

Hyperhomocysteinemia in Type 2 Diabetes

Relationship to macroangiopathy, nephropathy, and insulin resistance

MARTIN BUYSSCHAERT, MD
ANNE-SOPHIE DRAMAIS, MD

PIERRE E. WALLEMACQ, MD
MICHEL P. HERMANS, MD

OBJECTIVE — The aim of this study was to determine the distribution of plasma total homocysteine (tHcy) concentrations in type 2 diabetic patients and to assess whether high tHcy values were related to chronic complications (particularly macroangiopathy and nephropathy) and/or the degree of insulin resistance.

RESEARCH DESIGN AND METHODS — Fasting tHcy levels were measured in 122 type 2 diabetic patients in whom the presence of chronic complications (e.g., macroangiopathy, microalbuminuria, macroproteinuria, decreased creatinine clearance, hypertension, retinopathy, and neuropathy) was recorded alongside an assessment of insulin resistance by the homeostasis model assessment (HOMA).

RESULTS — We found that 31% of the cohort (group 1) had raised tHcy (mean \pm 1 SD) values (20.8 ± 5.1 $\mu\text{mol/l}$), whereas 69% (group 2) had normal values (10.2 ± 2.0 $\mu\text{mol/l}$). The prevalence of macroangiopathy was higher in group 1 than in group 2 subjects (70 vs. 42%, $P < 0.01$); the prevalence of coronary artery disease was particularly higher in group 1 (46 vs. 21%, $P < 0.02$). The prevalence of impaired renal function, evidenced by decreased creatinine clearance, was higher in group 1 (32 vs. 10%, $P < 0.005$). Other clinical and biological characteristics of both groups were comparable, although group 1 had lower levels of folic acid than group 2 (5.2 ± 2.9 vs. 7.0 ± 3.4 ng/ml, $P < 0.01$). No differences were found for microalbuminuria (33 vs. 31%), retinopathy (45 vs. 42%), or neuropathy (70 vs. 59%) between groups 1 and 2, respectively. The degree of insulin resistance was similar in groups 1 and 2 (46 ± 21 and $42 \pm 20\%$ of HOMA–insulin sensitivity) as was the assessment of β -cell function (63 ± 28 and $65 \pm 46\%$, respectively). No differences in tHcy levels were found between subjects receiving metformin and those not receiving metformin. In contrast, the plasma tHcy level was higher in diabetic patients treated with fibrates ($P = 0.0016$).

CONCLUSIONS — Elevated plasma tHcy levels in type 2 diabetes is associated with a higher prevalence of macroangiopathy and nephropathy when assessed from creatinine clearance indexes and is not associated with different degrees of insulin resistance.

Diabetes Care 23:1816–1822, 2000

The elevated plasma total homocysteine (tHcy) concentration is considered an independent risk factor for atherosclerotic disease in subjects with normal glucose tolerance (1–3).

Although type 2 diabetes is definitely associated with premature atherosclerosis (4,5), only a handful of studies have dealt with the association between hyperhomocysteinemia and macro- or microangiopathic

complications (6–15). Plasma tHcy can be affected by both glomerular hyper- and hypofiltration, two conditions not uncommon in type 2 diabetes that can respectively decrease and increase the tHcy value (6, 16,17). The significance of hyperhomocysteinemia in type 2 diabetes is further complicated by the multiple ways of considering impaired renal function: decreased creatinine clearance, albuminuria, or both (6,7,11,14,16–18). Even less information is available on the relationship between tHcy and the degree of insulin resistance, which is another cardiovascular risk determinant in type 2 diabetes.

Therefore, we measured plasma tHcy in a cohort of type 2 diabetic patients and assessed whether high values were related to chronic complications, particularly macroangiopathy and nephropathy. We also analyzed the relationship between tHcy and the degree of insulin resistance, as measured by the homeostasis model assessment (HOMA).

RESEARCH DESIGN AND METHODS

— A total of 122 consecutive type 2 diabetic in- and outpatient subjects (40 men and 82 women) participated in the study. Their age and diabetes duration (mean \pm 1 SD) were 63 ± 10 and 14 ± 9 years, respectively. Their BMI was 30 ± 6 kg/m². Medical conditions known to increase tHcy (e.g., dysthyroidism) were excluded. Glycemic control, assessed by the level of HbA_{1c}, was $8.6 \pm 2.0\%$. The C-peptide level was 0.96 ± 0.44 pmol/ml. Metformin or insulin therapy was administered (either alone or in combination with sulfonylureas) in 53 and 45% of the patients, respectively. Sulfonylureas were administered to 58% of the subjects. The cohort was divided in two groups according to their fasting plasma tHcy levels: group 1 had increased tHcy (>15.0 $\mu\text{mol/l}$, $n = 38$, 31% of the cohort), whereas group 2 had normal laboratory values (between 5.0 and 15.0 $\mu\text{mol/l}$, $n = 84$). This threshold was selected from published data that defined high and normal tHcy levels (3,19,20).

From the Service d'Endocrinologie et Nutrition (M.B., A.S.D., M.P.H.) and the Service de Biologie Clinique (P.E.W.), Cliniques Universitaires St. Luc, Université Catholique de Louvain, Brussels, Belgium.

