Insulin Clearance Contributes to the Variability of Nocturnal Insulin Requirement in Insulin-dependent Diabetes Mellitus

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SUMMARY

We have previously described, in insulin-dependent diabetic subjects (IDDM), a small, but significant, increase in the insulin clearance rate (ICR) during 0600–0800 h as compared with 0100-0300 h. To determine whether this increase was also seen at more physiologic levels of insulin replacement, we calculated ICR during euglycemic clamp studies in 13 patients with IDDM with a constant infusion of insulin at 20 mU/min/m² and during insulin replacement from the Biostator GCIIS without exogenous glucose.

During the euglycemic clamp study with constant insulin infusion at 20 mU/min/m², the ICR was 16% higher at 0600–0800 h than at 0100–0300 h (264 ± 50 ml/min/m² versus 228 ± 51 ml/min/m²; $P < 0.005$). During insulin replacement by the Biostator, the mean insulin infusion rate increased by 92 ± 27% (7.5 ± 1.1 to 13.5 ± 1.2 mU/min/m²; $P < 0.001$) and ICR increased by 123 ± 30% (130 ± 24 to 268 ± 51 ml/min/m²; $P < 0.01$) during the prebreakfast period when compared with 0100–0300 h. There was a highly significant correlation ($r = 0.97$) between the increment in insulin infusion rate and the increment in ICR.

Measurement of insulin concentration in saline solutions, delivered by the Biostator at a same rate and under similar conditions to those in this study, showed that insulin delivery was stable for the 8-h period of this study.

We conclude that, at levels of insulin replacement that maintain blood glucose between 90 and 100 mg/dl, variation in the ICR is an important contributor to the "dawn phenomenon" of increasing early morning blood glucose concentration and insulin requirements in diabetic subjects. DIABETES 1985; 34:1260–65.

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Patients with diabetes mellitus commonly have rising prebreakfast blood glucose concentrations without antecedent nocturnal hypoglycemia.[1–3] This has been called the "dawn phenomenon,"[4] and has been shown to occur in patients with insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM).[5,6] The phenomenon persists during continuous subcutaneous insulin infusion (CSII) during the night[7] and when endogenous ACTH and cortisol production are suppressed using constant infusions of glucocorticoids,[8] or when endogenous cortisol production is blunted with metyrapone.[9] The variability in nocturnal insulin replacement rates needed to maintain euglycemia during the night has obvious clinical implications, especially when attempts are made to achieve normal blood glucose concentrations overnight using various intensive methods of insulin therapy such as CSII.[2,3,7]

The mechanism of the dawn phenomenon is not known. In previous studies, we infused insulin at a rate of 40 mU/min/m² and found a small (8%) but consistent increase in calculated insulin clearance rates (ICR) during the prebreakfast period (0600–0800 h) in comparison with that seen earlier in the early morning (0100–0300 h).[10] However, since the previous study was conducted at a supraphysiologic rate of insulin infusion, our results might not be applicable at lower, more physiologic, rates of insulin delivery. We wanted to test the hypothesis that at lower rates of insulin replacement, a more substantial increase in insulin clearance would be seen that might provide an explanation for the dawn phenomenon.

In a preliminary report,[11] it was suggested that a continuous and significant loss of insulin activity occurred during prolonged use of the Biostator. This insulin loss presumably occurred in the tubing system, since the insulin concentration within the insulin reservoir was stable. The insulin concentration was reduced by 15% after the first 8 h and by 31% after 24 h. Since calculations of insulin requirements, insulin clearance, and insulin sensitivity depend on accurate infor-

mation about actual insulin delivery rates, instability or loss of activity of insulin during an infusion could result in a substantial error in these calculations and the conclusions derived from them. Therefore, we have also measured recovery of insulin from saline solutions prepared for use in the Biostator GCIIS or Harvard infusion pump under conditions identical to those used in this and our previous studies.[1,8,10,12]

