Sleep disturbances, daytime sleepiness and quality of life in adults with growth hormone deficiency

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ABSTRACT

Low energy and fatigue are frequent complaints in subjects with GH deficiency (GHD). Since interrelations between sleep and GH regulation are well documented, these complaints could partly reflect alterations of sleep quality. Therefore we sought to determine objective and subjective sleep quality and daytime sleepiness in adult GHD patients compared to age, gender and BMI-matched controls, and in a subset of these GHD patients undergoing recombinant human GH (rhGH) therapy compared to placebo.

Thirty patients, aged 19-74 yr, with untreated GHD (primary pituitary defects confirmed or likely in 26 patients, hypothalamic origin in 4 patients), and 30 healthy controls individually matched for gender, age and body mass index were enrolled in the study. Patients with associated pituitary hormonal deficiencies were on appropriate replacement therapy. Polygraphic sleep recordings were performed at baseline and after 4 months on recombinant human GH or placebo. Subjective sleep quality and quality of life were evaluated by means of the Pittsburgh Sleep Quality Index (PSQI) and Quality of Life-Assessment for GHD in Adults (QoL-AGHDA). Irrespective of etiology, GHD patients had a PSQI score above the clinical cut-off for poor sleep and lower QoL-AGHDA scores than controls, with tiredness being the most affected domain. Patients with pituitary GHD spent more time in slow-wave sleep (SWS) and had a higher intensity of SWS than their controls. Amongst these patients, older individuals obtained less total sleep than controls and their late sleep was more fragmented. Contrasting with pituitary GHD,
the 4 patients with hypothalamic GHD had lower intensity of SWS than their controls. Thirteen patients were reevaluated after 4 months rhGH and 4 months placebo. Compared to placebo, SWS duration was decreased in younger patients after rhGH, and a trend for a decrease in SWS intensity was observed in the whole group. PSQI scores decreased, while QoL ratings improved. In conclusion, GHD is associated with sleep disorders that may be caused by specific hormonal alterations, as well as with poor subjective sleep quality and daytime sleepiness. Disturbed sleep is likely to be partly responsible for increased tiredness, a major component of QoL in GHD. Partial reversal of the sleep alterations was observed after 4 months of rhGH treatment, which was paralleled by an improvement in QoL and reports of tiredness, as well as subjective sleep quality.
INTRODUCTION

*Growth hormone deficiency*

Growth hormone (GH) is secreted by the pituitary somatotrophs under the stimulatory influence of hypothalamic GH-releasing hormone (GHRH). Acylated ghrelin, a peptide produced mainly by the stomach, is another potent endogenous GH secretagogue, but its contribution to the control of spontaneous GH secretion under physiological conditions has not been fully clarified yet. GH secretion is inhibited by somatostatin, and by negative feedback from IGF-I and GH itself. Recently, the orexin/hypocretin system, initially identified as a regulator of food intake, and subsequently as a key modulator of the sleep-wake cycle, was also implicated in the control of the somatotropic system. Finally, GH secretion is influenced by a variety of other metabolic, neural and hormonal factors. GH exerts a broad spectrum of effects, either directly or via the stimulation of the release of insulin-like growth factor-I (IGF-I) from the liver. These effects include the promotion of linear growth in children, and the regulation of carbohydrate, protein, lipid, and mineral metabolism throughout the lifetime.

Adult GH deficiency (aGHD) was recognized as a distinct syndrome in the late 1980s. While childhood-onset GHD is more frequently due to genetic causes or idiopathic, aGHD is usually a consequence of a pituitary or sellar insult (pituitary adenoma, craniopharyngioma, neurosarcoidosis) or their treatment (surgery, radiation therapy). Other, less frequent but increasingly recognized, causes of aGHD are traumatic brain injury, empty sella syndrome, and lymphocytic
hypophysitis. At variance with childhood-onset cases, in adults, GHD, because of its etiology, is frequently associated to other pituitary hormone deficiencies, while it is more frequently isolated in children.9-11

Clinical picture

From a clinical point of view, adults with GHD present with alterations of:

Table 1: Clinical picture of the adult GH deficiency syndrome (NO nitric oxide, SMC smooth muscle cells, VO2max aerobic capacity).

| Body composition12-14 | ↑ subcutaneous fat  
|                      | ↑ visceral fat  
|                      | ↓ lean mass (trunk and limbs)  
| Bone mass12, 15, 16   | ↓ bone mineral density  
|                      | ↓ markers of bone turnover  
| Lipid profile12-13, 17| ↑ total cholesterol  
|                      | ↑ LDL cholesterol  
|                      | ↑ HDL cholesterol  
|                      | ↑ or = triglycerides  
| Glucose tolerance18,19| Insulin-resistance  
| Heart structure and function12, 20| ↓ thickness of left ventricular posterior wall and interventricular septum  
|                      | ↓ or = ejection fraction  
|                      | Impaired left atrium diastolic filling  
| Endothelial function20-21| ↓ endothelial production of NO  
|                      | Resistance of vascular SMC to NO  
|                      | ↑ intima-media thickness  
|                      | ↑ greater arteries rigidity  
|                      | ↑ or = C-reactive protein  
| Muscle strength and endurance to exercise12, 22| ↓ isometric strength  
|                      | ↓ VO2 max  

Diagnosis

The diagnosis of GHD is based on the clinical history, clinical picture and provocative tests of somatotropic function. The gold standard is the insulin tolerance test (ITT) which investigates the integrity of the hypothalamic-pituitary axis. The glucagon test relies on a similar mechanism. Other tests, such as the combined GHRH+arginine test or the GHRH+growth hormone releasing peptide test, are based on the administration of compounds that maximally stimulate the GH secretory reserve of the pituitary. The ITT can be contraindicated in patients with ischemic heart disease, seizures and elderly. In adults, one provocative test is usually sufficient to establish the presence of GHD. The cut-off for the diagnosis of GHD varies with the test used. For the ITT the validated cut-off for GHD in adults is a peak GH response of <3 mg/l. The following cut-off levels have been validated for GHRH+arginine: for those with a body mass index (BMI) <25 kg/m², a peak GH <11 mg/l; for BMI between 25 and 30 kg/m², a peak GH<8 mg/l; and for BMI >30 kg/m², a peak GH<4 mg/l. Of note, the latest clinical practice guidelines published by the Endocrine Society report the results of a study comparing 6 different provocative tests for the diagnosis of adult GHD, in which the cut-off for peak GH response to GHRH+arginine with highest sensitivity (95%) and specificity (91%) was found to be 4.1 mg/l.

IGF-I is also an indicator of GH status; however, in adults, normal levels do not rule out GHD since only approximately 50% of middle-aged adults with severe GHD defined by GH tests have a pathologically low IGF-I level (below –2 SDS). IGF-I measurement, however, was proven to be a more sensitive marker of severe GHD in
young adults compared with middle-aged adults, being especially sensitive in young adults with childhood onset GHD. Attention must be paid to other factors that can influence IGF-I levels, such as obesity, malnutrition, hepatic diseases, hypothyroidism. In the appropriate clinical context, very low IGF-I levels are nevertheless considered indicative of severe GHD and do not require further investigation with provocative testing.

Quality of life in adult GHD patients

As more and more patients were evaluated, another significant aspect of the syndrome emerged: aGHD patients frequently complained of reduced quality of life (QoL), the most consistent complaints being related to energy levels, vitality, mental fatigue and emotional reactions, as well as social isolation, anxiety, reduced self-confidence, dissatisfaction with body image and poor memory. In 1999 a specific questionnaire was developed, based on interviews with patients from 5 different European countries (United Kingdom, Germany, Italy, Spain, Sweden). The interviews investigated the following topics: work, household chores, sleep, hobbies, leisure time pursuits, family life, intimate relationships, sex, memory and concentration, mood, energy, body image, self confidence and self-esteem, social activities, friendships, expectations and hopes. Twenty-five statements were selected that best described issues experienced by patients. The possible answers were yes or no, and scores ranged from 0 (best) to 25 (worst). This questionnaire, called “Quality of Life Assessment of Growth Hormone Deficiency in Adults” (QoL-AGHDA) was validated against previously available, generic, quality of life
questionnaires, and in several languages, and subsequently used in several clinical studies evaluating the effect of recombinant human GH (rhGH) treatment. Murray et al. 32 studied 65 patients with aGHD at baseline, and after 3 and 8 months of rhGH treatment, and reported a significant improvement of QoL scores during treatment, particularly in males. A significant correlation was demonstrated between the impairment of QoL at baseline and its improvement during rhGH therapy. Similar results were obtained by other authors in patients on rhGH for up to 8 years 33-38; most authors observed a greater improvement in the first year of follow-up, but the improvement was sustained in the following years. Koltowska-Haggstrom et al. 37, using data from the KIMS database, reported that QoL scores, after long-term rhGH treatment, approximated those of the general population, in 4 different European countries. Normalization of QoL as determined by using the QoL-AGHDA questionnaire was also observed by the same group in German patients after 12 months treatment 38. Conversely, Malik et al.39 compared QoL scores of 89 aGHD patients on rhGH treatment for an average of 3 years with those obtained from 83 age- and gender-matched healthy control subjects from the general population, and reported significantly higher scores in patients despite therapy. These conflicting results could be linked to the different duration of follow-up, etiology of GHD, and scores obtained in the general population 37. There also appears to be a chronology in the improvement of the different dimensions explored by the questionnaire, the least affected (socializing issues) improving earlier than the most affected (tiredness, memory and concentration) 37. Maiter et al. 40 looked at the impact of previous radiation therapy and found that the
improvement in QoL scores was similar in irradiated and non-irradiated aGHD patients.

Sleep

Sleep is divided into two distinct states of brain activity which are defined based on the appearance of specific features in the electroencephalogram (EEG), the electrooculogram and the electromyogram: rapid eye movement (REM) sleep, characterized by mixed low-amplitude high frequency EEG activity, muscle atonia and rapid eye movements, and non-REM (NREM) sleep. During the deeper stages of NREM sleep, EEG frequency becomes progressively slower and well-defined high amplitude slow waves appear (Fig. 1).

Fig. 1: brain waves recorded by EEG during relaxed wakefulness and sleep.

NREM sleep and REM sleep alternate cyclically, with a periodicity of about 90 minutes, and a typical night comprises 4 to 6 of such NREM-REM cycles (Fig. 2).
NREM sleep is more prevalent in the first half the night, while REM sleep episodes are more represented in the second half. NREM sleep is subdivided into four stages: I, II, III, IV; stages III and IV are also called slow-wave sleep (SWS) because of the prevalence of low frequency, high-amplitude “delta” waves 41. In young people, SWS represents about 20% of sleep duration, stages I and II about 50% and REM about 25%. Older individuals display an increased number and duration of awakenings and decreased amounts of SWS, while REM sleep appears to be relatively preserved 42.

Fig. 2: Hypnogram of a typical night recorded in a young subject.

