Original Article

Retinal Vessel Diameters and Risk of Impaired Fasting Glucose or Diabetes

The Rotterdam Study

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The association between a smaller retinal arteriolar-to-venular ratio (AVR) and incident diabetes may be due to arteriolar narrowing, venular dilatation, or both. We investigated associations between baseline vessel diameters and incident impaired fasting glucose or diabetes in a population-based cohort (aged ≥55 years). Baseline retinal vessel diameters (1990–1993) were measured on digitized images of 2,309 subjects with a normal glucose tolerance test (postload glucose <7.8 mmol/l). At follow-up (1997–1999), impaired fasting glucose was defined as 6.1–7.0 mmol/l and diabetes as ≥7.0 mmol/l and/or antidiabetic medication use. Odds ratios (ORs) per SD increase in venular diameters were 1.13 (95% CI 1.00–1.29) for impaired fasting glucose and 1.09 (0.90–1.33) for diabetes. ORs per SD decrease in arteriolar diameters were 1.12 (0.98–1.27) and 1.08 (0.89–1.31) and per SD decrease in AVR were 1.29 (1.13–1.46) and 1.19 (0.98–1.45). After adjustment for cardiovascular risk factors, the associations were unaltered for venules and disappeared for arterioles. After stratification on age, associations between venular dilatation and impaired fasting glucose (1.23 [1.02–1.47]) or diabetes (1.18 [0.89–1.56]) were mainly present in participants aged <70 years. In conclusion, in our study, the risk of impaired fasting glucose and diabetes with AVR was explained by venular dilatation rather than arteriolar narrowing, warranting more focus on the causes of this dilatation. *Diabetes* 55:506–510, 2006

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AVR, arteriorlar-to-venular ratio.

Epidemiological studies have shown that high BMI, parental history of diabetes, and impaired glucose tolerance are some of the factors involved in the development of diabetes (1,2). Microvascular changes such as impaired reactivity and disturbed blood flow in microvessels have also been hypothesized to contribute to the pathogenesis of diabetes (3,4). Several cross-sectional studies have shown these abnormalities not only in people with diabetes (3) but also in those at high risk of developing diabetes, including people with impaired glucose tolerance or first-degree relatives of diabetic patients (4).

Until recently, prospective population-based data regarding the role of microvascular abnormalities in diabetes were lacking due to difficulties in noninvasively assessing the microcirculation in vivo. A semiautomated system was developed to measure retinal vessel diameters (5). It was suggested that the retinal arteriolar-to-venular ratio (AVR) thus obtained was a marker of generalized arteriolar narrowing and that a smaller AVR was related to incident diabetes (6). Due to the nature of ratio measures like this one, it is difficult to judge whether this relation is due to arteriolar narrowing or venular dilatation. Studying the arteriolar and venular diameters separately may be important for elucidating the microvascular complications of diabetes, such as retinal venular dilatation that has been reported to be present already in the pre-diabetic stage of hyperglycemia (7–9).

Therefore, we investigated in the population-based Rotterdam Study whether the AVR was associated with an increased risk of impaired fasting glucose or diabetes and whether this was explained by retinal arteriolar narrowing, venular dilatation, or both.

RESEARCH DESIGN AND METHODS

The present study was performed as part of the Rotterdam Study, a prospective population-based cohort study (10). A total of 7,983 inhabitants of a district of Rotterdam, aged ≥55 years, agreed to participate in the study (response rate of 78%). Because the ophthalmic part became operational after the screening of randomly invited participants had already started, a smaller number (n = 6,780) underwent the ophthalmic examination (11). The study was conducted according to the tenets of the Declaration of Helsinki, and the appropriate medical ethics committees approved the protocol. Written informed consent was obtained from all participants. Baseline interviews and examinations took place from 1990 to mid-1993, and the follow-up screening occurred between 1997 and 1999.

