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Impact of insulin on microvascular blood flow and endothelial cell function in the postprandial state in patients with Type 1 diabetes

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Abstract

The aim of the present study was to investigate postprandial microvascular blood flow following a standardized test meal in nondiabetic subjects and in patients with Type 1 diabetes after regular insulin or insulin lispro. In this open-label, randomised cross-over study, 20 nondiabetic participants and 20 patients with Type 1 diabetes were enrolled. To valuate the postprandial time course of skin microvascular blood flow, laser Doppler flux (LDF) readings were obtained at baseline and every 30 min following a standardized test meal. Furthermore, the microvascular response to acetylcholine (Ach) was measured, and blood was collected for the measurement of serum insulin and blood glucose levels. Patients with Type 1 diabetes received single doses of regular insulin or insulin lispro, respectively, in a randomised sequence, while in nondiabetics, no insulin substitution was performed. In nondiabetic participants, skin microvascular blood flow showed an early increase in LDF by median 6.0 arbitrary units (AU; interquartile range: 1.8–14.0 AU) within the first postprandial hour. The microvascular response to Ach also increased with a median response of 26.0 (19.0–49.3) AU at 30 min pp and 50.0 (31.7–65.1) AU at 60 min pp. In patients with Type 1 diabetes, the time course of postprandial LDF measurements observed after the administration of insulin lispro was nearly similar to the one observed in nondiabetic controls and differed from that after subcutaneous regular insulin treatment. The postprandial microvascular response to Ach was stronger following insulin lispro compared with regular insulin [30 min pp: 26.0 (19.0–49.3) vs. 20.9 (9.7–26.1) AU, $P = .0001$]. Postprandial microvascular blood flow is disturbed in patients with Type 1 diabetes. Improvement of postprandial metabolic control was found to improve postprandial microvascular function.

Keywords: Insulin; Microvascular blood flow; Postprandial metabolism

1. Introduction

Metabolism in man is regulated by complex hormonal signals and substrate interactions, and for many years, the clinical focus has centred on the metabolic and hormonal picture of an overnight fast. More recently, the postprandial state, that is, the period that comprises and follows a meal, has received increasing attention. Besides increases in blood glucose levels, an increase in plasma insulin level is a typical feature of the postprandial state. Both glucose and insulin interact with endothelial function and might influence the regulation of postprandial microvascular blood flow.

This study was conducted to investigate postprandial microvascular blood flow in nondiabetic participants and in patients with Type 1 diabetes after a standardized test meal. In addition, it should be evaluated if there is a difference in postprandial microvascular function according to the absorption kinetics of subcutaneous insulin.

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2. Patients and methods

2.1. Study design

This was a single-centre, open-label cross-over study in 20 patients with Type 1 diabetes without microvascular complications and 20 nondiabetic control participants. The local ethics committee approved the study protocol, and every patient gave a written informed consent. Patients received a single dose of regular insulin or insulin lispro, respectively, in randomised sequence, with each dose followed by a standardized test meal. The nondiabetic control group received an identical test meal without any external insulin supplementation.

2.2. Methods

Microvascular and endothelial cell responses to a standardized test meal were assessed in nondiabetic controls (without insulin supplementation) and in patients with Type 1 diabetes receiving prandial insulin lispro or regular insulin. Skin microvascular blood flow was measured by laser Doppler fluxmetry, and endothelial function was assessed by iontophoresis of acetylcholine (Ach). Insulin lispro was injected immediately before the test meal, while regular human insulin was given 20±5 min before the test meal. Insulin doses were adjusted individually according to the patients' regular prandial insulin requirements. The patients had to finish the complete test meal (75.0 g carbohydrates, l0.3 g protein, and 7.0 g fat) within 20 min. At baseline and every 30 min following the meal, microvascular blood flow was measured, and blood samples for the measurement of glucose and insulin were collected.

2.3. Laser Doppler fluxmetry

Laser Doppler flux (LDF) was recorded using a laser Doppler device with a temperature-controlled sensoring probe (MonitorTTC-45, Moor Instruments, Devon, UK). LDF readings are given in arbitrary units (AU).

