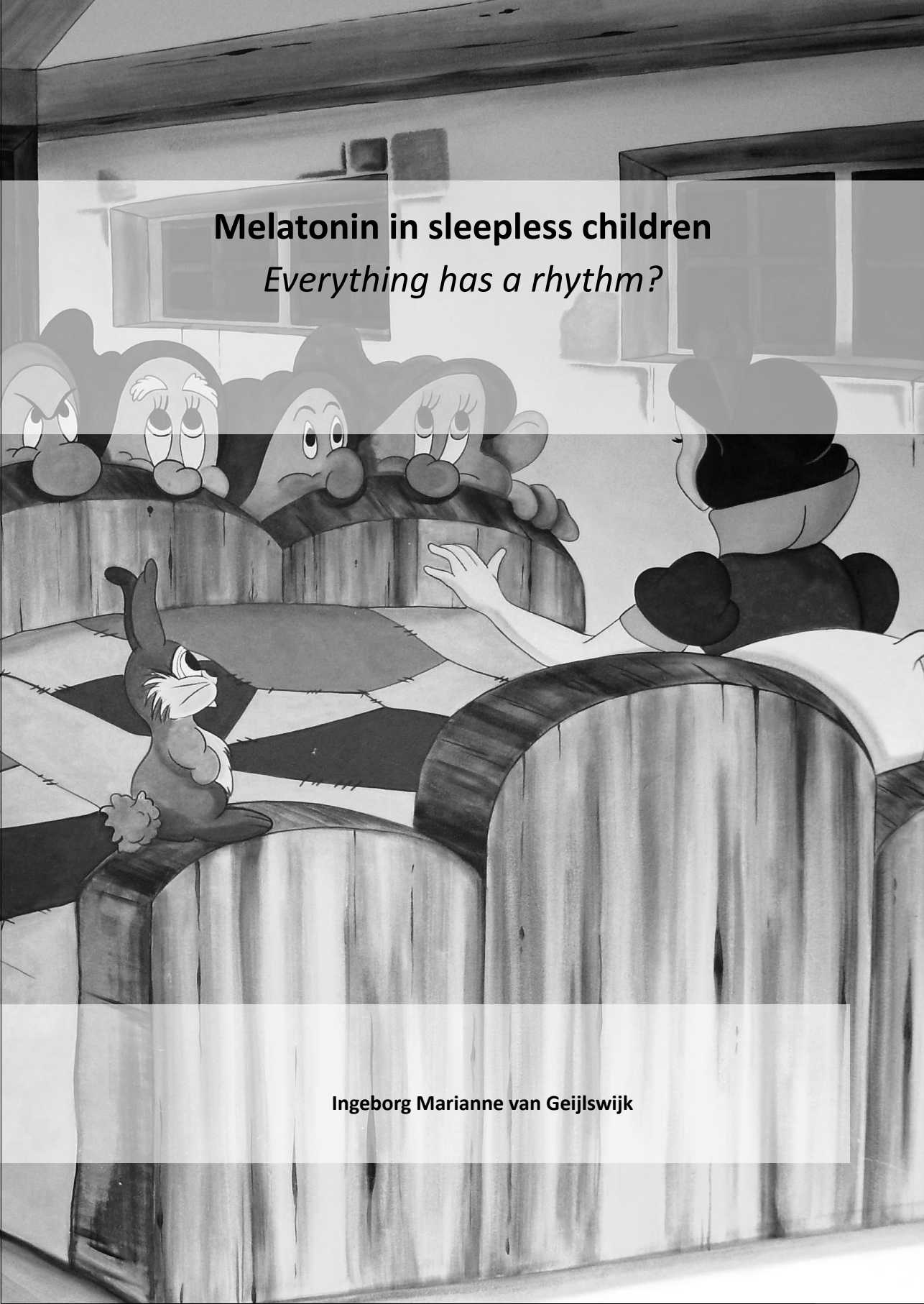
Melatonin in sleepless children

Everything has a rhythm?



Ingeborg Marianne van Geijlswijk

The work presented in this thesis was performed at the Department of Clinical Pharmacy of Gelderse Vallei Hospital Ede and at the Phamacy Department of the Faculty of Veterinary Medicine of Utrecht University, in affiliation with the Department of Pharmacoepidemiology and Clinical Pharmacology of the Utrecht Institute for Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht University in collaboration with the Department of Sleep-wake Disorders and Chronobiology of Gelderse Vallei Hospital Ede.

Parts of this thesis were performed in collaboration with
- the Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University
- the Department of Clinical Neurophysiology of Gelderse Vallei Hospital Ede
- the Department of Clinical Chemistry and Hematology of Gelderse Vallei Hospital Ede

MIX
Paper from responsible sources
FSC® C014810

Thesis, Utrecht University

ISBN	978-90-393-5524-4
Cover	Wallpainting Mascha van de Boom, photography Ed Moret
Lay-out	Marjolein Smithuis, Communication and Design, Faculty of Science, Utrecht University; www.uu.nl/beta/comvorm
Printed by	Ipskamp Drukkers BV, Enschede, The Netherlands

Melatonin in sleepless children

Everything has a rhythm?

Melatonine bij slapeloze kinderen

Alles heeft een ritme?

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen
op woensdag 27 april 2011 des middags te 2.30 uur

door

Ingeborg Marianne van Geijlswijk

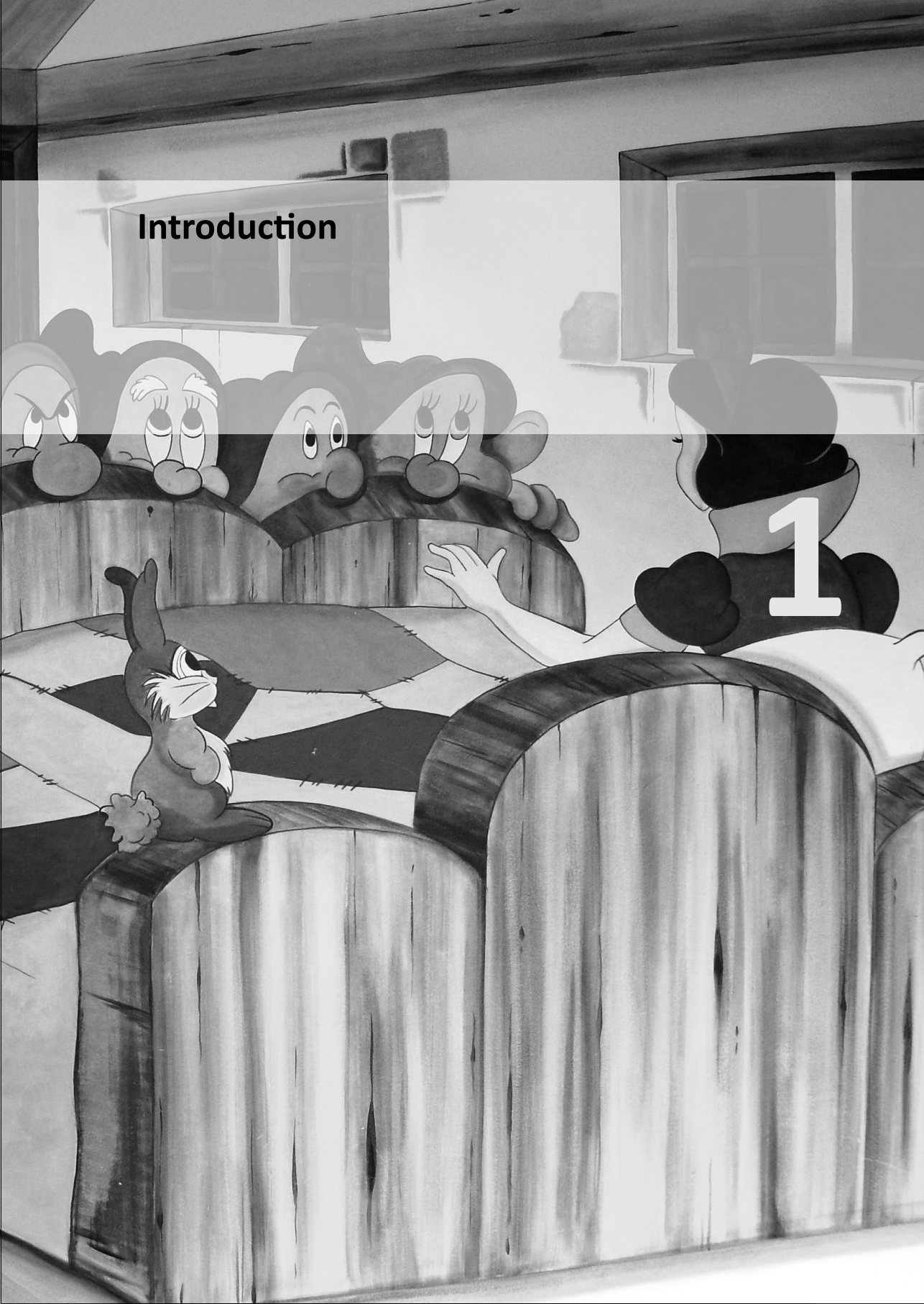
geboren op 7 mei 1965
te Amsterdam

Promotoren: **Prof.dr. A.C.G. Egberts**
Prof.dr. H. Vaarkamp
Co-promotor: **Dr. M.G. Smits**

In liefdevolle herinnering aan mijn vader

INDEX

Introduction



1

Every living organism has endogenous rhythms regulated by endogenous substances (eg melatonin, cortisol). These rhythms are synchronized by the master circadian clock, which resides in humans within the paired suprachiasmatic nuclei (SCN) of the anterior hypothalamus (Hannibal and Fahrenkrug. 2006). The daily patterns of most physiologic and hormonal processes stem from this master pacemaker in the SCN (Gachon et al. 2004). The endogenous clock gets entrained by exogenous impulses like daylight, length of day, temperature, exercise and feeding with the overarching aim to allow preparation for anticipated metabolic and physical activities (Kalsbeek et al. 2010). This anticipation of and subsequent adaptation to the outside world varies between species, age and sex, but usually concerns physiologic processes, behaviour and/or hormone regulation (Hastings and Maywood. 2000; Falcon et al. 2009b). For instance Scherbarth and Steinlechner (2010) concluded that the *normal fluctuation* in melatonin levels resulted in *different results* of gonadotrophic effect and reproduction, depending on the animal species involved. Melatonin is the mediator for the environmental cue that activates the seasonal breeder organism in a species appropriate way to the seasonal changes. The production of melatonin in the pineal gland is common in all vertebrates, but the regulation of the pineal gland in vertebrates shows an impressive evolutionary development from ectotherms (fish and frogs) to mammals (Figure 1), from a full circadian organ localised underneath a thin part of the skull consisting of photoreceptor, circadian clock and melatonin production to a collaborative system of eyes, SCN and pineal gland (which is localised in most central part of the brain).
Although the daily rhythm of melatonin is equal in nocturnal and diurnal mammals, in man the rise of endogenous melatonin, the so called dim light melatonin onset (DLMO) is associated with the initiation of the sleep phase. The endogenous melatonin rhythm may determine the distinctive preference of individuals for an early or late rhythm, so called morning- or eveningness (Griefahn. 2002).

Delayed sleep phase disorder (DSPD) is a problem in which the circadian clock does have the 24-hour rhythm entrainment but at a delayed phase angle (Weitzman et al. 1981), resulting in a sleep- wake timing that is late with respect to societal norms (Pandi-Perumal et al. 2007). It has been estimated that chronic insomnia diagnosed according to the official DSM-IV classification has a prevalence in adults of 6% (Ohayon. 2002) and that approximately 10% of patients with chronic insomnia have DSPD (Regestein and Monk. 1995). One of the symptoms of DSPD is sleep onset insomnia and although it is not an official diagnostic criterion, DSPD is often recognized by a delayed DLMO.
Parents of children with DSPD symptoms often describe ultimate despair when trying to cope with the bed ritual of their children, because sleep onset is delayed and the children get up and come out repeatedly until very late. On the long run, the children as well as their parents get exhausted, because of the obligatory school times, and the children tend to show poor school performances due to insufficient sleep quality and quantity.

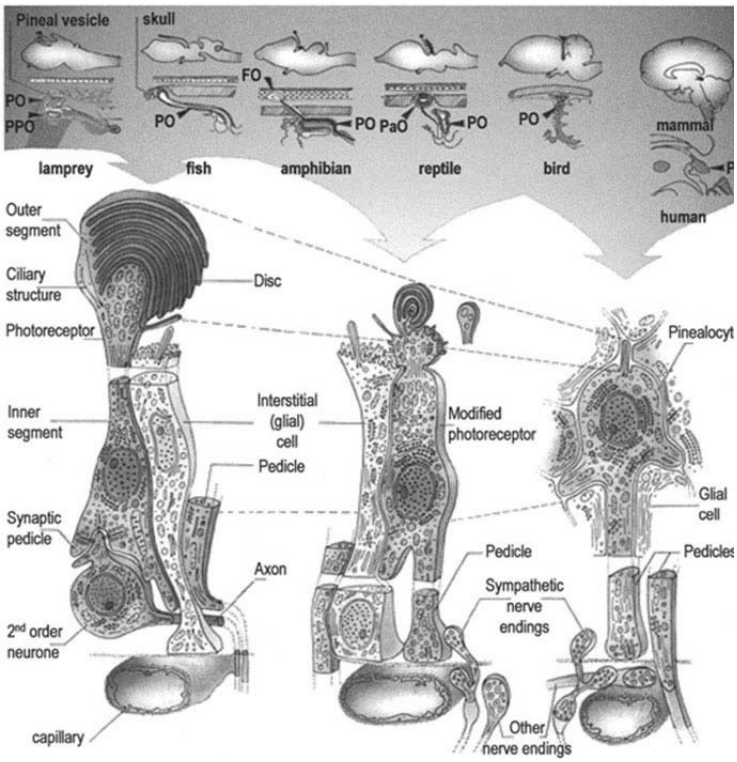


Figure 1. Evolution of the pineal organ in vertebrates. The upper panel shows the anatomical location of the pineal gland in the vertebrate brain (in black). The lower panel shows the cell types of the epithelium in different representatives from fish to mammals. Note the disappearance of the photoreceptive pole and of the second-order neurons as well as the appearance of the sympathetic innervations. FO, frontal organ; PaO, parietal organ; PO, pineal organ; PPO, parapineal organ.(Falcon et al. 2009a)

Sleep onset insomnia has often been associated with Attention Deficit Hyperactivity Disorder (ADHD) and it has been suggested that in part of the ADHD patients there either is a shared underlying pathophysiology or misinterpretation of daytime consequences of DSPD as ADHD symptoms (Szeinberg et al. 2006). Additionally, in South Europe most physicians are sceptical towards sleep medication for DSPD in children: they wonder whether the northern way of life implies frequent usage of pharmacological bondage of children to fit the societal needs (Jenni. 2005). Is it appropriate to have a young child take medication for its probably genetically determined sleep/wake habits, a healthy child that is just not able to adapt to the needs of our society? Are we medicating this child for its own good, or for the good of others? Is this child better off with an adjusted sleep/wake rhythm, or should we simply accept its unadjusted state?

Exogenous melatonin, administered 5-6 hours before DLMO, produces the biggest advancement of the melatonin and sleep-wake rhythm (Lewy et al. 2004). Exogenous melatonin administration before DLMO to children with sleep onset insomnia has successfully been applied to change the sleep pattern and ameliorate associated consequences thereof (Smits et al. 2003; van der Heijden et al. 2007; Braam et al. 2009). Drug holidays of one week in the children who took melatonin for sleep-onset insomnia resulted in the former sleep problem in more than 90% of the patients (Hoebert et al. 2009). This suggests that the chronobiotic effects of melatonin can only be sustained through continued use.

Treatment of children with melatonin is also controversial, because of its effects on reproduction in animals (Arendt. 1997; Szeinberg et al. 2006). Thirty years ago reproduction was the main subject of research with melatonin. Impressive effects of seasonal changes in reproductive activity of most mammals are associated with the direction of the shift of DLMO due to lengthening or shortening of the days.

As the endogenous production of the hormone melatonin is a response to external *Zeitgebers*, one could theorise that a melatonin rhythm in accordance with the environment might be an indicator for the harmonisation of the individual to its environment, and a parameter for its wellbeing. For instance, the association between the severity of major depressions and the eveningness chronotype gets more and more substantiated (Selvi et al. 2010). Thus, amongst medical professionals opposite opinions about the efficacy and safety of melatonin in patients with CSOI in general and in CSOI in children in particular exist and therefore different levels of willingness to prescribe melatonin to children with CSOI are found.

Very little data are available to determine the optimal dosing and timing strategy, including duration of therapy, in the vulnerable paediatric population. Professionals with years of experience with melatonin prescription have already accepted this long term use in children and do entitle it as safe. However by most professionals with less experience, melatonin is regarded as a hypnotic at best, but more likely as a hormone. It seems to have become an unwritten rule for many general practitioners to limit melatonin therapy in a child to six months. As melatonin is associated with reproduction many general practitioners have a deeply felt concern that long term usage of melatonin in pre-pubertal children will lead to postponed puberty.

OBJECTIVES

- To investigate the relationship between endogenous circadian melatonin production rhythm and the ability to cope with and adapt to the circumstantial situation
- To establish the most appropriate dosing and timing of melatonin to treat children with CSOI with respect to optimal sleep onset improvement

- To establish the efficacy and safety of melatonin therapy on the short and the long run

OUTLINE

Do individuals ad liberty to adapt and to anticipate to the environment have an undisturbed melatonin rhythm as compared to individuals without natural exogenous impulses or even manipulated with an unnatural multiphase light schemes? Because determination of melatonin rhythm in pigs is feasible according to several publications, we decided to study two populations of pigs: one population in a traditional farm with natural light exposition, one population in a large pig production stable without natural light. The results of this study are described in chapter 2.

Melatonin, being a natural human hormone, exhibits a complex pharmacodynamic and pharmacokinetic profile (Zhou et al. 2009). Its short half-life in normal persons may cause efficacy problems when timing of exogenous administration is not right. In individuals with a compromised Cytochrome P450 metabolism efficacy problems of a completely other nature might emerge. In chapter 3 three case reports demonstrate the therapeutic consequences of the complex kinetics of melatonin in poor metabolisers.

Chapter 4 is a meta analysis of the efficacy of melatonin in DSPD. An earlier review (Buscemi et al. 2006) did not find a sleep improving effect of melatonin. However, in that study DLMO was not taken into account as to the time of its administration. Therefore we performed a meta-analysis of studies with melatonin treatment in DSPS patients, who took melatonin at a time related to DLMO.

Two previously performed studies in children at hospital Gelderse Vallei collected sleep results of melatonin therapy during four weeks. Both studies reported endpoint results. At what point during those four weeks was the full effect of melatonin attained, and how stable was it thereafter? In chapter 5 this post hoc analysis is presented.

Chapter 6 describes Meldos, a randomised, placebo controlled double blind trial, comparing three bodyweight related doses of melatonin to placebo in the treatment of CSOI. The aim of this trial was to establish the optimal dose of melatonin in children between six and twelve years of age. Based on the results of chapter 5 the therapy results were evaluated after just one week of therapy. We collected sleep parameters and DLMO in the week before and during this one week of melatonin therapy. The objective was to perform a simple, non invasive, short termed trial in a group of basically healthy children with or without ADHD suffering from CSOI to establish individual responses to three melatonin dosages or placebo.

Previous studies and experience had taught us that melatonin therapy shifts the endogenous melatonin onset, but that this effect wears off in several days. Since we attribute the positive effects of melatonin to this chronobiotic effect, we know that to

maintain a healthy sleep pattern in children with DSPD, exogenous melatonin will be needed during a longer period to prevent the return of the delayed rhythm.
The children included in the Meldos trial were melatonin naïve at enrolment. Most children were given a prescription of melatonin after finishing the trial to continue therapy. Mean (SD) 3.1 (.87) years after finishing MELDOS we asked all those former participants to participate in a follow up study, to document their experiences with melatonin. We inquired about their emotional development, their sleep habits and their pubertal development. This systemic evaluation of the consequences of long term melatonin usage is addressed in chapter 7.
In the general discussion the social implications of the results of our studies are discussed.

REFERENCES

Arendt J (1997) Safety of melatonin in long-term use (?). J Biol Rhythms 12:673-81.

Braam W, Smits MG, Didden R, Korzilius H, Van Geijlswijk IM, Curfs LM (2009) Exogenous melatonin for sleep problems in individuals with intellectual disability: a meta-analysis. Dev Med Child Neurol 51:340-9.

Buscemi N, Vandermeer B, Hooton N, Pandya R, Tjosvold L, Hartling L, Vohra S, Klassen TP, Baker G (2006) Efficacy and safety of exogenous melatonin for secondary sleep disorders and sleep disorders accompanying sleep restriction: meta-analysis. BMJ 332:385-93.

Falcon J, Besseau L, Fuentes M, Sauzet S, Magnanou E, Boeuf G (2009a) Structural and functional evolution of the pineal melatonin system in vertebrates. Ann N Y Acad Sci 1163:101-111. DOI: 10.1111/j.1749-6632.2009.04435.x.

