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Hyperhomocysteinemia Is Associated With the Presence of Retinopathy in Type 2 Diabetes Mellitus

The Hoorn Study

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Background: Retinopathy is the leading cause of blindness among patients with type 2 diabetes mellitus (DM). Hyperhomocysteinemia is a recently recognized risk factor for cardiovascular disease, independent of established risk factors.

Objective: To study the association between the homocysteine level and retinopathy among subjects with and without DM.

Methods: We studied an age-, sex-, and glucose tolerance–stratified random sample of a 50- to 75-year-old general white population in the Hoorn Study (N=625). Retinal vascular changes (retinopathy) were assessed using ophthalmoscopy and/or fundus photography. Hyperhomocysteinemia was defined as a serum total homocysteine level greater than 16 µmol/L.

Results: The prevalence of retinopathy was 9.8% (28/285) in subjects with normal glucose tolerance, 11.8% (20/169) in those with impaired glucose tolerance, 9.4% (10/106) in those with newly diagnosed type 2 DM, and 32.3% (21/65) in those with known type 2 DM. The prevalence of retinopathy was 10.3% (39/380) in subjects without hypertension and 16.3% (40/245) in subjects with hypertension; it was 12.0% (64/534) in subjects with a serum total homocysteine level of 16 µmol/L or less and 16.5% (15/91) in those with a serum total homocysteine level of more than 16 µmol/L. After stratification for DM and adjustment for age, sex, glycosylated hemoglobin, and hypertension, the odds ratio (95% confidence interval) for the relation between retinopathy and hyperhomocysteinemia was 0.97 (95% confidence interval, 0.42-2.82) in patients without DM and 3.44 (95% confidence interval, 1.13-10.42) in patients with DM (P=.08 for interaction).

Conclusion: The findings suggest that hyperhomocysteinemia may be a risk factor for retinopathy in patients with type 2 DM, but probably not in patients without DM.

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DIABETIC retinopathy is the leading cause of blindness among patients with type 2 diabetes mellitus (DM).[1,2] About one third of all cases of impaired visual function in patients with type 2 DM is attributable to retinopathy, the remainder to cataract, glaucoma, macular degeneration, and other causes. Blindness has been estimated to be 25 times more common in persons with DM than in those without DM.[3] After 20 years of having DM, more than 60% of the patients with type 2 DM have some degree of retinopathy.[2] The pathogenetic mechanisms of diabetic retinopathy are incompletely understood.

Diabetic retinopathy seems to be essentially a retinal vascular disorder, probably beginning in the capillary bed. Epidemiological studies have shown that the risk and severity of diabetic retinopathy are strongly related to the duration of DM, hyperglycemia, and hypertension, and also, but less consistently, to hypercholesterolemia and smoking.[2,4-9] Furthermore, there is a close relation between the presence of diabetic retinopathy and the risk of microalbuminuria. Patients with type 2 DM, compared with those without diabetic retinopathy, have about a 2-fold increased risk of developing microalbuminuria.[10,11] Conversely, patients with DM who have microalbuminuria or macroalbuminuria, compared with those with normoalbuminuria, have a higher prevalence and an increased severity of diabetic retinopathy, as well as an increased risk of developing this complication.[12-15] Together these findings suggest that diabetic retinopathy and microalbuminuria have certain pathogenetic mechanisms in common.

Hyperhomocysteinemia is a recently recognized risk factor for cardiovascular disease independent of major cardiovascular risk factors.[16-18] In addition, hyper-

SUBJECTS AND METHODS

STUDY POPULATION

The Hoorn Study, conducted from 1989 to 1992, is a cross-sectional survey of glucose tolerance and other cardiovascular risk factors in a 50- to 75-year-old general white population.[21] A random sample of all men and women aged 50 to 75 years was drawn from the municipal population registry office of Hoorn, the Netherlands; 2484 subjects participated (response rate, 71%). All subjects, except previously diagnosed diabetic subjects with DM who were treated with oral glucose-lowering agents or insulin, underwent a 75-g oral glucose tolerance test and were classified according to the World Health Organization criteria.[22] A second oral glucose tolerance test (participation rate, 93%) was performed, for reasons of efficiency, in a random subsample (n=1122), stratified by 2-hour glucose values of the first test, age, and sex. Finally, from this subsample another age-, sex-, and glucose tolerance–stratified random sample (n=708) was drawn. The presence of retinal vasculopathy (as defined below) was investigated (n=625; response rate, 88%) by 2 experienced ophthalmologists. The examination included both ophthalmoscopy and fundus photography (detailed below). Glucose tolerance was divided into 4 categories on the basis of the mean of the 2 oral glucose tolerance test results: subjects with normal glucose tolerance (n=285), subjects with impaired glucose tolerance (n=169), subjects with newly diagnosed DM (n=106), and subjects with known DM(n=65). The Hoorn Study was approved by the Ethical Review Committee of the University Hospital Vrije Universiteit, Amsterdam, the Netherlands. Informed consent was obtained from all participants.

