CLINICAL SCIENCE

Insulin Therapy does not Interfere with Venous Endothelial Function Evaluation in Patients with Type 2 Diabetes Mellitus

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INTRODUCTION: Endothelium-dependent dilation is improved in insulin-treated diabetic patients, but this effect is probably due to improved glycemic control. The objective of the present study was to compare endothelium-dependent dilation in patients with well-controlled type 2 diabetes who are or are not using insulin as part of their therapy.

METHODS: We studied 27 patients with type 2 diabetes (11 women, 60.3 years $\pm$ 6 years, with HbA1c $<$ 7% and no nephropathy), including 16 patients treated with anti-diabetic agents (No-Ins, 8 women) and 11 patients treated with insulin alone or in combination with anti-diabetic agents (Ins, 3 women). Endothelial function was evaluated by the dorsal hand vein technique, which measures changes in vein diameter in response to phenylephrine, acetylcholine (endothelium-dependent vasodilation) and sodium nitroprusside (endothelium-independent vasodilation).

RESULTS: Age, systolic blood pressure (No-Ins: 129.4 mmHg $\pm$ 11.8 mmHg, Ins: 134.8 mmHg $\pm$ 12.0 mmHg; P= 0.257), HbA_{1c}, lipids and urinary albumin excretion rate [No-Ins: 9 mg/24 h (0-14.1 mg/24 h) *vs.* Ins: 10.6 mg/24 h (7.5-14.4 mg/24 h), P=0.398] were similar between groups. There was no difference between endothelium-dependent vasodilation of the No-Ins group (59.3% $\pm$ 26.5%) *vs.* the Ins group (54.0% $\pm$ 16.3%; P=0.526). Endothelium-independent vasodilation was also similar between the No-Ins (113.7% $\pm$ 35.3%) and Ins groups (111.9% $\pm$ 28.5%; P=0.888).

CONCLUSIONS: Subcutaneous insulin therapy does not interfere with venous endothelial function in type 2 diabetes when glycemic and blood pressure control are stable.

KEYWORDS: Diabetes mellitus, type 2; Diabetes complications; Vascular diseases; Vascular endothelium; Insulin.

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INTRODUCTION

The high cardiovascular risk posed by diabetes mellitus (DM) can be attributed to both classic risk factors[1] and also to hyperglycemia and its consequences: oxidative stress and endothelial dysfunction.[2,3] These abnormalities are related to insulin resistance, which is detrimental to endothelial function and leads to a pro-inflammatory and pro-coagulant state that plays an important role in mediating atherogenesis and cardiovascular disease.[4] Diabetes mellitus[2] and hyperglycemia[5] are independent determinants of endothelial dysfunction.

Short-term improvement of glucose control in diabetic patients cannot reverse endothelial dysfunction,[6,7] but long-term treatment of diabetes may be able to achieve this goal.[8,9] It is not known if the treatment of diabetic patients with subcutaneous insulin can interfere with the evaluation of endothelial function. No existing studies have evaluated the possible effect of subcutaneous insulin use on venous endothelial function, a method that, in some instances, can be more sensitive to subtle endothelial abnormalities.[10] The

aim of this study was to compare endothelium-dependent dilation in patients with type 2 DM with good metabolic and blood pressure control according to whether they used insulin as part of their therapy.

METHODS

Patients

We studied 27 patients with type 2 diabetes (11 women; age at diagnosis $>$ 35 years old, no previous history of ketoacidosis, insulin independence for at least 12 months after diagnosis), including 16 patients treated with diet alone and/or anti-diabetic agents (No-Ins group) and 11 patients treated with subcutaneous insulin (NPH and/or regular insulin; 38.2 $\pm$ 11.6 U/patient) with or without anti-diabetic agents (Ins group). Patients were selected from the outpatients visiting the internal medicine ward of Irmandade Santa Casa de Misericórdia de Porto Alegre (ISCMPA) or Hospital de Clínicas de Porto Alegre (HCPA). We excluded patients older than 70 years and those patients with high blood pressure levels (systolic arterial pressure $>$ 180 mmHg and/or diastolic $>$ 100 mmHg); microalbuminuria (24 h urinary albumin $>$ 30 mg/24 h); creatinine $>$ 1.2 mg/dL; body mass index $>$ 30 kg/m^2; acute coronary syndromes; stable angina; current smokers; those patients under treatment with nitrates or alpha-receptor antagonists; and/or those patients who were unable to understand and sign the consent form. The recommended carbohydrate consumption was limited to 60% of the total caloric intake for all patients.