Falcon J, Besseau L, Fuentes M, Sauzet S, Magnanou E, Boeuf G (2009b) Structural and functional evolution of the pineal melatonin system in vertebrates. Ann N Y Acad Sci 1163:101-111. DOI: 10.1111/j.1749-6632.2009.04435.x.

Gachon F, Nagoshi E, Brown SA, Ripperger J, Schibler U (2004) The mammalian circadian timing system: from gene expression to physiology. Chromosoma 113:103-112. DOI: 10.1007/s00412-004-0296-2.

Griefahn B (2002) The validity of the temporal parameters of the daily rhythm of melatonin levels as an indicator of morningness. Chronobiol Int 19:561-577.

Hannibal J, Fahrenkrug J (2006) Neuronal input pathways to the brain's biological clock and their functional significance. Adv Anat Embryol Cell Biol 182:1-71.

Hastings M, Maywood ES (2000) Circadian clocks in the mammalian brain. Bioessays 22:23-31. DOI: 2-Z.

Hoebert M, van der Heijden KB, van Geijlswijk IM, Smits MG (2009) Long-term follow-up of melatonin treatment in children with ADHD and chronic sleep onset insomnia. J Pineal Res 47:1-7.

Jenni OG (2005) Sleep onset insomnia during childhood or poor fit between biology and culture: comment on van der Heijden et al. 'Prediction of melatonin efficacy by pre-treatment dim light melatonin onset in children with idiopathic chronic sleep onset insomnia'. J Sleep Res 14:195-7; discussion 197-9.

Kalsbeek A, Yi CX, La Fleur SE, Fliers E (2010) The hypothalamic clock and its control of glucose homeostasis. Trends Endocrinol Metab 21:402-410. DOI: 10.1016/j.tem.2010.02.005.

Lewy AJ, Emens JS, Bernert RA, Lefler BJ (2004) Eventual entrainment of the human circadian pacemaker by melatonin is independent of the circadian phase of treatment initiation: clinical implications. J Biol Rhythms 19:68-75.

Ohayon MM (2002) Epidemiology of insomnia: what we know and what we still need to learn. Sleep Med Rev 6:97-111.

Pandi-Perumal SR, Smits M, Spence W, Srinivasan V, Cardinali DP, Lowe AD, Kayumov L (2007) Dim light melatonin onset (DLMO): a tool for the analysis of circadian phase in human sleep and chronobiological disorders. Prog Neuropsychopharmacol Biol Psychiatry 31:1-11.

Regestein QR, Monk TH (1995) Delayed sleep phase syndrome: a review of its clinical aspects. Am J Psychiatry 152:602-8.

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. DOI: 10.1007/s00360-010-0498-2.

Selvi Y, Aydin A, Boysan M, Atli A, Agargun MY, Besiroglu L (2010) Associations between chronotype, sleep quality, suicidality, and depressive symptoms in patients with major depression and healthy controls. Chronobiol Int 27:1813-1828. DOI: 10.3109/07420528.2010.516380.

Smits MG, van Stel HF, van der Heijden K, Meijer AM, Coenen AM, Kerkhof GA (2003) Melatonin improves health status and sleep in children with idiopathic chronic sleep-onset insomnia: a randomized placebo-controlled trial. J Am Acad Child Adolesc Psychiatry 42:1286-93.

Szeinberg A, Borodkin K, Dagan Y (2006) Melatonin treatment in adolescents with delayed sleep phase syndrome. Clin Pediatr 45:809-18.

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 46:233-41.

Weitzman ED, Czeisler CA, Coleman RM, Spielman AJ, Zimmerman JC, Dement W, Richardson G, Pollak CP (1981) Delayed sleep phase syndrome. A chronobiological disorder with sleep-onset insomnia. Arch Gen Psychiatry 38:737-46.

Zhou SF, Yang LP, Zhou ZW, Liu YH, Chan E (2009) Insights into the substrate specificity, inhibitors, regulation, and polymorphisms and the clinical impact of human cytochrome P450 1A2. The AAPS journal 11:481-94.

Melatonin rhythm in pigs kept under two different light regimen conditions

2

Ingeborg M. van Geijlswijk, PharmD [a,b]; Arie van Nes, DVM, PhD [c]; Maaike Pannekoek [a,c]; Silvia C. Endenburg, PhD [d]; Marcel G. Smits, MD, PhD [e]; Henk Vaarkamp, DVM, PhD [a]

[a] Utrecht University, Faculty of Veterinary Medicine, Pharmacy Department, Utrecht, Netherlands
[b] Utrecht Institute for Pharmaceutical Sciences (UIPS), Department of Pharmacoepidemiology and Clinical Pharmacology, Faculty of Science, Utrecht, Netherlands
[c] Utrecht University, Faculty of Veterinary Medicine, Department of Farm Animal Health, Utrecht, Netherlands
[d] Gelderse Vallei Hospital, Department of Clinical Chemistry and Hematology, Ede, Gelderland, Netherlands
[e] Gelderse Vallei Hospital, Department of Sleep-wake disorders and Chronobiology, Ede, Gelderland, Netherlands

Disclosure statement
This was not an industry-supported study

Conflict of interest
All authors state they have no conflicts of interest to declare.

submitted

ABSTRACT

Inappropriately applied bright light disturbs 24 hour melatonin rhythm and consequently desynchronizes biological rhythms, resulting in decrease of human and animal well-being. Some large swine producers apply artificial light, for instance three times a day for three hours. Consequently, the 24 hour melatonin rhythm of these pigs might differ from pigs living in farms with natural light schemes and if so, might contribute to difference in well-being. To contribute to the discussion whether or not large swine production units decrease well being of pigs, 24 hour melatonin rhythm of pigs in a large swine production unit (Farm B) was compared with pigs of a farm with natural light schemes (Farm A). Salivary samples were collected hourly for 24 hours at both farms. Mean scotophase and mean photophase MEL levels were not statistically significant different, neither on Farm A nor on Farm B. No statistically significant differences were found comparing mean MEL levels of Farm A and Farm B during scotophase and photophase, the MEL levels one hour after lights off and one hour after lights on, MEL levels just before and after feeding. The controversy in existing literature about domestic pigs having an entrained melatonin rhythm or not, is not adjourned by our study. Whether or not the lack of an entrained melatonin rhythm is an indicator for quality of life of the domestic pig is not elucidated by our results.

Key Words: Melatonin rhythm, pigs, stabling

INTRODUCTION

Every living organism has an endogenous rhythm, the biological clock. This endogenous rhythm is synchronized (entrained) by exogenous impulses like daylight, length of day, temperature and feeding to adapt the organism to the environment. The necessity of this adaptation to the outside world varies with animal species, age and gender, but often concerns physiologic processes, behaviour and hormone regulation(Hastings. 1991; Falcon et al. 2009). For instance, Schertbarth et al. (Scherbarth and Steinlechner. 2010) concluded that the normal variation in melatonin levels resulted in different results of gonadotrophic effect and reproduction, depending on the animal species involved. Melatonin is the mediator for the environmental cue that activates the seasonal breeder organism in a species appropriate way to the seasonal changes. As the production of the hormone melatonin is a response to external *Zeitgebers*, one could theorize that a melatonin rhythm in accordance to the environment might be an indicator for the harmonization of the individual to its environment, and a parameter to measure its wellbeing.

Recently, in The Netherlands, a governmental point of view concerning large swine production units was postulated. The public discussion on this topic is predominated by animal welfare and is especially engaged in the ethical and emotional aspects of this type of housing. While the technicalities like feeding, heating, ventilation and square meters can be measured, validated and reported, the ethical question "is the animal happy?"' remains unanswered. Is appropriate growth enough to guarantee the animals' wellbeing, or should we look for a parameter that is sensitive for more subtle discomforts?

During the last twenty years researchers have been measuring serum concentrations of melatonin in pigs under different environmental conditions, and there seems to be a controversy as to whether pigs have nocturnal rises in serum melatonin (Diekman et al. 1992; Bubenik et al. 1996; Bollinger et al. 1997; Andersson. 2001). This controversy with regard to an assumed universal phenomenon (nocturnal rises in serum melatonin) in all vertebrates (Falcon et al. 2009) raises the intriguing question: could melatonin be an indicator for the balance of an animal and its housing?

Mack and Unshelm. (1997) for instance concluded that housing of pigs with low artificial light intensities, 50 lx at day (photophase) and 8 lx at night (scotophase) prevents the development of a biological endogenous rhythm. Others (Tast et al. 2001b) found that pigs do develop a day/night melatonin rhythm if the photophase light intensity is 40 lx and the scotophase light intensity is less than 1 lx. Higher photophase light intensity did not influence the scotophase (peak) melatonin response or basal melatonin concentrations during the photophase. Andersson. (2001) studied several litters of pigs, and concluded that in all pigs a night and day difference could be found as from ages of ten weeks, but that the amplitude differed between gender and between litters, suggesting a genetic factor. Comparison of the melatonin secretion patterns of the European wild boar under

its natural environment with the patterns of cross-bred (Yorkshire×Finnish Landrace) domestic pigs demonstrated the existence of a seasonal circadian rhythm in melatonin secretion in both the European wild boar and the domestic pig (Tast et al. 2001a). The results suggested no difference in photoperiodic-melatonin transduction between the European wild boar and domestic pig whether due to altered genotype or reduced light environment.

In Dutch pig husbandry it is obligatory to have a minimum of eight hours light per day with an intensity of at least 40 lx. In order to obtain optimal feed conversion a strategy is developed of giving smaller feed portions more frequently and stimulating sleep shortly after. It consists of introducing dark periods after feeding: some pig owners divide the obligatory eight light hours in several periods per day (multiphase light regime), before and during feeding. This strategy is in compliance with Dutch law.
We compared the 24 h melatonin rhythm of pigs kept under a multiphase light regimen with the rhythm of pigs housed under the natural day-night regimen. The reference husbandry (farm A) was a traditional stable with outside windows, and 12 hours of natural light during the measurements in September. The test husbandry (farm B) was a highly organized modern stable with a three times three hour artificial light regimen during three feeding times. The objective of our study was to test whether endogenous melatonin production qualifies as an indicator for animal welfare.

METHODS AND MATERIALS

Animals and housing

Farm A

Six cross-bred (Dutch Landrace×Yorkshire) female pigs of three litters, aged five months, weighing 50-60 kg, kept under a single phase light regime with at least ten hours of artificial and natural light per day. The photophase lasted from 7:00 a.m. to 5:00 p.m.. Due to outside windows in the stable there was a dim light phase until sunset (about 8:30 p.m. at the time of the study). The three pens, housing 2 to 6 pigs per pen, (Fig.1) we sampled had different light intensities because of these windows and the corridor windows at the opposite wall. The pigs in the pen near the window were exposed to a light intensity, as measured by a Lux-meter (lx-93, Nieuwkoop BV, Aalsmeer, Netherlands), varying from 700 lx in the shady parts of the pen to 7000 lx in the sunbeams. The pen in the middle had a light intensity of 140 lx and the third pen had a light intensity of 240 lx, due to the bulb lights in the corridor. The light intensity during the dim light phase was 25 lx in pen number 1, pen number 2 and 3 had a dim light intensity of 0.5 lx. After sunset the light intensity was reduced to 0.5 lx in all pens. From every pen two pigs were sampled. The

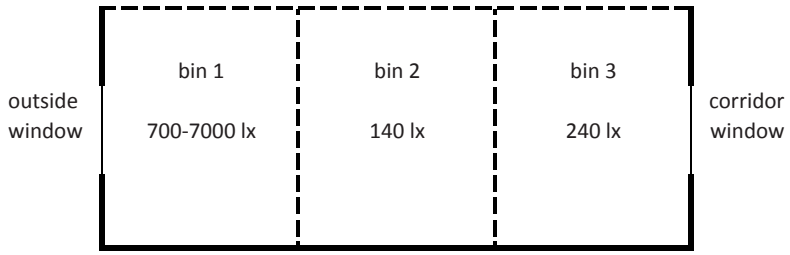


Figure 1. Light conditions in the pig stable of Farm A

pigs were fed commercial pellets, restrictedly at 7:00 a.m. and 3:45 p.m. The pigs emptied the feed-trough immediately.

Farm B

Six cross-bred (Dutch Landrace×Yorkshire) female pigs of two litters, aged five months, weighing 50-60 kg, kept under a multiple phased light regimen, three periods of three hours of artificial light of 40 lx. The first period lasted from 5:30 a.m. to 8:30 a.m., the second period from 1:00 p.m. to 4:00 p.m. and the third period from 8:00 p.m. to 11:00 p.m. Twenty to twenty-five minutes after the start of the photophase the pigs were fed. The scotophase set in abruptly, without a dim light phase like Farm A, and was completely dark, 0 lx. The pigs were fed slurry, restrictedly at 5:45 a.m., 1:20 p.m. and 8:25 p.m. The pigs emptied the feed-trough immediately.

The stable was divided in eight pens, each containing thirteen pigs of the same age and all pigs entered the stable at the same time as is required in an all in – all out system. The sampled pigs were housed in one pen.

Sample collection

On both farms, during the four days preceding the sampling period, the pigs were trained by playful test sampling and animal handling a couple of times per day. After the trainings days the pigs were eager to cooperate, on the experiment day, during which saliva samples were taken of every pig every hour during 24 hours.

The samples were collected by having the pigs chew on cotton saliva collectors salivettes® (Sarstedt bv Etten Leur, the Netherlands) during 30 seconds. To prevent suppression of melatonin secretion by bright light (Bojkowski et al. 1987) during the collections in the dark periods, flashlights were used to assist the presentation of the salivette to the pigs, while avoiding shining in the eyes of the animals (Brainard et al. 2000).

The collector tubes were immediately stored at a temperature of 4˚C and centrifuged within 24 hours and then stored at a temperature of -18˚C and analyzed within 8 weeks. Salivary melatonin concentrations were measured as described elsewhere for human samples (Nagtegaal et al. 1998). The use of the applied method (Bühlmann Laboratories AG, Schönenbuch, Switserland) in pigs was described earlier (Mack and Unshelm. 1997).

Statistical methods

Three variables were derived for analysis. The absolute melatonin saliva levels (MEL levels) in pg/ml (A), the relative individual melatonin concentrations (relative to the highest melatonin level in each pig) (R) and the increase or decrease of melatonin levels (difference between a level and the preceding level) (D) were analyzed in three models per farm. For every comparison the independent sample T-test (normal distribution) or the nonparametric Mann-Whitney U-test was used.

In model 1, L/D, melatonin levels during scotophase were compared to melatonin levels during photophase. In model 2, light change, the MEL levels after the lights were switched on were compared to MEL levels after the lights were switched off, the first photophase hour in comparison with the first scotophase hour. Model 3, feeding time, compared MEL levels before feeding with MEL levels after feeding.

Correlations per farm for melatonin levels and L/D, light change and feeding time were calculated with Pearson correlation. All calculations were performed in SPSS 15.0 for Windows (SPSS inc. 2006).

RESULTS

MEL levels of pigs kept in two different housing conditions did not show a distinct day and night rhythm. Mean scotophase and mean photophase MEL levels were not statistically significant different, neither on Farm A nor on Farm B. When the mean MEL levels of Farm A and Farm B during scotophase and photophase were compared, no differences were

Table 1. Mean melatonin (MEL) levels of farm A and farm B during Dark and Light hours

Farm A

	Total (143 samples)		Dark (65 samples)		Light (78 samples)	
	Mean	SD	Mean	SD	Mean	SD
MEL level A (pg/mL)	12.43	8.76	11.15	6.08	13.50	10.41
MEL level R (%)	34.1%	24.5%	32.0%	22.9%	35.8%	25.8%
In-/decrease D (pg/mL)			-0.80	7.71	0.65	12.94

Farm B

	Total (144 samples)		Dark (90 samples)		Light (54 samples)	
	Mean	SD	Mean	SD	Mean	SD
MEL level A (pg/mL)	12.95	7.49	12.43	6.62	13.81	8.75
MEL level R (%)	38.6%	22.9%	37.2%	21.2%	40.9%	25.6%
In-/decrease D (pg/mL)			-1.97	9.55	3.28	9.89

found (Table 1). Pigs of both farms showed a mean decrease during scotophase versus a mean increase during photophase. The difference between mean change of MEL levels of Farm B during scotophase and photophase was not statistically significant with the Mann Whitney U test.

Figure 2 and 3 show the mean melatonin curves of the pigs of Farm A and Farm B. The grey areas depict the scotophases. No nocturnal rise was detected in either farm.
Time-trends for MEL levels were best described by sixth-order polynomial equations (R^2 =.48 for Farm A and .54 for Farm B).

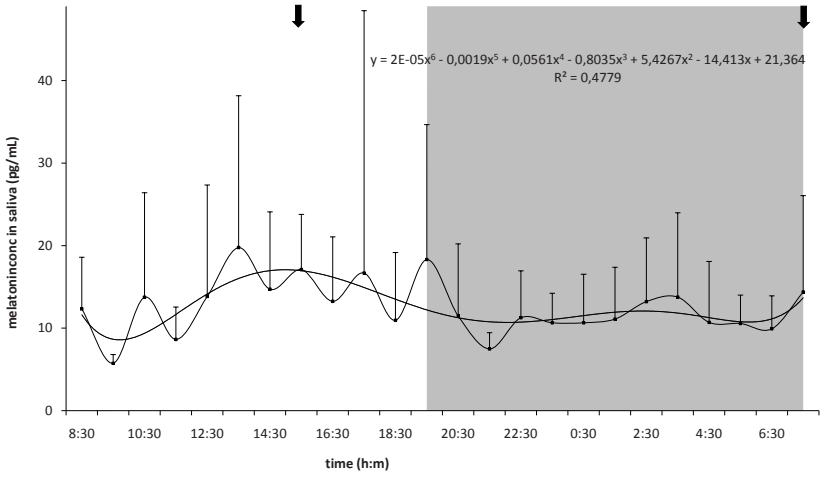


Figure 2. Mean Melatonin concentration curve farm A (n=6)
white area = photophase period; grey area = scotophase period; ⬇ = feeding time

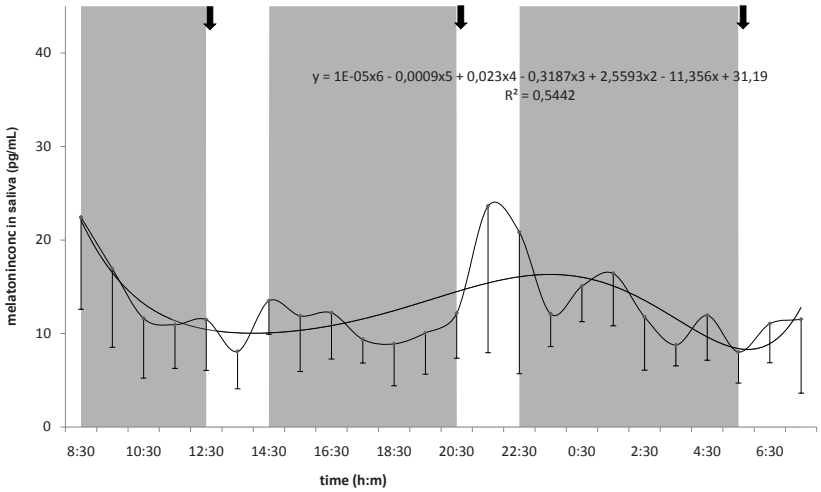


Figure 3. Mean Melatonin concentration curve farm B (n=6)
white area = photophase period; grey area = scotophase period; ⬇ = feeding time

Light change effects

Although daytime fluctuations in melatonin levels in individual pigs were observed, the overall pattern was not related to scotophase/photophase changes. The MEL levels one hour after lights off were compared with MEL levels one hour after lights on. No statistically significant differences were found (Table 2).

Feeding time effects

For farm B in the independent sample T-test a significant correlation between feeding time and MEL levels was found. The Levene's Test for equality of variances was also significant. Subsequent analysis with a non parametric test (Mann-Whitney) revealed no significant correlation. We initially analyzed MEL levels of 1-4 hours after feeding, but identified two interfering processes. All feeding times at Farm B and the morning feed at farm A coincided with lights on. Furthermore, if food-derived-melatonin attributed to rising of the MEL levels, this phenomenon can not be separated from the lights off within 2.5 hours after finishing the meal at farm B.

The afternoon feeding (at 3:45 p.m.) at Farm A was not accompanied by a change in light. No correlation of feeding and MEL levels could be detected with this feeding. MEL levels of farm B one hour after feeding were lower than the MEL levels of farm A, but the difference was not statistically significant. The difference between MEL levels just before and after feeding did not show statistically significant differences.

Table 2. Mean melatonin (MEL) levels of farm A and farm B one hour after light change

Farm A				
	1 hour after lights off (6 samples)		1 hour after lights on (6 samples)	
	Mean	SD	Mean	SD
MEL level A (pg/mL)	11.48	8.73	14.38	11.67
MEL level R (%)	30.3%	20.7%	36.2%	26.7%
Last hour change D	-6.83	19.78	4.47	13.18
Farm B				
	1 hour after lights off (18 samples)		1 hour after lights on (18 samples)	
	Mean	SD	Mean	SD
MEL level A (pg/mL)	16.67	10.42	9.73	4.14
MEL level R (%)	47.8%	28.7%	29.0%	15.7%
Last hour change D	-2.64	13.95	0.26	5.44

DISCUSSION

We did not find any circadian melatonin rhythm in pigs, neither under normal day-night light regime nor under multiphase light regime.

