Glargine and Regular Human Insulin Similarly Acutely Enhance Endothelium-Dependent Vasodilatation in Normal Subjects

Jukka Westerbacka, Robert Bergholm, Mirja Tiikkainen, Hannele Yki-Järvinen

**Objective**—Human insulin enhances the vasodilatory effect of acetylcholine (ACh), an endothelium-dependent vasodilator, in normal subjects. Structural changes in a long-acting insulin analog, insulin glargine, may change its binding properties to insulin receptor and structurally homologous receptors, such as the insulin-like growth factor-1 receptor, and thereby alter its vascular effects. In the present study, we compared effects of glargine and regular human insulin on blood flow responses to endothelium-dependent and endothelium-independent vasoactive agents in vivo in normal subjects.

**Methods and Results**—Ten healthy men (age: 33±9 years [mean±SD]; BMI: 23±2 kg/m²) were studied on two separate occasions in a double-blind, randomized, crossover fashion. In each study, blood flow responses to intrabrachial artery infusions of ACh and SNP were determined during infusion of saline and intravenously maintained normoglycemic hyperinsulinemia. Hyperinsulinemia (120 minutes; infusion rate: 1 mU/kg per minute) was created by infusing either insulin glargine or human regular insulin. Glargine and human regular insulin similarly stimulated whole-body glucose metabolism and suppressed serum free-fatty acid (FFA) concentrations. Endothelium-independent blood flow responses to low (3 μg/min) and high (10 μg/min) doses of SNP were unaffected by insulin glargine (12.2±2.6 versus 13.4±4.6 and 19.1±4.2 versus 19.6±5.1 mL/dL per minute, saline versus insulin, low- and high-dose) and regular human insulin (11.2±3.4 versus 12.0±5.2 and 16.8±5.7 versus 18.4±7.7 mL/dL per minute, respectively). In contrast, endothelium-dependent blood flow responses to low (7.5 μg/min) and high (15 μg/min) doses of ACh increased significantly and similarly by insulin glargine, 13.9±4.8 versus 19.3±6.5 mL/dL per minute (saline versus insulin, +39%; P<0.01) for low-dose ACh and 17.3±6.3 versus 23.2±9.2 mL/dL per minute (+34%; P<0.02) for high-dose ACh, and regular human insulin, 11.5±6.0 versus 15.8±8.0 mL/dL per minute (+38%; P<0.05) and 14.0±7.5 versus 21.1±10.4 mL/dL per minute (+51%; P<0.01).

**Conclusion**—Insulin glargine and regular human insulin have similar acute stimulatory effects on endothelium-dependent vasodilation in humans. (Arterioscler Thromb Vasc Biol. 2004;24:320-324.)

**Key Words:** arteries ■ insulin-like growth factor-1 ■ vasculature

Endothelial dysfunction, defined as an impairment of endothelium-dependent vasorelaxation caused by loss of nitric oxide (NO) bioactivity in the vessel wall, is considered an early functional change preceding atherosclerosis. Previous studies have shown that intravenous infusion of regular human insulin induces slow vasodilatation in peripheral resistance vessels, which can be abolished by L-NMMA, an inhibitor of endothelial NO synthesis. Insulin also acutely enhances the vasodilatory effect of acetylcholine (ACh), an endothelium-dependent vasodilator, but not that of sodium nitroprusside (SNP), an endothelium-independent vasodilator, but not that of sodium nitroprusside (SNP), an endothelium-independent vasodilator. Such vascular effects, which have also been described in the heart, could have contributed to beneficial cardiovascular effects of insulin therapy in the DIGAMI study and studies reporting benefits from glucose-insulin-potassium infusions. All these studies have used unmodified regular or intermediate-acting insulins.

