original article

Assessing glycemia in type 1 diabetic patients using a microdialysis system for continuous glucose monitoring

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BACKGROUND: Continuous glucose monitoring systems can monitor moment-to-moment changes in blood glucose concentration, which cannot be detected by intermittent self-monitoring. Continuing monitoring systems may lead to improved glycemic control. We evaluated a microdialysis technique for improving glycemic control in type 1 diabetes patients treated by different means of basal insulin substitution.

PATIENTS AND METHODS: Fifty-two type 1 diabetic patients on twice daily NPH and pre-meal aspart insulin were randomized in two groups: the continuation of NPH (n=26) (group 1) or once daily glargine (n=26) (group 2). 48-hour *GlucoDay* registrations were started at the beginning and after 4 months.

RESULTS: At baseline, time spent in the euglycemic range (glucose between 3.9 and 8.0 mmol/L) was 37.96±6.81% for the NPH group and 35.83±6.24% for the glargine group. At endpoint, time in the euglycemic range increased in both groups (51.02±7.22% and 57.29±10.27%, $P<0.001$ vs. before treatment for both groups). Time spent in the hypoglycemic range (glucose <3.9 mmol/L) was 9.98±2.57% for the first group and 10.24±3.55% for the second group at baseline. At endpoint, time in the hypoglycemic range decreased in both groups (8.00±2.13% and 6.59±2.04%, $P<0.001$ vs. before treatment for both groups).

CONCLUSION: The analysis of the *GlucoDay* data gave us information about glycemia other than HbA1c and self-monitoring of blood glucose, such us a peakless activity profile and the lower percentage of time spent in the hypoglycemic range in the glargine-treated group.

Worldwide, the number of cases of diabetes mellitus is expected to increase exponentially, with current estimates suggesting an increase from 171 million in 2000 to 371 million sufferers by 2030.[1] In Croatia, type 2 diabetes mellitus accounts for about 90% of cases and type 1 diabetes mellitus for about 7%.[2] To prevent or delay the onset of microvascular complications, intensive insulin therapy is needed and recommended for treatment of type 1 diabetes. The Diabetes Control and Complications Trial (DCCT) was designed to demonstrate the importance of intensive care for optimal glycemic control to reduce the risk of developing microvascular complications.[3] In broad terms, for every 1% fall in glycosylated hemoglobin (HbA1c) there is a reduction in microvascular risk by about 25%, irrespective of whether the patient has type 1 or type 2 diabetes.[4] The targets for blood glucose control are now much clearer. There are no thresholds within the diabetic range of blood glucose for risk of microvascular complications.[5] To reduce risk, the goal should be to achieve normal blood glucose levels.

The assessment of blood glucose can be done in various ways. Continuous glucose monitoring systems have considerable potential for monitoring moment-to-moment changes in blood glucose concentration which cannot be detected by intermittent blood glucose self monitoring, and may lead to improved glycemic control.[6,7,8] NPH is no longer considered as only basal replacement insulin in type 1 diabetes because of the risk of nocturnal hypoglycemia.[9,10,11] The novel recombinant insulin analog insulin glargine is a modification of human insulin in which two arginines are added to

the B-chain and glycine is substituted for asparagine at the A21 position of the insulin molecule. These changes cause a shift of the isoelectric point to a neutral pH, precipitation at physiologic tissue pH, and increased hexamer stability, resulting in delayed absorption and a flat profile after injection, compared with the shorter duration of action and early peak of NPH insulin.[12]

To assess glycemia in type 1 diabetics treated by two different basal insulin replacement options we used *GlucoDay*, a microdialysis-based continuous subcutaneous glucose monitor that collects glycemia values every 3 minutes and thus enables calculation of parameters.

