Hyperhomocysteinemia Following a Methionine Load in Patients With Non–Insulin-Dependent Diabetes Mellitus and Macrovascular Disease

Medha N. Munshi, Angie Stone, Louis Fink, and Vivian Fonseca

In the setting of an outpatient diabetic clinic, we determined whether macrovascular disease in patients with diabetes mellitus is associated with hyperhomocysteinemia (elevated plasma homocysteine [H(e)] concentrations) following a methionine load. Methionine-load tests were performed in 18 healthy controls, 11 diabetics without vascular disease (five insulin-dependent [IDDM] and six non–insulin-dependent [NIDDM]), and 17 diabetics with vascular disease (five IDDM and 12 NIDDM). All subjects were male, and there was no significant difference in mean age among the three groups. We measured plasma H(e) concentrations before and 2, 4, 6, 8, and 24 hours after an oral methionine load. Hyperhomocysteinemia (peak plasma H(e) concentration > control mean ± 2 SD) occurred with significantly greater frequency (seven of 18, 39%) in patients with NIDDM as compared with age-matched controls (7%), being more common in those with macrovascular disease (five of 12, 41%). The area under the curve (AUC) over 24 hours, reflecting the total period of exposure to H(e), was also elevated with greater frequency in patients with NIDDM and macrovascular disease (33%) as compared with controls (0%). We conclude that hyperhomocysteinemia is associated with macrovascular disease in a significant proportion of patients with NIDDM. Further investigation of this association may determine whether hyperhomocysteinemia contributes to the increased frequency and accelerated clinical course of vascular disease in patients with diabetes mellitus.

HOMOCYSTEINE [H(e)] is a thiol-containing amino acid formed by demethylation of methionine. Greatly elevated plasma H(e), as occurs in homocystinuric children, is known to predispose to premature vascular disease involving all major vessels. Hyperhomocysteinemia (moderate elevation in plasma H(e)) both basally and following a methionine load is being increasingly recognized as an independent risk factor for development of macrovascular diseases (cerebral, coronary, and peripheral arterial disease).[1,2] We conducted a study to determine whether hyperhomocysteinemia is associated with macrovascular diseases in patients with diabetes mellitus.

SUBJECTS AND METHODS

Twenty-eight patients with diabetes mellitus (<60 years old) and 18 healthy volunteers were studied after provision of informed consent. Diabetes was diagnosed and classified according to the National Diabetes Data Group.[3] Only male subjects were studied because of the difficulty in interpretation of data in perimenopausal women.[4] Patients with renal insufficiency (plasma creatinine > 1.4 mg/dL), hepatic dysfunction, and vitamin B_{12} or folate deficiency were excluded.

Subjects were divided into the following three groups. Group I. Healthy controls (n = 18): mean age, 42.8 ± 4 years. Group II. Diabetic patients without vascular disease (n = 11): mean age, 45.8 ± 6 years; mean duration of diabetes, 15.5 ± 5 years (nine on insulin and two on sulfonylureas). None of these patients had symptoms of claudication, foot ulceration, angina, or cerebral ischemia, and Doppler ankle-brachial pressure index was greater than 1.0. Group III. Diabetic patients with vascular disease (n = 17): mean age, 49.3 ± 8 years; mean duration of diabetes, 13.1 ± 7 years (13 on insulin, three on sulfonylureas, and one on diet restriction). Eight patients had symptoms of intermittent claudication and a Doppler ankle-brachial pressure index less than 0.9; two also had a history of myocardial infarction. Seven patients had a history of myocardial infarction or had undergone coronary artery bypass surgery. Two patients had suffered cerebrovascular accidents and had abnormal carotid Doppler examinations.

Clinical characteristics of the subjects are summarized in Table 1.

Methionine-Load Test

After an overnight fast, venous blood samples were obtained and methionine 0.1 g/kg body weight was administered orally. Blood samples were collected at 2, 4, 6, 8, and 24 hours after the methionine load. During the test, patients were given breakfast and lunch, with methionine content restricted to 35 mg. Total plasma H(e) (homocystine, homocysteine-cysteine mixed disulfides, and protein-bound homocysteine) level was measured by a high-performance liquid chromatograph coupled with a fluorescence detectometer.[5]

Statistical Analysis

Statistical analysis was performed with ANOVA and the chi-square test.

