Insulin Therapy Improves Endothelial Function in Type 2 Diabetes

Satu Vehkavaara, Sari Mäkimattila, Anna Schlenzka, Juha Vakkilainen, Jukka Westerbacka, Hannele Yki-Järvinen

Abstract—A total of 75 in vivo endothelial function tests (intrabrachial artery infusions of endothelium-dependent [acetylcholine] and -independent [sodium nitroprusside] vasoactive agents) were performed in 18 type 2 diabetic patients (aged 58±2 years, body mass index 28.5±0.6 kg/m², and fasting plasma glucose 229±11 mg/dL) and 27 matched normal subjects. These tests were performed before and 6 months after combination therapy with insulin and metformin and before and 6 months after metformin therapy only. Before insulin therapy, blood flow responses to acetylcholine (15 μg/min) were significantly blunted in type 2 diabetic patients (7.5±0.7 mL dL⁻¹ min⁻¹) compared with normal subjects (11.6±0.9 mL dL⁻¹ min⁻¹, P<0.01). During insulin therapy, the acetylcholine response increased by 44% to 10.8±1.6 mL dL⁻¹ min⁻¹ (P<0.05). Insulin therapy also significantly increased the blood flow responses to both low and high doses of sodium nitroprusside. We conclude that insulin therapy improves endothelium-dependent and -independent vasodilatation. These data support the idea that insulin therapy has beneficial rather than harmful effects on vascular function. (Arterioscler Thromb Vasc Biol. 2000;20:545-550.)

Key Words: endothelium ▪ atherosclerosis ▪ vasodilatation ▪ insulin therapy ▪ type 2 diabetes

The UK Prospective Diabetes Study (UKPDS) showed that intensive blood glucose control with insulin or sulfonylureas, both of which significantly increase circulating free insulin concentrations, retards the development of microvascular complications.¹ The incidence of myocardial infarction decreased by 16%, which was almost statistically significant (P=0.052). Neither insulin nor sulfonylureas had adverse effects on cardiovascular outcome.¹ In contrast to these drugs, a substudy of the UKPDS suggested metformin to be cardioprotective in overweight patients.² The patients treated with metformin were not better controlled but gained less weight and had lower serum free insulin concentrations than did those treated with other regimens.² Endothelium-dependent vasodilatation is diminished in atherosclerotic coronary arteries³,⁴ and characterizes patients at risk of developing atherosclerosis, such as those with hypercholesterolemia, type 2 diabetes, and hypertension.⁵,⁶,⁷ In patients with type 2 diabetes, the impaired vasodilator response to endothelium-dependent vasodilators, such as acetylcholine (ACh), has been a rather consistent finding.⁸–¹² In addition, responses to endothelium-independent vasodilator agents, such as sodium nitroprusside (SNP), have been found to be impaired in many⁸,¹¹ although not all⁹,¹³ studies. These data suggest that the function of endothelial or vascular smooth muscle cells or both may be abnormal in type 2 diabetic patients. It is also possible that inactivation of exogenous and endogenous nitric oxide is increased.¹⁴ It is currently unknown, however, whether these defects are inherent features of type 2 diabetes or secondary to metabolic alterations such as chronic hyperglycemia,¹⁵,¹⁶ increases in free fatty acid (FFA) concentrations,¹⁷–²¹ or other lipid abnormalities.¹⁸,²²,²³ Regarding insulin, acute studies have shown that although physiological changes in circulating insulin concentrations do not alter blood flow, they potentiate ACh-induced vasodilatation.²⁴ The only treatment study hitherto performed in type 2 diabetes examined effects of antioxidants on endothelial function.¹⁰

The present study was undertaken to determine whether insulin therapy changes in vivo endothelial function in patients with type 2 diabetes. Because metformin diminishes weight gain during insulin therapy and may improve cardiovascular outcome,²⁵ we chose to study the effects of 6 months of combination therapy with insulin and metformin on endothelial function. These data were compared with a group of normal subjects and with a group of type 2 diabetic patients chronically treated with metformin.

