Homocysteine and Diabetic Retinopathy

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OBJECTIVE — Homocysteine is an emerging risk factor for cardiovascular and nondiabetic ocular vaso-occlusive diseases. However, studies of the relationship between homocysteine and diabetic retinopathy have reported inconsistent results. The purpose of this study was to evaluate the relationship between plasma total homocysteine concentration and diabetic retinopathy.

RESEARCH DESIGN AND METHODS — We assessed the homocysteine-retinopathy relationship in 168 men and women with type 2 diabetes in a community-based, cross-sectional study. We photodocumented diabetic retinopathy status and measured plasma total homocysteine concentration using a commercial fluorescence polarization immunoassay enzymatic kit. Data for selected clinical/demographic variables and established risk factors for diabetic retinopathy were obtained from fasting blood samples and an interviewer-assisted lifestyle questionnaire.

RESULTS — A higher mean plasma total homocysteine concentration was observed in diabetic individuals with retinopathy than in those without retinopathy (11.5 μmol/l [95% CI 10.4–12.5] vs. 9.6 μmol/l [9.1–10.2], $P = 0.001$). Furthermore, the relationship between homocysteine and diabetic retinopathy was not explained by renal dysfunction and was independent of the other major risk factors for diabetic retinopathy (duration of diabetes, A1C, and systolic blood pressure) and determinants of higher homocysteine concentrations (age, sex, and red cell folate) (odds ratio 1.20 [95% CI 1.023–1.41], $P = 0.024$).

CONCLUSIONS — Plasma total homocysteine concentration may be a useful biomarker and/or a novel risk factor for increased risk of diabetic retinopathy in people with type 2 diabetes.

Diabetes Care **31:50–56, 2008**

Homocysteine has generated considerable interest in recent years as both a sensitive biomarker of folate deficiency and an emerging risk factor for cardiovascular disease (even within the normal range of homocysteine concentrations) (1) and has been linked to vaso-occlusive diseases in the eye (2). The known determinants of higher fasting plasma homocysteine levels are older age, male sex, and certain genetic abnormalities, while the major risk factors for hyperhomocysteinemia (elevated plasma total homocysteine concentration) are impaired renal function and poor vitamin B status (particularly folate status but also vitamin B6 and B12 status). In the elderly (age >75 years), hyperhomocysteinemia is generally associated with low folate status or renal impairment (3).

In addition, a multitude of physiological, lifestyle, and drug associations with higher homocysteine concentrations have been reported, including positive associations with smoking and alcohol and coffee intake (4). Numerous studies have evaluated the diabetic retinopathy–homocysteine relationship (Table 1) but have yielded inconsistent results (5–28), possibly as a result of methodological differences and residual confounding. We hypothesized that homocysteine is associated with diabetic retinopathy in type 2 diabetes, independent of the major determinants of both retinopathy and homocysteine levels, and that homocysteine concentrations would be higher in diabetic individuals with than in those without retinopathy.

RESEARCH DESIGN AND METHODS

Diabetes status and subject selection.

Self-reported diabetes status was confirmed biochemically according to World Health Organization diagnostic criteria for classification of diabetes (29). To broaden the range of dietary intakes and lifestyle exposures, we sourced subjects from the Melbourne Collaborative Cohort Study (MCCS), a community-based prospective cohort of 41,528 male and female volunteers aged 40–69 years at baseline (1989–1994) and recruited from the electoral roll, ethnic radio, clubs, and churches (30). We invited 248 men and women with type 2 diabetes from the MCCS to participate in this study, of whom we excluded three (two men with type 1 diabetes and one man with ungradable photographs). Of the eligible subjects ($n = 245$), 68% ($n = 168$) participated in the study. Ethics approval was obtained from the MCCS scientific committee, Deakin University, and Monash University (Melbourne, Australia), and written informed consent was obtained from every participant.

Diabetic retinopathy.