RESULTS

Basal, peak, and 24-hour plasma H(e) concentrations in the three groups are summarized in Table 2. Peak plasma H(e) occurred at 6 or 8 hours. Peak and 24-hour plasma H(e) and the trapezoidal area under the curve (AUC) for the 24-hour period were significantly higher in group III as compared with controls ($P < .01$).

There was a wide variation in plasma H(e) after a methionine load in diabetic patients, with some patients being low-normal and some very high (Figs 1 and 2).

Hyperhomocysteinemia occurred with significantly greater frequency in diabetics than in controls. Peak plasma H(e) was elevated (>control + 2 SD) in nine of 28 (32%) diabetics (six in group III [35%] and three in group II [27%]), but in only one of 18 (5.5%) controls ($P < .02$, χ^2 test). The 24-hour AUC was elevated in seven of 28 (25%) diabetics, but in none of the controls ($P < .02$). Both peak H(e) and AUC were elevated in six of 28 (21%) diabetics and in none of the controls ($P < .03$). Four of these six

From the Division of Endocrinology and Metabolism and Division of Pathology, John L. McClellan Veterans Administration Hospital and University of Arkansas for Medical Sciences, Little Rock, AR.

Submitted March 20, 1995; accepted May 10, 1995.

Address reprint requests to Vivian Fonseca, MD, Division of Endocrinology and Metabolism, 4301 W Markham, Slot 587, Little Rock, AR 72205.

0026-0495/96/4501-0020$03.00/0

Table 1. Clinical Characteristics of Controls and Patients

Characteristics	Group I†	Group II	Group III
No. of subjects	18	11	17
Age (yr)	42.8 ± 4	45.8 ± 6	49.3 ± 8
IDDM/NIDDM (n)		6/6	5/12
Body weight (lb)*	161 (134-235)	175 (134-268)	185 (142-256)
Hemoglobin A_{1c} (%)	—	10.7 ± 1.8	9.4 ± 2.4
Proteinuria or microalbuminuria (n)	—	6/12	5/17
Smoking (n)	3/18	4/11	5/17
Cholesterol (mg/dL)*	196 (146-270)	243 (65-293)	209 (157-449)
Triglycerides (mg/dL)*	111 (34-166)	118 (66-1,012)	192 (47-1,401)
Vitamin B_{12} (pg/mL)*	453 (214-928)	601 (326-1,105)	554 (284-914)
Red blood cell folate (ng/mL)*	576 (361-1,244)	688 (469-907)	663 (343-1,657)

NOTE. Group I, normal volunteers; group II, diabetics without vascular disease; group III, diabetics with vascular disease.

*Values are the median and range, since data are not normally distributed.

†Five patients were age-matched for patients with IDDM and 13 were age-matched for patients with NIDDM.

patients were in group III, and the fifth patient, who was in group II, has subsequently had a myocardial infarction. Six of 17 (35%) patients in group III but none of the controls had elevations of both peak H(e) and AUC ($P < .01$).

When the data were analyzed according to type of diabetes, hyperhomocysteinemia occurred in two of 10 patients with insulin-dependent diabetes mellitus ([IDDM] one of five with vascular disease and one of six without) and in none of five younger age-matched controls. Hyperhomocysteinemia occurred in eight of 18 patients with non–insulin-dependent diabetes mellitus ([NIDDM] six of 12 with vascular disease and two of six without vascular disease) and in only one of the remaining 13 control subjects matched for age with NIDDM patients ($P < .05$).

There was no correlation between hyperhomocysteinemia and age, duration of diabetes, hemoglobin A_{1c} level, cholesterol level, smoking status, or proteinuria. In diabetic patients, proteinuria was present in four of nine with hyperhomocysteinemia and in seven of 19 with normal peak H(e) (nonsignificant).

DISCUSSION

Our data demonstrate that plasma H(e) concentrations following a methionine load are significantly higher in diabetics with macrovascular disease than in controls. Some diabetic patients without overt vascular disease also have high plasma H(e) concentrations. It is possible that these patients have subclinical disease, as exemplified by one patient from group II who developed a myocardial infarc-

Table 2. Plasma H(e) After Methionine Load in Diabetes (mean ± SD)

H(e) (μmol/L)	Group I	Group II	Group III
Basal	9.2 ± 3.7	9.3 ± 3.2	9.5 ± 2.5
Peak	26.6 ± 5.7	27.2 ± 10.6	33.6 ± 13.3*
24-hour	13.5 ± 4.2	14.7 ± 7.3	19.2 ± 7.5*
AUC (μmol · L).	411 ± 84	418 ± 140	512 ± 189*

NOTE. Group I, normal volunteers; group II, diabetics without vascular disease; group III, diabetics with vascular disease.