In this study we sampled every hour, in contrast to all earlier published studies (every two hours). With the very short half life of melatonin (30 minutes) the chances to overlook peak levels are substantially decreased with shorter intervals.

The factors that could have hampered our observations to find a day-night rhythm in contrast to other groups were the way of sampling (saliva instead of serum), the analytical method, the gender of the pigs (exclusively female), the age of the pigs (pre-puberty), and/or the type of food (pellets in farm A and slurry in farm B) or the restricted way of feeding(Mendoza et al. 2005; Feillet et al. 2008).

Saliva levels are related to peripheral blood levels. Although not established in pigs, in men this relationship is rather well defined, 10 pg/mL in serum correlates to 4 pg/mL in saliva (Nagtegaal et al. 1998). Saliva of pigs is rather alkaline, pH=8.5, compared to human saliva of pH=7. As melatonin has two weak pK_a's: 4.7 (alkaline) and 12.3 (acid) the fraction of unionized melatonin does not differ substantially when comparing saliva of pH=7 with pH=8.5.

The concentrations we found in saliva of our pigs, mean 12.5-13 pg/mL would correspond to 30 pg/mL in serum. Mack (Mack and Unshelm. 1997) reported mean saliva levels between 9.875 and 18.03 pg/mL. The corresponding venous blood levels were indeed found by Bubenik et al. (2000) and Bollinger et al. (1997) in gilts, both groups reported

Table 3. Mean melatonin (MEL) levels of farm A and farm B one hour after feeding

Farm A				
	morning feed (6 samples)		afternoon feed (6 samples)	
	Mean	SD	Mean	SD
MEL level A (pg/mL)	14.38	11.67	13.25	7.81
MEL level R (%)	36.2%	26.7%	34.5%	18.6%
Last hour change D	4.47	13.18	-3.85	4.60
Farm B				
	feed following long scotophase (6 samples)		feed following short scotophase (2 times. 12 samples)	
	Mean	SD		SD
MEL level A (pg/mL)	11.05	4.17	10.13	4.72
MEL level R (%)	32.5%	15.8%	31.3%	19.5%
Last hour change D	3.02	6.82	-0.65	4.77

a lack of nocturnal rise like we did. The group of Minton and Griffith used boars (Minton et al. 1989) and barrows (Griffith and Minton. 1991). The last group reported constant levels of 26 pg/mL under constant light or 7.4 pg/mL under constant dark circumstances. Neither sampling method nor female gender appears to be causing deviant results.

On the other hand, Andersson. (2001) and Tast et al. (2001b; 2001c) reported a rhythmic melatonin secretion in domestic pigs, the venous blood levels they found were substantially lower: basal levels about 1 pg/mL and peak levels 10 pg/mL.

Earlier papers attributed the differing outcomes of research on rhythmic melatonin secretion in pigs to inadequate analytic methods (Tast et al. 2001c). Andersson. 2001) (found nocturnal rise) reported some daytime peak levels, which he attributed to contamination with tissue factors due to the venapunction, causing cross reactions in the RIA assay. The method of analysis applied was the same in all groups, a radioimmunoassay, and the more recent studies all applied the commercially available RIA (Bühlmann Laboratories AG, Schönenbuch, Switserland), the way of sampling differed. But for the same way of sampling, for instance saliva collection, both outcomes (with or without rhythm) were found (this study and Mack and Unshelm. (1997)).

Bubenik et al. 1996) concluded in 1996 that melatonin peaks in domestic pigs probably originate from feeding. Additional thorough research failed to identify the main regulator of melatonin concentrations in peripheral blood (Bubenik et al. 2000).

In this study four pigs of farm A showed a peak melatonin level between 11:00 a.m. and 2:00 p.m., not obviously related to light change or feeding (individual data not shown). One pig showed a peak at the end of the afternoon around 7:30 p.m., about the time the scotophase started.

Four of the pigs of farm B showed a peak melatonin level; three pigs showed a peak at 10:00 p.m., the end of the last photophase of the day (individual data not shown). One pig showed a peak at 8:00 a.m., the end of the first photophase of the day. These peaks of the farm B pigs might be correlated to feeding, as these pigs were fed 15-25 minutes after the lights went on, at 5:45 a.m., 1:20 p.m. and 8:25 p.m.. As a causal relationship between food intake and peak levels was described (Bubenik et al. 1996), we compared the melatonin levels before and after feeding. Analysis with a non parametric test (Mann-Whitney U test) could not detect a significant correlation. However, In suprachiasmatic-lesioned rats, in other words rats without the internal master circadian clock, restricted feeding (for rats meaning 6 hrs per 24 hrs access to food) in constant dim light resulted in restored rhythmic pineal gland melatonin production, levels start rising 14 hrs after start of food access(Feillet et al. 2008). This timespan of 14 hours was also found in mice with a hypocaloric diet kept in constant light conditions (Mendoza et al. 2005). Circadian time 14 (CT14) in humans is 14 hours after wake-up, and normally also coincides with melatonin rise (DLMO).

Another incentive for these peaks might have been the internal biological clock of these pigs, entrained to the strict light – feeding regulation, anticipating lights off within an hour.

In 1991, Griffith and Minton. (1991) found elevated levels of melatonin in pigs kept under constant light, as compared with pigs kept under constant dark conditions. They concluded that for some unknown reason MEL levels in pigs kept under constant light condition responded in the opposite direction compared to the expected one. Later research of their group revealed that higher light intensity might be needed to show a "normal" day-night melatonin rhythm (Griffith and Minton. 1992), which in turn was contradicted by Tast et al. (2001b).

Due to the observation in this study that direct daylight delivers at least a tenfold of light intensity to an animal as compared with normal inside daylight, we might conclude that the domestic pig with indoor housing circumstances does not receive as much light as the wild boar living outdoors that does have an entrained circadian melatonin rhythm. Influence of feed might depend on the source of the feed; farm A and farm B differed substantially in sort of feeding but failed to show a melatonin difference there.

In conclusion, the results of the present study indicate that in cross-bred (Dutch Landrace×Yorkshire) domestic pigs: 1) high MEL levels are present throughout the day and night, 2) peak levels seem regulated by neither the photoperiod nor the alimentation process, 3) the light environment typical for a traditional farm and a large swine production farm attribute in the same extend to the absence of a "normal" variation of MEL levels.

Whether or not the lack of an entrained melatonin rhythm is an indicator for quality of live of the domestic pig is not elucidated by our results. The lack of difference between both farm management systems does not allow any conclusions about animal welfare to be drawn. The existing controversy in the melatonin production rhythm in domestic pigs might be elucidated by an experiment under controlled feeding conditions like the rat experiments of Feillet et al. (2008), as the feeding regimen might be a determinative factor.

ACKNOWLEDGEMENTS

We thank the farmers for their contribution to this study. This research would not have been possible without the support of the management of the Faculty of Veterinary Medicine Utrecht University. We thank Prof Toine Egberts for his encouragement and his comments during the conduct of the trial and the preparation of this manuscript.

REFERENCES

Andersson H (2001) Plasma melatonin levels in relation to the light-dark cycle and parental background in domestic pigs. Acta Vet Scand 42:287-94.

Bojkowski CJ, Aldhous ME, English J, Franey C, Poulton AL, Skene DJ, Arendt J (1987) Suppression of nocturnal plasma melatonin and 6-sulphatoxymelatonin by bright and dim light in man. Horm Metab Res 19:437-40.

Bollinger AL, Wilson ME, Pusateri AE, Green ML, Martin TG, Diekman MA (1997) Lack of a nocturnal rise in serum concentrations of melatonin as gilts attain puberty. J Anim Sci 75:1885-1892.

Brainard GC, Rollag MD, Hanifin JP, van den Beld G, Sanford B (2000) The effect of polarized versus nonpolarized light on melatonin regulation in humans. Photochem Photobiol 71:766-770.

Bubenik GA, Pang SF, Cockshut JR, Smith PS, Grovum LW, Friendship RM, Hacker RR (2000) Circadian variation of portal, arterial and venous blood levels of melatonin in pigs and its relationship to food intake and sleep. J Pineal Res 28:9-15.

Bubenik GA, Pang SF, Hacker RR, Smith PS (1996) Melatonin concentrations in serum and tissues of porcine gastrointestinal tract and their relationship to the intake and passage of food. J Pineal Res 21:251-256.

Diekman MA, Brandt KE, Green ML, Clapper JA, Malayer JR (1992) Lack of a nocturnal rise of serum melatonin in prepubertal gilts. Domest Anim Endocrinol 9:161-167.

Falcon J, Besseau L, Fuentes M, Sauzet S, Magnanou E, Boeuf G (2009) Structural and functional evolution of the pineal melatonin system in vertebrates. Ann N Y Acad Sci 1163:101-111. DOI: 10.1111/j.1749-6632.2009.04435.x.

Feillet CA, Mendoza J, Pevet P, Challet E (2008) Restricted feeding restores rhythmicity in the pineal gland of arrhythmic suprachiasmatic-lesioned rats. Eur J Neurosci 28:2451-2458. DOI: 10.1111/j.1460-9568.2008.06538.x.

Griffith MK, Minton JE (1992) Effect of light intensity on circadian profiles of melatonin, prolactin, ACTH, and cortisol in pigs. J Anim Sci 70:492-498.

Griffith MK, Minton JE (1991) Free-running rhythms of adrenocorticotropic hormone (ACTH), cortisol and melatonin in pigs. Domest Anim Endocrinol 8:201-208.

Hastings MH (1991) Neuroendocrine rhythms. Pharmacol Ther 50:35-71.

Mack AC, Unshelm J (1997) Circadian rhythm of the light-dependant hormone melatonin as an indicator for the photic requirement of domestic pig. In: Saloniemi H (ed) Proceedings [of the] 9th International Congress in Animal Hygiene, ISAH'97, 17 - 21 August, Helsinki, Finland. Helsingin yliopisto, Helsinki, pp 216-220.

Mendoza J, Graff C, Dardente H, Pevet P, Challet E (2005) Feeding cues alter clock gene oscillations and photic responses in the suprachiasmatic nuclei of mice exposed to a light/dark cycle. J Neurosci 25:1514-1522. DOI: 10.1523/JNEUROSCI.4397-04.2005.

Minton JE, Davis DL, Stevenson JS (1989) Contribution of the photoperiod to circadian variations in serum cortisol and melatonin in boars. Domest Anim Endocrinol 6:177-181.

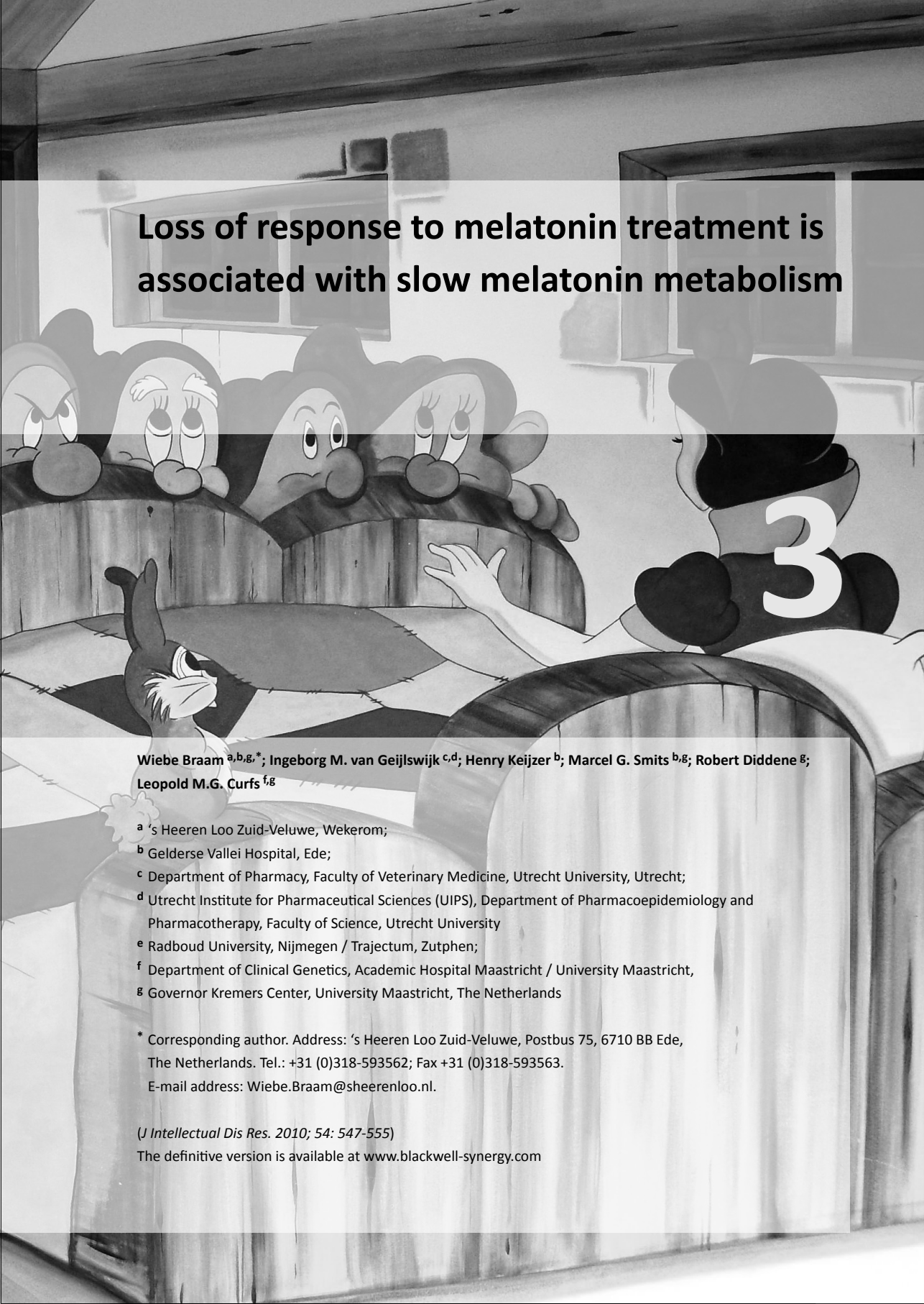
Nagtegaal E, Peeters T, Swart W, Smits M, Kerkhof G, van der Meer G (1998) Correlation between concentrations of melatonin in saliva and serum in patients with delayed sleep phase syndrome. Ther Drug Monit 20:181-3.

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. DOI: 10.1007/s00360-010-0498-2.

Tast A, Halli O, Ahlstrom S, Andersson H, Love RJ, Peltoniemi OA (2001a) Seasonal alterations in circadian melatonin rhythms of the European wild boar and domestic gilt. J Pineal Res 30:43-9.

Tast A, Love RJ, Evans G, Andersson H, Peltoniemi OA, Kennaway DJ (2001b) The photophase light intensity does not affect the scotophase melatonin response in the domestic pig. Anim Reprod Sci 65:283-90.

Tast A, Love RJ, Evans G, Telsfer S, Giles R, Nicholls P, Voultsios A, Kennaway DJ (2001c) The pattern of melatonin secretion is rhythmic in the domestic pig and responds rapidly to changes in daylength. J Pineal Res 31:294-300.



Loss of response to melatonin treatment is associated with slow melatonin metabolism

3

Wiebe Braam [a,b,g,*]; Ingeborg M. van Geijlswijk [c,d]; Henry Keijzer [b]; Marcel G. Smits [b,g]; Robert Diddene [g]; Leopold M.G. Curfs [f,g]

[a] 's Heeren Loo Zuid-Veluwe, Wekerom;
[b] Gelderse Vallei Hospital, Ede;
[c] Department of Pharmacy, Faculty of Veterinary Medicine, Utrecht University, Utrecht;
[d] Utrecht Institute for Pharmaceutical Sciences (UIPS), Department of Pharmacoepidemiology and Pharmacotherapy, Faculty of Science, Utrecht University
[e] Radboud University, Nijmegen / Trajectum, Zutphen;
[f] Department of Clinical Genetics, Academic Hospital Maastricht / University Maastricht,
[g] Governor Kremers Center, University Maastricht, The Netherlands

[*] Corresponding author. Address: 's Heeren Loo Zuid-Veluwe, Postbus 75, 6710 BB Ede, The Netherlands. Tel.: +31 (0)318-593562; Fax +31 (0)318-593563.
E-mail address: Wiebe.Braam@sheerenloo.nl.

(*J Intellectual Dis Res. 2010; 54: 547-555*)
The definitive version is available at www.blackwell-synergy.com

ABSTRACT

Background

In some of our patients with intellectual disability (ID) and sleep problems, the initial good response to melatonin disappeared within a few weeks after starting treatment, while the good response returned after considerable dose reduction. The cause for this loss of response to melatonin is yet unknown. We hypothesize that this loss of response is associated with slow metabolisation of melatonin.

Method

In this pilot study we determined melatonin clearance in two female (aged 61 and 6 years) and one male (aged 3 years) patients who had chronic insomnia, late melatonin onset and mild ID, and whose sleep quality worsened a few weeks after initial good response to melatonin treatment, suggesting melatonin tolerance. After a 3 week washout period, patients received melatonin 1.0, 0.5 or 0.1 mg respectively at 11 a.m. Salivary melatonin levels were measured just before melatonin administration, and 2 and 4 hours thereafter. After this melatonin clearance test, melatonin treatment was resumed with a considerably lower dose.

Results

In all patients melatonin concentrations remained >50 pg/ml at 2 and 4 hours after melatonin administration. After resuming melatonin treatment sleep problems disappeared. The same procedure was followed in 3 patients who did not show loss of response to melatonin after 6 months of treatment. In all patients of this control group melatonin concentrations decreased between 2 and 4 hours after melatonin administration with a mean of 76%.

Conclusion

We hypothesize that loss of response to melatonin treatment can be caused by slow metabolisation of exogenous melatonin. As melatonin is metabolised in the liver almost exclusively by cytochrome P450 enzyme CYP1A2, this slow metabolisation of melatonin is probably due to decreased activity/inducibility of CYP1A2. In patients with loss of response to melatonin, a melatonin clearance test should be considered and a considerably dose reduction is advised.

Keywords: melatonin, metabolism, tolerance, poor metabolizer, CYP1A2

INTRODUCTION

In our double-blind, placebo-controlled, parallel study in children with Angelman syndrome, some parents reported loss of response (i.e. return of night wakes) after 4 weeks of melatonin (5.0 mg ≥ 6 year and 2.5 mg < 6 year) treatment (Braam et al. 2008). Evening salivary melatonin levels were measured at baseline and after 4 weeks of treatment on a day no study medication was given. Melatonin levels in 3 of the 4 patients who received melatonin, were extremely high (>50 pg/ml) after 4 weeks of treatment, while they were very low at baseline. In response to these high levels we lowered the melatonin dose to 0.1mg, which resulted in substantially improved sleep. We postulated that these patients with loss of response to melatonin treatment, possibly as a result of enduring high melatonin levels, were poor metabolisers of CYP1A2.

From that time we have seen several patients with an intellectual disability (ID) who responded initially well on melatonin in the adequately timed dose (3-5 mg in adults and 1-2.5 mg in children aged 5-12 yr). The initial good response disappeared after a few weeks of treatment, but returned when the dose was lowered considerably (0.1 – 0.5 mg), while time of administration did not change.

Exogenous melatonin is a chronobiotic drug with some hypnotic properties (Zhdanova et al. 1997). It advances sleep onset in adults (Nagtegaal et al. 1998) and children (Smits et al. 2003; van der Heijden et al. 2007) with chronic sleep onset insomnia and late endogenous dim light melatonin onset (DLMO), and it improves sleep in patients with ID (Braam et al. 2009). Melatonin advances sleep-wake and other circadian rhythms maximally in adults when it is administered 5 to 6 hours before DLMO (Lewy et al. 1992), while soporific effects occur within 30 to 60 minutes after intake (Zhdanova. 2005).

The effective dose of melatonin still remains a matter of discussion. When the dose is too low, melatonin does not influence circadian rhythmicity. When the dose is too high melatonin does not work anymore because melatonin levels remain high and lose rhythmicity (Lewy et al. 2005). Dollins et al. (1994), in a study comparing the effects of a wide range of melatonin doses (0.1 – 10 mg), reported that the efficacy of low (0.1 – 0.3 mg) 'physiological doses' (i.e. doses resulting in serum melatonin levels within normal nocturnal range), did not significantly differ from the efficacy of pharmacological doses (1.0 – 10 mg) in promoting sleep when administered during the day to young healthy subjects. According to Zhdanova. (2005) doses to induce physiologic circulating melatonin levels (0.1 – 0.5 mg) are sufficient to promote sleep and to induce circadian phase shift, whereas too high doses may cause side effects, i.e. circadian rhythm alterations and possibly desensitise melatonin receptors.

