# Sleep Release of Human Growth Hormone in Treated Juvenile Diabetics

## Similarity to Normal Subjects and Nonsuppression by Hyperglycemia

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SUMMARY

**The effect of diabetic nocturnal hyperglycemia upon HGH release in sleep was studied in five insulin-treated, stable, nonobese juvenile diabetics on eleven nights and compared to mean release in thirteen normal young men. All were studied by sleep polygraphy. Samples were drawn every twenty minutes from indwelling venous catheters without disturbing sleep. HGH was measured by RIA and plasma glucose concentration by 0-toluidine. Despite hyperglycemia of 195-382 mg. per 100 ml. on nine out of eleven nights at the time of sleep HGH peak, the repetitive nightly pattern of HGH release in diabetics resembled those of normals. The diabetic group's mean HGH concentration during sleep was 4.0 ± 0.6 ng./ml. (± S.E.) and mean peak sleep HGH concentration was 11.2 ± 1.3 ng./ml., which were not significantly different from those of normals (3.4 ± 0.3 and 12.5 ± 1.4 ng./ml. ± S.E. respectively). Thus, diabetic hyperglycemia, similar to constant glucose infusion hyperglycemia in normals, does not result in suppression of HGH release in sleep. This indicates that hyperglycemia is not an important regulator of rhythmical HGH release in sleep and suggests a primary neural character of this rhythm. HGH release in sleep was not enhanced in treated juvenile diabetes.** DIABETES *20*:691-95, October, 1971.

Peaks of plasma human growth hormone (HGH) concentration in early sleep of normal subjects[1-3] represent circadian maxima.[3-5] This HGH release is a sleep-dependent rhythm rather than nychthemeral variation as shown by sleep-reversal studies.[6] The pattern of release in a given subject is repetitive night after night and is usually associated with the cyclic occurrence of slow wave or deep sleep.[3] Release is prevented or lessened by slow wave sleep deprivation.[7] In normal subjects during wakefulness HGH release induced by insulin hypoglycemia,[8] arginine infusion,[9] or late in oral glucose tolerance test[10] is suppressed by concurrent hyperglycemia. In contrast, HGH release in sleep is not suppressed by acute hyperglycemia achieved by constant glucose infusion.[11,12] These observations suggest that HGH release in sleep may result from a primary neural rhythm which is not acutely regulated by glucose concentration.

We reasoned that juvenile diabetes mellitus afforded a further opportunity for inquiry into the relationship of HGH release in sleep to blood glucose concentration. The purpose of the present study was to learn if the rhythmic release of HGH in sleep occurred in diabetes, and if so, whether it was comparable to that in normals or was influenced by nocturnal hyperglycemia.

### MATERIALS AND METHODS

Table 1 lists identifying data on five juvenile diabetic volunteers. All felt well, were without recent ketoacidosis, and were in reasonable control on their usual insulin-diet program, though three required insulin twice a day. The girls menstruated regularly. Diabetic subjects (S) were studied on three consecutive nights ($N_1$-$N_3$) except for single night studies in $S_1$ and $S_5$. All were at stable weight except for $S_1$, who was actively growing. All were on their usual insulin-diet program during the study, though evening snack or insulin were altered if hypoglycemia was encountered on $N_1$.

Mean normal HGH data during polygraphically monitored sleep were obtained from studies of thirteen normal nonobese, young men age 18-31, each studied on two to three control nights totaling thirty-one baseline studies.

Sleep polygraphy and scoring employed standardized technics.[13] Plasma glucose by an ortho-toluidine method[14]

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TABLE 1

Diabetic subjects

| | Sex | Age (yr.) | % Ideal Weight | Duration Diabetes (yr.) | Insulin Dose a.m./p.m.* | Fast/ 3 hr. p.c. Glucose (mg. per 100 ml.)† |
|---|---|---|---|---|---|---|
| 1 | M | 10 | + 11 | 2 | 20L + 18R | 172/107 |
| 2 | M | 21 | — 3 | 5 | 26L + 14R / 20L | 102/150 |
| 3 | M | 18 | + 5 | 9 | 20NPH + 20R / 15NPH + 15R | 127/162 |
| 4 | F | 18 | + 10 | 4 | 40NPH | 218/144 |
| 5 | F | 16 | 0 | 6 | 30NPH + 20R / 10NPH | 195/83 |

* Insulin dose in units: R = Regular, L = Lente, and NPH = Neutral Protamine of Hagedorn. Numerator = a.m. dose, denominator = p.m. dose.