Protocol

The protocol was approved by the Research Unit Ethics Committees at Hospital de Clínicas de Porto Alegre (HCPA), Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) and Irmandade Santa Casa de Misericórdia de Porto Alegre (ISCMPA), and informed consent was obtained from each patient. All subjects were instructed to avoid caffeinated drinks and alcohol for the 12-h period preceding their appointment. The studies started about 3 h after the participant consumed a light breakfast.

Dorsal hand vein technique

Venous endothelial function evaluation was performed in a quiet room at a constant temperature of 21℃ $\pm$ 1℃. Venous endothelial function was assessed in all patients via the dorsal hand vein technique, [11] a method for which our group has published details elsewhere.[10,12,13] Briefly, a 23-gauge butterfly needle was inserted into a suitable vein on the back of the hand, and a continuous infusion of physiologic saline solution (0.3 mL/min) was started. A tripod holding a linear variable differential transformer (LVDT; Shaevitz Engineering, Pennsauken, NJ) was mounted on the hand, with the central aperture of the LVDT that contained a movable metallic core located 10 mm downstream from the tip of the needle. The signal output of the LVDT, which is linearly proportional to the vertical movement of the core, provided a measurement of the vein diameter. Readings were taken at a congestive pressure of 40 mmHg by inflating a blood pressure cuff placed on the upper portion of the arm being studied. The vein was pre-constricted by infusing increasing doses of the α1-adrenergic selective agonist phenylephrine (25 ng/min to 8333,3 ng/min) until the dose that produced approximately 70% constriction of the vein was found (ED_{70}). This degree of pre-constriction was defined as 0% venodilation. The endothelium-dependent venodilation was assessed with incremental infusions of acetylcholine (3.6 ng/min to 3600 ng/min), and endothelium-independent venodilation with sodium nitroprusside (495.3 ng/min to 990.6 ng/min) was calculated as a percentage of the range between 100% and 0% vasodilation. Drugs were infused with a Harvard infusion pump (Harvard Apparatus Inc., South Natick, MA). Blood pressure and heart rate were monitored in the contra-lateral arm with a sphygmomanometer.

Biochemical measurements

Venous blood and urine samples were obtained during the morning hours after an overnight fast. Albuminuria was determined by immunoturbidimetry (MicroAlb; Ames-Bayer, Tarrytown, NY, USA); plasma glucose was determined by the glucose-peroxidase colorimetric enzymatic method (Biodiagnostica, Pinhais, Brazil); and total cholesterol, HDL cholesterol and triglycerides were determined by a colorimetric method and HbA_{1c} by high-performance liquid chromatography (Merck-Hitachi L-9100; Merck, Darmstadt, Germany). LDL cholesterol was calculated according to the Friedwald formula. Serum creatinine was measured by the Jaffé method. Serum insulin was determined by enzyme immunoassay commercial kits (Abbot-Murex, Park, IL, USA,), and high-sensitivity C-reactive protein (CRP) was measured by nephelometry.

Statistical analyses

Analyses were performed using SPSS® Base 13.0 (SPSS Inc., Chicago, IL, USA). The results are presented as the means $\pm$ standard deviations (SD) or medians (25th-75th percentile). Variables that did not have a Gaussian distribution (Kolmogorov-Smirnov test) were $\log_{10}$-transformed before additional analyses. Differences between the groups were tested with unpaired Student's *t*-tests and chi-square tests. The differences were recognized as statistically significant when $P < 0.05$.