Address correspondence and reprint requests to Prof. M. Buysschaert, Service d'Endocrinologie et Nutrition, Cliniques Universitaires St. Luc, Avenue Hippocrate 54, UCL 54.74, B-1200 Bruxelles, Belgium. E-mail: buysschaert@diab.ucl.ac.be.

Received for publication 6 April 2000 and accepted in revised form 5 September 2000.

Abbreviations: BP, blood pressure; HOMA, homeostasis model assessment; HPLC, high-performance liquid chromatography; SAH, S-adenosyl-L-homocysteine; S_i, insulin sensitivity index; tHcy, total homocysteine.

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

Table 1—Clinical characteristics of type 2 diabetic patients with and without hyperhomocysteinemia

	Group 1 (with hyperhomocysteinemia)	Group 2 (without hyperhomocysteinemia)	P
n	38	84	—
Homocysteinemia ($\mu\text{mol/l}$)	20.8 \pm 5.1	10.2 \pm 2.6	—
Age (years)	66 \pm 10	61 \pm 10	<0.02
Men/women	66/34	68/32	
Duration of diabetes (years)	16 \pm 9	13 \pm 8	NS
Family history			
Diabetes	48	58	NS
Cardiovascular disease	32	31	NS
Past/current smoking	35/19	42/11	NS
BMI (kg/m^2)	31 \pm 6	29 \pm 6	NS
Waist-to-hip ratio	0.99 \pm 0.08	0.98 \pm 0.09	NS
BP (mmHg)	154/86	152/87	NS
Metformin	39	59	NS
Sulfonylurea	53	60	NS
Insulin	56	40	NS
Lipid-lowering drugs	43	23	<0.05
BP-lowering drugs	84	65	0.05

Data are means \pm SD or % unless otherwise indicated.

Assays

Homocysteine was measured on heparinized plasma using a fluorescence polarization immunoassay on an IMx analyzer (Abbott Diagnostics). The samples had been centrifuged within 2 h and kept frozen until analysis. For homocysteine assaying, protein bound homocysteine and mixed disulfide forms of homocysteine were reduced to free homocysteine by dithiothreitol. Homocysteine was converted to S-adenosyl-L-homocysteine (SAH) by use of hydrolase and excess adenosine. SAH and fluorescein-labeled S-adenosyl-cysteine were used as markers in the competition immunoassay with binding to mouse monoclonal antibody. Assay analytical performance was evaluated by running in duplicate two series of homocysteine control samples (7, 12.5, and 25 $\mu\text{mol/l}$) on 5 consecutive days, resulting in interassay reproducibility (coefficient of variation) ranging from 1.61 to 2.01%, with the limit of detection estimated at 0.4 $\mu\text{mol/l}$ (95% confidence). This rapid and automated assay correlated well with a reference high-performance liquid chromatography (HPLC) method ($r > 0.90$). The normal range for an adult control population ($n = 103$) was 9.3 \pm 4.7 $\mu\text{mol/l}$, with a median value of 8.5 $\mu\text{mol/l}$. HbA_{1c} levels were determined by ion-exchange HPLC. Vitamin B₁₂ and folic acid levels were determined by radioimmunoassay methods, as were plasma insulin and C-peptide concentrations.

HOMA assessment of insulin resistance and β -cell function

This noninvasive method is based on a structural computer model of a glucose/insulin feedback system that incorporates mathematical descriptions of the function and interactions of various organs involved in plasma glucose control. It is based on empirical data, and reproduces physiological reality in a "reference individual," in that it will find the equilibrium point of fasting plasma glucose, insulin, C-peptide, and proinsulin, and it can predict the plasma glucose, insulin, C-peptide, and proinsulin concentrations for any possible combination of these parameters in the fasting state. The model is applicable to nondiabetic, glucose-intolerant, and diabetic individuals. It was described by Matthews et al. (21) and Hosker et al. (22), and the part of the model dealing with insulin and C-peptide secretion and kinetics was described by Rudenski et al. (23). The mathematical structure of the model was upgraded in the aspects relating insulin and glucose sensitivity (24) to the influence of proinsulin in the model (allowing its use with nonspecific or specific insulin assays) and to the contribution of glycosuria to plasma glucose (25,26). Final validation and performance comparison with other methods currently available were recently published (27). HOMA samples were drawn from an antecubital cannula, the

sampled arm being wrapped in electric blankets to provide arterialized blood. Immediately after insertion of the cannula, three fasting samples were taken at 5-min intervals for both insulin and glucose assays used for modeling, with oral antidiabetic drugs and/or insulin withdrawn 24 h before sampling.

HOMA was obtained at the time of the study in 22 (58%) and 56 (67%) subjects in groups 1 and 2, respectively. The clinical and biological characteristics of the subjects in both groups were not different in regard to whether they had a HOMA test performed (data not shown).

