Relative Roles of Insulin Clearance and Insulin Sensitivity in the Prebreakfast Increase in Insulin Requirements in Insulin-dependent Diabetic Patients

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SUMMARY

During continuous subcutaneous or intravenous insulin infusion therapy, many patients with insulin-dependent diabetes (IDD) require more insulin in the prebreakfast period (0600–0800 h) than earlier in the morning (0100–0300 h). This study was designed to assess whether variations in insulin clearance or insulin sensitivity might contribute to overnight variations in insulin requirements. Euglycemic insulin clamp studies were performed in random sequence from 2400 to 0300 h and from 0500 to 0800 h on successive nights in 10 subjects with IDD. Insulin was infused at a rate of 40 mU/min/m² and plasma glucose concentration was maintained at 100 mg/dl by a variable rate glucose infusion from a Biostator GCIIS (Miles Laboratories, Elkhart, Indiana). Insulin clearance was (mean ± SEM) 277 ± 41 ml/min/m² between 0700 and 0800 h compared with 256 ± 41 ml/min/m² between 0200 and 0300 h ($P < 0.05$), while glucose infusion rates were the same [3.86 ± 0.52 mg/kg/min from 0730 to 0800 h versus 3.99 ± 0.51 mg/kg/min from 0230 to 0300 h (P = NS)]. All eight patients with a previously documented prebreakfast increase in insulin requirements had higher insulin clearance at this time. These results indicate that differences in insulin clearance between the prebreakfast period and the early morning may account partially for the higher prebreakfast insulin requirements in some subjects with IDD, and the variations in insulin requirements during the night are not due to variations in insulin sensitivity. DIABETES 33:60–63, January 1984.

In some patients with insulin-dependent diabetes mellitus (IDD), basal insulin requirements are substantially higher in the prebreakfast period (0600–0800 h) than during the early morning (0100–0300 h).[1–3] Although this change is temporally related to increases in plasma cortisol that occur from 0600 to 0800 h,[4,5] recent studies have suggested that this phenomenon is not dependent on the normal diurnal variation in ACTH or cortisol secretion.[6,7] During conventional insulin therapy with one or two daily subcutaneous injections of insulin, the prebreakfast increase in plasma glucose concentration has been associated with decreasing plasma free insulin concentrations,[4] suggesting that this 0600–0800-h increase in plasma glucose concentrations might result from waning effects of previous subcutaneous insulin injections. However, a similar increase in prebreakfast blood glucose concentrations and insulin requirements during continuous subcutaneous insulin infusion therapy has also been described.[8]

We have observed previously that plasma free insulin concentrations fail to increase despite the higher insulin infusion rates needed to maintain euglycemia between 0600 and 0900 h during continuous intravenous "closed-loop" insulin infusions in subjects with IDD.[7] This suggested to us that changes in insulin clearance or degradation might contribute to higher prebreakfast insulin requirements. In order to determine a possible role of changes in insulin clearance or insulin sensitivity in the variability of overnight basal insulin requirements, we used the euglycemic insulin clamp technique to compare insulin clearance and sensitivity between 0100–0300 h and 0600–0800 h in 10 subjects with IDD.

METHODS

Ten normal-weight, C-peptide–negative subjects with IDD, between 20 and 40 yr old (mean age: 30 ± 2 yr), with a mean duration of diabetes of 15 ± 2 yr were studied. Values for glycosylated hemoglobin varied from 8.3 to 13.4% (mean: 9.6 ± 0.7%) in an assay where normal values do not exceed 8.4%. All subjects had normal renal and hepatic function. Studies were performed at the Clinical Research Center of Washington University and informed consent was obtained before participation in the study.

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The subjects were withdrawn from intermediate-acting insulin at least 30 h before, and subcutaneous short-acting insulin at least 12 h before the study period. Blood glucose was controlled using a modified closed-loop intravenous insulin delivery system before the study periods.[9] Since we wished to evaluate insulin clearance and sensitivity relative to time of day, not duration of fast, the subjects ate their last meal 12 h before the euglycemic insulin clamp studies, which were performed from 2400 to 0300 h and 0500 to 0800 h on consecutive nights in random sequence.