A battery-powered iontophoresis controller (MIC 1, Moor Instruments) with an indirect ion chamber (ION 3, Moor Instruments) was used to investigate the LDF response to Ach at the dorsum of the foot (Forst, Pfutzner, et al., 1998; Parkhouse & LeQuesne, 1988). Ach (1% solution; Miochol E, CibaVision, Weßling, Germany) was delivered using an anodal current of 200 μA for a period of 60 s.

2.4. Glucose and insulin

Venous blood glucose levels were measured using an automatic analyser (Super GL, Dr. Müller Gerätebau, Freital) for the determination of glucose in haemolysed blood samples (NaF). Serum insulin levels were determined using a commercially available Immuno-Chemiluminescence Assay (MLT Insulin Assay, MLT Research, Wales, UK).

2.5. Statistical analysis

All statistical analyses were performed on an exploratory basis.

Following an intent-to-treat approach, all randomised patients who received at least one dose of active study medication were included in the analysis.

LDF measurements are presented as median and interquartile ranges. In case that no carryover effects were presumed, data from both periods were combined to evaluate treatment differences. To explore treatment differences regarding the time courses of postprandial microvascular blood flow, treatment (insulin lispro and human regular insulin) by time (time points 30, 60, 90, 120, 150, and 180 min) interactions were assessed using a nonparametric approach for longitudinal data in factorial experiments (Brunner, Donhof, & Langer, 2002). Furthermore, treatment differences at each time point were investigated using Wilcoxon Sign Rank Test.

Table 1
Glucose and insulin levels at baseline and after the standardised test meal in the different groups

	Glucose (mmol/l)			Insulin (mIU/ml)		
Minutes	Nondiabetic	Type 1 regular	Type 1 lispro	Nondiabetic	Type 1 regular	Type 1 lispro
0	4.4±0.6	6.2±2.0	5.3±2.1	7.9±14.5	14.9±18.9	16.4±19.5
30	6.0±1.3	8.3±2.4	7.7±2.2	50.2±31.4	36.6±18.6	57.0±28.6***
60	5.0±0.9	9.6±2.8	8.0±2.2**	36.2±21.8	37.7±19.2	48.4±25.8**
90	4.7±0.9	9.9±3.1	8.1±2.4**	26.0±15.5	38.5±17.8	40.1±23.3
120	4.7±0.8	9.6±3.1	7.8±2:3	24.4±20:9	37.0±18.8	33.5±24.0
150	4.6±0.5	8.5±3.1	7.9±2.5	18.6±17.1	35.6±18.8	29.9±23.9*
180	4.6±0.4	7.4±3.0	7.4±2.2	14.1±8.3	34.0±26.0	27.0±27.2**

Mean±S.D.

* $P<.05$ vs. Type 1 regular.

** $P<.01$ vs. Type 1 regular.

*** $P>.001$ vs. Type 1 regular.

3. Results

Twenty nondiabetic controls (10 males, 10 females; mean age±S.D. = 31.9±5.9 years) and 20 patients with Type 1 diabetes (6 males, 14 females; mean age 30.4±5.9 years; mean duration of diabetes 7.8±6.4 years; mean HbA_1c 6.7±0.7%) participated in the study. All healthy and diabetic participants completed the study and were included in the full analysis set.

3.1. Nondiabetic controls

In the nondiabetic control group, the highest serum insulin concentrations were reached 30 min following the test meal and, thereafter, consistently declined until the end of the observational period (Table 1). The insulin/glucose ratio increased rapidly within the first postprandial hour and, thereafter, declined steadily during the investigation (Fig. 1).

In parallel, following an initial decline, a sharp increase in microvascular blood flow could be observed within the first 60 postprandial minutes (Fig. 2). The microvascular response to Ach increased to median 35.6 AU (10.4 – 56.6 AU) at 30 min pp and to median 50.0 AU (31.7 – 65.1 AU) at 60 min pp.