† Blood glucose values represent fasting and three-hour postbreakfast results just prior to study.

and HGH by radioimmunoassay[3,15] with Wilhelmi HS 1216-C* as standard were done on samples obtained every twenty minutes from venous intracath and extramural extension tubing. The sleep laboratory was not entered for sampling nor was the patient's sleep stage altered by sampling. The tubing was filled with dilute (5 U./ml.) heparin-saline between samples. Blood samples (3 ml.) were immediately centrifuged, separated, frozen, and plasma held at —20° until assayed. Maximum sensitivity of the HGH assays varied from 0.6-1.0 ng./ml. All samples in a single subject were tested in the same assay.

## RESULTS

All subjects slept readily and normally in regard to per cent of time in sleep spent in the various stages, infrequency of awakening, and repetition of a subject's per cent of sleep in stage pattern over several nights.[16] Hyperglycemia, ranging from 195-382 mg. per 100 ml. at the time of HGH peak, occurred early in sleep in all five diabetic subjects on nine of eleven nights (table 2). The degree of nocturnal hyperglycemia in this relatively healthy group of diabetics with acceptable fasting and three-hour postcibal daytime glucose concentrations was unexpected. Hyperglycemia could not be predicted from the electroencephalographic, -myographic, or -oculographic recordings.

All five diabetics released HGH in early sleep each night comparable to release in normal subjects (figures 1, 2; table 2). The patterns of a diabetic subject's HGH peaks were also similar over the three nights (figure 1), again comparable to those in normals.[3,11,17] The mean HGH concentration during sleep in the five diabetics was 4.0 ± 0.6 ng./ml. (± S.E.) and was not significantly different from the mean of 3.4 ± 0.3 ng./ml. of the group of normal males. The mean sleep peak HGH concentration of the diabetic group was 11.2 ± 1.3, again not significantly different from the 12.5 ± 1.4 ng./ml. (mean ± S.E.) of the normal group. Plots of mean HGH by time after sleep onset (figure 2) were also similar in the two groups except for a slight delay in onset of HGH release in sleep and a tendency to slightly higher values late in sleep in the diabetics.

Diabetic hyperglycemia did not suppress early sleep HGH concentrations into our 0-5 ng./ml. basal fasting 8 a.m. normal range. The pattern of a diabetic subject's sleeping hyperglycemia as stable, rising, or falling during the various study nights did not appear to affect the similarity of his nightly HGH release patterns (figure 1), nor did the HGH peaks appear to raise subsequent glucose concentrations in later sleep. Normoglycemia prior to sleep onset or during early sleep occurred in two subjects and did not result in enhanced HGH release when compared to the peaks of their hyperglycemic

TABLE 2

Peak HGH value in sleep and coincident glucose concentration in five juvenile diabetics over eleven nights

| Diabetics | HGH (ng./ml.) | Plasma Glucose (mg./100 ml.) |
|---|---|---|
| $S_1N_1$ | 6.5 | 195 |
| $S_2N_1$ | 24.0* | 30* |
| $N_2$ | 7.6 | 243 |
| $N_3$ | 13.0 | 131 |
| $S_3N_1$ | 10.0 | 382 |
| $N_2$ | 15.6 | 330 |
| $N_3$ | 12.4 | 273 |
| $S_4N_1$ | 11.6 | 274 |
| $N_2$ | 16.0 | 234 |
| $N_3$ | 9.5 | 255 |
| $S_5N_1$ | 14.0 | 255 |
| Mean | 11.2* | 244* |
| SD | 2.9 | 57 |
| Normal males n = 13 | | |
| Mean | 12.5 | |
| SD | 5.1 | |

* Hypoglycemic sleep data not included in calculations.

*Gift, Endocrinology Study Section, National Institutes of Health.

