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ABSTRACT

Purpose: To examine the association between retinal vessel diameters and retinopathy in participants with and without type 2 diabetes in a Danish population-based cohort.
Methods: The study included 878 persons aged 30 to 60 years from the Inter99 Eye Study. Retinopathy was defined as a presence of one or more retinal hemorrhages or one or more microaneurysms. Vessel diameters were expressed as central retinal artery equivalent diameter (CRAE) and central retinal vein equivalent diameter (CRVE). Multiple linear regression analyses were performed.
Results: Among participants with diabetes, CRAE was 6.3 μm (CI 95%: 1.0 to 11.6, p = 0.020) wider and CRVE was 7.9 μm (CI 95%: 0.7 to 15.2, p = 0.030) wider in those with retinopathy compared to those without retinopathy, after adjusting for age, gender, HbA1c, blood pressure, smoking, serum total and HDL cholesterol. In all participants, CRAE increased with presence of retinopathy (p = 0.005) and with smoking (p = 0.001), and CRAE decreased with hypertension (p < 0.001), high HDL cholesterol (p = 0.016) and age (p < 0.001). Central retinal vein equivalent diameter increased with presence of retinopathy (p = 0.022) and with smoking (p < 0.001), and decreased with higher HDL cholesterol (p < 0.001) and age (p = 0.015). Female gender was associated with wider CRVE (p = 0.029).
Conclusions: Wider retinal vessel diameters were associated with the presence of retinopathy in participants with diabetes, but not in participants without diabetes. The associations between retinal vessel diameters and known retinopathy risk factors were confirmed. These results suggest that information obtained by non-invasive imaging of the interior of the eye can contribute to a better understanding of systemic disease processes.

Introduction

Diabetes is now regarded as an epidemic with the population of patients expected to rise to 380 million in the world by 2025 [1]. The presence of either intraretinal hemorrhages or the localized

outpouchings of capillaries known as microaneurysms defines the lower threshold of retinopathy. Retinopathy is estimated to affect between 1% and 15% of people without diabetes mellitus [2]. There are few epidemiologic data describing the incidence of retinopathy and associated risk factors in non-diabetic populations [3,4]. Previous studies have shown a relationship between wider retinal vessel diameter and retinopathy development in a diabetic population [5–7]. The presence of retinopathy increases the risk of coronary heart disease (CHD) mortality in people with and without diabetes [8].

The retinal blood vessels are accessible to direct non-invasive visualization providing the opportunity to investigate microvascular changes before reaching a stage of proliferative retinopathy.

The development of semi-automatic retinal vessel measurement techniques, in digital fundus photographs, has made it possible to analyze even modest retinal vessel narrowing and dilatation which simple ophthalmoscopy has been unable to detect. This was first published in the Atherosclerosis Risk in Communities Study 20 years ago [9]. Improvements in digital retinal photography and imaging technology can now allow objective documentation of retinal vascular characteristics [10].

The aim of this study was twofold: 1) to examine the associations between retinal vessel diameters and retinopathy in participants with and without diabetes type 2, and 2) to assess the associations between retinal vessel diameters and known retinopathy risk factors in a Danish population-based cohort.

Subjects, materials and methods

Study population

The study population of the Inter99 study comprised an age- and sex-stratified sample of 13.016 participants residing in 11 suburban municipalities of the south-western part of Copenhagen County. The study sample was randomized from the Danish Civil Registration System that comprises all participants permanently residing in Denmark. A total of 6784 participants aged 30–60 years underwent the screening-program at the Research Center for Prevention and Health at Copenhagen University Hospital in Glostrup, Denmark.

A subgroup of 1437 participants was invited for the Inter99 Eye Study and 970 (67.5%) participated in the eye examination. The subgroup was chosen based on the following criteria: 1) an age- and sex-stratified control group polled to match the background population (n = 500), and 2) participants with known or screen-detected diabetes, and participants with a high risk of ischemic heart disease (n = 470). All study participants with missing data or ungradable images were excluded (n = 92). Thus, a total of 878 participants were included in the present study.

The prevalence and associations of retinopathy in participants without diabetes, participants with type 2 diabetes, and participants with impaired glucose regulation in Inter99 Eye Study population was reported elsewhere [4,13,14].