Methods

Study Design

In vivo endothelial function was measured in 18 type 2 diabetic patients previously treated with metformin (1000 mg BID, n=14; 500 mg BID, n=4) before and 6 months after combination therapy with bedtime human isophane insulin and metformin (effect of insulin therapy). A control group of 27 normal subjects was studied to determine whether endothelial function was abnormal in the type 2 diabetic patients.
Inclusion and Exclusion Criteria

The type 2 diabetic patients were recruited from diabetes outpatient clinics in the Helsinki area on the basis of the following criteria: (1) aged 40 through 70 years, (2) treatment with metformin alone, (3) glycated hemoglobin (HbA1c) >8.5% (reference range 4.0% to 6.0%), (4) current body mass index <35 kg/m2, (5) duration of diabetes >3 years, and (6) no history of ketoacidosis. Exclusion criteria included the following: (1) clinically significant cardiovascular, hepatic, neurological, endocrinologic, or other major systemic disease, (2) retinopathy requiring laser treatment, (3) an elevated serum creatinine concentration, (4) history of drug or alcohol abuse, and (5) mental illness rendering the subjects unable to understand the nature, scope, and possible consequences of the study. Informed written consent was obtained after the purpose, nature, and potential risks were explained to the subjects. The experimental protocol was designed and performed according to the principles of Helsinki Declaration and was approved by the Ethical Committee of the Helsinki University Central Hospital.

Insulin Therapy

Patients considered eligible to participate in the study met with the doctor and the diabetes nurse 4 weeks before the start of insulin treatment. At this visit, the patients underwent a complete history and physical examination. The patients were instructed to measure their fasting blood glucose concentrations and to record any episode of symptomatic hypoglycemia daily. The patients then visited the laboratory for measurement of fasting blood glucose, HbA1c, serum concentrations of creatinine, liver enzymes, and the urinary albumin excretion rate. An ECG was also recorded. The results of the laboratory tests were checked, and if acceptable, an endothelial function test was performed before the start of insulin treatment with bedtime human isophane. Treatment with metformin was continued without changing the metformin dose. The patients were taught self-adjustment of the insulin dose on the basis of fasting plasma glucose measurements. The patients were asked to increase the dose by 2 and 4 IU if the fasting plasma glucose exceeded 144 and 180 mg/dL on 3 consecutive measurements. The goal was to lower fasting plasma glucose to ≤108 mg/dL and HbA1c to <7.5%. The patients visited the hospital outpatient clinic monthly for 3 months after start of insulin therapy and then at 3-month intervals. The second endothelial function test was performed after the outpatient visit at 6 months. To determine the degree of variation in endothelial function attributable to the method and continued treatment of type 2 diabetic patients, 6 type 2 diabetic patients who had been treated with metformin for 1.2±0.4 years (6 men, aged 53±2 years, body mass index 28.1±1 kg/m2, duration of diabetes 5±1 years, and HbA1c 9.1±0.4%) were studied twice with a 6-month interval.

Measurements

In Vivo Endothelial Function Test

In vivo endothelial function was determined by measuring forearm blood flow responses to intra-arterial infusions of endothelium-dependent (ACh) and -independent (SNP) vasodilators. The study was begun after a 10- to 12-hour fast at 7:30 AM. Venous blood samples were withdrawn for measurement of plasma glucose and serum free insulin, HbA1c, FFA, triglyceride, HDL, and total cholesterol concentrations. A 27-gauge unmounted steel cannula (Coopers Needle Works) connected to an epidural catheter (Portex) was inserted into the left brachial artery. Drugs were infused at a constant rate of 1 mL/min with infusion pumps (Braun AG). Subjects rested in a supine position in a quiet environment for 30 minutes after needle placement before blood flow measurements. Normal saline was first infused for 18 minutes. Drugs were then infused in the following sequence: 3 (low dose) and 10 (high dose) μg/min SNP (Roche) and 7.5 (low dose) and 15 (high dose) μg/min ACh (Iolab Corp). Each dose was infused for 6 minutes, and the infusion of each drug was separated by infusion of normal saline for 18 minutes, during which blood flow returned to basal values. Forearm blood flow was recorded for 10 seconds at 15-second intervals during the last 3 minutes of each drug and saline infusion period with mercury-in-rubber strain-gauge venous occlusion plethysmography (EC 4 Strain Gauge Plethysmograph, Hokanson), which was connected to a rapid cuff inflator (E 20, Hokanson), an analog-to-digital converter (McLab4e, AD Instruments Pty Ltd), and a personal computer, as previously described.26 Blood flow measurements were performed simultaneously in the infused (experimental) and control arm. Means of the final 5 measurements of each recording period were used for analysis. Metformin was discontinued for 2 days before the endothelial function studies to avoid any acute effects on vascular function.