The timing, duration and quality of sleep during any given 24-hour period ultimately results from the activity of two interacting time-keeping mechanisms in the central nervous system: endogenous circadian rhythmicity and sleep-wake homeostasis; the former is generated by a feedback loop of gene transcription/translation in the suprachiasmatic nuclei of the hypothalamus that results in a self-sustained near 24-hour oscillation, while the latter relates the timing and intensity of sleep to the duration of prior wakefulness 43-45.
Neurophysiological basis of sleep

From a neurophysiological point of view, normal waking is associated with neuronal activity in several chemically defined ascending arousal systems, including monoaminergic neurons in the brainstem and posterior hypothalamus, cholinergic neurons in the brainstem and basal forebrain, and hypocretin (orexin) neurons in the lateral hypothalamus. The activity of these neuronal populations is reflected in the low-voltage fast-frequency EEG pattern of wakefulness, and declines rapidly at sleep onset. Non-rapid eye movement (NREM) sleep is generated by neurons located in the preoptic region of the hypothalamus and adjacent basal forebrain. These so-called “sleep-active neurons” have been shown to be gamma-aminobutyric acid (GABA)-ergic neurons which have direct projections to the “wake-active” areas and most likely inhibit them by releasing GABA, resulting in a progressive slowing of EEG frequencies. When the firing activity of the sleep-active neurons is synchronized, delta (slow) waves, which are considered a marker of sleep depth, appear on the EEG. Finally, REM sleep is generated by the activation of neurons in the pons and adjacent portions of the midbrain.

Power spectral analysis of the EEG

Power spectral analysis, a methodology that describes how the power of a signal or time series is distributed across frequencies is used to further characterize sleep and gives information about the quality of sleep. It is based on the assumption that the EEG can be mathematically decomposed into an infinite number of pure sinusoidal
components, each of a different frequency, which when added together yield the original signal. Applying a Fast Fourier Transform to the EEG signal allows to obtain an approximation of these frequency components separately \(^48\). The most investigated frequency band is the delta band (0.75-4 Hz), which reflects the low-frequency waves typically observed in SWS. Delta power is normally highest at the beginning of the sleep period and subsequently decreases throughout the night \(^44, 49\). Delta power, also referred to as “slow-wave activity” (SWA), is considered as a marker of sleep pressure \(^43, 49-50\) and is highly reproducible in the same individual \(^51-52\), although it decreases with age \(^53\). The other frequency bands are theta (4-7 Hz), alpha (7-12 Hz), sigma (12-15 Hz), beta (15-30 Hz) and gamma (>30 Hz). Sigma power is generated by the occurrence of sleep spindles, alpha power is a marker of the synchronization of cortical oscillations in high frequency ranges, and beta and gamma power reflect intra-sleep arousal.

**Relationships between the somatotropic axis and sleep**

**GH secretion and sleep**

In healthy young men, the major GH secretory episode occurs after sleep onset, and may account for as much as 70% of the total 24-hr GH secretion. Daytime GH levels are generally low, with few bursts of secretion that can be associated with post-prandial periods, exercise or stress \(^54-55\). In contrast, in healthy young women, daytime pulses are more frequent and the sleep onset-associated surge, although consistently present, does not generally represent the majority of the 24-hr GH secretion \(^56-57\) (Fig. 3).
Average 24-hour GH concentrations, as well as trough (interpulse) levels, are higher in women compared to men\textsuperscript{58-60}. Some authors have described an increase in pulse amplitude and mass secreted per burst\textsuperscript{60-62} in women while others observed an increase in pulse frequency\textsuperscript{59, 63}. However, few studies have focused on gender differences in nighttime GH secretion; either a greater nocturnal GH production in men compared to age- and BMI-matched women\textsuperscript{59, 62}, or no difference in integrated GH concentrations\textsuperscript{63} were reported. In the latter study women displayed a different secretory pattern, with a pre-sleep pulse and at least one more pulse during the night. The lower nocturnal GH release in women as compared to men could in fact be related to a higher GH secretory activity before sleep onset, reflecting a negative feedback inhibition of GH itself. The sexual dimorphism in the pattern of GH secretion is thought to be related to sex differences in GHRHergic\textsuperscript{60, 64-66}, somatostatinergic\textsuperscript{59-60, 67} and/or ghrelinergic\textsuperscript{68} tone; however, no consensus has been reached so far. A role for sex steroids in the feedback mechanisms controlling
GH secretion at the pituitary level has also been hypothesized \(^{59, 69-70}\). To our knowledge only one study \(^{65}\) looked into the mechanisms underlying sex differences in nocturnal GH secretion and the results suggest a lower endogenous GHRH tone in women.

**Fig. 4:** Plasma GH profile during normal nocturnal sleep, sleep deprivation and daytime recovery sleep \(^{71}\)

The sleep-onset-associated GH surge is mostly maintained and in subjects living in free-running conditions, i.e. without any time cues \(^{72}\), as well as in subjects exposed to a 3-hour sleep-wake cycle for 10 days \(^{73}\). Similarly, night shift workers exhibit a GH secretory peak in the first half of their daytime sleep period \(^{74}\). These results were confirmed by delaying sleep by 8 or 12 hours in young, healthy, day-active men \(^{74-75}\) (Fig. 4).

Conversely, awakenings interrupting sleep appear to interrupt GH release. Indeed, two independent studies have found that the GH response to GHRH administration is inhibited when sleep is interrupted, either spontaneously or experimentally \(^{76-77}\). This inhibition of GHRH-stimulated GH release by awakening could be linked to a
concomitant pulse in cortisol secretion, which occurs consistently during full nighttime awakenings, as CRH administration has been shown to inhibit the GH response to GHRH 76.

More in particular, sleep-onset GH release appears consistently associated with SWS. The temporal coincidence between the onset of SWS and increasing concentrations of GH was already noted in the late 1960s 55, 78-79. A remarkable study published in 1991 showed that, when plasma GH levels are sampled at very frequent intervals (30 seconds), the release of GH immediately follows the appearance of delta waves in the EEG 80. Subsequently, a direct and robust correlation between the amount of GH secreted and the duration of SWS episodes was reported 81, as well as between pulses of GH secretion and “pulses” of SWA 82. The latter relationship is best evidenced when GH secretory rates are estimated by mathematical deconvolution of GH concentrations, as illustrated in Fig. 5. Indeed, a close temporal concomitance between GH secretory pulses and peaks of SWA becomes apparent.
Pharmacological interventions that increase SWS and SWA also increase GH release, thus demonstrating the existence of common underlying mechanisms. Figure 6 illustrates the findings from two separate placebo-controlled studies which involved administration of a drug enhancing SWS/SWA at bedtime (a) gamma-hydroxybutyrate, b) ritanserin). In both studies, GH secretory pulses were coincident with peaks of SWA in the majority of the subjects, both in the placebo and in the active drug conditions\textsuperscript{82-83}. Further, in both studies, there were significant correlations between the increase in SWS/SWA and the increase in GH release. The findings are consistent in indicating that the drug-induced increase in
SWA is associated with increased GH release, even though these two drugs have very different mechanisms of action.

**Fig. 6:** profiles of SWA and GH secretory rate under placebo and a) gamma-hydroxybutyrate, b) ritanserin (adapted from 82-83). The differences in the SWA scale between the 2 studies are due to different amplifier settings.

![Graphs showing SWA and GH secretory rate under placebo and gamma-hydroxybutyrate, ritanserin](image)

Lastly, GH release is observed more frequently during daytime naps when they occur in the afternoon, a time when SWS propensity is higher, than when they occur in the early part of the day 84-85.

Despite the strong evidence for a link between SWS regulation and nocturnal GH release, several authors have reported significant elevations of GH levels prior to sleep onset or during other stages of sleep 86-88 in men as well as in women. Such pre-sleep GH pulses could reflect the presence of a sleep debt 71 and/or a circadian effect (see below). Dissociations between SWS and nocturnal GH release have also been reported. In one study, partial SWS deprivation failed to suppress or delay the
sleep-onset GH pulse. In another study, in which nocturnal GH secretion was correlated to EEG delta power, about 50% of the subjects displayed increased GH secretion before sleep onset or in absence of concomitant delta activity. As discussed below, the association between GH release and SWS appears to be primarily mediated by GHRH. However, because GH secretion is also under the control of multiple negative regulatory mechanisms, occasional dissociations between pulses of GH levels and SWS are not unexpected.

**Circadian influences**

Although GH secretion is consistently stimulated by sleep onset and SWS, it is also under the influence of circadian rhythmicity, as demonstrated by several studies in which the sleep-wake cycle was manipulated: a higher propensity for GH secretion was observed when sleep occurred in the afternoon and evening. However, careful experiments conducted in a “forced desynchrony protocol” where subjects lived on a 28-hr sleep-wake and light-dark cycle (9h20:18h40 dark:light) only revealed a minimal non-significant circadian modulation of SWA. The higher propensity for GH secretion in the late evening and early night could also be related to the circadian nocturnal decline in somatostatin secretion. In rats, a circadian rhythm of somatostatin has been observed, with higher levels during the dark (active) phase compared to the light (inactive) phase and a similar trend was inferred for humans. However, peripheral somatostatin levels are difficult to measure in humans and may not reflect somatostatin concentrations in the hypophysial portal blood. The role of this peptide in the generation of GH pulses in
humans is suggested by experiments showing the persistence of GH pulses during continuous GHRH infusion\textsuperscript{93}; furthermore, repeated boluses of GHRH produced a greater GH response to GHRH starting from the late afternoon hours, suggesting a decline in somatostatin action before habitual bedtime\textsuperscript{94}. However, the same group later reported the persistence of daytime and nighttime GH pulses during continuous octreotide infusion\textsuperscript{95-96}, and argued that a decline in somatostatin levels is not the initiating event of GH pulses. Nevertheless, these findings do not exclude the possibility of a declining somatostatinergic tone at the time of GH pulses, since the amplitude of the GH increments was lower under octreotide infusion\textsuperscript{96}.

**Molecular and neural mechanisms underlying the relationship between GH release and sleep**

Rodent and human studies have identified GHRH as the primary factor underlying sleep-associated GH secretion.

In rats, hypothalamic GHRH mRNA expression exhibits a circadian rhythm with maximum levels at the onset of the light period (i.e. the sleep period), followed by a progressive decrease to reach a nadir at the beginning of the dark (i.e. active) period\textsuperscript{97}. An opposite pattern was described for hypothalamic GHRH protein content\textsuperscript{98}. Injection of GHRH in rodents, either intracerebroventricularly (i.c.v.), into the medial preoptic area or intravenously, increases the duration of SWS\textsuperscript{99-101}. Consistent with these observations, administration of a GHRH receptor antagonist or of anti-GHRH antibodies decreases NREM sleep\textsuperscript{102-103}. Furthermore, mice and rats with nonfunctional GHRH receptor (lit/lit mice and dw/dw rats) have lower
amounts of spontaneous NREM sleep than wild types 104-105. Conversely, spontaneous dwarf rats, characterized by GH deficiency due to a mutation of the GH gene, have a longer duration of spontaneous NREM sleep, as well as a higher hypothalamic GHRH mRNA content compared to control rats 106, consistent with increased central GHRH tone due to the absence of feedback inhibition of GH. The majority of GHRH-producing neurons are located in the arcuate nucleus of the hypothalamus but another, smaller, GHRHergic neuronal population is present in the ventromedial nucleus and in the parvicellular portion of the paraventricular nucleus. These neurons have been shown to project to the anterior hypothalamus/preoptic area, and in particular to the ventrolateral preoptic (VLPO) nucleus, which is very active during SWS 57, 107. Recently, Peterfi et al. 108 evaluated the effect of i.c.v. GHRH, octreotide or GHRH antagonist administration, on sleep patterns and activation of sleep-active neurons in the hypothalamus in male rats. GHRH given at the beginning of the dark period, i.e. the active period, increased the duration of NREM sleep and SWA; immunohistochemistry staining of hypothalamic sections showed an increased activation of GABAergic neurons (Fos+GAD-positive cells) in the VLPO and in the median preoptic nucleus (MnPN), which is consistent with the sleep-related activity of these areas. Similar immunohistochemistry results were observed when the animals underwent sleep deprivation. Conversely, octreotide and the GHRH antagonist injected i.c.v. at the beginning of the light phase (i.e. the resting period), decreased the duration of NREM sleep and SWA, as well as the number of Fos+GAD-positive cells. These
data provide functional and anatomical evidence for a role of GHRH in the regulation of NREM sleep.