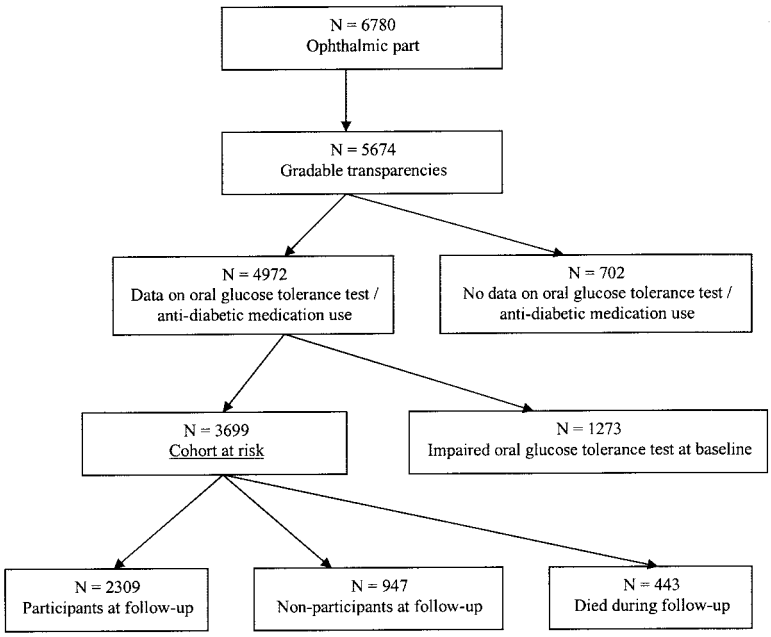

FIG. 1. Flowchart of the study population.

Retinal vessel diameter measurements at baseline. Retinal vessel diameters were measured on fundus color transparencies. These transparencies, covering a 20° field centered on the optic disc, were digitized with a high-resolution scanner (Nikon LS-4000; Nikon, Tokyo, Japan) (12). For each person, the image of one eye with the best quality was chosen and analyzed with a semiautomated system (Retinal Analysis; Optimate, Madison, WI) (Department of Ophthalmology & Visual Science, University of Wisconsin-Madison, Madison, WI) by four trained graders masked for the end points (11). We used the improved Parr-Hubbard-Knudtson formula to compute the summary vessel measures (13) and additionally adjusted for the differences in magnification in case of refractive errors of the eye (14). Sum values were calculated for the arteriolar and venular blood column diameters (in μm), the AVR being the ratio of these. In a random sample of 100 participants, we found no differences between right and left eyes for the arteriolar and venular diameters.

Ascertainment of incident cases of impaired fasting glucose and diabetes. At baseline, a nonfasting blood sample was drawn followed by a 75-g oral glucose tolerance test in participants who were not on antidiabetic medication (15). Two hours later, a second blood sample was obtained. Glucose levels were measured by the glucose hexokinase method (15).

At the follow-up examination, the oral glucose tolerance test was not performed; instead, blood samples were drawn at the research center after overnight fasting (8–14 h). Definitions for incident cases of impaired fasting glucose and diabetes were adapted from the American Diabetes Association guidelines (16,17). For the current study, only subjects who had a normal oral glucose tolerance test (postload glucose value <7.8 mmol/l) and did not use antidiabetic medication at baseline were included. Incident impaired fasting glucose was defined as having a fasting glucose value of 6.1–7.0 mmol/l and incident diabetes as fasting blood glucose value ≥7.0 mmol/l. Also, subjects who started using antidiabetic medication during follow-up were classified as having incident diabetes.

Assessment of confounders. Blood pressure was measured in sitting position at the right brachial artery with a random-zero sphygmomanometer. In the analyses, we used the average of two measurements taken at one occasion. Information on smoking habits was derived from the baseline interview. Smoking was categorized as never, former, or current. BMI was computed as weight (in kilograms) divided by the square of height (in meters) (kg/m^2). Nonfasting serum total cholesterol and HDL was determined by an enzymatic procedure (18). Serum levels of C-reactive protein were determined by the rate near infrared particle immunoassay method (Immage high sensitive C-reactive protein; Beckman Coulter). Atherosclerotic plaques, assessed by ultrasound at the bifurcation, common, and internal carotid artery on both sides, were defined as focal thickening of the vessel wall (of at least 1.5 times the average intima-media thickness) relative to adjacent segments with or without calcified components. The carotid plaque score (range 0–6) reflected the number of these locations with plaques (19).

Study sample. Of 6,780 participants in the ophthalmic part of the baseline study, 5,674 had gradable fundus transparencies for retinal vessel measurements. Data on glucose tolerance test and antidiabetic medication were available for 4,972 individuals. Of these, 3,699 participants had a normal glucose tolerance test and did not use antidiabetic medication at baseline and thus formed the cohort at risk for impaired fasting glucose or diabetes. During follow-up, 443 participants died and 947 refused or were unable to participate at the follow-up examination, leaving 2,309 participants for the current analyses (Figure 1).

