Influence of Growth Hormone on Overnight Insulin Requirements in Insulin-dependent Diabetes

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SUMMARY

After a 0100–0300 h nadir, the insulin requirements to maintain blood glucose at 90–110 mg/dl increase substantially in the prebreakfast (0600–0800 h) period in some insulin-dependent diabetic patients (IDDMs). Early insulin-like and delayed insulin-antagonistic effects of physiologic early morning increases in growth hormone (hGH) secretion may account for this variability of overnight insulin requirements. To assess the role of hGH, we studied five IDDMs using a closed-loop insulin infusion device (Biostator, GCIIS). Either saline (C) or somatostatin plus glucagon (SRIF + G) was infused during separate overnight (2400–0800 h) study periods. An infusion of hGH from 2400 to 0130 h was added to SRIF + G infusion during an additional study period (SRIF + G + hGH). In comparison to 0100–0300 h, mean insulin infusion rates required to maintain blood glucose values between 105 and 120 mg/dl during the prebreakfast period increased by 66 ± 25% during C, and 42 ± 12% during SRIF + G when serum growth hormone was suppressed to ≤0.75 ng/ml. During SRIF + G + hGH, the mean prebreakfast insulin infusion rate increased by 42 ± 11% with a mean peak hGH level of 14.7 ± 5.4 ng/ml at 0130 h. Mean plasma free insulin levels remained constant during the night despite the significantly higher insulin infusion rates between 0600 and 0800 h.

During SRIF + G, insulin requirements remained constant overnight before 0600 h, whereas during both C and SRIF + G + hGH conditions, a nadir was noted between 0100 and 0300 h. Data pooled from 10 overnight, saline infusion studies suggest that the early morning nadir in insulin requirements occurs within 60 min of the peak hGH concentration. We conclude that the physiologic early morning increase in hGH is not an essential component of the prebreakfast increase in insulin requirements. Nocturnal growth hormone secretion may, however, contribute to the early morning nadir in insulin requirements seen in IDDM. DIABETES 1985; 34:135–39.

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In some insulin-dependent diabetic patients (IDDMs) treated with multiple daily injections or continuous insulin infusions, overnight insulin requirements increase substantially during the prebreakfast period between 0600 and 0800 h in comparison with insulin requirements needed to maintain euglycemia between 0100 and 0300 h.[1-7] Free insulin concentrations in this prebreakfast period have been shown to be decreased, or fail to increase appropriately in spite of higher insulin infusion rates.[8-10] In addition, the metabolic clearance rate of insulin has been shown to be increased significantly in these patients between 0600 and 0800 h compared with the earlier morning period.[11] Although variable insulin clearance, binding, or degradation may be a partial explanation, the fact that prebreakfast glucose increments occur despite comparable plasma insulin concentrations suggests that glucose counterregulatory factors may also contribute to the "dawn phenomenon."

Previous studies have demonstrated normal overnight patterns of the known glucose counterregulatory hormones, including cortisol, growth hormone, glucagon, epinephrine, and norepinephrine in these patients.[9,12] Although it has been shown that the pituitary-adrenocortical axis is not an essential component in the prebreakfast increase in insulin requirements,[9,13] the role of the other counterregulatory hormones has not been adequately studied. In normal subjects, an acute increase in growth hormone (hGH) concentration within the physiologic range is associated with an early, transient insulin-like effect, followed in 4–6 h by a hyperglycemic effect.[14] In addition, sustained increases in hGH concentration are associated with increased hyperglycemia in IDDMs treated with continuous insulin infusions.[15] Since IDDMs have a normal or exaggerated diurnal pattern of hGH secretion with spikes occurring during the early hours of sleep,[12] we

designed studies to test the hypothesis that hGH could contribute both to the 0100–0300 h nadir as well as the 0600–0800 h increment in insulin requirements.

