

## Adaptation of the 24-h growth hormone profile to a state of sleep debt

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**Spiegel, K., R. Leproult, E. F. Colecchia, M. L'Hermite-Balériaux, Z. Nie, G. Copinschi, and E. Van Cauter.** Adaptation of the 24-h growth hormone profile to a state of sleep debt. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R874–R883, 2000.—In normal men, the majority of GH secretion occurs in a single large postsleep onset pulse that is suppressed during total sleep deprivation. We examined the impact of semichronic partial sleep loss, a highly prevalent condition, on the 24-h growth hormone profile. Eleven young men were studied after six nights of restricted bedtimes (0100–0500) and after 7 nights of extended bedtimes (2100–0900). Slow-wave sleep (SWS) was estimated as the duration of stages III and IV. Slow-wave activity (SWA) was calculated as electroencephalogram power density in the 0.5- to 3-Hz frequency range. During the state of sleep debt, the GH secretory pattern was biphasic, with both a presleep onset “circadian” pulse and a postsleep onset pulse. Postsleep onset GH secretion was negatively related to presleep onset secretion and tended to be positively correlated with the amount of concomitant SWA. When sleep was restricted, both SWS and SWA were increased during early sleep. Unexpectedly, the increase in SWA affected the second, rather than the first, SWA cycle, suggesting that presleep onset GH secretion may have limited SWA in the first cycle, possibly via an inhibition of central GH-releasing hormone activity. Thus neither the GH profile nor the distribution of SWA conformed with predictions from acute sleep deprivation studies, indicating that adaptation mechanisms are operative during chronic partial sleep loss.

growth hormone secretion; slow-wave activity; sleep deprivation

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GROWTH HORMONE (GH) is secreted as a series of pulses throughout the 24-h cycle. In normal adult subjects, the most reproducible and often the largest GH pulse occurs during early sleep, in temporal association with the first phase of deep slow-wave sleep (SWS) (10, 29, 33, 37). The amount of GH secreted during the first SWS episode is quantitatively correlated with the duration of SWS (9, 38) as well as with the intensity of SWS, as estimated by slow-wave activity (SWA), i.e.,

spectral power of the electroencephalogram (EEG) in the 0.5- to 3.5-Hz frequency range (7). Pharmacological stimulation of SWS is associated with increased GH secretion with a significant dose-dependent relationship (7, 39), indicating that common mechanisms underlie SWS and GH release. Rodent as well as human studies have identified GH-releasing hormone (GHRH) as the primary factor controlling sleep-associated GH release (24, 26) and have indicated that GHRH may promote SWS by central mechanisms (13, 15, 21–23, 25, 32).

In men, GH release in early sleep normally accounts for 50–70% of the total daily output throughout adulthood (37). Numerous studies involving acute total sleep deprivation have shown that, in the absence of sleep, nocturnal GH secretion is drastically diminished. When modest amounts of GH are secreted during nocturnal sleep deprivation (38, 42), they are thought to be due to a circadian rhythm in somatostatin tone, with lower activity and thus decreased inhibition of pituitary GH release in the evening and early part of the night (11). Recovery from total sleep deprivation, whether initiated in the morning or later in the day, is invariably associated with a robust increase in GH secretion. Similarly, when bedtime is acutely delayed by a few hours, nocturnal GH levels remain low as long as the subject is awake and rebound as soon as sleep is initiated (reviewed in Ref. 37).

Although the effects of acute total or partial sleep deprivation on the temporal organization of human GH release have been well described, the impact of the much more common condition of semichronic partial sleep deprivation has never been evaluated. Voluntary sleep curtailment has become a hallmark of modern society. To maximize the time available for work and/or leisure, many individuals curtail sleep to the minimum tolerable (4, 6). Workers on the night shift typically sleep on average <5 h/day (2). Whether chronic sleep curtailment affects the amount and temporal distribution of GH secretion is not known. In view of the importance and multiplicity of the metabolic actions of

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GH, even modest alterations of the 24-h secretory profile could be associated with significant peripheral effects.

The present study was thus designed to evaluate the impact of 1 wk of sleep curtailment, i.e., a state of sleep debt, on 24-h GH secretion and its relationship to the duration and intensity of SWS. We calculated GH secretory rates and the amount and intensity of SWS in normal young men studied after 1 wk of sleep curtailment to 4 h/night and repeated these measurements after 1 wk of sleep extension to 12 h/night, a schedule that returned to the subjects the number of bedtime hours lost during the period of sleep restriction.

## SUBJECTS AND METHODS

### Subjects

The subjects were 11 healthy young nonobese men (body mass index:  $23.4 \pm 0.5 \text{ kg/m}^2$ ), aged  $22 \pm 1 \text{ yr}$ , with no personal history of endocrine, metabolic, neurological, or psychiatric disorders and taking no medication. They were self-described as good sleepers, were nonsmokers, and in good physical condition obtained by regular moderate exercise. Positive criteria for selection included regular life habits (i.e., stable bedtimes  $\pm 1 \text{ h}$ ) and a habitual sleep length of  $\sim 8 \text{ h}$  ( $\pm 30 \text{ min}$ ). Shift and/or night workers or subjects having traveled across time zones fewer than 4 wk before the beginning of the study were excluded.

### Experimental Protocol

The experimental protocol was approved by the Institutional Review Board of the University of Chicago. All studies were carried out in the Clinical Research Center (CRC) of the University of Chicago after written informed consent had been obtained from the subjects.

During the week preceding the study, the subjects were asked to conform to a standardized schedule of bedtimes (2300–0700) and mealtimes (0900, 1400, and 1900). They were asked not to deviate from this schedule by more than 30 min and wore a wrist activity monitor (Gaehwiler Electronics, Hombrechtikon, Switzerland) during the entire duration of the study to allow for the monitoring of compliance with scheduled bedtimes and the detection of unintentional naps.