A primed[10] continuous infusion (40 mU/m²/min) of regular porcine insulin (Eli Lilly Co., Indianapolis, Indiana) was administered by Harvard pump (Harvard Apparatus Co., Inc., South Natick, Massachusetts), and a Biostator GCIIS (Miles Laboratories) was used in mode 7:1 to deliver the variable dextrose infusion rate necessary to maintain plasma glucose concentrations at 100 mg/dl. Samples for plasma glucose, free insulin, and C-peptide concentrations were collected from a catheter placed in a hand vein contralateral to the infusion catheter.

Plasma glucose concentrations were measured at 10–15-min intervals at the bedside by a glucose-oxidase method (Beckman Glucose Analyzer, Beckman Instruments, Fullerton, California) to ensure the accuracy of the Biostator glucose measurements.

Samples for plasma free insulin and C-peptide levels were drawn every 30 min. Plasma free insulin was measured by radioimmunoassay after a 2-h incubation in a metabolic shaker at 37°C and polyethylene glycol precipitation, as described by Kuzuya et al.[11] All samples from this study were measured in the same assay with an intraassay coefficient of variation of 7%. Plasma C-peptide was also measured by radioimmunoassay[12] and glycosylated hemoglobin was determined using a minicolumn (Isolab Inc., Akron, Ohio).

Steady-state mean plasma free insulin (FI) concentrations were determined over the final 60 min of each study period. The glucose infusion rate (M) was determined over the final 30 min of each study. Insulin sensitivity was defined by the M/FI ratio (mg/kg/min ÷ μU/ml × 100) as an assessment of the effectiveness of insulin on tissue glucose uptake.[13] Insulin clearance was calculated by dividing the insulin infusion rate by the mean free insulin concentration during the final 60 min of each study period.[13] Since C-peptide concentration was less than 0.03 pmol/ml at all times during the study period, endogenous insulin production was assumed to be negligible in the calculation of insulin clearance.

All data are presented as mean ± SEM. All statistical comparisons are between the different time periods in a given patient, and were done using the paired *t* test. Coefficients of variation were determined using standard analytic procedures.[14]

RESULTS

During the euglycemic insulin clamp studies, the plasma glucose concentration was maintained at 99 ± 2 mg/dl from 0230 to 0300 h, and at 101 ± 2 mg/dl from 0730 to 0800 h (P = NS). The coefficient of variation for the plasma glucose concentration was 3.0 ± 1.4 from 0230 to 0300 h and 1.8 ± 0.4% from 0730 to 0800 h. The mean plasma free insulin concentrations were 210 ± 32 μU/ml from 0200 to 0300 h and 187 ± 26 μU/ml from 0700 to 0800 h (Table 1). These were significantly different (P < 0.01) despite identical insulin infusion rates. Insulin clearance between 0700 and 0800 h was 277 ± 41 ml/min/m² compared with 256 ± 41 ml/min/m² between 0200 and 0300 h (P < 0.05). Nine of the ten subjects had higher (3.3–21.5%) insulin clearance between 0600 and 0800 h than between 0100 and 0300 h. The only subject without an increase (no. 8) had stable overnight basal insulin requirements during continuous subcutaneous insulin infusion therapy. The mean glucose infusion rate (M) required to maintain the plasma glucose concentration at 100 mg/dl was similar during the two time periods (3.99 ± 0.52 mg/kg/min from 0230 to 0300 h versus 3.86 ± 0.51 mg/kg/min from 0730 to 0800 h; P = NS), as was insulin sensitivity determined by the M/FI ratio (2.62 ± 0.67 from 0230 to 0300 h versus 2.71 ± 0.64 from 0730 to 0800 h; P = NS). Thus, insulin clearance was an average of 8% higher during the prebreakfast period than during the early morning, but insulin sensitivity was unchanged.