Statistical analysis. ANCOVA was used to compare baseline characteristics of participants to nonparticipants at follow-up. Arteriolar and venular diameters and AVR were analyzed both in quartiles and linearly (per SD difference) with logistic regression models. Odds ratios (ORs) and 95% CIs were calculated for incident impaired fasting glucose and diabetes. Participants who developed impaired fasting glucose during follow-up were excluded from analyses where diabetes was the outcome, and vice versa. For additional adjustments we included cardiovascular risk factors that we previously showed to be associated with retinal vessel diameters (11) and that also have been implicated in the pathogenesis of diabetes. We adjusted for age, sex, follow-up time, and additionally for other cardiovascular risk factors. We repeated the analyses after stratifying on age. The cutoff value of 70 years was chosen to secure enough cases in both strata. All analyses were performed with SPSS Windows version 11.0 (SPSS, Chicago, IL).

RESULTS

Table 1 shows that nonparticipants were on average older, more often women and smokers, and had higher blood pressures and more plaques in the carotid arteries. People who died during follow-up had a worse cardiovascular risk profile compared with participants. After a mean follow-up time of 6.4 years (range 5.2–9.5), 305 participants developed impaired fasting glucose and 118 developed diabetes. In people who developed impaired fasting glucose, the mean arteriolar diameter at baseline was 146.3 ± 13.5 μm, venular diameter 224.8 ± 20.6 μm, and AVR 0.65 ± 0.05, whereas in those who developed diabetes these values were 146.4 ± 13.5, 224.1 ± 19.8, and 0.66 ± 0.06 μm, respectively. In the remaining participants the mean arteriolar diameter was 147.9 ± 14.2, venular diameter 222.4 ± 19.7, and AVR 0.67 ± 0.06 μm.

The lowest quartile of arteriolar diameters compared with the highest one was neither related to an increased risk of impaired fasting glucose nor to the risk of diabetes (Table 2). However, comparing the highest quartile of venular diameters to the lowest one showed a 46% increased risk of impaired fasting glucose. There was no consistent trend between quartiles of venular diameters

TABLE 1
Baseline characteristics

	Participants	Nonparticipants*	Adjusted differences†‡ (95% CI)	Died§	Adjusted differences†‖ (95% CI)
n	2,309	947		443	
Age (years)	65.0 ± 6.5	68.7 ± 8.1	3.7 (3.1–4.2)#	74.4 ± 8.4	9.4 (8.7–10.1)#
Sex (% female)	57.0	62.0	4.4 (0.6–8.2)#	47.0	−12.3 (−17.7 to −6.8)#
Systolic blood pressure (mmHg)	133.7 ± 20.0	139.4 ± 22.9	3.2 (1.6–4.8)#	143.8 ± 23.5	3.3 (1.0–5.6)#
Diastolic blood pressure (mmHg)	73.3 ± 10.8	74.2 ± 11.4	1.3 (0.4–2.2)#	73.6 ± 12.3	1.1 (−0.2 to 2.3)
Current smoking (%)	21.8	28.2	10.2 (7.0–13.5)#	27.9	13.9 (9.2–18.6)#
BMI (kg/m^2)	26.2 ± 3.4	26.3 ± 3.7	0.0 (−0.3 to 0.3)	25.8 ± 3.7	−0.6 (−0.9 to −0.2)#
Total serum cholesterol (mmol/l)	6.66 ± 1.16	6.68 ± 1.17	0.02 (−0.07 to 0.11)	6.30 ± 1.28	−0.22 (−0.34 to −0.09)#
Serum HDL cholesterol (mmol/l)	1.38 ± 0.35	1.39 ± 0.36	0.01 (−0.02 to 0.03)	1.31 ± 0.36	−0.03 (−0.06 to 0.01)
C-reactive protein (mg/l)	2.42	2.74	0.33 (−0.06 to 0.72)	5.01	2.44 (1.88–3.00)#
Number of carotid artery plaques ≥4 (%)	11.0	17.1	3.9 (1.0–6.8)#	24.0	5.6 (1.5–9.8)#
Retinal AVR	0.67 ± 0.06	0.66 ± 0.06	−0.002 (−0.006 to 0.003)	0.66 ± 0.06	−0.009 (−0.016 to −0.003)#
Retinal arteriolar diameter (μm)	147.7 ± 14.1	146.4 ± 14.4	−0.4 (−1.5 to 0.7)	145.0 ± 15.0	−0.5 (−2.1 to 1.1)
Retinal venular diameter (μm)	222.8 ± 19.9	220.9 ± 20.8	0.1 (−1.5 to 1.6)	221.1 ± 20.5	2.5 (0.3–4.7)#

