Altered sleep patterns in patients with non-functional GHRH receptor

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Abstract

Objectives: GH-releasing hormone (GHRH) exerts hypnotic actions increasing the non-rapid eye movement (NREM) sleep. Conversely, GH stimulates the REM sleep. GH deficiency (GHD) often leads to sleep problems, daytime fatigue and reduced quality of life (QoL). GHD may be due to lack of hypothalamic GHRH or destruction of somatotroph cells. We have described a cohort with isolated GHD (IGHD) due to GHRH resistance caused by a homozygous *null* mutation (c.57+1G>A) in the GHRH receptor gene. They have normal QoL and no obvious complaints of chronic tiredness. The aim of this study was to determine the sleep quality in these subjects.

Methods: A cross-sectional study was carried out in 21 adult IGHD subjects, and 21 age- and gender-matched controls. Objective sleep assessment included polygraphic records of the awake, stages NREM [N1 (drowsiness), N2 and N3 (already sleeping)] and REM (R). Subjective evaluation included the Pittsburgh Sleep Quality Index, the Insomnia Severity Index and the Epworth Sleepiness Scale.

Results: IGHD subjects showed a reduction in sleep efficiency ($P=0.007$), total sleep time ($P=0.028$), duration of N2 and R in minutes ($P=0.026$ and $P=0.046$ respectively), but had increased duration and percentage of N1 stage ($P=0.029$ and $P=0.022$ respectively), wake ($P=0.007$) and wake-time after sleep onset ($P=0.017$). There was no difference in N3 or in sleep quality questionnaire scores.

Conclusion: Patients with IGHD due to GHRH resistance exhibit objective reduction in the sleep quality, with changes in NREM and REM sleep, with no detectable subjective consequences. GHRH resistance seems to have a preponderant role over GHD in the sleep quality of these subjects.

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Introduction

Humans spend approximately one-third of their life sleeping (1). Human sleep is characterized by cyclic occurrence of periods of non-rapid eye movement (NREM) sleep, nearly three-quarters of the sleep, and the REM sleep (2, 3). Body size is important for environmental adaptation. Body size and sleep architecture are evolutionary regulated by reciprocal neuronal and humoral mechanisms (4). The relationship between sleep and stature is complex, and may include hormonal, metabolic and respiratory issues.

Sleep problems coexists with short stature in several conditions such as hypothyroidism, achondroplasia, mucopolysaccharidoses, fragile X, Down's and Prader–Willi syndromes (5). Down's syndrome subjects have low sleep efficiency, increased percentage of wakefulness after sleep onset (WASO), increase of the stage N1 (drowsiness) of NREM sleep and reduction of REM sleep (6). Patients with Prader–Willi syndrome have alteration in REM sleep, obstructive sleep apnea syndrome and severe

daytime sleepiness (7, 8, 9). Few studies in children with GH deficiency (GHD) suggest sleep fragmentation with decrease in total sleep time (TST), sleep efficiency and REM sleep and increase in N1 and WASO (10, 11).

Adult-onset GHD (AOGHD) individuals often complain of impaired quality of life (QoL) (12, 13, 14) with frequent daytime fatigue associated to sleep problems, suggesting a relation between sleep and the somatotrophic axis. Both GH and GH-releasing hormone (GHRH) secretion and action seem to be important in regulating sleep pattern (15, 16). Hypothalamic GHRH, together with interleukin 1B, tumor necrosis factor-alpha, and prostaglandin D, stimulates NREM sleep (17). GHRHergic neurons of the arcuate nucleus are involved in GH release from the pituitary, and the ones of the hypothalamus/preoptic region (18) in NREM sleep regulation (3, 4, 19). GHRH has hypnotic actions by increasing NREM sleep (15, 20, 21), even in the absence of GH, which may stimulate the REM sleep (22, 23, 24, 25). Therefore, sleep disturbance may differ depending from the origin of GHD: in pituitary disease, there is potentially excessive compensatory hypothalamic GHRH activity, whereas in hypothalamic disorders GHRH activity is insufficient (12). Hypophysectomized rats after GHRH injection show an increase in NREM, but not in REM sleep (22). In mice with non-functional GHRH receptor (GHRHR) (little mouse) the duration of recovery after sleep deprivation is reduced (18). Similarly, inhibition of brain GHRH by GHRH antibodies inhibits spontaneous NREM sleep in rats (26, 27). In agreement with animal data, Copinschi *et al.* have shown that patients with GHD due to pituitary disease (with supposedly increased GHRH activity) spend more time in NREM sleep than their controls (12).

