3.5 Years of Insulin Therapy With Insulin Glargine Improves In Vivo Endothelial Function in Type 2 Diabetes

Satu Vehkavaara, Hannele Yki-Järvinen

Objective—To determine long-term effects of insulin glargine on vascular function in patients with type 2 diabetes.

Methods and Results—A total of 49 in vivo endothelial function tests, intrabrachial artery infusions of endothelium-dependent (acetylcholine [Ach]) and endothelium-independent (sodium nitroprusside [SNP]) vasoactive agents, were performed in 11 patients with type 2 diabetes (age: 59±2 years; BMI: 29.7±0.9 kg/m²; fasting plasma glucose: 226±14 mg/dL) and 16 matched normal subjects. The tests in the type 2 diabetic patients were performed before and after 6 months and 3.5 years of combination therapy with insulin glargine and metformin. A control group of type 2 diabetic patients not treated with insulin was studied twice at 6-month intervals. Before treatment, blood flow during infusions of low and high doses of Ach were significantly lower in the type 2 diabetic patients than in the normal subjects (P=0.021 for ANOVA). In the patients with type 2 diabetes, blood flow during infusion of the low dose of Ach averaged 7.1±0.8 mL/dL per minute at baseline, 8.8±1.0 mL/dL per minute at 6 months (NS), and then increased compared with baseline by 87±29% to 11.6±1.4 mL/dL per minute at 3.5 years (P<0.02 versus baseline). Blood flow during infusion of the high dose of Ach increased from 8.8±0.9 at baseline to 13.0±1.9 mL/dL per minute at 6 months (P<0.05) and by 86±25% to 14.7±1.6 mL/dL per minute at 3.5 years (P<0.01 versus baseline), which was not different from normal subjects. Blood flow during infusion of low (blood flow at 0 months: 7.7±0.5; at 6 months: 9.9±0.6; P<0.01 for 6 versus 0 months) and high (blood flow at 0 months: 11.6±1.1 mL/dL per minute; P<0.02 for 3.5 years versus 0 months) and high (blood flow at 0 months: 10.7±0.9; 6 months: 13.4±1.0; P<0.05 for 6 versus 0 months; and 3.5 years: 16.6±1.5 mL/dL per minute; P<0.05 for 3.5 years versus 0 months) doses of SNP also increased significantly during insulin therapy.

Conclusions—We conclude that insulin glargine therapy improves endothelium-dependent and endothelium-independent vasodilatation. These data support the idea that long-term insulin therapy has beneficial rather than harmful effects on vascular function in type 2 diabetes. (Arterioscler Thromb Vasc Biol. 2004;24:1-7.)

Key Words: hyperglycemia • circulation • blood vessels • insulin therapy

The two perhaps most important trials addressing effects of insulin therapy on cardiovascular outcome in type 2 diabetes are the United Kingdom Prospective Diabetes Study (UKPDS)5 and the Diabetes Mellitus, Insulin Glucose Infusion in Acute Myocardial Infarction (DIGAMI) study.6 The UKPDS showed an almost statistically significant (P=0.052) 16% reduction in the risk of myocardial infarction in patients allocated to receive intensive glucose control using either insulin or sulfonylurea compared with standard therapy. In the DIGAMI study, patients with an acute myocardial infarction, who received insulin acutely and chronically, had a 28% reduction in mortality rate compared with those of patients who continued their existing therapies.

Coronary endothelial dysfunction, defined as impaired vasodilatory response to intracoronary infusion of acetylcholine (Ach), is an independent predictor of vascular events even after adjustment for traditional cardiovascular risk factors.7,8 Recently, these findings were extended to measurements performed across the forearm vascular bed.5,6 Regarding insulin, acute studies have shown that a physiological increase in the circulating insulin concentration potentiates Ach-induced but not sodium nitroprusside (SNP)-induced vasodilatation.7,8 In studies addressing effects of long-term insulin therapy on endothelial function, we have previously shown that endothelium-dependent and endothelium-independent blood flow in forearm resistance vessels improves during 6 months of combination therapy with bedtime neutral protamine Hagedorn (NPH) insulin and metformin, while continuation of long-term metformin therapy had no effect on vascular function.9 In another study of patients with type 2 diabetes, 2 months of insulin therapy tended (P=0.09) to increase the blood flow response to Ach and restored the ability of insulin to acutely potentiate Ach-induced vasodilatation.8 There are no data on effects of insulin therapy on vascular function beyond 6 months.
Insulin glargine is a long-acting insulin analogue. Its vascular effects have not been studied. Compared with regular human insulin in vitro, insulin glargine was, in one study, reported to have a 6.5-fold higher affinity for insulin-like growth factor 1 (IGF-1) receptors in transfected baby hamster kidney cells. In contrast to these results, a recent study found equivalent binding of insulin glargine and regular human insulin to insulin and IGF-1 receptors in skeletal muscle cells. IGF-1 is a potent stimulator of blood flow.

