Comparison of Acute Daytime and Nocturnal Insulinization on Diurnal Glucose Homeostasis in NIDDM

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OBJECTIVE — The question of whether to use insulin in the evening or in the morning during combination therapy in patients with non-insulin-dependent diabetes mellitus (NIDDM) is controversial. We compared the acute effects of 12-h nocturnal or daytime insulin infusions on the 24-h glucose profile in 20 patients with NIDDM.

RESEARCH DESIGN AND METHODS — NIDDM patients were 56 ± 2 (mean ± SE) years of age and had a body mass index of 29.6 ± 1.1 kg/m^2; fasting plasma glucose concentration of 12.2 ± 0.5 mM; and fasting C-peptide concentration of 0.9 ± 0.2 nM. Each patient was studied twice. On one occasion, the patient received a 12-h intravenous infusion of insulin (mean 1.5 ± 0.1 IU/h) during the day, and on the other occasion an identical dose of insulin was infused during the night. Blood glucose, insulin, c-peptide, and free fatty acid concentrations were determined for 24 h.

RESULTS — The mean 24-h free insulin concentrations were similar in both studies (150 ± 12 vs. 162 ± 12 pM, daytime versus nocturnal insulin infusion). The mean 24-h free fatty acid concentration was 18% lower in the nocturnal than in the daytime (309 ± 30 vs. 376 ± 30 μM, $P < 0.001$) insulin infusion study. The mean 24-h C-peptide concentration was less suppressed if insulin was infused overnight than during the day (1.3 ± 0.2 vs. 1.1 ± 0.2 nM, $P < 0.01$). The mean 24-h plasma glucose concentrations were identical in both studies (11.1 ± 0.6 vs. 11.4 ± 0.7 mM, daytime versus nocturnal insulin infusion). We also searched for factors predicting the decrease in the blood glucose concentration during the nocturnal insulin infusion. The best predictors were a high initial blood glucose concentration at 2200 and a low fasting C-peptide concentration. These factors explained, independent of each other, 50% of the rate of decrease in the plasma glucose concentration.

CONCLUSIONS — Despite better suppression of lipolysis and less suppression of endogenous insulin secretion by nocturnal than daytime insulinization, the hypoglycemic effect of these two treatments is similar.

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NIDDM, non-insulin-dependent diabetes mellitus; HDL, high-density lipoprotein; FFA, free fatty acid; RIA, radioimmunoassay.

Regardless of the primary cause of non-insulin-dependent diabetes mellitus (NIDDM), hyperglycemia results from excessive hepatic glucose production in the overnight fasted state and from impaired suppression of endogenous glucose production by insulin in the postprandial state (1,2). Although patients with NIDDM exhibit insulin resistance under normoglycemic conditions, the absolute rate of glucose utilization is increased both after and between meals because of the mass-action effect of glucose (1). Inhibition of hepatic glucose production, therefore, represents a logical target for treatment of patients with NIDDM.

Because endogenous glucose production is maximal during the night, nocturnal insulinization might be at least as effective or perhaps even more effective than daytime insulinization (3). In our multicenter study comparing combination therapy regimens with either evening insulin or morning insulin, we found comparable improvements in glycemic control using both regimens (4). However, the plasma free insulin concentrations were lower after a bedtime than after a morning NPH insulin injection, which suggests that nocturnal administration of insulin might have had a greater hypoglycemic effect. Perhaps because of unnecessary hyperinsulinemia, combination therapy with evening insulin induced less weight gain than combination therapy with morning insulin both in the Finnish study and in a recent Swedish multicenter insulin treatment study (4,5). Whether nocturnal insulin is indeed more potent in lowering the blood glucose concentration than a similar dose of insulin given during the day has not, however, been directly tested.

This study was undertaken to compare directly the hypoglycemic effect of nocturnal and daytime insulinization. We also searched for factors that might predict the hypoglycemic effect of insulin in an individual patient. To avoid possible

differences in insulin absorption, insulin was administered intravenously.