Insulin glargine is a long-acting human insulin analogue that differs from human insulin by three amino acids. Two positively charged arginine molecules added to the C-terminus of the B-chain shift the isoelectric point from a pH of 5.4 to 6.7, making the molecule more soluble at a slightly acidic pH and less soluble at a physiological pH. Replacement of the acid-sensitive asparagine at position 21 in the A chain by glycine is needed to avoid deamination and dimerization of the arginine residue. These structural changes and addition of zinc result in a peakless and prolonged time-action profile of glargine compared with regular human insulin. Although these changes have proven to be useful in clinical practice in decreasing the frequency of hypoglycemia, they have the potential to change binding to the insulin receptor and structurally homologous receptors like the insulin-like growth factor-1 (IGF-1) receptor. In vitro
studies have yielded conflicting data regarding the binding affinity and mitogenic properties of insulin glargine as compared with regular human insulin. In rat fibroblasts overexpressing the human insulin receptor, regular human insulin and insulin glargine appear similar with respect to insulin receptor binding, activation of early insulin signaling events, and stimulation of mitogenesis. Insulin glargine and human regular insulin also appear to have comparable effects on multiple metabolic parameters in rat cardiomyocytes and cultured human skeletal muscle cells. However, in human osteosarcoma cells expressing exclusively IGF-1 receptors, insulin glargine binds with a 6- to 8-fold greater affinity to the IGF-1 receptor than human insulin. IGF-1 is a potent endothelium-dependent vasodilator in vivo in humans when infused into the brachial artery in vivo in humans. If glargine stimulated IGF-1-dependent actions such as vascular growth more than did regular human insulin, this could influence the long-term effects of the two insulins on diabetic vascular complications. In registration studies comparing human NPH and glargine insulin, more patients had a greater than a 3-step progression of retinopathy in 1 of 4 phase III studies (7.5% versus 2.7%; P < 0.05; data on file, Aventis). Although this finding has not been confirmed in any subsequent studies and was attributed to chance alone by an independent panel of evaluators, it raised some concern.

Ideally, vascular effects of glargine and human insulin should be compared in a long-term outcome study in patients with type 2 diabetes. However, in such a study, multiple factors other than those resulting from interaction with the IGF-1 receptor could be responsible for different outcomes. In the present study, we wished to compare the acute vascular effects of glargine and regular human insulin. This was performed by measuring blood flow responses to the endothelium-dependent vasodilator ACh and the endothelium-independent vasodilator SNP during maintenance of hyperinsulinemia with either human regular insulin or glargine. In addition, we compared effects of the two insulins on glucose metabolism and serum FFA concentrations, effects that are known to be mediated via the insulin receptor.

**Methods**

**Subjects and Study Design**

This investigator-initiated study, which was not supported by the manufacturer of insulin glargine, was performed in a randomized, double-blind fashion using a two-way crossover parallel group study design. Ten normal male subjects aged 33 ± 9 years (mean ± SD) with BMI 23 ± 2.4 kg/m² and waist circumference 85 ± 3 cm were studied. Their fasting plasma glucose (5.1 ± 0.4 mmol/L), glycosylated hemoglobin A₁c (5.2 ± 0.4%), serum triglyceride (0.9 ± 0.3 mmol/L), total (4.7 ± 0.8 mmol/L), HDL (1.3 ± 0.3 mmol/L), and LDL (3.1 ± 0.8 mmol/L) cholesterol concentrations were within the normal range.

The subjects were healthy, as judged by medical history and physical examination, ECG, and routine laboratory tests. None of the subjects was using any medications and all were non-smokers. For 2 days before the study, the subjects consumed a weight-maintaining diet containing at least 200 g of carbohydrate per day. The study was conducted in accordance with the guidelines in The Declaration of Helsinki. Written informed consent was obtained after the purpose, nature, and potential risks had been explained to the subjects. The experimental protocol was approved by the Ethics Committee of the Department of Medicine at Helsinki University Central Hospital.

The subjects were studied on two separate occasions with a 1-week interval when either insulin glargine or human regular insulin was used to create hyperinsulinemia.