Melatonin is metabolised in the liver almost exclusively by cytochrome P450 enzyme CYP1A2 to its main primary metabolite 6-hydroxymelatonin, than conjugated to sulphate, and excreted in urine (Claustrat et al. 2005). In most individuals exogenous melatonin has a half-life between 35 and 45 minutes (Fourtillan et al. 2000). Caffeine clearance is

considered as the gold standard for assessment of the CYP1A2 activity, because more than 90% of the primary metabolism of caffeine depends on CYP1A2 (Hartter et al. 2001; Hartter et al. 2006). However, as melatonin is metabolised more exclusively by CYP1A2, melatonin has been proposed as an alternative probe drug for CYP1A2 activity (Hartter et al. 2001).

There are large inter-individual differences in plasma levels after oral administration of melatonin as high as 37-fold, as well as a 2.5-fold difference in bioavailability of melatonin between females and males. These differences can be attributed to inter-individual variation of the first-pass effect through the liver and the activity of gastrointestinal CYP (Fourtillan et al. 2000).

Reports on loss of response, after initial good response to melatonin treatment are scarce, and in some reports the development of tolerance to melatonin was suggested, because sleep improved temporary when increasing the melatonin dose. We hypothesise that, what we call loss of response to melatonin treatment can be explained by slow metabolism of melatonin, resulting in such an increase of melatonin levels that melatonin rhythmicity disappears. Consequently melatonin loses its chronobiotic and hypnotic effects. Therefore the melatonin dose has to be reduced instead of being increased. To evaluate this hypothesis, we studied melatonin metabolism in three patients showing loss of response to melatonin treatment, and in three patients that did not show signs of loss of response after 6 months of melatonin use.

METHODS

We studied three patients with loss of response to melatonin treatment, i.e. patients who initially responded very well to melatonin therapy, but in whom this effect was lost after several weeks of therapy and sleep problems eventually became worse than before therapy started. They visited our expert centre for patients with sleep disturbances and ID.

In these patients parents and other caregivers completed daily a sleep log to assess sleep. The rate of melatonin metabolisation was assessed with a clearance test. At 11 a.m. 1 p.m. and 3 p.m. saliva samples were collected in Salivette® tubes (Sarstedt, Nümbrecht, Germany) by chewing on a cotton swab for 1-2 minutes. Immediately after the first sample collection melatonin (1mg, 0.5 mg or 0.1 mg respectively) was taken. Melatonin levels were measured in saliva with a Radio Immuno Assay (RIA) (Bühlmann laboratories, Schönenbuch, Switzerland) as previous described (Nagtegaal et al. 1998). Radioactivity was counted with the Perkin-Elmer 1470 Wizard gamma counter (Perkin-Elmer Nederland B.V., Groningen, the Netherlands). The linear range is between 0.5 and 50 pg/mL. When exceeding the linear range the samples were diluted, provided that there was sufficient sample amount.

From the time we became aware of possible slow melatonin metabolism, we perform the melatonin clearance test in all patients who are going to be treated with melatonin. Patients who did not show loss of response to melatonin after 6 months of treatment were considered normal melatonin metabolisers. Initial data from three patients, who did not show signs of loss of response to melatonin after 6 months of treatment, were used as controls in this study.

RESULTS

Case 1

Case 1 is a 61 year old woman with a mild ID, for which no cause had been identified, who was admitted to our sleep clinic because of severe sleep onset problems. She lived in a group home for over 20 years and had never been able to fall asleep before 1 a.m. In the morning however, she was sleepy when she had to get up at 8 a.m. There were no physical problems that could account for her sleep onset problems. She suffered from epilepsy which was treated by carbamazepine (800mg), lamotrigine (200mg) and phenobarbital (35mg). Despite this medication, she had a generalised epileptic attack two or three times a year on average.

DLMO occurred at 10:58 p.m. (Table 1), consistent with a delayed sleep phase syndrome. She was prescribed 5 mg melatonin at 9:00 p.m., the usual time evening medication was given by her caregivers. She was advised to go to bed at 10:00 p.m., but she refused to do so as this would result in a major change in her lifestyle. So we compromised at 11:00 p.m. and to go to bed at midnight. Two weeks later she told us that she was very satisfied with the result. She now took her melatonin at 10:00 p.m. and fell asleep before 11.00 p.m. Moreover, she told, she felt no longer sleepy the next morning.

Three months later she returned to our sleep clinic, complaining that since 4 weeks “her sleeping pill did not work anymore”. She told us that she could not fall asleep before 00:30 h and felt sleepy again in the morning. A caregiver accompanying her, informed us that she was very irritable and had mood swings at daytime.

The results of the melatonin measurements are summarised in Table 1. Melatonin levels at the end of the evening, on an evening we asked her to take no melatonin, and in the middle of the next day, were > 50 pg/ ml. The melatonin clearance test, using 1 mg melatonin, performed three weeks after stopping the melatonin treatment, showed that salivary melatonin concentration remained > 50 pg/ml during 6 hours after administration of melatonin (Table 2; Fig. 1). We concluded that she was a poor metaboliser of CYP1A2. Therefore we lowered the melatonin dose to 0.5 mg. As a result her sleep onset problems disappeared, as did her complaints about feeling sleepy in the morning. Her irritability and mood swings also had disappeared. Six months later positive effect of melatonin was still present with 0.5 mg melatonin at 10 pm.

Table 1. Melatonin in saliva before treatment start and during treatment when response to melatonin treatment disappeared

	Prior to treatment start			After loss of response		
	Case 1	Case 2	Case 3	Case 1	Case 2	Case 3
12:00 a.m.				>50	>50	>50
4:00 p.m.				>50	>50	†
6:00 p.m.		<0.5	*		>50	>50
7:00 p.m.		<0.5	*		>50	>50
8:00 p.m.		†	*		>50	>50
9:00 p.m.	2.6	2.0	*	>50	>50	>50
10:00 p.m.	1.1	8.8	*	>50	>50	†
11:00 p.m.	4.1			>50		
0:00 a.m.	5.1			>50		
1:00 a.m.	7.6			>50		
	10:58 p.m.	9:18 p.m.	*			

* No melatonin levels measured prior to start melatonin treatment.
†Not enough saliva collected for assessment melatonin levels.

Case 2

Case 2 is a 6 year old girl, who was referred to our sleep center because of sleep onset and sleep maintenance problems that existed for several years. She had a mild ID, for what a comprehensive genetic examination had not revealed a cause. She was put to bed at 8:30 p.m., and could only fall asleep when her mother was sitting at her bedside. Even then it took over one hour for her to fall asleep. One or two times every night she went to her mother's bedroom and tried to get into her bed. Her mother had to force her to go back to her own bed, and it took another hour to put her back to sleep.

At admission, DLMO occurred at 9:18 p.m. (Table 1) melatonin 2.5 mg was administered at 7:30 p.m. Four weeks after start of melatonin treatment, her mother informed us that she was very satisfied with the results. Her daughter became sleepy within half an hour after taking melatonin and fell asleep before 8:30 p.m. Only two or three nights a week she went to her mother's bed in the middle of the night, but could easily be brought back to her own bed. At daytime she played with more concentration and was not hyperactive anymore.

One month later, however, her mother complained that her daughter's sleep maintenance problems had slowly returned. She even woke up more often during the night, and earlier in the morning, than before melatonin treatment. Also, sleep latency had become longer and at daytime she again showed hyperactive behaviours. Her mother had doubled melatonin dose on her own initiative, because she thought her daughter had developed tolerance to the treatment, but this failed to have an effect on her daughter's sleep

Table 2. Melatonin clearance test in case 1, 2 and 3 and in 3 control patients.

	Melatonin clearance test					
	Case 1	Case 2	Case 3	Control 1	Control 2	Control 3
Melatonin dose (mg)	1	0.5	0.1	1	0.5	0.5
Melatonin (pg/ml) in saliva						
11:00 a.m.	5.1	5.0	3.4	2.7	0.5	0.4
1:00 p.m.	>50	>50	>50	49.1	15.3	>100
3:00 p.m.	>50	>50	>50	7.6	2.6	19.6
Melatonin half time (min)	#	#	#	44	46	<50

T½ can not be determined

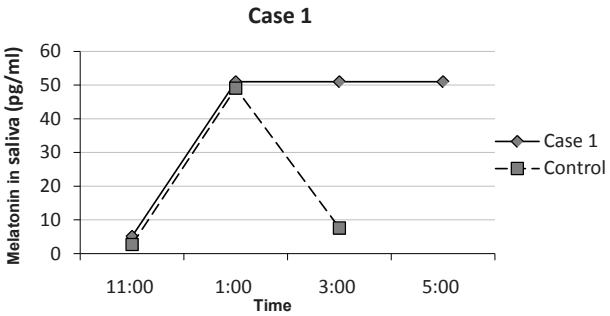


Figure 1. Melatonin clearance test in Case 1 and control. Melatonin in saliva before and after 1mg melatonin at 11:00 h

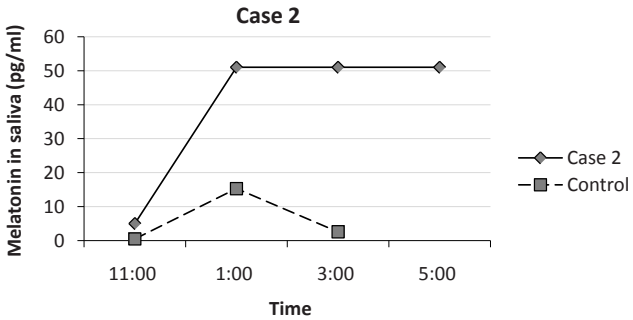


Figure 2. Melatonin clearance test in Case 2 and control. Melatonin in saliva before and after 1mg melatonin at 11:00 a.m.

problems. Salivary melatonin levels during the day and night were all > 50 pg/ml, on a day no exogenous melatonin was taken (Table 1). After a wash-out period of 2 weeks, the melatonin clearance test showed that salivary melatonin levels remained > 50 pg/ml during 6 hours after administration of melatonin 0.5 mg (Table 2; Fig. 2). Therefore we

concluded that she was poor metaboliser of CYP1A2. Melatonin treatment was resumed with 0.5 mg at 8:30 p.m. This resulted in advancement of sleep onset and better sleep maintenance. Her mother told that she had not slept as well as now since many years. She also was not hyperactive anymore. These positive effects were still present 6 months later.

Case 3

Case 3 is a 3-year old boy with Down syndrome, who attended the sleep centre because of sleep maintenance problems and early waking since one year. He also had settling problems since his mother had stopped breastfeeding when he was 6 months old. He could only fall asleep when in the arms of his mother. Because of recommendations of friends, who also had a disabled child, his mother had asked her general practitioner for a melatonin prescription. Melatonin 1 mg had an instant success. Unfortunately, frequent night wakes returned after four weeks, and the boy's parents stopped giving him melatonin. But because he did not fall asleep before 10:00 p.m., parents started to give him melatonin again, in spite of the sleep maintenance worsening. At our sleep centre we saw a hyperactive boy with Down syndrome. We advised to stop melatonin medication, and asked parents to take salivary samples. Two days after discontinuing melatonin treatment, melatonin levels were >50 pg/ml at noon as well as in the afternoon and evening (Table 1). Two weeks later, without melatonin, parents told that night wakes had disappeared, but that sleep onset problems still existed. At that time melatonin levels had returned to normal low levels. The melatonin clearance test, performed 3 days later, using 0.1mg melatonin, showed that salivary melatonin levels remained > 50 pg/ml during 6 hours after administration of melatonin (Table 2; Fig. 3). Therefore we concluded that he was poor metaboliser of CYP1A2.

Two weeks later sleep latency became longer than 2 hours and parents asked for medication. We resumed melatonin in a lower dose (0.1mg). As a result of this, sleep latency was reduced to 15 minutes or shorter. Only once a week their son woke up in the

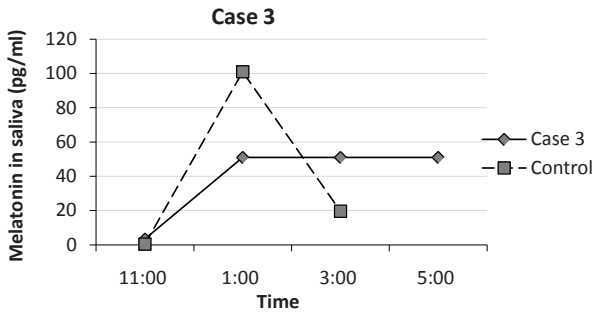


Figure 3. Melatonin clearance test in Case 3 and control. Melatonin in saliva before and after 1mg melatonin at 11:00 a.m.

middle of the night, but could be brought back to his bed easily. At follow up 6 months later, these results were still preserved.

Control subjects

Three patients that visited our sleep centre, aged 40, 12 and 8 years of age respectively, that did not show signs of loss of response to melatonin treatment after 6 months of treatment, served as control patients. Their melatonin clearance tests prior to the start of the melatonin treatment, showed a normal profile with a mean 76% decline in melatonin levels. Half-life of melatonin was 44, 46 and <50 minutes respectively (Table 2).

DISCUSSION

Melatonin metabolism was measured in three ID patients that showed a remarkable return of sleep problems after an initial about 4-weeks lasting good response to melatonin treatment. In these patients sleep improved again after lowering the melatonin dose considerably. The melatonin clearance test in these three patients showed very high levels of melatonin even 6 hours after intake of melatonin, supporting the view that exogenous melatonin was metabolised much slower than in control patients who remained good melatonin responders during at least 6 months of melatonin treatment. Therefore we concluded that these three patients are poor metabolisers of melatonin, probably due to CYP1A2 dysfunction.

For CYP1A2, metabolism of most substrates can be described using the Michaelis-Menten equation, demonstrating saturation kinetics. For some substrates in higher concentration the model seems inadequate, suggestive of a two binding sites model, either inhibitory or cooperative (Lin et al. 2001; Miller and Guengerich. 2001). Saturation will fortify the effects of exogenous melatonin in poor metabolisers. Poor metabolisers will initially experience disproportional long lasting higher serum levels after normal doses resulting in effective therapy. During the first stage of saturation they will experience disproportional increase in serum levels after a normal dose, thus still resulting in effective therapy. Finally such a high level will be reached, that a next dose won't result in an effective increase of serum level. This could be the explanation for delayed onset of these effects and the huge impact of dosage reduction. The delay in the loss of response to melatonin treatment and the effectiveness of dose reduction with restoration of rhythmicity can be attributed to the saturation phenomenon, with eventually much extended elimination half life values for melatonin.

Several reports indicate that single nucleotide polymorphisms in the CYP1A2 gene are associated with increased inducibility, decreased activity or inducibility or even loss of activity of the CYP1A2 enzyme as compared tot the wild type (Nakajima et al. 1999; Sachse et al. 1999; Chevalier et al. 2001; Zhou et al. 2009a; Zhou et al. 2009b). The (sub)

variant alleles associated with decreased of absent activity of CYP1A2 are *1C, *1K, *3, *4, *5, *6 and *7.The CYP1A2 locus is found on chromosome 15q24.1 ((National Center for Biotechnology Information.). The proportion of individuals with the slow phenotype narrowly ranges from 12 – 14% (Butler et al. 1992; Nakajima et al. 1994), but varies among ethnic populations (Zhou et al. 2009a).

As far as we know this is the first study on the association between loss of response to melatonin treatment, and slow metabolisation of melatonin. Until now, there are only a few anecdotal reports that melatonin can lose its effect during long term use, all in ID persons. McArthur and Budden. (1998), in a double-blind, placebo-controlled, crossover study in 9 children (age 4 - 17 years) with Rett syndrome, found melatonin (2.5 - 7.5 mg depending on age and body weight) to be effective in reducing sleep latency during the first 3 weeks of the study. However, this positive effect was lost in the fourth treatment week, when mean sleep latency became even worse than with placebo. Also Ishizaki et al. (1999), in a study on 50 children and young adults with ID, reported that in some cases the effectiveness of melatonin was diminished in the course of the study. Jan et al. (2000), in a study to examine effective doses of controlled-release melatonin in 42 children with chronic sleep–wake cycle disorders and severe neurodevelopmental difficulties, reported that 4 children developed tolerance to the treatment, but that this was difficult to prove because in patients with neurodevelopmental disorders other unrecognized causes of sleep disturbance can emerge, incorrectly suggesting tolerance. Andersen et al. (2008), in a retrospective study on 107 children with autism spectrum disorders that were prescribed melatonin for insomnia, found 7 cases in which sleep initially improved, but sleep problems returned, despite dose escalation. In these four studies, however, possible causes were not studied. In some reported cases of loss of response to melatonin treatment the development of tolerance to melatonin was suggested, because sleep improved temporarily when increasing the melatonin dose. Results from our pilot study, however, show that loss of response to melatonin treatment is not caused by melatonin tolerance, but by slow metabolising of melatonin. In tolerance (also called tachyphylaxis, i.e. with nitrates) there is a diminution of the response to a drug after continued use, necessitating larger doses to restore the response. Wearing-off is normally spoken used in context of end of dose phenomenon: e.g. in Parkinson's disease, prior to the next dose of levodopa. In the last hours before the next dose of levodopa, especially the kinetic symptoms of Parkinson worsen seriously. In our patients, however, a lasting melatonin effect only returned after lowering of the dose; therefore we probably best describe this observation as loss of response.

Results from this pilot study indicate that loss of response to melatonin treatment is associated with slow melatonin metabolisation. This may result in increasing daily melatonin levels. Consequently after some time this will lead to highly cumulated melatonin levels and the circadian melatonin rhythm is lost. This loss of circadian rhythm might explain why exogenous melatonin in our cases lost its effectiveness. Lewy et

al. (2002), describing a blind patient with a free-running circadian rhythm, who could be entrained to 0.5 mg of melatonin but not to 20 mg, was the first who showed that melatonin loses its chronobiotic activity when the dose is too high.

There are several other possible causes of the loss of response to melatonin treatment. When the patients do not take melatonin the treatment will not be effective. Therefore we always ask parents to be sure that their child takes melatonin indeed. The timing of melatonin administration is very important. To advance sleep-wake rhythm and consequently advance sleep onset, melatonin should be administered 5-6 hours before DLMO in adults (Lewy et al. 2002; van der Heijden et al. 2007). Melatonin advances the DLMO. When melatonin is administered close to the DLMO, DLMO cannot be advanced. Consequently melatonin loses its chronobiotic activity. In that case exogenous melatonin should be administered earlier. In case 1 melatonin was administered about 2 hours before DLMO. Therefore it cannot be ruled out that exogenous melatonin lost its effectiveness because it was administered too close to the advanced DLMO. Another explanation could be a probable desensitization of melatonin receptors during prolonged elevation of circulating melatonin to supraphysiologic levels, as was suggested earlier by Zhdanova. (2005). However, the results of the melatonin clearance test and the improvement of the sleep after considerably lowering the melatonin dose strongly suggest that the increased melatonin levels, due to the slow metabolisation of melatonin, were the main cause of the recurrence of the sleep problems in our cases

Some limitations of this pilot study need consideration. First, we only studied three patients and three controls. However our robust results can be explained by a clear well funded theory. Furthermore, the time at which melatonin was admitted needs discussion. This time was not in accordance with usual recommendations i.e. 5 hours before DLMO in adults (Pandi-Perumal et al. 2007). However in children the optimal time of melatonin administration has not yet been established. The results of the melatonin clearance test and the improvement of the sleep after considerably lowering the melatonin dose strongly suggest that the slow metabolisation of melatonin was the main cause of the recurrence of the sleep problems, and not the time of administration. And lastly our patients had mild ID.

However, to our knowledge, there are no reports on altered pharmacokinetics of melatonin in individuals with an ID.

In many cases melatonin is prescribed by non-melatonin specialised family physicians. Observations like described above will be attributed to inadequate dosing and wrongly lead to dose escalation. Experience with the relationship of medication timing with DLMO and the awareness of the complicated pharmacokinetics are required for appropriate evaluation of therapy. Therefore melatonin therapy should be initiated and initially be controlled by experienced sleep professionals. This way only we can extend effectively our knowledge of this chronobiotic, and prevent negative experiences and resulting prejudices. Melatonin is in some countries available as an over-the-counter drug. A

considerable number of people are poor metaboliser of CYP1A2 and unaware of this. These circumstances are strong arguments for the need of further research to verify our findings.

Based on the results of this pilot study we are performing melatonin clearance tests in all patients who are going to be treated with melatonin. Furthermore we study CYP1A2 polymorphisms in all these patients. When the clinically relevant slow melatonin metabolisation phenotypes can be associated with polymorphisms of the CYP1A2 gene, it will be possible to determine the optimal melatonin dose individually by genotyping CYP1A2 alone (without the melatonin challenge). We already knew that the timing of melatonin should be individually determined by the individual DLMO (van der Heijden et al. 2005; van der Heijden et al. 2007). The present study shows that also the appropriate dose probably should be individually determined, by any means for a substantial part of the population with above described polymorphisms. We expect to be able to determine CYP1A2 status in the same saliva samples as in which DLMO is measured. This makes it possible to individualize timing and dose of melatonin easily.

Pending the results of future studies on the significance of melatonin metabolisation, clinicians who treat patients with melatonin should be aware of the possibility of slow melatonin metabolisation. When it is not possible to perform a melatonin clearance test, it is strongly advised to lower the melatonin dose instead of increasing the melatonin dose, in case melatonin treatment effectiveness decreases considerably after an initially few weeks lasting good response to melatonin treatment.

