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ORIGINAL ARTICLE

# Phase-shifts of 24-h rhythms of hormonal release and body temperature following early evening administration of the melatonin agonist agomelatine in healthy older men

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## Summary

**Objective** Older adults are less responsive to the phase-shifting effects of light than younger subjects and may have difficulties adapting to abrupt time shifts. This study aims to determine whether the potent melatonin agonist agomelatine (S-20098) is capable of phase-shifting overt circadian rhythms in older adults.

**Subjects and design** Eight healthy elderly men participated in a double-blind, two-period, cross-over study of 15 days of daily administration of either agomelatine (50 mg) or placebo at 1830 h.

**Measurements** At the end of each treatment period, the 24-h profiles of body temperature and of the plasma levels of GH, PRL, cortisol and TSH were collected and sleep was monitored polygraphically.

**Results** Phase-advances, averaging nearly 2 h, were observed for the temperature profile and for the variables characterizing the temporal organization of cortisol secretion following agomelatine administration. A similar trend was observed for the circadian rise of plasma TSH. There was no effect of agomelatine on any of the sleep variables. Agomelatine stimulated GH secretion during the wake period and was associated with a transient elevation of PRL levels.

**Conclusions** Melatonin agonists such as agomelatine may be useful to phase-shift at least some overt circadian rhythms in older adults.

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## Introduction

In ageing, sleep becomes shallow and fragmented and wake-up time tends to advance.[1] Many 24-h hormonal rhythms, including those of melatonin, PRL, cortisol, TSH and GH, are dampened and/or advanced, suggesting alterations in the processes involved in the entrainment of overt rhythms.[2–5] Studies in both rodents and humans have indeed demonstrated that ageing is associated with a reduced responsiveness of the circadian clock to both photic and nonphotic entraining agents.[2,6–8] Older adults may thus have greater difficulties adapting to abrupt time shifts such as those occurring during jet lag or after shift work rotations.

Phase-shifting effects of melatonin on human circadian rhythms have been demonstrated in young adults.[9,10] Whether melatonin or melatonin agonists can produce consistent phase-shifts in older men has not been systematically studied. Studies in hamsters have indicated that agomelatine (S-20098), a powerful melatonin agonist, administered in the evening is able to restore the sensitivity of the suprachiasmatic nucleus (SCN) to external zeitgebers, but that the magnitude of the shifts is reduced by approximately 30% in older as compared to young animals.[11,12] A human study[13] showed that phase-advances, but not phase-delays, were attenuated in response to light exposure in older adults. Melatonin could also facilitate circadian adaptation through its putative hypnotic properties.[14] The effects of melatonin on sleep are, however, controversial.[15–18]

Agomelatine (S-20098) is an antidepressant that is a powerful agonist of melatonin[19,20] and has 5-hydroxytryptamine$_{2C}$ ($5HT_{2C}$) antagonist properties.[21] In healthy young adults, large phase-advances of circadian rhythms – similar to those elicited by melatonin – have been observed following acute early evening administration of agomelatine.[22] The present study was designed to determine whether 2 weeks of early evening administration of agomelatine would affect the overt rhythms of body temperature, hormonal release and sleep quality in healthy older adults.

## Materials and methods

### *Subjects*

Eight normal men (51–76 years old, mean 60 years; body mass index (BMI) 21–29 kg/m$^2$, mean 25 kg/m$^2$) participated in the study. The eligibility of each subject was evaluated during a prestudy period that included a complete clinical examination, a biological screening and

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two nights of sleep recording. Subjects with alcohol abuse, other substance abuse, personal history of physical or psychological illness, endocrine disease, sleep complaints, or other conditions that could interfere with circadian rhythms and/or the sleep/wake cycle were excluded. Shift workers or subjects who had travelled across time zones during the month preceding the study were excluded.

Screening levels of IGF-I in enrolled subjects ranged from 106 to 175 μg/l. The duration of slow wave sleep (SWS) ranged from 7 to 52 min.

All subjects gave written informed consent after receiving a complete explanation of the aims and means of the study.