3.2. Patients with Type 1 diabetes

3.2.1. Insulin and glucose

As shown in Table 1, postprandial serum insulin and blood glucose profiles were closer to the control group after the injection of insulin lispro than after the injection of human regular insulin. Serum insulin levels 30 min pp were markedly higher after subcutaneous application of insulin lispro compared with regular insulin ($P < .0001$). In the late postprandial absorption phase (180 min pp), significantly higher insulin levels could be observed following regular insulin administration compared with insulin lispro ($P < .01$). Blood glucose levels after insulin lispro were significantly lower compared with regular insulin within the first 120 min following the test meal ($P < .01$). As shown in Fig. 1, the insulin/glucose ratio after subcutaneous application of insulin lispro was much closer to the control group than after the application of regular insulin.

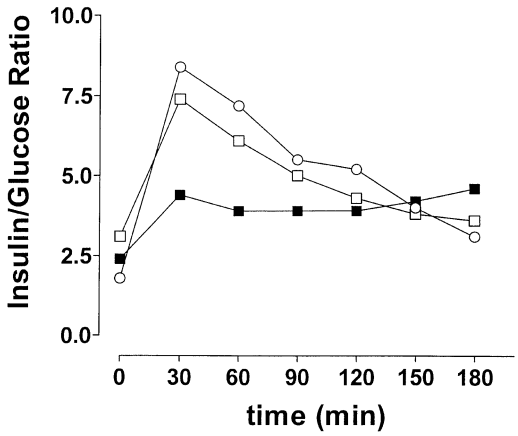

Fig. 1. Time course of mean insulin/glucose ratio (○: nondiabetic control; □: patients with Type 1 diabetes lispro; ■: patients with Type 1 diabetes, regular insulin).

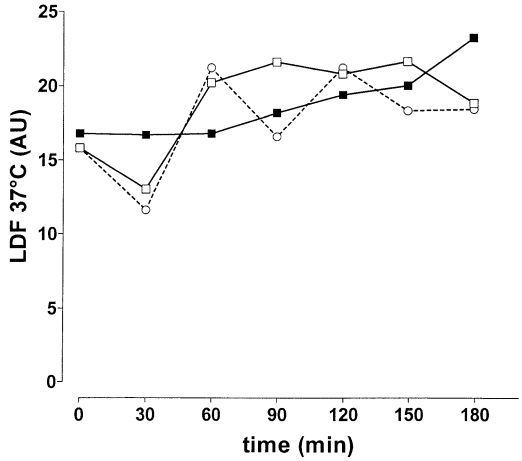

Fig. 2. Postprandial skin microvascular blood flow: time course of median LDF readings at a sensory probe temperature of 37 °C (○: nondiabetic control; □: patients with Type 1 diabetes lispro; ■: patients with Type 1 diabetes, regular insulin).

3.2.2. Microvascular blood flow response

The postprandial microvascular skin blood flow measurements are illustrated in Fig. 2. In parallel with the nondiabetic control group, microvascular blood flow after the subcutaneous application of insulin lispro markedly increased within the first postprandial hour. In contrast, the subcutaneous application of regular insulin was followed by a delayed and prolonged increase of microvascular blood flow. Nonparametric analyses evaluating the treatment-by-time interactions revealed a trend towards a difference in the postprandial microvascular blood flow profile ($P = .09$).

3.2.3. Response to Ach

In addition to the microvascular blood flow, the early postprandial microvascular response to Ach was more pronounced after insulin lispro compared with regular insulin. Thirty minutes postprandial, LDF readings after exposure to Ach had increased by 26.0 AU (19.0 – 49.3) following insulin lispro versus 20.9 AU (9.7 – 26.1) after regular insulin ($P = .0001$), 60 min pp, and LDF increased by 38.8 AU (21.4 – 82.2) after insulin lispro versus 33.1 AU (19.0 – 44.7) after regular insulin ($P = .09$).

4. Discussion

Microvascular function and the microvascular exchange of fluid and nutrients in the postprandial state are highly

sophisticated mechanisms under the control of several physiological systems, such as endothelial function, neurovascular control, rheological factors, and vasoactive hormonal regulation, all of which have been reported as being disturbed in diabetes mellitus (Corbin et al., 1987; Forst et al., 1997; Shami & Chittenden, 1991).