RESEARCH DESIGN AND METHODS

— Twenty patients (12 men, 8 women) with NIDDM participated in the study. Their mean age was 56 ± 2 years; body mass index was 29.6 ± 1.1 kg/m^2; HbA_{1c} was 9.3 ± 0.3% (range 6.4–12.2%, reference range 4–6%); fasting plasma glucose concentration was 12.2 ± 0.5 mM (7.3–16.9 mM); fasting serum C-peptide concentration was 0.9 ± 0.1 nM (0.4–1.4 nM); and fasting serum insulin concentration was 78 ± 12 pM. Fasting serum triglyceride concentration was 3.3 ± 0.5 mM (0.6–8.3 mM); serum cholesterol concentration was 5.7 ± 0.3 mM, and the serum high-density lipoprotein (HDL) cholesterol concentration was 1.0 ± 0.1 mM. Eighteen patients used glyburide, and 12 patients used both glyburide and metformin. Drug administration was continued unchanged during the two studies in each patient. None of the patients was or had been treated with insulin. Patients with clinically significant nephropathy, retinopathy, neuropathy, or cardiovascular disease were excluded from the study.

The purpose, nature, and potential risks of the studies were explained to the patients before their informed consent was obtained. The experimental protocol was approved by the ethical committee of the Helsinki University Hospital.

Each patient was studied twice within a week. On both occasions, blood glucose, insulin, C-peptide, and free fatty acid (FFA) concentrations were determined for 24 h. On one occasion, the patient received a 12-h intravenous infusion of insulin during the day, and on the other occasion an identical dose of insulin was infused during the night (Fig. 1). The order of the two studies were randomized. The patients were admitted to the hospital 1 day before the study and placed on a weight-maintaining diet containing 25 kcal/kg with 50% of calories from carbohydrate, 30% from fat, and 20% from protein. The diet was similar on both study occasions and consisted of breakfast (20–25% of total calories) at 700, lunch (30–35%) at 1130, an afternoon snack (5%) at 1400, and dinner (30–35%) at 1630. An evening snack (10%) was served at 2000. Blood samples were drawn for measurement of fasting plasma glucose, serum C-peptide, serum insulin, blood HbA_{1c}, serum triglyceride, and total cholesterol and HDL cholesterol concentrations before the start of the daytime insulin infusion.

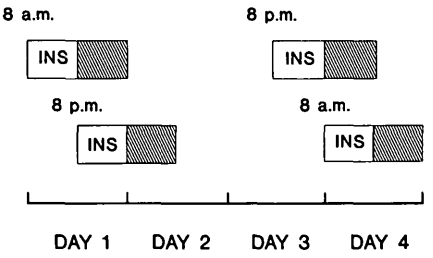

Figure 1—*Design of the study. INS, the 12-h intravenous insulin infusion;* ▨, *the 12-h period after discontinuation of the insulin infusion.*

Daytime insulin infusion

The patients fasted after the evening snack, which was served at 2000. At 0745, two indwelling catheters were inserted in antecubital veins, one for infusion of regular insulin and the other for blood sampling. At 0800, an infusion of insulin (Insulin Actrapid Human®, Novo-Nordisk, Bagsvaerd, Denmark) was started and continued for 12 h. The insulin dose was 25 IU/12 h for the first patient, 15 IU/12 h for the next 6 patients, and 20 IU/12 h for the remaining 13 patients (mean 1.5 ± 0.1 IU/h). Blood was withdrawn for 24 h (for 12 h during the insulin infusion and for 12 h thereafter) for measurement of plasma glucose concentrations hourly, insulin concentrations every 2 h, and serum FFA concentrations every 4 h. Serum C-peptide concentrations were measured every 2 h in seven patients.

Nocturnal insulin infusion

The patients received their meals until 1945. An infusion of insulin was started at 2000 and continued for 12 h. Blood samples were withdrawn as described above during the 12 h of the nocturnal insulin infusion and for 12 h the next day. The meal schedule and insulin doses were identical to those in the daytime insulin study.

Measurements

Plasma glucose concentrations were measured with the glucose oxidase method using the Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Serum FFA concentrations were measured by a fluorometric method (6). Serum free insulin concentrations were determined after precipitation with polyethylene glycol (7) by radioimmunoassay (RIA) using the Phadeseph Insulin RIA kit (Pharmacia, Uppsala, Sweden). Blood HbA_{1c} was determined by high-performance liquid chromatography (BioRad, Richmond, CA) (8). Serum triglyceride and total and HDL cholesterol concentrations were determined as previously described (4). Serum C-peptide concentrations were determined by RIA using the RIA-mat C-peptid II kit (BYK-Sangtec Diagnostica, Frankfurt, Germany) (9).

Statistical analysis

The 24-h mean metabolite and hormone concentrations were compared using simple pairwise comparisons with 0 using Student's *t* test. Simple linear and multiple regression analyses were calculated using the Statsgraphics statistical package (STSC and Statistical Graphics, Rockville, MD).

