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Effects of insulin analogs as an add-on to metformin on cutaneous microcirculation in type 2 diabetic patients

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ABSTRACT

Background: A single insulin injection was shown to improve microcirculatory blood flow. Our aim was to examine the effects of 4 weeks of insulin therapy by three randomly assigned insulin analog regimens (Detemir, Aspart, and their combination) on cutaneous blood flow (CBF) and microcirculatory endothelial function as an add-on to metformin in type 2 diabetic patients poorly controlled on oral antidiabetic treatment.

Methods: Fourty-two type 2 diabetic patients with no history of cardiovascular disease in secondary failure to oral antidiabetic agents had CBF measurements before and after acetylcholine (Ach) iontophoretic administration. CBF measurements were performed at fasting and after a standardized breakfast during the post-prandial period. Before randomization (Visit 1, V1) during the tests, participants took only metformin. The same tests were repeated after 4 weeks of insulin treatment (Visit 2, V2).

Results: Thirty-four patients had good quality recordings for both visits. During V1, CBF and CBF response to Ach increased in the post-prandial period. After 4 weeks of insulin treatment, metabolic parameters improved. Compared to V1, CBF at fasting did not increase at V2 but there was an improvement in endothelial function at fasting after Ach iontophoresis, without difference across insulin regimens. Oxidative stress markers were not modified, and *E*-selectin and vascular cell adhesion molecule 1 levels decreased after insulin treatment, without differences between insulin groups.

Conclusions: A strategy of improving glycemic control for 4 weeks with insulin analogs improves microcirculatory endothelial reactivity and reduces endothelial biomarkers at fasting, whatever the insulin regimen used. Insulin therapy associated to metformin is able to improve fasting microvascular endothelial function even before complete metabolic control.

1. Introduction

Cutaneous blood flow (CBF) is an accessible model for the study of microvascular disease. CBF is measured in a non-invasive way and represents an index of the microcirculatory function not only of the skin but also other vascular beds (Holowatz et al., 2008). CBF has been used to assess peripheral vascular (Rossi et al., 2006) and atherosclerotic coronary artery disease (Khan et al., 2008).

Insulin induces capillary recruitment in human skin in healthy normoglycemic subjects, a process that increases both vasodilatation in skin microcirculation and local endothelial activity (Rossi et al., 2005). The induced post-prandial vasomotion then provides necessary substrates for all tissue layers (Serne et al., 2002). However, post-prandial CBF changes may vary depending on the approach used during each study. For example, microvascular perfusion increased during steady-state hyperinsulinemia elicited by an insulin clamp, but perfusion did not change after a mixed meal in healthy individuals or in obese patients (Jonk et al., 2011b). It has been suggested that hyperinsulinemia resulting from insulin resistance may reduce microvascular function even before the appearance of diabetes (Jaap et al., 1997; Wiernsperger N., 2000). In obese patients, post-prandial microvascular function is impaired due to a defect in insulin-stimulated microvascular

Abbreviations: CBF, Cutaneous blood flow; INSUVASC, INSUlin Regimens and VASCular Functions; Ach, Acetylcholine; AUC, area under the curve; VCAM-1, Vascular cell adhesion molecule-1; ANOVA, analysis of variance

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vasomotion (Jonk et al., 2011a). Obesity seems to alter microcirculation independently from hypertension and type 2 diabetes as well as in the absence of endothelial dysfunction, thus underlining the precocious nature of these microcirculatory changes (Czernichow et al., 2010; Rossi et al., 2011).

Metformin and insulin combination is known to improve glycemic control in type 2 diabetes (Aviles-Santa et al., 1999; Giugliano et al., 1993; Wulffele et al., 2002) and can be used early in the course of the disease (Inzucchi et al., 2012). In patients with metabolic syndrome, metformin treatment improved nutritive skin microcirculatory reactivity (Kraemer de Aguiar et al., 2007). In addition, in patients with type 2 diabetes treated with regular insulin, metformin as compared to a placebo improved plasma markers of endothelial function independently from changes in glycemic control (De Jager et al., 2005). Furthermore, the pleiotropic vascular effects of metformin are known to be independent from its antihyperglycemic effects (Valensi et al., 1995; Wiernsperger N. F., 2000).

Fast-acting insulin analogs have a more rapid onset than regular insulin and can better reduce postprandial glycemic excursions. These formulations are more efficacious for the treatment of acute hyperglycemia, inhibit the production of free radicals and counteract oxidative stress (Forst et al., 2010; Gale, 2000; Hohberg et al., 2008). A single dose of rapid insulin analog, compared to regular insulin, can also restore post-prandial microvascular blood flow in patients with type 1 diabetes (Forst et al., 2005).

Longer term effects on microcirculation of insulin analog treatment as an add-on to metformin have not been studied in type 2 diabetic patients, although their association is widely used for the treatment of diabetes.

The aim of this study was to examine the changes in CBF and microcirculatory endothelial function in poorly controlled type 2 diabetic patients at fasting and after a standardized breakfast, with three different insulin analog regimens. We hypothesized that insulin treatment with long-acting, fast-acting or basal bolus insulin as an add-on to metformin would provide additional improvement of CBF either at fasting, after a meal, or both.