OPHTHALMOLOGIC INVESTIGATION

Retinopathy was assessed by ophthalmoscopy and/or fundus photography. In each participant, both eyes were dilated with 0.5% tropicamide and 5% phenylephrine hydrochloride eye drops. After an average period of 15 minutes, indirect and direct ophthalmoscopy (N=625) was carried out by 1 of 2 ophthalmologists, and findings regarding the retinal status were reported on standard forms. Thereafter, two 45° standard field, 35-mm black-and-white fundus photographs (Kodak Tri-X 400 ASA; Eastman Kodak, Rochester, NY; Kowa Pro 1 fundus camera; Kowa Optical Industry, Tokyo, Japan) were taken of each eye. Photographs were taken with a green filter (to improve the contrast), centered on the macular area and the optic disc. Fundus photographs of 148 subjects were inadvertently lost. (The fundus photographs were randomly lost with regard to age, sex, hypertension, glucose tolerance category, and serum total homocysteine [tHcy] level of the subjects [data not shown].) Thus, for the present analysis fundus photographs of 477 subjects were available.

Both ophthalmoscopic and fundus photographic findings were graded according to the modified Airlie House classification.[23,24] The fundus photographs were independently graded by 2 ophthalmologists. The independent judgment of a third ophthalmologist was taken to be decisive in case of disagreement about the grading of retinopathy on the fundus photograph. For the present analysis "the worst eye" of each subject according to ophthalmoscopy or fundus photography was used.[24] Any retinopathy (yes/no) was defined as the presence of 1 or more hemorrhages, microaneurysms, soft or hard exudates, neovascularization, and/or laser coagulation scars in 1 or both eyes. Diabetic retinopathy was defined as presence of 1 or more microaneurysms and/or laser coagulation scars in 1 or both eyes (there were no subjects with neovascularization), regardless of other abnormalities.

MEASUREMENT OF SERUM tHcy LEVEL

Fasting blood samples were centrifuged within 1 hour following collection. Serum samples were stored at –20°C for 6 years. cThere is good evidence that serum tHcy levels in frozen samples are stable for 10 years or longer.[25] The serum tHcy (free plus protein-bound) level was measured using tri-*n*-butylphosphine as the reducing agent and ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate as the thiol-specific fluorochromophore, followed by high-performance liquid chromatography with fluorescence detection.[26] The intra-assay and interassay coefficients are 2.1% and 5.1%, respectively.

OTHER MEASUREMENTS

Subjects were classified as either a current cigarette smoker or nonsmoker. The body mass index was calculated as weight

Continued on next page

homocysteinemia seems to be a risk factor for microalbuminuria independent of major determinants, ie, DM, hypertension, protein intake, and renal function.[19] It is unknown whether hyperhomocysteinemia is also associated with and, thus, a possible contributing cause of retinopathy in the general population. We therefore investigated this issue in the 50- to 75-year-old general white population of the Hoorn Study.[20]

RESULTS

Of 5 right and 8 left eyes, ophthalmoscopic findings were missing. In addition to the photographs of 148 subjects that were lost, photographs of 3 right and 4 left eyes were missing. Moreover, because of poor quality, photographs of 18 right and 20 left eyes were ungradable for retinopathy. Thus, ophthalmologic data of 625 subjects were available for further analysis; 76% (477 of 625 subjects) were based on both ophthalmoscopic and fundus photographic findings.

With regard to the fundus photographs, the κ (95% CI) between the ophthalmologists was 0.87 (0.78-0.96) for the right eyes (n=437) and 0.95 (0.89-1.00) for the left eyes (n=428), indicating a good agreement; between ophthalmoscopic and fundus photographic findings, the κ was 0.39 (range, 0.22-0.56) for the right eyes (n=450) and 0.39 (range, 0.21-0.58) for the left eyes (n=443), indicating moderate agreement.