MATERIALS AND METHODS

Five normal weight IDDMs with mean (± SEM) age of 27 ± 2 yr and mean duration of diabetes of 17 ± 2 yr were studied. All subjects had been treated with insulin since the time of diagnosis and gave a history of ketoacidosis. All subjects were being treated with continuous subcutaneous insulin infusion (CSII) using highly purified porcine insulin for at least 6 mo before the study. The mean glycosylated hemoglobin value for the group was 7.6 ± 0.4% in an assay where normal values range between 4.0% and 8.3%. This assay employs a minicolumn (Isolab Inc., Akron, Ohio) and saline-dialyzed samples. Despite their successful therapy using CSII, these subjects continued to clinically manifest a dawn phenomenon with increasing prebreakfast, basal insulin requirements.

CSII therapy was discontinued at least 6 h before, and the last mealtime bolus dose was delivered at least 12 h before the 2400–0800 h study period. Subjects ate their last meal at least 6 h before the study period, and blood glucose was controlled using a modified, closed-loop intravenous insulin (Iletin II; Eli Lilly and Company, Indianapolis, Indiana) delivery system[16] before being connected to the Biostator GCIIS (Miles Laboratories, Elkhart, Indiana) at 2300 h. The Biostator was operated using mode 1:1 with constants set to deliver insulin at 10–20 mU/min (RI) with the blood glucose at 110 mg/dl (BI) so that the insulin infusion rate (IR) increased twofold at a blood glucose concentration of 122 mg/dl (QI = 30 mg/dl) according to the following algorithm: $IR = RI([G - BI/QI] + 1)^2$. Dextrose was not given during these studies. The static 1:1 mode was used to reduce the minute-to-minute variability in insulin infusion rates resulting from small changes in the glucose concentrations.

Each subject was studied over a 2–3 mo period on three separate nights in random sequence. On one night, an infusion of saline accompanied Biostator control. On another night, the subjects received an infusion of somatostatin (Beckman Instruments, Inc., Bioproducts Division, Stanford, California) (250 μg/h) and glucagon (Eli Lilly and Company) (1 ng/kg/min in 2 subjects, 1.25 ng/kg/min in 3 subjects). On a third night, somatostatin and glucagon were infused as above, and human growth hormone (hGH) (National Pituitary Agency, lot P-20 or P-21) was infused at 100 ng/kg/min from 2400 to 0130 h to simulate the usual pattern of increased circulating growth hormone concentration during the early hours of sleep. A continuous infusion pump (Harvard Apparatus, Millis, Massachusetts) was used for all infusions. All studies were performed during hospitalization in the Clinical Research Center of Washington University after obtaining informed written consent.

Between 2400 and 0800 h, blood samples were collected every 30 min through an indwelling venous catheter for measurement of plasma glucose, free insulin, growth hormone, glucagon, and cortisol. Plasma glucose was measured by the glucose-oxidase method using a Beckman glucose analyzer (Beckman Instruments, Fullerton, California). Growth hormone,[17] glucagon,[18] (30K antibody) and cortisol[19] were measured by radioimmunoassay. Plasma free insulin concentration 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.[20] All samples from each patient were run in the same assay with an intraassay coefficient of variation of 7%.

The results of five additional overnight studies during saline infusion alone in three additional IDDMs (age 28 ± 3 yr, duration 14 ± 4 yr) using identical Biostator conditions were also included in the analysis of the temporal relationship between overnight peak hGH concentrations, blood glucose concentration, and insulin requirements.

The mean blood glucose concentrations and insulin infusion rates were calculated as the means of minute-to-minute blood glucose concentrations and insulin infusion rates for 30-min periods during Biostator control. The paired *t*-test was used to compare data in individual subjects from one time interval to another and between study nights. All results are expressed as mean ± SEM.