Study procedures

All participants showed up for a clinical examination after an overnight fast. Blood pressure, height, weight, waist and hip circumference, total cholesterol, HDL-cholesterol, triglyceride and plasma glucose were measured and a standard 75 g oral glucose tolerance test was performed. Questionnaires including information on lifestyle, chronic diseases and family history of chronic diseases were collected in all participants. A detailed description of Inter99 Study has been published previously [12].

Fundus photographs were captured using 60° digital grey-scale (red-free) fundus photographs and 60° colour fundus photographs on transparency film centred on the macula and optic disc. A green filter for red-free photographs was used to enhance the sharpness and contrast of the blood vessels. Retinal vessel diameters were measured according to international standards (Vessel Measurement System, IVAN protocol, version 2, University of Wisconsin), using semi-automatic software for vessel measurement developed in Denmark [15].

Absolute distances were calculated assuming a uniform vertical optic disc diameter of 1800 μm. A standard grid containing three concentric circles was placed centred at the papilla on the images. The grader then identified all the arteries and veins between the outer two circles, using red and blue lines for arteries and veins,

respectively, to delineate the borders of the blood vessels, which were defined, in this context, as the edges of the blood column the vessel wall being translucent. The program identified the six largest arteries and the six largest veins and calculated the central retinal artery equivalent (CRAE) and the central retinal vein equivalent (CRVE) according to the formulas described by Knudtson et al. [16].

We tested the IVAN software against our Danish custom-developed semi-automatic software in 20 red free fundus photographs. We found intraclass correlation coefficients (ICC) of 0.8 (95% CI: 0.5 to 0.9) for CRAE and 0.9 (95% CI: 0.8 to 0.9) for CRVE and there were no significant differences between measurements of CRAE (p = 0.448) and CRVE (p = 0.828). The inter grader reliability between 2 independent masked graders using our Danish program was 0.9 (ICC) for arteries and 0.8 (ICC) for veins, measured on 45 fundus photographs. The mean difference in vessel diameter among the two graders was 3.9 μm (0.024%) for arteries and 6.2 μm (0.025%) for veins.

The photographs were graded for retinal lesions and classified according to the retinopathy severity scale from the early treatment diabetic retinopathy study (ETDRS) [17]. The maximum grade in any of the seven standard photographic fields in any of a subjects two eyes determined the retinopathy level [4].

Definitions

We used the glycaemia thresholds from the World Health Organization (WHO) 1999 criteria [11]. Participants who reported being unaware of having diabetes and were later found to have a fasting plasma glucose concentration ≥7.0 mmol/L or a 2-h plasma glucose concentration ≥11.1 mmol/L were diagnosed as having screen-detected diabetes. Subjects with self-reported diabetes were classified as having known diabetes. Hypertension was defined as blood pressure over 140/90 mmHg.

Retinopathy was present, by definition, in participants where one or both eyes were classified as ETDRS level 15 or higher (retinal hemorrhages present without any definite micro aneurysms or other lesions) [4]. Mild retinopathy or worse was classified as ETDRS level ≥35 (More than just micro aneurysms but less than severe non-proliferative Diabetic Retinopathy (NPDR)).

Statistical analyses

Retinal vessel variables were found to be normally distributed. These variables are therefore presented as means with standard deviations.

Since we had very few cases with moderate to severe retinopathy it was appropriate to include retinopathy as a dichotomous variable (≥15).

First, we compared retinal vessel variables between participants with and without retinopathy. For this analysis we used a two-tailed, unpaired Student's samples *t*-test.

In the second analysis, we studied associations between retinal vessel diameters as dependent variables (CRAE and CRVE) and retinopathy as independent variable using multiple linear regression analyses. The assumptions underlying such analysis were checked, and found to be adequately met (linearity, normal distribution of residuals, and acceptable collinearity using the VIF statistic).

Linear regression analysis was also used to identify possible interactions between selected pairs of independent variables. There was no significant interaction between retinopathy and diabetes status on the association with CRAE and CRVE. However, stratified analyses were performed because of support in biological theories.

For additional adjustments we included cardiovascular risk factors (age, gender, HbA1c concentration, systolic blood pressure, smoking, serum total and HDL cholesterol) which previously have been shown to be associated with retinal vessel diameters, and have been implicated in the pathogenesis of diabetes (Table 2).