The baseline characteristics of the study population are presented in **Table 1**. The standardized prevalence of any retinopathy was 10.7%. The prevalence was 9.8% (28/285) in subjects with normal glucose tolerance, 11.8% (20/169) in subjects with impaired glucose tolerance, 9.4% (10/106) in subjects with newly diagnosed DM, and 32.3% (21/65) in subjects with known

(in kilograms) divided by height (in meters), squared. Blood pressure (BP) was measured, in total, as the mean of 4 measurements, performed on 2 different occasions, with a random 0 sphygmomanometer under standardized conditions. Hypertension was defined as a BP of 160 mm Hg or more systolic and/or 95 mm Hg or more diastolic and/or the current use of antihypertensive medication. Fasting and 2-hour postload venous plasma glucose levels were measured with a glucose dehydrogenase method (Merck, Darmstadt, Germany) and the glycosylated hemoglobulin (HbA_{1c}) level by ion-exchange, high-performance liquid chromatography, using a DM monitoring system (Modular Diabetes Monitoring System; Bio-Rad, Veenendaal, the Netherlands). The fasting serum total cholesterol level was measured by enzymatic techniques (Boehringer-Mannheim, Mannheim, Germany). Hypercholesterolemia was defined as a total cholesterol level of 6.5 mmol/L or higher (≥251 mg/dL) and/or the current use of cholesterol-lowering medication. The serum creatinine level was measured by the modified Jaffé method. Estimation of the serum creatinine clearance was done according to the formula described by Cockcroft and Gault.[27] Immunospecific insulin was measured in serum by a double-antibody radioimmunoassay (lot SP21; Linco Research, St Louis, Mo). The interassay coefficient of variation was 6%. The lower limit of sensitivity was 12 pmol/L. Ophthalmologic investigations and laboratory measurements were carried out in a masked fashion for glucose tolerance status and other clinical data.

STATISTICAL ANALYSIS

Variables are presented as mean (SD), number (percentage of the total), or, in case of skewed distribution, median and interquartile range. For age, sex, and glucose tolerance category, standardized prevalence of any retinopathy and hyperhomocysteinemia was calculated, as described previously in detail.[18] Briefly, the frequency was determined in 24 strata (age, 3; sex, 2; and glucose tolerance, 4) of the subsample. To assess the standardized prevalence in the original population-based sample (standard, N=2484), the prevalence was calculated from the magnitude of each age, sex, and glucose tolerance category stratum. κ Coefficients were calculated to assess agreement with regard to the presence of retinopathy on fundus photographs between 2 ophthalmologists, and of the presence of retinopathy between fundus photographic and ophthalmoscopic examinations. Differences between subjects with and without any retinopathy were tested using the *t* test or Wilcoxon rank sum test for continuous variables and Pearson χ^2 test for frequency measures. Associations of risk factors for retinopathy with serum tHcy level (logarithmically transformed) were studied by calculation of the Pearson product-moment correlation coefficients.

We performed logistic regression analyses to study the relation between the serum tHcy level and the presence of any retinopathy. The number of cases in the separate classes of retinopathy was insufficient to allow a more detailed analysis. We chose 2 different approaches to investigate the nature of the relation between the serum tHcy level and retinopathy, because it is unclear whether this relation, if any, is linear or has a certain threshold. We calculated odds ratios (ORs) and 95% confidence intervals (CIs) both for the serum tHcy level as a categorical (to allow for a nonlinear dose-response relation and to reduce the influence of outliers) and as a continuous variable, the latter expressed per 5-µmol/L (about 1 SD) increment of serum tHcy level (restricted to the range from the minimum tHcy value to 35 µmol/L). For analysis with the serum tHcy level as a categorical variable, we calculated ORs for the tHcy value divided in 2 (>16 µmol/L vs ≤16 µmol/L) and in 3 categories (≤9.0 µmol/L, 9.1-16.0 µmol/L, and >16.0 µmol/L). To test for trend, the 3 categories were entered in the model as an ordinal variable. We used multiple logistic regression analysis to control for potential confounders, ie, age, sex, glucose tolerance category, known duration of DM of more than 10 years (in the present study a duration effect was seen after 10 years, which is in agreement with other studies[2,5,28]), HbA_{1c} level, and hypertension. To exclude the possibility of residual confounding as thoroughly as possible, we also tested models that, in addition, included serum creatinine level, creatinine clearance, hypercholesterolemia, current smoking, body mass index, and/or fasting insulin level. Possible interaction between the tHcy level and DM for risk of retinopathy was assessed in a stratified analysis and with an interaction term. All analyses were performed using SPSS for Windows 95 version 7.5.2 (SPSS, Chicago, Ill). A 95% CI not including 1.0 was considered to indicate statistical significance.

