Endothelial Dysfunction Is Detectable in Young Normotensive First-Degree Relatives of Subjects With Type 2 Diabetes in Association With Insulin Resistance

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Background—Endothelial dysfunction (ED) is regarded as an early step in the development of atherosclerosis. Among the pathogenetic factors leading to atherosclerosis, the role of insulin resistance and hyperinsulinemia as independent risk factors is still under debate. In this study, we examined the association between ED and insulin resistance in normotensive and normoglycemic first-degree relatives (FDRs) of patients with type 2 diabetes mellitus (DM).

Methods and Results—Endothelium-dependent and -independent vasodilation of the brachial artery was measured with high-resolution ultrasound (13 MHz) in 53 normotensive FDRs (21 men, 32 women; mean age, 35 years) with normal oral glucose tolerance, 10 age- and sex-matched normal control subjects, and 25 DM patients (mean age, 57 years). According to the tertiles of the clamp-derived glucose metabolic clearance rate (MCR), the FDRs were further classified as insulin resistant with an MCR $\leq$5.8 mL·kg$^{-1}$·min$^{-1}$, insulin sensitive (IS) with an MCR $\geq$7.8 mL·kg$^{-1}$·min$^{-1}$, and borderline with an MCR of 5.9 to 7.7 mL·kg$^{-1}$·min$^{-1}$. Flow-associated dilation was 4.1±0.9% in insulin-resistant FDRs, 6.7±1.1% in borderline FDRs, 9.0±1.2% in insulin-sensitive FDRs ($P=0.002$), 7.7±2.9% in control subjects ($P=NS$ versus FDRs), and 3.8±1.0% in DM patients ($P=0.03$). In multiple regression analysis, low MCR was significantly correlated with ED independent of age, sex, smoking, body mass index, percent body fat, serum insulin, and lipids.

Conclusions—There is a significant association between ED and insulin resistance in young FDRs of DM subjects independent of the classic cardiovascular risk factors. (Circulation. 2000;101:1780-1784.)

Key Words: endothelium $\n$ vasodilation $\n$ risk factors $\n$ atherosclerosis $\n$ insulin

The early manifestation of vascular disease in type 2 diabetes mellitus, often before hyperglycemia becomes evident,1 raised the question about the pathogenetic factors that initiate the development of vascular derangements in the prediabetic population. It is under debate whether insulin resistance, independent of confounding variables such as hypertension and hyperlipidemia, is an independent risk factor for cardiovascular disease. The aim of our study was to assess whether insulin resistance (quantitatively measured by glucose clamp) is correlated to the presence of early signs of vascular disease in individuals with a family history of type 2 diabetes. It is believed that endothelial dysfunction plays a central role in the development of atherosclerosis.2,3 Thus, a disturbed flow-associated (endothelium-dependent) vasodilation (FAD) is regarded as an early marker in the development of vascular disease.4 We determined FAD in a metabolically well-defined cohort of normoglycemic and normotensive first-degree relatives (FDRs; mean age, 35 years) of subjects with type 2 diabetes (DM) and studied whether the prevalence of decreased FAD correlates independent of other classic cardiovascular risk factors with glucose clamp-assessed insulin sensitivity.

Methods

Subjects and Laboratory Findings
We included 88 persons in the study. Fifty-three normotensive subjects (mean age, 35 years) with $\geq$1 parent with type 2 diabetes (subgroup of the recently described Tübingen Familien Früherfassung study6), and 10 age- and sex-matched control subjects (negative family history of diabetes) without evidence of manifest metabolic or cardiovascular disease were recruited via their parents from our outpatient clinic. In addition, 25 DM patients (mean age, 57 years) were also recruited in the outpatient clinic. This group was included in a previously published study6 comparing type 2 and 1 diabetic patients.

Patients with hypercholesterolemia (cholesterol >6.5 mmol/L) were excluded. Written informed consent was obtained from all subjects. The study protocol was approved by the Ethical Committee of the University of Tübingen. The subjects had been on a weight-maintaining diet containing $\geq$40% carbohydrate for 3 days before

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1780
the tests. On the first visit, an oral glucose tolerance test was performed. Venous blood was sampled at 0, 15, 30, 60, 90, and 120 minutes to determine plasma glucose and serum insulin. According to ADA criteria, the oral glucose tolerance test results had to be within the normal range in FDRs and control subjects.