PATIENTS AND METHODS

The study was approved by the local ethics committee, and participants gave written informed consent. All patients with type 1 diabetes mellitus (fasting C-peptide concentration<200 pmol/L), classified according to the revised American Diabetes Associations guidelines,[13,14] admitted to the Day Care Hospital of the Clinical Hospital Split in Croatia, during January 2005, were included. All 52 type 1 diabetic patients were educated on treatment skills and changes in their daily lifestyles in the course of a 5-day educational program. They took active part in self-monitoring, exercise, menu planning and assessment of their own participation in the treatment of their disease. Initially, they were treated with a combination of three daily doses of a fast-acting analogue aspart insulin pre-meal and two daily doses of NPH insulin. After a 5-day educational program in Day Care Hospital, the first 26 patients continued their previous insulin regimen (group 1), and other 26 patients (group 2) were asked to replace basal insulin in the form of glargine at bedtime. Endocrinologists assisted patients in titrating the dose of the basal insulin to achieve fasting glucose values of 4.4-6.6 mmol/L, whilst avoiding hypoglycemia. Subjects previously using twice-daily NPH and randomized to receive insulin glargine were advised to reduce the insulin glargine dosage 10% compared with total NPH dosage. Endocrinologists titrate insulin glargine according to a treat-to-target algorithm.[15] The titration period was continued until adequate glycemic control in the endocrinologist`s judgment had been achieved. Insulin glargine is a clear solution and is easily distinguished from NPH insulin, requiring an open-label design. The aspart insulin was used in both groups as bolus insulin during the whole observation period.

From the beginning of the study and after four months, we used a microdialysis-based continuous subcutaneous glucose-monitoring device (*GlucoDay*, A. Menarini Diagnostics, Florence, Italy) in all patients. The microdialysis probe of the *GlucoDay* was inserted subcutaneously in the periumbilical region. The *GlucoDay* device consists of a peristaltic pump that pumps Dulbecco solution at a rate of 10 microL/min along the microdialysis fiber (0.17 mm internal diameter and 18.000 Dalton molecular weight cut-off) and transports the dialysate derived from the subcutaneous interstitial fluid to the glucose sensor. The glucose sensor (immobilized glucose oxidase) takes a glucose measurement from the dialysate every second and stores an average value every 3 minutes until the device is removed. The lag time between subcutaneous and intravenous glucose values has been estimated to be less than 3 minutes.[16] Before insertion of the *GlucoDay* device, the sensitivity of the glucose sensor was checked in vitro using a standard D-glucose solution (90 mg/L), which gives a signal of 6 to 40 nAmp. Subcutaneous glucose was monitored every 3 minutes via the device. Blood glucose concentration was calculated from the data collected by the device (which measured glucose concentrations in the dialysate, expressed as nAmp) by calibration with the capillary values 120 minutes after probe insertion and 10 minutes before end of monitoring. When calibrating by the capillary value, the correlation coefficient obtained was r=0.97. No complications at the site of implantation were observed and there were no complaints of discomfort associated with *GlucoDay*. In addition, because of the ability of the system to communicate with a computer through an infrared port, online glucose variations were detected in real time, but we used the option of blind recording for patients to exclude any possible additional treatment intervention. Data collected from the patients included age (years), sex, body mass index (kg/m^2), diabetes duration (years), half-day glycemic profiles of capillary blood (mmol/L), and HbA1c (%).

Capillary blood glucose levels were analyzed by the enzymatic colorimetric method (Glucose GOD-PAP, Chronolab AG, Switzerland, on Olympus chemistry analyzer AU 400, Japan). All blood glucose probes for calibration were collected and managed by a nurse to avoid the subjective reactions of the endocrinologists to the patients. HbA1c was measured by ion-exchange chromatography based on separating hemoglobin adducts according to their charge (Chronolab AG, Switzerland). The normal values for capillary blood glucose were 4.2-6.1 mmol/L (coefficient of variation 1.4%), and for HbA1c were 4.2-6.2% (coefficient of variation 1.5%). The evaluation of the curves obtained with subcutaneous glucose recording, when compared to measurements on venous blood performed by the reference laboratory system showed a correlation coef-

Table 1. General characteristics of the type 1 diabetic patients treated with NPH two times/day (group 1) and once daily glargine at bedtime (group 2).

	Group 1 (n = 26)	Group 2 (n = 26)
Sex		
Female	10	14
Male	16	12
Age (years)	36.92±8.82	36.5±9.10
Duration of diabetes (years)	13.12±8.78	11.15±7.56
Body mass index (kg/m^2)	24.35±2.7	24.38±2.78
HbA1C before treatment	8.56±1.33	8.01±1.47
HbA1C after 4 months of treatment	8.00±1.04	7.13±1.08*

Values are expressed as means±SD. *P<0.05 for group 1 vs. group 2.