MATERIALS AND METHODS

We studied 13 patients with IDDM between 21 and 39 yr of age. Informed consent was obtained from all subjects according to a protocol approved by the Washington University Human Studies Committee. The mean duration of diabetes was 15 ± 5 yr. All had normal weight and were C-peptide negative (C-peptide < 0.03 pmol/ml) before breakfast. Renal and liver function tests were normal. Before study, the patients were withdrawn from intermediate-acting insulin for at least 36 h and from short-acting subcutaneous (s.c.) insulin injections for at least 12 h. Their last meal was 6 h before starting the study. For at least 8 h, plasma glucose was maintained between 80 and 110 mg/dl by a modified, closed-loop i.v. insulin delivery system.[13] Insulin infusion rates were modified hourly based on blood sugar measurements and for the last 5 h before the study, infusion rates were (mean ± SD) 3.4 ± 0.5, 1.6 ± 1.3, 1.2 ± 1.1, and 0.9 ± 0.8 U/h. Pyrogen-free 3-^{3}H-glucose (New England Nuclear, Boston, Massachusetts) was diluted to the appropriate concentration in sterile 0.9% saline and passed through a sterile Millipore filter before initiating a primed (11 μCi), continuous (0.11 μCi/min) infusion at 2300 h. Subjects were connected to a Biostator GCIIS (Miles Laboratories, Elkhart, Indiana) for the 9-h study period.[14] The first hour served for calibration and equilibration of the Biostator.

In patients 1–5 (group 1), insulin was infused between 2400 and 0300 h at a fixed i.v. rate of 20 mU/min/m^2 using a continuous infusion pump (Harvard Apparatus Co., Millis, Massachusetts). During this infusion, the Biostator delivered dextrose at a varying rate to maintain blood glucose at 100 mg/dl, according to the following algorithm (mode 9:1): DR = Wt([BC − GY]/[3 + M]) + RC, where: DR = dextrose infusion rate (mg/min), Wt = body weight (kg), BC = desired blood glucose level, GY = current blood glucose concentration determined by the Biostator each minute, M = slope of blood glucose change over the preceding 5 min, and RC = glucose utilization (mg/kg·min). This algorithm is designed to perform euglycemic clamp studies with minimal variation in minute-to-minute dextrose infusion rates.

Between 0300 h and 0500 h, the fixed-rate insulin infusion was interrupted and the Biostator was reprogrammed to provide insulin at a variable infusion rate to maintain euglycemia without exogenous dextrose. At 0500 h, the CSII and euglycemic clamp were resumed and continued until 0800 h. In this way, similar conditions were created during the two study periods (2400–0300 h and 0500–0800 h).

In patients 6–13 (group 2), insulin was provided by the Biostator at a variable infusion rate using an algorithm designed to vary insulin delivery rate each minute as needed to maintain the desired blood glucose of 100 mg/dl (mode 1:1): IR = RI([Gy − BI]/QI + 1)^2, where: IR = insulin infusion rate (mU/min), BI = desired blood glucose concentration (mg/dl), GY = current blood glucose concentration determined by the Biostator each minute, QI = constant called static gain, and RI = desired insulin infusion rate (mU/min) when blood glucose is equal to BI.

Subjects in both groups were asleep or remained resting comfortably in bed throughout the study period. Blood was obtained at 30-min intervals for measurement of plasma-free insulin concentration and isotope enrichment.

To assess the stability of insulin delivery from saline solutions, we performed studies according to two protocols. In the first, four 50-cc Plastipak disposable syringes (Becton-Dickinson, Rutherford, New Jersey) were filled with 50 cc of 0.9% saline. To each of two syringes, 14 U of regular porcine insulin (Iletin II, Eli Lilly and Company, Indianapolis, Indiana) was added (expected concentration 280 mU/ml) while to the other two, 38 U was added to each (expected concentration 760 mU/ml). These solutions were gently agitated for 1 min. A 30-in. extension tube with a capacity of 3.5 cc (Seamless Hospital Products Co., Wallingford, Connecticut) was attached to each syringe and 10 cc of solution was discarded through the tubing. The syringes were fixed into a Harvard infusion pump that was operated to deliver 0.078 ml/min (about 1.3 and 3.6 U/h, respectively). Samples for measurement of insulin concentration were obtained from the end of the extension tubing at 60-min intervals for 8 h.