### *Protocol*

The protocol, approved by the Institutional Review Board of the Université Libre de Bruxelles, was designed as a randomized, double-blind, placebo-controlled, cross-over study. The subjects were asked to follow their usual bedtimes, that is going to bed at around 2300 h and getting up at around 0700 h during the two treatment periods.

The subjects were studied during two 15-day periods, separated by a wash-out period of 2 weeks. During the study periods, they received daily, at around 1830 h, a tablet of either 50 mg of agomelatine or placebo. The sequence of administration was randomized: three subjects received placebo first and five subjects received agomelatine first. During the wash-out period, all subjects received placebo.

At the end of each treatment period, the subjects were admitted at about 1700 h in the Sleep Laboratory for 48 h.

During each inpatient period, indoor light intensity was less than 300 lux during the daytime and no naps were permitted. Subjects were kept recumbent in total darkness from 2300 h until 0700 h and sleep was polygraphically recorded on both nights. On the second day of hospitalization, an intravenous 10% glucose infusion was started at 0900 h and administered at a constant rate of 5 g/kg/24 h until 1500 h the next day. The subjects remained fasted throughout the duration of glucose infusion but could drink water *ad libitum*. This procedure prevented possible effects of meal ingestion on hormonal profiles. A sterile heparin-lock catheter was inserted in a contralateral forearm vein at 1300 h. Blood samples were obtained from 1400 h at 15-min intervals for 25 h. Thereafter the rate of glucose infusion was progressively tapered until discontinued at 1700 h. The total amount of blood withdrawn was less than 300 ml for each inpatient study. Data collected during the first hour are illustrated in the figures but were not included in calculations. The i.v. line was kept patent by a slow drip of heparinized saline (750 IU heparin in 100 ml 0·9 g NaCl). Blood samples were collected using a plastic syringe connected to the lateral arm of a three-way stopcock. During waking hours, the stopcock was directly attached to the antecubital catheter. During the sleep period, the indwelling catheter was connected to plastic tubing extending to an adjacent room through a hole in the wall. To prevent sample dilution, before collecting a blood sample through the plastic tubing, 6 ml of the saline solution was removed together with 2 ml of blood. After having removed the blood sample, the dead space saline mixed with blood was returned to the subject and the tubing was slowly rinsed using heparinized saline drip. Protein levels were measured in each plasma sample to detect any artefactual dilution.

Temperature was recorded with a rectal probe (Mini-Logger, Mini-Mitter Co., Inc., Sunriver, OR, USA) every 5 min for 24 h. Valid recordings were obtained for both study periods in seven of the eight subjects.

### *Sleep analysis*

Polysomnographic recordings were scored according to the criteria of Rechtschaffen and Kales.[23] Sleep onset and final awakening were, respectively, defined as the time of occurrence of the first and of the last 20-s interval scored 2, 3, 4 or REM. Sleep period time (SPT) was defined as the time interval separating sleep onset and final awakening. Total sleep time (TST) was defined as the SPT minus the minutes of awakenings. Sleep efficiency and sleep maintenance were defined as the TST, expressed as percentage of the time spent in bed and as percentage of the SPT, respectively. Sleep latency was defined as the time interval from lights out until sleep onset. The number of awakenings was defined as the total number of awakenings occurring during the SPT.

### *Hormonal assays*

For each hormone, all samples from a given subject were analysed in the same assay. GH, PRL, cortisol and TSH levels were measured using a chemiluminescence assay (Immulite, Diagnostic Products Corporation, Los Angeles, CA, USA), and lower limits of sensitivity were 0·05 μg/l, 0·5 μg/l, 27·6 nmol/l, 0·002 mU/l, respectively. Mean intra-assay coefficients of variation (CVs) were 6, 6, 7 and 4·2%, respectively. IGF-I levels were measured at 0900 h using a radioimmunoassay (Nichols Institute Diagnostics, USA) that includes an acid–ethanol extraction procedure, with a lower limit of sensitivity of 15 μg/l and a mean intra-assay CV variation of 3%.

