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Melatonin Treatment Effects on Adolescent Students' Sleep Timing and Sleepiness in a Placebo-Controlled Crossover Study

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During the last few decades, the incidence of sleep-onset insomnia, due to delay of circadian phase, has increased substantially among adolescents all over the world. We wanted to investigate whether a small dose of melatonin given daily, administered in the afternoon, could advance the sleep timing in teenagers. Twenty-one students, aged 14–19 yrs, with sleep-onset difficulties during school weeks were recruited. The study was a randomized, double blind, placebo (PL)-controlled crossover trial, lasting 5 wks. During the first 6 d in wks 2 and 4, the students received either PL or melatonin (1 mg) capsules between 16:30 and 18:00 h. During the first 6 d of wk 5, all students received melatonin. Wks 1 and 3 were capsule-free. In the last evening of each week and the following morning, the students produced saliva samples at home for later melatonin analysis. The samples were produced the same time each week, as late as possible in the evening and as early as possible in the morning. Both the student and one parent received automatic mobile text messages 15 min before saliva sampling times and capsule intake at agreed times. Diaries with registration of presumed sleep, subjective sleepiness during the day (Karolinska Sleepiness Scale, KSS) and times for capsule intake and saliva samplings were completed each day. Primary analysis over 5 wks gave significant results for melatonin, sleep and KSS. Post hoc analysis showed that reported sleep-onset times were advanced after melatonin school weeks compared with PL school weeks ($p < .005$) and that sleep length was longer ($p < .05$). After the last melatonin school week, the students fell asleep 68 min earlier and slept 62 min longer each night compared with the baseline week. Morning melatonin values in saliva diminished compared with PL ($p < .001$) and evening values increased ($p < .001$), indicating a possible sleep phase advance. Compared with PL school weeks, the students reported less wake up ($p < .05$), less school daytime sleepiness ($p < .05$) and increased evening sleepiness ($p < .005$) during melatonin weeks. We conclude that a small dose of melatonin given daily, administered in the afternoon, could advance the sleep timing and make the students more alert during school days even if they continued their often irregular sleep habits during weekends. (Author correspondence: arne.lowden@stress.su.se)

Keywords: Adolescence, Circadian rhythms, Delayed sleep phase, Melatonin, Teenagers

INTRODUCTION

Since 1980, sleep problems among adolescents have increased about three times in Sweden, most markedly during the last decade (Swedish Government Official Reports, 2006). At least part of the reason for this is probably a change in young people's evening habits, causing a delay of their circadian rhythm in relation to the rhythm of nature, school or work (Shochat et al., 2010). This development of sleep patterns and problems seems to be almost worldwide (Gradisar et al., 2011). The major problem is that the onset of sleep is very late and that sleep is strongly reduced because of the need to rise early for school. Insufficient sleep and effects of delayed sleep during school weeks can have a profound negative effect on school performance, cognitive function and mood (Curcio et al., 2006; Millman, 2005; Thorpy et al., 1988).

One important contributor to sleep-onset insomnia in the young may be that they are "night owls." The incidence of eveningness (late chronotype) increases steeply during the second decade of life, after which it declines slowly during the rest of life (Roenneberg et al., 2007). The mechanism behind differences in chronotype is that the circadian clock has a different acrophase (time of maximum) for different chronotypes and thus promotes alertness and sleep differently, with a strong delay for evening types, resulting in the characteristic difficulties in sleep onset and ability to rise in

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the morning (Zee & Manthena, 2007). On average, late chronotypes have a longer circadian period than intermediate and early types (Brown et al., 2008) and the nadir of body temperature and cortisol production occurs later during sleep (Bailey & Heitkemper, 2001). Consequently, they are less alert and more prone to stay in bed in the morning, thereby missing the phase-advancing effect (Dijk et al., 1995) of early morning light. In contrast, late chronotypes experience stronger circadian alerting signals in the evening, which may lead to more phase delaying light exposure during late hours (Emens et al., 2009). Even the modest light from a computer or ordinary room light is sufficient to phase delay the internal clock (Zeitzer et al., 2000). The phase delay is due to a combination of genetic components in the circadian system (Jones et al., 2007), pubertal maturation (Frey et al., 2009) and social components (Shirayama et al., 2003), like society's demands and the student's daily habits. In extreme cases, the diagnosis "delayed sleep phase disorder" (DSPD) may be applied (American Academy of Sleep Medicine, 2001).

Theoretically, there are four different ways to help teenagers who have delayed sleep phase problems (Czeisler et al., 1981; Okawa et al., 1998): (1) Information about its cause and about sleep hygiene. (2) Chronotherapy, i.e., progressive forced delay of sleep. (3) Light treatment. (4) Melatonin treatment. The first should always be used but is as a single treatment often insufficient (Moseley & Gradisar, 2009). The second is impractical and the third often difficult to implement in the school situation. This study is focused on the usefulness of melatonin.

Melatonin has an important role in the circadian regulation of sleep. Its production in the pineal gland is regulated by the SCN and synchronized to the light–dark cycle by signals from the eye. In its turn, melatonin is acting on the SCN in a feedback system, which is thought to stabilize the sleep/wake cycle (Pandi-Perumal et al., 2008b).

Melatonin has two different effects on the SCN, mediated by two different receptors (Pandi-Perumal et al., 2008a). By binding to MT_1 receptors, it has an inhibitory effect on SCN wake promoting activities. As these activities increase during waking hours to compensate for increasing sleep debt, the inhibitory, soporific effect of melatonin is most evident towards the end of the day. Taken at that time, it is often used as a sleep aid (Wyatt et al., 2006).

By binding to MT_2 receptors, melatonin acts by phase shifting the circadian firing rhythm in the SCN. This phase shift, however, is possible only at certain periods of sensitivity, i.e., dusk and dawn. If melatonin is taken some hours before dim light melatonin onset (DLMO), it will advance the sleep timing (Lewy et al., 1998).

Both time and dose of melatonin administration determine its phase shifting effect. Given after DLMO, as is the case when it is used as a sleep aid, it has no or minimal such effect (Burgess et al., 2008). Given before DLMO to advance the sleep timing, but in a high dose, it can produce less of the sleep timing shift as it may still have an effect at night's end, but in the opposite direction (spillover effect, Lewy et al., 2006).

The optimal time for melatonin administration in order to accomplish a phase advance is also dose-dependent, since melatonin has a short elimination half-life and exogenous melatonin levels should be continuous with the beginning of the endogenous melatonin profile. A dose of 3 mg is most effective when given about 5 h before DLMO, whereas .5 mg is best given 2–4 h before DLMO (Burgess et al., 2010).