Assessment of chronic complications

Hypertension was considered in all patients treated with antihypertensive drugs and/or in subjects previously diagnosed with high blood pressure (BP) levels (systolic BP > 140 and diastolic BP ≥ 90 mmHg). Macroangiopathy was considered in subjects who met the following three conditions: 1) having a history of a cardiovascular event and/or the presence of angina and/or permanent ischemic electrocardiogram abnormalities at rest or ischemic abnormalities in a stress test (usually combined with a cardiac noninvasive imaging technique); 2) having claudication and/or abolished peripheral pulses and/or foot lesions due to vascular disease demonstrated by Doppler echography and/or angiography; or 3) having carotid vascular disease, as assessed by Doppler echography. Microalbuminuria was defined as urinary albumin concentrations between 20 and 200 mg/l, assessed through immunonephelometry (BN II; Dade Behring, Deerfield, IL), or as urinary albumin excretion between 30 and 300 mg/24 h. Proteinuric nephropathy was considered in the presence of gross albuminuria and/or macroproteinuria (Albustix [Ames] positive). Creatinine clearance values were calculated by the Cockcroft-Gault formula. Retinopathy was diagnosed on the basis of direct ophthalmoscopy (through a dilated pupil) by an experienced ophthalmologist and/or by fluorescein angiography. Peripheral neuropathy was assessed by questioning patients about symptoms of neuropathy, including paresthesia, dulled sensation, and pain in legs and feet, and was based on clinical examination (i.e., measuring abnormal knee/ankle reflexes and a Semmes-Weinstein monofilament) and confirmed by measurement of conduction velocities of ulnar (motor and sensory), tibial, peroneal, and sural nerves.

Table 2—Biological characteristics of type 2 diabetic patients with and without hyperhomocysteinemia

	Group 1 (with hyperhomocysteinemia)	Group 2 (without hyperhomocysteinemia)	P
n	38	84	—
Homocysteinemia ($\mu\text{mol/l}$)	20.8 \pm 5.1	10.2 \pm 2.6	—
HbA _{1c} (%)	8.2 \pm 1.7	8.8 \pm 2.0	NS
Cholesterol (mg/dl)	207 \pm 160	239 \pm 244	NS
Triglycerides (mg/dl)	203 \pm 45	214 \pm 46	NS
LDL cholesterol (mg/dl)	120 \pm 33	125 \pm 34	NS
HDL cholesterol (mg/dl)	42 \pm 12	45 \pm 14	NS
Cholesterol-to-HDL ratio	5.2 \pm 2.0	5.1 \pm 1.7	NS
Thyroid-stimulating hormone ($\mu\text{U/ml}$)	1.2 \pm 0.7	1.6 \pm 0.9	NS
Folic acid (ng/ml)	5.2 \pm 2.9	7.0 \pm 3.4	<0.01
Vitamin B ₁₂ (pg/ml)	414 (263–470)	434 (340–672)	NS

Data are means \pm SD or median (range [25th to 75th percentile]) unless otherwise indicated.

Statistics

Results are presented as means \pm 1 SD or median (25th to 75th percentile). The significance of differences between group means were assessed by a two-sided Student's *t* test or an alternate *t* test and by Fisher's exact test for differences in proportions between groups. Logistic regression was performed with backward selection of variables, using macroangiopathy as a dependent variable. Results were considered significant or nonsignificant for $P \leq$ or >0.05 , respectively.

RESULTS — Table 1 shows the clinical characteristics of both groups of patients with and without increased tHcy. Group 1 subjects were older by an average of 5 years (66 \pm 10 vs. 61 \pm 10 in group 2, $P < 0.02$). The subjects were otherwise comparable in regard to their sex ratio, diabetes duration, positive family history for diabetes or cardiovascular disease, blood pressure levels, and smoking status. BMIs and waist-to-hip ratios were also similar. There was no significant difference in diabetes treatment allocation between groups, although group 1 subjects were less frequently treated with oral antidiabetic drugs and more frequently given exogenous insulin. Patients in group 1 were more frequently treated with lipid- and/or BP-lowering drugs (43 vs. 23%, $P < 0.05$; 84 vs. 65%, $P = 0.05$, respectively). As shown in Table 2, HbA_{1c} levels were 8.2 \pm 1.7 and 8.8 \pm 2.0% in patients with hyper- and normohomocysteinemia, respectively (NS), whereas fasting lipids were comparable in the two groups.

The prevalence of macroangiopathy was significantly higher in group 1 (with hyperhomocysteinemia) than in group 2 (70 vs. 42%, $P < 0.01$) (Fig. 1). The prevalence of coronary artery disease was significantly higher in group 1 (46 vs. 21%, $P < 0.02$), while peripheral and carotid atherosclerotic disease prevalence, although higher in the presence of hyperhomocysteinemia, did not reach significant levels (36 vs. 20% and 14 vs. 6% for groups 1 and 2, respectively). Hypertension was present in 84 and 63% of the patients in groups 1 and 2, respectively ($P < 0.05$).

The proportion of patients with microalbuminuria or albuminuria was comparable in both groups, whereas the prevalence of impaired renal function, evidenced by decreased calculated creatinine clearance,

was significantly higher in group 1 (Table 3). The relationship between tHcy and creatinine clearance in both groups is illustrated in Fig. 2 ($r = -0.372$; $P < 0.001$). When group 1 subjects were compared with an age- and creatinine clearance-matched subgroup with normal tHcy (drawn from group 2, $n = 35$), the prevalence of macroangiopathy was still higher (70 vs. 50%, $P = 0.05$).

Multiple logistic regression with macroangiopathy (dependent entry) against eight variables (tHcy, age, diabetes duration, smoking, BMI, creatinine clearance, hypertension, and the total-to-HDL cholesterol ratio) showed an independent association of macroangiopathy with tHcy ($P = 0.05$), diabetes duration ($P < 0.01$), and smoking status ($P = 0.014$).

On the other hand, no differences were found for retinopathy (45 vs. 42% in groups 1 and 2, respectively) or peripheral neuropathy (70 vs. 59%) (Table 3).