Methods

Study Design

The study was investigator-initiated and not supported by the manufacturer of glargine insulin. In vivo endothelial function tests were performed by the same investigator (S.V.) in 11 type 2 diabetic patients who were previously treated with metformin (500 mg twice per day; n=10, 500 mg twice per day; n=1) and after 6 months and 3.5 years of combination therapy with bedtime glargine insulin and metformin, and in 6 type 2 diabetic patients (age: 50±2 years; BMI: 28±1 kg/m²) who had been treated with long-term metformin (500 mg twice per day). However, the latter group was as

Inclusion and Exclusion Criteria

The type 2 diabetic patients were recruited from diabetes outpatient clinics in the Helsinki area based on the following criteria: (1) age 40 to 70 years; (2) treatment with metformin alone; (3) HbA₁c >8.5% (reference range 4.0% to 6.0%); (4) current BMI <35 kg/m²; (5) duration of diabetes more than 3 years; and (6) no history of ketoacidosis. Exclusion criteria included: (1) clinically significant cardiovascular, hepatic, neurologic, endocrine, or other major systemic disease; (2) retinopathy requiring laser treatment; (3) an

Other Measurements

Plasma glucose concentrations were measured in duplicate with the glucose oxidase method, using the Beckman Glucose Analyzer II (Beckman Instruments). HbA₁c was measured by HPLC using the fully automated Glycosylated Hemoglobin Analyzer System (Bio-Rad). Serum-free insulin concentrations were measured by radioimmunoassay (Phadeseph Insulin RIA; Pharmacia & Upjohn Diagnostics) after precipitation with polyethylene glycol. Serum HDL cholesterol and triglyceride concentrations were measured with respective enzymatic kits from Roche Diagnostics, using an autoanalyzer (Roche Diagnostics Hitachi 917; Hitachi Ltd). Whole-body fat and fat-free mass were measured by a single-frequency bioelectrical impedance device (model BIA-101A; Bio-Electrical Impedance Analyzer System).

Statistical Analysis

Data between the type 2 diabetic patients and control subjects were compared using Student unpaired t test. Changes in endothelial function during insulin therapy were analyzed using ANOVA for repeated measures. Horizontal contrasts were thereafter calculated using the paired t test. Correlation analyses were performed using Spearman nonparametric correlation coefficient. All calculations were made using the Systat statistical package (Systat, Evanston, IL). All probability values are two-tailed. A P<0.05 was considered statistically significant. Data are expressed as mean±SEM.
Results

Glycemic Control, Body Composition, and Lipids
During insulin therapy, HbA1c decreased from 9.1% ± 0.4% to 7.5% ± 0.2% at 6 months and remained at this level (7.5% ± 0.2%) until 3.5 years (Table 1). The bedtime insulin dose averaged 40 ± 5 IU at 6 months (range: 12 to 60 IU) and increased to 60 ± 10 IU at 3.5 years (range: 14 to 120 IU). Body weight remained unchanged during insulin therapy (mean change versus baseline 1.2% ± 0.8 kg and 2.4 ± 1.4 kg at 6 months and 3.5 years, respectively; NS). Serum-free insulin concentrations increased significantly (Table 1). The percent body fat tended to decrease slightly (29.8% ± 2.0%, 28.5% ± 2.1%, and 28.9% ± 1.9% at 0, 6 months, and 3.5 years; NS), whereas fat-free mass increased from 61.2% ± 2.5% at baseline to 63.2% ± 3.0% (P < 0.01 versus baseline) at 6 months, and to 63.7% ± 3.1% at 3.5 years (P < 0.05 versus baseline). Serum HDL cholesterol concentrations increased significantly and triglyceride concentrations decreased slightly but not significantly (Table 1).

Endothelial Function

Reproducibility Study
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 per minute; NS). Blood flow responses to the low (8.4 ± 1.2 and 7.7 ± 0.3 mL/dL per minute; NS) and high (10.8 ± 2.0 and 10.5 ± 2.7 mL/dL per minute; NS) doses of SNP also remained unchanged, as did those to the low (7.2 ± 1.0 and 7.3 ± 1.1 mL/dL per minute; NS) and high (8.9 ± 1.4 and 9.1 ± 1.0 mL/dL per minute; NS) doses of ACh. The coefficients of variation for the two 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, respectively.

