Effect of Insulin Glulisine on Microvascular Blood Flow and Endothelial Function in the Postprandial State

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ABSTRACT

OBJECTIVE: To investigate the effect of insulin glulisine on postprandial microvascular blood flow in T2DM.

RESEARCH DESIGN AND METHODS: Fifteen patients with T2DM received insulin glulisine or human insulin before a liquid meal test. Thereafter, skin microvascular blood flow was measured by the use of laser Doppler fluxmetry (LDF), and blood samples were taken for the measurement of plasma levels of glucose, insulin, intact proinsulin, ADMA, nitrotyrosine, IL-18, MMP-9, oxLDL, and FFA.

RESULTS: Insulin glulisine resulted in higher postprandial insulin levels (mean±SEM; AUC₀₋₁₂₀: 51.0±6.8 vs. 38.2±5.4 mU/L; p=0.004), while plasma glucose (AUC₀₋₂₄₀: 158±9 vs. 180±9 mg/dl; p<0.05) and intact proinsulin (AUC₀₋₂₄₀: 26.2±3.5 vs 31.2±4.3 pmol/L; p=0.002) were lower. Microvascular blood flow increased after insulin glulisine (27.9±3.1 to 51.7±9.9 AU; p<0.05), while only a minor increase was found during human insulin (27.9±3.1 to 34.4±7.8; n.s.). ADMA and Nitrotyrosine levels were reduced after insulin glulisine (p<0.05, respectively).

CONCLUSIONS: Insulin glulisine is superior to human insulin in restoring postprandial metabolic and microvascular physiology.
Several epidemiological studies have demonstrated an association between glucose spikes and the development of vascular complications in patients with type 2 diabetes mellitus. Postprandial generation of oxidative stress and impaired endothelial function are major contributors in the development of early vascular damage and atherosclerosis. Recent studies have shown that microvascular blood flow increases after a meal in several tissues like gut, skin, adipose tissue or heart (1-5). Postprandial regulation of microvascular blood flow is a complex process, inversely affected by postprandial glucose and insulin excursions (2,6,7).

Diminished prandial insulin secretion and an increase in postprandial plasma glucose excursions with an increased postprandial oxidative stress and impaired endothelial function are early features of type 2 diabetes mellitus. Insulin glulisine attenuate the postprandial increase in plasma levels of intact proinsulin compared with regular human insulin, which may lead to a corresponding reduction in cardiovascular risk and beta-cell protection. Therefore, the pharmacokinetic of prandial insulin formulations may be important not only in controlling postprandial glucose excursions, but also in the maintenance of normal endothelial function and microvascular blood flow.

The aim of this study was to compare the effect of insulin glulisine with regular human insulin in terms of postprandial microvascular blood flow and several laboratory markers of endothelial function and oxidative stress in patients with type 2 diabetes.

PATIENTS AND METHODS

This investigation was a single-centre, open-label, randomised, two-way crossover study of patients with type 2 diabetes who were on oral antidiabetic treatment with insufficient metabolic control (HbA1c >6.5–9.9%). Male and female patients (aged 40–70 years; body mass index <40 kg/m²) were included in the study if they were on a stable dose of sulfonylurea alone or combined with metformin for ≥3 months. Patients were excluded if they had been treated with insulin, peroxisome proliferator-activated receptor-γ agonists, glinides or glucosidase inhibitors within the last 4 weeks prior to screening. All other concomitant treatment had to be identical on both treatment days. Other exclusion criteria were evidence of major micro- or macrovascular complications and impaired cardiovascular, respiratory, hepatic and renal function.