As early as 1991, Dahlitz et al. showed that melatonin, given for some weeks 5 h before habitual sleep-onset time, could advance the sleep timing more than 1 h in patients with DSPD (Dahlitz et al., 1991). This was confirmed in a recent meta-analysis including nine randomized controlled trials, comparing melatonin with placebo (PL; Van Geijlswijk et al., 2010). Four of the studies included children (Smits et al., 2001, 2003; Van Der Heijden et al., 2007; Weiss et al., 2006). None of them included teenagers, however, and to the best of our knowledge, no such controlled studies exist.

Children under 12 yrs of age, as in the studies mentioned above, usually have sleep habits, controlled by their parents. Teenagers, in contrast, tend to set their own limits, especially those suffering from DSPD. There is also an overrepresentation of neuropsychiatric problems among these students who are often both less able to cooperate and in greater need of help, as sleep debt aggravates their handicaps in school (Van Der Heijden et al., 2005).

It should be emphasized that in most European countries, melatonin is a prescription-only medication. The European Panel on Dietetic Safety Authority (EFSA, 2011) have recommended 1 mg of melatonin to be used close to bedtime in the general population. In Sweden, a special license from the Medical Products Agency is required for each prescription of ordinary melatonin, as it is not yet classified as a medication. According to the Agency, more convincing studies on the effect of ordinary melatonin are necessary, before the license requirement can be abolished (Wallenbeck & Forslund, 2008).

Nowadays, a consensus exists that melatonin is a safe treatment with appropriate dosage and timing (Arendt et al., 2008). There might still be some concern, however, about its long-term use during childhood and adolescence, as melatonin is known to be involved in the seasonal regulation of animals' reproduction (Srinivasan et al., 2009). The gonadotrophic effects differ among species, however (Scherbarth & Steinlechner, 2010), and in a recent PL -controlled study, no treatment-related changes of hormones, including testosterone and estradiol, were detected after long-term treatment of humans (Wade et al., 2010). The only study to follow pre-pubertal children with regard to their development (Van Geijlswijk et al., 2010) found

that puberty onset seemed to be undisturbed after 3.1 yrs of melatonin usage.

The aim of this study was to investigate the effect of an intake of 1 mg of melatonin late in the afternoon, as the sole intervention on sleep onset, sleep duration, daytime sleepiness and melatonin in teenage students with sleep-onset insomnia.

SUBJECTS AND METHODS

Participants

In December 2009, all students, in grades 8, 10 and 11 in the schools of a mid-Swedish town with around 15 000 inhabitants, completed a questionnaire about sleep habits and sense of fatigue during daytime. Out of 456 students, 58 answered "yes" to the question whether they had sleep-onset insomnia and confirmed that they wanted help with that.

Criteria for participation selection included a motivation to be helped, being unable to go to sleep before 01:00 h at least two out of five nights every school week, and a substantial sense of morning fatigue. Exclusion criteria were prior use of melatonin and use of light therapy.

Thirty-four students who satisfied the criteria were contacted of whom, 23 were willing and allowed by their parents to participate. One decided to withdraw after a few days, as he could not wait for help. One was excluded because of non-compliance. No reward was promised but after having finished the study, each student was given two cinema tickets.

The participants included consisted of 10 boys and 11 girls between 14 and 19 yrs of age. Informed consent was obtained from all parents and students after personal meetings with the school doctor.

Design/Protocol

The study started on a Thursday in the middle of January 2010 and covered a period of 5 consecutive weeks. Parents were instructed to help the students with sleep–wake diaries and remind them to take melatonin–PL capsules and deliver saliva samples at agreed times. Other measures to advance sleep timing, like day light lamps or more strict sleep hygiene than before, were not to be taken as long as the study lasted.

The melatonin and PL used in the study was obtained from Natural Pharma International, NPI AB. The melatonin and PL were administered to the students as hard-gelatin capsules, which were indistinguishable from one another by appearance, taste and smell. The capsules were dispensed in numbered sets of three bottles, labelled 1, 2 and 3, each containing six capsules. The sets had been randomized and were delivered together with sealed data in envelopes, separate for each set. In half of the sets, bottle 1 contained capsules with 1 mg melatonin and bottle 2 PL, in the other sets, bottle 1 contained PL and bottle 2 melatonin. Bottle 3 contained melatonin capsules in all sets, and this was known to all participants. The randomization of the first two bottles was blind to students and study team and the code was broken only after all study procedures were terminated. The purity of the melatonin substance and the accuracy/assay of the melatonin dose in capsules for the clinical study were verified to fulfil specifications by independent quality control units within the supplying pharmaceutical company.

During the 1st and 3rd study wks, no capsules were to be taken. During the 2nd, 4th and 5th wks, the students took the capsules from bottles 1, 2 and 3, respectively. Wednesdays were always capsule-free. The design of the study is illustrated in Figure 1.

As there were no practical possibilities to measure DLMO, the optimal timing of melatonin intake had to be approximately calculated from the sleep habits, as reported by the students in the questionnaire. A time, 3–4 hrs before estimated DLMO (Crowley et al., 2006), was chosen but had to be adapted to the students' and parents' daily habits. Individually adapted times between 16:30 and 18:00 h were chosen at the initial meeting with the school doctor. Both the student and one parent received an automatic mobile text message (SM-Ace, Pixie data 1.9.0) 15 min before intake time agreed upon, reminding them of the capsule intake. The exact time for the intake was then noted in the diary.

MEASURES

During all study weeks, students completed diaries with registration of sleep (presumed sleep-onset time, time of awakening), naps and subjective sleepiness during

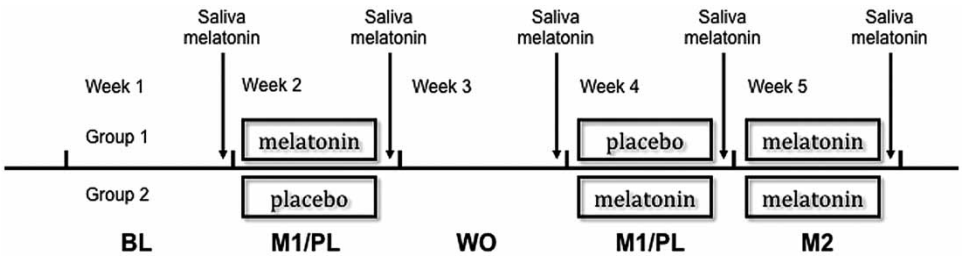

FIGURE 1. Design of study. A group of students ($n = 21$) were randomised into two groups and studied across 5 experimental weeks that included a BL week, 2 M1/PL weeks, a WO week and a week when both groups had melatonin (M2). Each experimental week started on a Thursday and ended on Wednesday that was medication free and subjects took saliva melatonin samples in the evening and in the morning.

the day (KSS, which quantifies sleepiness four times a day on a nine-grade scale, Kaida et al., 2006). The exact times for capsule intakes and saliva samplings were also registered in the diaries.