Data are unadjusted means ± SD or %. *Unable or refused at follow-up. †Age and sex adjusted if applicable. ‡Nonparticipants versus participants. §Subjects who died before the follow-up examination. ‖Deceased subjects versus participants. #Significant ($P < 0.05$).

and the risk of diabetes. Finally, the lowest quartile of AVR compared with the highest one gave a significantly increased risk of 93% for impaired fasting glucose and of 80% for diabetes.

Table 3 shows that each SD decrease in arteriolar diameters increased the risk of impaired fasting glucose by 12%, whereas 1 SD increase in venular diameters resulted in a 13% increased risk. The corresponding nonsignificant increases in risk for diabetes were 8 and 9%. A decrease in the resulting AVR at baseline was associated with significantly increased risk of impaired fasting glucose and borderline significantly with diabetes. For each SD decrease in AVR, the risk of impaired fasting glucose increased by 29% and of diabetes by 19%.

Table 4 shows that after adjusting for other known cardiovascular risk factors, the associations of smaller arteriolar diameters with impaired fasting glucose and diabetes completely disappeared. The strength of the association between larger venular diameters and impaired fasting glucose remained the same, albeit borderline significant. Even for diabetes the nonsignificant OR remained the same.

Stratification on age revealed that the associations of larger venular diameters with impaired fasting glucose or diabetes were stronger in younger and absent in the older participants (Table 5).

TABLE 2
ORs of incident impaired fasting glucose or incident diabetes per quartile of baseline retinal vessel diameters or AVR

Quartiles (range)	Impaired fasting glucose (*n* = 305)	Diabetes (*n* = 118)
Arteriolar diameter (μm)		
4. (156.4–204.7)	1.0 (reference)	1.0 (reference)
3. (147.1–156.3)	1.04 (0.73–1.49)	1.42 (0.82–2.46)
2. (137.8–147.0)	1.29 (0.92–1.82)	1.58 (0.92–2.72)
1. (95.0–137.7)	1.17 (0.82–1.66)	1.29 (0.73–2.28)
Venular diameter (μm)		
1. (135.1–209.0)	1.0 (reference)	1.0 (reference)
2. (208.9–222.1)	1.33 (0.94–1.90)	1.24 (0.73–2.12)
3. (222.0–235.4)	1.20 (0.84–1.72)	0.91 (0.52–1.62)
4. (235.3–313.6)	1.46 (1.02–2.08)	1.41 (0.83–2.38)
AVR		
4. (0.70–0.87)	1.0 (reference)	1.0 (reference)
3. (0.66–0.69)	1.37 (0.94–2.00)	1.33 (0.76–2.33)
2. (0.63–0.65)	1.64 (1.13–2.36)	1.30 (0.73–2.30)
1. (0.49–0.62)	1.93 (1.35–2.77)	1.80 (1.05–3.08)

Data are ORs (95% CI) adjusted for age, sex, and follow-up time.

DISCUSSION

Our data provide evidence that retinal venular dilatation rather than arteriolar narrowing explains the associations between smaller AVR and impaired fasting glucose or diabetes. The associations with venular dilatation were more pronounced in the younger participants.

It has been suggested that venular dilatation is one of the earliest changes in the microcirculation of diabetic

TABLE 3
ORs of incident impaired fasting glucose or incident diabetes per SD difference in baseline retinal vessel diameters or AVR

	Impaired fasting glucose (*n* = 305)	Diabetes (*n* = 118)
Arteriolar narrowing*	1.12 (0.98–1.27)	1.08 (0.89–1.31)
Venular dilatation†	1.13 (1.00–1.29)	1.09 (0.90–1.33)
AVR‡	1.29 (1.13–1.46)	1.19 (0.98–1.45)

Data are ORs (95% CI) adjusted for age, sex, and follow-up time. *Per SD decrease in arteriolar diameter. †Per SD increase in venular diameter. ‡Per SD decrease in AVR.