The degree of insulin resistance estimated by HOMA was similar in groups 1 and 2, respectively (insulin sensitivity index [S_I] 46 \pm 21 and 42 \pm 20%, NS), as was the assessment of β -cell function (63 \pm 28 and 65 \pm 46%, NS) (Fig. 3). Likewise, there was no difference in values when patients in both groups treated exclusively with oral antihyperglycemic drugs (i.e., without insulin administration) were considered (HOMA of S_I 50 \pm 22% vs. 44 \pm 22% [NS] and HOMA of β -cell function 85 \pm 75 vs. 69 \pm 25% [NS] in groups 1 and 2, respectively).

Folic acid levels, although still in the normal range, were significantly lower in patients with hyperhomocysteinemia than in those with normal tHcy (5.2 \pm 2.9 vs. 7.0 \pm 3.4 ng/ml, $P < 0.01$). Vitamin

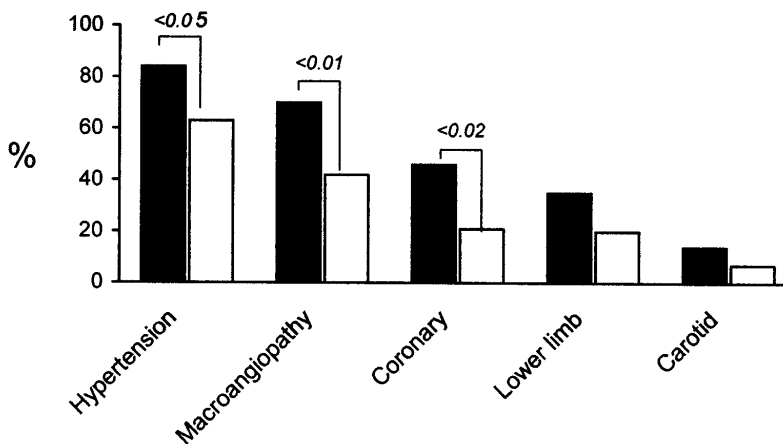


Figure 1—Prevalence of hypertension and macroangiopathy in subjects with hyperhomocysteinemia (group 1, ■, $n = 38$) or normal levels of plasma tHcy (group 2, □, $n = 84$).

Table 3—Neurological and microangiopathic complications in type 2 diabetes with and without hyperhomocysteinemia

	Group 1 (with hyperhomocysteinemia)	Group 2 (without hyperhomocysteinemia)	P
n	38	84	—
Polyneuropathy	70	59	NS
Retinopathy	45	42	NS
Microalbuminuria	33	31	NS
Macroproteinuria	11	15	NS
Creatinine clearance (ml/min)*	67 ± 25	91 ± 36	<0.001
Creatinine clearance <50 ml/min	32	10	<0.005

Data are means ± SD or % unless otherwise indicated. *Values were reached using the Cockcroft-Gault formula.

B₁₂ was slightly (but not significantly) lower in patients in group 1 than in patients in group 2.

When subjects in both groups with creatinine clearance ≥50 ml/min were combined and divided according to whether they were treated with fibrates (23%) or not (77%), higher tHcy levels were found in fibrate-treated patients than in non-fibrate-treated patients (17 ± 5 vs. 13 ± 6 μmol/L, P = 0.0016). However, folic acid and vitamin B₁₂ were comparable (6.3 ± 4.0 vs. 6.5 ± 4.0 ng/ml and 460 ± 202 vs. 582 ± 393 μg/ml, respectively). The prevalence of macroangiopathy (36 vs. 47%) and coronary (28 vs. 28%) and peripheral (8 vs. 21%) vascular disease were not significantly different in both fibrate-treated and non-fibrate-treated groups.

Finally, when patients in both group 1 and group 2 with creatinine clearance ≥50 ml/min were combined and divided according to whether they were treated with metformin (66%) or not (34%), no differences in tHcy levels were found between the metformin-treated patients and the non-metformin-treated patients (12.3 ± 5.3 vs. 13.2 ± 6.2 μmol/L, respectively). Similarly, folic acid levels (6.4 ± 3.0 vs. 6.1 ± 3.6 ng/ml) and vitamin B₁₂ status (501 ± 347 vs. 596 ± 352 μg/ml) were comparable in metformin-treated patients and non-metformin-treated groups, respectively.

CONCLUSIONS— The overall prevalence of hyperhomocysteinemia was high in type 2 diabetic subjects; 31% of the subjects had values >15 μmol/L. There is still debate over the significance of hyperhomocysteinemia in type 2 diabetes and its association with the risk for developing macroangiopathy. Chico et al. (11) reported comparable levels of tHcy in a group of 90 patients with and without macroangiopathy.

By contrast, other studies found a significant association between fasting (and/or post-methionine loading) hyperhomocysteinemia and vascular disease (7,14,15). Our data are in agreement with the latter observations; we found that high levels of fasting tHcy are associated with a higher prevalence of macroangiopathy, and, like Hoogeveen et al. (28), we observed that vascular disease in these patients affected the coronary as well as the peripheral vessels.