All subject wore actigraphs (Actiwatch™, Cambridge Neurotechnology, Cambridge, UK), but due to software problems only about half of the subjects (n = 13) could be analysed and actigraphy was thus excluded from the analysis. Within the subsample diary data, comparisons were performed to check for correspondence. Actigraphy data were scored without reference to the sleep diary data. The correlation reached .77 (Pearson) for sleep onset, .87 for sleep offset and .75 for sleep length. These levels are comparable to similar studies of adolescents comparing actigraphy data and survey data on sleep timing (Wolfson et al., 2003) and support the validity of using diary data in this study.

Starting and ending days of the study were Thursdays. Every week, each student produced two saliva samples (Salivette, Sarstedt®, Rommelsdorf, Germany) at home at exactly the same time Wednesday evenings and Thursday mornings. Every Thursday, the samples were sent by post in prepaid envelopes to the laboratory in Stockholm, where they were centrifuged and stored at –20°C for later melatonin analysis. Salivary melatonin was determined through Radio Immunoassay (RIA, Bühlmann Laboratories AG®, Schönenbuch, Switzerland)

A dose of 1 mg was chosen in this study in order to minimize the soporific effect, which is probably dose-dependent (Van Den Heuvel et al., 1998). Times for saliva samples were individually adapted to each student and parent's evening and morning routines. Both the student and one parent received an automatic mobile text message (SM-Ace, Pixie data 1.9.0) 15 min before sampling as a reminder. They were taken as late as possible on Wednesdays and as early as possible on Thursdays. This optimized the chance to have a sample from both the rise and descent of the melatonin secretion profile. A single melatonin value says little about circadian phase but as melatonin secretion patterns are individually fairly stable (Selmaoui & Touitou, 2003), weekly changes of melatonin values from the same clock time will reflect delays or advances of sleep timing, provided the samples are taken during the beginning and end of the melatonin secretion profile.

The Ethics Committee, Faculty of Medicine, Uppsala University and the Medical Products Agency, Uppsala approved the study. The experimental protocol was conformed to international ethical standards (Portaluppi et al., 2010).

Statistical Analysis

Means and standard errors were integrated across each experimental week and used in figures (mean values for timing in text using clock times). Mean values based on raw data were analysed with a two-way repeated measurement analysis of variance (ANOVA). The analyses were performed in three steps, a primary analysis

TABLE 1. Mean values (±SE) for sleep, melatonin and sleepiness (KSS) on weekdays and weekends

	BL	M1	PL	WO	M2	Statistical analysis
Sleep (weekdays)						
Sleep length (hrs)	7.03 ± .09	7.84 ± .12	7.54 ± .11	7.08 ± .12	8.07 ± .13	4, 5, 6, 7, 8
Sleep onset (h)	00:25 ± .06	23:25 ± .10	00:11 ± .09	00:25 ± .07	23:17 ± .11	1, 2, 4, 5, 6, 7, 8
Sleep offset (h)	07:26 ± .09	07:32 ± .08	07:43 ± .10	07:14 ± .08	07:17 ± .10	4, 5
Melatonin						
Evening (pmol/ml)	18.95 ± 6.43	29.20 ± 4.95	2.99 ± 4.88	17.56 ± 5.22	35.83 ± 6.30	2, 4, 5, 6, 7
Morning (pmol/ml)	5.71 ± 11.57	24.74 ± 4.98	34.13 ± 1.45	34.41 ± 6.82	13.61 ± 4.11	2, 5, 6, 7, 8
Sleep (weekend)						
Sleep length (hrs)	8.67 ± .16	9.76 ± .28	8.70 ± .20	8.68 ± .25	9.30 ± .25	2, 4, 6
Sleep onset (h)	01:25 ± .18	00:27 ± .27	01:36 ± .18	01:43 ± .21	00:39 ± .23	1, 2, 4, 6
Sleep offset (h)	10:05 ± .16	10:13 ± .20	10:18 ± .14	10:27 ± .21	09:56 ± .20	1
KSS (weekdays)						
Wake up time	7.37 ± .12	6.47 ± .16	7.11 ± .14	7.04 ± .13	6.10 ± .18	2, 5, 6, 7
Morning	5.93 ± .12	5.27 ± .14	5.66 ± .14	5.72 ± .14	5.09 ± .15	2, 5, 6, 7, 8
Afternoon	5.72 ± .17	5.09 ± .15	5.32 ± .18	5.66 ± .16	5.06 ± .17	2, 6
Evening	4.54 ± .18	6.29 ± .17	5.18 ± .19	5.34 ± .17	6.78 ± .15	2, 4, 5, 6, 7
KSS (weekend)						
Wake up time	6.15 ± .24	5.22 ± .18	5.97 ± .24	6.20 ± .22	5.70 ± .27	4, 6
Morning	5.48 ± .26	4.92 ± .26	5.00 ± .21	5.57 ± .23	5.04 ± .25	
Afternoon	5.28 ± .24	4.62 ± .27	4.87 ± .27	5.25 ± .25	4.78 ± .29	
Evening	4.51 ± .30	6.55 ± .27	5.09 ± .31	5.23 ± .32	6.89 ± .26	1, 2, 4, 5, 6, 7

For ANOVA differences, see below.

1, significant effect of day (ANOVA including 5 weekdays or 2 weekend days). 2, significant effect of week (ANOVA including 5 wks – BL, M1, PL, WO, M2). 3, significant interaction effect day × week (ANOVA). 4, PL × M1 (primary analysis, ANOVA – significant effect of week). 5, PL × M2 (ANOVA – significant effect of week). 6, BL × M1 (ANOVA – significant effect of week). 7, BL × M2 (ANOVA – significant effect of week). 8, BL × PL (ANOVA – significant effect of week).

for the difference between PL and Melatonin weeks that included the strongest control in the design and a secondary analysis using the factors Week (5 wks) and Day. In the third step, post hoc analyses were calculated between single weeks. All analyses separated weekdays (5 d, Sunday evening–Friday evening) and weekends (2 d Friday evening–Sunday evening). Main effects and interactions of Week and Day were calculated and presented in Table 1. The procedure included a Huyhn–Feldt correction for unequal variances. Direct hypothesis were posed for sleep onset and offset time, sleep length and melatonin changes. These variables were treated with a one-sided ANOVA for the primary analysis (analyse 4 in Table 1), the overall analysis (factor Week, analyse 2) and post hoc analysis PL × M2 (analyse 5, see Table 1). For saliva melatonin, transforming data to the best fit, the inverted square root, before analysis, reduced skewness. The significance level was set to 5% in all analyses.