Insulin sensitivity was measured with standard euglycemic hyperinsulinemic glucose clamp technique with a continuous infusion rate of 1 μU·kg⁻¹·min⁻¹ insulin according to the European Group for the Study of Insulin Resistance (EUGR) protocol. The last 40 minutes of clamp time were taken as a steady-state period. Insulin sensitivity was expressed as glucose MCR (in mL·kg⁻¹·min⁻¹). Lean body mass was determined by body composition analysis with tetrapolar impedance (BIA-101, RJL Systems).

Endothelial function was measured 1 hour before the clamp was started. All subjects were chemically euthyroid, had no sign of concurrent disease, and were not taking pharmacological agents known to affect carbohydrate/insulin metabolism or vascular tone. In women, the investigations were undertaken during the first week of the menstrual cycle because different phases of the cycle may affect endothelium-dependent vasodilation. Tests were done at 8 AM after an overnight fast of ≥10 hours. The probands also were asked to refrain from smoking for the same period. Blood samples were taken from the cubital vein for estimation of fasting plasma glucose, glycosylated hemoglobin, lipoprotein fractions, and triglycerides.

**Endothelial Function Test**

An important mechanism in activating the endothelial nitric oxide synthase is shear stress, which causes vasodilation after release of nitric oxide and thereby enhances local blood flow. We used a method described in 1992 by Celermajer et al. This noninvasive method to evaluate endothelial function uses postischemic (forearm) vasodilation, caused by increased flow in the proximal (brachial) artery and consequently a shear stress–induced vasodilation. Because this vasodilation can be mainly blocked by N-monomethyl-L-arginine, an inhibitor of endothelial nitric oxide synthase, this flow-associated dilation is regarded as endothelium dependent. This technique, which currently is widely used, has been shown to be reliable and reproducible. Concerning this method, it could also be shown that a disturbed flow-associated dilation of peripheral arteries is associated with coronary and carotid atherosclerosis.

We examined endothelium-dependent vasodilation using high-resolution ultrasound (AUS Idea, Exaote Biomedica) with an integrated ECG package. The diameter of the brachial artery was measured from 2-dimensional ultrasound images with a 13-MHz linear-array transducer with an axial resolution of 0.12 mm and a maximum penetration depth of 4.5 cm. The brachial artery was scanned in longitudinal sections 2 to 10 cm above the elbow. Lumen diameter was assessed according to the sonomorphological definition of Wendelhag et al. Subjects had to rest for ≥10 minutes before the first scan was recorded. Following the criteria published by Celermajer et al., the first scans were taken at rest and during reactive hyperemia. Increased flow was induced by deflating a pneumatic cuff after a 5-minute suprasystolic arterial forearm compression. The postischemic scan was performed 45 to 60 seconds after cuff deflation. To test the endothelium-independent dilation capacity, further scans were performed at rest and 3 to 4 minutes after sublingual administration of 0.4 mg GTN as a direct nitric oxide donor. The time span between the first and the second scans had to be ≥15 minutes for vessel recovery.

The ECG was monitored continuously. Vessel diameter was analyzed with the use of electronic calipers on frozen images over a length of the artery of ≥1 cm. Three measurements were taken at each scan for 3 cardiac cycles at the end of the diastole (incident with the R wave on the ECG), and the mean was then calculated. Because the time window for an exact measurement of the vessel diameter during reactive hyperemia is small, we did not measure blood flow or Doppler velocity. Concerning induced blood flow, we have never observed a significant difference between groups in previous studies.

The glucose clamp was released in each case after the ultrasound examination; therefore, the observer was unaware of the degree of insulin sensitivity. The difference in lumen diameter between rest and reactive hyperemia, expressed as percent change, was regarded as endothelium-dependent vasodilation (FAD%); GTN%, as endothelium-independent vasodilation.

**Statistical Analysis**

All calculations and statistical analyses were performed with the Statistical Package for Social Sciences for IBM-PC (SPSS Inc.). Data are presented as mean±SEM. Comparison between groups (univariate analysis) was done with χ² and Mann-Whitney U–Wilcoxon rank-sum tests for independent samples as appropriate. The Mann-Whitney test was used to exclude any influence from a nonparametric distribution. Stepwise multiple regression analysis was used in multivariate analysis with endothelial dysfunction as dependent variable.