RESULTS

During the control (saline infusion) study, the insulin infusion rate increased from 15.2 ± 2.9 mU · kg^{-1} · h^{-1} between 0100 and 0300 h to 22.0 ± 2.8 mU · kg^{-1} · h^{-1} between 0600 and 0800 h ($P < 0.001$) in response to a slight increase in the glucose concentration from 109 ± 3 to 115 ± 3 mg/dl (P = NS) (Figure 1). All five subjects demonstrated a normal pattern of circulating hGH with spikes of 5–25 ng/ml during the night. The mean hGH concentration reached a peak of 9.8 ± 3.5 ng/ml at 0200 h (Figure 2). There was a nadir of the mean glucose concentration (104 ± 3 mg/dl) and insulin infusion rate (11.2 ± 3.0 mU · kg^{-1} · h^{-1}) 30 min after the

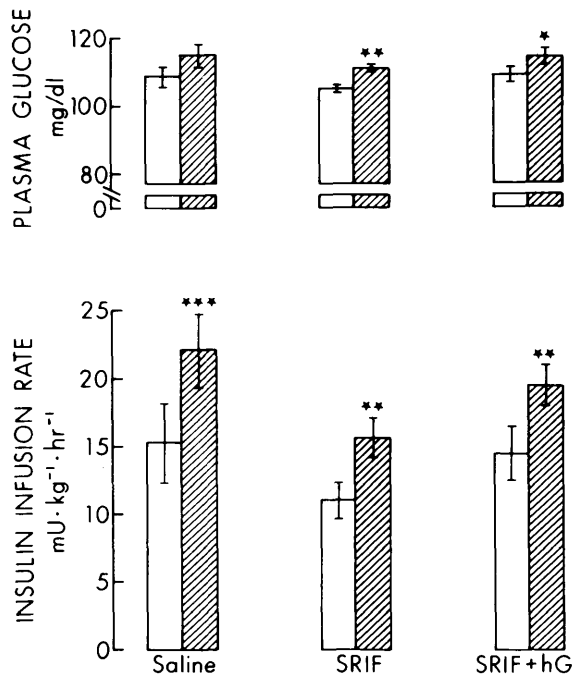

FIGURE 1. Blood glucose (upper panel) and insulin infusion rate (lower panel) during overnight Biostator control along with saline, suppression of endogenous growth hormone (SRIF), and SRIF with growth hormone replacement (SRIF + hGH) (see text for details). Open bars represent 0100–0300 h time period, hatched bars represent 0600–0800 h time period. Results are expressed as the mean ± SEM. *P < 0.05; **P < 0.01; and ***P < 0.001.

mean peak of hGH. This nadir represents a 26% fall in insulin infusion rate.

During inhibition of hGH secretion with somatostatin, the mean insulin infusion rate also increased significantly, from 11.0 ± 1.3 mU · kg^{-1} · h^{-1} between 0100 and 0300 h to 15.6 ± 1.5 mU · kg^{-1} · h^{-1} between 0600 and 0800 h ($P < 0.01$), in response to an increase in the glucose concentration from 105 ± 1 to 111 ± 1 mg/dl ($P < 0.005$) (Figure 1). Concentrations of hGH remained <0.75 ng/ml throughout the study period (Figure 2) and no decrease in the glucose concentration or insulin infusion rate was observed between 0100 and 0300 h.

During somatostatin infusion with hGH replacement between 2400 and 0130 h, the mean insulin infusion rate increased from 14.4 ± 1.9 mU · kg^{-1} · h^{-1} between 0100 and 0300 h to 19.4 ± 1.5 mU · kg^{-1} · h^{-1} between 0600 and 0800 h ($P < 0.01$) in response to an increase in the glucose concentration from 109 ± 2 to 114 ± 2 mg/dl ($P < 0.05$) (Figure 1). The mean hGH concentration initially rose to 10.2 ± 3.5 ng/ml at 0030 h, and reached a peak of 14.7 ± 5.4 ng/ml at 0130 h; this peak was similar to (P = NS) the peak hGH concentration during the control study (9.8 ± 3.5 ng/ml), indicating physiologic hGH replacement during this study period (Figure 2). A nadir of the glucose concentration (105 ± 3 mg/dl) and insulin infusion rate (11.8 ± 2.1 mU · kg^{-1} · h^{-1}) occurred at 0100 h, 30 min after the initial rise in circulating hGH concentration. This nadir represents an 18% fall in insulin requirements.