AOGHD individuals have often gonadal, adrenal and thyroid deficiency, which themselves (or their replacement therapy) may alter the quality of the sleep. In addition, some AOGHD patients have undergone brain surgery (and possibly receive anticonvulsants) or radiotherapy, all potentially able to influence the brain activities and sleep (28). Therefore, to assess the consequences of GHD on sleep, it would be ideal to have subjects with isolated GHD (IGHD). Few studies have investigated sleep quality in IGHD patients, and little is known about how sleep is altered in chronic GHRH deficiency (10, 29). In Northeastern Brazil, we have described a cohort of patients with IGHD due to GHRH resistance caused by a homozygous null mutation (c.57+1G>A) in the GHRHR gene (*GHRHR*) (30). The adult IGHD individuals from this cohort who have not received GH replacement therapy have normal longevity (31) and QoL, and no obvious

complaints of chronic tiredness (32). The aim of this study was to determine objectively and subjectively the sleep quality in these subjects.

Subjects and methods

Subjects

In a cross-sectional study, adult IGHD GH-naïve subjects, and age and sex-matched controls were recruited by the local Dwarfs Association, among inhabitants of Itabaianinha by a posted add and by word of mouth. Inclusion criterion for IGHD was genotype-proven homozygosity for the (c.57+1G>A) *GHRHR* mutation, whereas controls were normal statured individuals homozygous for the wild-type *GHRHR* allele. Exclusion criteria were: previous GH treatment, age under 18 and above 65 years, alcoholism or drug abuse, pregnancy, lactation, recent history (3 months) of serious illnesses or hospitalization, major psychiatric disorders, shift work, renal failure, chronic liver diseases, malignant neoplasms and current treatment with glucocorticoids, or medications that might affect their sleep. Twenty-one (10 males) IGHD and 21 (10 males) normal statured individuals volunteered and were enrolled. Individuals were transported by car in groups of three to Aracaju (approximately 150km from Itabaianinha) in the afternoon, and were admitted to a specialized clinic at 1830h. They received a standard meal at 2000h, and were allowed to walk within the unit until 2300h, when lights were turned off, and the room was insulated from light. They were allowed to wake up spontaneously, and the exam was considered sufficient when there was a record of at least 6h of sleep. The local sunrise during the realization of the protocol was at 0600h. The Federal University of Sergipe Institutional Review Board approved these studies and all subjects gave written informed consent.

Questionnaires for the assessment of sleep quality

The Pittsburgh Sleep Quality Index (PSQI), the Insomnia Severity Index (ISI), and the Epworth Sleepiness Scale (ESS) were translated into Portuguese. They were administered before the sleep studies and filled by the same investigator (F T O), as some individuals in both groups were illiterate. PSQI is an adult validated 19-item instrument grouped in seven components: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications and daytime dysfunction over the last month. Each component has a

range of zero to 3, and the sum of all the fields provides a global PSQI ranging from 0 to 21, with the highest score indicating poorer sleep quality. A global PSQI greater than 5 has 89.6% sensitivity and 86.5% specificity to identify patients with poor sleep quality versus those with good quality (33). ISI assesses the severity of insomnia perceived in the previous 2 weeks, consisting of seven items, each ranging from 0 (no symptoms) to 4 (severe symptoms). Total score categories were thus coded, 0 to 7, no clinical significant insomnia; 8 to 14, sub-threshold insomnia; 15 to 21, moderate insomnia; 22 to 28, severe insomnia (34). The Epworth Sleepiness Scale (ESS) is a self-reported score that measures daytime sleepiness as well as the likelihood of falling asleep in eight situations, scored from 0 (the least sleepy) to 24 (the most sleepy). Scores equal to or above 10 are consistent with excessive daytime sleepiness (35).