**Results**

**Baseline Characteristics and Laboratory Findings**

Endothelial function was measured in 53 FDRs (mean age, 35 years; range, 18 to 50 years), 10 age- and sex-matched normal control subjects (mean age, 30 years; range, 23 to 47 years), and 25 DM patients (mean age, 57 years; range, 38 to 70 years).

**Comparison Between FDRs and Control Subjects**

All study participants were extensively metabolically characterized as reported previously. There was no difference in body mass index (BMI) and percent body fat content but a lower mean value of insulin sensitivity in FDRs compared with normal control subjects (Table). According to that finding, FDRs had still normal (American Diabetes Association [ADA] criteria) but already higher values for blood glucose than control subjects 2 hours after oral glucose load. The insulin levels were not different under fasting conditions but were significantly higher in FDRs in the oral glucose tolerance test. The lipoprotein profiles were similar concerning HDL-cholesterol and triglycerides, but the control subjects had significantly lower LDL-cholesterol levels compared with FDRs. No difference was found in 24-hour blood pressure measurements.

**Comparison Between Insulin-Resistant and Insulin-Sensitive FDRs**

According to the tertiles of the metabolic clearance rate (MCR) for glucose, 18 FDRs were classified as insulin resistant (IR) with an MCR ≤5.8 mL·kg⁻¹·min⁻¹, 18 as insulin sensitive (IS) with an MCR ≥7.8 mL·kg⁻¹·min⁻¹, and 17 as borderline (BL) with an MCR of 5.9 to 7.7 mL·kg⁻¹·min⁻¹. The IS subgroup was matched for sex, whereas in the IR and BL subgroups, women were predominant (Table). There was no significant difference between IS and IR in distribution of sex and age. The percentage of active smokers was lower (16.7% versus 44.4%) in the IR than the IS group. Both BMI and percent body fat were strongly associated with insulin resistance. Among the laboratory findings, fasting plasma glucose and glycosylated hemoglobin HbA1c were not statistically significant different in IS, IR, and BL subjects, whereas analysis of the data from the oral glucose tolerance test showed that IR had already higher (but still normal) 120-minute plasma glucose, higher fasting, and higher 120-minute insulin values, reflecting hyperinsulinemia. There were no statistically significant differences in
24-hour ambulatory blood pressure and plasma lipids, despite lower HDL-cholesterol in IR.

**Endothelial Dysfunction**

**Comparison Between the Whole Group of FDRs and Normal Control Subjects**

It is known that differences in the baseline diameter of the brachial artery have an influence on the degree of flow-mediated dilation.13 In our cohort, the baseline diameter of the brachial artery was similar between FDRs and normal control subjects (3.62 ± 0.08 and 3.86 ± 0.18 mm, P = NS).

There was also no difference in glyceryl trinitrate (GTN)–induced vasodilation (18.0 ± 1.1% and 16.5 ± 1.8%, P = NS) evident in examinations of endothelium-independent dilation capacity (Figure 1). FAD% (Figure 2) was slightly but not significantly higher in control subjects (7.7 ± 2.9% versus 6.6 ± 0.6%, P = NS). According to the degree of insulin sensitivity, FAD% in control subjects was nearly as high as in the IS subgroup of FDRs (7.7 ± 2.9% versus 9.2 ± 1.2%, respectively) but with a broader range.

**Comparison Between IR and IS FDRs**

The baseline diameter of the brachial artery (3.65 ± 0.14 versus 3.77 ± 0.14 mm, P = NS) and vasodilation after application of GTN (18.8 ± 2.4% versus 15.1 ± 1.0%, P = NS) were not different between IS and IR. However, there was a weak but statistically significant correlation between reduced flow-associated vasodilation and degree of insulin sensitivity (r = 0.38, Figure 3). Postischemic dilation (FAD%) of the brachial artery was 4.1 ± 0.9% from the baseline vessel

![Figure 1. GTN-induced vasodilation in FDRs of DM subjects (n=53), normal control subjects (n=10), and DM patients (n=25).](image1)

![Figure 2. Flow-associated vasodilation in FDRs of DM subjects (n=53), normal control subjects (n=10), and DM patients (n=25).](image2)
vascular risk factors but without morphological coronary function was already demonstrated in patients with cardio-

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53). subjects (n

in independent (GTN%) vasodilation in IR, IS, and BL FDRs of DM

Figure 4. Endothelium-dependent (FAD%) and endothelium-independent (GTN%) vasodilation in IR, IS, and BL FDRs of DM subjects (n=53).

diameter in IR compared with 9.2±1.2% in IS (P=0.002) and 6.7±1.1% in BL (Figure 4) subjects.