### *Analysis of individual profiles*

The waveshape of individual profiles of body temperature and of plasma TSH and PRL was quantified by a best-fit curve obtained using a robust locally weighted regression procedure proposed by Cleveland[24] with a regression window of 4 h. Periodogram calculations were used to quantify circadian variations of cortisol profiles.[25] The values (and timings) of the acrophase and the nadir were defined as the levels (the timings) corresponding to the maximum and the minimum of the best-fit curve, respectively. The amplitude was defined as 50% of the difference between the acrophase and the nadir values. The quiescent period of cortisol concentrations was defined as starting (ending) when concentrations lower (higher) than 50% of the 24-h mean were observed for at least three consecutive samples. The end of the quiescent period of cortisol secretion corresponds to the beginning of the circadian nocturnal rise towards the morning maximum.

Significant pulses of GH secretion were identified using a modification of the computer algorithm ULTRA.[26] The threshold for significance of a pulse was set at twice the intra-assay CV. For each significant pulse, the amount of GH secreted was estimated by deconvolution based on a one-compartment model for GH clearance and variable individual half-lives, as described previously.[26]

**Table 1.** Sleep variables (mean ± SEM)

| | Placebo | Agomelatine | *P*-level |
|---|---|---|---|
| Sleep onset (clock time) | 2324 h ± 11 min | 2317 h ± 5 min | ns |
| Final awakening (clock time) | 0703 h ± 5 min | 0654 h ± 11 min | ns |
| Total sleep time (min) | 391 ± 11 | 382 ± 16 | ns |
| Wake (min) | 68 ± 7 | 75 ± 14 | ns |
| Stage 2 (min) | 231 ± 14 | 204 ± 14 | ns |
| Slow wave sleep (min) | 44 ± 9 | 47 ± 11 | ns |
| REM (min) | 71 ± 6 | 65 ± 5 | ns |
| REM latency (min) | 60 ± 7 | 63 ± 10 | ns |
| Sleep efficiency (%) | 81 ± 3 | 80 ± 3 | ns |
| Sleep maintenance (%) | 85 ± 2 | 84 ± 3 | ns |
| Sleep latency (min) | 14 ± 7 | 16 ± 6 | ns |
| Number of awakenings | 67 ± 8 | 67 ± 10 | ns |

On average, the half-disappearance time was 14 ± 0·6 min (mean ± SEM). A volume of distribution of 7% of body weight was used in these calculations. The total amount of GH secreted over a given time interval was determined by summing the amounts secreted in each of the significant pulses occurring during that time interval.

### *Tests of significance*

Because sleep quality is altered by the presence of the sampling catheter,[27] possible effects of agomelatine on sleep were evaluated during the nights without blood sampling.

Differences between values obtained under placebo and under agomelatine administration were evaluated using the Wilcoxon non-parametric test (StatView SE+ software for Macintosh computers; Abacus Concepts, Inc., Berkeley, CA, USA). Detection of outliers was performed using the Grubbs test.[28,29]

All group values were summarized by the mean ± SEM.

## Results

### *Effects of agomelatine on sleep*

As shown in Table 1, there was no significant effect of agomelatine on any of the sleep variables. The sleep variables were consistent with sleep characteristics of healthy older adults in the same age range.[30]

### *Phase-shifting effects of agomelatine*

*Body Temperature* (Fig. 1). Under placebo treatment, the rhythm of body temperature was as expected for normal elderly subjects, with maximum temperatures in the late evening followed by a sharp decrease towards minimal values in the middle of the night. Under agomelatine, the timing of the acrophase was markedly advanced in comparison with the placebo study (1813 h ± 27 min vs. 2301 h ± 42 min, P < 0·02). This advance of the acrophase averaged approximately 5 h, and could reflect the combination of an intrinsic circadian phase advance and an earlier decline of body temperature due to the well-documented hypothermic action of melatonin and agomelatine.[22,31,32]