Insulin Therapy Study
Basal blood flow in the experimental arm averaged 2.1 ± 0.3 mL/dL per minute at baseline and 2.0 ± 0.2 mL/dL per minute at 6 months (NS) and increased slightly to 2.5 ± 0.2 mL/dL per minute at 3.5 years (NS versus baseline and 6 months). Basal blood flow was not different from that in the normal subjects (2.1 ± 0.2 mL/dL per minute) at any time point. Blood flows in the control arm were similar to those in the experimental arm basally in patients with type 2 diabetes (2.1 ± 0.3 mL/dL per minute, 2.0 ± 0.2 mL/dL per minute, and 1.9 ± 0.1 mL/dL per minute at baseline, 6 months, and 3.5 years) and normal subjects (2.0 ± 0.2 mL/dL per minute; NS) throughout the study.

Before treatment, blood flow during the low (7.1 ± 0.8 mL/dL per minute versus 9.8 ± 1.1 mL/dL per minute, insulin-treated versus normal group) and high (8.8 ± 0.9 mL/dL per minute versus 12.0 ± 1.1 mL/dL per minute, insulin-treated versus normal group) dose infusions of ACh were significantly lower in the patients with type 2 diabetes than in the normal subjects (P < 0.001 for ANOVA; Figure 1). In patients with type 2 diabetes, blood flow during infusion of the low dose of ACh increased 2.1 ± 0.3 mL/dL per minute at baseline and 2.0 ± 0.2 mL/dL per minute at 6 months (NS) and then increased significantly to 2.5 ± 0.2 mL/dL per minute at 3.5 years (NS versus baseline and 6 months). After 3.5 years of treatment, blood flow during the low dose of SNP increased from 7.7 ± 0.5 mL/dL per

| TABLE 1. Physical and Biochemical Characteristics Before and After 6 Months and 3.5 Years of Insulin Therapy in Patients With Type 2 Diabetes and in Normal Subjects |
|--------------------|-----------------|-----------------|-----------------|-----------------|
|                    | Before (n=11)   | 6 Months (n=11) | 3.5 Years (n=11) | Normal Subjects (n=16) |
| Sex (F/M)          | 2/9             | —               | —               | 4/12             |
| Age (y)            | 59 ± 2          | —               | —               | —                |
| Weight (kg)        | 87.5 ± 3.4      | 88.7 ± 3.8      | 89.7 ± 3.9      | 87.3 ± 2.5       |
| Fasting plasma glucose (mg/dL) | 226 ± 14†      | 137 ± 9|        | 118 ± 10††      | 102 ± 2          |
| HbA1c (%)          | 9.1 ± 0.4‡      | 7.5 ± 0.2       | 7.5 ± 0.4‡      | 5.7 ± 0.1        |
| Fasting plasma free insulin (mU/L) | 17 ± 3†       | 21 ± 3§       | 21 ± 3          | 7 ± 1            |
| Insulin dose (IU)  | —               | 40 ± 5          | 60 ± 5§         | —                |
| Insulin dose (IU/kg) | —             | 0.43 ± 0.05     | 0.63 ± 0.09§    | —                |
| Serum triglycerides (mmol/L) | 2.0 ± 0.4      | 1.7 ± 0.2       | 1.8 ± 0.3       | 1.5 ± 0.2        |
| HDL cholesterol (mmol/L) | 1.10 ± 0.06*    | 1.16 ± 0.03     | 1.33 ± 0.08**   | 1.32 ± 0.08      |

*P < 0.01 and †P < 0.01 for type 2 diabetic patients before insulin therapy vs normal subjects.
†P < 0.05 and ††P < 0.01 for type 2 diabetic patients before vs after 6 months of insulin therapy.
‡P < 0.01 for type 2 diabetic patients before insulin therapy vs normal subjects.
§P < 0.01 and ‡P < 0.001 for type 2 diabetic patients 6 months vs 3.5 years of insulin therapy. **P < 0.05 and †††P < 0.001 for type 2 diabetic patients before vs after 3.5 years of insulin therapy.
The improvement in vascular function may not be specific to glargine, because we did not study a control group using NPH. We chose, however, to use combination therapy with bedtime insulin glargine and metformin as the insulin treatment regimen, because effects of glargine on vascular function have not previously been studied. Although use of glargine is associated with less nocturnal hypoglycemia and dinner-time hyperglycemia than NPH insulin in patients with type 2 diabetes, the ultimate usefulness of any drug used to treat type 2 diabetes is determined by its effects on cardiovascular disease. The present study is limited by the small group of patients studied; because of the 3.5 years’ duration of treatment, we were not able to include a time-control group beyond 6 months. The latter is because glycemic control in patients with oral agents alone progressively tends to worsen, and patients often have to be transferred to insulin. Also, it was not possible to perform a randomized study because use of placebo insulin injections was not considered ethical. A positive control was not included; however, over the same time period that this study was