In nondiabetic controls and in patients with Type 1 diabetes, the regulation of microvascular blood flow was found to be influenced by the ingestion of glucose (Khodabandehlou, Zhao, Vimeux, Aouane, & Le Devehat, 1998; Forst, Kunt, Pohlmann, Goitom, Löbig, et al., 1998). Postprandial metabolism and regulation of blood flow is a complex and dynamic process regulated by increasing blood glucose, insulin, and C-peptide concentrations (Flynn, Boolell, Tooke, & Watkins, 1992; Forst, Kunt, Pohlmann, Goitom, Engelbach, et al., 1998; Steinberg, 1999). These haemodynamic effects are caused by a direct impact on endothelial-derived vasoactive substances like an increased release of nitric oxide (Poldermann, Stehouwer, vanKamp, & Gooren, 1996; Steinberg, Brechtel, Johnson, Fineberg & Baron, 1994) or a stimulation of Na–K-ATPase activity (Tack, Lutterman, Vervoort, Thien, & Smits, 1996). Insulin has been shown to stimulate endothelial NO secretion and to increase microvascular skin blood flow (Scherrer, Randin, Vollenweider, Vollenweider, & Nicod, 1994; Tooke, Lins, Ostergen, Adamson, & Fagrell, 1985). Acute increases in blood glucose levels stimulate free radical production, activate thrombin formation, fibrinolysis, and coagulation and have been shown to affect postprandial lipid metabolism (Shaw, van Schie, Carrington, Abbott & Boulton, 1955). Hyperglycaemia activates proteinkinase C (PKC) in endothelial cells, which results in an increased expression of adhesion molecules, an increased release of vasoconstrictive agents such as endothelin and platelet-derived growth factor (PDGF), and a decreased release of vasodilatating substances like nitric oxide (NO) or prostacyclin (Lefebvre & Scheen, 1998).

In patients with Type 1 diabetes, the dynamics of postprandial microvascular blood flow was found to be disturbed compared with that of nondiabetic controls (Khodabandehlou et al., 1998; Forst, Kunt, Pohlmann, Goitom, Löbig, et al., 1998; Le Devehat, Khodabandehlou, Zhao, & Vimeux, 1997). The impact of postprandial insulin levels or different postprandial blood glucose concentrations is still a matter of discussion.

Insulin lispro has been shown to result in a faster increase in serum insulin and to reduce postprandial blood glucose excursions compared with human regular insulin (Lalli et al., 1999; Gale, 2000; Anderson et al., 1997; Feinglos, Thacker, English, Bethel, & Lane, 1997). In agreement with these observations, our study confirmed an earlier and stronger increase in serum insulin levels and reduced postprandial blood glucose excursions following insulin lispro compared with human regular insulin. In addition to the tighter blood glucose control, insulin lispro resulted in a postprandial time course of microvascular blood flow dynamics, which was much closer to the control group than that followed by regular insulin application (Fig. 2). Following an initial decrease in microvascular blood flow in the nondiabetic control group and in patients receiving insulin lispro, skin blood flow increased to its peak value within the first postprandial hour. In contrast, the application of regular insulin in patients with Type 1 diabetes resulted in a delayed and prolonged increase in microvascular blood flow.

Ach produces its vascular effects through a complex sequence of events resulting in an increased release of nitric oxide by endothelial cells (McVeigh et al., 1992; Smits, Kapma, Jacobs, Lutterman, & Thiel, 1993). Several studies revealed an impaired hyperaemic response to Ach in patients with diabetes mellitus reflecting impaired endothelial function in patients suffering from diabetes mellitus (Parkhouse & LeQuesne, 1988; Arora et al., 1998; Walmsley & Wiles, 1991). In accordance with previous results (Forst, Kunt, Pohlmann, Goitom, Löbig, et al., 1998), our recent data confirm a reinforcement of the hyperaemic response to Ach in the postprandial state. Within the first postprandial hour, the microvascular response to Ach was found to be more pronounced following insulin lispro treatment than following the application of regular insulin. Nevertheless, none of the insulin treatments used in our study was able to completely restore endothelial function in patients with Type 1 diabetes.

In conclusion, our study was the first to prove an altered time course of postprandial microvascular blood flow in patients with Type 1 diabetes. The restoration of postprandial insulin and glucose dynamics using fast acting insulin analogs results in a more physiologic regulation of microvascular blood flow than substitution with regular insulin.

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