A schematic representation of the experimental protocol is given in Fig. 1. The subjects spent 16 consecutive nights in the CRC, including 3 nights with 8-h bedtime from 2300 to 0700 (B1–B3; baseline), 6 nights with bedtime limited to a 4-h period from 0100 to 0500 (R1–R6; sleep restriction), and 7 nights with 12-h bedtime from 2100 to 0900 (E1–E7; sleep extension). The center of the sleep period was kept constant throughout the study. During bedtime hours, the lights were turned off. Except during the last 2 days of each study condition, the subjects were allowed to leave the CRC from 0900 to 1900 to carry out their usual daytime activities. Naps were not allowed.

During the last 60 h of each study condition, the subjects remained continuously at bedrest in the CRC. The subjects received identical carbohydrate-rich meals (62% carbohydrates) at 5-h intervals (i.e., 0900, 1400, 1900) and these constituted their only source of caloric intake. Only decaffeinated diet sodas were allowed in addition to water ad libitum. During the 60-h studies at the end of sleep curtailment (R6) and sleep extension (E7), blood was sampled through an

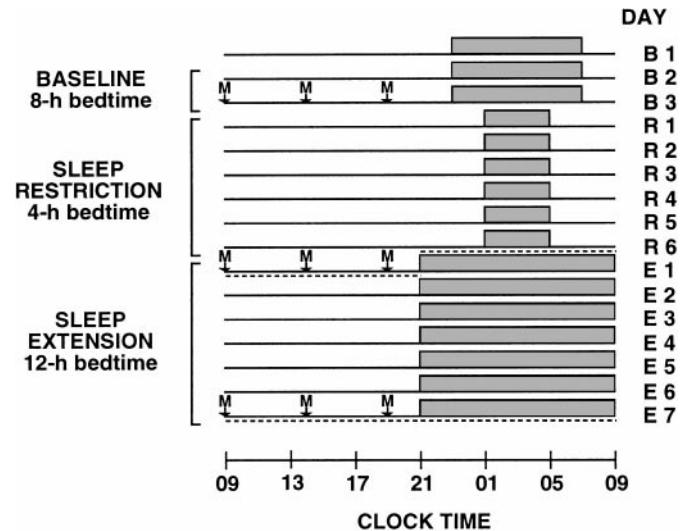


Fig. 1. Schematic representation of the study protocol. Shaded bars, sleep periods; M, carbohydrate-rich meals given at 5-h intervals. The study period included 3 nights with bedtime from 2300 to 0700 (B1–B3), 6 nights with bedtimes from 0100 to 0500 (R1–R6; sleep restriction), and 7 nights with bedtime from 2100 to 0900 (E1–E7; sleep extension). The dotted lines show the sampling periods at 10- to 30-min intervals used to determine the 24-h GH profiles at the end of sleep curtailment and sleep extension. Concomitant polygraphic sleep recordings were obtained.

indwelling catheter at 10- to 30-min intervals throughout the 24-h cycle for the measurement of hormonal levels and sleep was polygraphically recorded. The impact of sleep curtailment and sleep extension on glucose tolerance, thyroid function, and cortisol levels has been described in a separate report (31).

Limitations on amount of blood volume withdrawal did not permit us to sample blood for 24 h during the baseline condition preceding the period of sleep restriction. However, 8 of the 11 subjects agreed to participate in a separate “baseline” study with 8-h bedtimes that was performed one year after the sleep restriction/extension study in the same laboratory and using the same experimental procedures.

### Sampling Procedure

An intravenous sterile heparin-lock catheter was inserted in the forearm at least 12 h before the beginning of the 24-h blood sampling procedure. The line was kept patent with a slow drip of heparinized saline. The frequency of sampling was every 10 min during the first hour after meal presentation, every 15 min during the first 3 h of sleep, and every 30 min at all other times. During sleep hours, the catheter was connected to plastic tubing extending to an adjacent room and sampling was performed without disturbing the subject. Each blood sample was immediately centrifuged at  $4^{\circ}\text{C}$ . Plasma samples were frozen at  $-20^{\circ}\text{C}$  until assay.

In *subject #08*, the sampling catheter had to be reinserted during the sleep restriction condition. The stress of venipuncture was associated with a robust increase of GH levels and a concomitant surge of cortisol concentrations. GH data from this subject were therefore excluded from the analysis.

### GH Assay

Determinations of plasma GH levels were performed using a chemiluminescent enzyme immunoassay (Immulite, Diagnostics Products, Los Angeles, CA) with a limit of sensitivity

of 0.003  $\mu\text{g/l}$ , an intra-assay coefficient of variation of 5–6.5%, and an interassay coefficient of variation of 5.5–6.5%. All samples collected in the same subject during sleep restriction and sleep curtailment were analyzed in the same assay run.

#### *Analysis of Individual GH Profiles*

Because plasma was sampled at 10-, 15-, or 30-min intervals, plasma GH values were interpolated at 5-min intervals to facilitate the calculation of secretory rates.

GH secretory rates were estimated from plasma levels using a one-compartment model for GH clearance and subject-adjusted half-lives (i.e., from 21 to 13 min) to minimize the number of false-negative secretory rates, as previously described (38). The distribution volume was assumed to be 7% of the body weight (28). Amount of GH secreted was expressed either in micrograms (absolute amount) or as a percentage of the total amount secreted during the whole 24-h period (relative amount).

Significant GH secretory pulses were identified using the pulse detection program ULTRA (34, 35). The general principle of this algorithm is the elimination of all peaks for which either the increment (difference between the peak and the preceding trough) or the decrement (difference between the peak and the next trough) does not exceed a certain threshold related to measurement error. The standard deviation of the error associated with each calculated secretory rate was calculated following the theory of error propagation, assuming normally distributed errors on plasma levels. For each significant pulse, the amplitude was defined as the difference between the level at the peak and the level at the preceding trough.

#### *Sleep Recording and Analysis*

**Sleep stage scoring.** Polygraphic sleep recordings were scored at 20-s intervals in stages wake, I, II, III, IV, and rapid eye movement (REM) according to standard criteria (27). Sleep onset was defined as the time corresponding to the first 20-s interval scored II, III, IV, or REM provided the subsequent 20-s interval was not scored wake or stage I. Morning awakening was defined as the time corresponding to the last 20-s interval scored II, III, IV, or REM.