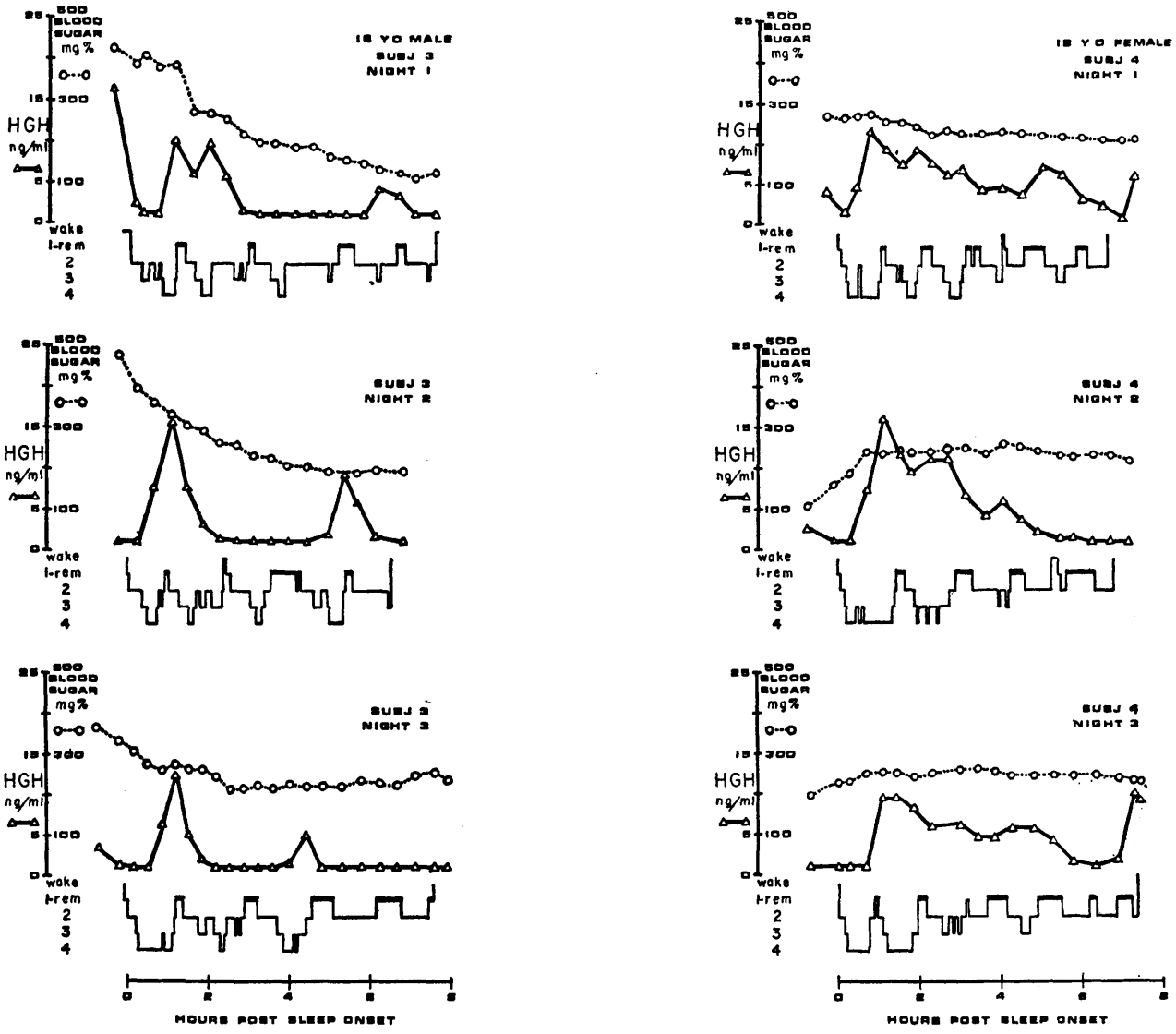


FIG. 1. HGH, glucose and sleep histograms in two insulin-dependent juvenile diabetic subjects over three nights. Sleep histogram stages: W (ake), 1-4 by depth of graph; REM by bar; Stage 3 and 4 represent slow wave or deep sleep.

nights. Hypoglycemia occurred only in subject 2, reaching a nadir of 30 mg. per 100 ml. in early sleep and remaining below 50 mg. per 100 ml. throughout the night. Sleep was not disrupted by this event. The sleep peak of HGH was greater in magnitude and duration on this night, though it retained its temporal relation to the first sleep cycle and its single peak character noted on the other two study nights. There were no new secondary HGH peaks in later sleep despite persistent hypoglycemia.

## DISCUSSION

We interpret these data to indicate that the rhythmic release of HGH in sleep of insulin-treated juvenile diabetics is comparable to that of normal subjects and is not readily suppressed by nocturnal hyperglycemia. The data, like those obtained from hyperglycemic normal subjects,[11,12] speak against glucose concentration as an important regulator of HGH release in sleep. The magnitude of release compared to that of normal subjects is such that no inference of a source of insulin antagonism during sleep different from that in normals[12] can be drawn, at least from this small group of relatively stable diabetics.