Discussion

In the present study, we examined effects of long-term insulin glargine therapy on vascular function in patients previously treated with metformin monotherapy. Before insulin therapy, the type 2 diabetic patients had blunted vasodilatory responses to the endothelium-dependent vasodilator ACh. After 6 months of therapy, the vasodilatory responses to ACh and SNP had improved significantly and were similar in the type 2 diabetic patients and in the normal subjects. In the group studied twice without insulin treatment at 6-month intervals, vascular function remained unchanged. After 3.5 years of therapy, the vasodilatory responses to ACh and SNP continued to improve, and responses to SNP even exceeded those in the normal subjects. The improvements in vascular function were significantly correlated with decreases in glucose and increases in insulin concentrations.

The improvement in vascular function may not be specific to glargine, because we did not study a control group using NPH. We chose, however, to use combination therapy with bedtime insulin glargine and metformin as the insulin treatment regimen, because effects of glargine on vascular function have not previously been studied. Although use of glargine is associated with less nocturnal hypoglycemia and dinner-time hyperglycemia than NPH insulin in patients with type 2 diabetes, the ultimate usefulness of any drug used to treat type 2 diabetes is determined by its effects on cardiovascular disease. The present study is limited by the small group of patients studied; because of the 3.5 years’ duration of treatment, we were not able to include a time-control group beyond 6 months. The latter is because glycemic control in patients with oral agents alone progressively tends to worsen, and patients often have to be transferred to insulin. Also, it was not possible to perform a randomized study because use of placebo insulin injections was not considered ethical. A positive control was not included; however, over the same time period that this study was
performed, we performed endothelial function tests in 47 women losing moderate amounts of weight with or without orlistat. In this study, the improvement in vascular function was closely correlated with the magnitude of LDL-lowering. Based on in vitro studies, insulin appears to have, at least acutely, beneficial effects on endothelial function. It increases NO production in cultured endothelial cells by increasing the expression and activity of endothelial nitric oxide synthase (eNOS). Activation of insulin receptor substrate-1 (IRS-1), phosphatidylinositol (PI)-3 kinase, and Akt appear necessary for this action of insulin, which results in phosphorylation of eNOS at a serine residue. The ability of insulin to induce vasodilatation increases slowly but steadily in vivo during several hours. This has been attributed to increased expression of the eNOS gene. It is presently unknown whether glargine regulates vascular function similar to human insulin in vitro in endothelial cells. Glargine has two positively charged arginines in the B-region of the insulin molecule, which could influence its receptor-binding specificity. In osteosarcoma cells expressing predominantly IGF-1 receptors and baby hamster kidney cells overexpressing the human IGF-1 receptor, the affinity of glargine for the IGF-1 receptor is 6- to 8-times higher than that of human insulin, whereas glargine and human insulin bind similarly to cultured human skeletal muscle cells. As can be expected from similar binding of glargine and human insulin to tissues predominantly expressing insulin receptors, there is no difference in mitogenicity as determined from thymidine uptake to human skeletal muscle cells, or in the ability of the two insulins to stimulate glucose uptake or

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Relationships (Spearman nonparametric correlation coefficient) between changes in fasting plasma insulin and blood flow during infusion of the high dose of acetylcholine at 3.5 years versus baseline (upper left panel), changes in fasting plasma insulin and blood flow (%) during infusion of the low dose of sodium nitroprusside at 3.5 years versus baseline (upper right panel), changes (%) in glycosylated hemoglobin and blood flow during infusion of the low dose of acetylcholine at 6 months versus baseline (lower left panel), and changes in fasting plasma glucose and blood flow (%) during infusion of the low dose of acetylcholine at 6 months versus baseline (lower left panel). SNP 10 indicates sodium nitroprusside at a rate of 10 μg/min; ACh 7.5, acetylcholine at a rate of 7.5 μg/min; ACh 15, acetylcholine at a rate of 15 μg/min; fP, fasting plasma; HbA1c, glycosylated hemoglobin.