**In Vivo Endothelial Function Test During Saline and Insulin Infusions**

Vascular function was assessed in forearm resistance vessels by measuring forearm blood flow responses to intra-arterial infusions of endothelium-dependent (ACh) and endothelium-independent (SNP) vasodilators, as previously described in detail. A 27-gauge unmounted steel cannula (Coopers Needle Works, Birmingham, UK) connected to an epidural catheter (Portex; Hythe, Kent, UK) was inserted into the left brachial artery. All drugs were infused at a constant rate of 1 mL/min with infusion pumps (B. Braun AG, Melsungen, Germany). The subjects rested supine in a quiet environment for 30 minutes after needle placement before blood flow measurements were begun. Normal saline was first infused for 18 minutes. Drugs were then infused in the following sequence: SNP (Nitropress; Abbott Labs, North Chicago, IL) 3 (low dose) and 10 (high dose) μg/min, ACh (Miochol; OMJ Pharmaceuticals, San Germán, PR) 7.5 (low dose) and 15 (high dose) μg/min. 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 time 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 using mercury-in-rubber strain-gauge venous occlusion plethysmography (EC4 Strain Gauge Plethysmograph; Hokanson, Bellevue, WA) and a rapid cuff inflator (E 20; Hokanson, Bellevue, WA). The strain-gauge was attached around the widest, most muscular segment of the forearm. Blood flow recordings were analyzed with Hokanson NIVP3 computer software, version 5.27b (Hokanson).

The endothelial function test was repeated under normoglycemic hyperinsulinemic conditions. These conditions were maintained using the euglycemic insulin clamp technique. Two 18-gauge catheters (Venflon; Viggo-Spectramed, Helsingborg, Sweden) were inserted, one in an antecubital vein for infusion of insulin and glucose and another retrogradely in a heated hand vein, to obtain arterialized venous blood for measurement of glucose concentrations every 5 minutes and serum FFA and insulin every 30 minutes. Either insulin glargine (Insulin Lantus; Aventis Pharmaceuticals, Frankfurt, Germany) or regular human insulin (Insulin Actrapid; Novo Nordisk, Denmark) was infused in a primed continuous fashion. The rate of the continuous insulin infusion was 1 μU/kg per minute. Whole-body glucose uptake was determined from the glucose infusion rate required to maintain normoglycemia between 30 to 120 minutes.

Blood pressure and heart rate were measured basally and every 60 minutes during the study.

**Analytical Procedures**

Plasma glucose concentration was measured in duplicate with glucose oxidase method using Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Serum insulin concentrations were determined by the Auto-DELFIA kit from Wallac (Turku, Finland). Using serial dilutions of human regular and glargine insulin showed that the antibody bound more avidly to glargine than to regular insulin by a factor of 1.34. The apparent insulin glargine concentrations were divided by this factor. Serum FFA were measured by a fluorometric method. HbA₁c was measured by high-pressure liquid chromatography using the fully automated Glycosylated Hemoglobin Analyzer System (BioRad, Richmond, CA). Serum total and HDL cholesterol, and triglyceride concentrations were measured with respective enzymatic kits from Roche Diagnostics using an autoanalyzer Roche Diagnostics Hitachi 917; Hitachi Ltd, Tokyo, Japan). LDL cholesterol concentration was calculated using the formula of Friedewald.

**Statistical Analyses**

Single measurements before and during the insulin infusion were compared using the paired t test. Comparison of blood flow re-
Metabolic and Hemodynamic Parameters Basally and During Infusion of Insulin Glargine and Human Insulin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
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<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>Insulin glargine 5.2±0.4</td>
<td>5.2±0.5</td>
<td>5.2±0.2</td>
<td>4.8±0.2</td>
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<td>Human insulin  5.0±0.4</td>
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<td>5.3±0.5</td>
<td>5.0±0.4</td>
<td>5.2±0.2</td>
</tr>
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<td>Insulin (mU/L)</td>
<td>Insulin glargine 4±2</td>
<td>67±18***</td>
<td>67±14***</td>
<td>66±16***</td>
<td>61±17***</td>
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<tr>
<td></td>
<td>Human insulin  3±1</td>
<td>66±10***</td>
<td>66±10***</td>
<td>64±9***</td>
<td>61±11***</td>
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<tr>
<td>FFA (µmol/L)</td>
<td>Insulin glargine 575±66</td>
<td>214±22**</td>
<td>165±15***</td>
<td>137±15***</td>
<td>121±13***</td>
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<tr>
<td></td>
<td>Human insulin  637±62</td>
<td>223±18***</td>
<td>164±11***</td>
<td>145±10***</td>
<td>143±13***</td>
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<tr>
<td>Whole-body glucose uptake (mg/kg per min)</td>
<td>Insulin glargine NA</td>
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<td>6.4±1.9</td>
<td>8.0±2.4</td>
<td>8.8±2.2</td>
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<tr>
<td></td>
<td>Human insulin NA</td>
<td>3.4±1.2</td>
<td>5.8±1.7</td>
<td>7.1±1.6</td>
<td>8.1±2.0</td>
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<td>Systolic blood pressure (mm Hg)</td>
<td>Insulin glargine 113±6</td>
<td>115±8</td>
<td>113±9</td>
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<td></td>
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<tr>
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<td>Human insulin  116±9</td>
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<td>118±7</td>
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<tr>
<td>Diastolic blood pressure (mm Hg)</td>
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<td>Human insulin  75±7</td>
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<td>Heart rate (bpm)</td>
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<td>55±5</td>
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<tr>
<td></td>
<td>Human insulin  56±6</td>
<td>55±5</td>
<td>56±5</td>
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</table>