Results of studies performed in humans are summarized in Table 2. The first study in which GHRH was administered to human subjects involved an i.v. injection in the morning or in the evening, one hour prior to bedtime; no change in sleep parameters was reported. Another group administered a single dose of GHRH to healthy male volunteers at sleep onset and did not observe any change in sleep architecture. In these 2 experiments, GH levels increased in response to GHRH administration. Several subsequent studies in humans have shown that single intravenous boluses of GHRH produce an increase in both GH and SWS or SWA. In one study the stimulation of SWS became apparent only at a time when the physiological propensity for SWS was low, i.e. in the second half of the night. Other authors did observe an increase in SWS after a single bolus of GHRH, however the dose administered was higher than in the latter report. In a few studies, GHRH was injected in a repetitive fashion starting before sleep onset, and a stimulatory effect of GHRH on SWS was observed in men, but not in women in whom GHRH had instead sleep-impairing effects consisting in a decrease of stage IV sleep and of REM sleep during the first half of the night. One group evaluated the effect of intranasal administration of GHRH, as little is known about the ability of GHRH to cross the blood-brain barrier, and the intranasal route bypasses the blood-brain barrier; an increase in SWS duration was observed as well, which was significant over the second half of the night, again at a time of lower SWS propensity. Surprisingly, GH concentrations were decreased after GHRH, an
effect that the authors attribute to direct GHRH action on the CNS \textsuperscript{117}. Contrasting with the evidence in support of a role of GHRH in both SWS and GH stimulation, a study in healthy young men showed that the blockade of endogenous GHRH receptors by continuous i.v. infusion of a selective GHRH antagonist had no effect on sleep parameters, and in particular on SWS, while it did suppress GH response to GHRH stimulation \textsuperscript{118}. However, it was not clear whether this GHRH antagonist could cross the blood-brain barrier.

Table 2: Human studies about the effect of exogenous GHRH on sleep.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Protocol</th>
<th>Results</th>
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<tr>
<td>Garry et al., 1985\textsuperscript{109}</td>
<td>50 µg GHRH i.v. at 9 am or 8 pm 6 healthy young men</td>
<td>No changes in GH secretion and sleep parameters observed</td>
</tr>
</tbody>
</table>
| Kupfer et al., 1991\textsuperscript{110} | 0.5 µg/kg GHRH i.v. at bedtime 10 healthy young men | Significant ↑ in GH secretion  
No change in SWS duration or delta activity  
No change in REM duration or intensity |
| Steiger et al., 1992\textsuperscript{119} | 50 µg GHRH x4 q1h starting at 10 pm 7 healthy young men | Significant ↑ in GH secretion  
Significant ↑ in SWS duration |
| Kerkhofs et al., 1993\textsuperscript{111} | 0.3 µg/kg GHRH iv (8 healthy young men)  
- at the beginning of the 1\textsuperscript{st} SWS or at the beginning of the 3\textsuperscript{rd} REM cycle (sleep from 11 pm to 7 am)  
- at the beginning of the 1\textsuperscript{st} SWS after sleep deprivation (sleep from 4 am to 12 pm) | No effect if administered at the beginning of SWS (either after normal sleep or after sleep deprivation)  
Significant ↑ in SWS duration and ↓ awakenings if administered at the beginning of REM |
| Marshall et al., 1996\textsuperscript{114} | 200 µg GHRH in continuous infusion or 50 µg x4 q1h starting at 10 pm 12 healthy young men | Repetitive administration ↑  
SWS more than continuous administration (first half of night)  
Similar ↑ in GH secretion |
Ghrelin has been identified as a potent GH secretagogue based on the effects of exogenous administration. However, so far, studies attempting to evaluate the physiological relationship of endogenous ghrelin levels to spontaneous GH secretion have yielded inconsistent results. A large body of evidence supports the notion that ghrelin acts on GH secretion at least in part via a stimulation of GHRH and thus a positive interaction between central ghrelinergic activity and SWS/SWA should be expected. Additionally, ghrelin acts as a functional somatostatin antagonist at both the pituitary and the hypothalamic level. Nevertheless, the interaction between ghrelin and sleep regulation is still poorly understood. Intracerebroventricular administration of ghrelin in rats was shown to increase arousal and food intake. However, systemic administration of ghrelin in mice had clear somnogenic effects, and ghrelin-KO mice display decreased NREM sleep, increased REM sleep and wakefulness. In humans,
systemic ghrelin administration at sleep onset was reported to promote SWS, as well as nocturnal GH secretion, in healthy young men. These findings were reproduced in 2 later studies in young and elderly men, but not young and elderly women. At the moment little is known about the molecular mechanisms underlying the modulatory effects of ghrelin on the sleep-wake cycle; the effects of systemic ghrelin could be mediated by the GHRH circuitry, while centrally administered ghrelin could act on different hypothalamic systems.

The orexin/hypocretin system is another newly identified player in the complex mechanisms underlying the relationship between sleep and GH secretion. Orexins/hypocretins, produced by neurons in the lateral hypothalamic area, were originally thought to be regulators of food intake. However, it emerged from subsequent studies that these peptides were also involved in the modulation of the sleep-wake cycle as an orexin deficiency is underlying most cases of animal and human narcolepsy. Orexins also appear to be participating in the control of all of the endocrine axes. In particular, orexinergic projections have been identified in the arcuate nucleus and the periventricular nucleus, where neuronal populations express orexin receptors. Interconnections between ghrelinergic and orexinergic neurons in the hypothalamus have also been reported. The somatotrophs express both orexin-A and the orexin-receptor. In rats, i.c.v. orexin-A administration was found to decrease both GH secretion and pulsatility, an effect thought to be due to either inhibition of GHRH tone and/or increasing somatostatinergic tone in the hypothalamus. Although early reports suggested that spontaneous GH secretion might be blunted in narcoleptics, these
were either not case-control studies, or patients and controls were not matched for age and BMI. A recent study by Overeem et al. \(^{149}\), in which patients and controls were carefully matched for age, gender, BMI and body fat, found that narcoleptic patients with CSF orexin deficiency are not GH deficient. Furthermore, the patients maintained a temporal relationship between GH release and SWS episodes, although the proportion of GH secreted during daytime was increased, as compared to controls. This altered circadian distribution of GH secretory events could be due to the lack of modulatory effect normally exerted by the orexin system on GHRH secretion during the daytime.

**Exogenous GH and sleep**

The impact of GH administration on sleep was evaluated in several animal studies: in mice, rats and cats, exogenous GH increases REM sleep duration \(^{150-151}\). In the first study performed in humans \(^{152}\), human GH obtained from pituitary extracts was administered intramuscularly (i.m.) to healthy volunteers 15 minutes before bedtime. Compared to saline, 2 UI of hGH i.m. did not alter sleep parameters, while at higher doses (5 UI) a significant increase in REM duration and a significant reduction in SWS duration were observed. The same authors failed to reproduce these results in a later study, in which, however, hGH (2UI i.m.) was administered twice daily (8 am and 5 pm) for 3 days before sleep was recorded; the lack of impact of GH on sleep might be related to the time lapse between the last administration and bedtime \(^{153}\). Kern et al. \(^{154}\) administered rhGH (either 5 UI i.m. before bedtime or in continous i.v. infusion overnight, or 48 UI i.v. over 30 minutes
at bedtime) to healthy male volunteers: no changes in sleep parameters were observed.

**Sleep in GHD patients**

Very few studies have objectively characterized sleep in GHD patients and the results, summarized in Table 3, have been inconclusive. The first ones were carried out in children: Orr et al. evaluated 10 children with isolated GHD, who displayed similar sleep parameters as their age-matched controls, except for REM sleep that was significantly shorter in the younger age group. Wu and Thorpy observed an increase in stage 1 and SWS in children with isolated GHD compared to an age-matched control cohort taken from literature. One later study involved young adults with childhood-onset isolated GHD and a decrease in SWA was reported in the patients. Finally, a study on women with Sheehan’s syndrome showed an increase in SWS duration in the patients compared to controls matched for age, BMI and menopausal status.

Sleep characteristics in GH deficient patients have also been studied during GH replacement therapy. Wu and Thorpy observed a decreased duration of stage 3 in children with isolated GHD treated for 1 to 2 weeks. In adults, SWS duration was reported to be shorter after 6 months on rhGH in young adult patients with isolated GHD, increased in middle-aged patients with GHD of variable origin, or unchanged, compared to placebo.
Table 3: studies evaluating sleep in GHD patients published in literature (CO childhood-onset, aGHD adult GHD, iGHD isolated GHD, PSG polysomnography).

<table>
<thead>
<tr>
<th>Authors</th>
<th>Protocol</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orr et al., 1977</td>
<td>10 children with iGHD vs controls</td>
<td>No difference in SWS duration</td>
</tr>
<tr>
<td>Wu and Thorpy, 1988</td>
<td>7 children with GHD</td>
<td>GH treatment: ↓ stage III duration Comparison with a cohort of normal</td>
</tr>
<tr>
<td></td>
<td>PSG study before and after 1-2 weeks of hGH treatment</td>
<td>age-matched children: ↑ stage I, III, IV in GHD patients</td>
</tr>
<tr>
<td>Astrom et al., 1989</td>
<td>7 young adults with CO-iGHD vs controls</td>
<td>↓ delta power in GHD patients</td>
</tr>
<tr>
<td></td>
<td>PSG study + power spectral analysis</td>
<td></td>
</tr>
<tr>
<td>Astrom et al., 1990</td>
<td>8 young adults with CO-iGHD</td>
<td>↓ total sleep time</td>
</tr>
<tr>
<td></td>
<td>PSG study before and after 6 months hGH treatment</td>
<td>↑ REM sleep</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No change in SWS duration</td>
</tr>
<tr>
<td>Nolte et al., 2002</td>
<td>5 patients with aGHD due to pituitary disease</td>
<td>On rhGH: normal duration of stage I, II and SWS, ↓ sleep efficiency, ↓</td>
</tr>
<tr>
<td></td>
<td>PSG study after 1 yr of rhGH treatment and after 6 months withdrawal</td>
<td>REM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After rhGH withdrawal: ↓ SWS</td>
</tr>
<tr>
<td>Schneider et al., 2005</td>
<td>18 aGHD patients</td>
<td>No difference in sleep parameters</td>
</tr>
<tr>
<td></td>
<td>PSG study after 6 months rhGH treatment vs placebo</td>
<td></td>
</tr>
<tr>
<td>Peker et al., 2006</td>
<td>19 aGHD patients</td>
<td>No difference in sleep parameters (↑ REM in patients with OSA)</td>
</tr>
<tr>
<td></td>
<td>PSG study before and after 6 months rhGH treatment</td>
<td></td>
</tr>
<tr>
<td>Ismailogullari et al., 2009</td>
<td>22 women with Sheehan’s syndrome vs controls and after 6 months rhGH</td>
<td>Vs controls: ↑ Stage IV, ↓ REM, ↓ sleep efficiency</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After rhGH: no difference in sleep parameters</td>
</tr>
</tbody>
</table>
AIM OF THE STUDY

Not a single study has assessed subjective sleep quality or daytime sleepiness in GHD as compared with normal controls. Furthermore, the studies looking at the impact of rhGH administration on sleep architecture and quality have yielded inconclusive results. The purpose of our study was therefore to:

1. characterize objective sleep quality, as well as subjective sleep quality, daytime sleepiness and QoL in a large cohort of untreated adult patients with GHD, individually matched for gender, age and body mass index (BMI) with control subjects.