We used a mydriatic retinal fundus camera (Kowa FX-500S; Kowa, Tokyo, Japan) to photodocument retinal status. Diabetic retinopathy grading was based on the EURODIAB protocol (validated against the Airlie House classification), in which the overall grading was that of the worse eye and nonproliferative diabetic retinopathy was defined as more than one microaneurysm and/or hemorrhage (31). A medical retina specialist (C.A.H.), masked to all other participant information, graded the slides on two separate occasions to assess internal validity, and agreement between gradings was excellent (κ value of 0.986).

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Received for publication 1 April 2007 and accepted in revised form 17 September 2007.

Published ahead of print at http://care.diabetesjournals.org on 26 September 2007. DOI: 10.2337/dc07-0632.

Abbreviations: ACR, albumin-to-creatinine ratio; MCCS, Melbourne Collaborative Cohort Study.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Studies of plasma homocysteine concentrations in populations with diabetes*

Authors	Year	Design	Country	Subjects			Homocysteine levels (μmol/l)				Association between DR and homocysteine	Other significant associations with homocysteine
				ND	T1D	T2D	ND	T1D	T2D	DR		
Hultberg et al.	1991	C, H	Sweden	46	79		11	10.4			Absent if normal renal	Nephropathy
Agardh et al.	1994	C, H	Sweden		76			8/9/13*			Absent	B folate, S-creatinine, S-urea, U-albumin, SBP, duration of diabetes, nephropathy (not microalbuminuria)
Neugebauer et al.	1997	C, H	Japan			112					Present (MTHFR)†	
Chico et al.	1998	C, H	Spain	56	75	90	7.4	7	9.2		Not investigated	AER, type 2 diabetes, presence and severity of nephropathy
Stabler et al.	1999	C, c	U.S.			452					Absent	Neuropathy, macroalbuminuria
Smulders et al.	1999	C, H	Netherlands			85					Absent	Microalbuminuria
Vaccaro et al.‡	2000	C, H	Italy	44	66		7.4			9.5	Present (proliferative)	MTHFR/C677T mutation, microalbuminuria (not type 1 diabetes)
Chiarelli et al.	2000	C, H	Italy		61					15.1	Present (PDR)	Microalbuminuria
Hoogeveen et al.§	2000	C, c	Netherlands	454		171					Present	Not investigated
Agardh et al.	2000	C, H	Sweden		49			10.4		13.9	Absent	Serum creatinine
Buysschaert et al.	2001	C, H	Belgium		71						Present (univariate)	Age, creatinine, folic acid were independently associated with homocysteine, SBP, cholesterol, duration, complications were univariate associations
Agullo-Ortuno et al.	2002	C, H	Spain	54	57	32	10.1	11.7	11.7		Present (type 1 diabetes)	Macroangiopathy and nephropathy but only in type 1 diabetes
Abdella et al.	2002	C, H	Kuwait			358			10.2		Absent	Male sex, CHD, A1C, creatinine, apoB (not microalbuminuria), neuropathy, smoking
Matteucci et al.	2002	C, H	Italy	133	79		12.2	9.2			Absent	Sex, age, smoking in control subjects; sex, age, smoking, creatinine, lipoprotein(a), nephropathy in type 1 diabetic subjects
Guo et al.	2002	C, H	China	28		32	8.9/22.3¶		11.6/25¶	13.9/37.3¶	Present	Type 2 diabetes, MTHFR genotype, metabolites of NO
Yang et al.‖	2002	C, H	China	19		55	9.7		11.3	14.7	Present	No association with type 2 diabetes
Sun et al.	2003	C, H	China	57		208					Present	Type 1 and type 2 diabetes, type 1 diabetes complications
Looker et al.	2003	P, c	U.S. (Pima)			396					Present (PDR)	Nephropathy
Goldstein et al.	2004	C, H	Israel	156		179	11.8		13.5	NPDR, 14.6; PDR, 15.9	Present	Not investigated
Saeed et al.	2004	C, H	U.K.		48			7.7		8.8	Present	Microalbuminuria
Yucel et al.	2004	C, H	Turkey	30		40					Present (NPDR and PDR)	Not investigated
Soedamah-Muthu et al.	2005	C, H	13 European countries		533						Present (NPDR and PDR)	Hypertension, macroalbuminuria, CVD, GFR
De Luis et al.	2005	C, H	Spain			155					Absent (homocysteine ≥15 μmol/l)**	Peripheral arteriopathy, nephropathy, fibrinogen, lipoprotein(a), SBP, DBP
Huang et al.	2006	C, H	Taiwan	204		257	10.0		12.9		Present	Duration of diabetes >10 years