TABLE 4
Multivariable adjusted ORs of incident impaired fasting glucose or incident diabetes per SD difference in baseline retinal vessel diameters or AVR

	Impaired fasting glucose (n = 305)	Diabetes (n = 118)
Arteriolar narrowing*	0.99 (0.86–1.15)	1.02 (0.82–1.27)
Venular dilatation†	1.15 (0.99–1.34)	1.08 (0.86–1.36)
AVR‡	1.14 (0.98–1.32)	1.09 (0.87–1.36)

Data are ORs (95% CI) adjusted for age, sex, follow-up time, diastolic and systolic blood pressure, smoking, BMI, serum total and HDL cholesterol, C-reactive protein, and carotid artery plaque score. *Per SD decrease in arteriolar diameter. †Per SD increase in venular diameter. ‡Per SD decrease in AVR.

patients (20), thought to result from retinal hypoxia and lactate accumulation (20,21). Successful treatment of proliferative diabetic retinopathy with retinal photocoagulation has been shown to be accompanied by a reduction in venular diameters, possibly due to less retinal hypoxia (21). In normoglycemic individuals, it has been observed that administration of intravenous dextrose resulted in retinal venular dilatation (7). In a case-control study, 266 patients with juvenile diabetes had significantly larger venular diameters (396.5 μm) compared with 129 control subjects (368.0 μm) (8). Also, in a population-based cohort study on 996 diabetic patients, larger venular diameters were associated with 4 years' progression of retinopathy (relative risk 4th vs. 1st quartile: 2.3 [95% CI 1.4–4.0]) and with 14-year incidence of proliferative retinopathy (4.3 [1.5–12.2]) (21). Venular and to a lesser extent arteriolar dilatation has also been described in other vascular beds, including the renal and cerebral circulation (22). However, the actual mechanisms underlying venular dilatation remain unclear.

An important limitation is the use of different methods to diagnose diabetes at baseline and follow-up. Because of its inconvenience to patients, the oral glucose tolerance test was not performed at the follow-up examination. Instead, it was decided to use glucose levels after overnight fast. It has been reported that diagnosing diabetes with fasting glucose levels would result in fewer cases than with oral glucose tolerance test, especially in the elderly (23,24). Some participants thus would have obtained a diagnosis of diabetes had an oral glucose tolerance test been performed at follow-up. They might currently have been misclassified as nondiabetic based on their fasting levels. Because retinal vessel measurements and diagnosis of diabetes were assessed independently from each other, this misclassification would have been random, resulting in broader CIs and underestimation of the associations.

TABLE 5
Multivariable adjusted ORs of incident impaired fasting glucose or incident diabetes per SD difference in baseline retinal vessel diameters or AVR stratified on age

	Impaired fasting glucose (cases)	Diabetes (cases)
Age <70 years	231	88
Arteriolar narrowing*	1.00(0.85–1.19)	0.98(0.76–1.28)
Venular dilatation†	1.23(1.02–1.47)	1.18(0.89–1.56)
AVR‡	1.21(1.01–1.45)	1.13(0.86–1.47)
Age ≥70 years	74	30
Arteriolar narrowing*	0.93(0.68–1.26)	1.08(0.71–1.65)
Venular dilatation†	1.02(0.76–1.37)	0.91(0.60–1.37)
AVR‡	0.95(0.70–1.29)	1.00(0.67–1.49)

Data are n or ORs (95% CI) adjusted for age, sex, follow-up time, diastolic and systolic blood pressure, smoking, BMI, serum total and HDL cholesterol, C-reactive protein, and carotid artery plaque score. *Per SD decrease in arteriolar diameter. †Per SD increase in venular diameter. ‡Per SD decrease in AVR.

Another potential limitation of our study is the number of nonparticipants at baseline. As we described previously, there were only small differences in baseline cardiovascular risk factors between individuals with and without gradable transparencies (11). Furthermore, individuals who had an oral glucose tolerance test were hardly different from those without these data with respect to their cardiovascular risk profile (data not shown). These comparisons suggest that selection bias, if any, played a limited role at baseline.

There are several explanations for the fact that the associations were stronger and more significant for impaired fasting glucose than for diabetes. We had three times more cases with impaired fasting glucose than with diabetes. There might be an under representation of diabetic cases in our study population, because nonparticipants at follow-up were significantly older, had higher blood pressures, were more often smokers, and had more carotid artery plaques. As these risk factors also increase the risk of diabetes, the nonparticipants would have more often developed diabetes. Furthermore, those who died during follow-up had an even worse cardiovascular risk profile, including significantly larger venular diameters after adjustment for age and sex. Because those who develop diabetes are more likely to die than those who have normal glucose, it is possible that there is selective nonparticipation of people who had larger venular diameters and developed diabetes.