RESULTS

Serum insulin and C-peptide concentrations

The mean 24-h serum insulin concentrations were comparable during daytime (150 ± 12 pM) and nocturnal (162 ± 12 pM) insulin infusion studies (Fig. 2).

The mean 24-h serum C-peptide concentration was 15% lower during the daytime (1.1 ± 0.2 nM) than during the nocturnal (1.3 ± 0.2 nM) insulin infu-

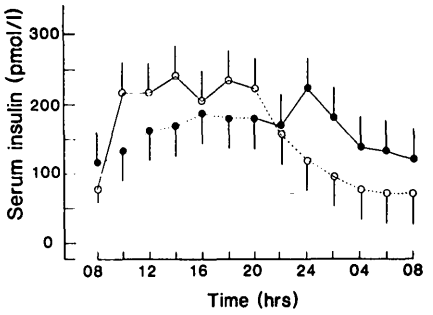

Figure 2—*Twenty-four-hour serum insulin concentrations in the nocturnal (●) and daytime (○) studies. ———, Insulin infusion; – – – –, no insulin. Data from both studies have been superimposed to allow better comparison of the profiles in the two studies.*

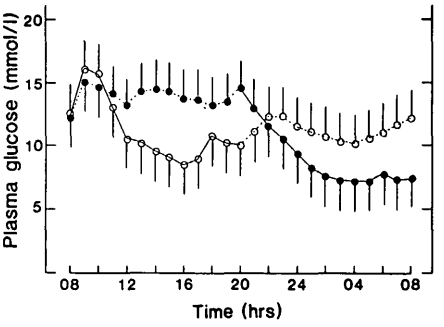

Figure 3—*Twenty-four-hour plasma glucose concentrations in the nocturnal (●) and daytime (○) studies. ———, Insulin infusion; – – – –, no insulin.*

sion. During the daytime insulin infusion, the mean 12-h C-peptide concentration was 32% lower than during the control day (no insulin infusion, 1.2 ± 0.2 vs. 1.7 ± 0.3 nM, $P < 0.001$). During the nocturnal insulin infusion, the mean 12-h C-peptide concentration (0.9 ± 0.1 nM) was 19% lower than during the control night (no insulin infusion, 1.1 ± 0.2 nM, $P < 0.05$).

Serum FFA concentrations

Mean 24-h serum FFA concentrations were 18% lower in the nocturnal (309 ± 30 μM) than in the daytime (376 ± 30 μM) insulin-infusion study ($P < 0.001$). The mean 12-h FFA concentration was 22% lower during the daytime insulin infusion (257 ± 29 μM) than during the control day (329 ± 36 μM; $P < 0.01$). The FFA concentrations averaged 296 ± 28 μM during the nocturnal insulin infusion, which was 40% lower than the concentration during the control night (497 ± 35 μM; $P < 0.001$).

Plasma glucose concentrations

The mean 24-h plasma glucose concentrations were almost identical during the daytime (11.1 ± 0.6 mM) and nocturnal (11.4 ± 0.7 mM) insulin infusions (Fig. 3).

Determinants of the rate of decline in plasma glucose during nocturnal insulin infusion

When the individual plasma glucose curves were analyzed, a linear decrease was observed between 22 and 2 h (mean r ± SE for individual regression lines was 0.86 ± 0.15). The decreases in plasma glucose during the 4-h period ranged between −0.3 and 12.3 mM.

We next determined which clinical or laboratory parameters were associated with the decrease in the plasma glucose concentration. In simple linear regression analysis, the initial plasma glucose at 22 h was directly ($r = 0.51$, $P < 0.05$) and the fasting plasma C-peptide concentration inversely ($r = -0.46$, $P < 0.05$) associated with the decrease in plasma glucose (Fig. 4). Parameters such as body mass index, serum triglycerides, HbA_{1c}, or the mean free insulin concentration during the 4-h period were not significantly associated with the decrease in plasma glucose.

In multiple linear regression analysis, the best predictors of the decrease in plasma glucose concentration in response to nocturnal insulin were the initial plasma glucose concentration (at 2200) and the fasting C-peptide concentration, which together explained 50% of the individual variations in the rate of decrease in the plasma glucose concentration ($P < 0.001$ for regression model, F ratio 10.6). Inclusion of the ambient serum insulin concentration did not improve the model ($P < 0.005$ for regression model, r^2 adjusted for degrees of freedom 48%, $P < 0.004$, F ratio 6.75; Fig. 5).