In the second protocol, two 500-cc plastic bags (Viaflex Travenol) of 0.9% saline were used. Seventy-two units of regular porcine insulin (Iletin II) was added into each bag (expected concentration 144 mU/ml). The bags were gently agitated for 1 min. To each bag, a Travenol solution administration set (approx. 2.1 m), a Biostator infusion pump tubing (Luer LK-9033B, 0.051 in. Gray), and a Biostator Luer manifold with cap (Life Science Instruments, Miles Laboratories) were connected in sequence, as is done in our studies during operation of the Biostator GCIIS (Miles Laboratories). One hundred milliliters of the solution was discarded through the tubing before operation. The Biostator's pump was operated to deliver 15 mU/min at a flow rate of 6.25 ml/h. Samples for insulin assay were obtained at the patient connection site at 60-min intervals for 8 h.

The concentration of free insulin in plasma was determined in quadruplicate by polyethylene glycol precipitation followed by radioimmunoassay as previously described.[15] Before assay of the insulin-saline solution samples, they were diluted 3000-fold in a buffer of saline phosphate (pH 7.4) containing 1% bovine serum albumin to bring them into the optimal range for our radioimmunoassay. All insulin determinations from each study protocol were run in a single assay with an intraassay coefficient of variation of 9.7%. C-peptide was measured by radioimmunoassay.[16] Since C-peptide was undetectable in these subjects, for purposes of calculation of insulin clearance, endogenous insulin production was assumed to be negligible. Insulin clearance was calculated by dividing the insulin infusion rate by the plasma free insulin concentration during each 30-min period.[17] Endogenous glucose production and utilization rates were calculated from the isotope dilution values using Steele's equations.[18] For patients in group 2, the mean insulin infusion rate for each 30-min interval is calculated as the mean of minute-to-minute values. This mean value was considered to be representative

TABLE 1
The measurement of insulin concentration from saline solutions

	Protocol A				Protocol B	
		Insulin concentration (mU/ml)			Insulin concentration (mU/ml)	
Time	280	280	760	760	144	144
1 + 2 h	198	249	679	702	98	128
3 + 4 h	233	294	693	656	79	97
5 + 6 h	226	206	730	759	92	103
7 + 8 h	261	318	694	698	92	131
Mean ± SEM	236 ± 13	261 ± 18	681 ± 31	729 ± 47	95 ± 6	126 ± 11

Two-hourly mean insulin concentrations, during Harvard pump (protocol A) and Biostator (protocol B) infusions, in three different saline-insulin solutions. The mean ± SEM of the eight hourly measurements are given for each experiment.

of insulin requirements at that time. Insulin requirements, plasma free insulin concentrations, and insulin clearance rates were compared for each patient during the two study periods using data from the last 120 min in each period. The paired Student *t*-test was employed for the comparison of the data from the two periods. Data are reported as mean ± SEM.

RESULTS

The measurement of insulin concentration from saline solutions shows that insulin concentrations are stable for 8 h during either Harvard pump or Biostator operation (Table 1). Comparing the mean values for each 2-h period (employing Student's *t*-test), no significant differences were found between any two consecutive measurements at any of the insulin concentrations. Insulin delivered during the first 2 h was similar to that during the last 2 h. The mean coefficient of variation for our insulin concentrations in these experiments was 18%, and was similar in both protocols (17.3% and 18.5%). These data indicate that under the conditions of Biostator GCIIS and Harvard infusion pump use employed in these studies, insulin delivery rate was relatively stable throughout an entire 8-h study period. There was no significant change in insulin delivery during the time of these studies.

In group 1 (Figure 1), the mean blood glucose concentration was 94 ± 4 mg/dl between 0100 and 0300 h and 97 ± 2 mg/dl between 0600 and 0800 h. These values were not significantly different. Mean plasma free insulin concentration was 121 ± 90 μU/ml at 0100–0300 h and 105 ± 33 μU/ml at 0600–0800 h. This difference was also not statistically significant. However, insulin clearance rate was 16% higher from 0600 to 0800 h (264 ± 58) than from 0100 to 0300 h (228 ± 51 ml/min/m²; $P < 0.05$).

Endogenous glucose production was suppressed to <0.5 mg/min/kg during this supraphysiologic rate of insulin delivery. Thus, the exogenous dextrose administered was equivalent to the glucose metabolized. The dextrose required to maintain euglycemia was 3.53 ± 0.6 mg/min/kg at 0100–0300 h, and 3.66 ± 0.8 mg/min/kg at 0600–0800 h. These values were not statistically different.

