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

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OBJECTIVE — To investigate the effect of insulin glulisine on postprandial microvascular blood flow in type 2 diabetes.

RESEARCH DESIGN AND METHODS — A total of 15 patients with type 2 diabetes received insulin glulisine or human insulin before a liquid meal test. Thereafter, skin microvascular blood flow was measured by laser Doppler fluxmetry and blood samples were taken for measurement of plasma levels of glucose, insulin, intact proinsulin, asymmetric dimethylarginine, nitrotyrosine, interleukin-18, matrix metalloproteinase-9, oxidized LDL, and free fatty acids.

RESULTS — Insulin glulisine injections resulted in higher postprandial insulin levels (means ± SEM area under the curve $[AUC]_{0-120}$ 51.0 ± 6.8 vs. 38.2 ± 5.4 mU/l; $P = 0.004$), while plasma glucose (AUC_{0-240} 158 ± 9 vs. 180 ± 9 mg/dl; $P < 0.05$) and intact proinsulin (AUC_{0-240} 26.2 ± 3.5 vs. 31.2 ± 4.3 pmol/l; $P = 0.002$) were lower. Microvascular blood flow increased after insulin glulisine injection (27.9 ± 3.1 to 51.7 ± 9.9 arbitrary units [AU]; $P < 0.05$), while only a minor increase was found during human insulin (27.9 ± 3.1 to 34.4 ± 7.8 AU; not significant). Asymmetric dimethylarginine and nitrotyrosine levels were reduced after insulin glulisine ($P < 0.05$).

CONCLUSIONS — Insulin glulisine is superior to human insulin in restoring postprandial metabolic and microvascular physiology.

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Several epidemiological studies have demonstrated an association between glucose spikes and the development of vascular complications in patients with type 2 diabetes. 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 gut, skin, heart, and adipose tissues (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 increased postprandial oxidative stress and impaired endothelial function are early features of type 2 diabetes. Insulin glulisine attenuates 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 β-cell protection. Therefore, the pharmacokinetics 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.

RESEARCH DESIGN AND METHODS

This investigation was a single-center, open-label, randomized, two-way crossover study of patients with type 2 diabetes who had insufficient metabolic control (A1C >6.5–9.9%) and were on oral antidiabetic treatment. Male and female patients (age 40–70 years; BMI <40 kg/m^2) 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 before 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 randomized to receive subcutaneous administration of a single 0.10 units/kg dose of either insulin glulisine immediately prior to or regular human insulin 15 min before consumption of a standardized liquid meal test (Ensure Plus; 56% carbohydrate, 29% fat, and 15% protein) on two distinct study days within 1 month. Patients entered the study center in the morning after an overnight fast (8 h), and an intravenous catheter was inserted into a superficial vein of one forearm. A laser Doppler probe was adjusted at the contralateral forearm for measurement of microvascular skin blood flow. At time points 0 (before liquid meal intake), 30, 60, 120, 180, and 240 min after consumption of the liquid meal,

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Abbreviations: ADMA, asymmetric dimethylarginine; AUC, area under the curve; IL, interleukin; LDF, laser Doppler fluxmetry; MMP-9, matrix metalloproteinase-9; oxLDL, oxidized LDL.

Table 1—*Results from the standardized liquid meal test*

	Human regular insulin						Insulin glulisine					
	0′	30′	60′	120′	180′	240′	0′	30′	60′	120′	180′	240′
Insulin (mU/l)	16.1 ± 3.0	35.5 ± 5.3*	46.8 ± 7.4*	39.0 ± 4.9*	32.1 ± 4.7*	23.1 ± 2.9*	15.8 ± 2.7	51.6 ± 7.5*†	60.1 ± 8.8*†	54.4 ± 25.6*†	40.4 ± 4.1*†	26.9 ± 3.2*
Glucose (mg/dl)	139 ± 7	176 ± 9*	207 ± 10*	204 ± 11*	170 ± 11*	136 ± 10	137 ± 7	171 ± 10*	185 ± 33*,§	179 ± 11*†	139 ± 11†	108 ± 10†
Intact proinsulin (pmol/l)	21.6 ± 3.7	25.,6 ± 4.5*	29.8 ± 4.7*	34.9 ± 4.1*	34.1 ± 4.5*	30.0 ± 4.3*	19.6 ± 3.5	23.0 ± 3.6*	26.7 ± 4.2*†	30.5 ± 4.0*†	27.0 ± 3.4*†	21.9 ± 3.2†
$LDF_{37°C}$ (AU)	23 ± 3	19 ± 2	34 ± 8	27 ± 6	19 ± 9	23 ± 3	28 ± 3	39 ± 7†	52 ± 10*†	31 ± 5	28 ± 3	36 ± 5†
ADMA (pmol/l)	33 ± 4	36 ± 3	35 ± 3	34 ± 4	33 ± 3	33 ± 3	39 ± 4	36 ± 4	37 ± 4	34 ± 4	32 ± 3*	33 ± 4*
Nitrotyrosin (nmol/l)	192 ± 33	312 ± 56*	466 ± 96*	407 ± 96*	210 ± 58	379 ± 58*	501 ± 81	394 ± 87	197 ± 42*†	238 ± 64*	370 ± 82	437 ± 111

Data are means ± SEM. *$P < 0.05$ vs. baseline. †$P < 0.05$ vs. human regular insulin.

microvascular skin blood flow was measured and blood taken for determination of plasma glucose, insulin, intact proinsulin, nitrotyrosine, asymmetric dimethylarginine (ADMA), matrix metalloproteinase-9 (MMP-9), free fatty acids, interleukin (IL)-18, and oxidized LDL (oxLDL).