2. assess the impact on sleep and subjective sleepiness of a 4-month period of rhGH treatment in aGHD patients, as compared to placebo, in a single-blind placebo-controlled design.
SUBJECTS AND METHODS

Patients

Thirty adults with GHD (26 males, 6 females), aged 45±18 years (19-74) and with a BMI of 27.4±1 kg/m² were enrolled in the study. These patients were recruited in four centers:

a. Sleep, Chronobiology and Neuroendocrinology Research Laboratory, Department of Medicine University of Chicago, USA;
b. Department of Endocrinology and Centre d’Etude des Rythmes Biologiques, Université Libre de Bruxelles, Belgium;
c. Department of Endocrinology, Université de Liège, Belgium;
d. Department of Endocrinology, Università di Pisa, Italy.

Diagnosis of GHD

The diagnosis of GHD was based on an intravenous insulin tolerance test or a GHRH+arginine test performed within the last 5 years, with a maximum GH response <3 ng/ml for ITT or according to BMI for GHRH+arginine (BMI <25 kg/m², peak GH <11 mg/l; 25≤BMI<30 kg/m², peak GH<8 mg/l; BMI ≥30 kg/m², peak GH<4 mg/l26). GHD was present for at least 1 year.
Exclusion criteria

Screening included a clinical examination and routine laboratory measurements. Patients with evidence of substance abuse, liver disease, renal insufficiency, heart failure, malignant disease, chronic infectious disease, neurological or psychiatric disease, clinically significant hyperprolactinemia, or diabetes requiring administration of insulin or sulfonylurea, were excluded from the study. Individuals employed as shift workers within the last 3 months and subjects having traveled across more than 2 time zones within the last 2 weeks were not included. All subjects were off hypnotic drugs for at least 3 months.

Patients characteristics

Six patients had childhood onset idiopathic GHD, while the remaining 24 patients had an adult onset GHD. The patients had either never received GH therapy or were off GH treatment for at least 6 months at the time of enrollment.

The individual diagnoses and additional pituitary hormone deficiencies, as well as treatment, are presented in Table 4.

In 12 of the 30 patients, the origin of the adult onset GHD was a primary pituitary defect without supra-pituitary involvement: surgical removal of a pituitary tumor without radiotherapy (10 patients); spontaneous necrosis of a pituitary tumor (1 patient); pituitary stalk section (1 patient). None of these patients presented with diabetes insipidus.

In 6 additional patients, the existence of primary pituitary lesions was confirmed but a supra-pituitary involvement could not be excluded: surgical removal of a pituitary
tumor with diabetes insipidus (2 patients); radiotherapy for a pituitary tumor (1 patient); surgical removal of a craniopharyngioma, without any adherence to the hypothalamus or the optic chiasm, with presence of diabetes insipidus (2 patients); spontaneous necrosis of a pituitary tumor with diabetes insipidus (1 patient). Both pituitary and hypothalamic lesions were possible in two other patients (1 neurosarcoidosis, 1 histiocytosis).

Consistent with known genetic causes for “idiopathic” pituitary deficiencies\(^{163}\), a primary pituitary origin of GHD was considered very likely in the 6 patients with childhood-onset idiopathic GHD. A primary pituitary defect was excluded in the remainder 4 patients (referred to as “hypothalamic”): 1 brain tumor (surgery and radiotherapy, diabetes insipidus), 1 cerebral trunk thrombosis, 1 olfactory duct tumor (surgery), 1 pineal gland tumor (radiotherapy, diabetes insipidus). Based on these considerations, patients were divided into 3 categories according to the origin of the GH deficit:

- pure pituitary (n=18),
- pituitary with likely hypothalamic involvement (n=8),
- hypothalamic (n=4).

Twenty-eight of the 30 patients had associated pituitary hormonal deficiencies and were on appropriate and stable replacement therapy, as assessed by at least two clinical and biological evaluations performed at intervals of at least 3 months. All 6 female patients were on estrogen (4 also on progesterone/progestagen) replacement therapy; 16 male patients with gonadal insufficiency were treated with testosterone (intramuscular injections of 200-250 mg every 2-3 weeks or transdermal
applications of 50 mg/day); 21 patients with thyroid insufficiency received oral L-thyroxine (50-250 μg/day); 24 patients with adrenal insufficiency received oral hydrocortisone (20-30 mg/day), or cortisone acetate (25-37.5 mg/day), averaging 12.0±0.7 mg/m²/day hydrocortisone or the
Table 4: Clinical characteristics of the patients (NFPA non functioning pituitary adenoma, surg surgery, RT radiotherapy, AO adult-onset, CO childhood-onset, CDI central diabetes insipidus)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>BMI</th>
<th>Diagnosis</th>
<th>Onset</th>
<th>Hypo-adrenalism</th>
<th>Hypo-thyroidism</th>
<th>Hypo-gonadism</th>
<th>CDI</th>
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<tr>
<td>Bxl01</td>
<td>M</td>
<td>64</td>
<td>25</td>
<td>NFPA + surg</td>
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<td>X</td>
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<td>Bxl04</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bxl05</td>
<td>M</td>
<td>69</td>
<td>30.1</td>
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<td>AO</td>
<td>X X X</td>
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<td>74</td>
<td>32.9</td>
<td>Cerebral thrombosis</td>
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<td>X X</td>
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<tr>
<td>Bxl07</td>
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<td>29</td>
<td>26</td>
<td>Pituitary stalk section</td>
<td>AO</td>
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<td>Bxl08</td>
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<td>CO</td>
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<td></td>
<td></td>
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<td>X X X</td>
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<td></td>
<td></td>
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<tr>
<td>Bxl10</td>
<td>M</td>
<td>36</td>
<td>44</td>
<td>Neurosarcoioidosis with hypothalamic infiltration</td>
<td>AO</td>
<td>X X X X</td>
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<td>24</td>
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<td>Chi03</td>
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<td>58</td>
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<td>Chi05</td>
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<td>Patient</td>
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<td></td>
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</tr>
</tbody>
</table>
equipotent dose of cortisone; 7 patients with diabetes insipidus were treated with desmopressin.

**Controls**

Thirty healthy controls were individually matched with the patients for gender, age and BMI. Women were also matched for formulations of estrogens and progesterone/progestagens. Whenever applicable, inclusion criteria were the same as for the patients.

**Experimental protocol**

The protocol was approved by the Institutional Review Boards of all participating universities. Written informed consent was obtained from all participants.

The protocol is illustrated in Fig. 7 and consisted of:

- a first 4-month phase during which either rhGH or placebo were administered to the GHD patients; objective and subjective sleep quality, as well as quality of life, were evaluated at baseline in patients and controls (**study A**) and at the end of the 4 months in patients (**study B1**)
- a 3-month wash-out period
- a second 4-month phase during which either placebo or rhGH were administered to the GHD patients; objective and subjective sleep quality, as well as quality of life, were evaluated at the end of the 4 months (**study B2**).
The outpatient visit and the inpatient study were performed in laboratories at the Universities of Chicago, Brussels (including the patients recruited at the University of Liege) and Pisa. The same equipment, instruments and recording techniques were used at each site.

**Outpatient visit**

All GHD patients and control subjects had an initial outpatient admission. This visit included:

- physical examination;
- administration of the QoL scale (QoL-AGHDA): 25 “yes or no” questions relative to specific complaints commonly reported by GHD patients. A higher score corresponds to lower QoL. The 25 complaints may be clustered in five domains: tiredness (7 questions), memory and concentration (6 questions), tenseness (3 questions), social isolation (5 questions), and self-confidence (4 questions)\(^{30,37}\). For each domain, the mean score per question was calculated.
- administration of the Pittsburgh Sleep Quality Index questionnaire (PSQI): a validated 19-item questionnaire that investigates subjective sleep quality, as well as subjective sleep latency, subjective sleep duration, subjective sleep efficiency, presence of sleep disturbances, use of hypnotic drugs and daytime sleepiness; scores can range from 0 and 21 and a score > 5 is indicative of impaired sleep\textsuperscript{164}.
- determination of plasma levels of IGF-I, free T\textsubscript{4} (FT4), Na, K, and fasting blood glucose.

**Ambulatory monitoring**

Patients and controls underwent 6 days of ambulatory sleep monitoring by wrist actigraphy (Actiwatch, Philips Respironics, Bend, OR), a method providing accurate estimations of sleep onset and offset\textsuperscript{165-166}. The median habitual bedtimes from these recordings were used to individually design the bedtime schedule during the inpatient study. Patients and controls also had to fill a questionnaire about the subjective quality of their sleep every morning (Karolinska Sleep Log) and keep a log of their caffeine and alcohol intake for those 6 days.

**Inpatient study**

**Fig. 8:** outline of the inpatient study (N1 night 1, N2 night 2)
The outline of the inpatient study is shown in Fig. 8. Within one week after the end of ambulatory monitoring, the subjects were admitted to the laboratory between 1700 h and 1900 h on day 1, and remained in the laboratory until discharge in the morning of day 3. Regular hospital meals were served at 08h00, 12h30 and 19h0. Lights were turned off 5 min before scheduled bedtime and turned on 5 min after scheduled wake time. During bedtimes, sleep was polygraphically recorded (DigiTrace Care Services, Boston, MA).

Upon awakening on day 2, a blood sample was taken for measurement of plasma IGF-I. Thereafter, subjects were maintained under normal indoor light (± 300 lux) until bedtime. During waking hours, they had sedentary activities (reading, watching TV and simple neurobehavioral tests) and were free to ambulate around the unit. Naps were not allowed.

During the second night, all experimental conditions were identical to those in the first night.

**Monitoring of rhGH treatment in patients**

Recombinant human GH (Genotropin, Pfizer, Inc, New York, USA) was administered as follows:

<table>
<thead>
<tr>
<th>Table 5: rhGH dosage instructions according to age and gender.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men &lt;45 y.o.</strong></td>
</tr>
<tr>
<td>Initial dose (mg/day)</td>
</tr>
<tr>
<td>Increments (mg/day)</td>
</tr>
<tr>
<td>Max. dose (mg/day)</td>
</tr>
</tbody>
</table>
Patients were instructed to inject rhGH subcutaneously approximately 30 minutes before bedtime. IGF-I levels were monitored monthly and rhGH dosage was titrated accordingly.