ACKNOWLEDGMENTS

This study was supported by a research grant by 's Heeren Loo Zorggroep Steunfonds and Governor Kremers Center. The authors had no conflict of interest, financial nor non-financial.

REFERENCES

Andersen IM, Kaczmarska J, McGrew SG, Malow BA (2008) Melatonin for Insomnia in Children With Autism Spectrum Disorders. J Child Neurol 23:482-485.

Braam W, Smits MG, Didden R, Korzilius H, Van Geijlswijk IM, Curfs LM (2009) Exogenous melatonin for sleep problems in individuals with intellectual disability: a meta-analysis. Dev Med Child Neurol 51:340-9.

Braam W, Didden R, Smits MG, Curfs LM (2008) Melatonin for chronic insomnia in Angelman syndrome: a randomized placebo-controlled trial. J Child Neurol 23:649-54.

Butler MA, Lang NP, Young JF, Caporaso NE, Vineis P, Hayes RB, Teitel CH, Massengill JP, Lawsen MF, Kadlubar FF (1992) Determination of CYP1A2 and NAT2 phenotypes in human populations by analysis of caffeine urinary metabolites. Pharmacogenetics 2:116-127.

Chevalier D, Cauffiez C, Allorge D, Lo-Guidice JM, Lhermitte M, Lafitte JJ, Broly F (2001) Five novel natural allelic variants-951A>C, 1042G>A (D348N), 1156A>T (I386F), 1217G>A (C406Y) and 1291C>T (C431Y)-of the human CYP1A2 gene in a French Caucasian population. Hum Mutat 17:355-356.

Claustrat B, Brun J, Chazot G (2005) The basic physiology and pathophysiology of melatonin. Sleep Med Rev 9:11-24. DOI: 10.1016/j.smrv.2004.08.001.

Dollins AB, Zhdanova IV, Wurtman RJ, Lynch HJ, Deng MH (1994) Effect of inducing nocturnal serum melatonin concentrations in daytime on sleep, mood, body temperature, and performance. Proc Natl Acad Sci U S A 91:1824-8.

Fourtillan JB, Brisson AM, Gobin P, Ingrand I, Decourt JP, Girault J (2000) Bioavailability of melatonin in humans after day-time administration of D(7) melatonin. Biopharm Drug Dispos 21:15-22.

Hartter S, Korhonen T, Lundgren S, Rane A, Tolonen A, Turpeinen M, Laine K (2006) Effect of caffeine intake 12 or 24 hours prior to melatonin intake and CYP1A2*1F polymorphism on CYP1A2 phenotyping by melatonin. Basic Clin Pharmacol Toxicol 99:300-304. DOI: 10.1111/j.1742-7843.2006.pto_491.x.

Hartter S, Ursing C, Morita S, Tybring G, von Bahr C, Christensen M, Rojdmark S, Bertilsson L (2001) Orally given melatonin may serve as a probe drug for cytochrome P450 1A2 activity in vivo: a pilot study. Clin Pharmacol Ther 70:10-16. DOI: 10.1067/mcp.2001.116512.

Ishizaki A, Sugama M, Takeuchi N (1999) [Usefulness of melatonin for developmental sleep and emotional/behavior disorders--studies of melatonin trial on 50 patients with developmental disorders]. No To Hattatsu 31:428-37.

Jan JE, Hamilton D, Seward N, Fast DK, Freeman RD, Laudon M (2000) Clinical trials of controlled-release melatonin in children with sleep-wake cycle disorders. J Pineal Res 29:34-9.

Lewy AJ, Emens JS, Lefler BJ, Yuhas K, Jackman AR (2005) Melatonin entrains free-running blind people according to a physiological dose-response curve. Chronobiol Int 22:1093-106.

Lewy AJ, Emens JS, Sack RL, Hasler BP, Bernert RA (2002) Low, but not high, doses of melatonin entrained a free-running blind person with a long circadian period. Chronobiol Int 19:649-58.

Lewy AJ, Ahmed S, Jackson JM, Sack RL (1992) Melatonin shifts human circadian rhythms according to a phase-response curve. Chronobiol Int 9:380-92.

Lin Y, Lu P, Tang C, Mei Q, Sandig G, Rodrigues AD, Rushmore TH, Shou M (2001) Substrate inhibition kinetics for cytochrome P450-catalyzed reactions. Drug Metab Dispos 29:368-374.

McArthur AJ, Budden SS (1998) Sleep dysfunction in Rett syndrome: a trial of exogenous melatonin treatment. Dev Med Child Neurol 40:186-92.

Miller GP, Guengerich FP (2001) Binding and oxidation of alkyl 4-nitrophenyl ethers by rabbit cytochrome P450 1A2: evidence for two binding sites. Biochemistry 40:7262-7272.

Nagtegaal E, Peeters T, Swart W, Smits M, Kerkhof G, van der Meer G (1998) Correlation between concentrations of melatonin in saliva and serum in patients with delayed sleep phase syndrome. Ther Drug Monit 20:181-3.

Nakajima M, Yokoi T, Mizutani M, Kinoshita M, Funayama M, Kamataki T (1999) Genetic polymorphism in the 5'-flanking region of human CYP1A2 gene: effect on the CYP1A2 inducibility in humans. J Biochem 125:803-808.

Nakajima M, Yokoi T, Mizutani M, Shin S, Kadlubar FF, Kamataki T (1994) Phenotyping of CYP1A2 in Japanese population by analysis of caffeine urinary metabolites: absence of mutation prescribing the phenotype in the CYP1A2 gene. Cancer Epidemiol Biomarkers Prev 3:413-421.

National Center for Biotechnology Information *http://www.ncbi.nlm.nih.gov/gene/1544?log$=activity* :1.

Pandi-Perumal SR, Smits M, Spence W, Srinivasan V, Cardinali DP, Lowe AD, Kayumov L (2007) Dim light melatonin onset (DLMO): a tool for the analysis of circadian phase in human sleep and chronobiological disorders. Prog Neuropsychopharmacol Biol Psychiatry 31:1-11.

Sachse C, Brockmoller J, Bauer S, Roots I (1999) Functional significance of a C-->A polymorphism in intron 1 of the cytochrome P450 CYP1A2 gene tested with caffeine. Br J Clin Pharmacol 47:445-449.

Smits MG, van Stel HF, van der Heijden K, Meijer AM, Coenen AM, Kerkhof GA (2003) Melatonin improves health status and sleep in children with idiopathic chronic sleep-onset insomnia: a randomized placebo-controlled trial. J Am Acad Child Adolesc Psychiatry 42:1286-93.

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 46:233-41.

van der Heijden KB, Smits MG, van Someren EJ, Boudewijn Gunning W (2005) Prediction of melatonin efficacy by pretreatment dim light melatonin onset in children with idiopathic chronic sleep onset insomnia. J Sleep Res 14:187-94.
Zhdanova IV (2005) Melatonin as a hypnotic: pro. Sleep Med Rev 9:51-65.
Zhdanova IV, Lynch HJ, Wurtman RJ (1997) Melatonin: a sleep-promoting hormone. Sleep 20:899-907.
Zhou SF, Liu JP, Chowbay B (2009a) Polymorphism of human cytochrome P450 enzymes and its clinical impact. Drug Metab Rev 41:89-295.
Zhou SF, Yang LP, Zhou ZW, Liu YH, Chan E (2009b) Insights into the substrate specificity, inhibitors, regulation, and polymorphisms and the clinical impact of human cytochrome P450 1A2. The AAPS journal 11:481-94.

The use of exogenous melatonin in delayed sleep phase disorder: a meta-analysis

4

Ingeborg M. van Geijlswijk, PharmD [a,b]; Hubert P. L. M. Korzilius, PhD [c]; Marcel G. Smits, PhD [d]

[a] Utrecht University, Faculty of Veterinary Medicine, Pharmacy Department, The Netherlands;
[b] Utrecht Institute for Pharmaceutical Sciences (UIPS), Department of Pharmacoepidemiology and Pharmacotherapy, Faculty of Science, Utrecht University, The Netherlands;
[c] Radboud University Nijmegen, Institute for Management Research, The Netherlands;
[d] Hospital Gelderse Vallei, Department of Sleep-wake Disorders and Chronobiology, The Netherlands

Disclosure statement: This was not an industry-supported study.

(Sleep 2010; 33:1605-1614)

ABSTRACT

Study objectives: To perform a meta-analysis of the efficacy and safety of exogenous melatonin in advancing sleep-wake rhythm in patients with delayed sleep phase disorder.

Design: Meta analysis of papers indexed for PubMed, Embase, and the abstracts of sleep and chronobiologic societies (1990-2009).

Patients: Individuals with delayed sleep phase disorder.

Interventions: Administration of melatonin.

Measurements and results: A meta-analysis of data of randomized controlled trials involving individuals with delayed sleep phase disorder that were published in English, compared melatonin with placebo, and reported 1 or more of the following: endogenous melatonin onset, clock hour of sleep onset, wake-up time, sleep-onset latency, and total sleep time. The 5 trials including 91 adults and 4 trials including 226 children showed that melatonin treatment advanced mean endogenous melatonin onset by 1.18 hours (95% confidence interval [CI]: 0.89-1.48 h) and clock hour of sleep onset by 0.67 hours (95% CI: 0.45-0.89 h). Melatonin decreased sleep-onset latency by 23.27 minutes (95% CI: 4.83 -41.72 min). The wake-up time and total sleep time did not change significantly.

Conclusions: Melatonin is effective in advancing sleep-wake rhythm and endogenous melatonin rhythm in delayed sleep phase disorder.

Keywords: Melatonin, delayed sleep phase disorder, meta-analysis

INTRODUCTION

Sleep-wake timing is regulated by the biologic clock in a circadian rhythm: a rhythm consisting of approximately 24 hours. Entrainment of the biologic clock is achieved by environmental light. The endogenous rhythm of melatonin production by the pineal gland is regulated by the suprachiasmatic nucleus and is suppressed by exposure to bright light. Endogenous melatonin starts to rise in dim light (the so-called dim light melatonin onset [DLMO]), normally between 7:30 p.m. and 9:30 p.m. in adults and between 7:00 p.m. and 9:00 p.m. in children 6 to 12 years of age (Pandi-Perumal et al. 2007). This DLMO can be determined for each individual, and it characterizes the individual's circadian timing.

Delayed sleep phase disorder (DSPD) is a problem in which the circadian clock is entrained in the 24-hour rhythm but at a delayed phase angle (Weitzman et al. 1981). This can result in sleep- wake timing that is late with respect to societal norms (Pandi-Perumal et al. 2007). It has been estimated that approximately 10% of patients with chronic insomnia have DSPD (Regestein and Monk. 1995).

Treatment of DSPD relies on the use of chronotherapy or, in other words, the shifting of sleep-wake schedules (Czeisler et al. 1981) using carefully timed "morning" light administration (Rosenthal et al. 1990) to phase advance the clock and "evening" melatonin treatment to advance the clock (Sack et al. 2007). Based on the principles of chronobiology, effective treatment is entirely dependent on the correct timing of light and melatonin in relation to the circadian clock (circadian phase)(Wagner. 1996).

Some characteristic circadian clock times are wake time, defined as circadian time 0 and DLMO, which is classically defined as circadian time 14(Lewy et al. 2004), the time at which a melatonin level of 10 pg/mL is attained in the blood. This level was chosen during a period in the past when blood melatonin levels lower than 10 pg/mL could not be detected. Later, when the lower limit of quantification dropped, it was possible to measure lower melatonin levels in both blood and saliva. Salivary melatonin levels appear to correspond with 30% to 40% of the melatonin level in the blood. Consequently, salivary DLMO has been defined as the time at which 3 pg/mL or 4 pg/mL is found in the saliva. Nowadays, it is possible to measure salivary melatonin levels of 0.5 pg/mL or even lower, which has led to different definitions of DLMO (Voultsios et al. 1997; van Someren and Nagtegaal. 2007; Benloucif et al. 2008).

All of the studies in which the effects of melatonin have been observed determined the shift of DLMO after an intervention; in these studies, this measure was not influenced by the method of determination. Nevertheless, in the included studies, the traditional method has been applied.

When exogenous melatonin is administered, it functions as a chronobiotic drug with hypnotic properties (Wirz-Justice and Armstrong. 1996). Exogenous melatonin is currently under investigation as a potential treatment for DSPD (Cardinali et al. 2006). Previous studies have shown exogenous melatonin to shift the internal biologic clock (Lewy et al.

2004; Revell et al. 2005) in addition to eliciting direct soporific effects that occur mainly during the daytime when endogenous melatonin levels are low (Luboshizsky and Lavie. 1998).

The chronobiotic mechanism becomes apparent when depicting the shift of the biologic clock as a phase-response curve. Changes from baseline are plotted as an advance or delay of sleep. Studies that examine phase-response curves support the circadian-phase effectiveness of melatonin by showing a persistent effect upon the sleep profile after a washout period of 24 hours following cessation of melatonin administration (Rajaratnam et al. 2004). The cessation of melatonin therapy in adults with DSPD results in the delay of sleep onset and a return to pretreatment values within a few days to 1 year (Dahlitz et al. 1991; Dagan et al. 1998). In the children who had sleep-onset insomnia and who took melatonin, the drug holidays lasting 1 week resulted in the former sleep problem returning in more than 90% of the cases (Hoebert et al. 2009). This suggests that the chronobiotic effects of melatonin can only be sustained through continued use, although the need to advance sleep onset did disappear in 8% of the children who had received treatment (Hoebert et al. 2009) during a 4-year period.

The greatest advancement can be observed when melatonin is administered 5 hours prior to both the traditionally determined DLMO (circadian time 9) (van der Heijden et al. 2005a) and the threshold-determined DLMO (Burgess et al. 2008). Delays are registered when melatonin is administered between 6 to 15 hours after DLMO (Lewy et al. 1992; Lewy et al. 2004; Lockley. 2005; Cardinali et al. 2006; Pandi-Perumal et al. 2007). In addition to when the drug is administered, the dose of the drug may also play a role in the effectiveness of melatonin. When the dose is too low, no concrete effects will occur; when the dose is too high, the chronobiologic effects may be lost, and only the somnolent actions remain (Lewy et al. 2002; Wise. 2006). Recently, an association between time of administration and dose of exogenous melatonin in relationship to endogenous melatonin onset was demonstrated (Burgess et al. 2008). Given that melatonin has a very short elimination half-life in most individuals (between 35 and 45 minutes) (Fourtillan et al. 2000), it is quite plausible that very low doses (ie, 0.5 mg or less) administered relatively early (ie, 5 hours prior to DLMO) will have already been cleared to subphysiologic levels before endogenous melatonin onset occurs, and, hence, no shift of DLMO will be observed.

A recent meta-analysis showed melatonin treatment to be ineffective for statistically significant adaptation of several sleep parameters, such as sleep-onset latency (SOL), sleep efficiency, wakefulness, total sleep time (TST), and percentage of rapid eye movement sleep (Buscemi et al. 2006). One plausible reason for this finding, however, is that the circadian timing of the melatonin treatment was not taken into consideration. (Arendt. 2006), therefore, suggested that only those studies in which circadian timing was either measured or accurately predicted prior to treatment should be included in further analyses. In the current meta-analysis, only those studies in which the timing of melatonin treatment in relationship to the circadian clock was mentioned were analyzed.

METHODS

Data Sources

We searched in the databases PubMed and Embase and in the abstracts of sleep and chronobiologic societies that were published between January 1990 and September 2009 for randomized, placebo-controlled, double-blind, clinical trials that used melatonin in (circadian rhythm) sleep (onset) disorders. We did not include trials with melatonin agonists or other comorbidities (see online supplement for details).

Altogether, 182 articles were found. The full text for all articles thought to be potentially relevant was retrieved by 2 reviewers (the first and third authors), and additional publications were also sought.

Study Selection and Quality Assessment

All randomized controlled trials meeting the following criteria were selected for further analysis: had to involve individuals who had DSPD, whether or not they also had attention-deficit/hyperactivity disorder (ADHD); had to be reported in English; had to compare melatonin to a placebo; and had to report 1 or more of the following: DLMO, sleep onset (SOT), wake-up time (WUT), SOL (ie, amount of time between lying down to sleep and onset of sleep), and TST (ie, amount of time between SOT and WUT).

We included patients who had been diagnosed with ADHD because it is thought that part of the current ADHD epidemic might be attributed to DSPD that has either gone undiagnosed or been misdiagnosed as ADHD (Szeinberg et al. 2006). Several theories linking ADHD and DSPD, previously published by others, have been discussed by (Owens. 2009), such as a pathology that involves the prefrontal cortex, which regulates both sleep and attention or arousal and can, thus, result in shared symptoms. A compensation mechanism to daytime sleepiness (as a result of DSPD) could in turn lead to clinical manifestations thought to be ADHD. In those studies that have been conducted with both children and adults, the sleep-onset insomnia in individuals with ADHD is characterized as chronobiologic disturbances in sleep onset (van der Heijden et al. 2005b; van Veen et al. 2010).

We excluded studies other than randomized controlled trials (such as reviews and case reports), trials with other patient groups or other indications (eg, trials in dementia, trials or focused on epileptic patients or children with neurodevelopmental disorders other than ADHD, and trials for the treatment of jetlag or nightshift effects), trials with other treatment or combination of treatments (eg, a melatonin agonist such as ramelteon, light therapy), and studies using other outcomes (such as the quality of sleep or life, biochemical measurements). Nine papers remained (Figure 1).

Two reviewers assessed the methodologic quality of these 9 studies independently by using the Jadad scale (Jadad et al. 1996) (quick scan) and the Downs and Black checklist (Downs and Black. 1998). The concealment of treatment allocation was assessed by using

the criteria of Schulz et al (Schulz et al. 1995). Papers with a Jadad scale result of 3 or more were judged as suitable for this meta-analysis. The result of the more extensive Downs and Black list is depicted in Table 1 (quality assessment score). The discussion of what should be done with the results of quality assessment still remains open (Kunz and Oxman. 1998; Juni et al. 1999; Verhagen et al. 2002).

Data Extraction

For this study, we extracted from each paper the number of patients in the placebo and in the melatonin groups (for the crossover studies this means seemingly the doubling of

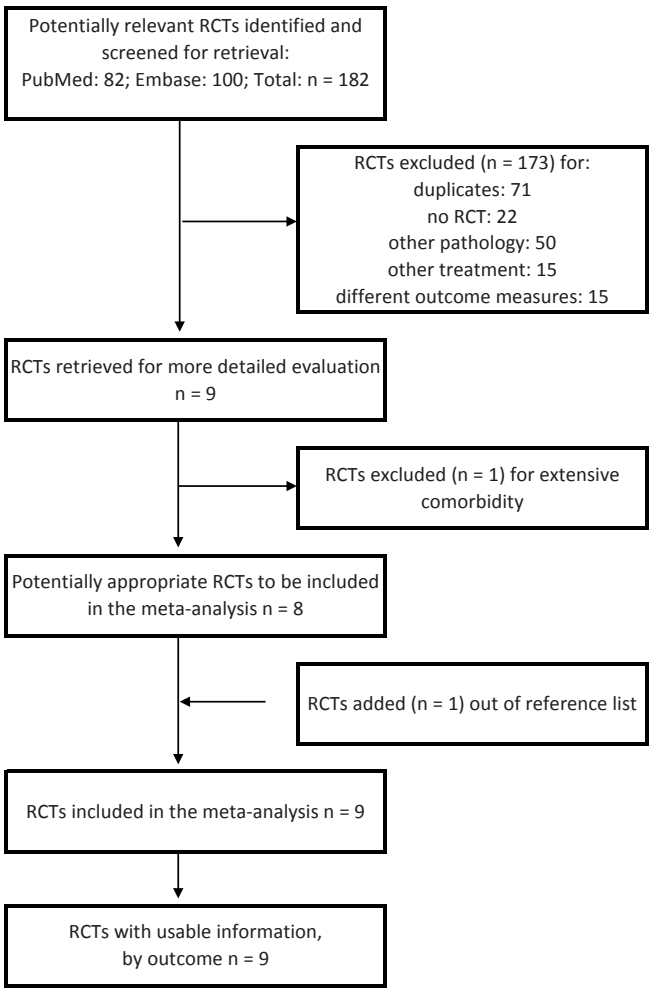


 Figure 1. Study selection

patients included in the study), the method of data collection (actigraphy, polysomnography or diary [also called somnolog or sleeplog]), the baseline DLMO (if available), study design, melatonin dose, duration of melatonin treatment, time of medication administration, and the available outcome measures after treatment (endpoints): DLMO, SOT, WUT, SOL, and TST. Extraction was checked by other authors.

For calculation reasons, clock times (applicable to DLMO, SOT, and WUT) were transformed to hour fractions, and, for clock times after 00:00, 24 was added; time spans (SOL and TST) were transformed to the number of minutes. The DLMO, SOT, and WUT results are therefore expressed in decimal hours, and SOL and TST results are expressed in minutes.

This meta-analysis was calculated with reported endpoints for melatonin treatment and placebo treatment for both crossover and parallel studies. This approach is different from the outcome measures in most of the original publications. All of the parallel and several of the crossover studies used the change of a parameter from baseline to treatment as the outcome measures for melatonin and placebo interventions. This subject will be further elucidated in the discussion section.