NA indicates not applicable; FFA, free fatty acids.

**P<0.01, ***P<0.001 for change from basal.

Data are mean±SD.

Responses to the two doses of vasoactive drugs and comparison between insulin preparations were performed using ANOVA for repeated measures.21 Period effect was analyzed as a period × treatment effect using repeated-measures ANOVA. A probability value of less than 0.05 was considered statistically significant. The calculations were performed using Systat version 10 (SPSS, Evanston, IL). All data are shown as mean±SD.

Results

Infusions of regular and glargine insulin increased serum insulin concentrations similarly (Table 1). Whole-body glucose uptake was similar during hyperinsulinemia created by infusing insulin glargine and regular human insulin (6.8±1.8 and 6.1±1.6 mg/kg per minute, respectively; NS) (Table 1). Serum FFA concentrations averaged 575±199 and 637±186 µmol/L during saline infusion in the glargine and regular human insulin studies (NS) (Table 1). Hyperinsulinemia created by glargine suppressed FFA to 159±43 and regular insulin to 168±28 µmol/L (average between 30 and 120 min; NS) (Table 1). Basal blood flows averaged 2.1±0.5 and 2.1±0.6 mL/dL per minute in the glargine and regular human insulin studies during saline infusions and 2.5±0.7 and 2.3±0.7 mL/dL per minute after 90 minutes of infusion of insulin glargine and regular human insulin before start of ACh (NS for both insulins versus saline).

Endothelium-dependent and endothelium-independent blood flows during insulin infusions are shown in Figure 1. Blood flows during infusion of the low (3 µg/min) and high (10 µg/min) dose of SNP were unaffected by insulin glargine (12.2±2.6 versus 13.4±4.6 and 19.1±4.2 versus 19.6±5.1 mL/dL per minute, saline versus insulin, low- and high-dose) and regular human insulin (11.2±3.4 versus 12.0±5.2 and 16.8±5.7 versus 18.4±7.7 mL/dL per minute, respectively). Blood flows during infusion of the low (7.5 µg/min) and high (15 µg/min) doses of ACh were significantly and similarly enhanced by insulin glargine: 13.9±4.8 versus 19.3±6.5 mL/dL per minute (saline versus insulin, +39%; P<0.01) for low-dose ACh and 17.3±2.1 versus 23.2±3.1 mL/dL per minute (+34%; P<0.02) for high-dose ACh and regular human insulin 11.5±6.0 versus 15.8±8.0 mL/dL per minute (+38%; P<0.05 for low-dose ACh) and 14.0±7.5 versus 21.1±10.4 mL/dL per minute (+51%; P<0.01 for high-dose ACh). There were no differences between the insulins during infusion of SNP (P=0.651 for treatment × blood flow) or ACh (P=0.501 for treatment × blood flow). Blood flow increased 0.3±0.2 (NS) and 0.5±0.2 (NS) mL/dL per minute above basal during infusion of insulin glargine and human
regular insulin, whereas ACh alone increased blood flow above basal by 11.4±4.9 and 9.2±5.7 mL/dL per minute (low dose) and 14.8±6.5 and 11.7±7.1 mL/dL per minute (high dose), respectively. Thus, during infusion of insulin glargine or human regular insulin and ACh, blood flow increased much more than by infusion of either insulin or ACh alone (vide supra). There was no period effect for changes in blood flow for either ACh ($P=0.797$ for period × treatment) or SNP ($P=0.245$ for period × treatment).