*$P < .01$ *v* group I by ANOVA.

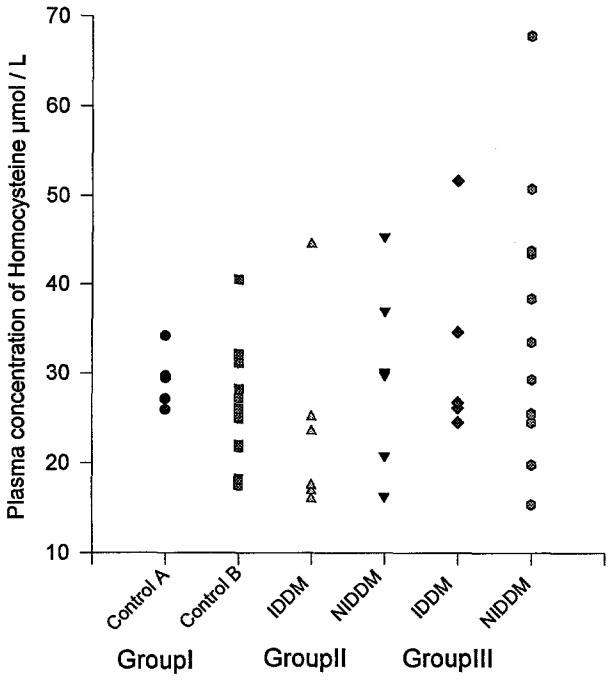

Fig 1. Peak plasma H(e) in controls (group I), diabetic patients without vascular disease (group II), and diabetic patients with vascular disease (group III). Control A, age-matched control for IDDM; control B, age-matched control for NIDDM.

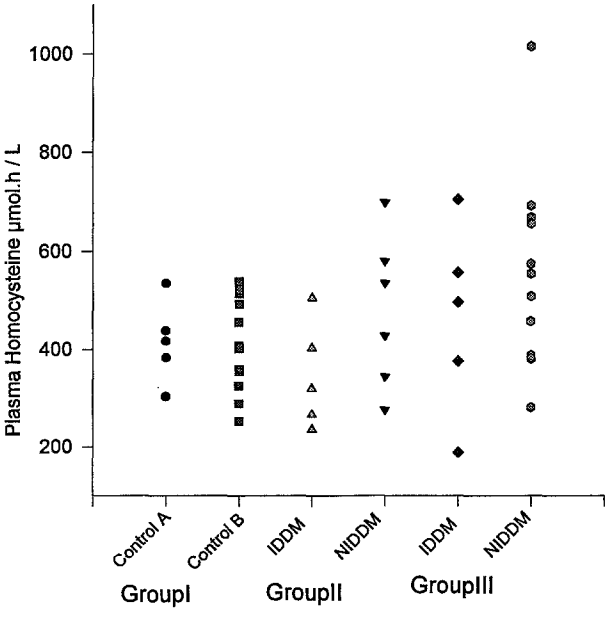

Fig 2. Trapezoidal AUC: plasma H(e) in controls (group I), diabetic patients without vascular disease (group II), and diabetic patients with vascular disease (group III). Control groups defined as in Fig 1.

tion at the age of 43 years, 6 months after the study was performed.

The prevalence of hyperhomocysteinemia in all diabetics in our study (35%) is similar to that previously reported in patients with vascular disease.[2] However, the prevalence in NIDDM subjects with vascular disease is much higher at 41%. These findings are compatible with the well-recognized fact that NIDDM is associated with a higher incidence of vascular disease. However, the incidence of vascular disease in IDDM subjects is also greater than in nondiabetics, and our observation that some patients with IDDM also have hyperhomocysteinemia is of interest.

Homocystinuria is a rare condition. The most common form of homocystinuria is that associated with a deficiency of cystathionine β-synthase, which is inherited in an autosomal recessive manner. On the basis of incidence of the homozygous condition, it has been estimated that the prevalence of the heterozygous state is 1% to 2% of the population. This is much lower than the prevalence of hyperhomocysteinemia in our series. Possible explanations for this increased incidence include (1) co-inheritance of diabetes and heterozygous homocystinuria, or (2) an acquired enzyme defect in the metabolism of methionine H(e). Many but not all patients with hyperhomocysteinemia and vascular disease in other studies have been shown to have defects in cystathionine β-synthase activity. The difficulty in labeling all these patients as heterozygotes has been highlighted in a review by McGill et al.[6] Since insulin has profound effects on amino acid metabolism, it is possible that an acquired defect in H(e) metabolism occurs in diabetics.