DISCUSSION

Based on previous studies, the prebreakfast increase in insulin requirements observed in many subjects with IDD cannot be explained solely by the physiologic diurnal variability

TABLE 1
Comparison of results from euglycemic clamp studies between 0100–0300 h and 0600–0800 h in 10 IDDs

	Glucose infusion rate (M) (mg/kg/min)		Plasma free insulin (FI) (μU/ml)		Tissue sensitivity to insulin (M/FI ratio)†		Insulin clearance (ml/min/m²)	
Patient no.	0100–0300 h	0600–0800 h	0100–0300 h	0600–0800 h	0100–0300 h	0600–0800 h	0100–0300 h	0600–0800 h
1	4.14	3.16	172	154	2.41	2.05	233	260
2	2.65	2.53	191	174	1.38	1.45	209	230
3*	6.87	6.72	212	176	3.24	3.82	377	455
4	2.61	2.35	428	354	0.61	0.66	93	113
5	4.36	4.56	111	99	3.92	4.61	360	404
6	4.43	4.55	132	128	3.35	3.55	303	313
7	2.71	3.12	286	254	0.95	1.22	140	157
8	6.01	5.90	79	85	7.61	6.94	506	471
9	4.57	4.02	213	185	2.14	2.17	188	216
10	1.62	1.71	272	260	0.60	0.66	147	154
Mean ± SEM	3.99 ± 0.52	3.86 ± 0.51 NS	210 ± 32	187 ± 26 P < 0.01	2.62 ± 0.67	2.71 ± 0.64 NS	256 ± 41	277 ± 41 P < 0.05

*Patient no. 3 inadvertently received insulin at 80 mU/m²/min.
†mg/kg/min ÷ μU/ml × 100.

of ACTH and cortisol.[6,7] Similarly, physiologic early morning spikes in growth hormone secretion are seemingly not essential determinants of this pattern.[15] Since peripheral plasma glucagon levels actually decrease slightly overnight,[6,7,16] it seems unlikely that portal glucagon levels are increasing sufficiently to account for the increasing insulin requirements between 0600 and 0800 h. The increases in epinephrine concentrations during the 0600–0800-h period[7] are small and probably insufficient to produce a hyperglycemic effect,[17] while increments in norepinephrine concentrations described with awakening in these patients are similar to normal subjects who do not manifest this overnight variability in basal insulin requirements.[7,18,19] Thus, it appears that the normal physiologic variability of circulating levels of known glucose counterregulatory hormones does not readily explain the mechanism for the so-called "dawn phenomenon."[3]

The original observation of Schmidt et al. that the prebreakfast increase in plasma glucose concentrations in IDDs could be attributed to a decrease in plasma free insulin concentrations might have been secondary to a waning insulin effect of the most recent subcutaneous insulin injection.[4] However, this pattern of increasing insulin requirements between 0600 and 0800 h has also been observed in IDDs treated with continuous "open-loop" or "closed-loop" insulin infusions,[2,7,8] and we recently found no increase in free insulin concentrations between 0600 and 0900 h in five IDDs, despite a twofold increase in mean intravenous insulin infusion rates at this time of day.[7]

The present study was designed to assess the role of insulin clearance as well as tissue sensitivity to insulin in the overnight variability in insulin requirements in IDD. The results indicate that insulin clearance is significantly higher between 0600 and 0800 h than in the early morning between 0100 and 0300 h. Without studies of insulin clearance at other times of the day it is impossible to determine whether this change is the result of an increased clearance of free insulin during the prebreakfast period, or a reduction of clearance during the early morning. We favor the former interpretation since basal insulin infusion rates needed to maintain euglycemia seem to be similar throughout the day except for an apparent increase between 0600 and 0800 h. Although the observed change in insulin clearance is small, all eight of the subjects with a known prebreakfast increase in basal insulin requirements had higher clearance in the prebreakfast period. The patient (no. 8) with similar insulin clearance in the early morning and the prebreakfast period clinically did not have the "dawn phenomenon."