Mean overnight glucose concentrations were similar during the three study nights, with levels of 113 ± 3 mg/dl during control, 108 ± 1 mg/dl during somatostatin alone ($P > 0.2$ versus control), and 112 ± 2 mg/dl during somatostatin plus hGH ($P > 0.3$ versus control, $P > 0.2$ versus somatostatin alone). The mean overnight insulin infusion rate was higher during the control study (19.2 ± 0.6 mU · kg^{-1} · h^{-1}) compared with somatostatin alone (13.0 ± 1.5 mU · kg^{-1} · h^{-1}, $P < 0.05$), but not compared with somatostatin plus hGH (16.8 ± 2.3 mU · kg^{-1} · h^{-1}).

Plasma glucagon concentrations remained relatively constant overnight during the control study with a mean level of 143 ± 20 pg/ml. During the somatostatin infusions, glucagon was infused at a constant rate, and the mean overnight glucagon concentration was 136 ± 38 pg/ml during somatostatin alone ($P > 0.2$ versus control) and 165 ± 17 pg/ml during somatostatin plus hGH ($P > 0.4$ versus control, $P > 0.2$ versus somatostatin alone) (Figure 2).

The mean plasma free insulin concentration during the control studies was 51 ± 10 μU/ml between 0100 and 0300 h, and remained constant overnight with a mean concentration of 55 ± 18 μU/ml between 0600 and 0800 h in spite of a 45% higher insulin infusion rate (Figure 2). Results were similar during somatostatin alone, and during somatostatin plus hGH. Plasma cortisol levels were similar during the three study nights and showed normal physiologic variability, increasing to 15–20 μg/dl after 0400 h (Figure 2).

Combining data from 10 overnight saline infusion studies to assess a possible temporal relationship between hGH spikes and plasma glucose concentrations or insulin requirements in IDDMs, we found that the nadir glucose concentration (105 ± 3 mg/dl) and insulin infusion rate (10.7 ± 1.3 mU · kg^{-1} · h^{-1}, $P < 0.02$ versus baseline) occurred 30–60 min after the peak hGH concentration (23.5 ± 3.7 ng/ml) (Figure 3).

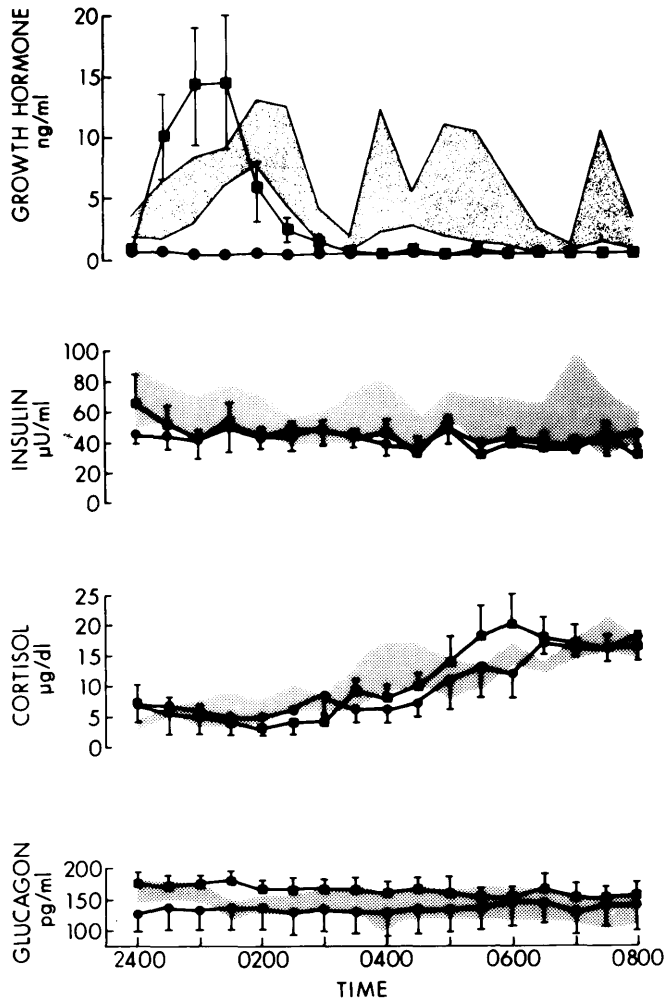

FIGURE 2. Concentrations (mean ± SEM) of growth hormone, glucagon, cortisol, and free insulin during overnight infusions of saline (shaded area), SRIF (circles), and SRIF + hGH (squares).