DM; it was 10.3% (39/380) in subjects without hypertension and 16.3% (40/245) in those with hypertension; it was 12.0% (64/534) in subjects with serum tHcy levels of 16 µmol/L or less and 16.5% (15/91) in those with serum tHcy levels higher than 16 µmol/L. The prevalence of diabetic retinopathy was 4.6% (13/285) in subjects with normal glucose tolerance, 5.9% (10/169) in subjects with impaired glucose tolerance, 4.7% (5/106) in subjects with newly diagnosed DM, and 23.1% (15/65) in patients with known DM. **Figure 1** shows the prevalence of any retinopathy according to absence or presence of hyperhomocysteinemia (>16 µmol/L) in patients with and without DM. We chose this cutoff value since risk of retinopathy increased markedly above this value among subjects with DM (**Table 2**).

The median serum tHcy level was 12.2 µmol/L (interquartile range, 10.0-15.3) in men and 10.7 µmol/L (interquartile range, 9.0-13.3) in women. Serum tHcy levels correlated with age ($r=0.17$; $P<.001$). After adjustment for age and sex, the serum tHcy level correlated with the serum creatinine level ($r=0.4$; $P<.001$), inversely with creatinine clearance ($r=-0.3$; $P<.001$), but there was no substantial correlation between the serum tHcy levels and the following variables: systolic BP ($r=0.06$; $P=.1$), diastolic BP ($r=-0.03$; $P=.5$), body mass index ($r=-0.02$; $P=.7$), fasting glucose level ($r=-0.07$; $P=.07$), fasting insulin level ($r=0.05$; $P=.2$), HbA_{1c} level ($r=-0.02$; $P=.6$), serum total cholesterol level ($r=0.04$; $P=.3$), or duration of DM in subjects with known DM ($r=-0.03$; $P=0.8$).

After adjustment for age and sex, the OR (95% CI) for any retinopathy was 1.37 (1.18-1.59) per percentage of increment of HbA_{1c} level, 1.76 (1.07-2.90) for DM (yes/no), 1.57 (0.97-2.55) for hypertension (yes/no), 1.46

Table 1. Characteristics of the Subjects*

	Subject With No Diabetic Retinopathy (n = 546)	Subjects With Diabetic Retinopathy (n = 79)	P†
Men, %	49.1	41.8	.3
Age, y	64.1 (7.3)	65.7 (6.7)	.06
Body mass index, kg/m^2	27.1 (3.9)	28.0 (4.5)	.07
Current smoker, %	28.7	28.2	.9
Blood pressure, mm Hg			
Systolic	138 (19)	147 (22)	.001
Diastolic	82 (10)	85 (11)	.04
Hypertension, %	37.5	50.6	.03
Glucose tolerance, %			
Normal	47.1	35.4	. . .
Impaired	27.3	25.3	. . .
Newly diagnosed diabetes mellitus, %	17.6	12.7	. . .
Known diabetes mellitus, %	8.1	26.6	<.001‡
Duration of diabetes mellitus, y§	5.9 (2.0-9.5)	9.4 (4.3-16.2)	.02
Fasting glucose level, mmol/L	6.5 (2.3)	7.8 (3.6)	.002
Glycosylated hemoglobin, % hemoglobin	5.8 (1.2)	6.6 (1.8)	<.001
Fasting insulin level, pmol/L	83 (62-116)	93 (72-143)	.01
Total serum cholesterol level, mmol/L	6.6 (1.2)	6.8 (1.2)	.08
Hypercholesterolemia, %	53.5	63.3	.1
Total serum homocysteine level, µmol/L	11.5 (9.3-14.1)	11.1 (9.4-14.0)	.99
Serum creatinine level, µmol/L	91 (17)	94 (30)	.5
Serum creatinine clearance, mL/min‖	75 (17)	74 (19)	.6

*Data are presented as mean (SD), percentage of the total or median (interquartile range). Ellipsis indicates not applicable.

†Tested with t test or Wilcoxon rank sum test for continuous variables and Pearson χ^2 test for frequencies.