RESULTS

Table 1 summarizes the results of the study and ANOVA statistics. Sleep and daytime sleepiness (KSS) data were registered separately for the 5 d in connection to school days (weekdays) and free days (two weekend days).

Weekday Sleep

Data derived from diaries showed an effect of Week for presumed sleep-onset time and sleep length. As shown in Figure 2, baseline (BL) mean presumed onset was at 00:25 ± .06 h. During the PL week, it was advanced to 00:11 ± .12 h during the first melatonin (M1) week to 23:25 ± .10 h and during the second melatonin (M2) week to 23:17 ± .11 h. The difference PL/M1 became significant ($p < .05$) as well as PL/M2 ($p < .005$).

Sleep offset times were stable across experimental weeks, means ranging from 07:14 ± .13 h at the wash out (WO) week to 07:43 ± .10 h at PL (Figure 3). However, waking occurred earlier at M1 (07:19 ± .08 h, $p < .05$) and at M2 (07:17 ± .10 h, $p = .0594$) than at PL (07:43 ± .10 h).

The effect of Day seen in Table 1 indicated that students had different sleep-onset times on schooldays as they went to bed shortly before midnight on Monday, Tuesday and Thursday but later on Wednesdays (00:16 ± .10 h).

From 7.0 ± .09 h during BL sleep length increased with 31 min at PL, with 49 min during M1 and 62 min during M2, see Figure 4. The difference PL/M2 was significant ($p < .05$). By the 5th wk, 89% of the students recorded longer sleep before school days than during the 1st wk.

Weekend Sleep

In connection to weekends, both sleep-onset time and sleep length changed across experimental weeks ($p < .01$ and $p < .05$, respectively). The condition

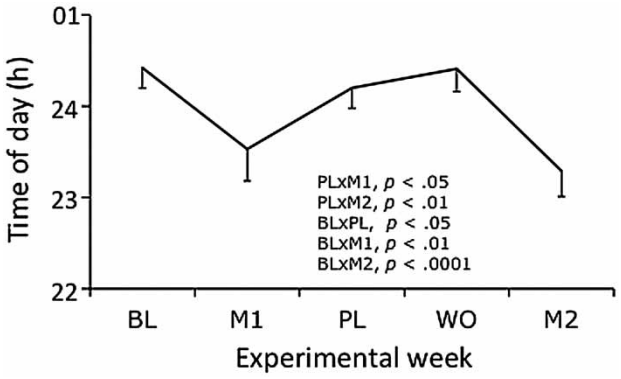

FIGURE 2. Sleep onset (diary). Means ± SE across experimental weekdays including BL = baseline, M1 = melatonin, PL = placebo, WO = washout and M2 = melatonin. Results of primary analysis (PL × M1) and other differences between weeks are indicated (p-values).

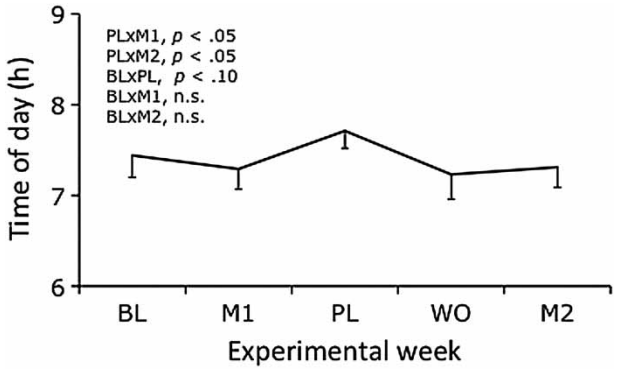

FIGURE 3. Sleep offset time (diary). Means ± SE across experimental weekdays including BL = baseline, M1 = melatonin, PL = placebo, WO = washout and M2 = melatonin. Results of primary analysis (PL × M1) and other differences between weeks are indicated (p-values).

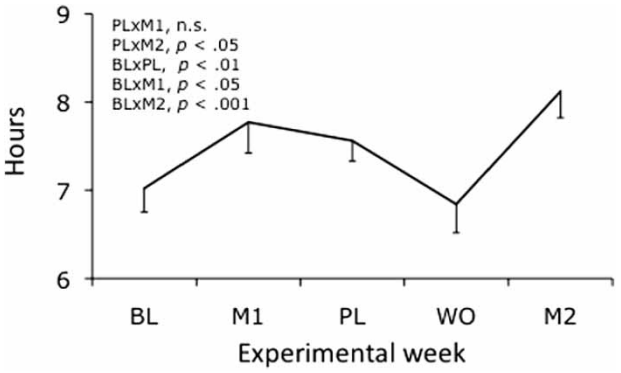

FIGURE 4. Sleep length (diary). Means ± SE across experimental weekdays including BL = baseline, M1 = melatonin, PL = placebo, WO = washout and M2 = melatonin. Results of primary analysis (PL × M1) and other differences between weeks are indicated (p-values).

differences resembled findings for weekday sleep, but sleep was initiated about 1 h later or more on each experimental week. Sleep-onset time was 01:25 ± .18 h during BL, 01:36 ± .18 h during PL, 00:27 ± .27 h during M1, 00:39 ± .23 h during M2 and at 01:43 ± .21 h at WO. The difference PL/M1 was significant ($p < .05$). Mean sleep offset time ranged from 09:56 ± .20 h at M2 to 10:27 ± .21 h at WO, the difference being significant ($p < .05$). Also the difference between BL (10:05 ± .25 h) and M2 was close to significant ($p = .0705$). Mean sleep length was 8.67 ± .16 hrs during BL and 2 min longer during PL (8.70 ± .20 hrs) and 65 and 38 min longer during M1 (9.76 ± .28 hrs) and M2 (9.30 ± .25 hrs), respectively. PL × M1 difference was significant ($p < .05$) and PL × M2 difference was almost significant ($p = .0675$).

