Cigarette Smoking and Plasma Total Homocysteine Levels in Young Adults With Type 1 Diabetes

OBJECTIVE — The purposes of this study were to compare plasma total homocysteine (tHcy) levels, a recognized cardiovascular risk factor, in nondiabetic subjects and type 1 diabetic patients, and to evaluate whether chronic cigarette smoking had a deleterious effect on plasma tHcy levels in type 1 diabetic patients.

RESEARCH DESIGN AND METHODS — Plasma tHcy concentrations were measured in 60 young type 1 diabetic patients without clinical evidence of macroangiopathy and in 30 healthy control subjects who were matched for age, sex, BMI, and smoking habit.

RESULTS — Plasma tHcy levels were significantly higher in type 1 diabetic patients than in control subjects (12.5 ± 4.8 vs. 10.3 ± 2.2 µmol/l, P < 0.01). After stratification by smoking status, diabetic smokers had values for age, sex, BMI, lipids, creatinine, blood pressure, glycometabolic control, diabetes duration, and microvascular complications that were superimposable on their nonsmoking counterparts. Nevertheless, plasma tHcy levels were markedly elevated in diabetic smokers versus nonsmokers (15.5 ± 5.7 vs. 10.6 ± 3 µmol/l, P < 0.0001) in a dose-dependent fashion (P < 0.0001, by analysis of variance when subjects were categorized for the number of cigarettes smoked daily).

CONCLUSIONS — Chronic cigarette smoking seems to adversely affect plasma tHcy levels in young adults with type 1 diabetes.

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Mild elevations of plasma total homocysteine (tHcy) levels have been recently demonstrated to be an established risk factor for atherosclerotic coronary heart disease, cerebrovascular disease, and lower-extremity occlusive disease in both nondiabetic and diabetic individuals (1–10). In particular, hyperhomocysteinemia appears to be a stronger risk factor for cardiovascular disease (CVD) in subjects with diabetes than in subjects with normal or impaired glucose tolerance (8).

There are now consistent data regarding the relationships between plasma tHcy levels and established CVD risk factors in nondiabetic subjects (4–12). However, there is limited and somewhat conflicting information regarding the effect of the diabetic state per se on plasma levels of tHcy, particularly in individuals with type 1 diabetes. Plasma tHcy concentrations have been found to be normal (13,14), lower (15,16), or higher (17) in type 1 diabetic patients compared with those in control subjects. Moreover, little is known about the relationships of plasma tHcy levels with established CVD risk factors in type 1 diabetic individuals (13,14,16). A recent large epidemiological study (4) showed that the CVD risk was especially high among smokers with mild hyperhomocysteinemia, thus supporting the evidence of a strong interaction between these risk factors for CVD. However, this recent study excluded diabetic subjects.

Although higher plasma levels of tHcy have been demonstrated in nondiabetic smokers versus nonsmokers (4,11,12), to our knowledge, there is a lack of available data regarding the impact of smoking on plasma tHcy levels in young adults with type 1 diabetes. On the other hand, the clarification of that may have important implications for risk management and our understanding of the pathophysiological mechanisms of CVD.

Thus, the main purpose of the present study was to evaluate whether chronic cigarette smoking had an adverse effect on plasma tHcy levels in a group of young type 1 diabetic patients with no clinical evidence of macroangiopathy.