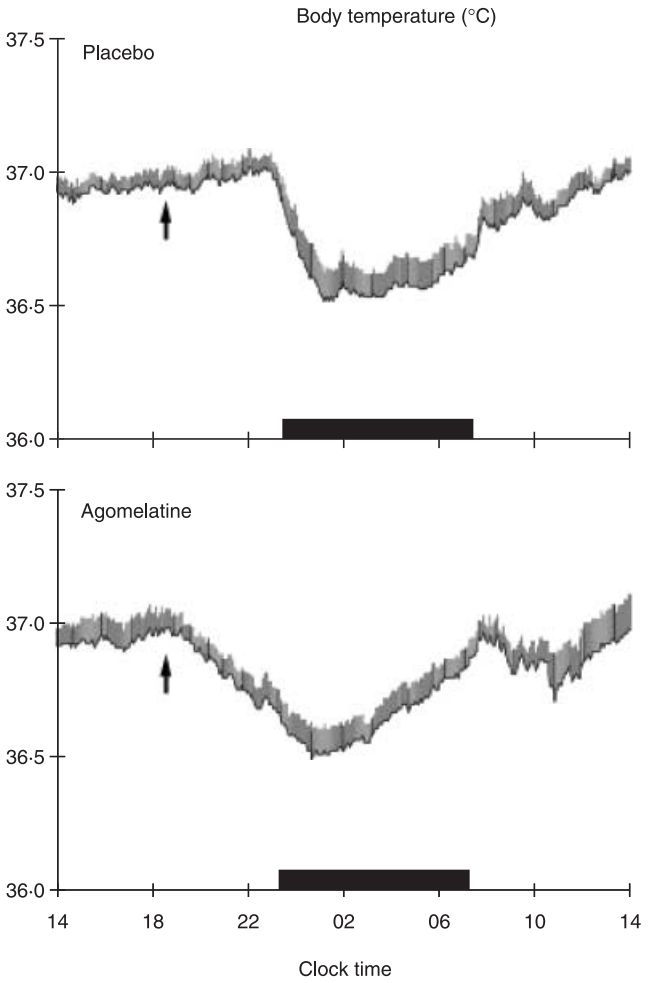


**Fig. 1** Mean (+ SEM, $n = 7$) profiles of body temperature under placebo and agomelatine treatments. The black bars indicate the bedtime periods. The arrows indicate the timing of the treatment administration (1830 h).

The timing of the nadir was also advanced under agomelatine as compared to placebo (0124 h ± 33 min *vs.* 0344 h ± 39 min, $P < 0·03$). The phase-advance averaged approximately 2 h. Although it could partly result from the hypothermic action of agomelatine, this phase-advance is likely to mainly reflect a phase-advance of the circadian clock, consistent with the well-known effects of melatonin on the circadian pacemaker.[9]

*Cortisol* (Fig. 2). In all subjects, the typical profile of plasma cortisol levels was observed under placebo treatment, with low concentrations in the evening and in the first part of the night, an abrupt elevation after the first few hours of the night, a morning maximum, and declining levels throughout the daytime. The rhythm of cortisol was advanced under agomelatine administration. Indeed, the timing of the acrophase (0628 h ± 21 min *vs.* 0739 h ± 30 min, $P < 0·05$), the timing of the nadir (2121 h ± 24 min *vs.* 2309 h ± 23 min, $P < 0·02$) and the end of the quiescent period (2343 h ± 44 min *vs.* 0136 h ± 18 min, $P < 0·03$) occurred earlier under agomelatine