Melatonin

Melatonin in saliva showed changes across conditions both in evening and morning measurements. As seen in Figure 5 evening values peaked after weeks with melatonin administration especially at M2 (35.8 ± 6.3 pmol/ml). The level at M1 reached 29.2 ± 5.0 pmol/ml and at PL, 21.0 ± 4.9 pmol/ml, a significant difference. The levels in the morning did not differ between conditions M1 and PL, but at M2 a clear drop had occurred, the levels across the experiment dropped from 34.1 ± 10.5 pmol/ml at PL to 13.6 ± 4.1 at M2.

The morning melatonin values from both the PL and WO weeks were lower than the BL value, whereas the evening values were less affected.

After the last study week, 94% of the students had lower morning melatonin values than after the 1st wk and 84% of the students had higher evening melatonin values after the last week.

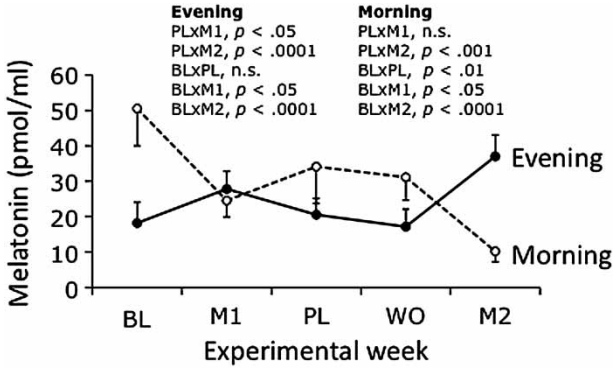

FIGURE 5. Saliva melatonin (pmol/ml). Means ± SE across experimental weekdays including BL = baseline, M1 = melatonin, PL = placebo, WO = washout and M2 = melatonin. Results of primary analysis (PL × M1) for evening (bold circles) and morning (open circles) samples and other differences between weeks are indicated (p-values).

KSS During School Days

The subjective evaluations of sleepiness (KSS ratings) are presented in Table 1 (analyse 2) and Figure 6. The figure shows means across each condition within each experimental week. The sleepiness patterns changed significantly between conditions (different weeks) for all four time points of measures across the day. The strongest effect was a change of sleepiness in the evening from PL (KSS = 5.18 ± .19) to increased levels during M1 (KSS = 6.29 ± .17) and during M2 (6.78 ± .15). The differences between PL and M1 and PL and M2 were significant ($p < .005$). Also sleepiness on awakening (KSS = 7.11 ± .14) and during morning hours (5.66 ± .14) for PL were significantly ($p < .05$) reduced at M2 (waking KSS = 6.1 ± 0.18; morning KSS = 5.09 ± .15).

KSS During Weekends

During weekends, evening sleepiness increased during M1 (KSS = 6.55 ± .27) and M2 (KSS = 6.89 ± .26) compared to PL (KSS = 5.09 ± .31) and reached significance ($p < .005$). Sleepiness also decreased ($p < .05$) during morning hours for M1 (PL KSS = 5.0 ± .21; M1 KSS = 4.92 ± .26). In general, mean sleepiness remained higher on Sundays (KSS = 5.47 ± .09) compared to Saturdays (KSS = 5.36 ± .09) throughout the study and this pattern was not affected by condition.

Side Effects

The participants were instructed to continuously note any suspected side effects following the intake of capsules, "for example tiredness within the first hours after capsule intake," as formulated in the diary. Only two participants noted tiredness 1 h after melatonin intake and one noted headache a few times following melatonin intake. No side effects were noted during PL weeks.

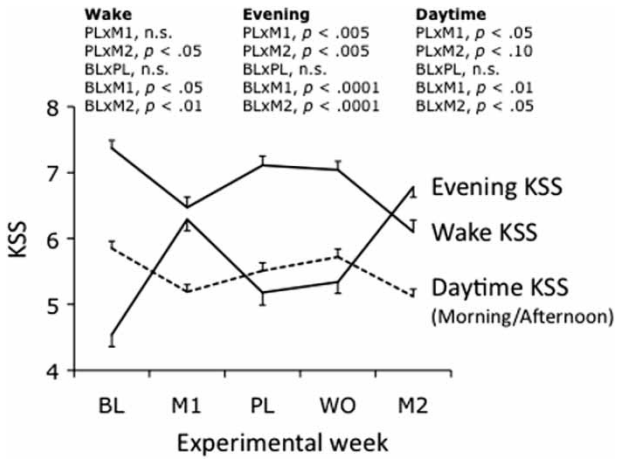

FIGURE 6. Evaluations of sleepiness (KSS). Means ± SE across experimental weekdays on three occasions during the day (wake, daytime – combined morning and afternoon ratings, and evening) including BL = baseline, M1 = melatonin, PL = placebo, WO = washout and M2 = melatonin. Results of primary analysis (PL × M1) and other differences between weeks are indicated (p-values).

At the end of the study, the students filled in a short inquiry form with questions about early evening tiredness for each capsule week. Now, 11 of the 21 participants confirmed some sense of fatigue already 1 or 2 hrs following capsule intake, 10 after melatonin and 1 after PL capsules. Six felt a little tired after melatonin some evenings during the 1st wk with melatonin only, four during both weeks.

In a short inquiry form at the end of the study 20 of 21 participants answered "yes" to the question "Did you benefit from the study – can you go to sleep earlier now?" and one answered "Don't know."

DISCUSSION

The results showed that a small dose of melatonin given to teenage students several hours before the usual bedtime will advance sleep onset and time of rising during school week and causes: increased sleepiness during the evening, increased saliva melatonin in the evening and decreased melatonin at wake up. The sleep-onset time remained advanced during the weekend and the evening and morning sleepiness remained increased and decreased, respectively. The pattern is interpreted as an advance of sleep timing.

To the best of our knowledge, the advanced sleep timing has not been demonstrated in teenagers before. As a group, teenagers have bedtimes that are difficult to regulate (Van Der Heijden et al., 2005). The results are very similar, however, to what has been demonstrated in adults (Dahlitz et al., 1991) and in children (Smits et al., 2001, 2003; Van Der Heijden et al., 2007; Weiss et al., 2006), whose sleep is more easy to influence.

Sleep-onset time, as well as time of awakening, was earlier during the M1 week compared to the PL week and evening KSS was higher during M1, as would have been expected if a circadian phase advance had occurred. An unexpected finding, however, was that sleep-onset time was significantly later on Wednesdays, more specifically the melatonin-free Wednesdays during M1 and the M2 week. This indicates that the earlier sleep-onset times during the other six evenings were at least partly due to the soporific effect of the melatonin capsule. The same effect was also reflected in the evening KSS values that tended to be lower on Wednesday than the other six evenings during M1.