Data Analysis

Because all of the studies compared melatonin with placebo, we considered our study to be a direct head-to-head comparison of 2 treatments (placebo vs melatonin).

The meta-analysis was performed using the free software program MIX (Meta-analysis with Interactive eXplanations, version 1.7 (Bax et al. 2006; Bax et al. 2008)). The descriptive method was used; the input was the results calculated for the DLMO, SOT, WUT, SOL, and TST, as described above. All of these measures could therefore be defined as continuous (noted by decimal hours or minutes), by utilizing the random-effects method. To make the adjustment correct for the variance in the number of participants, we used the inverse variance method (Sutton et al. 2000). The α level was set at .05 for each outcome.

We assessed, per parameter, the overall mean difference, the 95% confidence interval (CI), and the z score. First, those studies that had been conducted with adults (Dahlitz et al. 1991; Laurant et al. 1997; Nagtegaal et al. 1998; Kayumov et al. 2001; Mundey et al. 2005) and with children (Smits et al. 2001; Smits et al. 2003; Weiss et al. 2006; van der Heijden et al. 2007) were separately assessed, whereas the crossover and parallel studies were done simultaneously. Second, studies were assessed according to the data collection (polysomnography, diary or actigraphy), and they were combined once again for the 2 types of studies (crossover and parallel), irrespective of age.

We present the results in standard forest plots containing the mean differences, the 95% CI, the weight of each study, and the pooled analysis. SPSS 15.0 (SPSS inc. 2006) was used for analyzing the DLMO changes in relationship to to the time of melatonin administration. Linear analysis of variance regression and curve fit were applied.

Table 1—Characteristics of placebo-controlled studies using melatonin in delayed sleep phase disorder

Author, year of publication	QA score	n	Baseline DLMO level, h:min[a]	Study design	Melatonin dose, mg	Duration, wks
Adults						
Dahlitz et al (1991)	25	8	—	X	5	4
Laurant et al (1997)	26	25	22:35 (0:54)	X	5	2
Nagtegaal et al (1998)	28.5	25	23:17 (2:18)	X	5	2
Kayumov et al (2001)	25	22	—	X	5	4
Mundey et al (2005)	19	11	23:46 (1:62)	P	0.3/3	4
Children						
Smits et al (2001)	28	40	21:06 (1:16)	P	5	4
Smits et al (2003)	29.5	62	20:48 (0:59)	P	5	4
Weiss et al (2006)	30	19[b]	-	X	5	10 days
van der Heijden et al (2007)	31	105[b]	20:34 (0:55)	P	3/6	4

[a]Data are presented as mean (SD).
[b]Includes children with attention-deficit/hyperactivity disorder.

Abbreviations: QA, quality assessment; X refers to crossover studies; P, parallel-group studies;

RESULTS

Study Characteristics

In total, 9 placebo-controlled studies on melatonin in DSPD met our inclusion criteria, 5 of which involved 91 adults (Dahlitz et al. 1991; Laurant et al. 1997; Nagtegaal et al. 1998; Kayumov et al. 2001; Mundey et al. 2005) and 4 of which involved 226 children, aged 6 years to adolescence (Smits et al. 2001; Smits et al. 2003; Weiss et al. 2006; van der Heijden et al. 2007) (Table 1). The mean quality score was 4.0 out of 5 (range 3-5) based on the Jadad scale and 26 out of 32 (range 19-31) on the Downs and Black checklist. Concealment of allocation was adequate in all 9 studies. A funding source was described in 7 of the studies in which funding was received from public sponsors.

Four studies in adults and 1 stud (in children were crossover studies. In 2 crossover studies in adults, the participants received the trial medication during 2 subsequent periods of 4 weeks, separated by a 1-week washout period (Dahlitz et al. 1991; Kayumov et al. 2001). The other 2 crossover studies in adults supplied the trial medication during 2 subsequent

Table 1—

Time of melatonin administration	Measures and method of data collection					Author, year of publication
	DLMO	SOT	SOL	TST	WUT	
						Adults
22:00 h		LOG	PSG	LOG	LOG	Dahlitz et al (1991)
5 h ā DLMO (mean 17:35)	PL/SAL	LOG/ACT				Laurant et al (1997)
5 h ā DLMO (mean 18:17)	PL	ACT	PSG			Nagtegaal et al (1998)
19:00-22:00			PSG	PSG		Kayumov et al (2001)
1.5-6.5 h ā DLMO 15.00-21.30 (mean 17:15)	SAL	ACT	ACT	ACT	ACT	Mundey et al (2005)
						Children
18:00	SAL	LOG/ACT	LOG	LOG	LOG	Smits et al (2001)
19:00	SAL	LOG	LOG		LOG	Smits et al (2003)
20 min ā bedtime			LOG			Weiss et al (2006)
19:00	SAL	ACT	ACT	ACT	ACT	van der Heijden et al (2007)

Abbreviations: DLMO, dim-light melatonin onset; SOT, sleep-onset time; SOL, Sleep-onset latency; TST, total sleep time; WUT, Wake-up time;
SAL, saliva; PL, plasma; LOG, diary; ACT, actigraphy; PSG, polysomnography.

periods of 2 weeks, which were not separated by a washout period (Nagtegaal et al. 1998) or the washout period was for only 1 day (Laurant et al. 1997); in the crossover trial in children, the participants received melatonin for 2 ten-day periods, separated by 5 days of washout(Weiss et al. 2006). The remaining study in adults (Mundey et al. 2005) and the 3 studies in children were parallel-group studies, and the duration of the treatment in all of the parallel-group studies was 4 weeks.

Dose

In 7 of the studies, a 5-mg dose of melatonin was administered. One study in adults compared the efficacy of 0.3 mg of melatonin with the efficacy of 3 mg of melatonin and with placebo (Mundey et al. 2005). One study in children differentiated the dose according to bodyweight: children weighing less than 40 kg received 3 mg, those weighing 40 kg or more received 6 mg (van der Heijden et al. 2007). In this meta-analysis, the dose was not taken into account.

MEASUREMENTS

Dim-Light Melatonin Onset

DLMO was determined by measuring the melatonin in plasma in 2 studies (Laurant et al. 1997; Nagtegaal et al. 1998), in which 1 of these (Laurant et al. 1997) combined plasma samples with saliva samples; 4 studies exclusively used saliva for DLMO determination. Two studies evaluated endogenous melatonin production using an analysis of melatonin metabolites in urine (Dahlitz et al. 1991; Kayumov et al. 2001), 1 in combination with a 24-hour melatonin curve in plasma (Dahlitz et al. 1991). All of the baseline DLMOs are depicted in Table 1.

Melatonin significantly advanced DLMO: 1.69 hours in adults and 1.13 hours in children (Table 2); overall, 1.18 hours (Figure 2).

Sleep-onset Time

Three studies measured SOT using actigraphy, and 2 studies used data collected from a diary. Two studies applied both methods to assess SOT; the results of both measurements are used in this meta-analysis. Melatonin significantly advanced SOT: 0.70 hours in adults, and 0.64 hours in children (Table 2). Overall, an advance of 0.67 hours was found (Figure 3). The SOT outcomes assessed by actigraphy and diary were both statistically significant

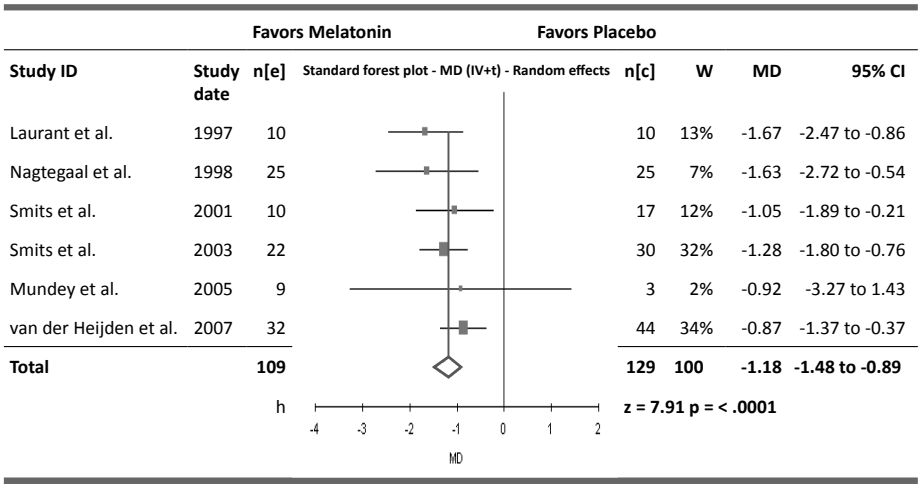
Table 2—Study outcomes, differentiated between studies with adults(Dahlitz et al. 1991; Laurant et al. 1997; Nagtegaal et al. 1998; Kayumov et al. 2001; Mundey et al. 2005) and those with children (Smits et al. 2001; Smits et al. 2003; Weiss et al. 2006; van der Heijden et al. 2007) with delayed sleep phase disorder

Outcome variable	Adults			Children		
	Studies, no./ participants, no.	Mean difference (95% CI)	z score	Studies, no./ participants, no.	Mean difference (95% CI)	z score
DLMO	3/82	-1.69 h (-2.31 to -1.07)	5.34[a]	3/155	-1.13 h (-1.47 to -0.80)	6.62[a]
SOT	5/111	-0.70 h (-1.04 to -0.36)	4.08[a]	4/193	-0.64 h (-0.93 to -0.36)	4.42[a]
WUT	2/27	-0.95 h (-3,25 to 1.36)	0.8[c]	3/168	-0.16 h (-0.33 to 0.02)	1.76[c]
SOL	4/111	-30.28 min (-63.29 to 2.74)	1.80[c]	4/206	-16.04 min (-23.77 to -8.32)	4.07[a]
TST	3/67	0.77 min (-33.87 to 35.42)	0.04[c]	3/168	28.39 min (13.06 to 43.72)	3.36[b]

Abbreviations: DLMO refers to dim-light melatonin onset; SOT, sleep-onset time; WUT, wake-up time; SOL, sleep-onset latency; TST, total sleep time.

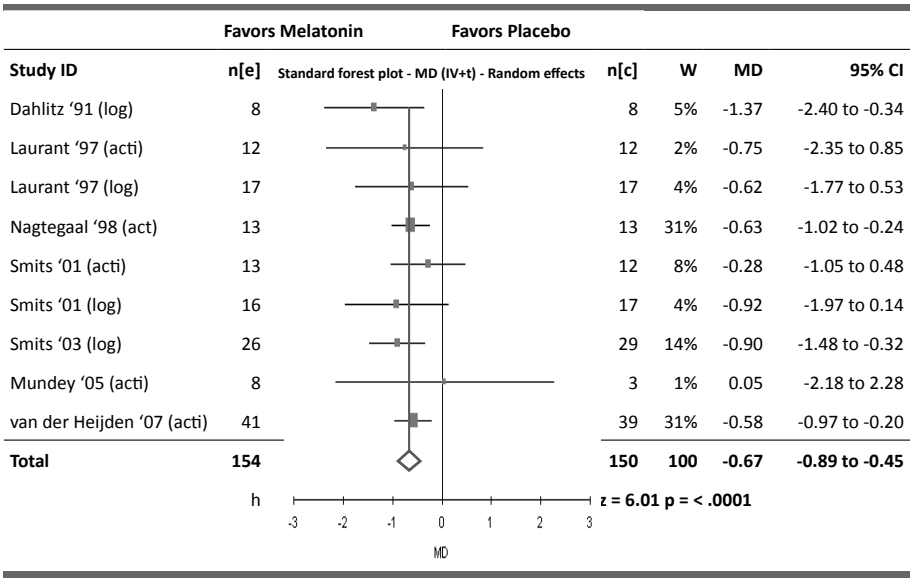
[a]P < .0001

[b]P < .001. [c]Not significant



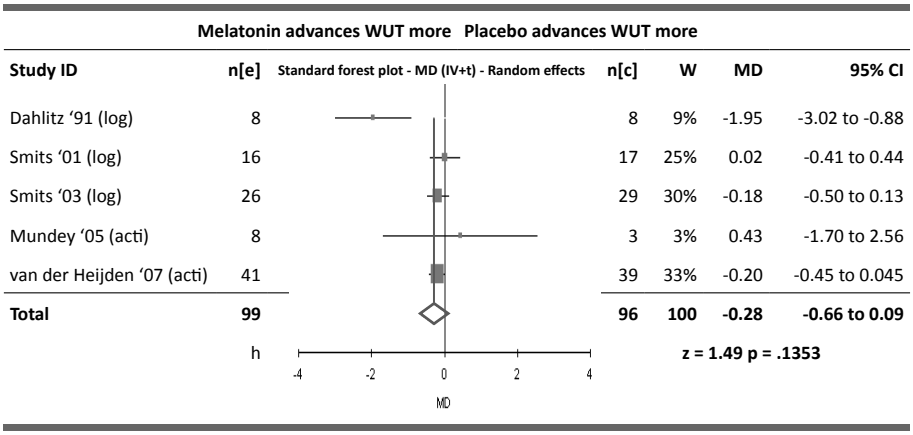
MD: Mean difference (h). IV: inverse variance. t: estimate of the between study variance where the weight (W) given to each study is calculated by the inverse sum of the within study and between study variance estimates.

Figure 2. Dim Light Melatonin Onset in patients with Delayed Sleep Phase Disorder.



MD: Mean difference. IV: inverse variance. t: estimate of the between study variance where the weight (W) given to each study is calculated by the inverse sum of the within study and between study variance estimates

Figure 3. Clock hour of sleep onset in patients with Delayed Sleep Phase Disorder.



MD: Mean difference. IV: inverse variance. t: estimate of the between study variance where the weight (W) given to each study is calculated by the inverse sum of the within study and between study variance estimates.

Figure 4. Wake up time in patients with Delayed Sleep Phase Disorder.

but differed clinically: 0.57 hours for actigraphy versus 0.94 hours using sleep-diary data (Table 3).

Wake-up Time

Two studies measured WUT by using actigraphy; 3 studies used data collected from a diary. Mean WUT was advanced in both adults (0.95 h) and children (0.16 h), but both

Table 3—Study outcomes, differentiated based on measures obtained with sleep diaries and with actigraphy of patients with delayed sleep phase disorder

Outcome variable	Diary			Actigraphy		
	Studies, no./ participants, no.	Mean difference (95% CI)	z score	Studies, no./ participants, no.	Mean difference (95% CI)	z score
SOT	4 / 138	-0.94 h (-1.37 to -0.52)	4.39[a]	5 / 166	-0.57 h (-0.82 to -0.31)	4.37[a]
SOL	3 / 126	-14.75 min (-24.21 to -5.29)	3.06[b]	2 / 91	-11.05 min (-26.93 to 4.83)	1.36[c]
TST	3 / 104	12.98 min (-32.98 to 58.94)	0.553[c]	2 / 91	17.25 min (-0.602 to 35.10)	1.89[c]

Abbreviations: SOT refers to sleep-onset time; SOL, sleep-onset latency; TST, total sleep time.
[a]$p < .0001$; [b]$p < .001$; [c]Not significant

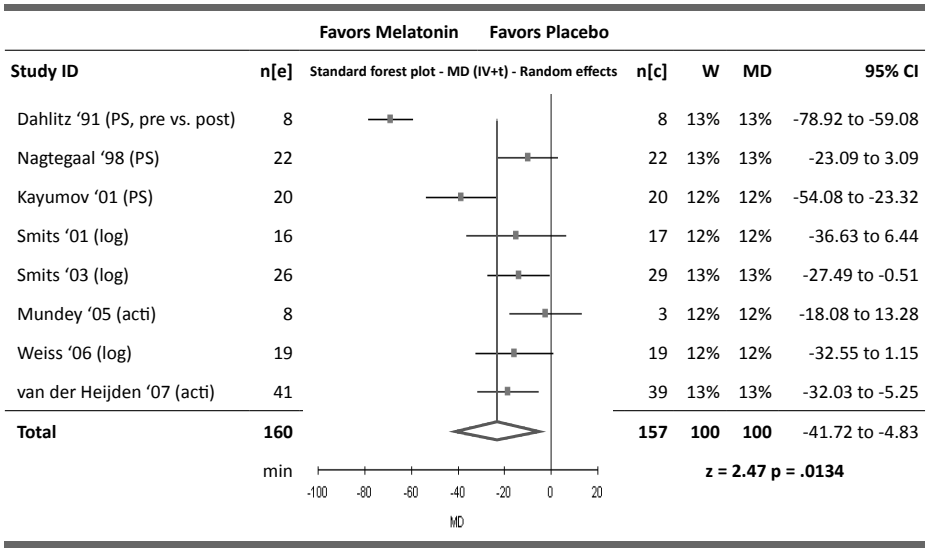
were found to be statistically insignificant (Table 2). Overall, an insignificant advance of 0.28 hours was found (Figure 4).

Sleep-onset Latency

Three studies measured SOL using polysomnography, 3 studies used data collected from a diary, and 2 studies measured SOL by using actigraphy. The reduction in SOL with melatonin was statistically significant in children (by 16.04 min) but insignificant in adults (by 30.28 min) (Table 2). Overall, a statistically significant reduction of 23.27 minutes was found when data from adults and children were combined (Figure 5). When measured with data from only sleep diaries, the reduction in SOL was statistically significant (a reduction of 14.75 min); with actigraphy, a statistically insignificant reduction of 11.05 minutes was found (Table 3).

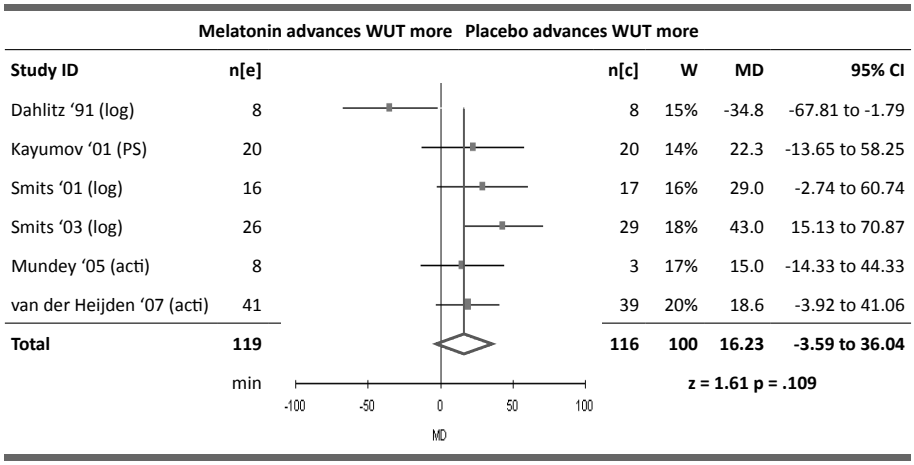
Total Sleep Time

One study measured TST with polysomnography, 2 studies used data collected from a diary, and 2 studies determined TST by actigraphy. Melatonin prolonged TST statistically significantly in children (by 28.39 min) but insignificantly (statistically and clinically) in adults (by 0.77 min) (Table 2). Overall, a statistically significant prolongation of TST of



MD: Mean difference. IV: inverse variance. t: estimate of the between study variance where the weight (W) given to each study is calculated by the inverse sum of the within study and between study variance estimates

Figure 5. Sleep onset latency in patients with Delayed Sleep Phase Disorder.



MD: Mean difference. IV: inverse variance. t: estimate of the between study variance where the weight (W) given to each study is calculated by the inverse sum of the within study and between study variance estimates.

Figure 6. Total sleep time in patients with Delayed Sleep Phase Disorder.

16.23 min was found (Figure 6). The TST outcomes of diary collection alone (by 12.98 min) or actigraphy alone (by 17.25 min) were all statistically insignificant (Table 3).

Safety and Adverse Events

Four studies did not report any adverse events (Laurant et al. 1997; Nagtegaal et al. 1998; Kayumov et al. 2001; Mundey et al. 2005). Headaches were reported in all 5 of the studies that reported patients experiencing adverse events: 2 out of 20 patients in the melatonin-treatment group had headaches (Smits et al. 2001); 3 out of 53 in the melatonin-treatment group, irrespective of dose (van der Heijden et al. 2007); 1 out of 8 participants (Dahlitz et al. 1991) and 1 with severe migraine (treatment not specified) out of 19 (Weiss et al. 2006). One study reported headaches exclusively in the placebo group (Smits et al. 2003). In general, 6% or 7% of subjects reported having headaches during melatonin treatment. Other adverse events during melatonin treatment, and not during placebo treatment, were feeling cold (8 out of 50 (Wasdell et al. 2008) and unspecified numbers (Smits et al. 2003)), a mood dip (numbers not specified) (Smits et al. 2003), and a decrease in appetite (numbers not specified) (Smits et al. 2003). Dizziness during melatonin treatment was reported in 2 studies: 2 out of 53 participants (van der Heijden et al. 2007) and not quantified (Smits et al. 2003).