RESULTS

No differences were observed between the groups studied concerning age ($P = 0.470$), body mass index ($P = 0.224$) or duration of diabetes ($P = 0.142$). These and other clinical and laboratory data are shown in Table 1. There were no differences between the groups concerning these characteristics.

Among the 16 patients of the No-Ins group, 10 patients used statins, 10 patients used aspirin, 10 patients used diuretics, 13 patients used angiotensin-converting enzyme inhibitors, 12 patients used metformin, 4 patients used sulfonylurea, and 5 patients used beta-blockers. Among the 11 patients of the Ins group, 5 patients used statins, 6 patients used aspirin, 6 patients used angiotensin-converting enzyme inhibitors, 6 patients used metformin, 2 patients used sulfonylurea, 4 patients used diuretics, and 5 patients used beta-blockers.

Venous endothelial function data are shown in Table 2. Endothelium-dependent venodilation ($P = 0.526$) as measured for maximum venodilation by acetylcholine, venoconstriction induced by phenylephrine ($P = 0.566$) and venodilation by sodium nitroprusside (endothelium-independent venodilation; $P = 0.888$) were similar between

Table 1 - Baseline clinical and laboratory characteristics by group.

	No-Ins (n = 16)	Ins (n = 11)	*P*
Male (n, %)	8 (50)	8 (72.7)	0.441
Age (years)	59.6 ± 4.8	61.3 ± 6.6	0.470
Weight (kg)	68.3 ± 10.3	69.0 ± 15.5	0.906
BMI (kg/m^2)	26.8 ± 1.9	25.2 ± 4.0	0.224
Systolic BP (mmHg)	129.4 ± 11.8	134.8 ± 12.0	0.257
Diastolic BP (mmHg)	81.3 ± 5.3	80.9 ± 4.9	0.866
Heart rate (bpm)	69.0 ± 8.9	71.5 ± 4.8	0.363
Diabetes duration (years)	6.8 ± 2.5	8.4 ± 5.5	0.142
24-h albuminuria (mg)*	9 (0-14.1)	10.6 (7,5-14,4)	0.398
Total cholesterol (mg/dL)	161.1 ± 33.6	157.2 ± 18.1	0.715
HDL cholesterol (mg/dL)	46.9 ± 9.3	49.1 ± 11.7	0.622
LDL cholesterol (mg/dL)	82.8 ± 37.3	75.7 ± 30.8	0.601
Triglycerides (mg/dL)	115 (92.3-167)	126 (112.5-140.8)	0.835
Plasma glucose (mg/dL)	124.7 ± 17.6	130.7 ± 11.6	0.326
HbA_{1c} (%)	6.6 ± 0.6	6.9 ± 0.9	0.317
Insulinemia (μU/mL)	9.9 ± 4.3	10.8 ± 3.3	0.561
CRP (mg/L)	2.3 ± 0.8	2.1 ± 1.0	0.704
Creatinine (mg/dL)	0.9 ± 0.2	0.9 ± 0.1	0.729

Data are means ± SDs, medians (25th-75th percentile) or n (%); BMI: body mass index; BP: blood pressure; HbA_{1c}: glycated hemoglobin; CRP: C-reactive protein; *values before log transformation (log_{10}). No-Ins: diabetic patients treated with diet and/or anti-diabetic agents; Ins: diabetic patients treated with diet and insulin, with or without anti-diabetic agents; Pearson chi-squared test or unpaired Student's *t*-test.

groups. The dose of phenylephrine to reach ED_{70} and the doses of acetylcholine and sodium nitroprusside needed to reach maximum venodilation were not different between the No-Ins and Ins groups. The diameter of the dorsal hand vein did not differ ($P = 0.942$) between the No-Ins group (1.2 mm ± 0.7 mm) and Ins group (1.3 mm ± 0.6 mm).

DISCUSSION

The results of the present study show for the first time that venous endothelial function is similar among type 2 diabetic patients with good metabolic control and well-controlled blood pressure levels, irrespective of their treatment with subcutaneous insulin.