The probable soporific effect on sleep-onset time was unexpected as the melatonin dose (1 mg) was smaller than in other studies and given around 6 hrs before sleep onset. Dahlitz et al. (1991) used 5 mg melatonin 5 hrs before estimated sleep-onset time and determined alertness by a self-rating scale completed every 2 hrs during the day, without significant change of acrophase. Evening sleepiness is not measured in other controlled studies, but the soporific effect of melatonin during some hours following its intake is sometimes reported (Smits et al., 2001). In a study by Cajochen et al. (1996), subjective sleepiness after intake of 5 mg melatonin lasted for 3 hrs if intake was at 13:00 h and for 5 hrs after intake at 18:00 h.

Sleep duration did not show an increase during the school week in the primary analysis, but did so during the weekend. It was expected that an earlier bedtime would yield a longer sleep duration also during the school week. As shown in a meta-analysis (Van Geijlwijk et al., 2010), this is usually the case in studies with children but not with adults. A possible explanation to the lack of significance during school week may be that the students were sleepier at awakening during PL (KSS-value 7.11) than during M1 (KSS-value 6.47), making them oversleep more often (sleep offset times 7:43 and 7:19, respectively).

As we could not determine DLMO, we only have indirect evidence that the circadian phase of melatonin was advanced by the treatment. When comparing M1 with PL, there was a significant difference between levels of evening melatonin (higher during M1), possibly suggesting that a phase advance had occurred. The difference between morning melatonin levels did not reach significance, however, which weakens the previous statement.

The mean levels of melatonin would vary greatly if time of sampling changed across experimental weeks. This was probably not the case in this study since all participants were instructed to take their saliva samples each week at exactly the same clock times, individually adapted to their habits, on Wednesday evenings and Thursday mornings. To ensure this, both the student and one parent received an automatic mobile text message, reminding them of the sampling, 15 min before time agreed on. The students or parents noted the actual sampling times in the sleep logs and with few exceptions, they were all less than 10 min early or late.

Apart from the main analysis of the PL/M1 difference, some information may also be obtained by comparisons between M2 and PL. These will suffer from an effect of sequence and of the knowledge that melatonin has been given during M2, but it also reflects the every-day situation of taking a medication as well as potential effects of accumulation of advanced sleep timing and decrease of sleep debt.

In any case, the PL/M2 analysis clearly showed that the effects of medication were slightly stronger in M2 in several respects. Saliva melatonin increased in the evening and decreased in the morning, now also significantly, which likely would support the notion of a continuing phase advance of melatonin, consistent with earlier studies using DLMO (Van Geijlswijk et al., 2010). The design of the study made it impossible to show if the advanced sleep timing would have continued during additional weeks with melatonin. Nor was it possible to show how stable sleep patterns and wakefulness would have been without medication.

Sleep onset occurred slightly earlier in M2 than in M1, while times of rising remained the same, resulting in significantly increased sleep duration also during school week. From BL week, the students' mean sleep length in connection to weekdays had increased by 49 min

during M1 and by 62 min during M2. In contrast, mean sleep length during weekends was 28 min shorter during M2 than during M1. This may have been a result of diminished sleep debt during M2, as they are usually paid during weekends (Millman, 2005).

The students reported less sleepiness at awakening during M2 than PL and were also significantly more alert during morning hours. As in the discussion above, this may have been effects of both a phase advance of melatonin and a diminished sleep debt. A tendency to more afternoon alertness may only be explained by diminished sleep debt, though. The results are in accordance with some earlier studies (Kayumov et al., 2001; Nagtegaal et al., 1998). The overall link between sleep duration and school performance is well established (Wolfson & Carskadon, 2004). As daytime sleepiness has been shown to be even closer connected to poor school performance than short sleep duration (Anderson et al., 2009), the increased alertness during school days is perhaps the most important finding of this study.

When comparing the mean evening KSS values and sleep-onset times of all melatonin-free evenings in the whole study, we found that the Wednesdays in M1 and M2 had the highest KSS values and the Wednesday in M2 was the only to have a sleep-onset time before midnight. These findings indicate that the increased evening sleepiness and earlier sleep onset during M1 and M2 were mostly due to a sleep phase advance.

This study has several limitations. One concerns the modest size of the sample. Several trends towards significance were observed, which may have become significant had a larger sample been used. Another weakness is that sleep data are mainly dependent on sleep logs due to software problems with the actigraphs. As the correlation between available actigraph data and sleep logs were comparable to similar studies of adolescents (Wolfson et al., 2003), we found it acceptable to use only sleep log data.

Also, the external lighting conditions can be a confounder. However, it should not have affected the PL/M1 comparison since that was counter-balanced. Confounding of outdoor light is also unlikely for several reasons. Thus, the study started in January and ended in February when sunrise occurs just 10 min before school start. Sunset, however, was delayed from half past three, when school attendance usually is about to end, to half past four. Consequently, the participants did not get more daylight in the morning during the last study week, but had the chance to have more of it on their way home after school. On the other hand, if the change of daylight timing had any influence on the circadian phase, it would have resulted in a delay (Figueiro & Rea, 2010).

A main limitation is that the WO time probably was too short. A pilot study from the same schools (Eckerberg, 2009) showed that the phase advancing effect of melatonin under ordinary school conditions may last more than 1 wk. The WO time might have been insufficient to prevent a carryover effect from M1 to PL in group 1. This is a possible explanation to the significantly lower morning melatonin values during PL compared to BL week and might also explain why the M1/PL comparison did not reach significance. The significantly earlier sleep onset, longer sleep duration and decreased morning sleepiness in the PL/BL comparison may be real PL effects but also carry-over effects.

This study did not compare melatonin administration in the afternoon with such in the evening, but focused on the former. Its main advantage is the more or less persistent circadian phase advance, not achieved after evening administration (Burgess et al., 2008). When the wake up time is fixed, as with school children, the patient will probably be more alert and more ready for the important breakfast meal (Micha et al., 2010), even if this, to our knowledge, is not yet shown in a study. Afternoon administration also permits intermittent medication without immediate relapse of delayed sleep onset (Eckerberg, 2009). Even a single weekend dose seems to have some effect (Yang et al., 2001). Even if no side effects of the continuous long-term use of melatonin has been noted up till now (Van Geijlswijk et al., 2011), some caution might be recommendable.

A clear disadvantage of early melatonin administration is the soporific effect. During the first few days with melatonin administration, nearly half of the students in this study had experienced some degree of fatigue during 1 or 2 hrs following capsule intake. This MT_1-mediated effect is more evident when the homeostatic pressure for sleep is high (Wyatt et al., 2006). Thus, during the M2 week, when much of the sleep debt, presumably, was paid off, only four of the students experienced some post-capsule fatigue.