The following parameters were determined: sleep period (i.e., time interval separating sleep onset from morning awakening), sleep onset latency (i.e., time interval separating lights-off from sleep onset), total sleep time (i.e., sleep period – duration of intrasleep wake periods), sleep efficiency (i.e., total sleep time/time allocated to sleep), duration of light non-REM sleep (i.e., stages I and II), duration of SWS (i.e., sleep stages III and IV), duration of REM sleep, and duration of intrasleep wake periods. Because the duration of active GH secretion after sleep onset was confined to the first 3 h for all subjects under both study conditions, the duration of each sleep stage was further determined during this time interval.

**Spectral analysis of the sleep EEG.** For all-night spectral analysis, the EEG signal (from electrode references Cz-A1) was high-pass filtered and converted from analog to digital at a sampling frequency of 50 Hz. Subsequently, a spectral analysis of the EEG was performed for consecutive 20-s periods using a fast Fourier transformation (FFT) algorithm. SWA, a measure of the intensity of SWS, was calculated as the absolute power in the frequency range 0.5–3 Hz, often referred to as the delta range. SWA values that exceeded the preceding and following values by 300% or more were considered artifacts and were replaced by linear interpolation.

For both study conditions, the profiles of SWA were quantified by calculating a smoothed curve using a 60-point moving average method (i.e., window width of 20 min), thus allowing the determination of the minimum, maximum, duration, and amplitude of each cycle of SWA. The acrophase and nadir of the two first SWA cycles were defined, respectively, as the maximum and minimum in the smoothed curve and the amplitude was defined as the difference between the maximum and the following minimum. The duration of a SWA cycle was defined as the time period separating two minima in the smoothed curve. The values of the acrophase and nadir were expressed as the level attained by the smoothed curve, respectively, at its maximum and minimum and were expressed in squared microvolts. The amount of SWA in each cycle was calculated as the sum of all SWA values during the cycle. The mean SWA in a cycle was calculated as the amount of SWA in the cycle divided by the number of 20-s intervals in the cycle.

Due to a technical failure, computerized EEG recordings could not be obtained in *subject #10* in the sleep restriction condition. In contrast to all other subjects who exhibited lower amounts of SWA during sleep extension compared with sleep restriction, *subject #09* exhibited unexplained higher values of SWA when studied with a 12-h bedtime period than with a 4-h bedtime period. In this subject, the difference in the amount of SWA between sleep restriction and sleep extension was a statistically outlying value according to the criteria of Grubbs ( $P < 0.05$ ) (8). Therefore, comparisons of SWA between the sleep restriction and sleep extension conditions were performed without the data of *subject #09*.

#### *Statistical Analysis*

All group values are expressed as means  $\pm$  SE. The comparisons between sleep restriction and sleep extension conditions were performed using the Wilcoxon test. Correlations between parameters of sleep and of GH secretion were calculated using the Spearman coefficient ( $r_s$ ). Before such correlations were calculated, outliers were first identified by the Grubbs method (8). All statistical calculations were performed using the StatViewSE<sup>+</sup> software package for Macintosh (Abacus Concepts, Berkeley, CA).

## RESULTS

### *Twenty-Four-Hour Profiles of GH Secretion*

As summarized in Table 1, the amounts of GH secreted during the daytime (0900–2100) or the nighttime (2100–0900) were not significantly affected by bedtime duration, although the total amount of GH secreted during the 24-h span tended to be slightly higher at the end of sleep restriction. Pulse analysis revealed no significant differences for the number of secretory pulses or for their amplitude. Individual (Fig. 2) and mean (Fig. 3) GH profiles revealed a consistent temporal relationship between GH secretory pulses and the timing of meals. In the sleep-debt condition, significant GH secretory pulses were detected at 4 h 14 min  $\pm$  18 min after breakfast presentation, at 4 h 22 min  $\pm$  20 min after lunch presentation, and at 4 h 1 min  $\pm$  19 min after dinner presentation. In the sleep extension condition, significant GH secretory pulses were detected at 4 h 26 min  $\pm$  18 min after breakfast presentation and at 4 h 12 min  $\pm$  24 min after lunch presentation. Postbreakfast and -lunch GH secretions

Table 1. *Characteristics of GH secretion*

	Time Allocated to Sleep, h		P Level
	4	12	
<i>Amount of GH secreted, <math>\mu\text{g}</math></i>			
During the 24-h period	915 $\pm$ 115	789 $\pm$ 94	<0.08
Daytime (0900–2100)	230 $\pm$ 35	204 $\pm$ 37	NS
Nighttime (2100–0900)	685 $\pm$ 113	587 $\pm$ 78	NS
<i>Last 3 h before sleep onset</i>			
Amount secreted, $\mu\text{g}$	272 $\pm$ 49	85 $\pm$ 24	<0.01
Relative (%24 h) amount	32 $\pm$ 5	10 $\pm$ 2	<0.02
<i>First 3 h of sleep</i>			
Amount secreted, $\mu\text{g}$	344 $\pm$ 107	414 $\pm$ 56	NS
Relative (%24 h) amount	34 $\pm$ 7	53 $\pm$ 3	<0.02
<i>First pulse after sleep onset</i>			
Amount secreted, $\mu\text{g}$	257 $\pm$ 84	347 $\pm$ 38	<0.08
Relative (%24 h) amount	24 $\pm$ 5	46 $\pm$ 3	<0.03
Amplitude, $\mu\text{g}/\text{min}$	5.3 $\pm$ 1.4	6.6 $\pm$ 0.9	NS
Duration, min	76 $\pm$ 6	134 $\pm$ 15	<0.02

Values are means  $\pm$  SE. GH, growth hormone; NS, not significant.

were similar in both study conditions. Sleep onset occurred on average 3 h 37 min  $\pm$  13 min after the evening meal in the sleep-extension condition, i.e., before a postdinner GH pulse could be observed while the subjects were still awake. In the sleep-debt condition, the amount of GH secreted at the expected time of a postdinner GH pulse, 4–4.5 h after dinner, i.e., at 2300 to 2330, was much higher (258  $\pm$  55  $\mu\text{g}$ ) than post-breakfast (129  $\pm$  39  $\mu\text{g}$ ,  $P < 0.03$ ) or postlunch (74  $\pm$  26  $\mu\text{g}$ ,  $P < 0.01$ ) pulses, suggesting that secretory stimuli unrelated to meal ingestion were operative.