CONCLUSIONS— These data demonstrate comparable hypoglycemic effectiveness of nocturnal and daytime insulin administration in patients with NIDDM. We found that, in an individual patient, the initial blood glucose concentration, and perhaps unexpectedly, a low fasting C-peptide concentration were predictors of a good hypoglycemic response to insulin. The initial blood glucose concentration and a low C-peptide concentration were significant predictors, independent

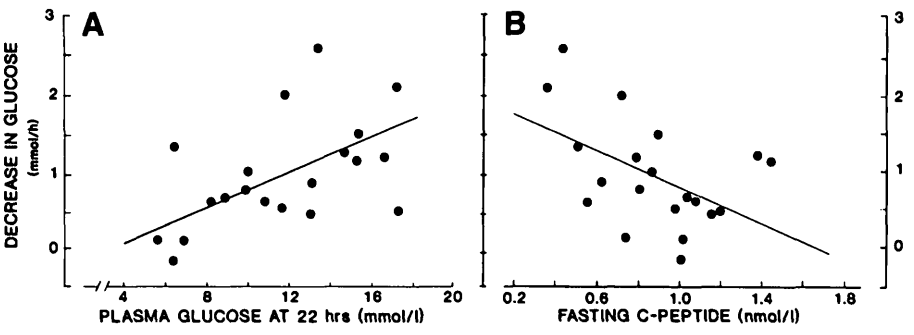

Figure 4—*The plasma glucose concentration at 22 h and the fasting C-peptide concentration plotted against the decrease in plasma glucose during the nocturnal insulin infusion (linear part between 22 and 2 h in Fig. 3). The simple correlation coefficients were 0.51 ($P < 0.05$) for the regression in A and −0.46 ($P < 0.05$) for B.*

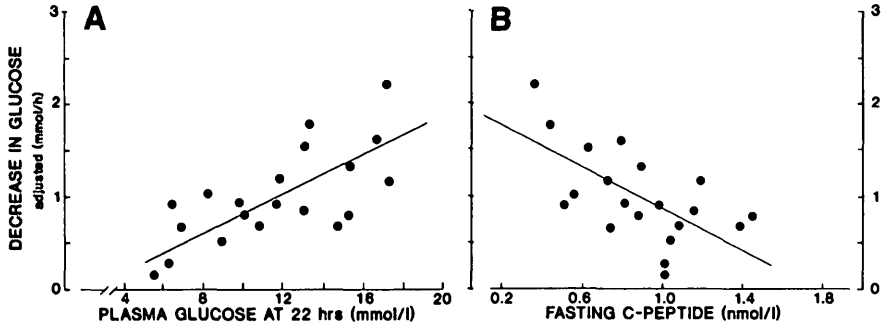

Figure 5—*The plasma glucose concentration at 22 h and the fasting C-peptide concentration plotted against the decrease in plasma glucose during the nocturnal insulin infusion (linear part between 22 and 2 h in Fig. 3). A: the decrease in plasma glucose has been related to the initial plasma glucose after adjustment for the individual C-peptide and the serum insulin concentrations. B: the decrease in plasma glucose has been plotted against the fasting C-peptide concentration after the decrease in plasma glucose has been adjusted for initial plasma glucose at 22 h and the insulin concentration. The multiple linear regression equation: decrease in plasma glucose (mmol/h) = 3.52 + a * [plasma glucose at 22 h] − b * [fasting C-peptide] − c * [serum free insulin between 22 and 2 h]. R^2 adjusted for the degrees of freedom = 48%, P < 0.004; a = 0.43 ± 0.12, P = 0.0027; b = 4.52 ± 1.9, P = 0.03; c = 0.019, NS.*

of each other and after adjusting for differences in insulin concentrations.