Although the soporific effect never was great enough to prevent the students to continue their activities, patients should be informed about it and advised to be extra careful with driving as long as they experience even slight post-capsule fatigue. As the effect is probably dose-dependent (Van Den Heuvel et al., 1998), it would have been preferable to give a lower dose of melatonin, for example 0.5 mg, which is enough to give a phase advance (Burgess et al., 2010). However, according to the producer, it was difficult to guarantee the conformity of capsule content with doses below 1 mg.

In summary, this study has shown that a small dose of melatonin, administered in the afternoon will result in a significant advanced sleep timing. The treatment was effective although the students continued with their often irregular sleep habits. We could also show that the treatment made the students feel more awake during the school day.

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REFERENCES

Anderson B, Storfer-Isser A, Taylor HG, Rosen CL, Redline S. (2009). Associations of executive function with sleepiness and sleep duration in adolescents. *Pediatrics* 123:701–707.

American Academy of Sleep Medicine (2005). *International classification of sleep disorders: diagnostic and coding manual.* 2nd ed. Westchester, IL: American Academy of Sleep Medicine.

Arendt J, van Someren E, Appleton R, Skene D, Åkerstedt T. (2008). Clinical update: melatonin and sleep disorders. *Clin. Med.* 8:381–383.

Bailey SL, Heitkemper MM. (2001). Circadian rhythmicity of cortisol and body temperature: morningness-eveningness effects. *Chronobiol. Int.* 18:249–261.

Brown SA, Kunz D, Dumas A, Westermark PO, Vanselow K, Tilmann-Wahnschaffe A, Herzel H, Kramer A. (2008). Molecular insights into human daily behavior. *Proc. Natl. Acad. Sci. USA* 105:1602–1607.

Burgess HJ, Revell VL, Eastman CI. (2008). A three pulse phase response curve to three milligrams of melatonin in humans. *J. Physiol.* 586:639–647.

Burgess HJ, Revell VL, Molina TA, Eastman CI. (2010). Human phase response curves to three days of daily melatonin: 0.5 mg versus 3.0 mg. *J. Clin. Endocrinol. Metab.* 95:3325–3331.

Cajochen C, Kräuchi K, von Arx MA, Möri D, Graw P, Wirz-Justice A. (1996). Daytime melatonin administration enhances sleepiness and theta/alpha activity in the waking EEG. *Neurosci. Lett.* 207:209–213.

Crowley SJ, Acebo C, Fallone G, Carscadon MA. (2006). Estimating dim light melatonin onset (DLMO) phase in adolescents using summer or school-year sleep/wake schedules. *Sleep* 29:1632–1641.

Curcio G, Ferrara M, De Gennaro L. (2006). Sleep loss, learning capacity and academic performance. *Sleep. Med. Rev.* 10:323–337.

Czeisler CA, Richardson GS, Coleman RM, Zimmerman JC, Moore-Ede MC, Dement WC, Weitzman ED. (1981). Chronotherapy: resetting the circadian clocks of patients with delayed sleep phase insomnia. *Sleep* 4:1–21.

Dahlitz M, Alvarez B, Vignau J, English J, Arendt J, Parkers JD. (1991). Delayed sleep phase syndrome response to melatonin. *Lancet* 337:1121–1124.

Dijk DJ, Boulos Z, Eastman CI, Lewy AJ. (1995). Campbell SS, Terman M. Light treatment for sleep disorders: consensus report. II. Basic properties of circadian physiology and sleep regulation. *J. Biol. Rhythms* 10:113–125.

Eckerberg B. (2009). *Melatonin treatment of sleep onset insomnia in adolescents: a pilot study*. www.eckerberg.se/pilotstudy. www.eckerberg.se/pilotstudy.

Emens JS, Yuhas K, Rough J, Kochar N, Peters D, Lewy A. (2009). Phase angle of entrainment in morning- and evening-types under naturalistic conditions. *Chronobiol. Int.* 26:474–493.

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (2011). Scientific opinion on the substantiation of a health claim related to melatonin and reduction of sleep onset latency (ID 1698, 1780, 4080) pursuant to Article 13(1) of Regulation (EC) No 1924/20061. *EFSA J.*9(6):16pp.

Figueiro MG, Rea MS. (2010). Evening daylight may cause adolescents to sleep less in spring than in winter. *Chronobiol. Int.* 27:1242–1258.

Frey S, Balu S, Greusing S, Rothen N, Cajochen C. (2009). Consequences of the timing of menarche on female adolescent sleep phase preference. *PLoS One.* 4(4):1–7.

Gradisar M, Gardner G, Dohnt H. (2011). Recent worldwide sleep patterns and problems during adolescence: A review and meta-analysis of age, region, and sleep. *Sleep Med.* 12:110–118.

Jones KH, Ellis J, von Schnatz M, Skene DJ, Dijk DJ, Archer SN. (2007). Age-related change in the association between a polymorphism in the PER3 gene and preferred timing of sleep and waking activities. *J. Sleep Res.* 16:12–16.

Kaida K, Takahashi M, Akerstedt T, Nakata A, Otsuka Y, Haratani T, Fukasawa K. (2006). Validation of the Karolinska Sleepiness Scale against performance and EEG variables. *Clin. Neurophysiol.* 117:1574–1581.

Kayumov L, Brown G, Jindal R, Buttoo K, Shapiro CM. (2001). A randomized, double-blind, placebo-controlled crossover study of the effect of exogenous melatonin on delayed sleep phase syndrome. *Psychosom. Med.* 63:40–48.

Lewy A, Bauer V, Ahmed S, Thomas K, Cutler N, Singer C. (1998). The human phase response curve (PRC) to melatonin is about 12 hours out of phase with the PRC of light. *Chronobiol. Int.* 15:71–83.

Lewy A, Emens J, Jackman A, Yuhas K. (2006). Circadian uses of melatonin in humans. *Chronobiol. Int.* 23:403–412.

Micha R, Rogers PJ, Nelson M. (2010). The glycaemic potency of breakfast and cognitive function in school children. *Eur. J. Clin. Nutr.* 64:948–957.

Millman RP. (2005). Excessive sleepiness in adolescents and young adults: Causes, consequences, and treatment strategies. *Pediatrics* 115: 1774–1786.

Moseley L, Gradisar M. (2009). Evaluation of a school-based intervention for adolescent sleep problems. *Sleep* 32:334–341.