Figure 2 shows individual 24-h profiles of plasma GH and GH secretory rates together with the corresponding profiles of sleep stages and SWA for three representative subjects after 1 wk of sleep restriction (Fig. 2, *left*) and after 1 wk of sleep extension (Fig. 2, *right*). The well-known sleep onset-associated GH pulse was observed in all individual profiles for both conditions. After 1 wk of sleep extension, the largest GH secretory pulse occurred during early sleep for all 10 subjects, as normally observed with a standard 8-h bedtime. Unexpectedly, after 1 wk of bedtimes reduced to 4 h, all subjects exhibited a GH pulse before sleep onset and this presleep onset pulse was the largest secretory episode of the 24-h cycle for 5 of 10 subjects. As a result, the mean 24-h profiles of plasma GH and GH secretory rates in the state of sleep debt exhibited a biphasic pattern of nocturnal release, with a large pulse occurring during waking around the usual time of sleep onset on a standard 8-h bedtime schedule followed by a second pulse after the onset of restricted sleep (Fig. 3, *left*). This secretory pattern contrasts with the mean profiles during sleep extension (Fig. 3, *right*) that present the usual single nocturnal GH pulse in early sleep.

The biphasic pattern of nocturnal GH release observed during sleep restriction is also qualitatively different from the mean 24-h profiles observed in a

subset of the same subjects in a separate study performed 1 year later with 8-h bedtimes from 2300 to 0700 (Fig. 4). Because of lifestyle changes (dietary and/or exercise habits) and a slight increase in body mass index of the subjects (23.4  $\pm$  0.4 vs. 23.1  $\pm$  0.4  $\text{kg}/\text{m}^2$ ,  $P < 0.07$ ), these “baseline” profiles cannot be submitted to quantitative within-subject comparisons with the profiles obtained 1 year earlier in the study with sleep restriction and extension. However, Fig. 4 clearly shows that the profiles observed with 8-h bedtimes are qualitatively similar to the profiles obtained with 12-h bedtimes. In contrast, the state of sleep debt was associated with a markedly different temporal organization of GH secretion.

#### *GH Secretion in Relation to Sleep Onset*

Figure 5 shows the mean profiles of plasma GH and GH secretory rates referenced to time of sleep onset, rather than clock time, to account for interindividual variations in sleep latency. Figure 5, *top*, shows the concomitant evolution of SWA. The profiles of mean secretory rates reveal that, on average, active GH secretion during sleep was limited to the first 3 h of the sleep period. The absolute amount of GH secreted during these first 3 h of sleep was similar for short and long bedtimes, but when expressed as percentage of the total 24-h output, it was lower during sleep restriction with only 34% of 24-h secretion occurring during early sleep compared with 53% during sleep extension ( $P < 0.02$ ; Table 1). During sleep extension, the percentage of the total 24-h secretion occurring during the first 3 h of sleep was actually similar to that normally observed with a standard 8-h bedtime. For comparison, in the baseline study illustrated in Fig. 4, this percentage averaged 59  $\pm$  5%.

During sleep restriction, the 3 h before sleep onset corresponded approximately to the interval 2200–0100, i.e., a window centered around the time of usual sleep onset on the baseline 8-h schedule. Remarkably, nearly one-third of the daily GH output was concentrated in this time interval (Table 1). In contrast, when bedtime was extended, negligible amounts of GH secretion were observed during the 3 h preceding sleep onset, which corresponded to a window centered at  $\sim$ 2100 (Table 1). Similarly, when standard 8-h bedtimes were enforced in a separate study, GH secretion was essentially absent during the 3 h preceding sleep onset (Fig. 4). The biphasic nature of the GH secretory pattern during sleep restriction resulted in a longer duration of exposure of peripheral tissues to elevated ( $>4 \mu\text{g}/\text{l}$ ) GH concentrations (4 h 12 min  $\pm$  25 min vs. 3 h 25 min  $\pm$  33 min during sleep extension,  $P = 0.02$ ).

The amount of GH secreted during the first pulse after sleep onset tended to be lower during the 4-h nights than during the 12-h nights ( $P < 0.08$ ) primarily because the duration of this first pulse was shorter ( $P = 0.02$ ; Table 1). Indeed, the amplitude of the sleep onset-associated pulse was not affected by the study condition (Table 1). During sleep restriction, but not sleep extension, a second smaller, but significant, GH