‡χ^2 Test for trend.

§Diabetes duration since diagnosis of those subjects with known diabetes mellitus.

‖Estimated creatinine clearance.

(0.89-2.39) for hypercholesterolemia (yes/no), 1.09 (0.64-1.86) for current smoking (yes/no), and (among subjects with known DM) 7.31 (2.69-19.85) for a DM duration of more than 10 years.

After stratification by DM (yes/no) and adjustment for age, sex, HbA_{1c} level, and hypertension, we observed a substantial difference between the 2 strata with regard to relative risk of retinopathy. The OR of retinopathy associated with hyperhomocysteinemia (>16 µmol/L) was 0.97 (95% CI, 0.42-2.82) in subjects without DM and 3.44 (95% CI, 1.13-10.42) in subjects with DM (*P*=.08 for interaction); after additional adjustment for serum creatinine clearance it was 1.01 (95% CI, 0.44-2.33) in subjects without DM and 3.33 (95% CI, 0.99-11.19) in subjects with DM, respectively. The results per category increment of serum tHcy level are shown in Table 2 and **Figure 2** (*P*=.03 for interaction). This indicates that hyperhomocysteinemia is associated with retinopathy in subjects with DM but not in subjects without DM. After adjustment for age, sex, HbA_{1c} level, and hypertension, the OR per 5-µmol/L increment of serum tHcy level was 0.89

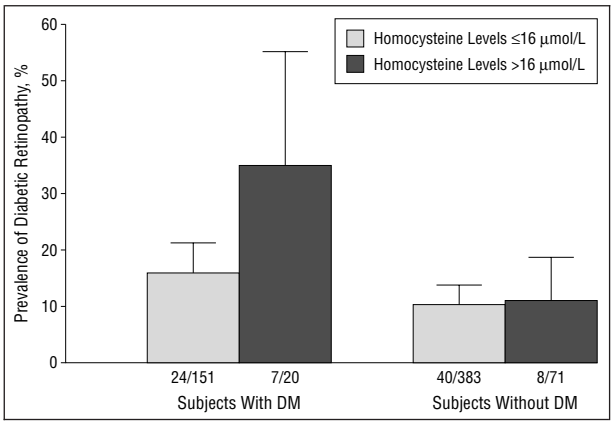

Figure 1. *Prevalence of retinopathy according to the absence or presence of hyperhomocysteinemia (>16 µmol/L) in subjects with and without diabetes mellitus (DM). Error bars represent the upper half of the 95% confidence intervals.*

(95% CI, 0.60-1.34) in subjects without DM and 1.50 (95% CI, 0.93-2.41) in subjects with DM. Additional adjustment for serum creatinine level, serum creatinine clearance, hypercholesterolemia, current smoking, body mass index, and/or fasting insulin level did not markedly change the results (data not shown).

To reduce the effect of possible misclassification, we repeated the analysis after classifying subjects with only hemorrhages in one or both eyes as having no retinopathy in an additional analysis. After adjustment for age, sex, and HbA_{1c} level, the OR for hyperhomocysteinemia was 1.11 (95% CI, 0.46-2.68) in subjects without DM and 5.28 (95% CI, 1.67-16.67) in subjects with DM, and per 5-µmol/L increment of serum tHcy level it was 0.96 (95% CI, 0.62-1.48) and 1.64 (95% CI, 1.00-2.71), respectively. If diabetic retinopathy (see "Subjects and Methods" section) was taken as the dependent variable, the OR for hyperhomocysteinemia was 1.11 (95% CI, 0.36-3.41) in subjects without DM and 4.45 (95% CI, 1.21-16.37) in subjects with DM, and per 5- µmol/L increment of serum tHcy level it was 0.96 (95% CI, 0.55-1.66) and 1.45 (95% CI, 0.84-2.53), respectively. Additional adjustment of the previous analyses for hypertension or BP revealed similar results (data not shown). Among subjects with DM, after stratification for hypertension and adjustment for age and sex, the OR for hyperhomocysteinemia was 6.00 (95% CI, 0.37-96.80) among subjects with normotension and 4.11 (95% CI, 0.95-17.79) among subjects with hypertension. These ORs did not differ significantly from each other, which suggests that the association between hyperhomocysteinemia and retinopathy is independent of the presence of hypertension.

