CLINICAL RESEARCH ARTICLE

Acute sleep disruption does not diminish pulsatile growth hormone secretion in pubertal children

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Short Title: Sleep disruption and growth hormone

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ABSTRACT

Study Objectives

In children, growth hormone (GH) pulses occur after sleep onset in association with slow wave sleep (SWS). There have been no studies in children to quantify the effect of disrupted sleep on GH secretion. This study aimed to investigate the effect of acute sleep disruption on GH secretion in pubertal children.

Methods

Fourteen healthy subjects (aged 11.3-14.1 yrs) were randomized to two overnight polysomnographic studies, one with and one without SWS disruption via auditory stimuli, with frequent blood sampling to measure GH.

Results

Auditory stimuli delivered during the disrupted sleep night caused a 40.0 ± 7.8% decrease in SWS. On SWS disrupted sleep nights compared to the undisrupted night, the rate of GH pulses during N2 sleep was significantly lower than during SWS (IRR=0.56, 95% CI: 0.32-0.97) but there were no differences in SWS and the other sleep stages. SWS sleep disruption had no effect on GH pulse amplitude and frequency or basal GH secretion.

Conclusion

In pubertal children, GH pulses were temporally associated with episodes of SWS. Acute disruption of sleep via auditory tones during SWS did not alter GH secretion. These results indicate that SWS may not be a direct stimulus of GH secretion.

Keywords: sleep, growth hormone, puberty, children, slow wave sleep

INTRODUCTION

During childhood, growth hormone (GH) plays a critical role in glucose homeostasis and linear growth. GH also contributes to bone mineral density, lean body mass, lipoprotein metabolism, and cardiovascular health (reviewed in (1)) across the lifespan. The neuroendocrine control of GH secretion is complex and involves the interplay of multiple neurotransmitters and hormones, including growth hormone releasing hormone (GHRH), somatostatin (SS), ghrelin, catecholamines, dopamine, and excitatory amino acids (2).

Sleep is also a powerful stimulus for GH secretion. In the 1960's, Takahashi et al. reported an association between the onset of deep sleep (also known as slow wave sleep; SWS) and GH pulse initiation in a small group of adult men (3); this temporal relationship was confirmed in more recent studies with frequent (every 30 second) blood sampling in healthy men that showed that maximal GH release occurs within five minutes of the onset of SWS (4). Sleep-associated GH pulses also occur in women, but GH in women is predominantly secreted during the daytime (5,6). Studies in children have similarly demonstrated GH secretory episodes during bouts of SWS (7), suggesting that sleep-state specific augmentation of GH secretion is also a feature of normal development.

While the temporal coincidence of bouts of SWS and GH pulses has now been firmly established, the few studies that have investigated the possibility of a *functional* relationship between SWS and GH secretion have been limited to adult male subjects and have produced mixed results. For example, while pharmacological augmentation of SWS with the GABA-agonist gamma-hydroxybutyrate caused a proportionate and contemporaneous increase in GH levels (8), SWS deprivation studies in adults have not consistently produced deficits in GH secretion (9-12).

Given the rising incidence of restricted sleep among adolescents (13) as well as sleep disorders such as obstructive sleep apnea (14) that cause sleep disruption, it is critical to determine whether sleep disruption during childhood may dysregulate the somatotropic axis and possibly alter linear growth and body composition.

To investigate whether undisrupted sleep is critical for GH secretion in children, we studied healthy children with and without SWS disruption using controlled auditory stimuli.

MATERIALS and METHODS

Participants

The 14 participants were pubertal children (7 male, 7 female), aged 11.3-14.1 years, whose clinical characteristics and reproductive and metabolic profiles have been previously reported (15,16). Subjects were euthyroid and were not on any medication known to interfere with sleep, growth, or puberty. The subjects had no known sleep disorder and underwent two sleep studies spaced two months apart, one with and one without SWS disruption, in random order (28 studies in total). GH secretion was a secondary outcome in these studies that were primarily powered to detect a significant change in LH secretion after sleep disruption.