**Objective sleep analysis**

Polygraphic recordings were visually scored at 30-sec intervals in stages wake, I, II, III, IV and REM using standardized criteria by the same experienced scorer who was blind to the subject’s condition. Sleep onset and morning awakening were defined as, respectively, the times of the first and last 30-sec intervals scored II, III, IV, or REM. The sleep period was defined as the time separating sleep onset from final morning awakening. Total sleep time was defined as the sleep period minus the total duration of wake after sleep onset (WASO). Sleep latency was defined as the time from lights off until sleep onset (Fig. 9). Sleep efficiency was calculated as the total sleep time, expressed as percentage of the time allocated to sleep. A spectral analysis on the central EEG lead was performed (PRANA, PhiTools, Strasbourg, France). Muscular, ocular and movement artifacts were eliminated prior to spectral analysis. Delta, theta, and alpha activities were calculated as the absolute spectral power in the frequency bands 0.5-4 Hz, 4.5-8 Hz, and 8.5-12 Hz, respectively. Mean power per 30-sec epoch was calculated for each band. Mean delta power in non-REM sleep quantifies the intensity of SWS. For illustrative purposes, the durations of NREM/REM cycles were also normalized to account for individual differences.
For study A, technical artifacts prevented sleep scoring for 3 of the 120 nights of recordings and spectral analysis for 9 of the 120 nights. All of the 52 nights recorded for study B were scored; technical artifacts prevented spectral analysis for 5 of them. With very few exceptions due to technical failures, comparisons between patients and controls used the second, rather than the first night of polysomnography, because all patients and controls were habituated to the experimental procedures and spent the preceding day in the same standardized and controlled environment.

**Statistical analysis**

For study A, the analysis principally compares the 26 GHD patients with confirmed or likely pituitary defects, and their individually matched controls. Because sleep quality changes in the course of normal aging\(^{42}\), we performed a median split of this group according to age (younger: 29±2 years, range 19-43 years, n=13; older: 60±3 years, range: 47–74 years, n=13) and compared patients and controls by ANOVA for repeated measures with age group as a between-subject factor. Differences in the prevalence of QoL symptoms were tested by the Chi-Square test with Yates’
correction for continuity. Correlations were explored using the Spearman coefficient.

We also compared the 12 patients with pure primary pituitary GHD, the 8 patients classified as pituitary GHD with possible hypothalamic involvement, the 6 patients with childhood onset idiopathic GHD and the 4 hypothalamic GHD patients. Differences between patients and their individually matched controls were compared by ANOVA with etiology of GHD as factor and age group as covariate.

For study B, differences between the 2 treatment periods were compared by ANOVA for repeated measures with treatment as a factor and age group as covariate.

All group values are expressed as means ± SEM.
RESULTS

Study A

Clinical characteristics for each GHD group and for matched controls are shown in Table 6. IGF-I values were lower in all GHD patients than in individually matched controls, averaging 72±7 ng/ml vs 194±11 ng/ml (p<0.001). GHD patients had normal plasma levels of fasting glucose, sodium and potassium. Plasma fT4 levels averaged 86±5 % of the median value of the normal range.

Table 6: Demographics of GHD patients and their age, gender and BMI-matched controls.

<table>
<thead>
<tr>
<th>Groups</th>
<th>M/F</th>
<th>Age (yr)</th>
<th>BMI (kg/m²)</th>
<th>Associated deficits (% patients)</th>
<th>Controls</th>
<th>Age (yr)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure pituitary (12)</td>
<td>9/3</td>
<td>55±5</td>
<td>26±1</td>
<td>67 67 92 0</td>
<td>54±5</td>
<td>25±1</td>
<td></td>
</tr>
<tr>
<td>Pituitary + possible hypothalamic (8)</td>
<td>7/1</td>
<td>39±5</td>
<td>31±2</td>
<td>100 100 100 63</td>
<td>39±5</td>
<td>31±2</td>
<td></td>
</tr>
<tr>
<td>Idiopathic (6)</td>
<td>6/0</td>
<td>33±5</td>
<td>26±3</td>
<td>67 67 67 0</td>
<td>33±5</td>
<td>25±3</td>
<td></td>
</tr>
<tr>
<td>Hypothalamic (4)</td>
<td>2/2</td>
<td>44±11</td>
<td>28±2</td>
<td>50 25 50 25</td>
<td>45±11</td>
<td>26±2</td>
<td></td>
</tr>
</tbody>
</table>

We first present detailed analyses for the 26 patients with pure pituitary defects, pituitary defects with possible hypothalamic involvement, or likely pituitary defects. An exploratory analysis of differences with patients with hypothalamic GHD follows.
Objective sleep quality

Table 7 presents the sleep parameters recorded in the 26 patients with pure pituitary defects, pituitary defects with possible hypothalamic involvement, and idiopathic GHD, and their gender, age and BMI-matched controls.

Table 7: Sleep variables recorded in 26 patients with pure pituitary defects, pituitary defects with possible hypothalamic involvement, and childhood-onset idiopathic GHD, and their individually matched controls (mean±SEM; SPT sleep period time, TST total sleep time, WASO wake after sleep onset).

<table>
<thead>
<tr>
<th></th>
<th>GHD patients</th>
<th>Controls</th>
<th>Condition GHD vs controls p level</th>
<th>Age young vs older p level</th>
<th>Age x condition interaction p level</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPT (min)</td>
<td>488±12</td>
<td>482±11</td>
<td>0.75</td>
<td>0.77</td>
<td>0.83</td>
</tr>
<tr>
<td>Sleep latency (min)</td>
<td>32±7</td>
<td>27±4</td>
<td>0.46</td>
<td>0.59</td>
<td>0.94</td>
</tr>
<tr>
<td>TST (min)</td>
<td>432±11</td>
<td>477±9</td>
<td>0.14</td>
<td>0.004</td>
<td>0.01</td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>83±2</td>
<td>88±1</td>
<td>0.05</td>
<td>0.001</td>
<td>0.03</td>
</tr>
<tr>
<td>WASO (min)</td>
<td>51±11</td>
<td>28±5</td>
<td>0.05</td>
<td>0.002</td>
<td>0.04</td>
</tr>
<tr>
<td>REM sleep (min)</td>
<td>91±7</td>
<td>100±5</td>
<td>0.28</td>
<td>0.002</td>
<td>0.05</td>
</tr>
<tr>
<td>Stages I+II (min)</td>
<td>220±11</td>
<td>267±14</td>
<td>0.02</td>
<td>0.93</td>
<td>0.70</td>
</tr>
<tr>
<td>Stage III (min)</td>
<td>47±5</td>
<td>37±4</td>
<td>0.06</td>
<td>0.67</td>
<td>0.17</td>
</tr>
<tr>
<td>Stage IV (min)</td>
<td>68±7</td>
<td>44±6</td>
<td>0.01</td>
<td>0.02</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Bedtimes, sleep period and sleep latencies were similar in GHD patients with primary pituitary lesions and controls, irrespective of age. While total duration of NREM sleep was not modified, GHD patients presented a shift of NREM sleep towards deeper stages, with lower amounts of stages I+II, and a more than 50% increase in the duration of stage IV. Differences between GHD patients and controls for total sleep time, sleep efficiency, WASO and REM were dependent on age (as revealed by a significant interaction between age and condition). Figure 10
illustrates the differences between GHD patients and controls for the sleep variables and the two age groups. The elevation in SWS (stages III+IV) relative to controls was significant in the entire group of GHD patients and in the younger group, but failed to reach statistical significance in the older group. Total sleep time was decreased and sleep fragmentation (as quantified by sleep efficiency and WASO) was increased in older, but not in younger GHD patients, as compared to controls. The increase in WASO in older subjects was not significant during the first 3 hours of sleep (GHD patients: 23±7 min; controls: 16±6 min; p=0.39) and reflected mainly sleep fragmentation during the later part of the night (hours 3-6 of sleep: 39±9 min vs 13±2 min, p=0.009). The reduction in total sleep time in older GHD patients was associated with lower amounts of REM sleep.

Fig. 10: Differences (mean±SEM) in total sleep time, sleep stages and delta activity between 26 GHD patients with pure pituitary defects, pituitary defects with possible hypothalamic involvement, or childhood onset idiopathic GHD, and their pair-matched healthy controls for the younger (n=13) and the older (n=13) age groups. Positive values indicate higher levels in GHD patients, negative values lower levels in GHD patients.
Mean profiles of delta, theta and alpha activities in both groups of 26 subjects are shown in Fig. 11. The normal homeostatic decline of delta activity occurred across the night in both groups. However, delta and theta activities were markedly higher in GHD patients than in controls.

**Fig. 11**: Mean profiles (+SEM) of absolute EEG spectral power in the delta, theta and alpha ranges during the first four NREM-REM cycles in GHD patients with pure pituitary defects, pituitary defects with possible hypothalamic involvement, or childhood-onset idiopathic GHD (left), and in their healthy controls (right).

Throughout the first 6 hours after sleep onset, mean delta activity in NREM sleep was increased by more than 50% in GHD patients as compared to controls. Theta power was also increased in patients compared to controls (Table 8). The elevation in delta power relative to controls was significant in the entire group of GHD patients and in the younger group, but failed to reach statistical significance in the
older group. EEG spectral power in the alpha range was not affected by GH deficiency.

**Table 8:** Mean spectral power levels in the delta, theta and alpha power, in GHD patients with pure pituitary defects, pituitary defects with possible hypothalamic involvement, or childhood-onset idiopathic GHD and in their healthy controls, over the first 6 hours of sleep.

<table>
<thead>
<tr>
<th>Condition</th>
<th>GHD patients</th>
<th>Controls</th>
<th>GHD vs controls p level</th>
<th>Age young vs older p level</th>
<th>Age x condition interaction p level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean delta power (µV^2)</td>
<td>1284±227</td>
<td>837±99</td>
<td>0.03</td>
<td>0.01</td>
<td>0.23</td>
</tr>
<tr>
<td>Mean theta power (µV^2)</td>
<td>146±27</td>
<td>96±12</td>
<td>0.05</td>
<td>0.47</td>
<td>0.94</td>
</tr>
<tr>
<td>Mean alpha power (µV^2)</td>
<td>58±9</td>
<td>51±6</td>
<td>0.53</td>
<td>0.95</td>
<td>0.27</td>
</tr>
</tbody>
</table>

**Subjective sleep quality and daytime sleepiness**

On average, the global PSQI was indicative of poor sleep (i.e. above the clinical cut-off of 5) in the 26 GHD patients with primary pituitary lesions while it was consistent with normal sleep in their controls (6.6±0.7 vs 3.6±0.5, p=0.04). The component assessing daytime sleepiness was also elevated in these patients as compared to their controls (1.1±0.2 vs 0.5±0.1, p=0.03 by paired Wilcoxon test).

