Plasma Homocysteine and Folate Are Related to Arterial Blood Pressure in Type 2 Diabetes Mellitus

Paolo Fiorina, Mario Lanfredini, Alberto Montanari, Maria Grazia Peca, Annamaria Veronelli, Alessandra Mello, Ettore Astorri, and Angelo Craveri

The aim of this study was to assess the relationship between homocysteine (tHcy), folate and vitamin B12 levels, urinary albumin excretion, and arterial blood pressure in patients with non–insulin-dependent diabetes mellitus (NIDDM). Our study was carried out in 33 NIDDM patients (16 men, 17 women) and 16 healthy volunteers as controls (seven men, nine women). Fasting and postmethionine load plasma tHcy levels were assessed, together with folate, vitamin B12, and urinary albumin excretion levels. In NIDDM patients, there were correlations between folate and mean arterial pressure ($r = 0.352, P = 0.046$), folate and systolic blood pressure ($r = 0.437, P = 0.013$), folate and vitamin B12 ($r = 0.499, P = 0.004$), tHcy and vitamin B12 ($r = -0.348, P = 0.04$), ln tHcy and ln folate ($r = -0.404, P = 0.01$), and, lastly, between tHcy, either fasting or postload, and urinary albumin excretion. Patients with elevated tHcy levels had significantly higher diastolic blood pressure ($P = 0.04$) and mean arterial pressure ($P = 0.03$). Otherwise, higher folate values were associated with lower systolic blood pressure ($P = 0.004$) and mean arterial pressure ($P = 0.02$). In addition, NIDDM patients with complications presented higher tHcy basal values than the group without complications ($P = 0.003$). A particular propensity of such patients towards endothelial dysfunction could explain the presence of correlations between these metabolic parameters and arterial blood pressure. Am J Hypertens 1998; 11:1100 –1107 © 1998 American Journal of Hypertension, Ltd.

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Folate and vitamin B12 are both involved in the determination of plasma homocysteine values. Homocysteine (tHcy) is an intermediate sulphydryl amino acid formed during the conversion of methionine to cysteine. Almost all of the population presented with tHcy plasma values ranging from 5 to 15 μmol/L, but often, due to genetic or acquired factors, the plasma tHcy level was enhanced. This enhancement might be responsible for atherogenic and thrombotic tendencies. Among the possible causes: an induction of cyclin A gene expression in vascular smooth muscle cells; an endothelial dysfunction; a reduced level of protein C, a natural anticoagulant involved in the blockage of factor V and VII of the coagulatory cascade; an inhibition of von Willebrand factor processing and secretion; an enhancement of lipid peroxidation; a direct endothelial damage caused by tHcy, due to a toxic sulphur-containing amino acid accumulation in endothelial cells.
an interaction between nitric oxide (NO) and tHcy\textsuperscript{11,12}, and finally a reduction in serum antithrombin activity, with a reduction of thrombomodulin, which binds with thrombin.\textsuperscript{13} A previous study has suggested that not only are plasma tHcy levels correlated with atherothrombotic events, but serum folate is also correlated with coronary artery disease.\textsuperscript{14} Increased risks were not related only to individuals with extremely low serum folate levels, but were also observed for individuals with normal levels, suggesting that current definitions of appropriate serum folate levels might be reassessed. Furthermore, dietary folate was inversely associated with carotid artery stenosis in the Framingham Study.\textsuperscript{14} None, previously, have demonstrated the presence of any relationship between arterial blood pressure and plasma folate or vitamin B\textsubscript{12} levels. Moreover, only a few studies have demonstrated an increase in plasma tHcy levels in hypertensive patients, whether asymptomatic\textsuperscript{15,16} or with cerebral infarction.\textsuperscript{17} Particularly, we thought that non–insulin-dependent diabetes mellitus (NIDDM) patients might represent a special group of subjects, in which a propensity towards endothelial dysfunction could lead to an enhanced endothelial vulnerability. Slight variations in plasma homocysteine values, or differences in folate or vitamin B\textsubscript{12} levels, which are correlated with the methionine-homocysteine pathway, could induce alterations in arterial regulatory tone. Our purpose was to assess the role of homocysteine, folate, vitamin B\textsubscript{12}, and increased urinary albumin excretion (as a sign of systemic disorders of endothelial function\textsuperscript{18}) in influencing ambulatorial function in NIDDM patients.