Polysomnography (PSG)

Nocturnal, laboratory based polysomnography (PSG) was carried out under standardized conditions (Fast-Poly 26i 26-Channel Digital Polygraph, São Paulo, Brazil). This included electroencephalography (EEG) to monitor sleep stage, electrooculography (EOG) to monitor eye movements, electrocardiography (ECG), surface submental electromyography (EMG) to record atonia during REM sleep, measurement of chest wall and abdominal breathing movements, and transcutaneous oxygen saturation (36). Sleep was divided into periods of 30s, and the sleep stages were scored according to the American Academy of Sleep Medicine Manual for the Scoring of Sleep and Associated Events (AASM) (37), in wake (W), NREM: N1, drowsiness; N2, characterized by theta EEG frequency, being the largest percentage of total sleep; N3, slow wave sleep; and REM (R), characterized by rapid eye movements and atonia (1). The following variables were analyzed:

- Total recording time (TRT), in minutes: time from lights-out to light-on.
- Sleep latency (SL), in minutes: time from lights-out to first epoch of any sleep.
- Total sleep time (TST), in minutes: the sum of sleep stages (N1, N2, N3, R).
- Sleep efficiency (SE): the percentage of the TST in relation the TRT.
- REM latency, in minutes: time from the start of the first epoch of any sleep until the start of the first epoch of stage R.
- Wake (W), in minutes: all the wake minutes during TRT.
- Wake after the sleep onset (WASO), in minutes: all the stage W from the start of the first epoch of sleep until lights-on.
- Time in each sleep stage (N1, N2, N3 and R) in minutes and percentage of TST.
- Arousal index: total number of arousals $\times 60$ /TST in minutes.

Abnormal breathing events during sleep as apnea or hypopnea were recorded and the total number of events per hour was the apnea-hypopnea index (AHI). AHI higher than 5 per hour defines obstructive sleep apnea syndrome (OSAS). The number of times per hour of sleep in which the oxygen saturation decreases of or greater than 3% corresponds to the oxygen desaturation index (ODI) (2, 38).

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Statistical analysis

Data are expressed as mean (standard deviation), except for sleep latency, TST, sleep efficiency, wake, WASO, N1/TST (%), N2/TST (%), N2 in minutes, R/TST (%), oxygen desaturation index (ODI) expressed as median (interquartile range). Student's *t*-test was used for variables with normal distribution, and Mann-Whitney *U* test for variables without Gaussian distribution. We performed a median split division of the whole group in younger (21 individuals under the age of 41 years) and older (21 individuals equal or above the age of 41 years). A further analysis by gender (22 women and 20 men) was also fulfilled. Statistical analysis was performed using the statistical software SPSS 19.0 version. *P* values under 0.05 were considered significant.

Results

Table 1 provides the demographic and subjective sleep questionnaires' quality data. The two groups have similar age and gender distribution. Only one subject in each group was older than 60 years. As expected, weight and

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Table 1 Anthropometric measures and scores of sleep quality assessed by the Pittsburgh Sleep Quality Assessment (PSQI), Insomnia Severity Index (ISI) and the Epworth Sleepiness Scale (ESS) in isolated GH deficiency (IGHD) subjects and controls. Data are presented as mean (S.D.).

	IGHD (n=21)	Controls (n=21)	P
Age (years)	43.5 (13.6)	42.52 (12.6)	0.8
Sex (male)	10	10	1.0
Weight (Kg)	39.3 (9.2)	76.4 (15.5)	<0.0001
Stature (meter)	1.25 (0.08)	1.68 (0.08)	<0.0001
Body mass index (Kg/m ²)	24.8 (5.6)	26.7 (4.8)	0.27
PSQI	6.29 (3.97)	6.24 (3.89)	0.969
ISI	6.86 (5.22)	7.86 (5.49)	0.549
ESS	7.29 (3.54)	8.95 (5.16)	0.230

stature were lower in IGHG. Both IGHG and control subjects had a PSQI of greater than 5, without significant difference between the groups. Questionnaire data were not influenced by age and sex.