Laser Doppler fluxmetry

Laser Doppler flux (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, U.K.). 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 min and the mean LDF was recorded for 3 min 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 20% (8,9).

Laboratory measurements

All laboratory measurements were done at the Institute for Clinical Research and Development (ikfe, 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, Möhnesee-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 ox-LDL: Immmundiagnostik, Bensheim, Germany). Insulin, intact proinsulin, and nitrotyrosine were measured by a chemoluminescence assay (insulins and intact proinsulin: Invitron, Monmouth, U.K.; nitrotyrosine: Upstate, U.S.). A1C was measured by high-performance liquid chromatography (Menarini Diagnostics, Neuss, Germany), while free fatty acids were determined photometrically (WAKO, Neuss, Germany).

Statistical analysis

Because this study was designed as a pilot study, no confirmatory analysis had been performed. All measurements are presented as means ± SEM. The area under the curve (AUC) was calculated for the first 120 min after consumption of the liquid test meal (AUC_{0-120}) and for the entire observation period (AUC_{0-240}) for plasma insulin, intact proinsulin, and glucose levels. Statistical comparison between fasting and postprandial values and between groups was performed using Student's *t* test (paired and unpaired as appropriate); $P < 0.05$ (two tail) was considered statistically significant. For correlation analysis, Spearman's correlation coefficients were calculated.

RESULTS — A total of 15 subjects (9 male, 6 female) with type 2 diabetes (baseline characteristics: mean ± SEM age 57.9 ± 2.1 years, BMI 32.2 ± 1.5 kg/m^2, A1C 7.1 ± 0.1%, duration of diabetes 11.2 ± 2.8 years, systolic blood pressure 129 ± 3 mmHg, and diastolic blood pressure 80 ± 2 mmHg) were randomized, and all completed the study. All patients were on combined treatment with sulfonylurea and metformin.

The results from liquid meal tests are shown in Table 1. Total insulin levels and the insulin AUC for the first 2 h were significantly higher for insulin glulisine compared with those for regular insulin (AUC_{0-120} 51.0 ± 6.8 vs. 38.2 ± 5.4 mU $\cdot l^{-1} \cdot min^{-1}$; $P = 0.004$). Corresponding to the higher insulin levels, glucose levels and the glucose AUC were significantly lower after insulin glulisine compared with those for regular insulin (AUC_{0-240} 158 ± 9 vs. 180 ± 9 mg $\cdot dl^{-1} \cdot min^{-1}$; $P < 0.05$). 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 min postprandially 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 (AUC_{0-240} 31.2 ± 4.3 vs. 26.2 ± 3.5 pmol $\cdot l^{-1} \cdot min^{-1}$; $P < 0.01$).

No significant difference in fasting LDF readings could be observed between the two treatment visits. The percentage increase in LDF over time is shown in Fig. 1. After glulisine injection, a significant increase in microvascular blood flow could be observed within 60 min 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 ($LDF_{44°C}$) resulted in maximal stimulation of microvascular skin blood flow, which was not different between the two treatment days (data not shown).

As shown in Figs. 2 and 3, an increase in nitrotyrosin and ADMA plasma levels

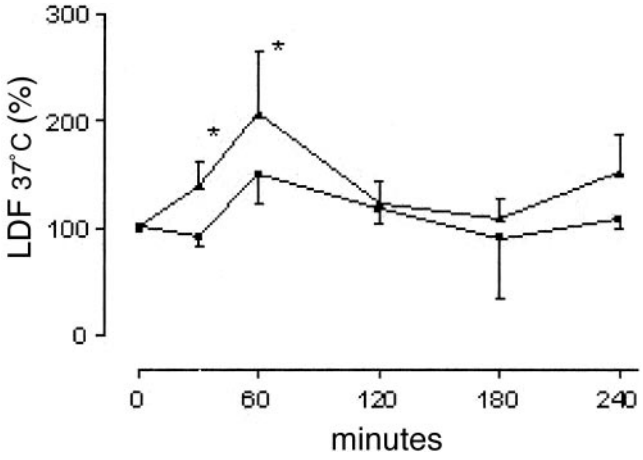

Figure 1—*$LDF_{37°C}$ at baseline and 30, 60, 120, 180, and 240 min after the liquid meal test. Baseline adjusted at 100%. *P < 0.05 insulin glulisine vs. regular human insulin. ■, regular human insulin; ▲, insulin glulisine.*

could be observed after regular insulin, whereas this increase was completely abolished after insulin glulisine treatment. No significant postprandial change or differences between the treatment groups could be found for free fatty acids, MMP-9, oxLDL, and IL-18 (data not shown).