In nondiabetic subjects, hyperhomocysteinemia is often a feature of end-stage renal disease (29,30). Similarly, in type 1 and

2 diabetes, it is associated with established nephropathy, particularly when the latter is defined according to filtration indexes (6,14,16–18). However, the relationship between hyperhomocysteinemia and both microalbuminuria and albuminuria is more problematic, with positive correlations being observed by several authors (8,11,12) but not confirmed by others (6,7,14,16). Our own data show that patients with hyperhomocysteinemia have a significantly higher prevalence of impaired renal function (as measured by creatinine clearance) than subjects with normal tHcy levels, while there was no difference when microalbuminuria or albuminuria was used as a surrogate marker of impaired renal function.

The precise relationship among hyperhomocysteinemia, macroangiopathy, and renal disease in type 2 diabetes has not yet been determined. In our series, the difference in macroangiopathy prevalence in subjects with hyperhomocysteinemia was associated with higher nephropathy prevalence; therefore, it could not be excluded that renal impairment per se partly contributed to the development of macroangiopathy. On the other hand, hypertension and the use of BP-lowering drugs were more frequent in the subjects with hyperhomo-

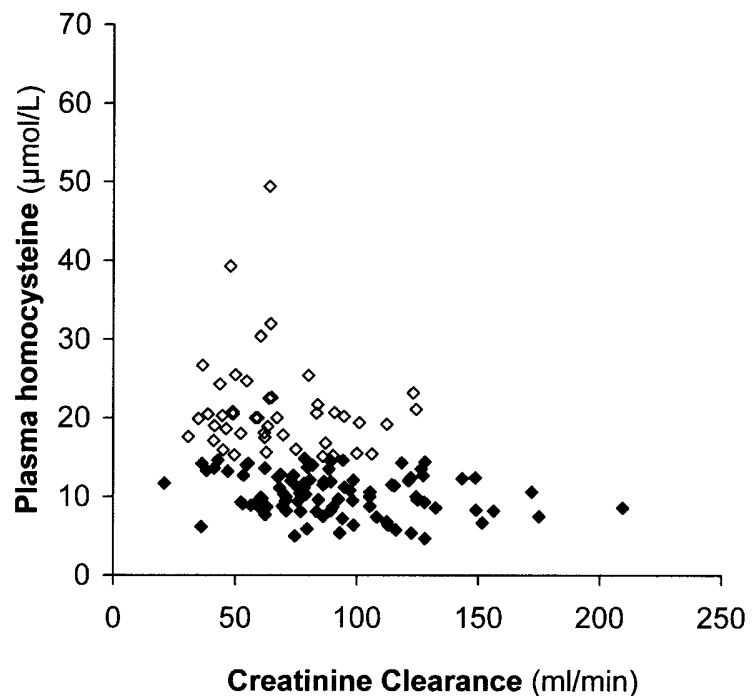


Figure 2—Relationship between plasma tHcy levels and creatinine clearance values (as calculated by the Cockcroft-Gault formula) in subjects with hyperhomocysteinemia (group 1, ◇) or normal levels of plasma tHcy (group 2, ◆). P < 0.001; r = -0.372.

cysteinemia, which could also account for the development of macroangiopathy, as suggested by Fiorina et al. (31). Finally, it can be anticipated from the use of hypolipidemic drugs, particularly fibrates, that patients with high tHcy levels were more often dyslipidemic. Nevertheless, multiple regression analysis disclosed an independent association between macroangiopathy and tHcy when major variables (e.g., creatinine clearance, hypertension, and dyslipidemia) were taken into account. Recent data from Donner et al. (32), Christen et al. (33), and Meleady and Graham (34) suggest that high levels of tHcy could be the result of macroangiopathy—not its causal factor. Our results suggest that elevated tHcy in type 2 diabetes are independently associated with macroangiopathy, although the study design was cross-sectional and does not allow us to define the causality direction for this association. Further studies will determine if high tHcy levels are a primary independent risk factor or a marker of atherosclerosis. Hyperhomocysteinemia could cause macroangiopathy directly or indirectly (via renal dysfunction and/or hypertension) through the hypothesized mechanism of endothelium damage (12,35,36).

Insulin resistance is considered as an independent risk factor (4,37), and a possible relationship between tHcy levels and features of insulin resistance has been suggested. Meigs et al. (38) concluded that marked hyperhomocysteinemia could be a feature of insulin resistance on the basis of fasting and post-oral glucose tolerance test insulin levels in nondiabetic subjects. This association was challenged by Bar-On et al. (39), who reported a negative correlation between tHcy and insulin levels. Our data showed a comparable decrease in insulin sensitivity in type 2 diabetic subjects with and without hyperhomocysteinemia. We used the HOMA model, which is validated for determining the degree of insulin resistance and β -cell function (21–27). Although the type of subjects and/or the methodology used for evaluating insulin resistance could eventually account for some of the observed differences between studies, our data suggest that hyperhomocysteinemia in type 2 diabetes is not associated with vascular damage via a mechanism involving dissimilar degrees of insulin sensitivity.