DISCUSSION

The 0600–0800 h increase in insulin requirements observed in some IDDMs may result in persistent fasting hyperglycemia, while the 0100–0300 h nadir may increase the risk for early morning nocturnal hypoglycemia.[1-8] Despite these important clinical observations, the pathophysiologic factors responsible for this overnight variability in insulin requirements have not been completely elucidated.

The normal diurnal variability of the pituitary-adrenocortical axis has been shown not to be an essential component in the prebreakfast increase in insulin requirements.[9,13] Normal overnight patterns of growth hormone, glucagon, epinephrine, and norepinephrine secretion have previously been observed in these patients.[9,12] This includes spikes in hGH concentration during the early hours of sleep in diabetic subjects that are similar to those observed in nondiabetic subjects. In the original observations of patients treated with subcutaneous insulin injections, the "dawn phenomenon" was associated with a decrease in circulating free insulin concen-

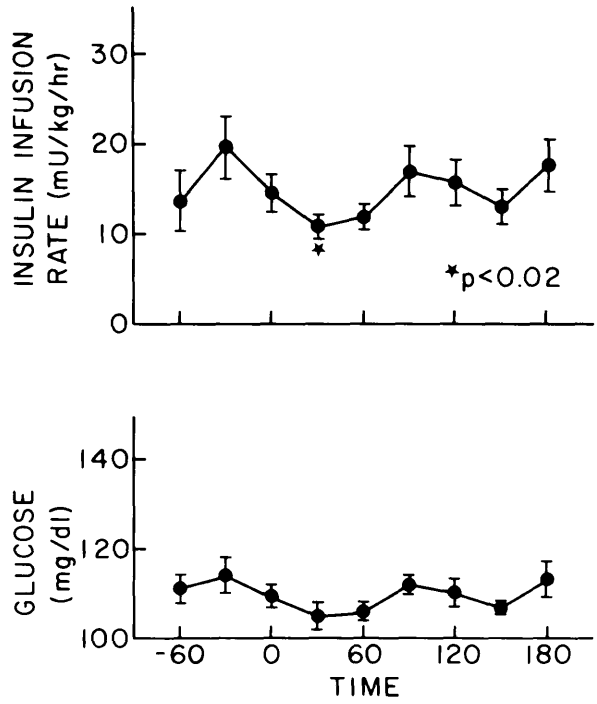

FIGURE 3. Relationship between mean ± SEM overnight nadir glucose concentrations and insulin infusion rates, relative to the time of peak growth hormone concentration (0 min) in 10 overnight studies in IDDMs. This decrease in insulin infusion rate was significant ($P < 0.02$ versus baseline).

trations.[8] Recent observations have confirmed that free insulin levels fail to increase during the prebreakfast period in spite of higher intravenous or subcutaneous insulin infusion rates.[9,10] In addition, insulin clearance appears to be increased between 0600 and 0800 h compared with the 0100–0300 h period, while insulin sensitivity is similar.[11] The overnight variability of plasma glucose concentrations in these patients may reflect variable insulin levels as the result of changing insulin clearance, antibody binding, or degradation, in combination with a heightened sensitivity to normal overnight patterns of known glucose counterregulatory factors.[21]

Early insulin-like and delayed insulin-antagonistic effects of hGH on glucose metabolism have been observed during the infusion of hGH in normal subjects.[14] The insulin-like effects occur within 1 h of the initiation of hGH infusion, and are attributed to both a decrease in hepatic glucose production and an increase in peripheral glucose utilization. These effects are transient, and by 4–6 h later, the insulin-antagonistic effects of hGH predominate. This hyperglycemic effect may be characterized by decreased insulin receptor binding affinity, as well as a probable postreceptor defect.[22-24] These data suggested the possibility that the normally occurring spikes in hGH secretion that occur during the early hours of sleep might contribute to both the early morning nadir and the prebreakfast increase in insulin requirements observed in some IDDMs.