Data are *n* unless otherwise indicated. *Normal/micro-/macroalbuminuria (clinical nephropathy). †MTHFR: retinopathy associated with homocysteine-related MTHFR genotype. ‡Excluded diabetes duration <10 years. §Adjusted for diabetes, age, sex, A1C, and hypertension. ¶Data before/after methionine loading. ‖Excluded abnormal renal and ACR. **Hyperhomocysteinemia defined as homocysteine ≥15 μmol/l. AER, albumin excretion rate; apoB, apolipoprotein B; c, community based; C, cross-sectional; CHD, coronary heart disease; CVD, cardiovascular disease; DBP, diastolic blood pressure; DR, diabetic retinopathy; GFR, glomerular filtration rate; H, hospital based; ND, nondiabetic retinopathy; NO, nitric oxide; NPDR, nonproliferative DR; P, prospective; PDR, proliferative DR; SBP, systolic blood pressure; T1D, type 1 diabetic; T2D, type 2 diabetic.

Table 2—*Characteristics of individuals with type 2 diabetes by retinopathy status* (n = 168)

	Retinopathy absent	Retinopathy present	*P*
n	120	48	
Age (years)	65.0 (59.0–69.0)	66.5 (60.3–69.0)	0.455
Fasting glucose (mmol/l)	10.0 (7.9–11.7)	10.4 (8.5–12.3)	0.266
A1C (%)	7.6 (6.6–8.7)	8.6 (7.1–10.2)	0.003
Diabetes duration (years)	7.0 (4.8–12.0)	12.0 (7.3–21.5)	<0.0001
Hypoglycemia medication (%)	63	90	0.001
BMI (kg/m^2)	30 (27–33)	28 (25–32)	0.122
Systolic blood pressure (mmHg)	142 (131–156)	148 (134–167)	0.186
Diastolic blood pressure (mmHg)	73 (69–81)	74 (69–79)	0.946
Pulse blood pressure (mmHg)	66 (58–79)	73 (62–89)	0.123
Current smoker (%)	9.1	4.3	0.292
Urinary ACR (mg/mmol)	1.1 (0.7–2.8)	1.9 (0.9–12.1)	0.017
Plasma total cholesterol (mmol/l)	5.5 (4.7–6.1)	5.1 (4.6–5.7)	0.079
Plasma HDL cholesterol (mmol/l)	1.1 (0.9–1.4)	1.1 (0.9–1.3)	0.659
Plasma triglycerides (mmol/l)	1.6 (1.2–2.5)	1.6 (1.1–2.5)	0.289
Plasma homocysteine (μmol/l)	9.2 (7.5–11.5)	10.5 (9.0–13.8)	0.003
Red cell folate (ng/ml)*	282 (199–438)	239 (181–402)	0.200

Data are median (25th–75th percentile) or percentages unless otherwise indicated. Current smoker and sex data are prevalence (%). *P* values reported are χ^2 for categorical variables (prevalence reported), ANOVA for parametric data, and Mann-Whitney for nonparametric data. *Adjusted for hematocrit.

Clinical measures and retinopathy risk factors.