Insulin has known physiological effects upon the vasculature, although the results of studies concerning these effects are discordant. Vasoconstriction and high vascular tonus have been described by some authors,[14,15] while other authors have demonstrated vasodilation induced by insulin infusion.[16,17] Perhaps these results are discordant because data were obtained from different vascular beds and from different species. In rabbit kidneys, insulin induced afferent arteriolar vasodilation, with no effects upon the efferent artery.[18] In diabetic patients, both regular and lispro insulin promoted the improvement of arterial endothelial function after a meal.[19,20] Short-term glucose control obtained with insulin and metformin[9] or with insulin only[8] can also induce better arterial endothelial function when compared with the same evaluation performed in hyperglycemic diabetic patients.

The present study was performed in type 2 diabetic patients with good metabolic control, as confirmed by their fasting plasma glucose levels and HbA1c. Their blood pressure levels were also very well controlled. Both hyperglycemia[21] and high blood pressure levels[22] can interfere with endothelial-dependent vasodilation. Although these factors were under control, there was still venous endothelial dysfunction in all of the patients in the group as compared to non-diabetic subjects, who showed 105.8% ± 9.5% of endothelium-dependent vasodilation in a previous study carried out by our group.[10] There were no differences between the groups studied except for the use of insulin, indicating that the subcutaneous use of insulin does not interfere with venous endothelial function, as was previously shown for arterial endothelium evaluation.[23]

Some limitations of this study should be mentioned, such as the small sample size in the Ins group. This group was not easy to recruit due to the necessary characteristics of the patients (i.e., very good glycemic and arterial pressure control). Additionally, because microcirculatory dysfunction is already present in subjects with metabolic syndrome without diabetes and is associated with the adiposity of these individuals, the characteristic inflammatory state of obesity may cause this abnormality; however, insulin resistance and hyperinsulinemia cannot be discarded as possible causes.[24] Although good reproducibility of venous and arterial endothelial function evaluation was previously shown,[25] arteries and veins have different biological activities in terms of the endothelium, probably due to marked regional and segmental heterogeneity in vascular endothelial function.[26] Future studies investigating the association between different vascular beds in response to subcutaneous insulin therapy may introduce new knowledge in this field of investigation.

The present data allow us to conclude that patients with type 2 diabetes treated with diet and anti-diabetic agents have similar venous endothelial function to those patients treated additionally with subcutaneous insulin. Subcutaneous insulin therapy does not interfere with venous endothelial function in type 2 diabetes given stable glycemic and blood pressure control.

Table 2 - Venous endothelial function by group.

Parameter	No-Ins	Ins	*P*
Venoconstriction (%, phenylephrine)	74.5 ± 8.3	72.5 ± 9.2	0.566
E_{max} (%, acetylcholine)	59.3 ± 26.5	54.0 ± 16.3	0.526
E_{max} (%, sodium nitroprusside)	113.7 ± 35.3	111.9 ± 28.5	0.888
Drug concentrations			
ED_{70} (ng/min, phenylephrine)	50 (25-100)	100 (25-100)	0.893
E_{max} (ng/min, acetylcholine)	3600 (630-3600)	3600 (1800-3600)	0.874
E_{max} (ng/min, sodium nitroprusside)	371.5 (247.7-495.3)	247.7 (247.7-495.3)	0.501

Data are means ± SDs or medians (25th-75th percentile). E_{max}: maximum effect; ED70: dose that produced approximately 70% constriction of the vein. Values of drug concentrations are given before log_{10} transformation.

AUTHORS' CONTRIBUTIONS:

AMVS was involved in the conception and design of the study, data collection, data analysis and interpretation, and drafting and editing the final document for publication. LMP was involved in patient selection and data analysis. MCB was involved in data analysis and interpretation. MCI was involved in data analysis and interpretation as well as the final write-up for publication. BDS was involved in the conception and design of the study, data analysis and interpretation, and writing, drafting and editing of the final document for publication. All authors read and approved the final manuscript.

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