**PATIENTS AND METHODS**

Our study was carried out in 33 NIDDM patients (16 men; 17 women; mean age, 60.9 ± 5.93 years; duration of disease, 7.75 ± 5.35 years), diagnosed as suggested in previous studies,\textsuperscript{19} and 16 healthy volunteers as controls, (seven men, nine women), matched for age, gender, and Quatelet index. Patients and controls were enrolled from a diabetic center. During enrollment, we tried to maintain a 1:1:1 ratio between the groups of microalbuminuric:normoalbumunuric:control. All the patients and controls gave their informed consent to be enrolled in our study. They were normotensive without antihypertensive treatment, according to World Health Organization criteria.\textsuperscript{20}

If systolic blood pressure was greater than 160 mm Hg, or diastolic blood pressure greater than 95 mm Hg, patients were excluded from the study. If they had overt renal dysfunction (creatinine > 1.5 mg/dL) or hypercholesterolemia (plasma cholesterol > 240 mg/dL), they were excluded from the study. Systemic blood pressure was determined at the arm after being in the supine position for at least 10 min, as the mean of at least three consecutive measurements by standard sphygmomanometry. Operators, during arterial blood pressure measurement, were blinded to patients’ or controls’ group, and, obviously, to plasma homocysteine levels.

Plasma homocysteine levels were assessed at baseline in both patients and controls, as was folate and vitamin B\textsubscript{12}. Furthermore, microalbuminuria, HbA\textsubscript{1c}, creatinine, total cholesterol, HDL, triglycerides, number of leukocytes, smoking, the presence of complications, and therapy, were analyzed in NIDDM patients. Vitamin B\textsubscript{12} and folate levels were determined simultaneously in a single tube by the ICN Pharmaceuticals (Chicago, IL) SimulTRACSNB Radioassay Kit. All patients and controls were subjected to the methionine load test, because in some patients with impaired homocysteine metabolism, fasting concentrations may be normal.\textsuperscript{21} Participants were phlebotomized after an overnight fast (10 to 14 h), and blood samples were taken at baseline and once again 5 h after receiving an oral load (0.1 g/kg body weight) of l-methionine.\textsuperscript{21}

All whole blood specimens for homocysteine analysis were drawn into vacutainers containing EDTA, and immediately refrigerated at 4°C. Within 2 h of collection blood samples were centrifuged, and then the EDTA plasma was separated and stored at −20°C. Total plasma homocysteine was determined by HPLC with fluorescence detection as previously described by Ubbink et al.\textsuperscript{22} Before reversed-phase HPLC analysis, the plasma thiols were derived with ammonium 7-flurobenzo-2-oxa-]-3-diazole-4sulphonate, a thiol-specific fluorogenic probe that is commercially available. The method is simple, sensitive, reproducible (between-run coefficient of variation of 6.6%), and very suitable for routine determination of tHcy levels. Urinary albumin excretion (UAE) was measured by nephelometry (Microalbuminuria Kit, Kone Diagnostics, Espoo, Finland), and microalbuminuria was defined as urinary albumin excretion greater than or equaling 30 mg/24 h and less than 300 mg/24 h. All the other parameters were analyzed as described in previous studies.\textsuperscript{3,21,23} A positive smoking history was recorded when the individual was still a smoker, or had quit less than 1 year before being interviewed. Atherothrombotic and cardiovascular complications were established on the basis of recorded documentation of myocardial infarction, stroke, coronary artery disease, cerebrovascular or peripheral vascular disease, retinopathy, nephropathy, and using standardized enzymatic/ECP, computed tomography scan, angiography, or B-mode/Doppler ultrasound.

Descriptive values were expressed as mean ± SD. Differences between groups, due to a not normal data distribution, were tested with Kolmogorov-Smirnov two-samples test. Spearman’s rank correlation coeffi-
Table 1. Characteristics of the NIDDM Patients

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Median</th>
<th>25th Percentile</th>
<th>75th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>177.9 ± 46.1</td>
<td>176</td>
<td>153</td>
<td>209</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>138 ± 13.2</td>
<td>135</td>
<td>130</td>
<td>150</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>80 ± 9.01</td>
<td>80</td>
<td>75</td>
<td>85</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>99.41 ± 9.27</td>
<td>100</td>
<td>90</td>
<td>106</td>
</tr>
<tr>
<td>Folate (ng/mL)</td>
<td>74.5 ± 36.5</td>
<td>68</td>
<td>51</td>
<td>85</td>
</tr>
<tr>
<td>B12 (pg/mL)</td>
<td>582.6 ± 207.6</td>
<td>559</td>
<td>435</td>
<td>690</td>
</tr>
<tr>
<td>tHcy (µmol/L)</td>
<td>8.12 ± 3.17</td>
<td>7.86</td>
<td>6.16</td>
<td>9.41</td>
</tr>
<tr>
<td>Post met tHcy (µmol/L)</td>
<td>26.5 ± 11.5</td>
<td>24.6</td>
<td>17.9</td>
<td>29.9</td>
</tr>
<tr>
<td>UAE (mg/24 h)</td>
<td>33.5 ± 25.7</td>
<td>30</td>
<td>17</td>
<td>38</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.04 ± 1.84</td>
<td>7.8</td>
<td>6.9</td>
<td>8.4</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.90 ± 0.29</td>
<td>0.9</td>
<td>0.8</td>
<td>1.0</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, systemic mean arterial pressure; B12, plasma vitamin B12 values; tHcy, fasting homocysteine values; Post met tHcy, postmethionine homocysteine values; HbA1c, glycosylated hemoglobin.