The studies were approved by the Partners Human Research Committee at Massachusetts General Hospital. Signed informed assent and consent was obtained from each subject and one parent, respectively. These studies were not registered at clinicaltrials.gov because they do not meet the definition of an "applicable clinical trial".

Experimental protocol

All overnight studies were conducted at the Clinical Research Center of the Massachusetts General Hospital. They included frequent blood sampling and PSG (15-17). PSG was performed according to standard methodology using an electro-encephalogram (total of 6 frontal, central and occipital leads), electro-oculogram, electrocardiogram, and pulse oximetry recordings (ALICE LE PSG system, Sleepware software, Phillips Respironics). An intravenous catheter with a long line was inserted upon admission to allow for blood sampling from outside the room while the subject slept. All subjects ate dinner before lights out. Caffeine was prohibited. PSG recording began before lights out and continued until spontaneous awakening the following morning. Lights were turned off between 9:00 and 10:30 pm, based on subject and parent reports of habitual bedtime. Blood samples were drawn every 10-minutes for 8 hours starting at or just before sleep onset. During SWS

disruption nights, auditory stimuli (3 sec, 1500 Hz tones, 40-100 dB followed by 18 sec of a 75 dB noise simulating a knock on the door) were delivered via a bedside speaker whenever the subject entered SWS (defined as ≥ 2 delta waves on EEG in a 15-sec recording interval) as determined by a registered polysomnographic sleep technician (rPSGT).

All samples were analyzed for GH using a quantitative sandwich enzyme immunoassay (Quantikine, R&D Systems, Minneapolis, MN, RRID: AB_2923238) that utilizes antibodies raised against the full-length 22 kDa GH isoform. The minimum detectable concentration (MDC) was 0.64 pg/ml with an inter-assay coefficient of variation (CV) of 7.1% for quality control serum (QCS) containing 1.3 ng/ml.

Data analysis

PSG recordings were visually scored by a rPSGT according to American Academy of Sleep Medicine criteria (18) in 30-sec epochs as stages of non-REM [(N1, N2, and N3 (i.e., SWS)], REM, or Wake. Sleep onset was defined as the first appearance of two consecutive 30-sec sleep epochs after Wake

The basal secretory rates and timing and amplitude of individual GH pulses were determined using a sparse deconvolution method that assumes a second order pharmacokinetic model for GH dynamics (19).

Five statistical analyses were performed: (i) Differences in sleep stages and GH secretion between the two study nights (with vs. without SWS disruption) were compared using paired t-tests or the Wilcoxon signed rank test. (ii) The associations of age, sex, and BMI percentile to GH pulse frequency and amplitude was determined using ANOVA (sex) or Pearson correlation coefficients (age, BMI percentile). (iii) The relationship between GH pulse rate per hour and sleep stage was evaluated using Poisson generalized estimating equations (GEE) with an unstructured covariance matrix for undisrupted and disrupted sleep nights separately to account for the repeated nature of the observations. These models were estimated using pulse count as the outcome and included an offset for time in each sleep stage to produce incidence rate ratio (IRR) estimates. Due to the small sample size, these models were not adjusted for potential confounders. (iv) The effect of SWS

disruption on the relationship between GH pulse onset and sleep stage was determined for each sleep stage or during wake after sleep onset (WASO) using Poisson GEE models restricted to each respective sleep stage. Data are expressed as mean ± SE unless otherwise indicated, and $P < 0.05$ is considered significant.

RESULTS

Baseline Characteristics

Seven pubertal girls (mean age 12.5 years ± 0.6 SD; Tanner II-IV breasts; all pre-menarchal) and 7 pubertal boys (mean age 12.9 years ± 1.0 SD; testicular volumes 4-15 cc) participated (**Table 1**). Subjects were predominantly (71.4%) non-Hispanic White and 28.6% were overweight or obese. There were no changes in subjects' Tanner stages or BMI percentiles between study visits.

