated with in-
creased risk of diabetes. This association persisted with different diabetes definitions, and was seen even among people at lower risk of developing this condition, including those without a family history of diabetes, those without impaired fasting glucose, those physically more active, and those with lower measures of adiposity.

Retinal arteriolar narrowing is a marker of microvascular damage from aging, hypertension, inflammation, and other processes.11 It reflects intimal thickening and medial hyperplasia, hyalinization, and sclerosis seen histopathologically.12 Because similar arteriolar changes associated with hypertension are well documented elsewhere in the body,22,23 the retinal arterioles appear to offer insights into the state of the systemic arterioles in health and disease.

Our finding provides prospective clinical evidence to support a key hypothesis in the pathogenesis of diabetes. The microcirculation, estimated using measures of microvascular reactivity and blood flow in the skin and skeletal muscles, is known to be insulin sensitive (i.e., insulin stimulates microvascular dilation and flow).24 Several cross-sectional studies have demonstrated microvascular alterations in persons with type 2 diabetes13 and in those at high risk of developing diabetes, including persons with impaired glucose tolerance, first-degree relatives of persons with diabetes, persons who are obese, and persons with hypertension.25,26 Because these microvascular alterations may result in a reduced ability of insulin to mediate glucose uptake in skeletal muscles, microvascular disease has been suggested to play a causal role in the development of diabetes.2,3 Our study now suggests that arteriolar narrowing precedes the onset of diabetes, and may even play a role in its initial development. This relationship was independent of impaired fasting glucose, a family history of diabetes, and obesity and hypertension.

Our data also offer insights for a number of diverse observations regarding the epidemiology of diabetes. Although hypertension28,29 and cigarette smoking30,31 are related to the risk of diabetes, the underlying mechanisms are unclear. Microvascular alterations (e.g., increased arteriolar resistance and reduced microvascular flow) have also been shown to precede hypertension26,27 and have therefore been hypothesized to explain the excess risk of diabetes in persons with hypertension.32 The risk of diabetes associated with cigarette smoking may be related to inflammation and consequent microvascular injury, since inflammation itself appears to play an important role in the development of diabetes.33,34 We have previously demonstrated that retinal arteriolar narrowing is related to long-term average blood pressure levels33 and independently related to cigarette smoking and markers of inflammation.14 Thus, it is possible that arteriolar narrowing, resulting from hypertension, cigarette smoking, inflammation, and other unmeasured processes, may be a common pathophysiological link to diabetesogenesis.

The strengths of the current study include its population-based design, the quantitative and masked evaluation of retinal arteriolar diameters, standardized identification of incident diabetes cases, and detailed information on risk factors. However, some limitations should be highlighted. First, given the imprecision of a single glucose determination and the relatively short follow-up, misclassification of diabetes may occur. Thus, arteriolar narrowing may be a marker of underlying diabetes in persons not yet meeting the diagnostic criteria. However, this association was independent of fasting glucose, insulin measured 6 years earlier, and other risk factors; persisted with a different diabetes definition; and was observed in persons without signs of diabetic retinopathy (a marker of underlying disease). Moreover, the fact that the associations were largely similar in those at higher and lower risk of diabetes probably minimizes the likelihood of misclassification biases. Second, selection bias may have masked some associations or accentuated others.

Retinal photographs were taken 6 years into the ARIC study, and if persons with arteriolar narrowing at risk of developing diabetes were more likely to die prior to photography, these associations could be falsely attenuated. Third, we have only shown a short-term association between AVR and incident diabetes; further study is required to determine whether longer-term associations exist. Finally, no data on the use of different vasodilator medications were available.

In conclusion, this population-based study documents a prospective association of retinal arteriolar narrowing to incident diabetes mellitus in middle-aged persons, independent of known risk factors. This finding suggests that microvascular processes may play a role in the development of diabetes.

**Author Contributions:** Study concept and design: Wong, R. Klein, Hubbard, Duncan. Acquisition of data: R. Klein, Couper. Analysis and interpretation of data: Wong, Sharrett, Schmidt, Pankow, Couper, B. Klein, Hubbard, Duncan. Drafting of the manuscript: Wong. Critical revision of the manuscript for important intellectual content: Wong, R. Klein, Sharrett, Schmidt, Pankow, Couper, B. Klein, Hubbard, Duncan. Statistical expertise: Couper. Obtained funding: R. Klein. Administrative, technical, or material support: Pankow. Study supervision: R. Klein.

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Health and intellect are the two blessings of life. —Menander (c 342-292 BCE)