Stepwise multiple regression analysis of all univariate significant variables, including variables that failed to reach significance (sex, smoking, triglycerides, and LDL cholesterol; P<0.1), basal vessel diameter, and age, with FAD% as dependent variable, showed MCR to be an independent risk factor for endothelial dysfunction (P=0.009).

Comparison Between FDRs and DM Patients Regarding endothelial-dependent vasodilation, there was a decrease in FAD% from 6.6±0.6% in the whole group of FDRs to 3.8±1.0% in the DM study patients (P=0.03; Figure 2). However, FAD% in the IR subgroup of the examined FDRs was nearly as low as in the DM patients (4.1±0.9% versus 3.8±1.0%). GTN% was significantly lower in the DM subgroup (14.3±1.0% versus 18.1±1.1%, P=0.007; Figure 1).

Discussion

It is believed that the endothelium plays a central role in the atherogenic process. Studies evaluating the relation between coronary heart disease and endothelial dysfunction clearly demonstrated that reduced endothelium-dependent vasodilation is an early functional disturbance in the development of atherosclerotic lesions.13,21–23 Indeed, altered endothelial function was already demonstrated in patients with cardiovascular risk factors but without morphological coronary atherosclerosis.24 Recent studies showed a correlation of endothelial dysfunction with increased age,25,26 active and passive smoking,11,27,28 hypercholesterolemia,11,29 essential hypertension,30 and poor metabolic control in type 1 and 2 diabetes.6,31,32 Our results, including former cross-sectional data from our study group,9 revealed a loss in endothelial function from the putative prediabetic stage to patients with overt type 2 diabetes. In addition, there was a trend toward a reduced flow-mediated dilation in FDR compared with a group of age- and sex-matched control subjects without a family history of diabetes. However, these differences were not statistically significant, indicating that a positive family history of diabetes, apart from the degree of insulin sensitivity, should not be overestimated in terms of endothelial dysfunction.

Within the group of FDRs, we found the IR group to behave differently than either BL or IS FDRs, indicating endothelial dysfunction in insulin-resistant offspring. The differences in FAD could not be attributed to other confounding variables, because we could not show any differences in the classic cardiovascular risk factors (age, sex, smoking habits, fasting blood glucose, glycosylated hemoglobin, and blood pressure) despite the higher BMIs and associated higher percent total body fat in IR subjects. Furthermore, women were studied in the first half of the menstrual cycle to account for the influence of estrogen. Even in the multiple stepwise regression analysis, there was an independent association between endothelial dysfunction and clamp-derived insulin sensitivity. These data are in good agreement with recent findings of Steinberg at al,33 who showed that obese (IR) subjects are characterized by a reduced increase in leg blood flow after graded intrafemoral artery infusions of methacholine chloride compared with lean control subjects.

The mechanisms of how insulin resistance and endothelial dysfunction might be connected are unclear. There is ongoing controversy over whether differences in blood flow might cause peripheral insulin resistance.33–36 Our data cannot contribute to this controversy. As far as potential mechanisms are concerned, the study of Petrie et al37 is important. Those authors found a positive relation between endothelial nitric oxide production and insulin sensitivity in 19 healthy young men. This is an interesting observation, and further studies may define a cross-talk between the nitric oxide signaling system and the insulin signaling chain, which might lead to a better understanding of the underlying mechanisms.

In conclusion, our results show a weak but significant correlation between endothelial dysfunction and insulin resistance in young FDRs of DM subjects independent of the classic cardiovascular risk factors. Therefore, noninvasive measurement of endothelial dysfunction could be useful for early identification of high-risk subjects for atherosclerosis with a positive family history of type 2 diabetes.

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References


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