Systolic and diastolic blood pressure was recorded using a Dinamap XL portable automated adult vital signs monitor (model 9300; Critikon, Tampa, FL). Blood pressure was recorded as the average of the last two of three consecutive readings, obtained from the right arm of seated subjects at 1-min intervals after a 10-min rest period. Weight was measured to within 0.1 kg before breakfast and following a 12-h fast, with subjects wearing light clothing and no shoes, using digital electronic scales (UC-300; A.N.D, Tokyo, Japan). Height was measured to within 0.1 cm using a wall-mounted stadiometer (Harpenden; Holtain Limited, Crymych, U.K.). BMI was calculated as weight in kilograms divided by the square of height in meters (2). A current smoker was defined as a subject who smoked at least seven cigarettes a week at the time of completing the questionnaire.

Plasma biochemistry.

Homocysteine concentration in EDTA-treated plasma was assayed using a commercially available Fluorescence Polarization Immunoassay (Abbott Diagnostics, Abbott Park, IL) (32). Plasma total homocysteine concentrations were read on an IMx System Analyser (Abbott Diagnostics). Plasma glucose concentrations were analyzed using an automatic analyzer (model 705; Hitachi, Tokyo, Japan) and a commercial enzymatic kit (Boehringer Mannheim Diagnostica, Mannheim, Germany) by the glucose oxidase method. Plasma cholesterol and triacylglycerol concentrations were also analyzed with the Hitachi model 705 analyzer using a commercial enzymatic kit (Boehringer Mannheim Diagnostica).

Erythrocyte folate concentration.

Erythrocyte folate was measured in hemolyzed whole blood (1/22 dilution) using a Bio-Rad Quantaphase Folate radioassay (detection range: 0.2–3.0 ng/ml, coefficients of variation [CVs] 9.8–12.1%) (33). Red cell folate concentration was calculated as follows: red cell folate (adjusted) = (22 × unadjusted red cell folate concentration)/hematocrit.

Urinary biochemistry.

Urinary albumin concentration was measured using immunonephelometry (Kallestadt QM300 or Beckman 360 Array nephelometers; interassay CV 3–5%). Urinary creatinine concentration was measured using an alkaline picrate method (Olympus AU800 autoanalyzer; interassay CV 2%). The urinary albumin-to-creatinine ratio (ACR) was then calculated as albumin (milligrams)/creatinine (millimoles).

Statistical analyses.

We used SPSS software for Windows (version 13; SPSS, Chicago, IL) to perform statistical analyses. The data were cross-sectional observations. Descriptive statistics for the exposure and outcome variables were obtained, and variables with distributions that were not normally distributed were log transformed before analysis. Associations between retinopathy and continuous variables were assessed using ANOVA for variables with homogeneous variances or the corresponding nonparametric test when variances were nonhomogeneous. Associations between categorical variables were analyzed using χ^2 tests.

Initially, variables assessed in univariate analyses, including known risk factors for diabetic retinopathy, were modeled using binomial logistic regression analysis to determine the best clinical predictors of diabetic retinopathy. Plasma total homocysteine concentration was then added to subsequent models that controlled for the major risk factors for diabetic retinopathy and the established determinants of homocysteine. The fit of each model was tested, and the Nagelkerke R^2 approximation was compared for each of the logistic regression models. $P < 0.05$ was considered statistically significant.

RESULTS — Characteristics of the participants are shown in Table 2. Diabetic retinopathy was identified in 28.6% of people with type 2 diabetes. As expected, A1C level was higher in participants with than in those without retinopathy, although both groups demonstrated poor metabolic control (A1C

Table 3—*Multivariate model of predictors of retinopathy in individuals with type 2 diabetes*