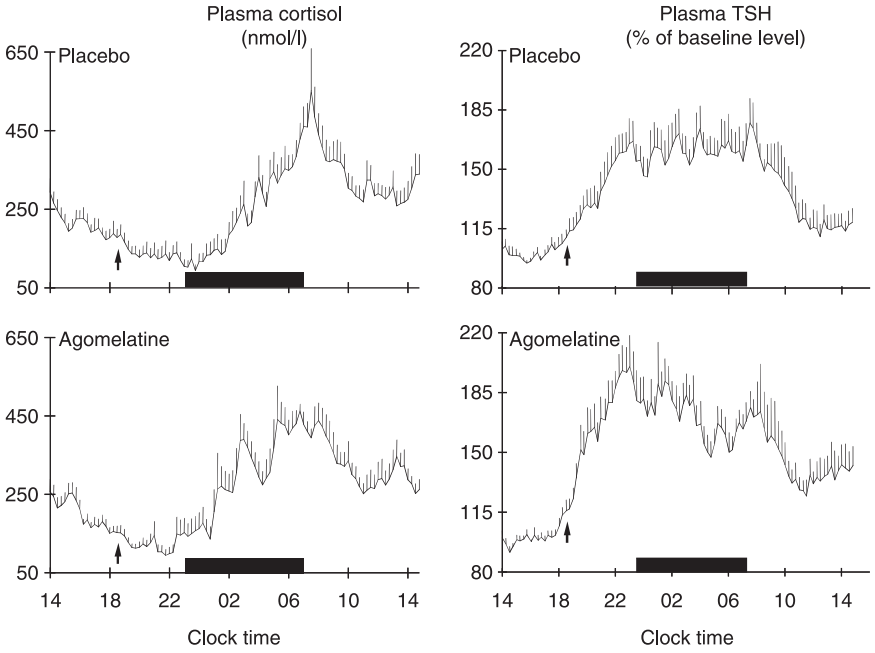


**Fig. 2** Mean (+ SEM, $n = 8$) profiles of cortisol and TSH concentrations under placebo and agomelatine treatments. To reduce interindividual variability, mean TSH profiles are shown after expressing each individual profile as a percentage of its mean value during the time interval 1500–1730 h (referred to as the 'baseline' level). The black bars indicate the bedtime periods. The arrows indicate the timing of the treatment administration (1830 h).

administration. These phase-advances averaged 1·5–2 h and were associated with a significant increase in the mean cortisol levels over the 2300–0300-h period (215 ± 36 nmol/l *vs.* 146 ± 19 nmol/l, $P < 0{\cdot}02$) and with a trend for an increase over the 0300–0700-h period (372 ± 39 nmol/l *vs.* 315 ± 25 nmol/l, $P < 0{\cdot}07$).

*TSH* (Fig. 2). Under placebo treatment, TSH concentrations followed the expected pattern, with low daytime levels followed by an increase before sleep onset. The slope of the linear regression of TSH values over the 2200–0700-h period was not significantly different from 0, suggesting that the inhibitory influence normally exerted on TSH levels during sleep in young adults[33] is no longer present in old age. Under agomelatine treatment, the timing of the onset of TSH circadian rise showed a trend for an advance, in comparison with the placebo study (1900 h ± 12 min *vs.* 2009 h ± 34 min, $P < 0{\cdot}07$). The relative values of the acrophase and of the amplitude of TSH profiles tended to be higher under agomelatine (193 ± 13% *vs.* 172 ± 10%, $P < 0{\cdot}10$ and 48 ± 7% *vs.* 39 ± 5%, $P < 0{\cdot}10$, respectively). Over the 2200–0700-h period, linear regression of TSH values revealed a negative slope that was significantly different from 0 (one group *t*-test: $P < 0{\cdot}04$), and significantly different from that observed under placebo treatment ($P < 0{\cdot}02$). Thus, agomelatine seemed to restore the normal sleep-associated inhibition of TSH secretion.

### *Agomelatine effects on GH and PRL secretion*

*GH* (Fig. 3). As expected in normal old men,[34] the 24-h profile of plasma GH under placebo treatment consisted of stable low levels abruptly interrupted by low-amplitude peaks.

Under agomelatine treatment, a significant peak of plasma GH was observed in all eight subjects following drug ingestion, over the time interval 1900–2100 h. Over that period, agomelatine administration was associated in all subjects with a marked increase in GH secretion, which averaged 350% of the secretion rate observed under placebo treatment ($P = 0{\cdot}01$).

The stimulatory effect of agomelatine on GH secretion was not transient, as a significant elevation in the amount of GH secreted was observed across almost the entire waking period, from 155 ± 41 μg under placebo treatment to 295 ± 75 μg under agomelatine treatment ($P < 0{\cdot}02$). This increase in GH secretion under agomelatine was due to an enhancement of the amplitude of the pulses and not to an increase in the number of pulses, and was observed in all eight subjects.