The exact mechanism by which both diabetes and hyperhomocysteinemia may contribute to vascular disease is not known. Both conditions have a number of similar abnormalities in hemostasis, platelet aggregation, fibrinolysis, and endothelial function that could contribute to the development of vascular disease.[7,8] These abnormalities are not present in all diabetic patients, being more common in patients with vascular disease. It is possible that in diabetics with vascular disease, these abnormalities are either mediated or exacerbated by H(e).

In the only other report of H(e) in diabetics, patients with retinopathy were studied exclusively. Basal plasma H(e) concentrations were higher only in patients with coexistent nephropathy and elevated plasma creatinine.[9] However, H(e) is known to be increased in renal failure.[10] Furthermore, methionine-load tests were not performed in the study reported by Hultberg et al.[9] Nevertheless, these findings are of interest because diabetic nephropathy is associated with rapid acceleration of macrovascular disease. We have not found an association between hyperhomocysteinemia and proteinuria in our study, although the number of patients studied is too small to draw a definite conclusion. It is possible that hyperhomocysteinemia in our patients reflects subclinical renal disease with decreased clearance of H(e). Even so, its recognition is important given the excess of cardiovascular morbidity in patients with nephropathy, the pathogenesis of which is poorly understood.

Recognition of hyperhomocysteinemia in patients with diabetes may also be of importance because it is potentially reversible. A number of therapeutic agents, including folic acid and pyridoxine, not only decrease plasma H(e) but also correct many of the associated hemostatic abnormalities.[7,11] Correction of post–methionine-load hyperhomocysteinemia in patients who have normal basal H(e) has recently been demonstrated.[12] If our results are confirmed in a large series of patients, it will be possible to conduct a clinical trial of pyridoxine and folic acid in the treatment of vascular disease in diabetes mellitus.

REFERENCES

1. Boers GHJ, Smals AG, Trijbels FJ, et al: Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. N Engl J Med 313:709-715, 1985

2. Clarke R, Daly L, Robinson K, et al: Hyperhomocysteinemia: An independent risk factor for vascular disease. N Engl J Med 324:1149-1155, 1991

3. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28:1039-1057, 1979

4. Boers GH, Smals AG, Trijbels, FJM, et al: Unique efficacy of methionine metabolism in premenopausal women may protect against vascular disease in the reproductive years. J Clin Invest 72:1971-1976, 1983

5. Ubbink JB, Hayward Vermaak WJ, Bissport S: Rapid high performance liquid chromatographic assay for total homocysteine levels in human serum. J Chromatogr 565:441-446, 1991

6. McGill JJ, Mettler G, Rosenblatt DS, et al: Detection of heterozygotes for recessive alleles. Homocyst(e)inemia: Paradigm of pitfalls in phenotypes. Am J Med Genet 36:45-52, 1990

7. Mudd SH, Levy HL, Skovby F: Disorders of transsulfuration, in Scriver CR, Beaudet AL, Sly WS, et al (eds): The Metabolic Basis of Inherited Disease (ed 6). New York, NY, McGraw-Hill, 1989, pp 693-734

8. Gwynne JT, McMillan DE: Summary and highlights of XIV International Diabetes Federation Satellite Symposium on Macrovascular Complications of Diabetes. Diabetes 41:116-119, 1992 (suppl 2)

9. Hultberg B, Agardh E, Andersson A, et al: Increased levels of plasma homocysteine are associated with nephropathy, but not severe retinopathy in type 1 diabetes mellitus. Scand J Clin Lab Invest 51:277-282, 1991

10. Wilcken DE, Gupta VJ: Sulphur containing amino acids in chronic renal failure with particular reference to homocystine and cysteine-homocysteine mixed disulfide. Eur J Clin Invest 9:301-307, 1979

11. Zweifler AJ, Allen RJ: An intrinsic blood platelet abnormality in an homocystinuric boy, corrected by pyridoxine administration. Thromb Diathes Haemorrh 26:15-21, 1971

12. Van den Berg M, Franken DG, Boers GHJ, et al: Combined vitamin B6 plus folic acid therapy in young patients with arteriosclerosis and hyperhomocysteinemia. J Vasc Surg 20:933-940, 1994