Correlations and Multivariate Analysis

The correlations between arterial blood pressure and all the other parameters were analyzed, and, due to the presence of a not-normal distribution, Spearman's rank coefficient was evaluated. There were negative correlations between folate and mean arterial pressure \( r = -0.352, P = .046 \) (Figure 1); folate and systolic blood pressure \( r = -0.436, P = .013 \) (Figure 1); and tHcy and vitamin B12 \( r = -0.348, P = .04 \). In addition, positive correlations appeared between vitamin B12 and folate \( r = 0.499, P = .004 \), glycosylated hemoglobin and glucose blood levels \( r = 0.374, P = .03 \), and fasting and postload tHcy and urinary albumin excretion. After a logarithmic transformation, the correlations between the parameters were reanalyzed by linear regression analysis, and an inverse correlation between ln tHcy and ln folate was revealed. Otherwise, a slight correlation (even if not significant) appeared between ln tHcy and ln MAP \( r = 0.25 \), and between ln tHcy and ln DBP \( r = 0.27 \). By using multiple regression analysis, all the arterial blood pressure parameters were tested for tHcy, UAE, folate, vitamin B12, glucose blood levels, cholesterol, and glycosylated hemoglobin. The parameter that appeared to exert an independent effect on arterial blood pressure was folate for MAP \( F = 3.24, P = .07 \).

Percentile Analysis

A first percentile analysis was made by splitting the NIDDM patients into two groups, depending on whether their levels of each parameter were above the 75th percentile.

Patients with homocysteine levels higher than 9.4 \( \mu \text{mol/L} \) had enhanced values of diastolic blood pres-
sure (86.25 ± 8.76 v 78.12 ± 8.44 mm Hg; P = .004) (Figure 2). Moreover, systolic blood pressure (144.37 ± 13.47 v 137.08 ± 12.59 mm Hg; P = .09) and mean arterial pressure (105.60 ± 8.95 v 97.75 ± 8.67 mm Hg; P = .09) appeared to be related to tHcy levels. Nevertheless, postmethionine load tHcy level was not related to arterial blood pressure.

Urinary albumin excretion is related to arterial blood pressure; NIDDM patients with higher UAE values had enhanced levels of systolic blood pressure (146.25 ± 12.17 v 135.43 ± 11.76 mm Hg; P = .02), diastolic blood pressure (84.37 ± 4.95 v 78.26 ± 9.00 mm Hg; P = .003), and mean arterial pressure (104.96 ± 6.36 v 97.29 ± 9.46 mm Hg; P = .02).

FIGURE 1. Regression plot of folate plasmatic levels on systemic mean arterial pressure (MAP), (Spearman’s rank correlation coefficient = −0.352, P = .046) and on systolic blood pressure (SBP), (Spearman’s rank correlation coefficient = −0.437, P = .013) respectively.

FIGURE 2. Plot of diastolic blood pressure (DBP) obtained by splitting the patients into two groups with homocysteine higher or lower than 75th percentile (86.25 ± 8.76 v 78.12 ± 8.44; F = 4.0657; P = .043.)
Among the parameters considered, folate appeared to be related to arterial blood pressure. Systolic blood pressure (142.60 ± 13.13 vs 126.87 ± 5.93 mm Hg; *P* = .001), DBP, and mean arterial pressure were lower in the NIDDM patients who presented with higher folate values.

Low vitamin B12 levels were associated with higher systolic (139.58 ± 13.74 vs 134.37 ± 12.37 mm Hg; *P* = .03) and diastolic blood pressure (Table 2). No significant differences were associated with glycosylated hemoglobin.

**Variance Analysis** Using the Kruskal-Wallis test, patients were examined to see whether parameters were above or below the 75th percentile of their own distribution curve. This analysis allowed us to closely evaluate any differences between the groups by considering intra- and intergroup variations. Homocysteine, folate, and urinary albumin excretion appeared to be strongly related to arterial blood pressure among the parameters considered. The role of vitamin B12 was slightly reduced if the groups were analyzed using this method. As regards the predictive values of ln diastolic blood pressure both for ln tHcy and ln folate using regression analysis, we obtained a remarkable effect, ie, an increase in tHcy levels from 6 to 12 μmol/L, associated with a rise in DBP values of 4.5 mm Hg. If tHcy levels increased threefold (from 6 to 18 μmol/L), we observed an increase of 8 mm Hg in DBP values. Moreover, if plasma folate values increased from 40 to 80 ng/mL, a decrease of 4 mm Hg in DBP values was observed.