In 1 patient, melatonin treatment was associated with an incidence of elevated alkaline phosphatase levels, although the elevation was almost reversed after 20 weeks of continued melatonin treatment (Dahlitz et al. 1991). One patient developed a mild case

of generalized epilepsy 4 months later after having started melatonin treatment and was treated with valproate (Smits et al. 2001).

Timing of Melatonin Administration in Relationship to the Effect Size

We analyzed the relationship between the mean time of medication administration and the mean difference in after-treatment DLMO between melatonin and placebo groups. Due to the very limited amount of data available from our studies (n = 5, we excluded the study of (Mundey et al. 2005) because of the very broad range of timing and the very small group size of placebo), the hypothesis that early administration enhances the DLMO shift properties of melatonin could not be statistically confirmed (P = 0.307). The resulting β coefficient of .578, representing the correlation coefficient for 2 variables, suggests that significance can be obtained by using an n of 12; indeed, more studies are needed.

When the pretreatment DLMO values were taken into account, as was the difference between the pretreatment and the posttreatment DLMO, the DLMO value (the DLMO shift) of the melatonin and placebo groups were evaluated in relation to the time of administration in all 6 studies, whereby the correlation coefficient increased to .614 but remained statistically insignificant (P = .065).

DISCUSSION

This meta-analysis concerns the effectiveness of exogenous melatonin in patients with DSPD, and it demonstrates that appropriately timed administration of exogenous melatonin does advance endogenous melatonin onset (DLMO) and sleep onset in both children and adults. Although melatonin decreased SOL in children, this was not the case in adults. Melatonin did extend sleep duration (TST) in children, but this was also not the case in adults. Finally, the WUT was not influenced.

Half of the studies assessed children in this review. We found differences between adults and children in melatonin efficacy in 2 measures: SOL and TST. This can be partly attributed to the disciplinary set routine in which children are brought to bed, whereas adults are more at liberty to choose their own bedtime, and, as a result, probably experience fewer problems regarding SOL and TST. The efficacy in children influenced the overall efficacy in this meta-analysis on both parameters.

Several methods of measurement were used to determine endpoints: polysomnography, actigraphy, and diary. Recently, Werner et al reported rates of agreement among actigraphy, a diary, and a questionnaire for recording sleep patterns in children (Werner et al. 2008). The method chosen did not significantly influence the results in regard to the SOL and TST. Sleep-onset results, however, did differ: sleep-onset amelioration was much better when it was recorded in a diary than with actigraphy. This might be due to the direct hypnotic effects of melatonin, noticeable during low endogenous melatonin

levels, and which can influence the subjective sense of being awake, while not influencing the more objective actigraphic measurement. Nevertheless, it reflects a positive feeling experienced by participants when falling asleep.

The use of melatonin was safe with respect to experienced adverse events in the treatment of DSPD, at least in the short-term treatment. This finding corresponds with the results of other studies that used melatonin (Buscemi et al. 2006). In a recently published evaluation of clinical experience in which melatonin treatment was prescribed by a pediatrician for 107 children (Andersen et al. 2008), parents reported, after 1.8 ± 1.4 years of treatment, on the adverse effects found in 3 children: morning sleepiness, "fogginess," and increased enuresis. There was no increase in the number of seizures that occurred in children with preexisting epilepsy nor were there any onset seizures (Andersen et al. 2008).

The evidence found in advancing the sleep-wake rhythm is contrary to the findings of Buscemi et al. (2006), who could not demonstrate the efficacy of melatonin. The present study included approximately the same number of studies (9) and participants (317) as the meta-analysis of Buscemi et al. (2006) (9 and 297, respectively). Consequently, the number of studies and participants does not account for the difference between our findings and those of Buscemi et al. (2006). This difference in findings might be attributed to the properties of melatonin. In our studies, we focused on the chronobiotic properties of melatonin (Wirz-Justice and Armstrong. 1996), which become evident when melatonin is administered only a few hours before endogenous melatonin onset. This chronobiotic effect was evidently expressed by the more than 1-hour advancement of endogenous melatonin onset and the advancement of the sleep-wake rhythm.

In the studies of Buscemi et al. (2006), the time of administration was not taken into account. When there is a short interval between the administration of melatonin and the endogenous melatonin onset, the advancement of biologic clock can be expected to be statistically and clinically insignificant. Therefore, they may have assessed the hypnotic properties of melatonin, which might even have been absent in some studies, if melatonin was administered after the onset of endogenous melatonin.

Another reason for suboptimal effects of melatonin treatment can also be found in the close relationship between the dose, the timing, and the resulting melatonin levels found in the blood. When too high a dose is administered and when it is given too late with respect to endogenous melatonin onset, this might result in melatonin levels persisting through the early morning. This could even result in delaying DLMO instead of advancing it! If these thoughts are taken into account, one might even theorize about enhancing the efficacy of melatonin treatment by advancing the administration of melatonin every few days to prolong the advancing effect until the optimal rhythm has been reached. This is comparable to the strategy that Lewy et al. (2004) applied in the entrainment of the blind.

A methodologic difference between our study and the meta-analysis of Buscemi et al. (2006) is that Buscemi and coworkers used endpoints for the crossover studies with their

own standard deviation (SD) and the changes from baseline for parallel studies with calculated SDs, when necessary.

The diagnosis DSPD remains a point of discussion. The clinical symptoms described as an "inability to fall asleep at conventional times" and in experiencing "difficulty in waking at conventional times in the morning" have not been precisely defined. The DLMO assessment might be helpful in diagnosing DSPD, as DLMO occurs late in this circadian-rhythm disorder. The American Academy of Sleep Medicine (Sack et al. 2007) did not adopt DLMO assessment as a diagnostic tool because this measurement had probably not yet become widely available. In addition, a delayed DLMO, as such, could result from several other conditions, such as shift work or traveling. Furthermore, in healthy subjects, melatonin levels and melatonin onset vary with age (Zhou et al. 2003), and DLMO can occur several hours apart among individuals who do not have any underlying pathology.

However, after a diagnosis of DSPD is made, assessment of the DLMO may be useful when evaluating the effects of initiated therapy. Furthermore, by establishing the most desirable timing for administering melatonin when considering the applied dose, DLMO assessment can also serve as a parameter for predicting the efficacy of a treatment using melatonin, thus preventing initiation in patients without anticipating any benefits.

The definition of DLMO is currently being discussed, as mentioned earlier (Benloucif et al. 2008). Consequently, a task force of the European Sleep Research Society was set up in 2006 to redefine DLMO. Once DLMO has been defined more precisely, it will be possible to agree on generally accepted standards.

A well-defined DLMO will not only help in diagnosing DSPD, but also will enable treatment to be more customized. A study in adults (Mundey et al. 2005) confirmed the finding of Lewy et al. (1992) that melatonin shifts the circadian rhythm the most when administered 5 to 6 hours before DLMO. Furthermore, a study in children (van der Heijden et al. 2005a) showed that the earlier melatonin was administered with respect to DLMO, the more both SOT and the DLMO were advanced. This study applied high doses. The current meta-analysis, which consists of studies in which similarly high dosages were administered, cannot confirm this finding, but an analysis made of the DLMO shift suggests that the DLMO shift is enhanced when administered earlier.

This is the first meta-analysis of melatonin studies in which circadian timing was measured or accurately predicted before treatment. The quality of the studies was thoroughly assessed. Nevertheless, several limitations still need to be considered. The number of studies conducted in adults, as well as the number of participants, was rather low. Moreover, most of the studies conducted in adults were crossover studies. A carry-over effect in these studies cannot be excluded. However, most of the findings obtained from these studies correspond with those of the parallel-group studies carried out in children. In the studies of (Smits et al. 2001; Smits et al. 2003), ADHD was diagnosed in 25% to 50% of the children, whereas, in the other 2 studies in children, ADHD was diagnosed in 100% of the participants. These individuals could fall under the category of circadian rhythm

sleep disorder co-morbid with ADHD. The separate DLMO and SOT results in children and adults were significant (Table 2). In children, the advancement of DLMO and SOT was less than in adults, which might be caused by the fact that the children represent a different clinical population than do the adults, who have a typical DSPD.

Because of the larger number of participants in the studies of children, the SDs were also lower, which therefore means that the children's groups contributed to a generally greater significant result for both DLMO and SOT (Figure 2 and Figure 4). For SOL, the general significant result (Figure 5) did not persist when data from children were excluded, as illustrated in Table 2 in the results of adults. Finally, 3 out of the 4 studies in children were performed by the same research group. This might indicate a sampling bias.

Only 3 studies used the gold standard, polysomnography, to establish the effects that melatonin had on sleep. However, the other methods, ie, actigraphy and keeping a sleep diary, are reliable methods for establishing shifts in sleep-wake rhythm (Littner et al. 2003).

This meta-analysis employed reported endpoints for melatonin treatment and placebo treatment. This differs from the original work conducted in all of the parallel and some of the crossover studies, in which the change of a parameter from baseline to treatment was used as the outcome measures for both melatonin and placebo interventions. For crossover studies, one can postulate that baseline corrections are not needed, since the same population underwent both treatments.

For the parallel studies, on the other hand, by reason of a presumed heterogeneity between both groups, it is common to depict the outcomes as changes from baseline. In reality, when pretreatment and posttreatment group results are used to determine this change instead of individual values, 2 SD values (SD of the pretreatment and posttreatment values in children) will determine the SD for the resulting change value.

In 1 paper, this value was given (Mundey et al. 2005); however, for the other 3 parallel studies, this value still needed to be calculated. This latter extensive method is comparable to the method of "adjusted indirect comparison of two different treatments versus placebo"(Gartlehner and Moore. 2008). It assumes heterogeneity between study populations. If one should decide to compute the changes according to the extensive method, then one should also perform the same calculations for the resulting SD of the calculated difference for the crossover studies, since the baseline values of those studies are also characterized by a SD. For all of the crossover studies, this value needs to be calculated.

Although it is possible to compute these SDs by making a few assumptions, we decided it would be more straightforward to use all of the endpoints from the studies included, since these were all available, including the SD, assuming there is homogeneity in the treatment groups of the parallel studies. Therefore, our approach relies more on published data and depends less on calculations that inevitably introduce measurement error. However, we also analyzed the results using the extensive method and subsequently compared them

to our method. We found no differences for the pooled DLMO result and only a small, but insignificant, difference in the pooled results of SOT and SOL. Nevertheless, the 2 methods showed different results regarding the effect of several studies on TST and WUT (although the significance of the pooled results of both methods was the same). This outcome was mainly attributable to very large baseline differences in 1 study using only 3 placebo patients (Mundey et al. 2005).

Another limitation was that the influence of melatonin on the daily health status was not assessed. More studies are needed to confirm the findings of the placebo-controlled trial in children (Smits et al. 2001) and the open-label study in adults (Nagtegaal et al. 2000), thus suggesting that melatonin improves the quality of life.

The relatively small change in clock-hour sleep onset of .67 hours could raise questions about its clinical significance. However, the general treatment effect is limited by several factors. Firstly, the optimal dose has not yet been established, and some of the previous studies applied high doses. Secondly, the optimal timing of the administration was not applied in most of the trials, nor was it adapted (further advancing the administration time) during therapy.

We suggest that an optimal melatonin therapy be based on 3 basic principles, namely, by identifying the appropriate patients (with a delayed biologic timing); by melatonin administration being based on biologic clock time, ie, 3 to 6 hours before DLMO (CT8-11); and by administering a small dose to avoid enhanced high melatonin levels during late night or early morning. To further optimize individual therapy, the results in which the administration time is advanced during the course of treatment should also be considered.

Currently, our findings can help to guide clinicians and patients in making decisions regarding the use of exogenous melatonin in the treatment of DSPD. The main conclusion we have been able to draw from our study is that, in cases in which a dose of melatonin has been administered in DSPD, it has been found to be effective, and that this dose should be kept as low as possible and administered as early as tolerable. Further research concerning the optimal timing of when to administer the dose in regard to DLMO shift would be warranted.

ACKNOWLEDGEMENTS

All of the authors contributed equally to this manuscript. No sponsorship was received, and no other outside source had any role in the design of the study or collection, management, or interpretation of the data, nor was there any external involvement in the decision to submit the manuscript for publication.

Ingeborg M. van Geijlswijk had full access to all of the data provided in the study, and she assumes responsibility for the integrity of the data and the accuracy of the data analysis.

We thank S.R. Pandi-Perumal and A.C.G Egberts for their helpful discussions and for critically reading the manuscript.

REFERENCES

Andersen IM, Kaczmarska J, McGrew SG, Malow BA (2008) Melatonin for Insomnia in Children With Autism Spectrum Disorders. J Child Neurol 23:482-485.

Arendt J (2006) Does melatonin improve sleep? Efficacy of melatonin. BMJ 332:550.

Bax L, Yu LM, Ikeda N, Tsuruta H, Moons KGM (2008) MIX: comprehensive free software for meta-analysis of causal research data. Version 1.7. *http://www.meta-analysis-made-easy.com/*.

Bax L, Yu LM, Ikeda N, Tsuruta H, Moons KG (2006) Development and validation of MIX: comprehensive free software for meta-analysis of causal research data. BMC Med Res Methodol 6:50.

Benloucif S, Burgess HJ, Klerman EB, Lewy AJ, Middleton B, Murphy PJ, Parry BL, Revell VL (2008) Measuring melatonin in humans. J Clin Sleep Med 4:66-9.

Burgess HJ, Revell VL, Eastman CI (2008) A three pulse phase response curve to three milligrams of melatonin in humans. J Physiol 586:639-47.

Buscemi N, Vandermeer B, Hooton N, Pandya R, Tjosvold L, Hartling L, Vohra S, Klassen TP, Baker G (2006) Efficacy and safety of exogenous melatonin for secondary sleep disorders and sleep disorders accompanying sleep restriction: meta-analysis. BMJ 332:385-93.

Cardinali DP, Furio AM, Reyes MP, Brusco LI (2006) The use of chronobiotics in the resynchronization of the sleep-wake cycle. Cancer Causes Control 17:601-9.

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.

Dagan Y, Yovel I, Hallis D, Eisenstein M, Raichik I (1998) Evaluating the role of melatonin in the long-term treatment of delayed sleep phase syndrome (DSPS). Chronobiol Int 15:181-90.

Dahlitz M, Alvarez B, Vignau J, English J, Arendt J, Parkes JD (1991) Delayed sleep phase syndrome response to melatonin. Lancet 337:1121-4.

Downs SH, Black N (1998) The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. J Epidemiol Community Health 52:377-84.

Fourtillan JB, Brisson AM, Gobin P, Ingrand I, Decourt JP, Girault J (2000) Bioavailability of melatonin in humans after day-time administration of D(7) melatonin. Biopharm Drug Dispos 21:15-22.

Gartlehner G, Moore CG (2008) Direct versus indirect comparisons: A summary of the evidence. Int J Technol Assess Health Care 24:170-7.

Hoebert M, van der Heijden KB, van Geijlswijk IM, Smits MG (2009) Long-term follow-up of melatonin treatment in children with ADHD and chronic sleep onset insomnia. J Pineal Res 47:1-7.

Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, McQuay HJ (1996) Assessing the quality of reports of randomized clinical trials: is blinding necessary? Control Clin Trials 17:1-12.

Juni P, Witschi A, Bloch R, Egger M (1999) The hazards of scoring the quality of clinical trials for meta-analysis. JAMA 282:1054-60.

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 63:40-8.

Kunz R, Oxman AD (1998) The unpredictability paradox: review of empirical comparisons of randomised and non-randomised clinical trials. BMJ 317:1185-90.

Laurant M, Nagtegaal JE, Smits MG, Kerkhof GA, Coenen AML (1997) Influence of melatonin on vigilance and cognitive functions in delayed sleep phase syndrome. Sleep-wake research in the Netherlands 8:79-82.

Lewy AJ, Emens JS, Bernert RA, Lefler BJ (2004) Eventual entrainment of the human circadian pacemaker by melatonin is independent of the circadian phase of treatment initiation: clinical implications. J Biol Rhythms 19:68-75.

Lewy AJ, Emens JS, Sack RL, Hasler BP, Bernert RA (2002) Low, but not high, doses of melatonin entrained a free-running blind person with a long circadian period. Chronobiol Int 19:649-58.
Lewy AJ, Ahmed S, Jackson JM, Sack RL (1992) Melatonin shifts human circadian rhythms according to a phase-response curve. Chronobiol Int 9:380-92.
Littner M, Kushida CA, Anderson WM, Bailey D, Berry RB, Davila DG, Hirshkowitz M, Kapen S, Kramer M, Loube D, Wise M, Johnson SF (2003) Practice parameters for the role of actigraphy in the study of sleep and circadian rhythms: an update for 2002. Sleep 26:337-41.
Lockley SW (2005) Timed melatonin treatment for delayed sleep phase syndrome: the importance of knowing circadian phase. Sleep 28:1214-6.
Luboshizsky R, Lavie P (1998) Sleep-inducing effects of exogenous melatonin administration. Sleep Med Rev 2:191-202.
Mundey K, Benloucif S, Harsanyi K, Dubocovich ML, Zee PC (2005) Phase-dependent treatment of delayed sleep phase syndrome with melatonin. Sleep 28:1271-8.
Nagtegaal JE, Laurant MW, Kerkhof GA, Smits MG, van der Meer YG, Coenen AM (2000) Effects of melatonin on the quality of life in patients with delayed sleep phase syndrome. J Psychosom Res 48:45-50.
Nagtegaal JE, Kerkhof GA, Smits MG, Swart AC, 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 7:135-43.
Owens JA (2009) A clinical overview of sleep and attention-deficit/hyperactivity disorder in children and adolescents. Journal of the Canadian Academy of Child and Adolescent Psychiatry = Journal de l'Academie canadienne de psychiatrie de l'enfant et de l'adolescent 18:92-102.
Pandi-Perumal SR, Smits M, Spence W, Srinivasan V, Cardinali DP, Lowe AD, Kayumov L (2007) Dim light melatonin onset (DLMO): a tool for the analysis of circadian phase in human sleep and chronobiological disorders. Prog Neuropsychopharmacol Biol Psychiatry 31:1-11.
Rajaratnam SM, Middleton B, Stone BM, Arendt J, Dijk DJ (2004) Melatonin advances the circadian timing of EEG sleep and directly facilitates sleep without altering its duration in extended sleep opportunities in humans. J Physiol (Lond) 561:339-51.
Regestein QR, Monk TH (1995) Delayed sleep phase syndrome: a review of its clinical aspects. Am J Psychiatry 152:602-8.
Revell VL, Kim H, Tseng CY, Crowley SJ, Eastman CI (2005) Circadian phase determined from melatonin profiles is reproducible after 1 wk in subjects who sleep later on weekends. J Pineal Res 39:195-200.
Rosenthal NE, Joseph-Vanderpool JR, Levendosky AA, Johnston SH, Allen R, Kelly KA, Souetre E, Schultz PM, Starz KE (1990) Phase-shifting effects of bright morning light as treatment for delayed sleep phase syndrome. Sleep 13:354-61.
Sack RL, Auckley D, Auger RR, Carskadon MA, Wright KP, Jr., Vitiello MV, Zhdanova IV (2007) Circadian rhythm sleep disorders: part II, advanced sleep phase disorder, delayed sleep phase disorder, free-running disorder, and irregular sleep-wake rhythm. An American Academy of Sleep Medicine review. Sleep 30:1484-501.
Schulz KF, Chalmers I, Hayes RJ, Altman DG (1995) Empirical evidence of bias. Dimensions of methodological quality associated with estimates of treatment effects in controlled trials. JAMA 273:408-12.
Smits MG, van Stel HF, van der Heijden K, Meijer AM, Coenen AM, Kerkhof GA (2003) Melatonin improves health status and sleep in children with idiopathic chronic sleep-onset insomnia: a randomized placebo-controlled trial. J Am Acad Child Adolesc Psychiatry 42:1286-93.
Smits MG, Nagtegaal EE, 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.
Sutton AJ, Abrams KR, Jones DR, Sheldon TA, Song F (2000) Methods for Meta-analysis in Medical Research. Wiley Europe, Chichester.
Szeinberg A, Borodkin K, Dagan Y (2006) Melatonin treatment in adolescents with delayed sleep phase syndrome. Clin Pediatr 45:809-18.
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 46:233-41.

van der Heijden KB, Smits MG, van Someren EJ, Boudewijn Gunning W (2005a) Prediction of melatonin efficacy by pretreatment dim light melatonin onset in children with idiopathic chronic sleep onset insomnia. J Sleep Res 14:187-94.
van der Heijden KB, Smits MG, Van Someren EJ, Gunning WB (2005b) Idiopathic chronic sleep onset insomnia in attention-deficit/hyperactivity disorder: a circadian rhythm sleep disorder. Chronobiol Int 22:559-70.
van Someren EJ, Nagtegaal E (2007) Improving melatonin circadian phase estimates. Sleep Med 8:590-601.
van Veen MM, Kooij JJ, Boonstra AM, Gordijn MC, Van Someren EJ (2010) Delayed circadian rhythm in adults with attention-deficit/hyperactivity disorder and chronic sleep-onset insomnia. Biol Psychiatry 67:1091-1096. DOI: 10.1016/j.biopsych.2009.12.032.
Verhagen AP, de Vet HC, Vermeer F, Widdershoven JW, de Bie RA, Kessels AG, Boers M, van den Brandt PA (2002) The influence of methodologic quality on the conclusion of a landmark meta-analysis on thrombolytic therapy. Int J Technol Assess Health Care 18:11-23.
Voultsios A, Kennaway DJ, Dawson D (1997) Salivary melatonin as a circadian phase marker: validation and comparison to plasma melatonin. J Biol Rhythms 12:457-66.
Wagner DR (1996) Disorders of the circadian sleep-wake cycle. Neurol Clin 14:651-70.
Wasdell MB, Jan JE, Bomben MM, Freeman RD, Rietveld WJ, Tai J, Hamilton D, Weiss MD (2008) A randomized, placebo-controlled trial of controlled release melatonin treatment of delayed sleep phase syndrome and impaired sleep maintenance in children with neurodevelopmental disabilities. J Pineal Res 44:57-64.
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 45:512-9.
Weitzman ED, Czeisler CA, Coleman RM, Spielman AJ, Zimmerman JC, Dement W, Richardson G, Pollak CP (1981) Delayed sleep phase syndrome. A chronobiological disorder with sleep-onset insomnia. Arch Gen Psychiatry 38:737-46.
Werner H, Molinari L, Guyer C, Jenni OG (2008) Agreement rates between actigraphy, diary, and questionnaire for children's sleep patterns. Arch Pediatr Adolesc Med 162:350-8.
Wirz-Justice A, Armstrong SM (1996) Melatonin: nature's soporific? J Sleep Res 5:137-41.
Wise ME (2006) Does melatonin improve sleep? Muddles with melatonin. BMJ 332:550.
Zhou JN, Liu RY, van Heerikhuize J, Hofman MA, Swaab DF (2003) Alterations in the circadian rhythm of salivary melatonin begin during middle-age. J Pineal Res 34:11-6.