Effect of Slow Wave Sleep (N3) Disruption on Sleep Stages

An average of 77.5 ± 13.6 (range 23-190) auditory stimuli were delivered to interrupt SWS (N3) during the night of sleep disruption (n=14 studies). By design, this intervention caused a 40.0 ± 7.8% decrease in the amount of time spent in SWS (N3) and a 30.7 ± 7.4% increase in the amount of time spent in lighter sleep stages (N1+N2) relative to the night of undisrupted sleep, whereas there was no change in %WASO or %REM sleep (**Figure 1, Figure 2**).

Pulsatile Growth Hormone Secretion During Sleep and Effects of SWS Sleep Disruption

During undisrupted sleep nights, subjects had 7.36 ± 0.58 GH pulses during the 8-hour sampling interval with an average pulse amplitude of 10.55 ± 1.22 ng/mL and average basal secretion rate of 0.29 ± 0.14 ng/ml per min. During SWS disrupted sleep nights, subjects had 6.29 ± 0.54 pulses with an average pulse amplitude of 9.69 ± 1.08 ng/mL and average basal secretion rate of 0.39 ± 0.11 ng/ml per min. There were no differences in GH secretory parameters in participants studied with vs. without SWS disruption (**Figure 3**). Participant age (undisrupted r=0.30, p=0.29; disrupted r=0.36, p=0.21), sex (undisrupted F=0.78, d.f.=1, p=0.39; disrupted F=0.10, d.f.=1, p=0.76), and BMI percentile (undisrupted r=-0.003, p=0.99; disrupted r=-0.15, p=0.61) were not related to GH pulse frequency in the undisrupted or SWS disrupted studies. Age (undisrupted r=0.44, p=0.11;

disrupted r=0.3, p=0.29) and BMI percentile (undisrupted r=0.19, p=0.53; disrupted r=-0.06, p=0.84) were also not related to average GH pulse amplitude. Sex was significantly related to average GH pulse amplitude on undisrupted (F=12.3, df=1, p=0.004) but not SWS disrupted nights (F=2.35, df=1, p=0.15), with boys having a higher average GH pulse amplitude (15.29 ng/mL, 95% CI: 8.30-28.18) than girls (3.58 ng/mL, 95% CI: 1.94-6.59) on the undisrupted nights.

Growth Hormone Secretion and Relationship to Sleep Stage

On nights without SWS disruption, GH pulses occurred at a rate of 1.4 (95% CI: 0.82, 2.40) pulses/hour of SWS, which was not significantly different than during any other sleep stages or bouts of WASO (all p-values >0.05) (**Table 2)**. During SWS sleep disruption nights, GH pulses occurred at a rate of 1.14 (95% CI: 0.82, 1.59) pulses/hour of SWS, but this was only statistically significant in comparison to the rate during N2 sleep (IRR=0.56, 95% CI: 0.32, 0.97) (**Table 2**). SWS disruption nights (compared with undisrupted nights) did not have different GH pulse rates during the various sleep stages or WASO (**Table 3**).

DISCUSSION

The current studies demonstrate that significant SWS disruption during one night does not diminish GH secretion in pubertal children; a 40% reduction in SWS did not affect basal or pulsatile GH amplitude or frequency in boys or girls. While this study is not without limitations (discussed below), this finding was unexpected given the traditional view that unperturbed SWS (i.e., deep sleep) is an important physiologic stimulus for GH secretion. Indeed, clinical research studies dating back to the 1960's have demonstrated that there is a large amount of GH secreted during the first entry into SWS following sleep onset and that the GH peak is delayed when sleep onset is delayed (3,12,20,21). Further, more recent sophisticated frequent (every 30 second) sampling studies with deconvolution of GH secretion and simultaneous PSG measurement (4) confirmed the temporal association between GH pulsatile secretion and episodes of SWS during undisrupted sleep in adults.