Nagtegaal JE, Kerkhof GA, Smits MG, Swart ACW, Van Der Meer YG. (1998). Delayed sleep phase syndrome: a placebo-controlled cross-over study on the effects of melatonin administered five hours before the individual dim light melatonin onset. *J. Sleep Res.* 7:135–143.

Okawa M, Uchiyama M, Ozaki S, Shibui K, Ichikawa H. (1998). Circadian rhythm sleep disorders in adolescents: clinical trials of combined treatments based on chronobiology. *Psychiatry Clin. Neurosci.* 52:483–490.

Pandi-Perumal S, Trakht I, Spence W, Srinivasan V, Dagarn Y, Cardinali D. (2008a). The roles of melatonin and light in the pathophysiology and treatment of circadian rhythm sleep disorders. *Nat. Clin. Pract. Neurol.* 4:436–447.

Pandi-Perumal S, Trakht I, Srinivasan V, Spence W. (2008b). Physiological effects of melatonin: role of melatonin receptors and signal transduction pathways. *Progr. Neurobiol.* 85:335–353.

Portaluppi F, Smolensky MH, Touitou Y. (2010). Ethics and methods for biological rhythm research on animals and human beings. *Chronobiol. Int.* 27:1911–1929.

Roenneberg T, Kuehnle T, Juda M, Kantermann T, Allebrandt K, Gordijn M. (2007). Epidemiology of the human circadian clock. *Sleep Med. Rev.* 11:429–438.

Selmaoui B, Touitou Y. (2003). Reproducibility of the circadian rhythms of serum cortisol and melatonin in healthy subjects: a study of three different 24-h cycles over six weeks. *Life Sci.* 73:3339–3349.

Scherbarth F, Steinlechner S. (2010). Endocrine mechanisms of seasonal adaptation in small mammals: from early results to present understanding. *J. Comp. Physiol. B.* 180:935–952.

Shirayama M, Shirayama Y, Iida H, Kato M, Kajimura M, Watanabe T, Sekimoto M, Shirakawa S, Okawa M, Takahashi K. (2003). The psychological aspects of patients with delayed sleep phase syndrome (DSPS). *Sleep Med.* 4:427–433

Shochat T, Flint-Bretler O, Tzischinsky O. (2010). Sleep patterns, electronic media exposure and daytime sleep-related behaviours among Israeli adolescents. *Acta Paediatr*. 99:1396–1400.

Smits MG, Nagtegaal JE, van der Heijden J, Coenen AM, Kerkhof GA. (2001). Melatonin for chronic sleep onset insomnia in children: a randomized placebo-controlled trial. *J. Child. Neurol.* 16:86–92.

Smits MG, van Stel HF, van der Heijden K, Meijer AM, Coenen AM, Kerkhof GA. (2003). Melatonin improves health status in children with idiopathic chronic sleep-onset insomnia: a randomized placebo-controlled trial. *J. Am. Acad. Child. Adolesc. Psychiatry* 42:1286–1293.

Srinivasan V, Spence WD, Pandi-Perumal SR, Zakharia R, Bhatnagar KP, Brzezinski A. (2009). Melatonin and human reproduction: shedding light on the darkness hormone. *Gynecol. Endocrinol.* 25:779–785.

Swedish Government Official Reports (2006), Ungdomar, stress och psykisk ohälsa. SOU 77:1–375.

Thorpy MJ, Korman E, Spielman AJ, Glovinsky PB. (1988). Delayed sleep phase syndrome in adolescents. *J. Adolesc. Health Care* 9:22–27.

Van Der Heijden KB, Smits MG, Van Someren EJ, Gunning WB. (2005). Idiopathic chronic sleep onset insomnia in attention-deficit/hyperactivity disorder: a circadian rhythm sleep disorder. *Chronobiol. Int.* 22:559–570.

Van Der Heijden KB, Smits MG, Van Someren EJ, Ridderinkhof KR, Gunning WB. (2007). Effect of melatonin on sleep, behavior and cognition in ADHD and chronic sleep-onset insomnia. *J. Am. Acad. Child Adolesc. Psychiatry* 46:233–241.

Van Geijlswijk IM, Korzilius HP, Smits MG. (2010). The use of exogenous melatonin in delayed sleep phase disorder: a meta-analysis. *Sleep* 33:1605–1614.

Van Geijlswijk IM, Mol RH, Egberts TC, Smits MG. (2011). Evaluation of sleep, puberty and mental health in children with long-term melatonin treatment for chronic idiopathic childhood sleep onset insomnia. *Psychopharmacology* 216:111–120.

Van Den Heuvel CJ, Kennaway DJ, Dawson D. (1998). Effects of daytime melatonin infusion in young adults. *Am. J. Physiol.* 275:19–26.

Wade AG, Ford I, Crawford G, McConnachie A, Nir T, Laudon M, Zisapel N. (2010). Nightly treatment of primary insomnia with prolonged release melatonin for 6 months: a randomized placebo-controlled trial on age and endogenous melatonin as predictors of efficacy and safety. *BMC Med.* 8:18pp.

Wallenbeck I, Forslund L. (2008). Slutreplik: Välkontrollerade studier saknas. *Läkartidningen* 105:1p.

Weiss MD, Wasdell MB, Bomben MM, Rea KJ, Freeman RD. (2006). Sleep hygiene and melatonin treatment for children and adolescents with ADHD and initial insomnia. *J. Am. Acad. Child Adolesc. Psychiatry* 45:512–519.

Wolfson AR, Carskadon MA, Acebo C, Seifer R, Fallone G, Labyak SE, Martin JL. (2003). Evidence for the validity of a sleep habits survey for adolescents. *Sleep* 15:213–216.

Wolfson AR, Carscadon MA. (2004). Understanding adolescent sleep patterns and school performance: a critical appraisal. *Sleep. Med. Rev.* 7:491–506.

Wyatt J, Dijk D-J, Ritz-De Cecco A, Ronda J, Czeisler C. (2006). Sleep-facilitating effect of exogenous melatonin in healthy young men and women is circadian-phase dependant. *Sleep* 5:609–618.

Yang C, Spielman A, D'Ambrosio P, Serizawa S, Nunes J, Birnbaum J. (2001). A single dose of melatonin prevents the phase delay associated with a delayed weekend sleep pattern. *Sleep* 24:272–281.

Zee PC, Manthena P. (2007). The brain's master circadian clock: implications and opportunities for therapy of sleep disorders. *Sleep Med. Rev.* 11:59–70.

Zeitzer JM, Dijk DJ, Kronauer R, Brown E, Czeisler C. (2000). Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. *J. Physiol.* 526:695–702.