**Analysis of Microalbuminuria** If we considered patients with microalbuminuria alone, all correlations were confirmed and enhanced: folate versus MAP (**r** = −0.463), folate versus SBP (**r** = −0.482), vitamin B12 versus folate (**r** = 0.739), tHcy versus vitamin B12 (**r** = −0.624), ln tHcy versus ln folate (**r** = −0.052), and tHcy versus UAE (**r** = 0.541). In addition, new correlations appeared, eg, vitamin B12 versus MAP (**r** = −0.581, *P* = .02).

**DISCUSSION**

The concentration of homocysteine in the plasma is regulated by several factors, genetic and acquired. Elevated concentrations of homocysteine, both under fasting conditions and postmethionine load conditions, were correlated with several atherothrombotic and cardiovascular disorders. Previous studies have discussed the potential interaction between tHcy and glucose intolerance as a risk factor for atherosclerosis. Also, as suggested in previous studies, no difference were observed between our diabetic patients and controls as regards plasma tHcy levels, folate, and vitamin B12 values. When the subjects were grouped in deciles of plasma vitamin concentration, the mean plasma tHcy levels were higher in the lowest deciles of vitamin B12 and folic acid concentrations, but it has been well established that folate and vitamin deficiencies may result in hyperhomocysteinemia. Otherwise, previous studies have suggested that plasma folate and vitamin B12 concentrations are related to an increased risk of coronary artery disease, but this risk disappeared after adjustment for plasma tHcy concentrations.

In our study a correlation between several parameters involved in the methionine-homocysteine pathways (such as plasma folate and tHcy levels) and
hemodynamic data was shown. Correlations were observed between folate and both MAP and SBP, respectively, whereas this was not confirmed with vitamin B₁₂. As regards the relationship between parameters and arterial blood pressure, it is important to distinguish between results that are identical and those that are not statistically significantly different. For example, MAP values in the groups above or below the 75th percentile of vitamin B₁₂ distribution are not statistically significant, despite a 5-mm Hg difference in mean arterial pressure. In a metaanalysis involving 420,000 patients, a 5-mm Hg difference in diastolic pressure was associated with 34% fewer strokes and 21% less coronary heart disease. After a multiple-stepwise regression analysis, only folate appeared to be independently linked to MAP, even if only slightly. This means that it is difficult to speculate whether folate and vitamin B₁₂ could be related to arterial regulatory tone only through the tHcy pathway.

It is likely that the control of vitamin B₁₂ and folate in NIDDM patients, either by increased dietary intake or vitamin supplement, can prevent an increase in tHcy levels. The correct treatment of an elevated plasma tHcy concentration by harmless vitamin supplements constitutes no health risk but may generate benefits in slowing atherothrombotic events, especially in microalbuminuric NIDDM patients. The Kruskal-Wallis analysis of percentile variance allowed us to observe how higher tHcy values were correlated with increased diastolic blood pressure and mean arterial pressure values, but not with systolic blood pressure values. A twofold increase in tHcy values (from 6 to 12 μmol/L) on a logarithmic scale was associated with 4.5-mm Hg increase in DBP values (this means three more strokes and two more coronary events per 10 patients). If a threefold increase was considered, on the regression analysis of predictive values, an increase of 8 mm Hg was observed. These plasma tHcy values were not so rare in a randomized cohort of patients.
Several potential mechanisms could be proposed for the relationship between folate, tHcy, vitamin B12, and microalbuminuria with blood pressure parameters, in NIDDM patients. These patients presented a decreased synthesis and an increased inactivation of endothelial nitric oxide by oxygen-derived free radicals or advanced glycosylated end products.\textsuperscript{35} NIDDM patients, particularly those with microalbuminuria, could represent a group of patients with a major propensity toward endothelial dysfunction, which could be revealed by slight alterations in plasma tHcy, folate, or vitamin B12 levels.

Recently a correlation between systolic blood pressure and plasma tHcy was demonstrated in a hypertensive geriatric population.\textsuperscript{36} It is possible that in conditions of reduced NO production, such as in NIDDM patients, a slight increase in tHcy (due to a decrease in folate or vitamin B12 levels) could scavenge residual endothelial nitric oxide and influence arterial regulatory tone. Homocysteine enhancement could be the link between high blood pressure, increased urinary albumin excretion, and elevated atherothrombotic events, which we observed in well-treated and compensated NIDDM patients (Figure 3).

REFERENCES