Independent variables	β	SE	Exp(β) (95% CI)	*P*
Age (years)	−0.06	0.04	0.94 (0.88–1.01)	0.082
Fasting glucose (mmol/l)	−0.01	0.09	0.99 (0.83–1.18)	0.922
Diabetes duration (years)	0.72	0.33	2.06 (1.08–3.96)	0.029
A1C (%)	0.20	0.15	1.22 (0.92–1.63)	0.172
Hypoglycemia medication (%)	0.58	0.60	1.79 (0.55–5.83)	0.332
BMI (kg/m^2)	−0.06	0.05	0.94 (0.85–1.04)	0.245
Systolic blood pressure (mmHg)	0.00	0.01	1.00 (0.98–1.03)	0.846
Diastolic blood pressure (mmHg)	0.00	0.03	1.00 (0.95–1.06)	0.916
Current smoker (%)	−0.82	0.89	0.44 (0.08–2.54)	0.360
Urinary ACR (mg/mmol)	0.40	0.17	1.50 (1.07–2.09)	0.018
Plasma total cholesterol (mmol/l)	−0.19	0.24	0.83 (0.52–1.32)	0.426
Plasma HDL cholesterol (mmol/l)	0.31	0.75	1.36 (0.31–5.95)	0.685
Plasma triglycerides (mmol/l)	−0.12	0.23	0.89 (0.57–1.40)	0.610

Log-transformed data were modeled for duration of diabetes and ACR. Model $R^2 = 0.30$.

>7%). Participants with diabetic retinopathy had a significantly longer duration of diabetes, were more likely to use hypoglycemia medication(s), and had a higher ACR. The majority in both retinopathy and nonretinopathy groups had systolic hypertension (systolic blood pressure >140 mmHg), with a trend evident for a higher systolic and pulse blood pressure in the retinopathy group. Lipid levels were not associated with diabetic retinopathy, and there were few smokers in the study population. Male sex was not associated with diabetic retinopathy (31 vs. 25% for women and men, respectively; $P = 0.388$), and we observed a nonsignificant sex difference in the median homocysteine concentration (9.0 vs. 9.9 μmol/l; $P = 0.063$).

A higher mean plasma total homocysteine concentration was observed in diabetic individuals with than in those without retinopathy (11.5 [95% CI 10.4–12.5] vs. 9.6 [9.1–10.2], respectively; $P = 0.001$). Hyperhomocysteinemia (plasma total homocysteine ≥12 μmol/l for a folate-fortified population) was present in 22% ($n = 37$) of individuals with type 2 diabetes, and 7.7% ($n = 14$) had a plasma total homocysteine concentration >15 μmol/l. Among those with diabetic retinopathy, 33% ($n = 17$) had hyperhomocysteinemia and 14.6% ($n = 8$) had a plasma total homocysteine concentration >15 umol/l.

In participants both with and without retinopathy, the mean red cell folate concentration was in the normal range and was not (statistically) significantly different between subjects with and without retinopathy (312 ng/ml [95% CI 252–373]) vs. 371 ng/ml [322–421], $P = 0.174$). However, folate depletion (red cell folate concentration <160 ng/ml) or deficiency (red cell folate concentration <120 ng/ml) was observed in 8.1% ($n = 12$) of participants. As expected, a higher red cell folate concentration (adjusted for hematocrit) was associated with lower homocysteine levels ($r = −0.359$, $P < 0.0001$).

Table 3 demonstrates that, of the established risk factors and clinical characteristics in Table 2, duration of diabetes and ACR ratio were the only independent predictors of diabetic retinopathy in our study group. In logistic regression models of homocysteine as a predictor of increased risk of diabetic retinopathy (Table 4), the significant association between homocysteine and diabetic retinopathy identified in univariate testing remained significant in multivariate testing (model 1). The increased risk of diabetic retinopathy predicted by higher homocysteine concentrations was not explained by renal dysfunction after controlling both for the other major risk factors for diabetic retinopathy (duration of diabetes, A1C, and systolic blood pressure) and for the other determinants of homocysteine concentrations (age, sex, and red cell folate) (odds ratio 1.20 [95% CI 1.023–1.41], $P = 0.024$), as shown in model 2. Model 3 demonstrates that biguanide (metformin) use did not explain the significant relationship between plasma homocysteine concentration and diabetic retinopathy. Age was inversely associated with diabetic retinopathy in the models that adjusted for systolic blood pressure.