Although intuitively one might predict that insulin therapy should mimic normal physiology, i.e., that more insulin should be given in the fed than in the fasted state, this may not be the case in the treatment of patients with NIDDM (2). First, insulin is needed only to correct excessive glucose production in a patient with NIDDM. The question, then, is whether the defects in glucose metabolism are greater during the day than during the night in these patients. When measured using double tracer and forearm catheterization techniques during an oral glucose tolerance test, both excessive basal hepatic glucose production and a defect in its suppression by oral glucose contribute to postprandial hyperglycemia (1). In absolute terms (total rate of glucose uptake), peripheral insulin resistance does not contribute to hyperglycemia, because the mass-action effect of glucose compensates for the defect in glucose utilization (1). In addition, the amount of glucose retained by the splanchnic bed is unaltered in patients with NIDDM (2). Thus, the question of when to give insulin depends on whether endogenous glucose production is most abnormal during the day or during the night. If there were no defect in suppression of endogenous glucose production during a meal, then one would need to administer insulin only between meals and during the night. If, on the other hand, there were a suppression defect, then insulin would be required only during meals. Because at least both defects exist in the average patient with NIDDM (2), insulin is required throughout a 24-h period to restore normoglycemia. The question then becomes whether insulin's hypoglycemic effect is similar during and between meals.

These data would indicate that nocturnal and daytime administration of insulin have, at least acutely, a comparable hypoglycemic effect. This was true even though the 24-h serum FFA concentration was significantly lower in the nocturnal than in the daytime study. These data are compatible with previous data demonstrating that acute alterations in FFA availability do not influence insulin suppression of hepatic glucose production in NIDDM (11). Also, even marked overnight suppression of FFA by a long-acting nicotinic acid analogue does not decrease overall hepatic glucose production, although gluconeogenesis decreases significantly (12). Thus, despite the close correlation between overnight FFA concentrations and hepatic glucose production rates in patients with NIDDM (13), nocturnal suppression of lipolysis does not seem to improve glycemic control in these patients.

This study does not allow identification of the mechanism by which insulin decreased the blood glucose concentration. During the night, we have previously demonstrated inhibition of gluconeogenesis and overall glucose production in a comparable group of NIDDM patients treated with evening insulin (14). Therefore, nocturnal insulinization probably lowered blood glucose via inhibition of hepatic glucose production. During the day, both inhibition of basal and enhanced suppression of hepatic glucose production during meals could have contributed to insulin's hypoglycemic effect (2).

These acute studies may not necessarily reflect the long-term effects of combination therapy with morning or bedtime NPH. For example, two recent multicenter studies found that, during prolonged treatment, combination therapy with evening insulin induced less weight gain than did combination therapy with morning insulin (4,5). In the Finnish study, in which plasma free insulin profiles were determined, the smaller weight gain was attributed to lower diurnal insulin concentrations during evening compared with morning insulin injections. In this study, the 24-h C-peptide concentrations were higher and the FFA concentrations lower during evening than during daytime insulin infusion. These differences could allow more efficient suppression of hepatic glucose production, provided they persist during prolonged therapy. This might then explain the equal hypoglycemic effectiveness of morning and evening insulin in the face of less hyperinsulinemia and consequently weight gain during evening insulin therapy.

Analysis of how fast the blood glucose concentration decreased in response to insulin was performed during the nocturnal insulin-infusion studies. A similar individual analysis was not possible during the daytime insulin infusion because of meals and the nonlinear decrease in blood glucose. We found that a high initial plasma glucose concentration predicted a good hypoglycemic effect. This finding is physiologically meaningful because glucose promotes, by glucose mass action, its own utilization (4). The other parameter that predicted a rapid decrease in blood glucose was a low C-peptide concentration. The effect of both factors was more evident after adjustment for the effect of the other (Fig. 5).

A low C-peptide concentration could be a marker of either insulin deficiency or good insulin sensitivity. Although even a normal C-peptide concentration in a hyperglycemic patient always is a signal of at least relative insulin deficiency, markedly insulin-deficient patients resembling those with insulin-dependent diabetes mellitus were not included in the study. The lowest individual C-peptide concentration was 0.4 nM, which is within the reference range for normal subjects (0.3–0.7 nM). Thus, the patients did not suffer from absolute insulin deficiency. If the C-peptide concentration reflected insulin deficiency, one would have predicted a poor rather than a good hypoglycemic response. Therefore, the serum C-peptide concentration more likely reflected insulin resistance being high in insulin-resistant individuals.

Taken together, these data indicate that, when the hypoglycemic effect of insulin is considered, insulin can be administered equally well during the night and during the day. Although better suppression of FFA concentration is achieved, at least acutely, with nocturnal insulin, this effect does not increase the hypoglycemic potency of this treatment. In an individual patient, a low fasting C-peptide concentration and a high initial blood glucose concentration predict a good glycemic response to intravenous insulin. These data can be interpreted to indicate that the mass-action effect of glucose compensates for insulin resistance in insulin-resistant individuals with poorly controlled NIDDM.

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