The large body of literature reporting a coincidence of GH secretion with SWS has led to the assumption that SWS is a direct stimulus of GH secretion. This concept has been well-received perhaps because it is consistent with the hypothesis that one of sleep's major functions is to facilitate anabolic processes such as linear growth. However, studies designed to demonstrate a functional relationship between SWS and GH secretion have produced mixed results. In relatively small studies conducted in adult men (n=2-10 per study) (9-12), SWS deprivation using either auditory or electrical stimuli did not consistently produce a decrement in GH secretion compared with baseline nor an increase in GH secretion during recovery nights, a time when there is typically a rebound in the amount of SWS. The current studies were also limited by a relatively small sample size, which prevented the adjustment for potential confounders, with heterogeneous characteristics (i.e., both boys and girls, different stages of puberty), and the study may have been underpowered to detect a small change in GH parameters since GH secretion was not the primary outcome of the study. In addition, the study design did include an adaptation night and did not prevent all SWS, rather it significantly shortened each episode of SWS. Given these limitations and the exploratory nature of this study, further investigation in a larger population is needed to confirm our findings.

Conversely, pharmacologic augmentation of SWS with gammahydroxybutyrate (GHB), a metabolite of gamma-aminobutyric acid (GABA), caused a proportional increase in GH secretion, raising the possibility that SWS directly stimulates GH secretion (8). However, as noted by those authors, GHB acts on multiple neurotransmitter systems (GABAergic, cholinergic, serotoninergic, and dopaminergic) and thus, it is possible that co-induction of SWS and GH secretion occurred through two, independent neural pathways. The observation that aging is associated with proportional declines in SWS and GH secretion has also been put forth as evidence of a functional relationship between SWS and GH (22). However, as noted by Feinberg (23), this relationship does not hold true during adolescence when there is an inverse relationship between GH and SWS: GH secretion is highest during puberty whereas there is a dramatic decline in delta frequency power in the EEG during puberty (large amplitude delta frequency waves in the EEG are a component of SWS). Finally, Van Cauter et al. reported that the longer an episode of SWS, the more likely it was to be associated with GH

secretion (24). However, this analysis did not control for the greater opportunity for GH secretion to occur over a longer duration of time, regardless of the sleep stage.

The ability of the current and previous studies to dissociate GH secretion from SWS suggest that SWS itself may not directly stimulate GH secretion. Rather, there may be a common upstream neural or hormonal signal that can, on occasion, simultaneously induce SWS and GH secretion. Therefore, interventions to disrupt SWS, as in the current protocol, would be acting downstream of this signal and would be incapable of interfering with signal transduction to the somatotrophs. There is anatomic and physiologic evidence from the rodent to suggest that growth hormone-releasing hormone (GHRH) is the signal that temporally links SWS and GH: GHRH neurons in the paraventricular nucleus (PVN) increase GHRH expression in response to sleep deprivation (25); GHRH directly activates GABA-ergic, sleep-active neurons in the preoptic area (26); and intra-preoptic injection of GHRH or a GHRH antagonist to rats increases or decreases SWS, respectively (27). GHRH neurons in the arcuate nucleus provide the primary input to the median eminence (28), driving pituitary GH secretion. Thus, one possibility is that GHRH neurons in the arcuate and PVN can, at times, coordinate GHRH secretion, resulting in the simultaneous induction of SWS and GH secretion.

An alternative interpretation of the current findings is that SWS does, in fact, stimulate pulsatile GH secretion but that there is no specific requirement that SWS be consolidated into discrete, long bouts, as typically occurs during normal sleep. That is, brief, fragmented bouts of SWS may be sufficient to trigger GH secretion during childhood. This hypothesis would predict that children with disturbed sleep from internal (e.g., untreated sleep apnea) or external (e.g., noise pollution) reasons should have normal linear growth; further studies are necessary to confirm that SWS disruption, when present chronically, does not diminish GH secretion during childhood. The current studies, however, demonstrate that SWS disruption, when it occurs acutely via auditory stimuli, does not interfere with normal pulsatile GH secretion in pubertal children.