Table 2 shows the sleep parameters. In comparison to the control group, IGHG subjects exhibited reduced sleep efficiency ($P=0.007$), total sleep time ($P=0.028$) and duration of N2 and R in minutes ($P=0.026$ and $P=0.046$ respectively). The percentage of R/TST also tended to be lower ($P=0.087$). Conversely, IGHG group had an increase in the duration of N1 stage and in the percentage of N1/TST ($P=0.029$ and $P=0.022$ respectively). Wake was longer in IGHG ($P=0.007$) probably due to the larger WASO ($P=0.017$). There was no difference in percentage and minutes of stage N3 between two groups. The differences between IGHG and controls persisted only in the older group (41–65 years of age). In this age group, the duration of N3 was lower in IGHG than that in controls: 39 (26.9) vs 62.4 (23.5) min, $P=0.049$. Regarding gender, the reduction of sleep efficiency, total sleep time and duration of N2 were only maintained in males. The increase in N1 in minutes and N1/TST was found in both genders. The difference in N1/TST was maintained only in females, but the significance for N1 in minutes was lost in both genders when they were analyzed individually, although there was a tendency toward a N1/TST difference in men ($P=0.08$). The reduction of R was similarly significant in both genders.

Discussion

Sleep is a rapidly reversible state of reduced responsiveness, motor activity and metabolism. Multiple theories exist

Table 2 Polysomnography parameters in 21 isolated GH deficiency (IGHG) subjects and 21 control subjects. Data are expressed as mean (S.D.), except for sleep latency, total sleep time (TST), sleep efficiency, stage wake, WASO, N1/TST (%), N2/TST (%), N2 (min), R/TST (%), oxygen desaturation index (ODI) expressed as median (interquartile range).

	IGHG	Controls	P
TRT (min)	451.0 (23.2)	442.9 (31.4)	0.954
Sleep latency (min)	16.0 (24.0)	9.0 (25.5)	0.623
TST (min)	330.0 (127.5)	385.5 (56.0)	0.028
Sleep efficiency (%)	77.7 (27.5)	87.5 (10.1)	0.007
REM sleep latency (min)	157.6 (95.2)	118.5 (44.5)	0.099
Stage wake (min)	96.5 (123.5)	54.3 (51.7)	0.007
WASO (min)	82.5 (116.5)	44.0 (46.5)	0.017
N1 (min)	51.3 (33.7)	31.8 (19.8)	0.029
N1/TST (%)	14.6 (18.9)	7.3 (8.1)	0.022
N2 (min)	190.0 (81.7)	220.0 (43.2)	0.026
N2/TST (%)	57.7 (15.6)	57.2 (8.9)	0.296
N3 (min)	51.1 (36.5)	61.9 (20.8)	0.247
N3/TST (%)	15.5 (9.5)	16.3 (4.8)	0.718
R (min)	45.6 (27.3)	61.5 (22.8)	0.046
R/TST (%)	11.9 (8.1)	14.7 (7.9)	0.087
Arousal index (/h)	12.2 (9.8)	14.7 (10.3)	0.437
ODI (events/h)	3.5 (8.9)	5.6 (11.9)	0.296
Apnea hypopnea index (/h)	10.2 (10.7)	13.0 (12.4)	0.446

TRT, total recording time; TST, total sleeping time spent in stages N1, N2, N3 and R; Sleep efficiency, the percentage of the TST in relation to the TRT; WASO, wake after the sleep onset; arousal index, total number of arousals $\times$ 60/TST in minutes; ODI, number of events per hour of sleep in which the oxygen saturation decreases by or more than 3%.

about its role, including restoration, energy conservation and memory consolidation (39). Within the last decade, GHRH has been put forward as a putative sleep-enhancing substance (40), extending NREM-time in rats and rabbits (41). Nevertheless, human studies are conflicting; some studies revealing significant increase in NREM after GHRH administration in healthy humans, whereas other studies have not confirmed this effect (20, 21, 42).

We here report our findings of sleep quality in subjects with IGHG due to GHRH resistance. The main findings of this work are the reduction in sleep efficiency, total sleep time and total minutes of stages N2 and REM. Furthermore, IGHG group showed an extension of the drowsiness period (percentage and minutes of N1 stage) and of the wakefulness probably due to the larger WASO in adult individuals with a null homozygous (C.57+1G>A) *GHRHR* mutation. These data suggest a shorter and more fragmented sleep in these subjects. However, this seems not to have subjective consequences, as these changes were not reflected in differences in quality of sleep questionnaires. Other studies had shown an increased

global PSQI in GHD, but these patients had associated multiple pituitary hormonal deficiency (12, 28) and confounders such as previous brain surgery, radiotherapy and hormonal replacement therapies may also play a role. The congenital nature of IGHD in our subjects may allow for adaptation mechanism(s) that may not be present in acquired GHD.