**TABLE 2.** Forearm Blood Flow Responses to Intra-arterial SNP and ACh Infusions in the Normal Subjects at Baseline and in the Type 2 Diabetic Patients at Baseline, 6 Months, and 3.5 Years

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>SNP 3</th>
<th>SNP 10</th>
<th>ACh 7.5</th>
<th>ACh 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls at baseline (n=16)</td>
<td>2.0±0.2</td>
<td>9.2±0.8</td>
<td>12.6±1.0</td>
<td>9.8±1.1</td>
<td>12.0±1.1</td>
</tr>
<tr>
<td>Type 2 at baseline (n=11)</td>
<td>2.1±0.3</td>
<td>7.7±0.5</td>
<td>10.7±0.9</td>
<td>7.1±0.8*</td>
<td>8.8±0.9*</td>
</tr>
<tr>
<td>Type 2 at 6 months (n=11)</td>
<td>2.0±0.2</td>
<td>9.9±0.6‡</td>
<td>13.4±1.0†</td>
<td>8.8±1.0</td>
<td>13.0±1.9†</td>
</tr>
<tr>
<td>Type 2 at 3.5 years (n=11)</td>
<td>2.5±0.2</td>
<td>11.6±1.1§</td>
<td>16.6±1.5§</td>
<td>11.6±1.4§</td>
<td>14.7±1.6§</td>
</tr>
</tbody>
</table>

*P<0.05 for type 2 diabetic patients before insulin therapy vs normal subjects.
‡P<0.05 and †P<0.01 for type 2 diabetic patients before vs after 6 months of insulin therapy.
§P<0.05 and ‡P<0.01 for type 2 diabetic patients before vs after 3.5 years of insulin therapy.

SNP 3 indicates sodium nitroprusside at a rate of 3 μg/min; SNP 10, sodium nitroprusside at a rate of 10 μg/min; ACh 7.5, acetylcholine at a rate of 7.5 μg/min; ACh 15, acetylcholine at a rate of 15 μg/min; controls, normal subjects; type 2, type 2 diabetic patients.
insulin signaling.13 Compared with our previous study in which we found 6 months of therapy with NPH insulin to increase endothelium-dependent and endothelium-independent vasodilatation, the present data are remarkably similar, although direct comparison of the two insulins are needed to firmly establish this conclusion.

Multiple metabolic changes could have contributed to the observed enhancement in vascular responses to ACh and SNP. Insulin therapy is known to increase LDL size22 and lower free fatty acid concentrations,23–25 which were not measured in the present study but which could improve endothelial function.26–28 We did observe significant relationships between decreases in glucose and increases in serum free insulin concentrations and improvement in endothelium-dependent and endothelium-independent vascular responses (Figure 2). There are no studies in humans examining effects of chronic hyperglycemia, per se, on vascular function, although recent in vitro studies have identified mechanisms underlying hyperglycemia-induced endothelial dysfunction. Hyperglycemia inhibits eNOS activity in cultured bovine aortic endothelial cells by activating the hexosamine pathway via mitochondrial overproduction of superoxide, which increases O-glycosylation of eNOS and thereby decreases its activity.29

We conclude that insulin glargine therapy induces a sustained improvement in glycemic control and markedly improves endothelium-dependent and endothelium-independent vasodilatation in forearm resistance vessels. The response to the endothelium-independent agent SNP became even supernormal, possibly as a consequence of hyperinsulinemia. The recent prospective data showing that the better the vasodilator responses in the forearm in patients with essential hypertension and coronary artery disease, the less likely the person is to experience a cardiovascular event, would suggest that the supernormal responses unlikely are harmful. However, anything deviating from normal physiology may have adverse effects in the long-term, which is why the present data should be viewed with caution, and the impression that successful insulin therapy is beneficial for vascular function should be confirmed in clinical trials with definite endpoints.

Acknowledgments

We gratefully acknowledge Kati Tuomola, Sari Haapanen, Katja Tuominen, and Maarit Toivonen for excellent technical assistance, diabetes nurses Paula Nikkanen and Pirko Salmi for excellent work, and the volunteers for their help. This work was supported by grants from the Academy of Finland (to S.V. and H.Y.-J.), the Sigrid Juselius Foundation (to H.Y.-J.), the Finnish Diabetes Research Society (to S.V. and H.Y.-J.), the Novo Nordisk Foundation (to H.Y.-J.), and the Finnish Medical Society Duodecim (to S.V.).

References


3.5 Years of Insulin Therapy With Insulin Glargine Improves In Vivo Endothelial Function in Type 2 Diabetes
Satu Vehkavaara and Hannele Yki-Järvinen

Arterioscler Thromb Vasc Biol. published online December 18, 2003;
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/early/2003/12/18/01.ATV.0000113817.48983.c5.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/