As far as other chronic complications of diabetes are concerned, we found no correlation between tHcy levels and retinopathy, which is in agreement with other data

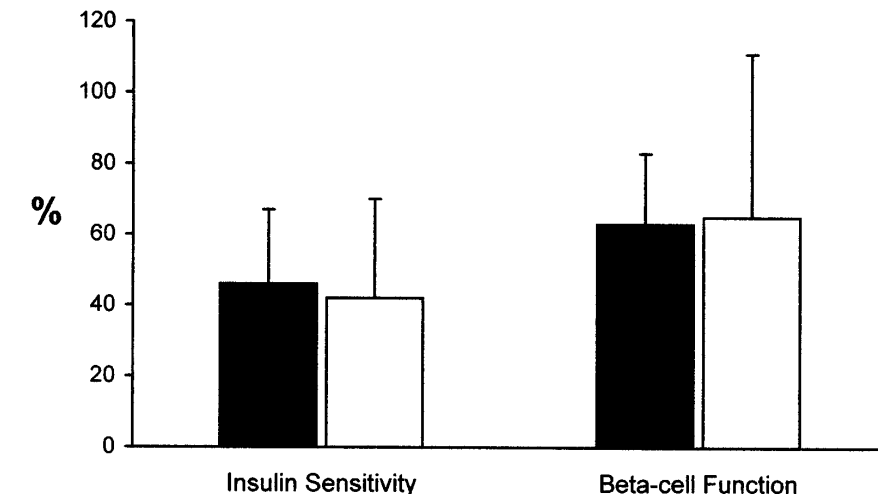


Figure 3—Insulin sensitivity and insulin secretion (assessed by HOMA) in 78 subjects with type 2 diabetes. Group 1 (■) subjects had hyperhomocysteinemia (n = 22), and group 2 (□) had normal plasma tHcy levels (n = 56). Error bars represent 1 SD.

reported in type 2 diabetes (11,18). Similarly, no relationship with neuropathy could be demonstrated, as already documented for type 1 and 2 diabetes (9,40). However, it is of interest to note that Stabler et al. (18) observed higher tHcy levels in type 2 diabetic patients when neuropathy was present.

Plasma folate is clearly the most important determinant of fasting tHcy. Thus, Guttormsen et al. (41) and others (42) have reported an association between the status of plasma folate and levels of tHcy. Our data are in agreement with those observations, because we found the folic acid concentrations to be lower (though still in the normal range) in patients with high tHcy levels.

The reason why plasma folate was lower in diabetic patients with hyperhomocysteinemia than in subjects with normal levels remains equivocal. No difference was observed between groups for alcohol and/or coffee consumption (data not shown), both of which are potentially able to account for the difference (43). Smoking habits were also comparable. The prevalence of gastroparesis (evaluated by gastric emptying studies) was also comparable between groups (data not shown). Therefore, our observations could be caused by other subtle dietary differences (especially cereal and/or vitamin intake) (44) and should thus be extended to include measurement of folates in erythrocytes, which could reflect the body's folate reserves better than values for plasma folate alone.

In agreement with and extending our observations, Boushey et al. (45), Mali-

now et al. (44), and Jacques et al. (46) suggested that dietary supplementation with folic acid and/or vitamin B₁₂ could be useful in patients with hyperhomocysteinemia, in particular when macroangiopathy was present. Preliminary data suggest that progression of carotid atherosclerotic plaques could be retarded by vitamin supplementation (47).

In nondiabetic individuals, the use of fibrates for treating hyperlipidemia could potentially be a confounding factor for hyperhomocysteinemia (48). In our diabetic patients, we also observed higher tHcy levels when fibrates were administered, although in fibrate-treated and non-fibrate-treated patients, folic acid and vitamin B₁₂ levels were comparable, as was the prevalence of macroangiopathy.

Another possible confounding factor is metformin usage. Carlsen et al. (49) reported that metformin therapy was associated with increased tHcy levels in nondiabetic subjects with cardiovascular disease. However, we found no significant difference between plasma tHcy concentrations in metformin-treated and non-metformin-treated diabetic patients, in accordance with Hoogeveen et al. (50).

In conclusion, the present study shows that elevation of plasma tHcy levels in type 2 diabetic subjects is associated with a higher prevalence of macroangiopathy and impaired renal function when assessed by a creatinine clearance index. The precise physiopathological mechanisms by which hyperhomocysteinemia is associated with

macroangiopathy is still being debated. Our data suggest that hyperhomocysteinemia is not associated with different degrees of insulin resistance. On the other hand, we report lower levels of folic acid in diabetic subjects with hyperhomocysteinemia, though still in the normal laboratory range. Further prospective studies are needed to determine whether dietary supplementation with this nutrient or other interventions aimed at decreasing plasma tHcy could reduce cardiovascular morbidity and mortality in type 2 diabetes.

Acknowledgments— The authors thank S. Meerkens for excellent secretarial assistance.