Patients were randomised to receive subcutaneous administration of a single 0.10 U/kg dose of either insulin glulisine immediately prior, or regular human insulin 15 minutes before the consumption of a standardised liquid meal test (Ensure Plus®; 56% carbohydrate, 29% fat and 15% protein) on 2 distinct study days within one months. Patients entered the study centre in the morning after an overnight fast (8 hours) and an intravenous catheter was inserted into a superficial vein of one forearm. A laser doppler probe was adjusted at the contralateral forearm for the measurement of microvascular skin blood flow. At timepoint 0 (before the liquid meal intake) and 30, 60, 120, 180 and 240 minutes after consumption of the liquid meal, the microvascular skin blood flow was measured and blood was taken for the determination of: plasma glucose, insulin, intact proinsulin, nitrotyrosine, asymmetric dimethylarginine (ADMA), matrix metalloproteinase-9 (MMP-9), free fatty acids (FFA), interleukin-18 (IL-18), and oxidized LDL (oxLDL).

Laser Doppler fluxmetry. Laser Doppler fluxmetry (LDF) was recorded using a laser Doppler probe with an incorporated skin heater at the median aspect of the lower forearm (Monitor TTC-45, Moor Instruments, Axminster, UK). The position of the probe was kept constant for the whole investigation. Before the start of the investigation, the patients rested in a supine
position for at least 15 minutes and the mean LDF was recorded for three minutes at a probe temperature of 37°C and 44°C. The mean individual coefficient of variation of microvascular blood flow measurement using this method is less than 20% (8,9).

**Laboratory measurements.** All laboratory measurements were done at the Institute for Clinical Research and Development (ikfe GmbH, Mainz, Germany). Blood samples were centrifuged and kept at -20°C until final analysis. Plasma glucose concentrations were determined by the glucose dehydrogenase method (Super GL, RLT, Möhness-Delecke, Germany). MMP-9, IL-18 and oxLDL were determined by enzyme-linked immunosorbent assay according to the manufacturers guidelines (MMP-9: R&D Systems, Wiesbaden, Germany; IL-18: IBL: Hamburg, Germany; ADMA and oxLDL: Immundiagnostik, Bensheim, Germany). Insulin, intact proinsulin and nitrotyrosine were measured by a chemoluminescence assay (insulins and intact proinsulin: Invitron, Monmouth, UK; nitrotyrosine: Upstate, US). HbA1c was measured by high-performance liquid chromatography (Menarini Diagnostics, Neuss, Germany), while FFAs were determined photometrically (WAKO, Neuss, Germany).

**Statistical analysis.** Since this study was designed as a pilot study, no confirmatory analysis had been performed. All measurements are presented as mean ± standard error of the mean (SEM). The area under the curve (AUC) was calculated for the first 120 minutes after consumption of the liquid test meal (AUC0–120min) as well as for the entire observation period (AUC0–240min) for plasma insulin, intact proinsulin and glucose levels. Statistical comparison between fasting and postprandial values and between groups was performed using the Student’s t test (paired and unpaired as appropriate); p<0.05 (two tail) was considered statistically significant. For correlation analysis, the Sperman's correlation coefficients were calculated.

**RESULTS**

Fifteen subjects (nine males and six females) with type 2 diabetes (baseline characteristics [mean ± SEM]: age 57.9 ± 2.1 years; BMI: 32.2 ± 1.5 kg/m²; HbA1c 7.1 ± 0.1%; duration of diabetes 11.2 ± 2.8 years; systolic blood pressure: 129 ± 3 mmHg; diastolic blood pressure: 80 ± 2 mmHg) were randomised and all of them completed the study. All patients were on combined treatment with sulfonylurea and metformin.

The results from the liquid meal tests are shown in table 1. Total insulin levels and the AUC-Insulin for the first two hours were significantly higher for insulin glulisine compared with regular insulin (AUC0–120: 51.0 ± 6.8 vs. 38.2 ± 5.4 mU/L/min; p=0.004). Corresponding to the higher insulin levels, glucose levels and the AUC for glucose were significantly lower after insulin glulisine compared with regular insulin (AUC0–240: 158 ± 9 vs 180 ± 9 mg/dL/min; p<0.05). A significant increase in intact proinsulin levels could be observed in both treatments, but intact proinsulin levels were significantly lower after insulin glulisine from 60 minutes pp onwards until the end of the observational period. The AUC for intact proinsulin was significantly higher after injection of regular human insulin compared with insulin glulisine (AUC0–240: 31.2 ± 4.3 vs 26.2 ± 3.5 pmol/L/min; p<0.01).