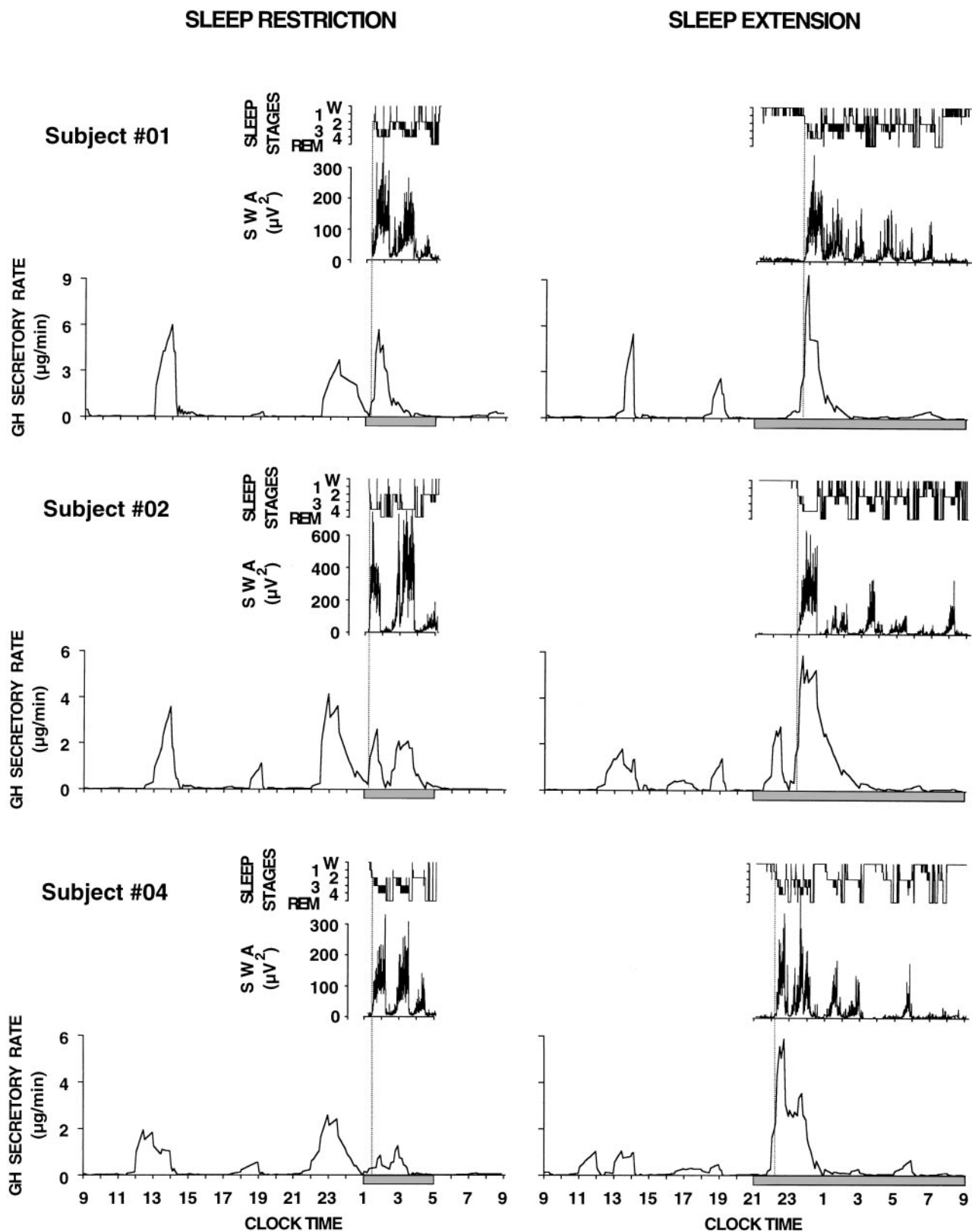


Fig. 2. Individual 24-h profiles of GH secretory rates together with the corresponding sleep stages pattern and slow-wave activity (SWA) for 3 representative subjects during the sleep restriction condition (*left*) and during the sleep extension condition (*right*). The shaded bars represent the sleep period. These profiles show that GH was preferentially secreted during slow-wave sleep (SWS) and increased SWA, with interruptions of secretory activity coinciding with the intervening rapid eye movement (REM) or wake stages.

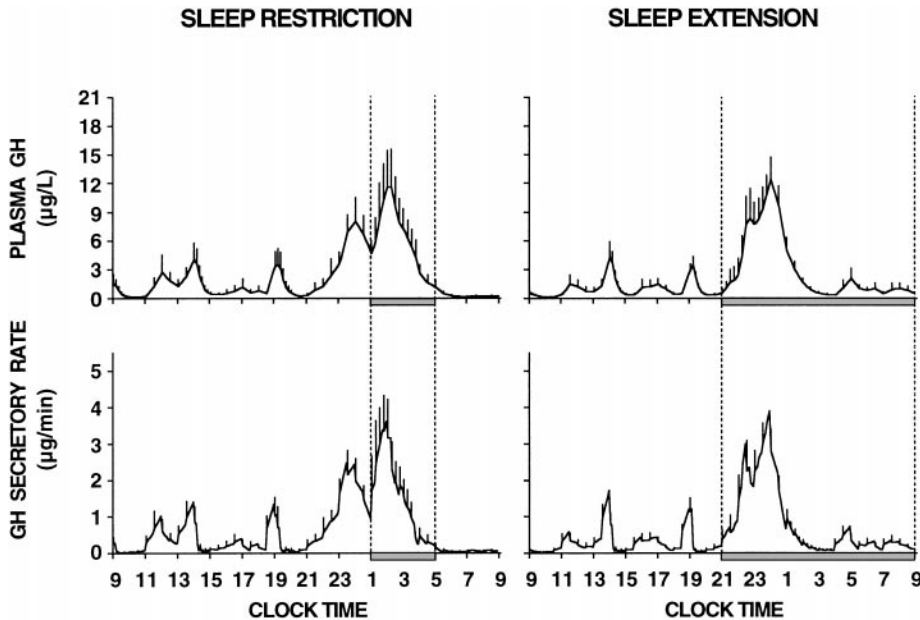


Fig. 3. Mean ( $\pm$ SE) 24-h profiles of plasma GH (*top*) and GH secretory rates (*bottom*) at the end of 1 wk of sleep restriction (*left*) and sleep extension (*right*). The GH profiles show a clear sleep-related component in both conditions. However, during sleep restriction, a second large pulse occurred before bedtime.

pulse immediately followed the sleep onset pulse in all subjects, in association with the second cycle of SWA. Examples are illustrated in Fig. 2.

#### Sleep Quality

Table 2 summarizes the sleep parameters during the last nights of sleep restriction and sleep extension. Sleep onset latency was remarkably short during sleep restriction and unusually long during sleep extension. When faced with chronic sleep curtailment, these young healthy adults were able to increase sleep efficiency to  $>95\%$  despite the presence of the sampling catheter ( $P = 0.005$ ). The adaptation to sleep restric-

tion and sleep extension was achieved by compression or extension of wake, stages I and II and REM sleep ( $P = 0.005$ ). In contrast, the total amount of SWS was not affected by restriction or extension of the bedtime period.

Because GH release during sleep was essentially confined to the first 3 h of sleep irrespective of the duration of the scheduled sleep period (Fig. 5), the amounts of each sleep stage were further determined during this time interval (Table 2). As observed for the total sleep period, the amounts of wake and stages I and II were lower during sleep restriction. In contrast, the amount of REM sleep was higher ( $P < 0.02$ ). During the first 3 h of sleep, the amount of SWS was higher during sleep restriction than during sleep extension ( $P < 0.01$ ), because the amount of sleep stage IV, i.e., the deepest stage of sleep, was higher ( $46 \pm 9$  vs.  $31 \pm 6$  min,  $P < 0.03$ ).