The results of this study indicate that the 66 ± 25% increase in insulin requirements between 0600 and 0800 h is not dependent on hGH secretion, since there was a similar 42 ± 12% increase ($P > 0.2$) when hGH secretion was totally suppressed with somatostatin. When hGH was replaced to physiologic levels between 2400 and 0130 h, the 0600–0800 h increment of 42 ± 11% was not altered significantly ($P > 0.1$ versus control, $P > 0.3$ versus somatostatin).

Somatostatin infusion with glucagon replacement, as performed in this study, was associated with a significant decrease in mean overnight insulin requirements compared with the control infusion (Figure 1). Although glucagon infusions during somatostatin resulted in mean peripheral glucagon levels in the physiologic range, relative hepatic glucagon deficiency might have been expected based on predicted portal vein:peripheral vein glucagon ratios of 1.5:1.[25,26] The observed decrease in insulin requirements during somatostatin alone may be attributed to relative hepatic glucagon deficiency. When hGH was replaced to physiologic levels during somatostatin, mean overnight insulin requirements increased to levels similar to the control study. These data are consistent with a predicted hyperglycemic effect of hGH.

The nadir of the mean glucose concentration and insulin infusion rates was observed at 0230 h during the control studies in these five patients, and this nadir occurred within 1 h after the initial spike in hGH concentration. When these data were combined with data from five additional control studies, a similar nadir in mean glucose concentrations and insulin infusion rates was observed 30–60 min after the mean hGH spike (Figure 3). Suppression of hGH with somatostatin abolished this nadir, while replacement of hGH caused its reappearance. Winter previously observed that the mean nocturnal peak in hGH concentration was associated with the early morning decrease in glucose concentration in six diabetic children with asymptomatic nocturnal hypoglycemia.[27] Although it was reasoned that hGH peaks occurred as the result of hypoglycemia (mean nadir glucose concentration 50 ± 17 mg/dl), alternatively these spikes in hGH secretion may have contributed to the decrease in blood glucose concentration. Together, these data suggest an important role for hGH in the early morning nadir in insulin requirements observed in many IDDMs. During the control studies, the individual patterns of growth hormone secretion are quite variable; this could contribute to erratic blood glucose during the overnight period in some subjects.

Although a hyperglycemic effect of hGH can occur at physiologic levels in IDDMs,[15] nocturnal variations in hGH secretion are not an essential component in the prebreakfast increase in insulin requirements. However, the spikes in hGH secretion that occur during the early hours of sleep may contribute to the 0100–0300 h nadir in insulin requirements observed in some subjects. This may be even more important during adolescence, when nocturnal spikes in hGH are greatly exaggerated. Finally, the failure of free insulin levels to increase between 0600 and 0800 h in spite of a 66% increase in insulin infusion rate further suggests the role of enhanced insulin clearance as an important etiologic factor in the "dawn phenomenon." However, the rising blood glucose concentrations seen in the prebreakfast period despite similar levels of insulinemia observed earlier during the night suggests that counterregulatory factors such as adrenergic activity or decreased hepatic sensitivity to insulin may also contribute to this pattern of insulin requirements.

In conclusion, the overnight variability in insulin require-

ments in some IDDMs may include either a 0100–0300 h nadir or an 0600–0800 h increase, or both. It appears that nadir insulin requirements are partially the result of normally occurring spikes in hGH during the early hours of sleep, while the 0600–0800 h increase is the result of relatively higher insulin clearance, and unidentified counterregulatory factors, and is independent of hGH secretion.

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