Associations between retinal vessel diameters (CRAE, CRVE) and known retinopathy risk factors (age, gender, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, hypertension, smoking, body mass index (BMI), diabetes mellitus and glucose regulation), were tested by multiple linear regression analyses with backward variable selection. Step 1 was to include all the variables that were significant in unadjusted analyses (age, gender, hypertension, total cholesterol, HDL cholesterol, smoking, diabetes, HbA1c, fasting p-glucose, 2-h OGTT p-glucose and BMI). Step 2 was to remove the variable with the highest P value, and to run the model once more. Step 3 was to repeat step 2 until all the remaining variables were significant (Table 3). Statistically, hypertension and antihypertensive treatment were treated as one.

There were no significant interactions between the two ascertainment groups (one control group polled to match the background population and one group with persons with known or newly diagnosed diabetes mellitus and persons with a high risk of ischemic heart disease) and the tested variables, meaning that the associations between retinopathy and risk factors (age, gender, fasting p-glucose and smoking) did not differ between the two ascertainment groups. Therefore, we presented the results of a combined analysis (878 persons) only.

A paired, two-tailed, Student's *T*-test was used when comparing IVAN software with Danish custom-developed semi-automatic software.

The statistical software package SPSS version 18 was used to perform the statistical analysis. A significance level of 0.05 was used throughout.

Results

This study included 878 participants, aged 30–60 years. Among the participants with diabetes (n = 199), 42 (21%) had retinopathy, whereas 57 (8%) had retinopathy among participants without diabetes (n = 679) (Fig. 1). In this study 3 participants had type 1 diabetes, and the 196 had type 2 diabetes.

Clinical characteristics of the study population are shown in Table 1. Participants with diabetes with retinopathy had significantly larger CRAE (p = 0.035), significantly larger CRVE (p = 0.039) and significantly higher fasting plasma glucose (p = 0.017) compared to the participants with diabetes without retinopathy. Participants without diabetes with retinopathy had significantly higher BMI (p = 0.022) compared to participants without diabetes without retinopathy. The prevalence of retinopathy in non-diabetic participants without hypertension was 7.5%, and in participants with hypertension 10.5%, as reported previously [4].

There were no significant interactions between the presence of retinopathy and the presence of diabetes on the effect of CRAE (p = 0.225) and CRVE (p = 0.350).

In the crude analysis there was statistical significant difference in CRAE between non-diabetic participants without and with hypertension (p < 0.001). There was no statistical significant difference in CRVE between non-diabetic participants without and with hypertension (p = 0.921).

Table 1
Clinical characteristics of the Inter99 Eye Study population.

	Total number (n = 878)	[c]All participants			Participants without diabetes			Participants with diabetes		
		No retinopathy (n = 779), 89%	[b]Any retinopathy (n = 99), 11%	p value	No retinopathy (n = 622), 92%	[b]Any retinopathy (n = 57), 8%	p value	No retinopathy (n = 157), 79%	[b]Any retinopathy (n = 42), 21%	p value
CRAE (μm)	878	163.0 (16.0)	166.0 (15.0)	0.101	164.0 (15.6)	165.0 (13.4)	0.338	161.0 (16.8)	167.0 (15.6)	0.035
CRVE (μm)	878	250.4 (21.0)	256.0 (22.3)	0.017	250.4 (20.6)	254.0 (24.0)	0.255	250,3 (21.4)	258.0 (20.3)	0.039
SBP (mmHg)	878	132.4 (17.3)	138.4 (19.1)	0.001	130.5 (16.3)	134.0 (20.2)	0.195	141.3 (18.5)	143.7 (16.7)	0.445
DBP (mmHg)	878	83.8 (11.2)	85.5 (10.8)	0.159	82.7 (11.0)	83.6 (11.0)	0.565	88.4 (11.3)	87.5 (10.2)	0.837
Age (year)	878	47.5 (7.6)	49.8 (7.2)	0.004	47 (7.6)	49 (7.5)	0.066	51 (7.2)	51.3 (6.7)	0.641
Gender (male/female), %	878	48%/52%	56%/44%	0.098	47% /53%	46%/54%	0.847	56%/44%	72%/29%	0.072
BMI (kg/m^2)[a]	878	26.0 (17.8–53.4)	28.4 (17.5–34.8)	0.010	26.1 (17.7–53.4)	27.3 (17.5–34.8)	0.022	29.6 (20.1–46.2)	29.4 (21.1–34.0)	0.210
Total cholesterol (mmol/L)	877	5.7 (1.2)	5.8 (1.1)	0.585	5.8 (1.2)	5.7 (1.5)	0.909	5.9 (1.0)	6.0 (1.1)	0.706
HDL cholesterol (mmol/L)	878	1.3 (0.3)	1.4 (0.4)	0.326	1.4 (0.4)	1.4 (0.4)	0.467	1.2 (0.3)	1.3 (0.4)	0.214
LDL cholesterol (mmol/L)	430	3.6 (1.0)	3.7 (1.0)	0.531	3.6 (1.0)	3.8 (1.1)	0.505	3.6 (1.1)	3.6 (1.0)	0.986
Fasting p-glucose (mmol/L)[a]	878	5.6 (3.0–20.9)	6.0 (4.8–19.7)	<0.001	5.5 (3.0–6.9)	5.6 (4.8–6.8)	0.103	7.0 (3.9–20.9)	7.4 (5.0–19.7)	0.017
2-h OGTT p-glucose (mmol/L)[a]	858	6.8 (2.3–30.6)	7.9 (3.9–25.9)	0.033	6.2 (2.3–11.0)	6.2 (3.9–10.9)	0.676	12.4 (3.9–30.6)	12.4 (4.2–25.9)	0.713
HbA1c (%)	877	6.0 (0.8)	6.4 (1.5)	0.006	5.8 (0.4)	5.8 (0.4)	0.878	6.7 (1.4)	7.2 (1.9)	0.128
Smoking (pack years)[a]	878	4.5 (0.0–112.5)	7.5 (0.0–90.0)	0.080	3.6 (0.0–76.0)	4.6 (0.0–5.5)	0.215	7.6 (0.0–112.5)	14.0 (0.0–90.0)	0.677