The difference in associations for impaired fasting glucose and diabetes might also be explained by the decreased capacity of the vessels to dilate at baseline in participants who developed diabetes due to higher age or blood pressures compared with those who developed impaired fasting glucose. In our population, both mean age and blood pressure at baseline were not significantly different between the two groups. What we did find, however, is that although all participants had a normal oral glucose tolerance test at baseline, subjects who developed incident diabetes (6.21 mmol/l) already had a significantly higher postload glucose level at baseline than those who developed impaired fasting glucose (5.98 mmol/l). A higher glucose level could result in an accelerated formation of advanced glycation end products, which can accumulate in various vascular beds including the retinal one leading to vascular wall stiffness and decreased endothelium-dependent vasodilatation (25).

Prospective data from the Atherosclerosis Risk in Communities Study showed that each SD decrease in the AVR was related to a 26% increased risk of diabetes (6). Other retinal arteriolar abnormalities such as arteriovenous nicking (OR 0.94 [95% CI 0.65–1.35]) and focal arteriolar narrowing (1.12 [0.78–1.59]) were not related to diabetes (6). Based on these data, the authors concluded that generalized arteriolar narrowing may play a role in its development (6). More recently, their conclusion was further supported by data from the Beaver Dam Eye Study showing that smaller arteriolar diameters increased the

risk of diabetes (OR per SD decrease in arteriolar diameters: 1.11 [95% CI 1.00–1.23]) (26). In the present study, we confirmed these findings with respect to the AVR. Our data showed that the associations with smaller arteriolar diameters disappeared after additional adjustments, pointing toward the involvement of inflammation and atherosclerosis in the pathogenesis of diabetes. We did not have information on other retinal arteriolar signs (focal arteriolar narrowing or arteriovenous nicking). With respect to venular diameters, we have previously shown that larger diameters were related to markers of atherosclerosis, higher levels of inflammation, and smoking (11). Because these factors also give an increased risk of diabetes, these factors could underlie the associations we have described. Although nonsignificant, the associations with venular dilatation remained unaltered after adjustment for these factors (Table 4). Taken together, both changes in venular and arteriolar diameters may be involved in the pathogenesis of diabetes.

Given that the Atherosclerosis Risk in Communities Study, which found stronger associations between AVR and diabetes (6), studied younger people, we decided to stratify on age. Our data also revealed that these associations were mainly present in the younger participants. In the older participants, venular dilatation was not related to impaired fasting glucose or diabetes probably due to decreased vasodilatory capacity in these individuals.

In conclusion, in our study the association of AVR with impaired fasting glucose and diabetes seemed to be largely explained by venular dilatation. These data support the view that venular changes are present already in the early stages of diabetes. In a clinical setting, venular changes such as dilatation could be assessed using a (semi) automated system to explore the full extent of microvascular abnormalities in diabetes. Further research needs to be conducted to elucidate the actual mechanisms that lead to these alterations.