References

1. Nygard O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug J, Ueland P, Kvale G: Total plasma homocysteine and cardiovascular risk profile: the Hordaland Homocysteine Study. *JAMA* 274:1526–1533, 1995
2. Perry IJ, Refsum H, Morris RW, Ebrahim SB, Ueland P, Shaper AG: Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. *Lancet* 346:1395–1398, 1995
3. Welch G, Loscalzo J: Homocysteine and atherothrombosis (Review). *N Engl J Med* 338:1042–1050, 1998
4. Lheto S, Rönnemaa T, Pyörälä K, Laakso M, Laakso M: Cardiovascular risk factors clustering with endogenous hyperinsulinaemia predict death from coronary heart disease in patients with type 2 diabetes. *Diabetologia* 43:148–155, 2000
5. Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M: Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 339:229–234, 1998
6. Agardh CD, Agardh E, Andersson A, Hultberg B: Lack of association between plasma homocysteine levels and microangiopathy in type 1 diabetes mellitus. *Scand J Clin Lab Invest* 54:637–641, 1994
7. Munshi M, Stone A, Fink L, Fonseca V: Hyperhomocysteinemia following a methionine load in patients with non-insulin-dependent diabetes mellitus and macrovascular disease. *Metabolism* 45:133–135, 1996
8. Hofmann MA, Kohl B, Zumbach MS, Borcea V, Bierhaus A, Henkels M, Amiral J, Fiehn W, Ziegler R, Wahl P, Nawroth PP: Hyperhomocyst(e)inemia and endothelial dysfunction in IDDM. *Diabetes Care* 20:1880–1886, 1997
9. Cronin C, McPartlin J, Barry D, Barry Ferriss J, Scott J, Weir D: Plasma homocysteine concentrations in patients with type 1 diabetes. *Diabetes Care* 21:1843–1847, 1998
10. van Leeuwen-Wintjens HR, Muls EE: The implications of hyperhomocysteinemia in a patient with type 1 diabetes. *Acta Clin Belg* 53:349–352, 1998
11. Chico A, Perez A, Cordoba A, Arcelus R, Carreras G, de Leiva A, Gonzalez-Sastre F, Blanco-Vaca F: Plasma homocysteine is related to albumin excretion rate in patients with diabetes mellitus: a new link between diabetic nephropathy and cardiovascular disease? *Diabetologia* 41:684–693, 1998
12. Lanfredini M, Fiorina P, Peca MG, Veronelli A, Mello A, Astorri E, Dall’Aglio P, Craveri A: Fasting and post-methionine load homocyst(e)in values are correlated with microalbuminuria and could contribute to worsening vascular damage in non-insulin-dependent diabetes mellitus patients. *Metabolism* 47:915–921, 1998
13. Okada E, Oida K, Tada H, Asazuma K, Eguchi K, Tohda G, Kosaka S, Takahashi S, Miyamori I: Hyperhomocysteinemia is a risk factor for coronary arteriosclerosis in Japanese patients with type 2 diabetes. *Diabetes Care* 22:484–490, 1999
14. Smulders Y, Rakic M, Slaats E, Treskes M, Sijbrands E, Odekerken D, Stehouwer C, Silberbusch J: Fasting and post-methionine homocysteine levels in NIDDM: determinants and correlations with retinopathy, albuminuria, and cardiovascular disease. *Diabetes Care* 22:125–132, 1999
15. Araki A, Sako Y, Ito H: Plasma homocysteine concentrations in Japanese patients with non-insulin-dependent diabetes mellitus: effect of parenteral methylcobalamin treatment. *Atherosclerosis* 103:149–157, 1993
16. Wollesen F, Brattstrom L, Refsum H, Ueland P, Berglund L, Berne C: Plasma total homocysteine and cysteine in relation to glomerular filtration rate in diabetes mellitus. *Kidney Int* 55:1028–1035, 1999
17. Hultberg B, Agardh E, Andersson A, Brattstrom L, Isaksson A, Israelsson B, Agardh CD: 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
18. Stabler S, Estacio R, Jeffers B, Cohen J, Allen R, Schrier R: Total homocysteine is associated with nephropathy in non-insulin-dependent diabetes mellitus. *Metabolism* 48:1096–1101, 1999
19. Kang SS, Wong PW, Malinow MR: Hyperhomocyst(e)inemia is a risk factor for occlusive vascular disease. *Ann Rev Nutr* 12:279–298, 1992
20. Ueland P, Refsum H, Stabler S, Malinow MR, Anderson A, Allen RH: Total homocysteine in plasma or serum: methods and clinical applications. *Clin Chem* 39:1764–1779, 1993
21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentration in man. *Diabetologia* 28:412–419, 1985
22. Hosker JP, Matthews DR, Rudenski AS, Burnett MA, Darling P, Bown EG, Turner RC: Continuous infusion of glucose with model assessment: measurement of insulin resistance and β -cell function in man. *Diabetologia* 28:401–411, 1985
23. Rudenski AS, Matthews DR, Levy JC, Turner RC: Understanding “insulin resistance”: both glucose resistance and insulin resistance are required to model human diabetes. *Metabolism* 40:908–917, 1991
24. Levy JC, Rudenski A, Burnett M, Knight R, Matthews DR, Turner RC: Simple empirical assessment of β -cell function by a constant infusion of glucose test in normal and type 2 (non-insulin-dependent) diabetic subjects. *Diabetologia* 34:488–499, 1991
25. Levy JC, Matthews DR, Hermans MP: Correct homeostasis model assessment (HOMA) evaluation uses the computer program (Letter). *Diabetes Care* 21:2191–2192, 1998
26. Hermans MP, Levy JC, Morris RJ, Turner RC: Comparison of tests of β -cell function across a range of glucose tolerance from normal to diabetes. *Diabetes* 48:1779–1786, 1999
27. Hermans MP, Levy JC, Morris RJ, Turner RC: Comparison of insulin sensitivity tests across a range of glucose tolerance from normal to diabetes. *Diabetologia* 42:678–687, 1999
28. Hoogeveen E, Kostense PJ, Beks PJ, Mackaay AJ, Jakobs C, Bouter LM, Heine R, Stehouwer CD: Hyperhomocysteinemia is associated with an increased risk of cardiovascular disease, especially in non-insulin-dependent diabetes mellitus: a population-based study. *Arterioscler Thromb Vasc Biol* 18:133–138, 1998
29. Bostom AG, Shemin D, Lapane K, Miller J, Sutherland P, Nadeau M, Seyoum E, Hartman W, Prior R, Wilson P, Selhub J: Hyperhomocysteinemia and traditional cardiovascular disease risk factors in end-stage renal disease patients on dialysis: a case-control study. *Atherosclerosis* 114:93–103, 1995
30. Chauveau P, Chadefaux B, Coude M, Aupetit J, Hannedouche T, Kamoun P, Jungers P: Hyperhomocysteinemia, a risk factor for atherosclerosis in chronic uremic patients. *Kidney Int* 41:72–77, 1993
31. Fiorina P, Lanfredini M, Montarani A, Peca M, Veronelli A, Mello A, Astorri E, Craveri A: Plasma homocysteine and folate are related to arterial blood pressure in type 2 diabetes mellitus. *Am J Hypertens* 11:1100–1107, 1998
32. Donner M, Klein G, Mathes P, Schwandt P,