RESEARCH DESIGN AND METHODS — The study population consisted of 60 young adults with type 1 diabetes who regularly attended the outpatient Diabetes Clinic of the University of Verona and the Diabetes Unit of the Hospital of Negrar (Verona, Italy) and 30 healthy volunteers who were recruited from hospital staff and relatives and were matched for age, sex, BMI, and smoking status. All participants underwent a medical history and a physical examination. None of the subjects had a history of recent acute illness or clinical evidence suggestive of any cardiovascular events or kidney or liver diseases. To exclude the presence of clinical macroangiopathy, a conventional 12-lead resting electrocardiogram, measurement of ankle brachial pressure index, and carotid ultrasoundography were performed in all of the diabetic subjects. Type 1 diabetic subjects...
had been treated with insulin and diet and had stable metabolic control; none of the participants, including the control subjects, were taking any other medications. Blood pressure was measured with a standard manometer by a trained staff member. Information on smoking habit was obtained from all of the participants through a questionnaire. Subjects were categorized into those who had never smoked and those who currently smoked. Smokers were then grouped into two categories: light smokers (1–10 cigarettes/day) and moderate-to-heavy smokers (>10 cigarettes/day). Venous blood was drawn in the morning (8:00–8:30 AM) after an overnight fast and ≥8 h of abstinence from smoking. Levels of plasma glucose, creatinine, lipids, and other basic biochemical blood measurements were determined by an automatic colorimetric method (DAX 96; Bayer Diagnostics, Milan, Italy). HbA1c concentrations were measured by an automated high-performance liquid chromatography (HPLC) analyzer (Bio-Rad Diamat, Milan, Italy) (18); normal range values in our laboratory were 3.0–5.5%. Blood samples for tHcy measurements were collected into EDTA tubes, immediately placed on ice, centrifuged at 1,600g for 15 min at 4°C within 30 min after drawing, and stored at −20°C. Plasma tHcy concentration was measured centrally (in Verona) by an automated HPLC analyzer with fluorescence detection (19). Both inter- and intra-assay coefficients of variation were <5%.

The plasma tHcy measurement represents a combined pool of four forms of homocysteine: ~1% circulates as the free thiol; 70–80% is disulfide-bonded to plasma proteins; and the remaining 20–30% combines with itself to form the dimer homocysteine or combines with other thiols, including cysteine, to form the homocysteine-cysteine-mixed disulfide. Urinary albumin excretion rate (AER) was determined as the mean from three different 24-h urine collections using a radioimmunoassay method, after excluding proteinuria due to urinary tract infection. According to AER values, subjects were classified as normoalbuminuric (<20 µg/min), microalbuminuric (20–200 µg/min), and macroalbuminuric (>200 µg/min). Of the study population, 12 (20%) patients were microalbuminuric, whereas most of them (80%, n = 48) had normal AER values. No patients had macroalbuminuria. Presence of retinopathy was diagnosed by fundoscopy by a single ophthalmologist after pupillary dilation. Approximately two-thirds of the patients (61.7%) had no diabetic retinopathy, whereas 23 (38.3%) patients had background (n = 20) or severe (n = 3) retinopathy. The prevalence of microvascular diabetic complications was similar to that described in other European and American populations with comparable age, diabetes duration, glycometabolic control, and smoking status (20,21).

Statistical analysis
All data are presented as means ± SD. The following statistical tests were performed: unpaired Student’s t test, Pearson’s product-moment correlation, one-way analysis of variance (ANOVA), analysis of covariance (ANCOVA), and χ2 test (for categorical variables). To improve skewness and kurtosis of the distributions, plasma tHcy and triglyceride levels were logarithmically transformed for statistical analyses and then back-transformed to their natural units for presentation in tables and figures. Distributions of all other variables were normal. Nonparametric statistical tests (i.e., Mann-Whitney U, Kruskal-Wallis, and Spearman’s rank correlation tests) were also performed, but because the results were very similar to those obtained with parametric procedures, only the former were presented. P values <0.05 were considered statistically significant.

RESULTS — As shown in Table 1, plasma tHcy levels were significantly higher (by ~20%) in type 1 diabetic subjects than in control subjects. The prevalence of moderate hyperhomocysteinemia, defined as plasma tHcy levels >14.6 µmol/l, which corresponded to the means ±2SD of values found in the control group, was significantly higher in diabetic subjects than in control subjects (23.3 vs. 3.3%, P < 0.02).