Other Measurements

Plasma glucose concentrations were measured in duplicate by the glucose-oxidase method27 with the use of a Beckman Glucose Analyzer II (Beckman Instruments). HbA1c was measured by high-performance liquid chromatography with the use of a fully automated Glycosylated Hemoglobin Analyzer System (Bio-Rad). Serum free insulin concentrations were determined by double-antibody radioimmunooassay (Pharmacia Insulin RIA kit) after precipitation with polyethylene glycol.28 Urine albumin was measured by an immunoturbidimetric (Hitachi Ltd) method with the use of an antiseraum against human albumin (Orion Diagnostica). Microalbuminuria was defined as an albumin excretion rate of 20 to 200 μg/min. FFA, serum total cholesterol, and triglycerides29 along with HDL cholesterol30 were measured as previously described. Whole-body fat and fat-free mass were measured by a single frequency bioelectric impedance device (model BIA-101A, Bio-Electrical Impedance Analyzer System, RJL Systems).31

Statistical Analysis

Data between the type 2 diabetic patients and control subjects were compared by the Student unpaired t test. Changes induced by insulin therapy in endothelial function were calculated by ANOVA for repeated measures because treatment groups were composed of the same subjects, as described by Ludbrook.32 Horizontal contrasts were thereafter calculated by the paired t test with the Bonferroni correction. Correlation analyses were performed by the Spearman nonparametric correlation coefficient. All calculations were made with the use of the Systat statistical package. All probability values are 2-tailed. A value of P<0.05 was considered statistically significant. Data are expressed as mean±SEM.

Results

Glycemic Control, Body Composition, Blood Pressure, and Lipids

During 6 months of insulin therapy, HbA1c decreased from 9.0±0.3% to 7.6±0.1% (P<0.001). The bedtime insulin dose averaged 32±4 IU (range 12 to 60 IU). Fasting serum free insulin concentrations increased from 11±2 to 14±1 mU/L (P<0.05). Body weight remained unchanged during insulin therapy (mean change 1.0±0.6 kg, P=NS). The percentage of body fat decreased from 29.5±1.6% to 28.4±1.7% (P<0.05), and fat-free mass increased from 58.0±2.2% to 59.6±2.4% (P<0.05). Serum FFAs decreased significantly during 6 months of insulin therapy from 746±46 to 574±30 μmol/L (P<0.001), and serum triglycerides decreased by 24% (P<0.01). Other lipid concentrations and blood pressure remained unchanged (Table 1).

Basal blood flow in the experimental arm was comparable before (2.2±0.2 mL · dL−1 · min−1) and after (2.1±0.2 mL · dL−1 · min−1) insulin therapy and was not different from that in the normal subjects (2.2±0.2 mL · dL−1 · min−1). Blood flows in the control arm were similar to those in the experimental arm basally in patients with type 2 diabetes (2.1±0.2 and 2.0±0.1 mL · dL−1 · min−1) and normal subjects (2.0±0.2 mL · dL−1 · min−1, P=NS) throughout the study.
Endothelial Function

Before insulin therapy, blood flow during infusion of the low (6.7±0.6 versus 9.3±0.8 mL · dL⁻¹ · min⁻¹, P<0.05) and high (7.5±0.7 versus 11.6±0.9 mL · dL⁻¹ · min⁻¹, P<0.01) doses of ACh was significantly blunted in the type 2 diabetic patients compared with the normal subjects. During insulin therapy, the blood flow response to the low dose of ACh did not increase significantly, whereas that to the high dose of ACh increased by 44% (7.5±0.7 versus 10.8±1.6 mL · dL⁻¹ · min⁻¹, before versus after; P<0.05). The responses to ACh were not different from those of the normal subjects (Figure).