In group 2 (Figure 2), the mean blood glucose concentration was slightly higher (115 ± 6 mg/dl) at 0600–0800 h than at 0100–0300 h (101 ± 2 mg/dl). This difference was significant ($P < 0.01$) and occurred despite a higher mean insulin infusion rate (13.5 ± 1.2 mU/min/m²) between 0600 and 0800 h than between 0100 and 0300 h (7.5 ± 1.1 mU/min/m², $P < 0.001$). Plasma free insulin concentration was similar (73 ± 20 μU/ml versus 70 ± 19 μU/ml) during the two time periods. Insulin clearance, as estimated from the

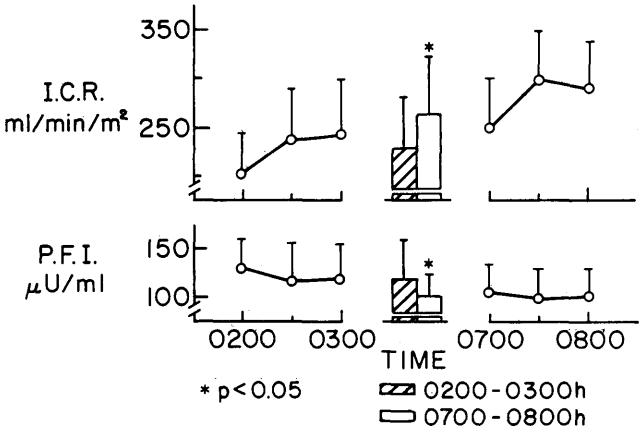

FIGURE 1. Insulin clearance rate (ICR) and plasma free insulin (PFI), during euglycemic clamp at a fixed-rate insulin infusion of 20 mU/min/m², in five IDDM subjects (group 1).

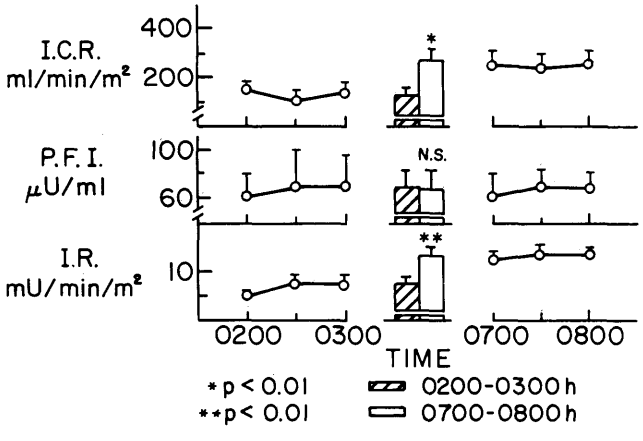

FIGURE 2. Insulin clearance rate (ICR), plasma free insulin (PFI), and insulin infusion rate (IR) during variable rate, "physiologic," insulin replacement using the Biostator in eight IDDM subjects (group 2).

plasma free insulin concentration and the mean insulin infusion rate, was 130 ± 24 ml/min/m² at 0100–0300 h compared with 268 ± 51 ml/min/m² at 0600–0800 h ($P < 0.01$).

The observed increase in insulin requirements at 0600–0800 h compared with 0100–0300 h (ΔIR) varied substantially among the subjects studied with values ranging from 13% to 260% with a mean of 92 ± 27%. The morning increase in insulin clearance rate (ΔICR) varied from 31% to 303% with a mean of 123 ± 30%. Comparing the ΔIR to the ΔICR values by regression analysis, a linear relationship was found with an r-value of 0.972 (Figure 3). Thus, as much as 95% of the variance of ΔIR can be attributed to ΔICR.

DISCUSSION

Although the dawn phenomenon has been demonstrated in both type I and type II diabetic subjects,[5] its pathogenesis is incompletely understood. Nocturnal variations in circulating levels of some glucose counterregulatory hormones have been investigated as a possible cause of the dawn phenomenon. The prebreakfast rise of plasma cortisol or ACTH cannot fully explain the dawn phenomenon.[8,9] Similarly, although the early nocturnal increases of growth hormone secretion may influence the early morning (0100–0300 h) nadir of insulin requirements, it appears to have no effect on the prebreakfast rise of insulin requirements under conditions in which endogenous secretion of growth hormone and glucagon are suppressed (and glucagon is replaced) with somatostatin infusion.[12] Plasma glucagon concentrations do not vary significantly overnight, and therefore cannot explain the "dawn" phenomenon.[8] Nocturnal and prebreakfast variation in sympathetic nervous system activity, expressed as changes in circulating epinephrine levels, could contribute to the dawn phenomenon;[8] however, the magnitude of increases in plasma epinephrine are below the threshold for metabolic effects of this hormone.[19]