- Richter W: Plasma total homocysteine levels in patients with early-onset coronary heart disease and a low cardiovascular risk profile. *Metabolism* 47:273–279, 1998
33. Christen W, Ajani U, Glynn R, Hennekens C: Blood levels of homocysteine and increased risk of cardiovascular disease. *Arch Intern Med* 160:422–434, 2000
34. Meleady R, Graham I: Plasma homocysteine as a cardiovascular risk factor: causal, consequential, or of no consequence (Review)? *Nutr Rev* 57:299–305, 1999
35. Colwell JA: Elevated plasma homocysteine and diabetic vascular disease (Editorial). *Diabetes Care* 20:1805–1806, 1997
36. Tawakol A, Omland T, Gerhard M, Wu J, Creager M: Hyperhomocyst(e)inemia is associated with impaired endothelium-dependent vasodilatation in humans. *Circulation* 95:1119–1121, 1997
37. Savage PJ: Cardiovascular complications of diabetes mellitus: what we know and what we need to know about their prevention (Review). *Ann Int Med* 124:123–126, 1996
38. Meigs JB, Jacques P, Selhub J, Murphy-Sheehy P, Nathan D, Singer D, D'Agostino R, Wilson P: Elevated plasma homocysteine levels are more prevalent among Framingham Offspring Study subjects with the insulin resistance syndrome (Abstract). *Diabetes* 48 (Suppl. 1):A165, 1999
39. Bar-On H, Kidron M, Friedlander Y, Ben-Yehuda A, Selhub J, Rosenberg I, Friedman G: Plasma total homocysteine levels in subjects with hyperinsulinemia. *J Intern Med* 247:287–294, 2000
40. Hoogeveen G, Kostense P, Valk G, Bertelsmann F, Jakobs C, Dekker J, Nijpels G, Heine R, Bouter L, Stehouwer C: Hyperhomocysteinemia is not related to risk of distal somatic polyneuropathy: the Hoorn Study. *J Intern Med* 246:561–566, 1999
41. Guttormsen A, Ueland P, Nesthus I, Nygard O, Schneede J, Vollset S, Refsum H: Determinants and vitamin responsiveness of intermediate hyperhomocysteinemia (≥ 40 $\mu\text{mol/liter}$): the Hordaland Homocysteine Study. *J Clin Invest* 98:2174–2183, 1996
42. Hultberg B, Agardh C, Agardh E, Lovestam-Adrian M: Poor metabolic control, early age at onset and marginal folate deficiency are associated with increasing levels of plasma homocysteine in insulin-dependent diabetes mellitus: a five-year follow-up study. *Scand J Clin Lab Invest* 57:595–600, 1977
43. Nygard O, Refsum H, Ueland P, Stensvold I, Nordrehaug J, Kvale G, Vollset S: Coffee consumption and plasma total homocysteine: the Hordaland Homocysteine Study. *Am J Clin Nutr* 65:136–143, 1997
44. Malinow M, Duell P, Hess D, Anderson P, Kruger W, Phillipson B, Gluckman R, Block P, Upson B: Reduction of plasma homocyst(e)ine levels by breakfast cereal fortified with folic acid in patients with coronary heart disease. *N Engl J Med* 338:1009–1015, 1998
45. Boushey C, Beresford S, Omenn G, Motulsky A: A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. *JAMA* 274:1049–1057, 1995
46. Jacques P, Selhub J, Bostom A, Wilson P, Rosenberg I: The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *N Engl J Med* 340:1449–1454, 1999
47. Peterson J, Spence J: Vitamins and progression of atherosclerosis in hyperhomocyst(e)inaemia. *Lancet* 351:263, 1998
48. de Lorgeril M, Salen P, Paillard F, Lacan P, Richard G: Lipid-lowering drugs and homocysteine. *Lancet* 353:209–210, 1999
49. Carlsen S, Folling I, Grill V, Bjerve KS, Schneede J, Refsum H: Metformin increases total serum homocysteine levels in nondiabetic male patients with coronary heart disease. *Scand J Clin Invest* 57:521–528, 1997
50. Hoogeveen E, Kostense P, Jakobs C, Bouter L, Heine R, Stehouwer C: Does metformin increase the serum total homocysteine level in noninsulin-dependent diabetes mellitus? *J Intern Med* 242:389–394, 1997