COMMENT

This population-based study indicates that hyperhomocysteinemia is associated with retinopathy in subjects with type 2 DM, independent of known determinants, ie, DM duration, glycemic level, and hypertension. We found a dose-response relation between the serum tHcy level and retinopathy among subjects with type 2 DM (Table 2). For each 5-µmol/L (about 1 SD) increment in serum tHcy level, the risk of retinopathy rose by about 50% (95% CI, −7 to 141) in the subjects with DM. Hyperhomocystein-

Table 2. Odds Ratios (95% Confidence Intervals) for Diabetic Retinopathy

Total Homocysteine Level, µmol/L	Prevalence of Diabetic Retinopathy, %*	Adjusted for Age and Sex	Adjusted for Age, Sex, HbA_{1c}† Level, and Hypertension	Adjusted for Age, Sex, HbA_{1c}, Hypertension, and Serum Creatinine Clearance	Adjusted for Age, Sex, HbA_{1c} Level, Hypertension, and Diabetes Duration >10 y
Subjects With Diabetes Mellitus					
≤9.0	10.0 (4/40)	0.53 (0.16-1.71)	0.56 (0.17-1.85)	0.56 (0.17-1.87)	0.54 (0.15-1.90)
9.1-16.0‡	18.0 (20/111)	1.00	1.00	1.00	1.00
>16.0	35.0 (7/20)	2.68 (0.92-7.80)	3.05 (0.99-9.42)	3.09 (0.92-10.38)	3.00 (0.95-9.51)
Per category increment		2.28 (1.09-4.79)	2.38 (1.10-5.17)	2.37 (1.02-5.53)	2.41 (1.09-5.31)
P for trend		.03	.03	.046	.03
Subjects Without Diabetes Mellitus					
≤9.0	12.1 (11/91)	1.38 (0.64-2.98)	1.52 (0.69-3.34)	1.47 (0.66-3.25)	
9.1-16.0‡	9.9 (29/292)	1.00	1.00	1.00	
>16.0	11.3 (8/71)	1.19 (0.52-2.74)	1.05 (0.45-2.46)	1.08 (0.46-2.54)	
Per category increment		0.91 (0.54-1.55)	0.82 (0.47-1.40)	0.85 (0.49-1.47)	
P for trend		.7	.5	.6	

**For parenthetic values, the numerator indicates the number of subjects; denominator, the total number in the subpopulation.*
†HbA_{1c} indicates glysosylated hemoglobin.
‡Indicates reference category.

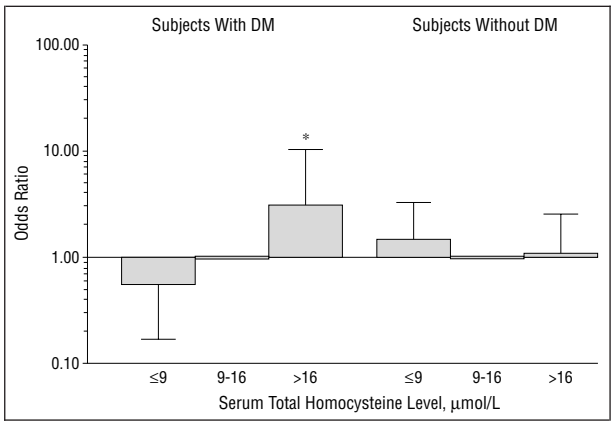

Figure 2. *Odds ratio for retinopathy among subjects with and without diabetes mellitus (DM), according to the serum total homocysteine level adjusted for age, sex, glycosylated hemoglobin level, creatine clearance, and hypertension. The reference category was serum total cholesterol values 9 to 16 µmol/L (>348 to >619 mg/dL). The error bars represent the upper or lower half of the 95% confidence intervals. Asterisk indicates* P<.05 *for trend.*

emia, defined as a serum tHcy level of 16 µmol/L or higher, was also related to retinopathy among subjects with type 2 DM (OR, 3.4 [95% CI, 1.1-10.6]). The results of the present study are in line with 2 studies that reported a higher serum tHcy level in subjects with type 1 and type 2 DM who have diabetic retinopathy than in those without DM.[29,30] However, these studies did not investigate the strength of the association between serum tHcy level and retinopathy, nor has additional adjustment for important risk factors for retinopathy been performed. Another study showed an association between the presence of diabetic retinopathy and C677T polymorphism of the methylenetetrahydrofolate reductase among patients with type 2 DM.[31] This mutation in the methylenetetrahydrofolate reductase gene was found in 5% to 15% of the general population. Persons who are homozygous for this mutation appear to have impaired enzyme activity, leading to an exaggerated hyperhomocysteinemic response to a low intake of folic acid. In contrast, Agardh et al,[32] Araki et al,[33] and Smulders et al[34] found no association between hyperhomocysteinemia and diabetic retinopathy in types 1 and 2 DM, respectively. However, none of these studies adjusted for the known determinants of retinal vasculopathy and one[32] was rather small.