No significant difference in fasting LDF readings could be observed between the two treatment visits. The percent increase in LDF over time is shown in figure 1. After glulisine injection a significant increase in microvascular blood flow could be observed within 60 minutes after the liquid meal. In contrast, only a very slight increase in microvascular blood flow could be observed with regular human insulin. Stimulation of skin microvascular blood flow by applying a heat stimulus of 44°C (LDF44°C) resulted in maximal stimulation of microvascular skin blood flow, which was not different between the two treatment days (data not shown).
As shown in figure 2 and 3, an increase in nitrotyrosin and ADMA plasma levels could be observed after regular insulin while this increase was completely abolished after insulin glulisine treatment. No significant postprandial change or differences between the treatment groups could be found for FFA, MMP-9, oxLDL and IL-18 (data not shown).

A linear correlation was found between plasma insulin levels and plasma glucose (r=0.263, p=0.0004), LDF<sub>37</sub> (r=0.148, p=0.05), intact proinsulin (r=0.342, p<0.0001), and ADMA levels (r=-0.268, p=0.0003), but not with plasma nitrotyrosin levels. As expected, postprandial plasma glucose levels showed a correlation with insulin (r=0.263, p=0.004) and intact proinsulin levels (r=0.436, p<0.0001), but not with LDF<sub>37</sub>, ADMA or nitrotyrosin plasma levels.

DISCUSSION

In recent years, attention has focussed on the causal relationship between the postprandial state and atherogenesis. There are considerable data, which indicate that postprandial glucose levels may be an independent risk factor for cardiovascular disease and are more predictive than fasting hyperglycaemia (10,11). It has been shown that acute hyperglycaemia affects endothelial function and impairs microvascular blood flow (4), which could be attributed to an increase in nitrotyrosine, leading to the generation of superoxide anions and an increase in oxidative stress (12). In addition, hyperglycaemia decreases the bioavailability of NO and reduces the ability of the vasculature to respond to NO, which leads to vasoconstriction and an increase in atherogenic potency (13).

In addition to the superior efficacy on postprandial plasma glucose excursions, the modified pharmacokinetics of rapidly-absorbed insulin analogues were found to have distinct effects in the regulation of postprandial microvascular blood flow (5,14,15). Our recent study showed an accentuated and rapid increase in microvascular skin blood flow with insulin glulisine compared with regular human insulin. This finding is in accordance with a previous study, which showed a faster and more physiological modulation of postprandial microvascular blood flow after injection with insulin lispro compared with regular human insulin (16). Therefore, the kinetics of insulin absorption from the subcutaneous tissue seems to have important implications in the regulation of postprandial microvascular skin blood flow. In a study by Scognamiglio et al., comparable microvascular effects of rapid acting insulin analogues were observed in the myocardial tissue (5).

Concurrent to the increase in microvascular blood flow, our study also revealed that the postprandial increases in the plasma levels of ADMA and nitrotyrosine were attenuated with insulin glulisine compared with regular human insulin. ADMA is a naturally occurring inhibitor of NO synthase and increased levels of ADMA are associated with endothelial dysfunction and an increased risk of cardiovascular disease (17). It has been reported that increased ADMA levels may contribute to the endothelial dysfunction observed in patients with insulin-resistant diabetes, which is most likely due to the reduction of the available NO pool (18). Insulin resistance has been associated with increased ADMA levels and insulin may directly affect the generation or degradation of ADMA. A study by Fard et al. showed that plasma levels of ADMA were accentuated after high-fat meals and accompanied by a decline in endothelial function, as indicated by a reduction in the flow-mediated vasodilation of the brachial artery (19).

The attenuated postprandial increase in plasma levels of nitrotyrosine found in our study is in agreement with previous results from a study by Ceriello et al., who observed a comparable effect after the injection of insulin aspart (12). Our results, within the context of the data obtained from Ceriello et al. and Scognamiglio et al.,
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indicate a significantly reduced oxidative stress and improved postprandial microvascular blood flow following the injection of rapid acting insulin analogues (such as insulin glulisine) compared with regular human insulin (4,12).