Spectral analysis of the EEG further emphasized the fact that SWS was of higher intensity during sleep restriction than during sleep extension. Mean SWA per 20-s epoch was indeed more than twofold higher when total bed time was 4 h than when bed time was 12 h ( $P < 0.02$ ). Unexpectedly, the distribution of SWA during sleep curtailment was not consistent with current models of SWS regulation that predict 1) increased SWA during the first cycle when the waking period is extended and 2) a progressive decrease of SWA with successive sleep cycles. Indeed, as illustrated in Fig. 5, the first SWA cycle was not of higher amplitude during sleep restriction than during sleep extension ( $157 \pm 32$  vs.  $147 \pm 36 \mu V^2$ ). Moreover, during sleep restriction, the amount of delta activity during the second SWA cycle was not lower than during the first SWA cycle (2nd cycle:  $20,485 \pm 6,606 \mu V^2$ ; first cycle:  $21,258 \pm 3,371 \mu V^2$ ). In fact, the major difference in distribution of SWA between the two study conditions concerned the amount of delta activity

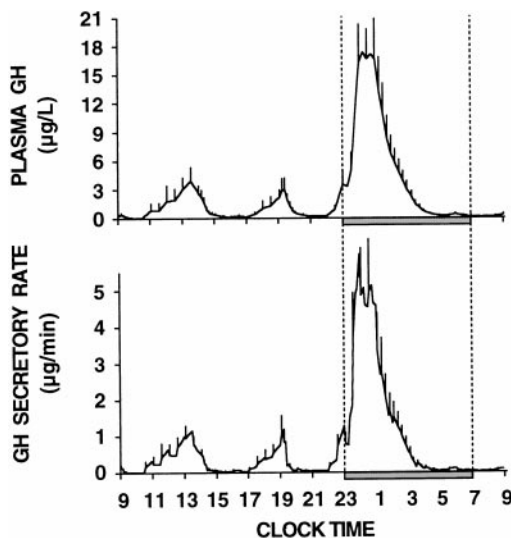


Fig. 4. Mean ( $\pm$ SE) 24-h profiles of plasma GH (*top*) and GH secretory rates (*bottom*) during a baseline condition performed 1 year later in 8 of 11 subjects. The shaded bars represent the 8-h sleep period with bedtimes from 2300 to 0700. Note that the most reproducible and largest GH secretory pulse occurs during early sleep as in the present study during the sleep-extension condition.

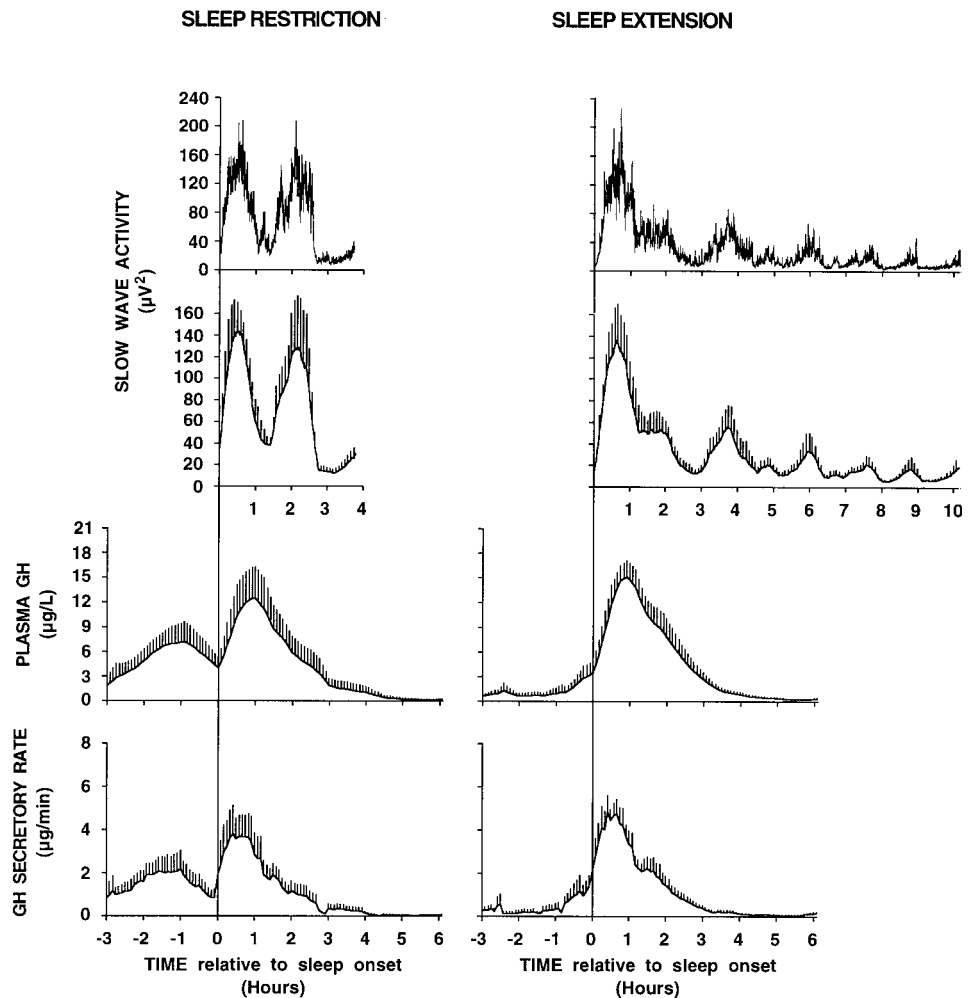


Fig. 5. Mean ( $\pm$ SE) profiles of raw SWA levels, smoothed SWA levels, plasma GH, and GH secretory rates (top to bottom, respectively) referenced to the time of sleep onset during sleep restriction (left) and sleep extension (right). Note that the main effect of the sleep condition on SWA was on the second, rather than the first, SWA cycle.

during the second cycle, which was markedly larger during sleep restriction ( $22,414 \pm 7,164 \mu V^2$ ) than during sleep extension ( $10,719 \pm 1,822 \mu V^2$ ,  $P < 0.03$ ).