No significant correlations were found between the global PSQI score and polysomnography-derived sleep variables. However, the daytime sleepiness component of the PSQI was associated with reduced sleep efficiency (r_s=-0.55, p=0.006) and longer WASO (r_s=0.47, p=0.02).
Quality of Life

Group data for QoL variables for the 26 patients with primary pituitary lesions and their matched controls are given in Table 8.

QoL scores were similar in U.S. and European patients. GHD patients had significantly higher total QoL-AGHDA scores than their controls, irrespective of age, but the scores were not indicative of a major impairment. Tiredness was the most frequent complaint and reached the highest score in both age groups. Only 30% of patients (8/26), as compared to 73% of controls (19/26), did not report any tiredness complaint (negative answer to each of the 7 questions; p<0.01). Memory problems were also more frequent in GHD patients than in controls.

Table 9: Global quality of life scores and scores for the different domains explored by the QoL-AGHDA questionnaire, in GHD patients with pure pituitary defects, pituitary defects with possible hypothalamic involvement, or childhood onset idiopathic GHD, and in their healthy controls. For each domain, the mean score per question was calculated.

<table>
<thead>
<tr>
<th></th>
<th>GHD patients</th>
<th>Controls</th>
<th>Condition</th>
<th>Age</th>
<th>Age x condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GHD vs controls</td>
<td>young vs older</td>
<td>interaction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p level</td>
<td>p level</td>
<td>p level</td>
</tr>
<tr>
<td>Global score</td>
<td>6.85±1.24</td>
<td>2.85±0.85</td>
<td><strong>0.006</strong></td>
<td>0.93</td>
<td>0.49</td>
</tr>
<tr>
<td>Tiredness</td>
<td>0.31±0.06</td>
<td>0.07±0.03</td>
<td><strong>0.001</strong></td>
<td>0.94</td>
<td>0.93</td>
</tr>
<tr>
<td>Memory and concentration</td>
<td>0.30±0.07</td>
<td>0.15±0.05</td>
<td><strong>0.0</strong></td>
<td>0.81</td>
<td>0.11</td>
</tr>
<tr>
<td>Tenseness</td>
<td>0.39±0.07</td>
<td>0.22±0.07</td>
<td>0.08</td>
<td>0.29</td>
<td>0.48</td>
</tr>
<tr>
<td>Social isolation</td>
<td>0.20±0.06</td>
<td>0.08±0.03</td>
<td>0.07</td>
<td>0.51</td>
<td>0.48</td>
</tr>
<tr>
<td>Self-confidence</td>
<td>0.18±0.05</td>
<td>0.11±0.05</td>
<td>0.26</td>
<td>0.43</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Figure 12 illustrates the differences between GHD patients and controls for the five domains of QoL and the two age groups. When each age group was analyzed
separately, tiredness was the only domain significantly affected in young patients, while deficits in memory and concentration, and a trend for more social problems were also found in older patients.

**Fig. 12:** Differences (mean±SEM) in quality of life scores for the five domains of the QoL-AGHDA scale between GHD patients with pure pituitary defects, pituitary defects with possible hypothalamic involvement, or childhood onset idiopathic GHD, and their pair-matched healthy controls for the younger (n = 13) and older (n = 13) age groups. Positive values indicate higher levels in GHD patients.

In the entire group of 26 patients, the score on the tiredness domain of QoL was associated with the daytime sleepiness component of the PSQI ($r_s=0.41$, $p=0.04$). In younger patients (n=13), a trend for a correlation between the tiredness score and the amount of SWS was detectable ($r_s=0.51$, $p<0.08$) (Fig. 13). No other correlations between sleep variables and global or partial QoL scores were found.

**Fig.13:** correlation between QoL-AGHDA tiredness score and SWS duration in younger patients (n=13) with aGHD.
Impact of etiology of GHD on objective sleep variables, subjective sleep quality and quality of life

Irrespective of GHD etiology (i.e. pure pituitary, pituitary with possible hypothalamic involvement, idiopathic childhood onset, hypothalamic), differences between patients and controls were qualitatively similar for all sleep variables derived from sleep staging as well as for subjective sleep quality and QoL. However, EEG spectral analysis revealed striking differences in the microarchitecture of non-REM sleep in patients with hypothalamic GHD as compared to those with pituitary GHD.

Fig. 14: Mean (+SEM) of absolute EEG spectral power in the delta range during the first four NREM-REM cycles in GHD patients as compared to their matched controls for the four diagnostic categories. From top to bottom: pure pituitary GHD (n=12), pituitary GHD with possible hypothalamic involvement (n=7), childhood onset idiopathic GHD (n=6), hypothalamic GHD patients (n=4). Differences in levels of delta activity across the four control groups are due to differences in sex, age and BMI distribution. For each diagnostic category, patients and controls are pair-matched for sex, age and BMI.
Indeed, as illustrated in Figure 15, the etiology of GHD was a significant predictor of the difference in delta activity between patients and controls after adjusting for age (p=0.05). In patients with hypothalamic GHD, the findings regarding delta activity were opposite from those observed in patients with “pure pituitary” GHD (p=0.005) or patients with childhood onset idiopathic GHD (p=0.05), with markedly decreased, rather than increased, delta activity in patients as compared to controls.

**Fig: 15:** Differences (mean±SEM) between patients and controls in total delta activity during the first 6 hours of sleep for: patients with pure pituitary GHD (n=12); patients with pituitary GHD with possible hypothalamic involvement (n=7; spectral analysis could not be performed for one patient); patients with childhood-onset idiopathic GHD (n=6); patients with hypothalamic GHD (n=4). Positive values indicate higher levels in GHD patients, negative values lower levels in GHD patients. **p<0.005 for difference between pure pituitary GHD and hypothalamic GHD, after controlling for age; * p=0.05 for difference between idiopathic GHD and hypothalamic GHD, after controlling for age.
**Study B**

13 patients completed phase B of the study (11M, 2F, age 46±5 years). Six patients were part of the “young” category and 7 were part of the “older” group. The average dose of rhGH was 0.4±0.1 mg/day. At the end of the rhGH period, average IGF-I was 227±30 ng/ml, vs 94±15 ng/ml at the end of the placebo period (p=0.001). Five patients started with the placebo phase, and 8 started with the rhGH phase. No side-effects were reported during rhGH replacement.

**Impact of rhGH treatment on anthropometric measures**

As shown in Table 10, no difference was observed in BMI at the end of the 4-months rhGH period, after controlling for the age group, compared to values obtained at the end of the placebo period. For the waist-to-hip ratio, data is available in both sessions for 9 patients. A trend for improvement was evident in this measure (p=0.10). Differences in body composition measures did not reach statistical significance, probably because data is available only for 6 patients; however the lean mass, and therefore the lean/fat ratio, increased with treatment.

**Table 10:** Anthropometric and body composition measures obtained for GHD patients after 4 months placebo or rhGH treatment (mean±SEM).

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>GH</th>
<th>Treatment</th>
<th>Age group</th>
<th>Treatment x age group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GH vs PL</td>
<td>young vs older</td>
<td>interaction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p level</td>
<td>p level</td>
<td>p level</td>
</tr>
<tr>
<td>BMI n=13</td>
<td>25.8±1.8</td>
<td>26±1.6</td>
<td>0.52</td>
<td>0.28</td>
<td>0.74</td>
</tr>
<tr>
<td>WHR n=9</td>
<td>0.92±0.02</td>
<td>0.90±0.02</td>
<td>0.10</td>
<td>0.45</td>
<td>0.13</td>
</tr>
<tr>
<td>Fat mass (kg) n=6</td>
<td>32.7±8.4</td>
<td>32.2±8.4</td>
<td>0.98</td>
<td>0.61</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>GH</td>
<td>Treatment</td>
<td>Age group</td>
<td>Treatment x age group</td>
</tr>
<tr>
<td>-----------------------------</td>
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<td>-----------</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GH vs PL</td>
<td>young vs older</td>
<td>interaction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p level</td>
<td>p level</td>
<td>p level</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>36.7±4.2</td>
<td>35.8±6.6</td>
<td>0.80</td>
<td>0.63</td>
<td>0.57</td>
</tr>
<tr>
<td>n=6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>51.7±6.3</td>
<td>54.8±5.8</td>
<td>0.54</td>
<td>0.63</td>
<td>0.89</td>
</tr>
<tr>
<td>n=6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean mass (%)</td>
<td>63.4±4.5</td>
<td>66.9±5.2</td>
<td>0.8</td>
<td>0.63</td>
<td>0.57</td>
</tr>
<tr>
<td>n=6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total body water (kg)</td>
<td>39.1±4.7</td>
<td>41.1±4.2</td>
<td>0.54</td>
<td>0.79</td>
<td>0.60</td>
</tr>
<tr>
<td>n=6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total body water (%)</td>
<td>47.8±3</td>
<td>50±3.9</td>
<td>0.87</td>
<td>0.18</td>
<td>0.78</td>
</tr>
<tr>
<td>n=6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean/fat ratio</td>
<td>1.8±0.3</td>
<td>2.5±0.7</td>
<td>0.86</td>
<td>0.45</td>
<td>0.75</td>
</tr>
<tr>
<td>n=6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Objective sleep quality**

**Result of the patient with GHD of hypothalamic origin**

We present the results of this patient (a 74-year old woman who suffered a cerebral trunk thrombosis) separately since she is the only one in this category.

**Table 11**: Sleep parameters, delta activity levels, PSQI score and AGHDA score of the patient with GHD of hypothalamic origin after 4 months placebo or rhGH treatment.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>GH</th>
</tr>
</thead>
<tbody>
<tr>
<td>TST (min)</td>
<td>390.5</td>
<td>382</td>
</tr>
<tr>
<td>Stages I+II (min)</td>
<td>314</td>
<td>194.5</td>
</tr>
<tr>
<td>Stage III +IV (min)</td>
<td>2</td>
<td>79</td>
</tr>
<tr>
<td>Stage REM (min)</td>
<td>74.5</td>
<td>108.5</td>
</tr>
<tr>
<td>WASO (min)</td>
<td>163.5</td>
<td>63</td>
</tr>
<tr>
<td>Delta activity in NREM (sum first 3 hours of sleep)</td>
<td>4391</td>
<td>95520</td>
</tr>
<tr>
<td>Theta activity in NREM (sum first 3 hours of sleep)</td>
<td>11486</td>
<td>20862</td>
</tr>
<tr>
<td>Alpha activity in NREM (sum first 3 hours of sleep)</td>
<td>7234</td>
<td>15328</td>
</tr>
<tr>
<td>PSQI global score</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>PSQI daytime sleepiness</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>GH</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>AGHDA global score</td>
<td>8</td>
<td>n.a.</td>
</tr>
<tr>
<td>AGHDA tiredness</td>
<td>0.71</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

**Results of the other GHD patients**

Of the 12 remaining patients, 5 had a “pure pituitary” origin of the GHD, 4 patients had an idiopathic GHD, 3 patients had a GHD of pituitary origin with likely hypothalamic involvement. While reviewing the results of the spectral analysis, we noticed that one patient (Bxl07), a 29-year old man with a diagnosis of transsection of the pituitary stalk, displayed results under placebo that were inconsistent with those obtained during study A, i.e. also in the absence of active treatment: the delta activity levels in study A were approximately 7 times higher than in the placebo session of study B. However delta activity is highly reproducible in a single individual even in recordings performed at several months distance. Indeed, the reproducibility of delta activity in each individual was confirmed by a highly significant correlation between the results of study A and those of the placebo session of study B when this patient was excluded ($r_s=0.9$, $p=0.002$; Fig. 16), while the correlation failed to reach statistical significance when this patient was included. Furthermore, the Grubbs test for detection of statistical outliers performed on the difference of both delta power and theta power during NREM between session A and PL identified this patient as a significant outlier ($p<0.05$). The most likely explanation for the difference in results observed in this patient is a technical artifact due to different amplifier settings. We are therefore presenting the results of the objective sleep analysis and of the spectral analysis for the other 11 patients.
**Fig. 16:** Correlation between delta power in NREM in sessions A and B-placebo (B-PL) including (left) and excluding (right) patient Bxl07.