CONCLUSIONS — Several interesting observations were made in this study. First, a higher plasma homocysteine concentration was associated with prevalent retinopathy in individuals with type 2 diabetes. Second, the difference in the mean plasma homocysteine concentration between subjects with and without retinopathy was relatively small (<2 μmol/l). Third, the mean plasma homocysteine concentration for both subjects with and without retinopathy was below the upper limit for normal for this age-group, i.e., 12 μmol/l for mean age <65 years (34). Finally, a 1 μmol/l increase in plasma homocysteine concentration was an independent predictor of increased risk of diabetic retinopathy of between 15 and 20% after adjusting for the established risk factors for diabetic retinopathy (duration of diabetes, A1C, and systolic blood pressure) and the major determinants of plasma total homocysteine concentration (age, sex, red cell folate concentration, and renal function).

Prospective studies are now needed to confirm our findings, which may have implications for the management of diabetes: Dietary modulation of homocysteine levels is possible (35). Interestingly, poor folate status did not account for the observed homocysteine-retinopathy relationship in our study, possibly due to the adequate folate status of the majority of participants. Future studies that evaluate the association between poor folate status and diabetic retinopathy may help clarify the basis of the observed homocysteine-retinopathy relationship.

Homocysteine may be a good biomarker for increased risk of diabetes complications, since retinopathy, nephropathy, and cardiovascular disease have all been linked to higher homocysteine levels. Our

Table 4—*Regression models of homocysteine as a predictor of retinopathy in type 2 diabetes*

	B	SE	Exp(B) (95% CI)	*P*
Model 1 ($R^2 = 0.25$)				
Duration (years)	0.85	0.33	2.34 (1.23–4.46)	0.009
A1C (%)	0.16	0.13	1.17 (0.91–1.52)	0.229
Age (years)	−0.05	0.03	0.95 (0.89–1.01)	0.113
Male sex	−0.57	0.64	0.57 (0.16–2.01)	0.380
Red cell folate (ng/ml)	−0.01	0.01	0.99 (0.97–1.02)	0.479
Homocysteine (μmol/l)	0.16	0.08	1.17 (1.01–1.37)	0.041
ACR (mg/mmol)	0.02	0.01	1.02 (1.00–1.05)	0.093
Model 2 ($R^2 = 0.24$)				
Duration (years)	0.90	0.33	2.46 (1.30–4.66)	0.006
A1C (%)	0.17	0.13	1.18 (0.91–1.53)	0.208
Age (years)	−0.07	0.04	0.93 (0.87–1.00)	0.049
Male sex	−0.31	0.65	0.74 (0.20–2.65)	0.638
Red cell folate (ng/ml)	−0.01	0.01	0.99 (0.97–1.02)	0.529
Homocysteine (μmol/l)	0.18	0.08	1.20 (1.02–1.41)	0.024
ACR (mg/mmol)	0.19	0.17	1.20 (0.87–1.67)	0.265
Systolic BP (mmHg)	0.02	0.01	1.02 (0.99–1.04)	0.153
Model 3 ($R^2 = 0.30$)				
Duration (years)	0.10	0.03	1.10 (1.03–1.17)	0.003
A1C (%)	0.22	0.14	1.25 (0.95–1.64)	0.111
Age (years)	−0.08	0.04	0.93 (0.86–1.00)	0.043
Male sex	−0.49	0.69	0.61 (0.16–2.38)	0.478
Red cell folate (ng/ml)	−0.01	0.01	0.99 (0.96–1.02)	0.439
Homocysteine (μmol/l)	0.18	0.09	1.19 (1.01–1.41)	0.036
ACR (mg/mmol)	0.01	0.01	1.01 (0.99–1.04)	0.310
Systolic BP (mmHg)	0.02	0.01	1.02 (0.99–1.04)	0.184
Biguanide (metformin)	−0.37	0.49	0.69 (0.26–1.81)	0.450

Duration of diabetes and urinary ACR data are log transformed. BP, blood pressure.