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DATA AVAILABILITY

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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FIGURE AND TABLE LEGENDS

Table 1. Subject demographics. Abbreviations: F, female; M, male. [a]Age- and sex-adjusted BMI percentile greater than 85 is classified as overweight and greater than 95 is classified as obese in children. [b]Tanner breast stage or testicular volume. All girls were premenarchal.

Table 2. Results of GEE Poisson models for GH pulse frequency as a function of sleep stage or wake after sleep onset during 'undisrupted sleep' and 'SWS disrupted sleep' studies. The intercept represents the GH pulse frequency per hour of SWS (i.e., N3). The other estimates in the table represent incident rate ratios of the GH pulse rate during that sleep stage in comparison to SWS sleep. For example, on the undisrupted night, the GH pulse rate during N1 sleep is 0.59 times the rate of GH pulses during SWS. Abbreviations: WASO, wake after sleep onset; SWS, slow-wave sleep.

Table 3. Tests of sleep disruption's effect on the GH pulse rate for each specific sleep stage and wake after sleep onset in the minimally adjusted models, shown with IRRs comparing the disrupted night to the undisrupted night and corresponding 95% confidence intervals. Abbreviations: WASO, wake after sleep onset; SWS, slow wave sleep; IRR, incidence rate ratio; CI, confidence interval.

Figure 1. Percent of time spent in each sleep stage in studies with or without slow wave sleep (SWS) disruption. WASO = wake after sleep onset. Boxplots indicate median, interquartile range, minimum, and maximum.

Figure 2. Polysomnogram (top) and pulsatile GH secretion (bottom) in two subjects, A and B, studied with (left) or without (right) slow wave sleep (SWS) disruption. GH levels represent model estimates. Vertical lines with circles indicate model-estimated timing and amplitude of GH pulses.

Figure 3. GH secretory parameters in participants studied with vs. without slow wave sleep (SWS) disruption. Boxplots indicate median, interquartile range, minimum, and maximum values for GH pulse frequency.

Table 1

subject	age	sex	BMI (kg/m2)	BMI%ile[a]	Pubertal stage[b]
1	11.6	F	16.5	29	III
2	12.3	F	19.2	70	II
3	12.3	F	19.8	73	III
4	12.3	F	17.9	44	III
5	12.5	F	18.6	53	III/IV
6	13.2	F	25.0	94	III
7	13.4	F	20.5	68	III
8	11.3	M	26.1	97	5cc
9	12.0	M	22.9	92	4cc
10	12.2	M	29.3	97	8cc/6cc
11	13.4	M	17.9	37	15cc
12	13.4	M	17.9	37	15cc
13	13.8	M	18.7	46	15cc
14	14.1	M	21.6	78	15cc

Table 2

	Undisrupted sleep			SWS disrupted sleep		
Sleep stage	Incidence Rate Ratios	CI	p	Incidence Rate Ratios	CI	p
Intercept	1.40	0.82-2.40	0.22	1.14	0.82-1.59	0.42
N1 sleep stage	0.59	0.30-1.15	0.12	0.71	0.31-1.64	0.42
N2 sleep stage	0.49	0.21-1.11	0.09	0.56	0.32-0.97	**0.04**
REM sleep stage	0.58	0.32-1.06	0.08	1.07	0.78-1.46	0.69
WASO	0.32	0.09-1.13	0.08	0.66	0.36-1.20	0.17

Table 3

Sleep stage	IRR (95%CI)	p
SWS	0.71 (0.42, 1.23)	0.22
N1	1.13 (0.47, 2.69)	0.79
N2	1.02 (0.71, 1.46)	0.92
REM	1.02 (0.65, 1.58)	0.95
WASO	0.86 (0.36, 2.01)	0.72