The main sleep variables are presented in table 12.

**Table 12:** Mean (±SEM) values of sleep variables in 11 GHD patients after 4 months placebo or rhGH treatment.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>GH</th>
<th>Treatment GH vs PL p level</th>
<th>Age group young vs older p level</th>
<th>Interaction treatment x age group p level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep period time (min)</td>
<td>484±11</td>
<td>427±22</td>
<td><strong>0.02</strong></td>
<td>0.92</td>
<td>0.34</td>
</tr>
<tr>
<td>Sleep latency (min)</td>
<td>16±5</td>
<td>22±10</td>
<td>0.28</td>
<td>0.21</td>
<td>0.60</td>
</tr>
<tr>
<td>Total sleep time (min)</td>
<td>435±14</td>
<td>385±24</td>
<td><strong>0.05</strong></td>
<td>0.36</td>
<td>0.24</td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>86±3</td>
<td>84±4</td>
<td>0.33</td>
<td>0.24</td>
<td>0.38</td>
</tr>
<tr>
<td>WASO (min)</td>
<td>49±3</td>
<td>42±11</td>
<td>0.56</td>
<td>0.07</td>
<td>0.79</td>
</tr>
<tr>
<td>REM sleep (min)</td>
<td>82±10</td>
<td>67±9</td>
<td>0.22</td>
<td><strong>0.03</strong></td>
<td>0.36</td>
</tr>
<tr>
<td>REM sleep (%)</td>
<td>19±2</td>
<td>17±2</td>
<td>0.51</td>
<td>0.14</td>
<td>0.42</td>
</tr>
<tr>
<td>Stages I+II (min)</td>
<td>272±15</td>
<td>249±19</td>
<td>0.16</td>
<td>0.62</td>
<td>0.50</td>
</tr>
<tr>
<td>Stages I+II (%)</td>
<td>64±5</td>
<td>66±5</td>
<td>0.18</td>
<td>0.33</td>
<td>0.07</td>
</tr>
<tr>
<td>Stage III (min)</td>
<td>30±8</td>
<td>30±11</td>
<td>0.47</td>
<td>0.93</td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td>Stage III (%)</td>
<td>6±2</td>
<td>8±2</td>
<td>0.64</td>
<td>0.99</td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td>Stage IV (min)</td>
<td>52±15</td>
<td>39±13</td>
<td>0.14</td>
<td>0.33</td>
<td>0.35</td>
</tr>
<tr>
<td>Stage IV (%)</td>
<td>12±3</td>
<td>9±3</td>
<td>0.09</td>
<td>0.24</td>
<td>0.38</td>
</tr>
</tbody>
</table>
Total sleep time was decreased by 50 min after rhGH treatment (385±24 vs 435±14 min), and the difference was significant after controlling for age (p=0.05). As illustrated in Fig. 17, in younger subjects, this difference was mostly due to a decreased duration of SWS (75±18 vs 114±24 min). A trend for significance was observed when SWS was expressed as percentage of total sleep time (18.0±3.5 vs 24.2±5.2%, p=0.07). REM sleep duration was also shorter but the difference did not reach statistical significance. In older patients, the decrease in sleep time was due to a shorter duration of the shallower stages of NREM sleep (stages I+II: 252±29 vs 278±23 min, p=0.04), while SWS duration was unchanged. An impact of age was also observed for sleep efficiency, wake after sleep onset (WASO) and REM sleep, as expected.

The average profiles of EEG delta, theta and alpha power in the 2 conditions are presented in Fig. 18.
During the first 3 hours after sleep onset, delta activity was decreased by rhGH treatment; after controlling for age group, the difference did not reach statistical significance (p=0.1), however, when age was taken into account as a continuous variable, the difference became significant (210166±62769 vs 283536±93402 µV², p=0.05), and this effect tended to persist when the first 6 hours of sleep were considered (p=0.07). As illustrated in Fig. 19, young and older patients behaved differently for this measure as well, as the decrease in delta activity was much more evident in younger patients.
Fig. 19: Mean difference (±SEM) in average EEG delta spectral power between GH and placebo over the first 3 and 6 hours of sleep in younger (n=4) and older (n=7) GHD patients. Negative values indicate lower levels under rhGH.

Theta activity decreased as well under rhGH, however the difference failed to reach statistical significance.

**Subjective sleep quality and daytime sleepiness**

PSQI scores are available in both sessions for 6 patients. PSQI global score was decreased after treatment with rhGH (4.5±0.7) compared to placebo (6±1.3), however the difference failed to reach statistical significance (p=0.23). Nevertheless, these results indicate that, on average, patients experienced an improved sleep quality on rhGH treatment, as scores were below 5, the cut-off for impaired sleep quality. In particular, 3/6 patients went from a score above 5 to a score below 5. No difference was observed for the scores obtained for the component of the questionnaire focusing on daytime sleepiness.
Quality of life

AGHDA scores in both sessions are available for 8 patients. Global scores were significantly lower, i.e. improved, after 4 months of rhGH s.c. compared to placebo (8.8±2.5 vs 4.8±1.6, p=0.02 by paired Wilcoxon test). Ratings for the tiredness were also lower (0.04±0.03 vs 0.37±0.12, p=0.06 by paired Wilcoxon test). Furthermore, only 2/8 patients reported tiredness-related complaints at the end of the rhGH period, vs 5/8 after placebo. Fig. 20 describes the differences between the 2 sessions in the single domains for both age groups: scores improved in all domains, except for the domain “social isolation” in older patients, but the differences failed to reach statistical significance, probably due to the limited number of subjects in each age group.

Fig. 20: Differences (mean±SEM) in quality of life scores for the five domains of the QoL-AGHDA scale between placebo and rhGH for the younger (n=4) and older (n=4) GHD patients. Negative values indicate lower levels (i.e. improvement) under rhGH.
DISCUSSION

The present study, performed in a large group of adult GHD patients individually matched for gender, age and BMI with control subjects, indicates that GHD due to a confirmed or likely primary pituitary defect is associated with an excess of high intensity SWS, poor subjective sleep quality and daytime sleepiness. Furthermore, quality of life appeared impaired in these subjects at baseline, consistent with what is reported in literature: several studies have shown that aGHD patients complain of an impaired quality of life, and in particular of tiredness and low energy levels that significantly impact their daily life\textsuperscript{30,32-38}.

Excessive amounts of SWS represent an unusual form of sleep disturbance as the vast majority of sleep disorders are characterized by reduced, rather than enhanced, SWS. However, a relatively high duration of SWS (29\% of total sleep time on average) was also reported in middle-aged GHD patients\textsuperscript{162}. Furthermore, a recent study performed in women with GHD due to Sheehan’s syndrome also found a longer SWS duration in patients compared to controls matched for age and BMI\textsuperscript{158}.

In our study, the enhancement of EEG power in adult GHD patients was specific to the delta and theta ranges, as alpha power was similar in patients and controls. GHRH has been extensively documented as having a stimulatory effect on SWS and delta activity\textsuperscript{111-114,117}. Furthermore, functional and anatomical evidence for a role of GHRH in the regulation of NREM sleep was obtained recently\textsuperscript{108}: indeed, the i.c.v. administration of GHRH to male adult rats produced an increase in NREM duration and SWA, as well as an activation of GABAergic neurons in sleep-active areas of the hypothalamus. One possible mechanism underlying the enhancement of
SWS and delta activity observed in GHD patients with no evidence of hypothalamic involvement could therefore be the lack of negative feedback regulation of GHRH by circulating GH, with a subsequent exaggeration of the stimulatory effect of GHRH on delta activity. Consistent with this putative mechanism, upregulation of hypothalamic GHRH activity, due to the absence of inhibitory control by GH, associated with increased amounts of NREM sleep has indeed been demonstrated in the spontaneous dwarf rat, an animal model of GHD due to a mutation of the GH gene. Conversely, mice and rats with nonfunctional GHRH receptor (lit/lit mice and dw/dw rats) have lower amounts of spontaneous NREM sleep than wild types. A reduction in the inhibitory control of hypothalamic GHRH by GH might contribute to an incomplete inhibition of SWS generating mechanisms during wake as well as during sleep and result in daytime sleepiness. In all our pituitary GHD patients, tiredness was the most consistent complaint on the QoL-AGHDA scale and was correlated with daytime sleepiness as assessed by a validated questionnaire that is not specific of GHD. Among our younger patients, those with higher amounts of SWS tended to have higher tiredness scores in the QoL-AGHDA. While these associations do not indicate causality, they suggest that sleep disturbances may contribute to daytime sleepiness and to tiredness complaints.

Our findings are at variance with those reported 20 years ago in eight subjects with isolated GHD: the authors observed a decrease in SWS duration and delta activity in their patients. In this study, controls were matched for sex and age, but not for BMI. Bedtimes and sleep periods were not reported, activity during waking hours was not controlled and daytime naps were not prohibited. Total sleep time
was surprisingly long in patients, ranging from 7h45 to 11h44 with a median of nearly 9 hours. Extended bedtimes and naps generally result in a shift of NREM sleep towards lighter stages which could explain the discrepancy with our findings. More recently, sleep profiles in a group of patients with adult onset GHD were reported to be comparable to a non-specified and non-individually matched healthy control group from the literature. Because of the high variability, especially with gender and age, of sleep characteristics among normal individuals, this study design was unlikely to detect differences between GHD patients and controls.

In remarkable contrast to findings in the two groups of patients in whom there was no evidence for a supra-pituitary involvement in the etiology of GHD, the patients with hypothalamic GHD had shallow non-REM sleep with markedly lower delta power than their controls, consistent with reduced GHRH activity. While these limited results await confirmation in a larger group of patients, they are consistent with a central role of GHRH activity in modulating sleep quality in GHD. Of note, a marked decrease in SWS duration was reported in children with psychosocial dwarfism, in whom the origin of the GH deficiency is thought to be hypothalamic in origin. When re-evaluated after a period of separation from their family, these children exhibited a recovery of GH secretion and the normalization of SWS duration.

Finally, delta activity results in our patients with pituitary defects and possible hypothalamic involvement were intermediate between those of pituitary and hypothalamic GHD patients, consistent with a mixed etiology of GHD.
The impact of rhGH replacement on sleep architecture and quality was evaluated after 4 months treatment, and a significant reduction in sleep duration was observed. This is unlikely to be due to an order effect or to seasonality, as placebo and rhGH were administered in a randomized cross-over fashion and the patients were studied all year long. The underlying change in sleep architecture was different in younger and older patients, as younger patients displayed a decrease in SWS, while in older adults SWS was preserved at the expense of the shallower stages of NREM sleep (stages I and II). Similarly, delta activity was lower after treatment, mostly in younger subjects.