Values are given as means (SD) unless otherwise stated.
[a] Values are given as medians (interquartile range).
[b] Grading for retinopathy was available for 878 participants. Ninety-two participants were excluded because fundus photographs were ungradable.
[c] The inclusion of a random sample of the background population made it possible to estimate the prevalence of retinopathy in the general population. We have no reason to think that the associations we found differed between the 2 ascertainment groups as test for interaction between associated variables and ascertainment group were insignificant.

Table 2
Associations between retinal vessel diameters (CRAE and CRVE) and retinopathy.

	All participants, any retinopathy Total number of cases (n = 99)			Participants without diabetes, any retinopathy Total number of cases (n = 57)			Participants with diabetes, any retinopathy Total number of cases (n = 42)		
	Coefficient (μm)	95% confidence interval	P Value	Coefficient (μm)	95% confidence interval	P Value	Coefficient (μm)	95% confidence interval	P Value
CRAE (μm)	4.2	1.1 to 7.2	0.007	2.8	−1.1 to 6.7	0.159	6.3	1.0 to 11.6	0.020
CRVE (μm)	4.9	0.7 to 9.2	0.021	2.4	−3.0 to 8.0	0.382	7.9	0.7 to 15.2	0.030

Retinal vessel diameters (CRAE and CRVE) were used as dependent variables and retinopathy as independent variable in multiple linear regression analyses.
Adjusted for age, gender, HbA$_{1c}$ concentration, systolic blood pressure, smoking, serum total and HDL cholesterol.

Table 3
Association between retinal vessel diameters (CRAE and CRVE) and individual risk factors for retinopathy onset, after multiple linear regression analyses.

Total number N = 878	Central retinal artery equivalent diameter (CRAE), μm		
	Coefficient (μm)	95% confidence interval	P Value
Hypertension (yes/not)	−8.5	−10.6 to −6.4	<0.001
Age (μm/1 year)	−0.3	−0.4 to −0.1	<0.001
Smoking (yes/no)	0.1	0.4 to 0.1	0.001
HDL (mmol/L)	−3.0	−5.5 to −0.5	0.016
Any Retinopathy	4.5	1.4 to 7.6	0.005
	Central retinal vein equivalent diameter (CRVE), μm		
Smoking (yes/no)	0.2	0.1 to 0.3	<0.001
HDL (mmol/L)	−6.0	−10.4 to −3.5	<0.001
Age (μm/1 year)	−0.2	−0.4 to −0.04	0.015
Gender (female)	3.1	0.3 to 6.0	0.029
Any retinopathy	4.7	0.7 to 9.1	0.022

The model initially included the variables: retinopathy, age, gender, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, hypertension, smoking, body mass index, diabetes mellitus and glucose regulation.