Forearm blood flows to both the low (7.8±0.4 versus 9.1±0.4 mL · dL⁻¹ · min⁻¹, P<0.05) and high (11.0±0.8 versus 13.0±0.7 mL · dL⁻¹ · min⁻¹, P<0.05) doses of SNP also increased significantly during insulin therapy. Blood flows during SNP infusion were not significantly different from those in normal subjects (Figure).

To determine the possible causes of enhanced endothelial functions during insulin therapy, simple correlation coefficients (Spearman) were calculated between changes in metabolic parameters and those of endothelium-dependent and -independent vasodilatation. No significant correlations were observed between metabolic parameters, which changed significantly during insulin therapy (HbA1c, fasting plasma glucose, serum FFAs, serum triglycerides, and serum free insulin concentrations), and the absolute or relative change in blood flow during SNP and ACh infusions (Table 2).

In the type 2 diabetic patients, who were studied twice during metformin therapy, basal flows were comparable (2.2±0.2 and 2.5±0.3 mL · dL⁻¹ · min⁻¹, P=NS). Blood flow responses to the low (8.4±1.2 and 7.7±0.3 mL · dL⁻¹ · min⁻¹, P=NS) and high (10.8±2.0 and 10.5±2.7 mL · dL⁻¹ · min⁻¹, P=NS) doses of SNP also remained unchanged, as did the responses to the low (7.2±1.0 and 7.3±1.1 mL · dL⁻¹ · min⁻¹, P=NS) and high (8.9±1.4 and 9.1±1.0 mL · dL⁻¹ · min⁻¹, P=NS) doses of ACh. The coefficients of variation for the 2 repeated measurements in these patients were 10±1%, 14±3%, 13±4%, 10±2%, and 15±4% for infusion of saline, low-dose SNP, high-dose SNP, low-dose ACh, and high-dose ACh.

Discussion

The present study is, to our knowledge, the first to examine the effect of insulin therapy on endothelial function in any type of diabetes. Before insulin therapy, endothelial function was impaired. Combination therapy with bedtime insulin and metformin resulted in correction of several metabolic abnormalities, including decreases in glucose, triglyceride,
and FFA concentrations, and in significant improvement of both endothelium-dependent and -independent blood flows in forearm resistance vessels. These changes were due to acute or chronic effects of insulin therapy rather than to metformin, in view of the fact that endothelial function remained unchanged in a control group studied twice during metformin therapy.

We chose to use combination therapy, with bedtime human isophane and metformin as the insulin treatment regimen. This regimen improves glycemic control without inducing weight gain. Lack of weight gain may be beneficial during insulin therapy, because weight gain is associated with increases in blood pressure and LDL cholesterol. In the UKPDS, patients treated with metformin gained less weight than did those treated with other agents, and the UKPDS also suggested that metformin might be cardioprotective in overweight patients. So far, however, it is unknown whether the decrease in cardiovascular events in the metformin-treated patients was due to differences in weight gain between the various treatment regimens. In the present study, endothelial function remained unchanged when measured twice in patients on chronic metformin therapy. Because the patients had already been treated for over a year with metformin before the first endothelial function test, these data do not exclude potential beneficial effects of metformin on endothelial function.

In all previous studies, except that of Avogaro et al, addressing endothelial function in type 2 diabetic patients by using either the invasive technique used in the present study or noninvasive measurement of the brachial artery diameter, endothelium-dependent vasodilatation has been impaired. This defect was also identified in the present study. The reason for normal endothelial function in the study of Avogaro et al is unclear but could be due to the small number of subjects studied (6 normal subjects and 10 patients with type 2 diabetes). Regarding endothelium-independent but guanylate cyclase– dependent vasodilatation, endothelial function in type 2 diabetic patients remained unchanged when measured twice in patients on chronic metformin therapy. Whether this reflects lack of a dominant effect of a single metabolic parameter or an effect of some factor not quantified in the present study, such as growth and hemostatic factors and LDL size, remains unclear.