Results published in literature about the effects of rhGH replacement on sleep in adult GHD patients are inconclusive. SWS duration was reported to be unchanged after 6 months on rhGH in young adult patients with isolated GHD, compared to baseline \textsuperscript{159}. Another group found a decrease in SWS duration in middle-aged patients with GHD of variable etiology after 6 months of rhGH withdrawal \textsuperscript{160}. Several authors report no impact of replacement therapy on sleep parameters, compared to placebo \textsuperscript{158,161-162}. However, none of these reports took the impact of age into account, when the age range of the patients studied spanned from 21 to 73 years.

Consistent with literature, the impairment in QoL was at least in part reversible with rhGH treatment: our patients obtained lower, i.e. improved, scores in the QoL-AGHDA questionnaire after 4 months treatment. The physiologic basis of the
improved well-being induced by rhGH treatment has not been fully elucidated. However it is likely that GH exerts direct effects on the central nervous system, as demonstrated by the high density of GH receptors in the hippocampus and choroid plexus. Animal studies have shown that systemic GH administration influences the metabolism of monoamines in the brain. Moreover, an increase in cerebro-spinal fluid (CSF) beta-endorphin concentrations has been reported in adult GHD patients after treatment. Another group reported a decrease in CSF concentrations of homovanillic acid, a dopamine metabolite, similar to what is observed after successful pharmacologic treatment of depressive episodes. After rhGH replacement, the frequency of tiredness complaints decreased from 5/8 patients to 2/8 compared to placebo. No association was observed between tiredness scores and sleep parameters, however the number of patients for whom data is available is limited (n=8). Subjective sleep quality was also improved by rhGH, as shown by the decrease of PSQI scores below the cut-off for impaired sleep quality; however the difference in scores was not significant and no correlation was observed between QoL-AGHDA tiredness scores and PSQI daytime sleepiness score, probably due to a lack of statistical power. To our knowledge, only one study evaluated subjective ratings of sleep quality in aGHD patients (n=12). Using the PSQI before and after 3 months treatment, the authors observed an improvement in the global score, the sleep quality and sleep latency scores but not daytime sleepiness. Unfortunately, sleep was not monitored in this study.
Older adults with pituitary GHD had less REM sleep than their controls. This loss of REM sleep, possibly due to GH deficiency 100-101, 152, 178, could be involved in the emergence of memory problems which were reported more frequently by older patients compared to their controls. There is indeed a growing body of evidence linking REM sleep and memory 179-180. Older adults with pituitary GHD also had more sleep fragmentation in the later part of the night and obtained less total sleep time than control subjects. While increased number and duration of awakenings, decreased total sleep time and decreased amounts of REM sleep are typical of normal adults in late life 42, pituitary GHD appears associated with an exacerbation of these sleep disturbances, suggestive of a more rapid senescence of sleep-regulating mechanisms in the absence of GH. The combination of increased duration and intensity of SWS in the early part of the night with increased sleep fragmentation in the later part of the night represents a peculiar sleep disorder as higher levels of SWS are generally associated with more consolidated sleep.

Nearly all GHD patients in our study received chronic replacement therapy (including hydrocortisone/cortisone acetate, L-thyroxine and sex steroids) to correct associated pituitary hormonal deficits. The substitution dose of glucocorticoid in some cases exceeded the normal endogenous cortisol secretion. However, the daily substitution dose (mean ± SEM) averaged 12.0±0.7 mg hydrocortisone per m² of body surface area (considering that 25 mg cortisone acetate is equivalent to 15 mg hydrocortisone). This dose is quite in agreement with current recommendations 181. The rationale for administering somewhat higher hydrocortisone amounts than the
normal endogenous secretion is that the absorption is never 100%, and quite variable from one patient to another, and that oral administration schedule cannot mimic the physiological pattern of hormonal secretion: the morning dose is given when the patient wakes up, i.e. several hours after the onset of the endogenous circadian rise of cortisol in normal subjects. Thus, despite appropriate replacement therapy, patients with primary adrenal insufficiency or with adrenal insufficiency secondary to a primary pituitary defect are likely to have supra-normal nocturnal CRH levels, while patients with adrenal insufficiency secondary to a hypothalamic defect will have low CRH levels.

Since CRH has been reported to inhibit SWS\textsuperscript{182}, elevated nocturnal levels of CRH in GHD patients with primary pituitary defects would favor a decrease of SWS, i.e. the opposite of what was observed. Thus, it is unlikely that glucocorticoid replacement therapy would be responsible for the higher amounts of SWS observed in our patients when compared to controls. On the other hand, CRH is a well-documented arousal factor that promotes wakefulness. Thus, elevated nocturnal levels of CRH in our patients could have promoted an increase in wake after sleep onset. Therefore, we cannot exclude that increased durations of wake in our patients with GHD of primary pituitary origin could be partly related to elevated CRH levels.
CONCLUSION

In conclusion, the present findings indicate that GHD may be associated with major sleep alterations, and suggests that “tiredness”, a major QoL complaint in GHD, may reflect poor sleep quality and daytime sleepiness. The sleep phenotype of GHD may be dependent on its etiology. In particular, patients with GHD of pituitary origin appeared to have an increased duration of SWS and enhanced SWA compared to age, gender and BMI-matched controls. These results are compatible with the hypothesis of a higher endogenous GHRH tone in these patients, as GHRH has been shown to enhance SWS and SWA. Conversely, patients with GHD of hypothalamic origin displayed lower SWA levels, although these findings need to be replicated in a wider population. Age-dependent differences in sleep structure were observed, and the effects of aging on sleep (sleep fragmentation, lower sleep quality, decreased REM sleep duration) appeared to be exacerbated in older GHD patients. Recombinant human GH treatment administered for 4 months appears to reverse some of the alterations observed in the macro- and micro-architecture of sleep, which likely contributes to the improved well-being reported by patients.
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REFERENCES


29. Shalet SM. Partial growth hormone deficiency in adults; should we be looking for it? *Clin Endocrinol (Oxf).* Apr 23 2010.


61. Vahl N, Moller N, Lauritzen T, Christiansen JS, Jorgensen JO. Metabolic effects and pharmacokinetics of a growth hormone pulse in healthy adults:


159. Aström C, Lindholm J. Growth Hormone deficient young adults have decreased deep sleep. *Neuroendocrinol.* 1990;51:82-84.


Appendix A: Quality of life Assessment of Growth Hormone Deficiency in Adults (QoL-AGHDA)

QoL - AGHDA

Quality of Life
Assessment of GH Deficiency in Adults

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LISTED BELOW ARE SOME STATEMENTS that people may make about themselves.

Read the list carefully and put a tick in the box marked YES if the statement applies to you.

Tick the box marked NO if it does not apply to you.

Please answer every item. If you are not sure whether to answer YES or NO, tick whichever answer you think is most true in general.

I have to struggle to finish jobs
I feel a strong need to sleep during the day
I often feel lonely even when I am with other people
I have to read things several times before they sink in

It is difficult for me to make friends
It takes a lot of effort for me to do simple tasks
I have difficulty controlling my emotions
I often lose track of what I want to say

I lack confidence
I have to push myself to do things
I often feel very tense
Now please go back to page 1 and make sure that you have answered "YES" or "NO" to every question, on all two pages of the questionnaire. Thank you for your help.
Appendix B: Pittsburgh Sleep Quality Index questionnaire

Pittsburgh Sleep Quality Index (PSQI)

Instructions: The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

During the past month,

1. When have you usually gone to bed? 
2. How long (in minutes) has it taken you to fall asleep each night? 
3. When have you usually gotten up in the morning? 
4. How many hours of actual sleep did you get that night? (This may be different than the number of hours you spend in bed) 

<table>
<thead>
<tr>
<th>5. During the past month, how often have you had trouble sleeping because you...</th>
<th>Not during the past month (0)</th>
<th>Less than once a week (1)</th>
<th>Once or twice a week (2)</th>
<th>Three or more times a week (3)</th>
</tr>
</thead>
</table>
a. Cannot get to sleep within 30 minutes |  |  |  |  |
b. Wake up in the middle of the night or early morning |  |  |  |  |
c. Have to get up to use the bathroom |  |  |  |  |
d. Cannot breathe comfortably |  |  |  |  |
e. Cough or snore loudly |  |  |  |  |
f. Feel too cold |  |  |  |  |
g. Feel too hot |  |  |  |  |
h. Have bad dreams |  |  |  |  |
i. Have pain |  |  |  |  |
j. Other reason(s), please describe, including how often you have had trouble sleeping because of this reason(s):  

6. During the past month, how often have you taken medicine (prescribed or “over the counter”) to help you sleep?

7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?

<table>
<thead>
<tr>
<th>9. During the past month, how would you rate your sleep quality overall?</th>
<th>Very good (0)</th>
<th>Fairly good (1)</th>
<th>Fairly bad (2)</th>
<th>Very bad (3)</th>
</tr>
</thead>
</table>

Component 1  
#9 Score  
Component 2  
#2 Score (≤15 min (0), 16-30 min (1), 31-60 min (2), >60 min (3))  
C1

Component 3  
#4 Score (>7 (0), 6-7 (1), 5-6 (2), <5 (3))  
C2

Component 4  
(total # of hours asleep)/(total # of hours in bed) x 100  
>85% (0), 75%-84% (1), 65%-74% (2), >65% (3)  
C3

Component 5  
# sum of scores 5b to 5j (0-0; 1-9-1; 10-18-2; 19-27-3)  
C4

Component 6  
#6 Score  
C5

Component 7  
#7 score + #8 score (0-0; 1-2-1; 3-4-2; 5-6-3)  
C6

Add the seven component scores together ______ Global PSQI Score _______
Appendix C: Karolinska Sleep Log

KAROLINSKA SLEEP LOG

Complete in morning within one hour of awaking:
1. At what time did you go to bed and turn the light off last night? _____ PM or AM
2. At what time did you wake up this morning? _____ PM or AM
3. How long did you sleep? _____ hours and _____ minutes
4. How long did it take you to fall asleep? _____ hours and _____ minutes
5. How many awakenings did you have last night? _____
6. How many total minutes were you awake after falling asleep last night? _____ minutes (Don't include time in bed before falling asleep)

Circle one per question only:

7. How did you sleep?
   1  2  3  4  5
   Very poorly  Very well

8. Did you feel refreshed after you arose this morning?
   1  2  3  4  5
   Not at all  Completely

9. Did you sleep soundly?
   1  2  3  4  5
   Very Restless  Very Soundly

10. Did you sleep throughout the time allotted for sleep?
    1  2  3  4  5
    Woke up much too early  Slept through the night

11. How easy was it for you to wake up?
    1  2  3  4  5
    Very Easy  Very Difficult

12. How easy was it for you to fall asleep?
    1  2  3  4  5
    Very Easy  Very Difficult

13. How much did you dream last night?
    1  2  3  4  5
    None  Very Much