When evaluating the association between glycemic indicators (fasting plasma glucose, 2-h plasma glucose and HbA1c) with CRAE and CRVE in participants with diabetes, the association between HbA1c and CRAE was statistically significant (p = 0.005; 95% CI: 0.8 to 4.8), as was the association between HbA1c and CRVE (p = 0.038; 95% CI: 0.1 to 5.6).

Associations between vessel diameters (CRAE and CRVE) and retinopathy after multiple linear regression analyses, adjusted for age, gender, HbA1c, systolic blood pressure, smoking, serum total and HDL cholesterol, are shown in Table 2.

Central retinal artery equivalent (CRAE)

All participants

Multiple linear regression analyses showed that CRAE was 4.2 μm (p = 0.007) wider in participants with retinopathy compared to participants without retinopathy.

Participants without diabetes

CRAE was wider in subjects with retinopathy compared to subjects without retinopathy but not significantly.

Participants with diabetes

CRAE was 6.3 μm (p = 0.020) wider in participants with retinopathy compared to participants without retinopathy.

Central retinal vein equivalent (CRVE)

All participants

Multiple linear regression analyses showed that CRVE was 4.9 μm (p = 0.021) wider in participants with retinopathy compared to participants without retinopathy.

Participants without diabetes

CRVE was wider in subjects with retinopathy compared to subjects without retinopathy but not significantly.

Participants with diabetes

CRVE was 7.9 μm (p = 0.030) wider in participants with retinopathy compared to participants without retinopathy.

Associations between retinal vessel diameters (CRAE and CRVE) and known retinopathy risk factors (age, gender, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, hypertension, smoking, body mass index, diabetes mellitus and glucose regulation) were tested by multiple linear regression analyses with backward variable selection. The results are presented in Table 3. Central retinal artery equivalent diameter increased with retinopathy (p = 0.005) and with smoking (0.001), and CRAE decreased with hypertension (p < 0.001), high HDL cholesterol (p = 0.016) and age (p < 0.001). Central retinal vein equivalent diameter increased with retinopathy (p = 0.022) and with smoking (p < 0.001), and CRVE decreased with high HDL cholesterol (p < 0.001) and age (p = 0.015). Female gender was associated with wider CRVE (p = 0.029).

Discussion

In this large population-based, cross-sectional study of adult Danes aged 30–60 years we have demonstrated a significant association between wider CRAE and wider CRVE and retinopathy in participants with diabetes. We found no significant association between retinopathy and retinal vessel diameters in participants without diabetes. Furthermore, CRAE increased with smoking, and CRAE decreased with hypertension, high HDL cholesterol and age. Central retinal vein equivalent diameter increased with smoking, and CRVE decreased with high HDL cholesterol and age. Female gender was associated with wider CRVE.

We defined retinopathy at the lowest possible threshold, namely as the presence of one or more retinal hemorrhages or one or more microaneurysms. This threshold was chosen as the presence of higher grades of retinopathy is rare among subjects without diabetes [4]. Also a threshold of having any hemorrhages or microaneurysm is more easy to administer in a clinical setting.