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REFERENCES

1. Hu G, Lindstrom J, Valle TT, Eriksson JG, Jousilahti P, Silventoinen K, Qiao Q, Tuomilehto J: Physical activity, body mass index, and risk of type 2 diabetes in patients with normal or impaired glucose regulation. *Arch Intern Med* 164:892–896, 2004
2. Chou P, Li CL, Wu GS, Tsai ST: Progression to type 2 diabetes among high-risk groups in Kin-Chen, Kinmen: exploring the natural history of type 2 diabetes. *Diabetes Care* 21:1183–1187, 1998
3. Tooke JE: Microvascular function in human diabetes: a physiological perspective. *Diabetes* 44:721–726, 1995
4. Caballero AE, Arora S, Saouaf R, Lim SC, Smakowski P, Park JY, King GL, LoGerfo FW, Horton ES, Veves A: Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. *Diabetes* 48:1856–1862, 1999
5. Hubbard LD, Brothers RJ, King WN, Clegg LX, Klein R, Cooper LS, Sharrett AR, Davis MD, Cai J: Methods for evaluation of retinal microvascular abnormalities associated with hypertension/sclerosis in the Atherosclerosis Risk in Communities Study. *Ophthalmology* 106:2269–2280, 1999
6. Wong TY, Klein R, Sharrett AR, Schmidt MI, Pankow JS, Couper DJ, Klein BE, Hubbard LD, Duncan BB: Retinal arteriolar narrowing and risk of diabetes mellitus in middle-aged persons. *Jama* 287:2528–2533, 2002
7. Falck A, Laatikainen L: Retinal vasodilation and hyperglycaemia in diabetic children and adolescents. *Acta Ophthalmol Scand* 73:119–124, 1995
8. Skovborg F, Nielsen AV, Lauritzen E, Hartkopp O: Diameters of the retinal vessels in diabetic and normal subjects. *Diabetes* 18:292–298, 1969
9. Grunwald JE, DuPont J, Riva CE: Retinal haemodynamics in patients with early diabetes mellitus. *Br J Ophthalmol* 80:327–331, 1996
10. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA: Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. *Eur J Epidemiol* 7:403–422, 1991
11. Ikram MK, de Jong FJ, Vingerling JR, Witteman JC, Hofman A, Breteler MM, de Jong PT: Are retinal arteriolar or venular diameters associated with markers for cardiovascular disorders? The Rotterdam Study. *Invest Ophthalmol Vis Sci* 45:2129–2134, 2004
12. Wolfs RC, Borger PH, Ramrattan RS, Klaver CC, Hulsman CA, Hofman A, Vingerling JR, Hitchings RA, de Jong PT: Changing views on open-angle glaucoma: definitions and prevalences: the Rotterdam Study. *Invest Ophthalmol Vis Sci* 41:3309–3321, 2000
13. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BE: Revised formulas for summarizing retinal vessel diameters. *Curr Eye Res* 27:143–149, 2003
14. Littmann H: [Determining the true size of an object on the fundus of the living eye] Zur Bestimmung der wahren Grosse eines Objektes auf dem Hintergrund eines lebenden Auges. *Klin Monatsbl Augenheilkd* 192:66–67, 1988
15. Stolk RP, Pols HA, Lamberts SW, de Jong PT, Hofman A, Grobbee DE: Diabetes mellitus, impaired glucose tolerance, and hyperinsulinemia in an elderly population: the Rotterdam Study. *Am J Epidemiol* 145:24–32, 1997
16. Expert Committe on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 26 (Suppl. 1):S5–S20, 2003
17. American Diabetes Association: Diagnosis and classification of diabetes mellitus (Position Statement). *Diabetes Care* 28 (Suppl. 1):S37–S42, 2005
18. van Gent CM, van der Voort HA, de Bruyn AM, Klein F: Cholesterol determinations: a comparative study of methods with special reference to enzymatic procedures. *Clin Chim Acta* 75:243–251, 1977
19. Bots ML, Hofman A, De Jong PT, Grobbee DE: Common carotid intima-media thickness as an indicator of atherosclerosis at other sites of the carotid artery: the Rotterdam Study. *Ann Epidemiol* 6:147–153, 1996
20. Ditzel J, Standl E: The problem of tissue oxygenation in diabetes mellitus: I. Its relation to the early functional changes in the microcirculation of diabetic subjects. *Acta Med Scand Suppl* 578:49–58, 1975
21. Klein R, Klein BE, Moss SE, Wong TY, Hubbard L, Cruickshanks KJ, Palta M: The relation of retinal vessel caliber to the incidence and progression of diabetic retinopathy: XIX: the Wisconsin Epidemiologic Study of Diabetic Retinopathy. *Arch Ophthalmol* 122:76–83, 2004
22. Zatz R, Brenner BM: Pathogenesis of diabetic microangiopathy: the hemodynamic view. *Am J Med* 80:443–453, 1986
23. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183–1197, 1997
24. Wahl PW, Savage PJ, Psaty BM, Orchard TJ, Robbins JA, Tracy RP: Diabetes in older adults: comparison of 1997 American Diabetes Association classification of diabetes mellitus with 1985 WHO classification. *Lancet* 352:1012–1015, 1998
25. Stitt AW: The role of advanced glycation in the pathogenesis of diabetic retinopathy. *Exp Mol Pathol* 75:95–108, 2003
26. Wong TY, Shankar A, Klein R, Klein BE, Hubbard LD: Retinal arteriolar narrowing, hypertension, and subsequent risk of diabetes mellitus. *Arch Intern Med* 165:1060–1065, 2005