Impairment of Coronary Circulation by Acute Hyperhomocysteinemia in Type 2 Diabetic Patients

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Hyperhomocysteinemia increases the risk of death in type 2 diabetic patients (1,2), although the causal relationship with regard to cardiovascular mortality still remains elusive. Acute elevations in circulating homocysteine levels have been shown to impair endothelial function in coronary microcirculation in normal subjects (3). The objective of the present study was to investigate the effect of acute hyperhomocysteinemia on coronary circulation in patients with type 2 diabetes. Coronary flow velocity reserve (CFVR) assessment by transthoracic Doppler echocardiography (TTDE) is a noninvasive method to evaluate coronary flow reserve and provides important information about coronary endothelial function (4).

RESEARCH DESIGN AND METHODS

— Twenty newly diagnosed (within 6 months of diagnosis) type 2 diabetic patients (14 men and 6 women, age 49 ± 8 years, BMI 26.1 ± 1.8 kg/m^2) and 20 healthy control subjects, who were matched for age, sex, and BMI, volunteered for this study after informed written consent was obtained. All subjects (both patients and control subjects) were taking no medication or vitamin supplements, and the protocol was approved by the local ethics committee. The diabetic patients were normotensive, had cholesterol levels within normal ranges, and were free from both micro- and macrovascular diabetes complications. Acute hyperhomocysteinemia was induced with the oral methionine load (100 mg/kg in fruit juice), as previously described (5). The CFVR was determined by TTDE before and 4 h after the load. A systematic approach was made to visualize coronary blood flow in the distal part of the left anterior descending (LAD) coronary artery by color Doppler echocardiography. With a sample volume (1.5 or 2.5 mm wide) positioned on the color signal in the LAD coronary artery, Doppler spectral tracings of flow velocity were recorded by fast Fourier transformation analysis. Dipyridamole was intravenously administrated (0.56 mg/kg for 4 min) to record spectral Doppler signals during hyperemic conditions. The coronary flow velocity was measured at baseline and at peak hyperemic conditions, and an average of the measurements was obtained in three cardiac cycles. CFVR was calculated as the ratio of hyperemic to basal coronary flow velocity. Blood samplings for determining homocysteine, total and HDL cholesterol, triglyceride, and glucose were collected at baseline and at 4 h after the oral load, immediately before each echocardiography study. The total plasma homocysteine level was measured by fluorescence polarization immunoassay (IMx System; Abbott Laboratories). Data are presented as means ± SD. Data were analyzed by ANOVA; individual means were compared using paired or unpaired Student's *t* tests, as appropriate. Linear regression analysis was used as appropriate. $P < 0.05$ was considered significant. All analyses were conducted using SPSS version 9.0 (SPSS, Chicago, IL).

RESULTS

— There was no significant difference between the diabetic patients and the control subjects in any of the parameters evaluated except for fasting glucose and HbA_{1c} concentrations (8.0 ± 1.0 vs. 4.7 ± 0.6 mmol/l for glucose, 7.9 ± 0.9 vs. 4.6 ± 7.9% for HbA_{1c}; $P < 0.001$ for both). Administration of the oral methionine load increased the total homocysteine level to 31.3 ± 4.2 μmol/l at 4 h in diabetic patients and to 26.2 ± 4.7 μmol/l in control subjects ($P < 0.001$). The basal coronary flow velocity was similar in the diabetic patients (23.7 ± 3.9 cm/s) and the control subjects (24.5 ± 4.4 cm/s). By contrast, coronary flow velocity in response to dypiridamole was significantly lower in diabetic patients than in control subjects (61.6 ± 16.1 vs. 75.6 ± 21.5 cm/s, $P = 0.023$). As a consequence, CFVR was significantly lower in diabetic patients (2.6 ± 0.6 vs. 3.1 ± 0.7, $P = 0.02$). Following the methionine load, basal coronary flow velocity did not show any significant change from premethionine values in both diabetic patients and control subjects. On the other hand, coronary flow velocity in response to dypiridamole was significantly and similarly reduced in both diabetic (43.2 ± 8.9 cm/s, $P < 0.001$) and control subjects (54.1 ± 8.5 cm/s, $P < 0.001$) compared with the respective premethionine values. As a consequence, postmethionine CFVR values were reduced in both groups (Fig. 1), with the lowest value recorded in the diabetic patients (1.8 ± 0.3 cm/s, $P < 0.001$). There was a significant inverse relationship between changes in CFVR after the methionine loads and changes

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Abbreviations: CFVR, coronary flow velocity reserve; LAD, left anterior descending; TTDE, transthoracic Doppler echocardiography.

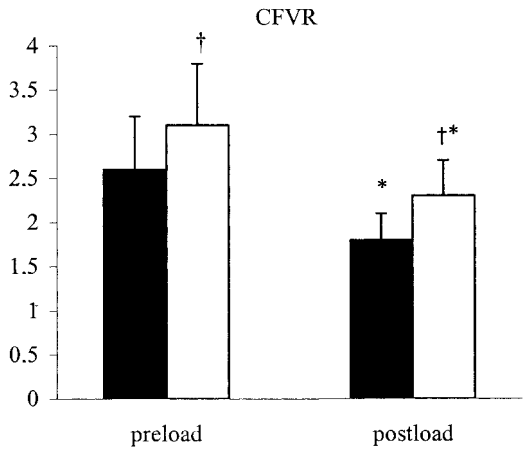

Figure 1—*CFVR in type 2 diabetic patients (■) and in control subjects (□) before and after methionine loads. *P < 0.001 for the comparison between preload and postload within each group; †P < 0.001 for the comparison between preload and postload between groups.*

in homocysteine levels (both groups, $r = -0.35$; $P < 0.01$).

CONCLUSIONS— The results of this study show that CFVR is lower in newly diagnosed, complication-free type 2 diabetic patients than in matched control subjects. Moreover, acute elevation in plasma homocysteine concentration impaired CFVR in both groups, with the greatest absolute reduction of coronary flow reserve in type 2 diabetic patients, and there was an inverse relation between postload homocysteine concentrations and CFVR impairment. Hyperhomocysteinemia is considered a potentially modifiable risk factor for cardiovascular disease (6). A relative risk of 1.4 for the difference between homocysteine levels 15 μmol/l and levels <10 μmol/l, after adjustment for other cardiovascular risk factors, seems to be the best estimate (8). In agreement with previous studies demonstrating normal plasma homocysteine concentrations in diabetic subjects without impaired renal function (9–11), we found no significant difference in the fasting concentration of plasma homocysteine between type 2 diabetic patients and control subjects. Although the absolute change in CFVR after the methionine load is similar between normal subjects and diabetic patients, type 2 diabetic patients seem more susceptible to the adverse vascular effect of raised plasma homocysteine concentrations, as they present reduced CFVR at baseline, and further impairment following acute hyperhomocysteinemia leads CFVR to the lowest level, which is maximal impairment of coronary flow.

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