By contrast, the amount of GH secreted during sleep was similar in both study conditions. The mean GH secretion dropped from 239 ± 160 μg under placebo treatment to 184 ± 82 μg under agomelatine treatment (ns), but this apparent decrease was entirely due to subject 6, an outlier according to the Grubbs test ($P = 0{\cdot}05$). In this subject, the sleep GH secretion under placebo treatment was 1352 μg, as compared with values ranging from 21 to 180 μg in the other seven subjects. If this outlier was excluded from the analysis, sleep GH secretion averaged 80 ± 21 μg under placebo treatment and 108 ± 35 μg under agomelatine (ns). Overall, over the 24-h span, when subject 6 was excluded, total GH secretion averaged 220 ± 52 μg under placebo treatment and 382 ± 93 μg under agomelatine ($P < 0{\cdot}03$).

No effect of agomelatine on IGF-I levels was detected (136 ± 13 μg/l after agomelatine treatment *vs.* 136 ± 12 μg/l after placebo administration, ns). These values were similar to those recorded during the screening process (134 ± 9 μg/l).

*PRL* (Fig. 3). Under placebo treatment, all subjects had a nocturnal elevation of PRL secretion, which occurred at the time usually found in normal men.[5,35] This nocturnal elevation of PRL averaged 219 ± 11% of baseline levels, that is a twofold elevation, and the acrophase occurred at 0345 h ± 36 min.

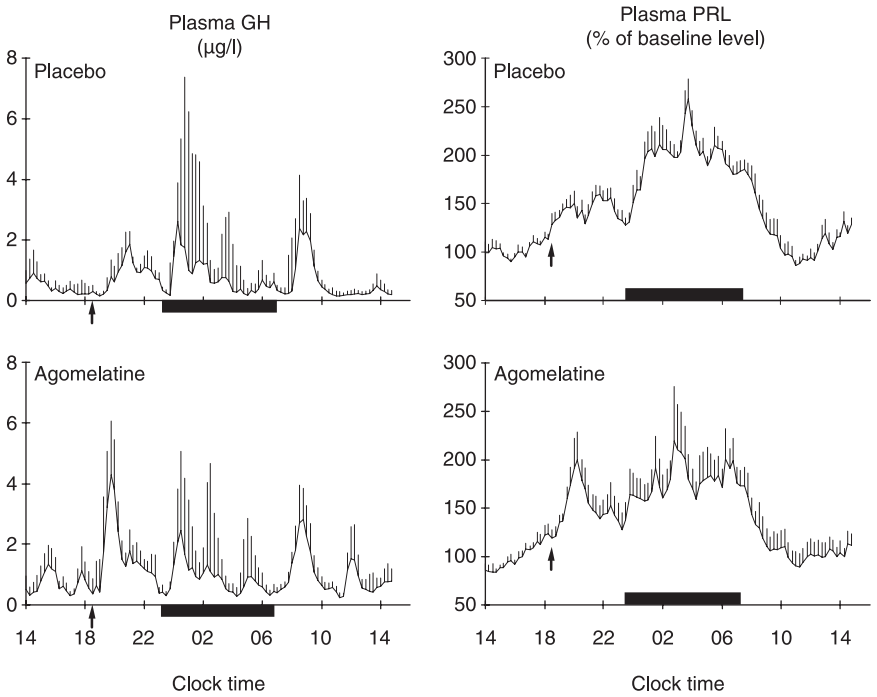


**Fig. 3** Mean (+ SEM, $n = 7$ for GH profiles and $n = 8$ for PRL profiles) profiles of GH and PRL concentrations under placebo and agomelatine treatments. To reduce interindividual variability, mean PRL profiles are shown after expressing each individual profile as a percentage of its mean value during the time interval 1500–1730 h (referred to as the 'baseline' level). The black bars indicate the bedtime periods. The arrows indicate the timing of the treatment administration (1830 h). One outlier (subject 6) was not included in the GH profiles (see text).

Agomelatine administration was associated within 2 h after drug ingestion with a modest elevation of PRL levels ($P < 0{\cdot}05$), but the concentrations remained well within the physiological range.