Data on systolic and diastolic blood pressure and heart rate are shown in Table 1. These parameters were comparable during saline infusion in the glargine and regular human insulin studies (Table 1). There was no change in blood pressure or heart rate during insulin infusions (Table 1).

**Discussion**

In the present study, we compared the acute vascular effects of the long-acting insulin analogue, insulin glargine, to those of regular human insulin. Insulin glargine was found to enhance endothelium-dependent vasodilation by ACh in vivo. The effect was similar when compared with that of human regular insulin. Neither insulin glargine nor human regular insulin altered the blood flow response to SNP, an endothelium-independent vasodilator. Thus, the amino acid differences between insulin glargine and regular human insulin do not appear to, at least acutely, influence vasodilatory properties of the two molecules. Both insulin preparations also similarly acutely stimulated glucose uptake and suppressed FFA concentrations.

We found both regular human insulin and insulin glargine to increase the blood flow response to ACh but not to SNP. Because the sum of the increases in blood flow above basal by insulin and ACh administered separately was much lower than blood flow during co-infusion of insulin and ACh, insulin not only increased but also potentiated the blood flow responses. These data confirm those of Taddei et al. In a recent study by Arcaro et al., contrary to present data and those of Taddei et al, acute hyperinsulinemia was suggested to abrogate endothelium-dependent vasodilation in normal subjects. The reason for these contradictory data is unclear. Possibly, methodological differences could have contributed. In the study by Arcaro et al, vasodilatation was induced by shear stress, and the changes in diameter of femoral and brachial arteries were followed-up. Data were expressed as the percent change in diameter by shear stress. In the femoral artery, insulin itself significantly increased the diameter as compared with saline. Therefore, any further dilatation by shear stress could have been influenced by the larger baseline diameter.

The present data extend the findings of Taddei et al to insulin glargine, which was found to have a potentiating effect identical to that of human insulin. IGF-1 has been shown in human clinical trials to promote progression of retinopathy and large-scale studies aiming at retarding progression of retinopathy by blocking growth hormone action by agents such as octreotide are ongoing after promising pilot studies. However, the dose of IGF-1 used in the trials showing retinopathy progression was 8 mg, i.e., ~10-fold lower than that of insulin glargine in phase III trials. Even in osteosarcoma cells, in which the greatest difference in binding of insulin glargine and human regular insulin has been documented, the affinity of insulin glargine to the IGF-1 receptor is ~200-fold lower than that of IGF-1. This means that signaling via the IGF-1 receptor by insulin glargine in clinical practice is more than 2000-fold less than IGF-1. For the same rate of glucose uptake, IGF-1 increases blood flow much more than glucose extraction compared with insulin. The similarity in the potentiation of blood flow responses to ACh by insulin glargine and regular human insulin support the idea that the slightly greater affinity of insulin glargine to IGF-1 than to insulin receptors is clinically meaningless. Insulin therapy with unmodified intermediate acting insulins has been shown in 3 independent studies to ameliorate or reverse endothelial dysfunction in patients with type 2 diabetes.

When infused intravenously at a rate of 2 mL/kg per minute, insulin glargine has had similar effects on counter-regulatory hormones and whole-body glucose uptake as regular human insulin in healthy subjects and patients with type 2 diabetes. The present data are in accordance with these previous observations and extend them to also show that insulin glargine acutely suppresses FFA similarly to human regular insulin.

In conclusion, these data demonstrate that insulin glargine and regular human insulin similarly acutely potentiate ACh-induced endothelium-dependent vasodilatation in normal human subjects in vivo. In addition, both insulins similarly stimulate glucose uptake and suppress serum FFA.

**Acknowledgments**

The study was supported by grants from the Academy of Finland (J.W., H.Y.-J.) and the Sigrid Juselius Foundation (H.Y.). We gratefully acknowledge Maarit Toivonen and Katja Tuominen for excellent technical assistance.

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Arterioscler Thromb Vasc Biol. 2004;24:320-324; originally published online December 4, 2003;
doi: 10.1161/01.ATV.0000110444.59568.56
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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