2. Material and methods

2.1. Patients

The INSUlin Regimens and VASCular Functions (INSUVASC) study was a pilot, randomized, open label three-arm study that was conducted at Jean Verdier University Hospital (Bondy, France). The study was approved both by the local ethics committee (Comité de Protection des Personnes, Reference Number: 090972), by the French National Agency for Drug Security (Agence Nationale de Sécurité du Médicament, A91140-20) and was registered as a clinical trial (NCT01022658).

The inclusion criteria were the following: patients with healthcare insurance and type 2 diabetes for at least 1 year, age between 30 years and 72 years, BMI ranging from 20 to 37 kg/m^2, HbA1c from 7.1 to 12% (53–108 mmol/mmol) under the maximal tolerated dose of sulfonylureas and metformin use during the last 2 months. If hypertension (defined as blood pressure ≥ 140/90 mmHg or current anti-hypertensive treatment) or dyslipidemia (defined as serum total cholesterol > 6.5 mmol/l, or triglycerides > 2.3 mmol/l, or current lipid-lowering treatment) was present, the participant should have had no changes in their treatment during the last 3 months. Exclusion criteria were the following: pregnancy or absence of contraception in women of childbearing age, anemia (hemoglobin level < 10 g/dl), proliferative or severe non-proliferative retinopathy requiring laser treatment, uncontrolled hypertension (systolic and/or diastolic blood pressure ≥ 160/100 mmHg), inability to check capillary blood glucose and to adjust insulin treatment, moderate to severe renal failure (glomerular filtration rate < 40 ml/min/1.73 m^2, calculated with the MDRD equation), hepatic failure (prothrombin time < 70%), chronic respiratory disease, cardiac arrhythmia, and lower limb arterial disease (intermittent claudication, absence of lower limb peripheral pulses or ankle brachial pressure index < 0.9). Patients should not have been treated with corticosteroids, thiazolidinediones, alpha glucosidase, dipeptidyl-peptidase 4 inhibitors or glucagon like peptide 1 analog treatment during the last 2 months and no insulin allergy.

Diabetic retinopathy was defined according to the Early Treatment of Diabetic Retinopathy Study severity scale. Nephropathy was defined as a 24-h urinary albumin excretion rate ≥ 30 mg/day in at least two assessments. The diagnosis of peripheral neuropathy was based on the presence of any two or more of the following: neuropathic symptoms, decreased distal sensation or decreased or absent ankle reflexes. Smoking was defined as present if patient continued to smoke or had stopped for < 3 years ago.

2.2. Study design

Participants had during the 2-month pre-inclusion period reinforced adherence to treatment and dietary advice and were treated with the maximal tolerated dose of sulfonylureas and metformin. All subjects were on metformin (2 g/day) and the maximal dose of sulfonylurea was for gliclazide 120 mg/day and for glimepiride 6 mg/day. Patients provided written informed consent and had a first visit (V1). Sulfonylurea treatment was given until 24 h before V1 and was stopped afterwards for the duration of the study. Patients took only metformin at H0 during V1 (Fig. 1), the usual antihypertensive (Supplemental Table 1) treatment was administered at the end of the V1. Participants were advised not to modify their daily physical activity during the study period.

After V1, patients were randomized to a 4-to-5-week treatment with one of three different insulin regimens (Aspart, Detemir, or Aspart combined with Detemir). Aspart was to be injected 15 min before each meal and Detemir once per day at 22:00 h. Participants were contacted for follow-up by telephone, and insulin treatment was adjusted to twice a week by two physicians (MF and KT) according to 6 daily glycemic measurements with the use of a prespecified protocol (Supplemental Table 2). There were no modifications for metformin, antihypertensive or lipid lowering treatment during the study. After 4–5 weeks, participants had their second visit (V2), which followed the same study protocol as V1.

At V1, all participants arrived at 07:30 in the morning after an overnight fast and were instructed to abstain from smoking, drinking alcohol-containing drinks, ingestion of coffee or tea during the morning prior to the study and to void their bladder. Measurements were conducted at an ambient temperature of 22 to 24 °C, 30 min after the insertion of a catheter for drawing blood in the right arm, in a room where noise and light were kept to a minimum. Patients remained in the supine position throughout the test. Fasting capillary glycemia was measured and laser Doppler measurements (Periflux System 5000®, Perimed, Stockholm, Sweden) were performed only if the glucose value was between 60 and 250 mg/dl at fasting (H0). Blood samples were collected at fasting.

Next, the patients ate a standardized breakfast consisting of 60 g bread, 30 g jam, 200 ml orange juice, and 100 ml coffee or tea (providing a total of 75 g of carbohydrates). After having their breakfast, participants took their usual metformin treatment. Laser Doppler measurements were repeated 1 and 2 h (H1 and H2) after breakfast. Blood samples were collected just after CBF measurements (H1 and H2).

During the second visit (V2), we used the same protocol and we provided metformin and Aspart injection (for the Aspart and Aspart/Detemir groups), 15 min before breakfast.

2.3. Laser Doppler measurements

The laser Doppler device had a 32 Hz sampling frequency, 780 nm wavelength, from 10 Hz to 19 kHz bandwidth, and a 0.1 s time