Table 2. *GlucoDay* registration glucose values for the type 1 diabetic patients treated with NPH two times/day (group 1) and once daily glargine at bedtime (group 2) before and after four months of treatment.

	Before treatment	After 4 months of treatment
Time spent in the euglycemic range (3.9-8.0 mmol/l) (%)		
Group 1	37.96±6.81	51.02±7.22*
Group 2	35.83±6.24	57.29±10.27*
Time spent in the hypoglycemic range (< 3.9 mmol/l) (%)		
Group 1	9.98±2.57	8.0±2.13 *
Group 2	10.24±3.55	6.59±2.04*

Values are expressed as means±SD. *P< 0.001, 4 months vs. before treatment.

ficient of 0.904 (r^2=0.817; P<0.001).The estimation standard error was -0.86 mmol/L and the error grid analysis gave values of 97% in the A/B zone, 2.5% in the C zone and 0.5% in the D zone.

The data was analyzed using SPSS for Windows (version 9.0., 2000). Results are expressed as the means±SD. Groups were compared by the t-test, with a two-tailed distribution and paired data.

RESULTS

There were no significant differences found for all baseline characteristics (age, duration of diabetes, BMI, HbA1c) as shown in Table 1. After 4 months there was no significant difference in BMI between the two groups. The duration of the study is one possible explanation for the lack of weight differences.

When comparing the two types of treatment according HbA1c, both groups showed a decrease in HbA1c (P<0.0000005 for the first group and P<0.000001 for the second group) (Table 1). At the end of the study, there was significant difference in the value of HbA1c between the groups (P<0.005071) (Table 1).

The values of HbA1c were significantly decreased within each group and were more homogeneous after treatment, which is shown from the reduced values of the standard deviations (1.33% to 1.04% for group 1; 1.47% to 1.08% for group 2). The biggest improvement was noticed in the group treated with a combination of three daily doses of aspart insulin and bedtime glargine insulin (Figure 1 and 2). Time spent at a glucose value between 3.9 and 8.0 mmol/L, confirmed by *GlucoDay*, increased in both groups (Table 2). Time spent at glucose below 3.9 mmol/L, confirmed by *GlucoDay*, decreased in both groups (Table 2). Both fiber insertion and the wearing of the device were well tolerated by all patients.

DISCUSSION

Studies in both pediatric and adult diabetic patients have conformed that intensive insulin therapy is needed and recommended to prevent the onset of microvascular diabetic complications.[3] Insulin secretion is a complex process requiring optimal coupling between glucose concentrations and insulin release. Insulin secretion is pulsatile, with release of bursts occurring every 4 to 6 minutes.[17]

At present, there are two models of physiological insulin replacement in type 1 diabetes mellitus. These models have in common the use of a rapid-acting insulin analog at each meal combined with basal insulin replaced in the form of either continuous subcutaneous insulin infusion or glargine once a day.[18,19,20]

In the past, ultralente or NPH insulin was commonly used to provide basal insulin concentrations.[10,11] More recently, glargine has been shown to be an effective basal insulin preparation.[15,21] Several studies, like our trial, have shown that when compared with NPH insulin, use of glargine results in comparable or lower HbA1c concentrations and a lower frequency of nocturnal hypoglycemia.[11,12,22]

Variation in glucose concentrations is frustrating to patients with type 1 diabetes and their health care providers.[23] An attempt to improve HbA1c in type 1 diabetes needs to incorporate assessment of glycemic variation. To measure glucose concentrations in two different basal insulin replacement options we used *GlucoDay*, which collects glycemia values every 3 minutes and thus enables us to calculate parameters. Continuous subcutaneous glucose monitoring can provide extremely useful

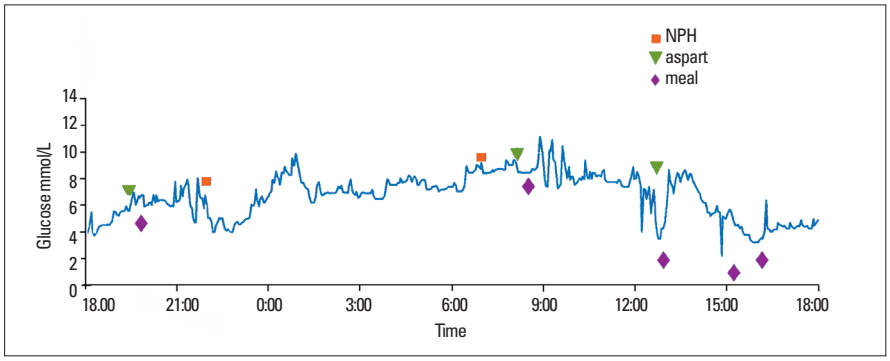

Figure 1. Example of *GlucoDay* registration showing glucose values in type 1 diabetic patient on two daily NPH doses and aspart insulin at each meal after 4 months of treatment.