In recent years, the role of intact proinsulin as a predictor of beta-cell function and progression of type 2 diabetes has attracted much attention. In patients with type 2 diabetes, increased intact proinsulin levels have been associated with several cardiovascular risk markers, including increased intima media thickness of the carotid artery (20,21), reduced fibrinolytic activity (22), and increased lipid and apolipoprotein concentrations (23). Epidemiological studies have identified intact proinsulin concentrations as an independent predictor of cardiovascular mortality (24,25). In patients with CHD without diabetes the application of a liquid meal test resulted in an excessive increase in plasma levels of intact proinsulin in the postprandial state (26). Therefore, it seems conceivable, that beside several other pathomechanisms, the postprandial increase in plasma levels of intact proinsulin may contribute to vascular dysfunction and the increased cardiovascular risk in insulin-resistant and type 2 diabetes patients. In this context, it seems noteworthy that our study showed a significantly smaller increase in intact proinsulin levels after injection of insulin glulisine compared with regular human insulin.

Besides improving metabolic control, treatment with insulin glulisine resulted in a significant reduction in oxidative stress with a concomitant improvement in postprandial microvascular blood flow compared with regular human insulin. Insulin glulisine attenuate the postprandial increase in plasma levels of intact proinsulin compared with regular human insulin, which may lead to a corresponding beta cell protection and a reduction in cardiovascular risk. Our data further strengthen the hypothesis that it is need to match the physiological postprandial kinetics of insulin secretion, as closely as possible, not only to improve metabolic control, but also to obtain a physiological postprandial regulation of tissue microcirculation.

**Study limitation.** This study has two important limitations. The first limitation is the limited number of patients included in the study, which stresses the need for larger confirmatory studies. The second limitation is the single application of both study medications, which only allows for the evaluation of acute prandial effects of insulin and is not appropriate to draw conclusions about the long term effects of fast acting insulin analogues on micro- or macrovascular complications. Further studies with larger patient numbers and longer treatment durations are necessary to prove the clinical evidence of this short term pilot study.

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REFERENCES


**TABLE 1.** Results from the standardized liquid meal test (mean±SEM; *p<0.05 vs. Baseline; §= p<0.05 vs. human regular insulin)

<table>
<thead>
<tr>
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<th>human regular insulin</th>
<th>insulin glulisine</th>
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<tbody>
<tr>
<td></td>
<td>0'</td>
<td>30'</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>16.1±3.0</td>
<td>35.5±5.3*</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>139±7</td>
<td>176±9*</td>
</tr>
<tr>
<td>Intact Proinsulin (pmol/L)</td>
<td>21.6±3.7</td>
<td>25.6±4.5*</td>
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<tr>
<td>LDF₃7 (AU)</td>
<td>23±3</td>
<td>19±2</td>
</tr>
<tr>
<td>ADMA (pmol/L)</td>
<td>33±4</td>
<td>36±3</td>
</tr>
<tr>
<td>Nitrotyrosin (nmol/L)</td>
<td>192±33</td>
<td>312±56*</td>
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Figure 1. LDF$_{37^\circ C}$ at baseline, 30, 60, 120, 180 and 240 minutes after the liquid meal test (○ regular human insulin, ■ insulin glulisine; baseline adjusted at 100%; *p<0.05 insulin glulisine versus regular human insulin)

LDF$_{37^\circ C}$=laser Doppler flux at 37°C
Figure 2. Plasma levels of nitrotyrosine at baseline, 30, 60, 120, 180 and 240 minutes after the liquid meal test (○ regular human insulin, ■ insulin glulisine; baseline adjusted at 100%; *p<0.05 insulin glulisine versus regular human insulin)
Figure 3. Plasma levels of ADMA at baseline, 30, 60, 120, 180 and 240 minutes after the liquid meal test (□ regular human insulin, ■ insulin glulisine; baseline adjusted at 100%; *p<0.05 insulin glulisine versus regular human insulin)