#### Relationships Between GH Secretion, SWS, and SWA

Because a previous study involving a standard 8-h bedtime showed that the amount of GH secreted after sleep onset is positively related with the amount of visually scored concomitant SWS (38) as well as with the amount of concomitant SWA (7), we sought to determine whether these relationships persisted during sleep restriction and sleep extension. To control for individual differences in total GH secretion and total SWA, the amounts of GH secreted were expressed in percentage of the total 24-h secretion and, similarly, the amounts of SWS and SWA were expressed in percentage of the total SWS and SWA during the sleep period.

The amount of GH secreted during the first pulse after sleep onset was positively correlated to the amount of concomitant SWS during sleep extension ( $r_S = 0.70$ ,  $P < 0.04$ ), but not during sleep restriction ( $r_S = 0.40$ ,  $P > 0.25$ ). When the intensity, not only the duration, of SWS was examined, the amount of sleep onset GH secretion correlated with concomitant SWA

Table 2. Sleep parameters during the last night of sleep restriction and sleep extension

	Time Allocated to Sleep, h		P Level
	4	12	
Sleep onset latency, min	6 $\pm$ 1	97 $\pm$ 13	< 0.02
Sleep efficiency, %	96.4 $\pm$ 0.6	71.0 $\pm$ 1.9	= 0.005
Total sleep time, min	227 $\pm$ 2	510 $\pm$ 14	= 0.005
<i>Total sleep period</i>			
Amount of wake, min	5 $\pm$ 1	134 $\pm$ 21	= 0.005
Amount of stages I + II, min	99 $\pm$ 7	315 $\pm$ 17	= 0.005
Amount of stages III + IV, min	74 $\pm$ 7	82 $\pm$ 12	NS
Amount of REM sleep, min	54 $\pm$ 5	114 $\pm$ 7	= 0.005
Mean SWA per 20-s epochs, $\mu V^2$	68 $\pm$ 15	30 $\pm$ 5	< 0.02
<i>First 3 h of sleep</i>			
Amount of wake, min	4 $\pm$ 1	17 $\pm$ 5	< 0.01
Amount of stages I + II, min	67 $\pm$ 7	88 $\pm$ 7	< 0.02
Amount of stages III + IV, min	72 $\pm$ 7	51 $\pm$ 6	< 0.01
Amount of REM sleep, min	38 $\pm$ 5	25 $\pm$ 4	< 0.02
Mean SWA per 20-s epochs, $\mu V^2$	83 $\pm$ 19	58 $\pm$ 11	< 0.02

Values are means  $\pm$  SE. REM, rapid eye movement; SWA, slow-wave activity.

during both sleep restriction ( $r_S = 0.67$ ,  $P < 0.06$ ,  $n = 9$ ) and sleep extension ( $r_S = 0.67$ ,  $P < 0.05$ ,  $n = 10$ ).

During sleep restriction, the amount of concomitant SWA was not the sole determinant of sleep onset GH secretion. Indeed, the amount of GH secreted in the first pulse after sleep onset was negatively related to the amount of GH secreted in the pulse preceding sleep onset ( $r_S = -0.86$ ,  $P = 0.01$ ). Relationships between presleep onset and postsleep onset GH secretion were not explored during sleep extension, because, under this condition, the majority of the subjects did not have a presleep onset GH pulse.

Multiple linear regression confirmed that, in the state of sleep debt, the amount of GH secreted in the first pulse after sleep onset was both positively correlated with the amount of concomitant SWA ( $P = 0.07$ ) and negatively related to the amount of presleep onset GH secretion ( $P < 0.01$ ). We also calculated the correlation between presleep onset GH secretion and amount of SWA during the first postsleep onset pulse to determine whether presleep onset GH secretion might have limited SWA in early sleep. The correlation was indeed negative but nonsignificant.

## DISCUSSION

In the state of sleep debt resulting from 1 wk of sleep curtailment achieved by delaying bedtime by 2 h and advancing wake time by 2 h, nocturnal GH secretion followed a biphasic pattern, with a first pulse occurring during wakefulness around the usual time of sleep onset on a standard 2300–0700 bedtime schedule and a second pulse after the onset of sleep. This pattern is at variance with the findings of many previous studies that examined the GH profiles during acute sleep deprivation achieved by delaying bedtimes (10, 29) (also reviewed in Ref. 37). Indeed, the secretory profile that has consistently emerged was characterized by absent or minimal secretion when the waking state was enforced during the usual sleep period, followed by a rebound of secretion during recovery sleep. A typical example of the mean GH profile from 18 normal young men studied in our laboratory during an 8-h period of nocturnal sleep followed by 28 h of continuous wakefulness and an 8-h period of recovery sleep is shown in Fig. 6 (data sources, Refs. 36 and 37). On the basis of such previous evidence, one would predict chronic sleep curtailment to be associated with minimal GH secretion during prolonged wakefulness and a large secretory pulse associated with high levels of SWA after the initiation of recovery sleep (5). Neither the pattern of GH secretion nor the distribution of SWA conformed with these expectations, indicating that adaptation mechanisms that are not apparent during acute sleep deprivation are operative during chronic partial sleep loss.