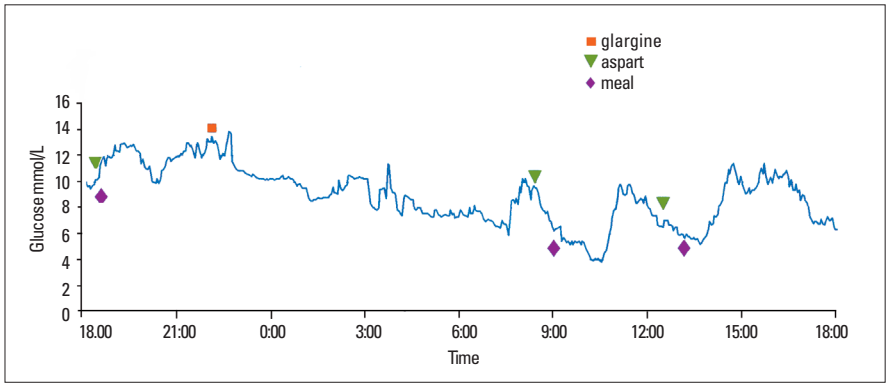

Figure 2. Example of a gylemic profile obtained with the *GlucoDay* device in type 1 diabetic patient on one dose of glargine and aspart insulin at each meal after 4 months of treatment.

information about an individual's glucose pattern and fluctuations during the day, which cannot be detected by intermittent blood glucose self monitoring.[24] This is particularly useful for detecting overnight glycemic excursions in intensively insulin-treated type 1 diabetic patients. Achieving HbA1c targets of <7 % HbA1c in type 1 diabetic patients in clinical practice involves long-term motivation and co-operation by patients and healthcare providers. Continuous glucose monitoring is an important adjunct to the overall care of the diabetic patient, particularly for the Day Care Hospital. Our findings demonstrated that the *GlucoDay* system was associated with little or no discomfort for the patient. It provided important information in real time that may lead to therapeutic adjustments, and the patient's glycemic control can be improved significantly.

It must be pointed out that glargine has a peakless time-action profile that lasts 24 hours, whereas NPH insulin has a distinct peak with a shorter duration of action.[25] Reviewing healthy subjects only, the analysis of within-day fluctuations of serum insulin levels shows that insulin glargine offers a more consistent serum level compared to NPH insulin.[26] Insulin glargine had a flat, prolonged action profile, with an onset of action later than NPH. Additionally intersubject variability was lower with insulin glargine than with human NPH insulin.[27] In a comparison study, Porcellati et al., compared once daily insulin glargine given in the evening with multiple daily injections of NPH insulin and continuous subcutaneous insulin infusion. The plasma glucose and insulin concentrations show that compared to NPH insulin, insulin glargine provided less variability in plasma glucose levels, without the glucose dip evident four hours after NPH administration. Plasma insulin levels were steady throughout the night, in contrast to the marked peak and trough associated with NPH insulin.[28,29,30]

Our study evaluated the microdialysis technique for improving glycemic control in type 1 diabetic patients with different basal insulin substitution. The availability of this technique may be helpful for the evaluation of the glucose profile in type 1 diabetic patients treated with different basal insulin supplementation.[7,8,30] This method confirms that basal insulin can be effectively supplied with either two doses of NPH or one dose

of glargine. However, glargine achieved a significantly lower HbA1c compared with NPH in patients with type 1 diabetes and glycemic variability measured by *GlucoDay* was also lower with glargine than NPH. The analysis of the *GlucoDay* data demonstrated high inter-individual variability. This type of monitoring supplies extra information on glycemic control that is not predictable from the glycated hemoglobin measurement or from intermittent blood glucose self-monitoring, and is easily used on a routine clinical basis.

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