Endothelium-independent vasodilatation, ie, vascular smooth muscle cell–dependent vasodilatation, also increased significantly during insulin therapy. Multiple factors, which could be either direct or indirect consequences of insulin therapy, could underlie the enhanced vascular smooth muscle–dependent vasodilatation. Regarding direct effects of insulin, insulin attenuates vascular contraction by inhibiting voltage-dependent calcium channels via a mechanism that appears coupled to the ability of insulin to increase glucose transport in vascular smooth muscle cells. The ability of insulin to attenuate angiotensin II–mediated calcium transients is blunted in cultured unpassaged vascular smooth muscle cells in insulin-resistant spontaneously hypertensive rats. Insulin therapy increases in vivo insulin sensitivity of glucose uptake in type 2 diabetic patients. If this would also happen in vascular smooth muscle, it might provide one mechanism to explain enhanced vascular smooth muscle cell–dependent vasodilatation after insulin therapy. Other possible mechanisms include increased bioavailability of exogenous (and endogenous) nitrates due to decreases in oxidative stress, advanced glycosylation end products, small dense LDL particles, and the susceptibility of circulating LDL to oxidation. Serum FFAs have also been suggested to enhance endothelium-dependent, and possibly

### Table 2. Interrelations Between Absolute and Relative Changes in Forearm Blood Flows During Intrabrachial Infusions of Endothelium-Dependent (ACh) and Endothelium-Independent (SNP) Vasodilators With Changes in Metabolic Parameters

<table>
<thead>
<tr>
<th></th>
<th>ΔSNP (10 μg/min)</th>
<th>%ΔSNP (10 μg/min)</th>
<th>ΔACh (15 μg/min)</th>
<th>%ΔACh (15 μg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔSerum triglycerides</td>
<td>−0.07</td>
<td>−0.07</td>
<td>−0.01</td>
<td>0.08</td>
</tr>
<tr>
<td>ΔSerum FFAs</td>
<td>−0.28</td>
<td>−0.22</td>
<td>0.17</td>
<td>0.25</td>
</tr>
<tr>
<td>ΔfP glucose</td>
<td>−0.10</td>
<td>−0.11</td>
<td>−0.25</td>
<td>−0.14</td>
</tr>
<tr>
<td>ΔHbA1c</td>
<td>0.03</td>
<td>0.05</td>
<td>0.16</td>
<td>0.18</td>
</tr>
<tr>
<td>ΔfP free insulin</td>
<td>−0.09</td>
<td>−0.08</td>
<td>0.13</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*F indicates fasting plasma levels.*

Data on possible causes of endothelial dysfunction are sparse in previous cross-sectional studies addressing endothelial function in type 2 diabetic patients. We recently performed a comprehensive search for such factors and found LDL size to be weakly correlated with endothelium-dependent vasodilatation. A similar relation was also described by Watts et al within a group of type 2 diabetic patients. In other studies, no significant correlations between cardiovascular risk factors and endothelium-dependent vasodilatation have been identified.

Insulin therapy induces several changes that potentially could enhance endothelial function. Such changes include decreases in serum triglyceride, FFA, and glucose concentrations, and all these parameters have been associated with endothelial function. Acute increases in insulin concentrations also enhance ACh-induced vasodilatation. In the present study, changes in none of these parameters during insulin therapy were significantly associated with enhanced endothelial function. Whether this reflects lack of a dominant effect of a single metabolic parameter or an effect of some factor not quantified in the present study, such as growth and hemostatic factors and LDL size, remains unclear.

Insulin therapy increases in vivo insulin sensitivity of glucose uptake in type 2 diabetic patients. If this would also happen in vascular smooth muscle, it might provide one mechanism to explain enhanced vascular smooth muscle cell–dependent vasodilatation after insulin therapy. Other possible mechanisms include increased bioavailability of exogenous (and endogenous) nitrates due to decreases in oxidative stress, advanced glycosylation end products, small dense LDL particles, and the susceptibility of circulating LDL to oxidation. Serum FFAs have also been suggested to enhance endothelium-dependent, and possibly
endothelium-independent,35 vasodilatation. FFAs are of interest because of the exquisite sensitivity of lipolysis to insulin.37,38

To conclude, endothelium-dependent and -independent vasodilatation improves during insulin therapy. Both improvements can be considered potentially antiatherogenic and support the view that insulin therapy, either via direct or indirect mechanisms, has beneficial rather than harmful effects on vascular function.

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References

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