The mechanisms underlying the increased nocturnal release of GH in the absence of sleep remain to be elucidated. A study with repeated injections of identical doses of GHRH across the 24-h cycle (11) indicated that the evening and early part of the night are

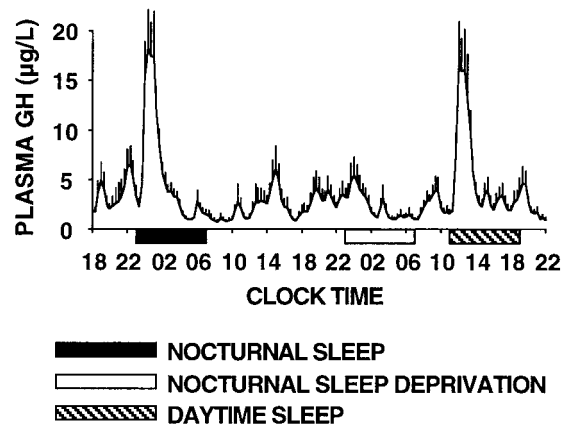


Fig. 6. Mean profile of plasma GH from 18 normal young men studied in our laboratory during an 8-h period of nocturnal sleep (black bar) followed by 28 h of continuous wakefulness, including sleep deprivation during usual bedtimes (open bar) and an 8-h period of recovery sleep (hatched bar). Note that acute sleep deprivation was not associated with a robust GH pulse around the usual bedtime. Data source: Refs. 36 and 37.

associated with increased GH responses, suggesting the existence of an intrinsic circadian variation of somatostatin inhibition independent of the sleep or wake state. Consistent with this concept, several studies indicated that the hours around midnight represent a period of increased propensity to secrete GH, even in the absence of sleep, compared with other segments of the waking period (1, 20, 30, 38, 42). However, this circadian modulation appeared to be of modest magnitude, because the amplitude of GH pulses during nocturnal waking, when detectable, was markedly lower than the amplitude of the sleep onset pulse. The appearance of a GH pulse during late night wakefulness as enforced for 6 consecutive days in the present study may therefore represent a progressive amplification of a circadian variation in somatostatin tone. This interpretation implies that chronic sleep curtailment achieved by advancing wake time only, without delaying sleep time, would be associated with a different pattern of GH release than that observed under the present study conditions. In the present study, the timing of the dinner meal could also have played a role in enhancing presleep GH secretion in the sleep-debt condition. Indeed, a small postprandial GH secretory pulse similar to that observed postbreakfast and postlunch was expected to occur ~4–4.5 h after dinner, i.e., at 2300 to 2330, at the time of the large presleep onset GH pulse. Other mechanisms than reduced nocturnal somatostatin tone, including increased GHRH activity and the involvement of another, as yet unidentified stimulatory pathway for GH secretion that can be activated by the synthetic GH-releasing peptide, could also be involved in facilitating GH secretion during late night wakefulness.

Despite the presence of significant presleep onset GH secretion, a robust sleep-onset GH pulse was nevertheless present after 1 wk of sleep curtailment, and the normal positive correlation with the concom-

itant amount of SWA was preserved. However, this second secretory pulse was not larger than when the subjects were allowed to satiate their sleep need. The finding of a highly significant negative correlation between presleep onset and postsleep onset GH secretion strongly suggests that, during chronic sleep curtailment involving a delay of bedtimes, presleep onset GH secretion partly inhibited postsleep onset GH release. Previous studies have indeed established that sleep-related GH secretion may be inhibited by elevations of waking GH levels (16) with a short time course consistent with the present observations (14). Studies in the rat have indicated that this negative feedback mechanism involves an increase in hypothalamic somatostatin release, which in turn may affect both pituitary GH release and the activity of GHRH neurons (41).

The amount of SWA in the first sleep cycle was also not increased during chronic partial sleep deprivation. This discrepancy with the predictions of current models of SWA homeostasis (5) could also reflect inhibitory effects of presleep onset GH secretion on GHRH-dependent mechanisms involved in the generation of SWS. Thus, although the initial response to the first night of sleep restriction most likely involved an increase in SWA and GH release in early sleep, it appears that in the course of adaptation to chronic sleep loss, increasing amounts of presleep onset GH secretion may have inhibited central GHRH activity, limiting both SWA and GH release in early sleep.

Sleep-onset GH secretion is thought to facilitate the maintenance of stable overnight glucose levels despite the prolonged fasting condition (40). Indeed, studies that have used bolus intravenous administrations of a low dose of synthetic GH to mimic physiological pulsatile release have shown that a primary effect is a rapid decrease in muscular glucose uptake (18, 19). In the present study, after 1 wk of sleep restriction, the biphasic nature of nocturnal GH release resulted in an extended period of elevated GH concentrations compared with fully rested conditions. This extended exposure of peripheral tissues to higher GH levels may have adversely affected glucose regulation. Indeed, as reported elsewhere (31), morning glucose tolerance was significantly decreased after 1 wk of sleep curtailment.

In older adults (20) and depressed patients (17), a presleep onset GH pulse is more commonly observed than in young healthy subjects. Both aging (3) and depression (12) are associated with marked decreases of total sleep duration due to increased sleep fragmentation and advanced morning awakening. The findings of the present study suggest that the alterations in temporal distribution of GH secretion in these conditions could partly reflect the impact of chronic sleep loss.

### Perspectives

Unexpectedly, the distribution of SWA and nocturnal GH secretion in the state of sleep debt did not

conform with the predictions from current theories of SWS homeostasis and with the findings of numerous studies of nocturnal GH secretion during acute sleep deprivation. Indeed, the increase in SWA during chronic sleep loss occurred during the second SWA cycle rather than at the beginning of the sleep period. Nocturnal GH secretion, instead of being consolidated in a single large pulse at sleep onset, was split into two pulses, one before sleep onset (i.e., at the usual bedtime before sleep curtailment) and one after sleep onset. We interpret these observations as reflecting the expression of an inherent circadian rhythmicity of GH release that is amplified in a state of sleep debt and further speculate that the extended duration of exposure of peripheral tissues to elevated GH levels could have played a role in the marked deterioration of glucose tolerance that occurred in our young healthy subjects (31).

These findings also predict that if sleep curtailment had been achieved by advancing wake time, without delaying bed time, a single GH pulse would have been observed after sleep onset, in concomitance with highest levels of SWA during the first SWA cycle. It is possible that this pattern of "early bird" sleep loss may have less severe deleterious consequences for glucose tolerance. In contrast, sleep restriction by delaying bedtime would be associated with a pattern of SWA and GH secretion similar to that observed in the present study and similar alterations of glucose tolerance. Exploring the impact of the timing of curtailed sleep on endocrine and metabolic alterations may have important implications for millions of individuals involved in shift work.

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