The clinical and biochemical characteristics of diabetic subjects grouped according to smoking status are reported in Table 2. The subjects were predominantly nonobese, normolipidemic, and normotensive. Although diabetic smokers had higher levels of plasma triglycerides, the two groups were comparable for other potential confounders. In particular, no significant differences were found in sex, age, BMI, total cholesterol, creatinine, glycometabolic control, blood pressure, diabetes duration, and chronic microvascular complications. Nevertheless, plasma tHcy levels were markedly elevated in diabetic smokers (by ~50%) versus non-smokers without any significant difference between sexes. The adjustment for plasma triglycerides did not significantly modify these results (data not shown). Similarly, healthy subjects who smoked had significantly higher plasma tHcy levels than healthy nonsmokers (12.2 ± 1.8 vs. 9.0 ± 1.5 µmol/l, P < 0.001). Because the diabetic group included individuals with microvascular complications (i.e., clinical evidence of retinopathy and/or microalbuminuria), a condition that might alter the plasma tHcy levels, statistical analyses excluding these participants (n = 24) were repeated. The results did not substantially change. Plasma tHcy levels remained significantly higher in diabetic smokers than in their nonsmoking counterparts (14.4 ± 4.1 vs. 10.6 ± 3.6 µmol/l, P < 0.001). As shown in Fig. 1, plasma tHcy levels increased markedly with the increase in the number of cigarettes smoked daily in the diabetic group. The relationship remained statistically significant, even after adjustment for potential confounders, such as age, sex, BMI, triglycer-
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Table 2—Clinical and biochemical characteristics of type 1 diabetic patients grouped according to smoking status

<table>
<thead>
<tr>
<th>Type 1 diabetic patients</th>
<th>Nonsmokers</th>
<th>Smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>37</td>
<td>23</td>
</tr>
<tr>
<td>Age (years)</td>
<td>31 ± 8</td>
<td>32.8 ± 9</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>17/20</td>
<td>15/8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.7 ± 3</td>
<td>24.2 ± 3</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>125 ± 13</td>
<td>125 ± 9</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80 ± 7</td>
<td>79 ± 6</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.7 ± 0.7</td>
<td>4.9 ± 0.8</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.92 ± 0.8</td>
<td>1.33 ± 0.9*</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>85 ± 15</td>
<td>94 ± 20</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>6.6 ± 1.2</td>
<td>6.8 ± 0.9</td>
</tr>
<tr>
<td>tHcy (µmol/l)</td>
<td>10.6 ± 3</td>
<td>15.5 ± 5.7†</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>15 ± 7</td>
<td>13 ± 9</td>
</tr>
<tr>
<td>Retinopathy (n)</td>
<td>13 (35%)</td>
<td>10 (43%)</td>
</tr>
<tr>
<td>Microalbuminuria (n)</td>
<td>6 (16%)</td>
<td>6 (26%)</td>
</tr>
</tbody>
</table>

Data are n and means ± SD. *P < 0.01; †P < 0.0001. All other differences were not statistically significant.

eride and creatinine concentrations, glycometabolic control, blood pressure, diabetes duration, and complication status (P < 0.0001 by ANCOVA). A significant dose-response relationship between plasma tHcy levels and the number of cigarettes smoked per day was found also in the control subjects (Fig. 1). When diabetic subjects were considered as a group, plasma tHcy levels did not significantly correlate with any of the study variables, even though there was a marginal correlation with plasma triglyceride and creatinine concentrations (r = 0.29 and 0.26, respectively, P < 0.05).

CONCLUSIONS — Case-control and prospective studies have reported that an elevated plasma tHcy level is a powerful risk factor for atherosclerotic vascular disease (1-10).

In the present report, we found that plasma tHcy levels were significantly higher in young adults with type 1 diabetes than in normal control subjects, and that chronic cigarette smoking had an adverse effect on plasma levels of tHcy in both groups. Additionally, when diabetic patients and control subjects were categorized according to the number of cigarettes smoked per day, the potentially deleterious effect of cigarette smoking on plasma tHcy levels was found to be clearly dose-dependent.