We found an association between wider CRAE and retinopathy in participants with diabetes. There may be two possible explanations for this. First, dilated arterioles may be seen as a breakdown of autoregulation in the eyes of participants with diabetes and that
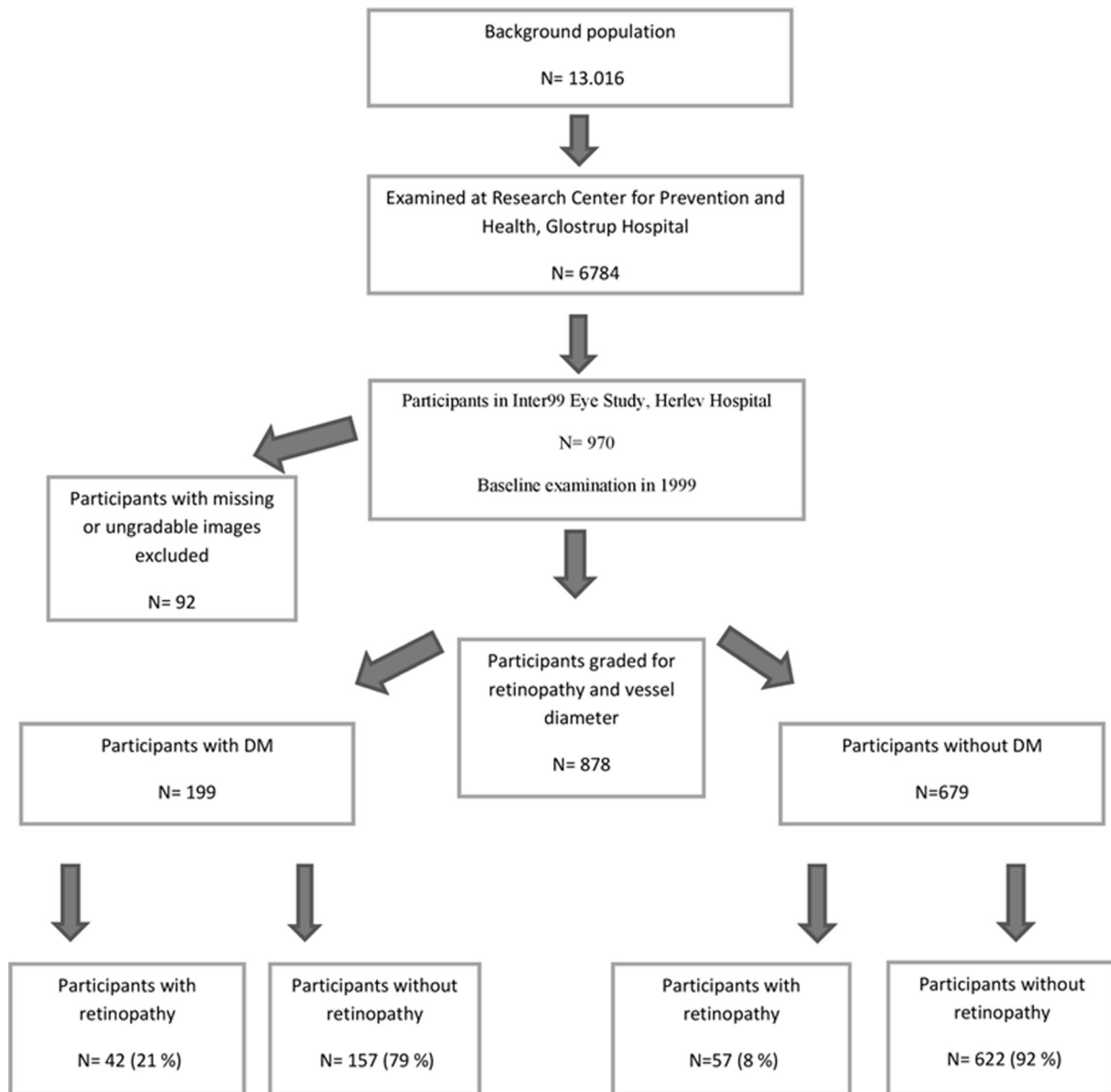
Fig. 1. Flow chart of the Inter99 Eye Study population.

would render them susceptible to incident retinopathy [18]. Second, arterioles could be wider because of peripheral ischemia and the autoregulation is intact which means that they will dilate to increase blood flow. In a few previous studies retinal artery diameter was not associated with development of more severe retinopathy stages [4–7,12,19–24]. On the other hand, in 2004 Klein R. et al. concluded that larger artery calibre independently of retinopathy severity level is related to the progression of diabetic retinopathy [6].

We found an association between wider CRVE and retinopathy in persons with diabetes, which can be explained by the hypothesis that vein dilatation in eyes can be a result of lactic acidosis associated with retinal hypoxia [25]. Others have found that larger vein diameter is an independent indicator of progression to either proliferative diabetic retinopathy or to non-proliferative diabetic retinopathy with high-risk characteristics [7].

Retinopathy and cardiovascular disease share many associated risk factors [8]. In our cohort we confirmed an association between retinopathy and CRAE widening and CRVE widening [26] as well as between smoking and CRAE widening and CRVE

widening [27]. Smoking-induced increase in nitric oxide production, potassium channel activation [28] and possible tissue degeneration might explain retinal arterial and vein dilatation. Higher serum HDL cholesterol and aging were independently associated with CRAE narrowing and CRVE narrowing in our study which is consistent with findings from previous studies [29,30]. High levels of serum cholesterol are associated with an increased risk of cardiovascular diseases [31], diabetic retinopathy [32], but not with retinopathy itself [33]. Therefore, the role of hyperlipidemia in retinopathy is not entirely clear. Thus, it is suggested that more may be learned from a closer inspection of those mechanisms.