The reduction in sleep efficiency and total sleep time fits with data showing a NREM sleep-promoting activity of GHRH mediated by the hypothalamus/preoptic region. Rats and mice with deficiencies of GHRH signaling sleep less than normal animals (43). For example, little mice (also lacking a functional GHRHR) have less spontaneous NREM and REM sleep, do not respond to GHRH and chronic GH infusion restores REM but not NREM sleep (18). Accordingly, our patients have lower duration in minutes of the stages N2 of NREM and in REM sleep. Normal human subjects after sleep deprivation, the administration of GHRH reduces N1 stage and improves sleep efficiency (44). There is scant data, raised from very small number of patients with pituitary macroadenomas and hypothalamic involvement exhibiting shorter sleep duration, sleep fragmentation and disturbed circadian movement rhythms, possible via alteration of suprachiasmatic nucleus (28, 45). This hypothalamic nucleus is the major regulator of sleep-wake rhythmicity and is located close to the optic chiasm (28). Indeed, patients with hypothalamic GH deficiency secondary to brain tumors and thrombosis have lower intensity of NREM than that in normal controls (12). The increase of NREM after administration of GHRH seems independent of peripheral GH increase (44). GHD patients with primary pituitary lesions have lower N1 and duration (12), consistent with the hypothesis of an effect of GHRH on this parameter (46). Based on all these observations, we speculate that the lack of GHRH action in our model is more important than GHD in affecting sleep quality, suggesting that GH has lower influence in sleep quality than GHRH in humans, as recently proposed (47, 48).

Stage N1 is the typical transition from wakefulness to sleep. In senescence (after 60 years of age) (36) the percentage of N3 decreases (49) and the number of arousals increases with consequent increase in N1 (50). Most IGHD (and control) subjects were below this age threshold, and therefore IGHD and not age is responsible for this 'aging' sleep pattern. When we analyze the data by age group, we found that the differences between IGHD and controls persisted only in the older subgroup. In addition, in this

subgroup we found a reduction in the duration of N3 in IGHD compared to controls, suggesting an additional effect of aging to GHRH receptor deficiency in this model. Interestingly, sleep, as skin ovary, and hearing seems to get older faster in IGHD than that in controls (51, 52), in contrast to bones, voice and blood vessels (53, 54, 55).

When we compared the data by gender, most of the abnormal parameters of sleep found in the IGHD were only maintained in males, suggesting a more important role of GHRH in sleep in males. Previous studies that have shown a relevant sleep-promoting effect of GHRH were performed in healthy young men, and therefore could not provide gender comparison (20, 21, 40).

Given the relative small number of subjects in each group after age and gender separation, more studies are needed to confirm a role of age and gender in sleep (25).

Differently from Laron syndrome (LS), a GH insensitivity condition with high circulating GH, we did not find a significant increase of obstructive sleep apnea syndrome (OSAS) in IGHD individuals. A narrow oropharynx secondary to a more severe obesity than the one of the IGHD subjects may predispose LS patients to OSAS (56). Interestingly, two LS patients showed a reduced percentage of REM during the IGF-1 therapy that seemed to occur simultaneously with decrease of GH levels. Since these patients are GH resistant, it is possible that the interactions between GH and REM sleep may be regulated by GH receptor-independent mechanisms (57).

Our study has some limitations. The major one is that—due to logistic limitations—we performed polysomnography only once, and a possible 'first night effect' cannot be ruled out. This however was the case for controls as well. Another limitation is that these patients, in addition to sharing the faulty *GHRHR* allele, may share other genetic traits that could influence the sleep. The recruitment of controls from the same geographical area reduces the risk of this possibility.

In conclusion, adult IGHD subjects lacking functional *GHRHR* exhibit objective reduction in sleep quality with changes in both NREM and REM sleep. The changes in NREM (longer duration of N1, and reduction of duration of N2), associated to increased WASO, seem more conspicuous than the mild reduction in the duration of REM sleep. These changes have minimal subjective consequences. Our data suggest a preponderant role of GHRH resistance over GHD in the sleep quality of these subjects.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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