Importantly, in the present study, we have evaluated the effect of smoking on plasma levels of tHcy in young type 1 diabetic individuals without any clinical evidence of macrovascular complications; the evaluation of patients with such complications would have confounded the interpretation of data. Moreover, the fact that the two groups of diabetic subjects (smokers vs. nonsmokers) were comparable for several factors known to adversely affect plasma tHcy levels (4,11,12) enhances the validity of our findings. Based on the present results, therefore, it seems reasonable to speculate that the observed difference between the two groups in plasma tHcy levels was secondary to cigarette smoking and, theoretically, could be caused by direct effects of nicotine, carbon monoxide, or other agents contained in tobacco smoke. However, the reason why plasma tHcy levels are increased in smokers is not fully understood. Although no straightforward explanation is available, smoking may directly inactivate enzymes of homocysteine remethylation, such as methionine synthase (22,23). Smoking is accompanied by changes in plasma thiol redox status, possibly due to a higher formation of reactive oxygen species (24). Furthermore, reduced intake of nutrients and vitamins and lower levels of plasma folate, vitamin B₁₂, and plasma pyridoxal 5'-phosphate have been demonstrated in smokers (25-27). Unfortunately, in this study, no information was available on nutritional parameters, especially plasma folate and vitamin B₁₂ levels, which are factors known to influence plasma tHcy levels (12,22). However, in previous studies (14,15), no significant differences in plasma folate and vitamin B₁₂ levels were found between control and type 1 diabetic subjects with or without hyperhomocysteinemia. Furthermore, in the Hordaland Homocysteine Study (11,12), the adjustment for cobalamin and folate intakes had only a marginal effect on the strong relationship observed between smoking and plasma tHcy levels in nondiabetic subjects.

Figure 1—Plasma tHcy levels in young adults with type 1 diabetes and in healthy control subjects (□) in relation to the number of cigarettes smoked daily. Data are means ± SE. P ≤ 0.001 in control subjects; P ≤ 0.0001 in diabetic subjects (for comparison by ANOVA)
In accord with previous studies (28,29), diabetic smokers showed significantly higher levels of plasma triglycerides than nonsmokers. Thus, one might postulate that the difference observed between smokers and nonsmokers in plasma tHcy levels is at least partly dependent on levels of plasma triglycerides. However, a role of cigarette smoking independent of plasma triglycerides is supported by the dose-response relationship found between plasma tHcy levels and the number of cigarettes smoked per day and by the results of the multivariate analysis, which included plasma triglyceride levels.

Overall, therefore, the evidence from this and other studies (17) suggests that type 1 diabetic patients, as compared with normal control subjects, have elevated plasma tHcy levels and that chronic cigarette smoking itself may be one of the major lifestyle determinants of plasma tHcy levels in both normal subjects (4,11,12) and type 1 diabetic individuals. However, diabetic nonsmokers had significantly higher plasma tHcy levels than healthy nonsmokers (10.6 ± 3.0 vs. 9.0 ± 1.5 µmol/l, P < 0.05) (Fig. 1). These results suggest the possibility that the increase of plasma tHcy levels found in diabetic subjects is explained only partly by cigarette smoking and that other specific and diabetes-related mechanisms may be involved.

The relatively small number of subjects examined did not allow us to do full analyses by complication status. In the present study, plasma tHcy levels did not substantially differ in diabetic individuals with microvascular complications (presence of clinical retinopathy and/or microalbuminuria) compared with those in individuals without complications (13.2 ± 5.6 vs. 12.1 ± 4.1 µmol/l, NS). At present, however, available data regarding the relationship between plasma tHcy levels and microvascular complications are still controversial in both type 1 and type 2 diabetes (13–17,30–32). Our findings are in accord with that of previous studies excluding any significant relationship between plasma tHcy levels and early stages of nephropathy or retinopathy in type 1 diabetes (13,14,16). On the other hand, although the evidence of a relationship between smoking status and diabetic complications is still conflicting, a number of studies have reported a strong relationship between cigarette smoking and the progression of microvascular diseases, thus supporting the possibility that quitting smoking would be effective in reducing the incidence of complications (33–35).

In conclusion, although this study is cross-sectional and cannot therefore prove a direct cause-effect relationship, the present results suggest that chronic cigarette smoking can exert a deleterious effect on plasma tHcy levels in young type 1 diabetic adults. These findings further support the clinical importance of discouraging the initiation of smoking and promoting its cessation in people with type 1 diabetes.

References
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