finding that a difference in homocysteine concentration of <2 μmol/l separated subjects with and without retinopathy suggests that a relatively small increase in the plasma homocysteine concentration, in the order of 1 μmol/l, may be a useful trigger for intensification of treatment of the major risk factors for diabetes complications (blood pressure, blood glucose, and blood lipids). Moreover, for the timely identification of individuals at greater risk of the vascular complications of diabetes, the upper limit of the normal range for plasma homocysteine (12 μmol/l for folate-fortified and/or adult populations and 20 μmol/l for folate-unfortified and/or older populations [age >65 years]) may need to be lowered, as has been the case for blood pressure and lipid target levels.

A limitation of many homocysteine studies, particularly homocysteine-lowering cardiovascular disease trials (36), has been their failure to control for renal disease. In the many studies that have evaluated the diabetic retinopathy–homocysteine relationship (Table 1), several did not control for impaired renal function. A number did not control for established retinopathy risk factors, and none controlled for metformin use. All of these factors were accounted for in the present study.

Many of the earlier studies evaluated homocysteine only as a categorical variable (proportion of study sample with hyperhomocysteinemia, i.e., homocysteine concentration above a designated cutoff). However, the cutoff for hyperhomocysteinemia is arbitrary and differed substantially between studies, ranging from 11.7 to 16 μmol/l. In our study, subjects with and without retinopathy had mean homocysteine levels <11 μmol/l, indicating that the cutoff used in earlier studies may have been too high. Therefore, both the variability in and the level of the selected cutoffs may have contributed to discordant findings between studies. Consequently, we evaluated the impact of homocysteine as a continuous variable.

Other methodological issues may have also contributed to the disparity between studies. Homocysteine can be determined either directly or after derivatization, making comparisons of findings between studies difficult. We measured plasma homocysteine levels directly, consistent with current expert opinion and recommendations. In addition, there are many different methods and classifications for assessment of diabetic retinopathy: we photodocumented retinopathy status according to the EURODIAB protocol, validated against the Airlie House classification, unlike many of the earlier homocysteine studies in which retinopathy was assessed using ophthalmoscopy (14). Nevertheless, it is possible that more accurate assessment of diabetic maculopathy would facilitate a better understanding of the nature of the retinopathy-homocysteine relationship.

The main limitation of this study was the use of a cross-sectional design, which precludes determination of temporal direction and therefore of causal inference. Although we controlled for retinopathy risk factors and the most important confounders of homocysteine in this population—specifically, age, sex, smoking status, red cell folate levels, metformin use, and renal status—many factors linked to homocysteine in other studies

were not assessed in our study, such as genetic and lifestyle factors (e.g., vitamin B12 and B6 intakes) and conditions associated with diabetes and aging, such as depression and dementia (32). However, in contrast to earlier studies, recruiting from a community-based cohort enabled evaluation of the homocysteine-retinopathy relationship in a more health-conscious group of individuals at lower risk of comorbidities and potential confounders than hospital-based populations.

In conclusion, our observations support a role for homocysteine in diabetic retinopathy at least as a biomarker and potentially as a risk factor if prospective studies confirm our observations. While we acknowledge that there is currently insufficient evidence to recommend routine screening of homocysteine for the purpose of treating elevated homocysteine concentrations in the wider adult population, almost 1 in 10 of our participants was folate depleted and thus at risk of folate deficiency and related functional deficits, suggesting that monitoring homocysteine and folate status in people with type 2 diabetes may have net health benefits. Finally, this study provides further support for recommending a folate-rich diet based on high intakes of fresh fruit and vegetables for people with type 2 diabetes.

Acknowledgments— The National Health and Medical Research Council of Australia partly funded this study (project no. 124317), which represents the collaboration and contribution of many, especially Connie Karschimkus, Mary Kaimakamis, and Melissa Cameron.

This work would not have been possible without the Melbourne Collaborative Cohort Study and the infrastructure support provided by the Cancer Council Victoria.

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