## Discussion

Although it has been shown that REM sleep propensity is increased following early evening oral administration of a single dose of either melatonin or agomelatine to normal young men,[36] no modifications of sleep were observed in the present study. This discrepancy could be due to differences in the population sample (young *vs.* old subjects), in the dosage of the melatonin agonist and/or in the duration of drug administration. Greater individual variability in the response to the drug in older subjects, than in young adults, may also be involved in the absence of significant effects of agomelatine on sleep. The absence of hypnotic effects of agomelatine in our older volunteers without sleep complaints is, however, consistent with the results of studies indicating that the sleep-promoting effects of melatonin in older adults are only observed in subjects who are insomniacs.[15–18]

This study shows that in older men, several phase reference points of various overt rhythms were advanced by about 2 h after 2 weeks of daily evening oral administration of agomelatine. These findings suggest, but do not demonstrate, that the effect is at the level of the central circadian clock, consistent with findings in rodents that indicated that agomelatine entrains circadian rhythms through an effect exerted on the SCN.[37] The direction and the magnitude of the phase-shifts were similar to those previously reported in normal young men after early evening administration of a single dose of either melatonin or agomelatine.[22]

Agomelatine is an antidepressant that has both melatonin agonist[19,20] and 5-$HT_{2C}$ antagonist properties.[21] Both melatonin and 5-HT inhibit light-induced phase shifts of circadian rhythmicity,[38] but it has been shown that these effects of 5-HT are mediated by the $5HT_{1b}$ receptor, but not by the 5-$HT_{2C}$ receptor.[39] Therefore, we speculate that the phase-shifting effects of agomelatine are due to its action as a melatonin agonist, rather than to its activity as a 5-$HT_{2C}$ antagonist. Taken together, these findings suggest that agomelatine could facilitate in older adults the realignment of at least some overt circadian rhythms, such as those occurring following transmeridian flights or shift work rotations. Studies of robust markers of circadian phase under constant routine conditions would be needed to definitely demonstrate that agomelatine affects the central circadian pacemaker in older humans. Further studies are also needed to determine whether shorter durations of treatment, say 1–3 days, have phase-shifting potency similar to that observed after 14 days of treatment. It is also possible that appropriately timed administration of agomelatine (e.g. in the early morning) might induce a phase delay and correct the intrinsic phase advance that is characteristic of ageing. These potential therapeutic implications are particularly interesting in view of the reduced responsiveness of the ageing circadian system to other synchronizing agents, including both photic and nonphotic stimuli.[2,6–8]

Agomelatine treatment resulted in a consistent increase in GH secretion during most of the waking period, and 24-h GH secretion was significantly elevated. The increase in daily GH output was not accompanied by an elevation in plasma IGF-I levels. Additional investigations would be needed to determine whether a more prolonged treatment and/or higher agomelatine doses would induce elevations of IGF-I circulating levels. If this was the case, daily treatment with agomelatine could have beneficial effects on metabolic variables dependent on the GH axis, such as body composition, muscle strength and bone metabolism. The mechanisms underlying these effects of agomelatine on GH secretion are not known. A stimulation of GH release following melatonin administration has been reported in some, but not all, previous studies.[40–44] Controversial results have

been reported about the effects on GH release of 5-HT, and of agonists and antagonists for the 5-HT receptors.[45]

Treatment with agomelatine was also associated with a short-term and modest elevation of PRL levels that occurred within 1 h following the agonist administration. This observation is consistent with previous studies on the effects of exogenous melatonin administration.[40,41,44,46–48] The mechanisms are still unknown. However, PRL release is stimulated by 5-HT and serotoninergic compounds and it has been shown that this action is partially mediated by the 5-$HT_{2C}$ receptor.[19] Thus, it is unlikely that the agomelatine effects on PRL observed in the present study are mediated by its 5-$HT_{2C}$ antagonist properties.

Finally, agomelatine seemed to restore in older adults the sleep-related inhibition of TSH secretion usually observed in young normal subjects.[50]

In conclusion, the present study demonstrates that it is possible to obtain large and consistent shifts of overt circadian rhythms of older adults using the melatonin agonist agomelatine and reveals noncircadian hormonal effects that are worthy of further investigation.

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