We have previously reported an 8% increase in insulin clearance rates during the prebreakfast period.[10] However, in that study an insulin infusion rate of 40 mU/min/m² resulted in a mean plasma free insulin of 210 ± 32 μU/ml. This is well above the levels of circulating plasma free insulin seen in nondiabetic subjects and most type I diabetic subjects during the night. Thus, our earlier studies of insulin clearance were conducted at higher plasma free insulin concentrations than those at which the dawn phenomenon is observed clinically. Since these supraphysiologic levels of plasma insulin might blunt changes in insulin clearance, and might generate data that are not relevant to more physiologic conditions, the present study was carried out to measure insulin clearance at lower, more physiologic insulin infusion rates. Administering insulin at 20 mU/min/m², which is only 30–100% above physiologic replacement, resulted in a doubling of the prebreakfast increment in insulin clearance rate (16%) in comparison with the 8% difference found when insulin was infused at 40 mU/min/m².

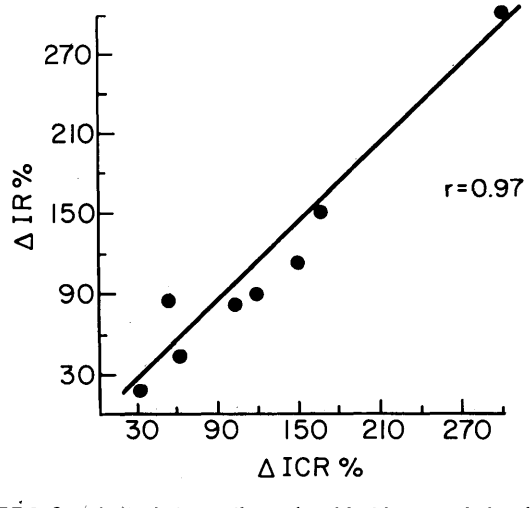

FIGURE 3. Correlation between the prebreakfast increase in insulin clearance rate (ΔICR) and insulin infusion rate (ΔIR) in eight IDDM subjects (group 2).

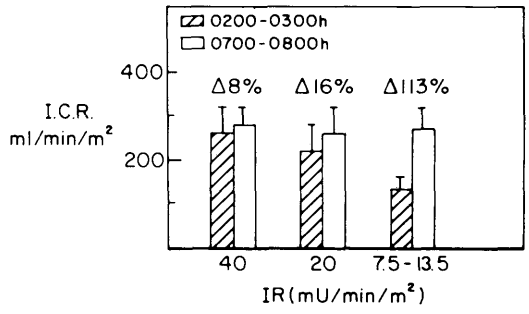

FIGURE 4. Comparison between insulin clearance rate (ICR) at 0200–0300 h and 0700–0800 h using different insulin infusion rates (IR). The infusion rates used were 40 mU/min/m² (from ref. 10), 20 mU/min/m² (group 1), and 7.5–13.5 mU/min/m² (group 2).

In patient group 2, when insulin was replaced at physiologic infusion rates, there was a 92 ± 27% higher insulin requirement and a 123 ± 30% higher insulin clearance rate between 0600 and 0800 h than between 0100 and 0300 h. This increase in insulin clearance rate suggests that under conditions of physiologic insulin replacement, insulin clearance is substantially higher during the prebreakfast period than earlier in the night. Because of the high linear correlation ($r = 0.97$) between ΔIR and ΔICR, it seems likely that this increase in morning insulin clearance plays an important role in the dawn phenomenon.

Despite comparable plasma free insulin levels during the two study periods in group 2, glucose concentrations increased only during the prebreakfast period. Thus, other factors, such as diurnal variations in adrenergic activity or other glucose counterregulatory factors, may also contribute to the dawn phenomenon.