Female gender was associated with wider CRVE in our study. Mean age for females in our study was 47 years, meaning that they were moving toward menopause. The role of gender as a contributing factor in retinopathy has long been debated. It appears that any treatment of diabetic retinopathy using sex hormones, or their blockers, may not be a "one size fits all" treatment, but may vary according to the life stage, level of retinopathy and the gender of an individual [34].

Hypertension was independently associated with CRAE narrowing in our study. In the Anglo-Scandinavian Cardiac Outcomes Trial involving 712 hypertensive individuals, despite similar blood pressure levels, persons randomized to receive the calcium channel blocker amlodipine besylate had a smaller arteriole length to diameter ratio, a measure of retinal arteriole narrowing, than those randomized to receive the -blocker atenolol [35].

Maybe lack of adjustment for antihypertensive treatment can explain associations in our study. Population-based studies which used retinal photographs to define signs of retinopathy found signs of retinopathy in 2–14% of non-diabetic population aged above 40 years [18,36–38] in line with the prevalence we found in our population [4]. To the best of our knowledge, there are no published histology studies of non-diabetic retinopathy. Thus, we do not know if non-diabetic retinopathy is a separate entity at the ultrastructural level.

Association of change in CRAE and CRVE with the incidence of diabetic retinopathy raises the question of whether measurement of CRAE and CRVE will detect even earlier clinically meaningful stages of diabetic retinopathy before the onset of microaneurysms and blot hemorrhages [39] . Since changes in retinal vessel diameter reflect a range of subclinical pathophysiologic responses to endothelial dysfunction, one can consider findings from our cohort as a contribution to understanding the etiology of retinal changes. Ophthalmologists may in the future be able to use desktop software, linked to a fundus camera, to measure retinal vessel diameter in the clinic, and then use the additional information to determine which patients will develop retinopathy and therefore be able to set in treatment to prevent it [10].

Strengths of the present study include a population-based, large sample of participants, a computer-assisted measurement of retinal vessel diameters from clear digital fundus images, and a standardized protocol.

There are a few noteworthy limitations. First, unknown sources of variability in vessel measurements cannot be excluded despite high reproducibility of measurements. Second, the fundus photography was not electrocardiograph synchronized, therefore potential variations in vessel diameters may have been introduced because of physiologic variations in cardiac cycle may influence the vessel calibers [25]. Third, dose-response relationship (worse retinopathy-thicker vessel) was impossible to test due to only few cases of moderate retinopathy. Fourth, the cross-sectional design is an important limitation that needs to be mentioned.

Furthermore, the ICC results in our study indicate that retinal vessel diameters calculated by the Danish custom-developed semi-automatic software are comparable with retinal vessel diameters calculated by the IVAN software and have a high inter-rate reliability. The lengths of 95% confidence intervals for the calculated ICC's are relatively short. This indicates that 20 pictures are significant for calculating these ICC's. Thus, the Danish custom developed software provides reliable research tool for objective assessment of structural vascular changes.

In conclusion, we have demonstrated that wider retinal artery and vein diameters were independently associated with the presence of retinopathy in participants with diabetes but not in participants without diabetes. The associations between retinal vessel diameters and known risk factors for retinopathy were confirmed. The possible clinical utility of our findings awaits future research addressing whether a single fundus photograph can be used in the early evaluation of an individual patient's retinopathy risk.

Declaration of competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

DD drafted the manuscript and performed the statistical analyses. ICM graded the retinopathy, revised the manuscript and contributed to the cleaning, analysis and interpretation of data. CG made contributions to the conception and design of the Inter99 Study and has been responsible for all the diabetes related measurements. KF have contributed intellectually to the manuscript. LK made substantial contributions to the conception and design of the Inter99 Eye Study, was involved in patient examination and data management and revised the manuscript. ML made substantial contributions to the conception and design of the Inter99 Eye Study and to the analysis and interpretation of data. NCBBV participated in data collection, measured the blood vessel diameters, did the data interpretation, and statistical analyses. All authors helped to draft the manuscript, and all authors read and approved the final manuscript.

Acknowledgements

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