The absolute values for insulin clearance in our study appear to be lower than previously described values for patients with IDD.[13] This may reflect higher free insulin concentrations commonly observed after a 2-h incubation period in the assay used in these determinations.[20] However, since all free insulin concentrations were determined in the same assay (intraassay coefficient of variation of 7%), values obtained in a given individual at different times of the night should be a reliable indicator of changes in free insulin clearance, even if the absolute values for free insulin are higher as a result of the assay technique.

With the euglycemic insulin clamp technique used in these studies, we also found that changing tissue sensitivity to insulin does not account for the variable overnight basal insulin requirements in these patients. The glucose infusion rate (M) and insulin sensitivity (M/FI ratio) were the same during both study periods. Although the absolute values for M are decreased compared with previously described values in normal control subjects, they are consistent with determinations in other patients with IDD, confirming the previous observation that some degree of insulin resistance is common in these patients.[20] Although insulin receptor studies were not performed as part of this study, substantial overnight variability in insulin receptor binding in subjects with IDD has not been observed.[21] It thus appears that the prebreakfast change in insulin requirements does not reflect an overnight variability in insulin sensitivity as determined by the euglycemic clamp techniques.

The conditions of the euglycemic clamp study result in hepatic and peripheral overinsulinization compared with the usual condition of mild to moderate hepatic underinsulinization in most subjects with IDD during overnight basal conditions. The relatively small differences that we have observed in peripheral free insulin concentrations and insulin clearance might be sufficient to contribute substantially to decreased portal insulin concentrations in the prebreakfast period under these more usual conditions. Also, to the extent that the previously described hyperglycemic responses of subjects with IDD to glucose counterregulatory factors[22] can be attributed to hepatic underinsulinization, variable insulin clearance may contribute to the overnight variability in insulin requirements in these patients.

The changes in insulin clearance observed during this study were not the result of differences in the duration of fasting, since clamps were performed on both study nights between 12 and 15 h after the last meal. Although our study was designed to eliminate length of fasting as a variable, in previous studies of the "dawn phenomenon," the duration of fasting was greater during the prebreakfast period than during the early morning period.[2,6,7] Since fasting may result in an increased removal of insulin by the liver,[23,24] an increased duration of fasting during the prebreakfast period may create greater increments in the differences in insulin clearance than those observed during the present study. Additional studies are needed to further address this possibility.

Although the increase in insulin clearance rates observed in this study is small, four points should be noted. First, 7 of the 10 patients had a greater than 10% (range: 10–22%) higher clearance during the prebreakfast period than during the early morning. Second, during this study insulin clearance was determined during a state of hepatic and peripheral overinsulinization. Receptor occupancy under these conditions is near maximal; the observed differences in clearance may be more pronounced at more physiologic insulin concentrations with lower receptor occupancy. Third, although small fluctuations in circulating insulin concentrations result in little change in glucose metabolism at the insulin levels achieved during this study, such small fluctuations in insulin concentration within a physiologic range may have marked effects on glucose production and utilization rates. Fourth, the changes in insulin clearance observed in

this study may be only one of many factors contributing to the apparent increase in insulin requirements observed in some patients with IDD.

In summary, this study demonstrates that insulin clearance is higher in the prebreakfast period than in the early morning in some patients with IDD. This occurs without any demonstrable change in insulin sensitivity. Variable overnight insulin clearance may reflect variations in hepatic or renal blood flow resulting in altered insulin degradation or variations in insulin binding to antibodies. This variability in clearance may contribute to the so-called "dawn phenomenon" of increasing prebreakfast blood glucose concentrations in IDDs, and variable basal insulin infusion rates should be considered when treating these patients with continuous subcutaneous or intravenous insulin infusion therapy.

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