Diabetic retinopathy involves both morphologic and functional changes of the retinal capillaries.[35,36] Hyperhomocysteinemia may induce endothelial dysfunction and injury followed by platelet activation and thrombus formation, possibly by increasing oxidative stress.[37] Therefore, it is conceivable that hyperhomocysteinemia is causally related to retinal vasculopathy through changes of the retinal vasculature and formation of microthrombi. Since oxidative stress is thought to be increased in type 2 DM,[38] this may make them more susceptible to hyperhomocysteinemia-induced oxidative damage.

We can think of 3 sources of disease misclassification that may have resulted in bias of the relation between hyperhomocysteinemia and retinopathy. Of 24% of all subjects, the fundus photographs were missing, and, therefore, in these subjects the diagnosis of retinopathy was solely dependent on the ophthalmoscopic examination findings. There is evidence that the sensitivity to detect retinopathy by ophthalmoscopy, even in the hands of an experienced ophthalmoscopist, is lower than that of fundus photography using color or black-and-white transparencies.[24,39-42] In addition, the method of detecting any retinopathy with 2 stereoscopic standard fields compared with 7 is slightly less sensitive (sensitivity about 0.85).[42] Finally, a number of early small lesions may be missed on 45° fundus photographs compared with photographs taken with a smaller angle. All 3 limitations of the present study may have introduced false-negative disease misclassification, which was, in all likelihood, nondifferential for the serum tHcy level. This would tend to underestimate the strength of the reported relation between hyperhomocysteinemia and retinopathy.[43]

In an additional analysis we showed, independent of hypertension, a positive association between hyperhomocysteinemia and diabetic retinopathy, with the presence of

microaneurysms as the defining characteristic. Although microaneurysms are also related to certain other diseases (eg, collagen vascular diseases or human immunodeficiency virus retinopathy), these diseases are rare compared with DM. Therefore, it is likely that our definition of diabetic retinopathy is somewhat more specific for DM-related retinal abnormalities than our definition for "any" retinopathy. As the results of the analyses with any and with diabetic retinopathy are quite similar, we believe that our conclusions are unaffected by this issue.

The Beaver Dam Eye Study[44] is a population-based study among individuals without DM, aged 43 through 84 years, that reported a prevalence of retinopathy of 7.8% as assessed by means of 2 standard photographic fields. The Rotterdam Study,[7] a population-based study of the elderly persons (aged ≥55 years), reported a prevalence of retinopathy of 4.8%, as detected by grading 1 standard photographic field, which is lower than the 10.7% we found. The difference in reported prevalences may partly be explained by less sensitive methods used to detect retinopathy in the Rotterdam Study. In the present study both ophthalmoscopic and photographic findings were used to asses the presence of retinopathy. The poor agreement we found between retinal photography and ophthalmoscopy is comparable with other studies.[45,46]

We evaluated a possible dose-response relation between the serum tHcy level and retinopathy, because it is unknown whether this relation is graded or has a certain threshold. The limited number of subjects with retinopathy, however, did not allow for a precise assessment of the presence of a possible threshold, which may be at 16 µmol/L among subjects with type 2 DM, but this result clearly needs to be confirmed in other studies. The boundaries of the serum tHcy level categories were quite broad and chosen post hoc. Another limitation is that, owing to the limited number of subjects with retinopathy, we could not explore the association between the serum tHcy level and the separate degrees of diabetic retinopathy. Since we did not assess B vitamins and the present study is cross sectional, we cannot rule out the possibility that low vitamin B levels may cause diabetic retinopathy or that diabetic retinopathy per se can raise serum tHcy levels, although the latter appears biologically implausible.

CONCLUSIONS

Hyperhomocysteinemia is associated with retinopathy among subjects with type 2 DM, but probably not in subjects without DM. If the finding of the present study can be confirmed in a prospective study, this may have implications for the clinical management of subjects with type 2 DM since the serum tHcy level can be lowered substantially with folic acid supplementation.[47]

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