A linear correlation was found between plasma insulin levels and plasma glucose ($r = 0.263$, $P = 0.0004$), $LDF_{37°C}$ ($r = 0.148$, $P = 0.05$), intact proinsulin ($r = 0.342$, $P < 0.0001$), and ADMA levels ($r = -0.268$, $P = 0.0003$) but not between plasma insulin and 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 $LDF_{37°C}$, ADMA, or nitrotyrosin plasma levels.

CONCLUSIONS — In recent years, attention has been focused on the causal relationship between the postprandial state and atherogenesis. There are considerable data indicating that postprandial glucose levels may be an independent risk factor for cardiovascular disease and are more predictive than fasting hyperglycemia (10,11). It has been shown that acute hyperglycemia 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, hyperglycemia decreases the bioavailability of nitric oxide (NO) and reduces the ability of the vasculature to respond to NO, which leads to vasoconstriction and an increase in atherogenic potency (13).

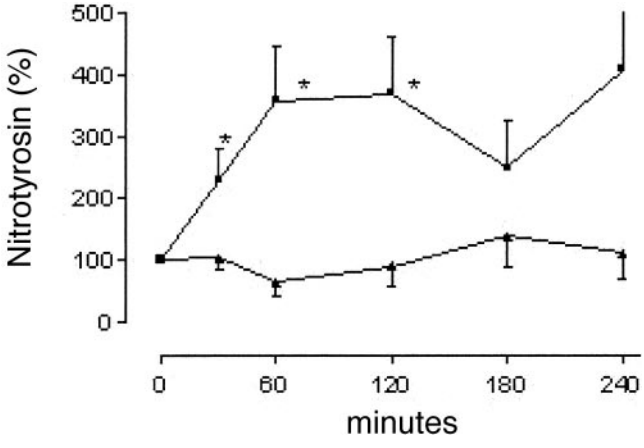

Figure 2—*Plasma levels of nitrotyrosine at baseline and 30, 60, 120, 180, and 240 min after the liquid meal test. Baseline adjusted at 100%. *P < 0.05 insulin glulisine vs. regular human insulin. ■, regular human insulin; ▲, insulin glulisine.*

In addition to their superior efficacy on postprandial plasma glucose excursions, the modified pharmacokinetics of rapidly absorbed insulin analogs 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 (16) that showed a faster and more physiological modulation of postprandial microvascular blood flow after injection with insulin lispro compared with regular human insulin. Therefore, the kinetics of insulin absorption from the subcutaneous tissue seem to have important implications in the regulation of postprandial microvascular skin blood flow. In a study by Scognamiglio et al. (5), comparable microvascular effects of rapid-acting insulin analogs were observed in myocardial tissue.

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, concurrent with the increase in microvascular blood flow. ADMA is a naturally occurring inhibitor of NO synthase; increased levels of ADMA are associated with endothelial dysfunction and 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 resistance, 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. (19) 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.

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. (12), who observed a comparable effect after the injection of insulin aspart. Our results, within the context of the data obtained from Ceriello et al. and Scognamiglio et al., indicate a significantly reduced oxidative stress and improved postprandial microvascular blood flow following injection of rapid-acting insulin

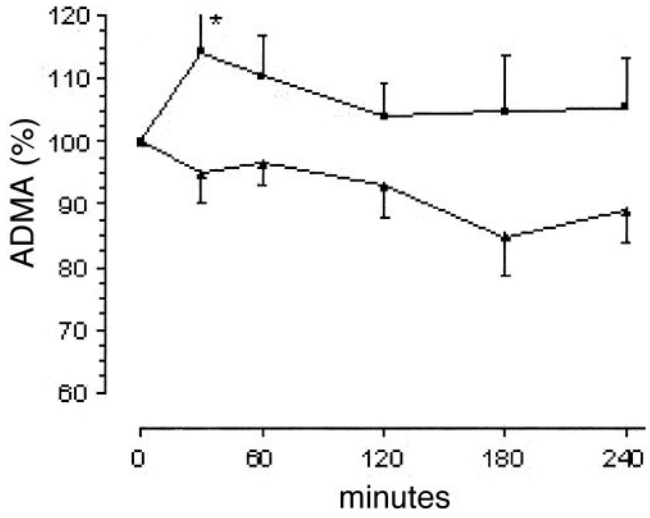

Figure 3— *Plasma levels of ADMA at baseline and 30, 60, 120, 180, and 240 min after the liquid meal test. Baseline adjusted at 100%.* *$P < 0.05$ *insulin glulisine vs. regular human insulin.* ■*, regular human insulin;* ▲*, insulin glulisine.*

analogs (such as insulin glulisine) compared with regular human insulin (4,12).

In recent years, the role of intact proinsulin as a predictor of β-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 coronary heart disease 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 diabetic 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 attenuates the postprandial increase in plasma levels of intact proinsulin compared with regular human insulin, which may lead to a corresponding β-cell protection and a reduction in cardiovascular risk. Our data further strengthen the hypothesis that there is a 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 limitations

This study has two important limitations. The first 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 for drawing conclusions about the long-term effects of fast-acting insulin analogs 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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