Mean HGH and glucose data plots contained in a study of diabetic diurnal variation by Hansen and Johan-

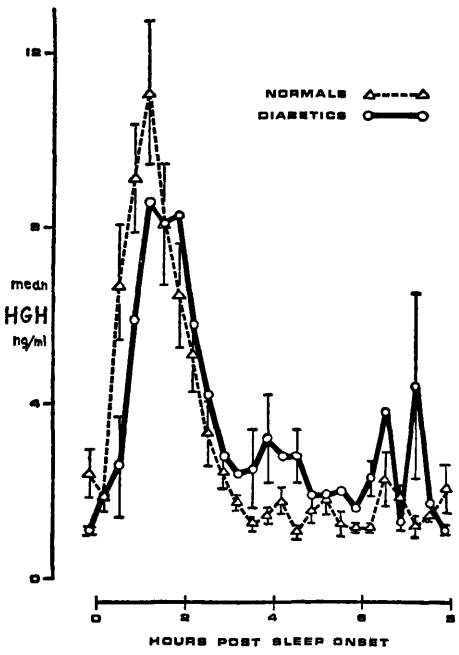


FIG. 2. Mean HGH concentration each twenty-minute interval after sleep onset in thirteen normal young men and five insulin-dependent juvenile diabetic subjects. Diabetic group: Solid line. Normals: Dotted line. Standard errors are given on the diabetic plot only at those points where no overlap with normal S.E. bars occurred.

sen also reflect nonsuppression of HGH release by diabetic nocturnal hyperglycemia.[18] Our data, however, do differ from theirs, which showed diabetic mean HGH concentration during behaviorally adjudged sleep to be greater than that of normals. Their study was done in newly diagnosed, untreated juvenile diabetics.[18,19] We have seen sleep patterns similar to those they reported in normal subjects during seventy-two-hour fasts.[20] Here basal elevation of HGH occurs throughout sleep upon which sleep peaks can still be seen to occur. This suggests that such augmented HGH values occur in association with neural events related to decreased glucose utilization or increased lipolysis and fat utilization rather than being characteristic of juvenile diabetes per se.

Others who have reported failure of diabetic hyperglycemia to suppress wakeful peak HGH release during arginine[9] or constant insulin [21] infusion have suggested this reflects either altered inhibition of HGH release by hyperglycemia in diabetes itself[9] or enhanced responsiveness to other glucose-independent stimuli to release such as stress.[21] Enhanced HGH release was suggested to be independent of age of onset and the presence or absence of retinopathy but perhaps to correlate with marked endogenous glucose lability in certain individual diabetics.[21] This lability persisted despite constant insulin infusion[21] or attempts at optimal regulation.[22] Though our study group did not appear to contain such diabetic subjects, in the study of Fatourechi et al.,[21] the HGH rises seen were not always in response to glucose fall. This also suggests that such patient's HGH responses are not principally regulated by glucose. Probably such HGH responses are to a variety of neural stimuli including falling glucose concentration, stress, and the pseudo-fasting of altered substrate utilization in uncontrolled diabetes. In addition it is also conceivable that each individual's wakeful HGH responses may also be rhythmical and characteristic for him similar to the rhythmical sleep release pattern. This might account for some of the presently unexplained daytime fluxes in HGH concentration. To test this hypothesis we are presently studying subjects over several forty-eight-hour periods for evidence of endogenous rhythmical diurnal HGH release.

Hypoglycemia in its single occurrence in our study was associated with enhanced HGH release, though it did not otherwise alter the basic sleep-HGH release rhythm of that subject despite his persistent hypoglycemia. Though this suggests the neural mechanism(s) of sleep release may be amplified by the neural set of hypoglycemia, further data on hypoglycemic sleep release are required to speak to this point.

It is clear, however, that sleep release of HGH is not suppressed by hyperglycemia in treated juvenile diabetics or in normals and that juvenile diabetic hyperglycemia apparently fails to inhibit wakeful HGH responses to a variety of stimuli as well. This points up the need to understand the central neural mechanisms basic to HGH release. Plasma substrates, which have been viewed as moment to moment HGH regulators, may simply act as neural modulators. Rhythmic sleep release of HGH, which is not modified by moderate exercise,[17] short-term high carbohydrate feeding[20] or acute hyperglycemia,[11] thus appears resistant to such modulation. The teleologically appealing explanation for this different order of response is that sleep release of HGH represents a genetically determined anabolic neural rhythm. However, positive data in this regard have not yet been offered.

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