In patient group 1, endogenous glucose production was suppressed to 0.2 ± 0.17 mg/kg/min by the infusion of insulin at 20 mU/min/m². This is similar to data reported by others.[20,21] Glucose disposal was similar during the two study periods, in agreement with our results using the higher insulin infusion rate.[10] In addition, glucose production and glucose utilization rates were similar between 0100 and 0300 h and 0600 and 0800 h during physiologic rates of insulin replacement using the Biostator (data not shown). Taken together, these data suggest that variation in tissue sensitivity to insulin does not play a significant role in the "dawn phenomenon."

Further investigation is needed to establish the mechanism of the apparent nocturnal variations in insulin clearance rates and its relationship to circulating free insulin concentrations.

Clearance of insulin by the kidney or liver could change during the night, as could binding of insulin to circulating antibodies. In addition, we have made no attempt in this study to investigate the role of supraphysiologic rates of insulin infusion on subsequent insulin clearance, or the effect of duration of fasting on insulin clearance. Although the results of studies designed to evaluate these factors would be of interest, our study is more clinically relevant to diabetic patients. During the average day, the increasing prebreakfast insulin needs are occurring after prolonged (8–10 h) constant insulin delivery and 3–4 additional hours since the last meal.

It is important to note that the plasma free insulin concentrations are somewhat higher and the insulin clearance rate somewhat lower than expected. This may reflect the higher free insulin concentrations commonly observed using the assay used in these determinations. However, this does not alter our conclusions of increasing clearance as an important contributor to the dawn phenomenon. We compare only changes in free insulin levels and clearance from one time period to another in the same patient, and these changes occur independently of the absolute value.

It is also important to comment on the recent suggestion of loss of insulin activity during prolonged use of the Biostator GCIIS.[11] We have investigated this possibility in our system. Our studies show that at insulin concentrations of 144–760 mU/ml, under conditions identical to those used during this and previous insulin infusion and Biostator studies,[1,8,10,12] insulin concentration and delivery rate do not change significantly during an 8-h study period. At an insulin delivery rate of 15 mU/min, which corresponds to a fluid flow rate of 6.25 ml/h at an insulin solution concentration of 144 mU/ml, we found no decrease in insulin delivered to the patient after 8 h of Biostator use. At a flow rate of 8.4 ml/h, a 30% loss of insulin from solution after 15 h of Biostator use has been found.[22] This loss has been found to decrease with increasing flow rates. In our studies, the flow rate during the prebreakfast period (which is after only 7–9 h of Biostator use) was 10.1 ± 1.2 ml/h (mean ± SEM). Even if such a loss were to occur, it would not explain the 92% increase in insulin infusion rate and the 123% increase in insulin clearance. In addition, in group 1, insulin was infused by a continuous infusion pump, not the Biostator. Thus, our previous[8,10,12] and current findings cannot be explained by loss of insulin from solution during prolonged Biostator use. In a recent study by Kerner et al.,[23] a similar morning increase in the insulin clearance rate was found employing either a syringe pump or the Biostator. This would also suggest that increased insulin clearance rates observed in IDDM subjects at dawn are not merely an artifact produced by the Biostator.

The lower 2400–0300 h insulin requirements and insulin clearance rates seen in patients treated with insulin during the night has obvious clinical implications. When insulin is given at a constant rate calculated to avoid hypoglycemia and activation of glucose counterregulatory mechanisms against overinsulinization, rising prebreakfast blood sugars are commonly seen. As one increases the rate of insulin replacement to meet the 0500–0800-h insulin needs, unacceptable glucose nadirs could be expected in those diabetic subjects with inadequate glucose counterregulation.

Last, it should be noted that the higher prebreakfast insulin clearance rate could reflect either an increased clearance at 0600–0800 h, or a reduced clearance between 0100 and 0300 h. The latter is suggested by the fact that the insulin clearance rate between 0600 and 0800 h is similar regardless of insulin infusion rate, whereas the clearance at 0100–0300 h decreases as insulin infusion rate decreases (Figure 4). Therefore, the increased nocturnal variation in clearance at physiologic insulin replacement results from a lower clearance at 0100–0300 h and not an increase at 0600–0800 h. However, our clinical experience, as well as the observations of other authors,[24] favors the hypothesis that the dawn phenomenon is due to a morning increase in insulin requirement rather than to a 0100–0300-h decrease. Further studies are needed to clarify this point.

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