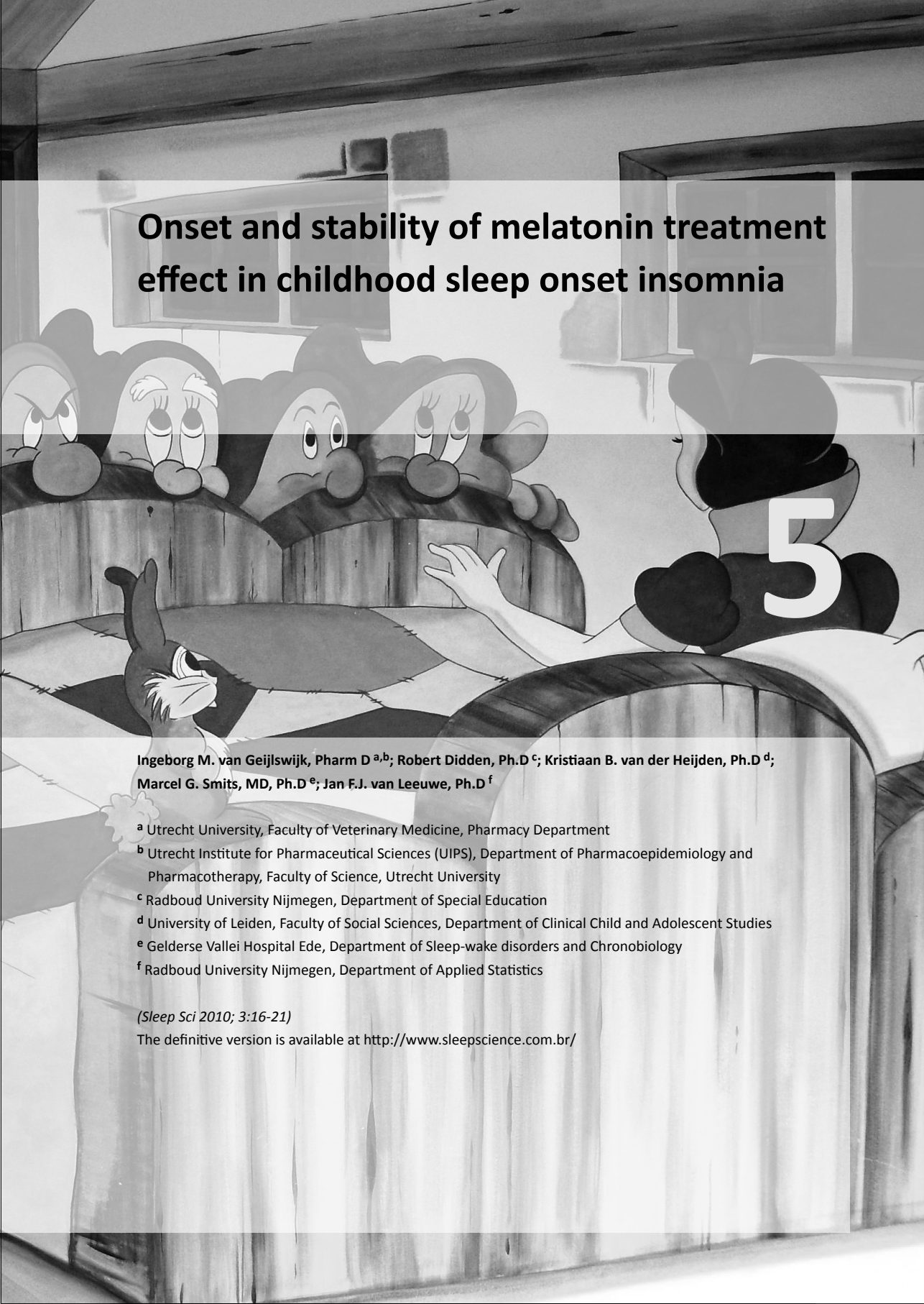
QUERIES

PubMed:

("melatonin"[MeSH Terms] OR melatonin[Text Word]) AND (("sleep disorders"[TIAB] NOT Medline[SB]) OR "sleep disorders"[MeSH Terms] OR sleep disorder[Text Word]) AND ((("placebos"[TIAB] NOT Medline[SB]) OR "placebos"[MeSH Terms] OR placebo[Text Word]) AND controlled[All Fields]) AND (("double-blind method"[TIAB] NOT Medline[SB]) OR "double-blind method"[MeSH Terms] OR double blind[Text Word]) OR ("circadian rhythm sleep disorders"[Text Word] OR "sleep disorders, circadian rhythm"[MeSH Terms] OR delayed sleep phase Disorder[Text Word]) AND ("melatonin"[MeSH Terms] OR melatonin[Text Word]) AND (("1990/01/01"[EDAT] : "2010/01/01"[EDAT]) AND "humans"[MeSH Terms] AND English[lang] AND ("randomized controlled trial"[Publication Type] OR "randomized controlled trials as topic"[MeSH Terms] OR "randomised controlled trial"[All Fields] OR "randomized controlled trial"[All Fields]) NOT ("ramelteon"[Substance Name] OR "ramelteon"[All Fields]) NOT Parkinson[All Fields] NOT ("autistic disorder"[MeSH Terms] OR ("autistic"[All Fields] AND "disorder"[All Fields]) OR "autistic disorder"[All Fields] OR "autistic"[All Fields]))

Embase:

"'randomized controlled trial'/exp OR 'randomised controlled trial'/exp AND 'melatonin'/exp OR 'melatonin' AND ('sleep disorders'/exp OR 'sleep disorders') AND 'placebo controlled' AND ('double-blind method'/exp OR 'double-blind method') OR ('circadian rhythm sleep disorders' OR 'sleep disorders, circadian rhythm'/exp OR 'sleep disorders, circadian rhythm' OR 'delayed sleep phase disorder' AND ('melatonin'/exp OR 'melatonin')) AND [english]/lim AND [humans]/lim NOT (case AND report) NOT ('ramelteon'/exp OR ramelteon) NOT ('dementia'/exp OR dementia) AND controlled AND ('study'/exp OR study) NOT autism NOT ('tasimelteon' OR tasimelteon) AND [1990-2010]/py"



Onset and stability of melatonin treatment effect in childhood sleep onset insomnia

5

Ingeborg M. van Geijlswijk, Pharm D [a,b]; Robert Didden, Ph.D [c]; Kristiaan B. van der Heijden, Ph.D [d]; Marcel G. Smits, MD, Ph.D [e]; Jan F.J. van Leeuwe, Ph.D [f]

[a] Utrecht University, Faculty of Veterinary Medicine, Pharmacy Department
[b] Utrecht Institute for Pharmaceutical Sciences (UIPS), Department of Pharmacoepidemiology and Pharmacotherapy, Faculty of Science, Utrecht University
[c] Radboud University Nijmegen, Department of Special Education
[d] University of Leiden, Faculty of Social Sciences, Department of Clinical Child and Adolescent Studies
[e] Gelderse Vallei Hospital Ede, Department of Sleep-wake disorders and Chronobiology
[f] Radboud University Nijmegen, Department of Applied Statistics

(Sleep Sci 2010; 3:16-21)
The definitive version is available at http://www.sleepscience.com.br/

ABSTRACT

Objectives

To assess onset and stability of therapeutic effect of 4-weeks melatonin treatment for chronic sleep onset insomnia in elementary school-aged children.

Methods

Retrospective analysis of unpublished data obtained in two previously published randomized, placebo-controlled, double-blind trials on melatonin efficacy for childhood insomnia in children with chronic sleep onset insomnia, age 6-12 yrs, n =49

Intervention consisted of placebo (n=25) or melatonin 5 mg (n=24) administered at 6 (n=9) or 7 p.m. (n=40) during 4 weeks.

Collected data were lights out time, sleep onset latency, sleep onset, total sleep time, wake up time, and subjective sleep measures recorded in a diary.

Results

Melatonin treatment showed a phase-advance of lights out of 9:15 p.m. (1.05) to 8:28 p.m. (1.07); sleep onset advanced from 10:05 p.m. (0.93) to 8:45 p.m. (1.09) and sleep latency decreased from 53 min (39) to 18 min (16). After the 4-week trial period these values were 8:44 p.m. (1,27), 9:09 p.m. (1.33), 25 min (39).

Conclusions

Melatonin advances sleep latency and sleep onset and increases total sleep time starting right from the first treatment night in children with chronic sleep onset insomnia. Evidence is provided that the onset of melatonin treatment effect can be expected within a few days after commencement and remains stable after that.

Key Words: Elementary school-aged children; Chronic sleep onset insomnia; Melatonin; Onset of treatment effects; Stability of treatment effects

INTRODUCTION

Sleep onset insomnia is a highly prevalent disorder among school-aged children. A chronic and severe presentation leading to long term sleep deprivation can seriously affect a child's physical and mental development. We have previously shown (Smits et al. 2001; Smits et al. 2003) in two randomized, placebo-controlled, double blind studies in elementary school children with chronic sleep onset insomnia suggestive of delayed sleep phase disorder, that melatonin (5 mg) advanced sleep-wake rhythm and lengthened total sleep duration. However, the data published merely included treatment measurements performed within the fourth treatment week. We have further analyzed these findings by considering *all* data obtained from day one to week four in the treatment period, instead of focusing on endpoint data. Knowledge of the time-course and stability of the treatment effects during therapy initiation is clinically relevant for clinicians and researchers for this can prevent unnecessary long administration of melatonin, for example when the appropriate dose of melatonin needs to be determined (Arendt and Skene. 2005). If, during the initiation phase, the dose effect is evaluable after days instead of weeks, the initiation phase of effective therapy can be shortened from weeks to days.

While long term treatment efficacy has already been established by several research groups (Carr et al. 2007; Wasdell et al. 2008), and direct soporific effects of melatonin were recently described in the context of sedation for EEG recording (Ashrafi et al. 2009), this is the first study to report on the time-course and stability of melatonin treatment effects for insomnia treatment.

This study's aim was to evaluate the acute, wanted effects of melatonin administration to children with sleep onset insomnia. The long-term safety, especially addressing the influence on puberty onset, is a yet still to be sufficiently answered issue. For this population long term effects including the adverse events are described elsewhere (Hoebert et al. 2009).

MATERIAL AND METHODS

Participants were elementary school children with chronic sleep onset insomnia, who participated in one of two randomized placebo-controlled studies published earlier (Smits et al. 2001; Smits et al. 2003). They were aged 6-12 years and in otherwise good general health; their sleep problems were not related to any other pathology than ADHD and suggestive of delayed sleep phase disorder. Other sleep pathology was excluded by ambulatory polysomnography with 24-hour cassette electroencephalography at the child's home and parental report.

The institutional review board on human research approved both studies and informed consent was obtained from the parents of all participants. A 1-week baseline phase preceded a 4-week treatment phase. Melatonin immediate release tablets (5 mg) or identical-looking

placebo were given at 6 p.m. (melatonin: n=19; placebo: n=19) daily in the first study (Smits et al. 2001) and at 7 p.m. (melatonin: n=35; placebo: n=36) in the second (Smits et al. 2003). Sleep onset insomnia was defined as (1) complaints of sleep-onset problems expressed by parents and/or child, (2) occurrence on at least 4 days/ week for longer than 1 year, (3) average sleep onset later than 8:30 p.m. for children at age 6 years and for older children 15 minutes later per year, and (4) average sleep onset latency exceeding 30 minutes. Exclusion criteria were disturbed sleep architecture as measured by ambulatory polysomnography, and sleep maintenance insomnia (one awakening >30 minutes or two or more awakenings >5 minutes summing up to at least 40 minutes, occurring on one or more nights a week, for a period of at least 4 weeks preceding the start of the trial). Further exclusion criteria were mental handicap, severe learning disabilities, liver disease, renal failure, chronic pain, and severe neurological or psychiatric disorders. Finally, any prior use of melatonin, use of hypnotics, antidepressants, and neuroleptics were exclusion criteria. Allowed medications were methylphenidate and salbutamol by inhalation.

In the present paper a sleep log was considered incomplete when it did not cover the entire 4 week treatment period (which is the period of interest in the present study). This is in contrast to the previously published articles, when it was considered incomplete when there were no data of the 4th treatment week (which was the period of interest in the previous studies). This complete coverage of the 4 weeks treatment was accomplished in 49% of the originally included participants. The 51% missing data during the first three treatment weeks is due to the fact that the decision to record sleep data over the entire 4-week trial period (instead of during the fourth treatment week only), was made at a time when both trials were already in progress.

Parents were asked to complete the following parameters in sleep logs daily: lights out time (time at which the light was turned off before sleeping), sleep onset time (estimated time at which the child fell asleep, assessed by checking on the child through listening or watching every 10 minutes, as non invasive as possible), wake up time (estimated time of waking up), get up time (time of getting out of bed). Means and standard deviations were calculated over the baseline week and each treatment week, resulting in 5 mean (SD) values per parameter. Within- group, between-group and interaction effects were tested using multivariate analyses of variance ($\alpha \leq .05$). Differences in means for four sleep parameters, i.e., sleep onset time (SO), sleep onset latency (SOL), wake up time and total sleep time (TST) were tested for each of the 34 pairs of consecutive days (1st and 2nd night.....34th and 35th night) using the Wilcoxon signed rank test. To account for multiple comparisons the α-level was set at .00147 (.05 was divided by the number of comparisons: 34).

Besides the mean values above mentioned, we tried to rate therapy success with an individual parameter. We defined as criterion for successful therapy the number of patients with a decreased sleep onset latency of at least 25 minutes (being the standard

deviation in sleep onset latency of both groups and even more important: a decrease expected to be experienced as a clinically relevant relief of sleep onset insomnia). We assessed this parameter for both groups (melatonin and placebo) after one, two, three and four weeks of therapy compared to baseline.

RESULTS

In the first study (Smits et al. 2001), there were 38 participants, of which 33 sleep logs were available on the 4th treatment week, however of those 33 only 9 sleep logs were completed during the entire four treatment period. In the other paper (Smits et al. 2003), there were 62 participants, of which 55 sleep logs were completed during 4th treatment week, however, only 40 sleep logs were completed during the entire four treatment weeks which data are used for the present study.
Of the total sample included in the present study (n=49), 25 children, 17 male and eight female, (six of trial (Smits et al. 2001) and 19 of trial (Smits et al. 2003)) were assigned to melatonin treatment and 24 children, 19 male and five female (three of trial (Smits et al. 2001) and 21 of trial (Smits et al. 2003)) to placebo.
Figure 1 depicts the mean values for lights out and sleep onset time over the course of treatment. As can be seen, robust phase advances occur within the first week of melatonin treatment, after which the values remain stable except for a slight delay within the third treatment week. The convergent direction of the lights out and sleep onset lines indicate a decrease of sleep onset latency within the first treatment week.

This sleep onset latency (SOL) is of importance, since this parameter clearly demonstrates the trouble falling asleep for children sent to bed. Mean (± SD) SOL changed in the treatment group from baseline 53 min (± 39) to 18 min (±16) in treatment week one to 16 min (±17) in week 3, to 19 min (±21) in week 4 and 25 min (±38) in week five.
On the contrary, lights out, sleep onset and therefore SOL did not significantly change in the placebo group, as illustrated by the white gap in figure 1 (62 (±43), 55 (±48), 58 (±47), 60(±51) and 49 (±52) min in the last treatment week) (see also figure 2).

Responders, defined as patients with a twenty-five minutes decrease in SOL, were compared between weeks and groups, the group effect tested by type III between-subjects tests. During the 1st treatment week, in the melatonin group 14 patients responded with a >25-minute decrease in mean SOL, while this was 4 in the placebo group (F=9.379; p = 0,004). Responders in the 2nd week were 16 patients in melatonin and 5 in placebo group (F=11.035; p = 0,002); 13 in melatonin and 4 in placebo in the 3rd week (F=7.505; p = 0,009), and 13 in melatonin and 7 in placebo in the 4th week (F=2.679; p = 0,108). In the melatonin

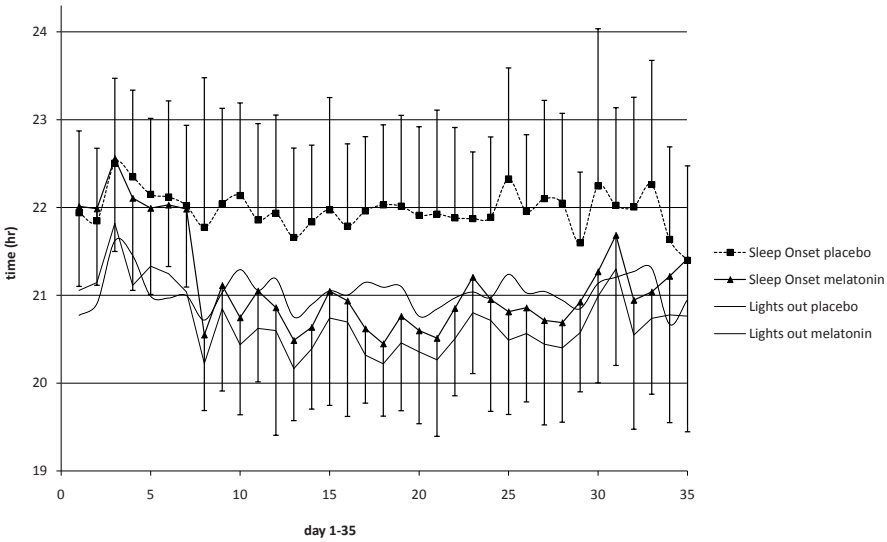


Figure 1. Mean lights out time and sleep onset time day 1-7 (i.e. baseline) and day 8-35 (i.e. intervention).

group, a number of patients of 11 met this criterion for success for each of the 4 weeks, against 1 in placebo (F=10.177; p = 0,003).

Noteworthy are two patients in the melatonin group without any response and one patient in the placebo group with a consistent high response.

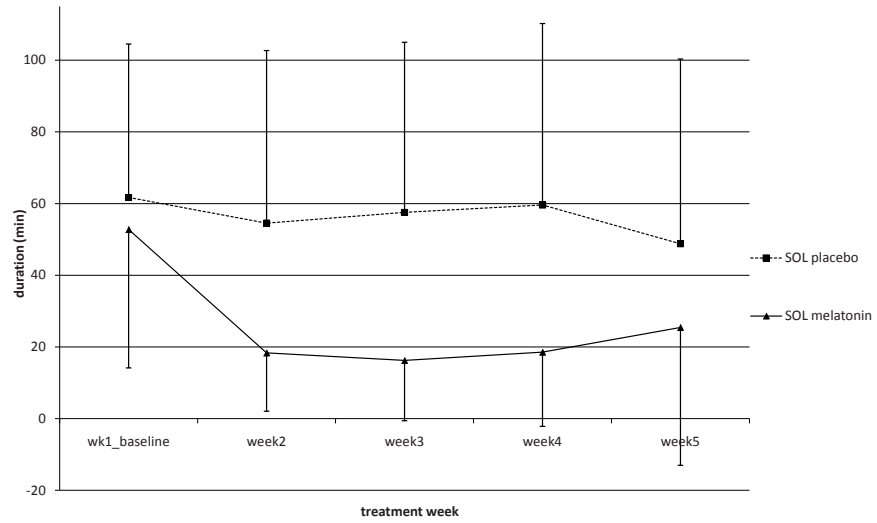


Figure 2. Mean sleep onset latency in week 1 (i.e. baseline) and week 2-5 (i.e. intervention).

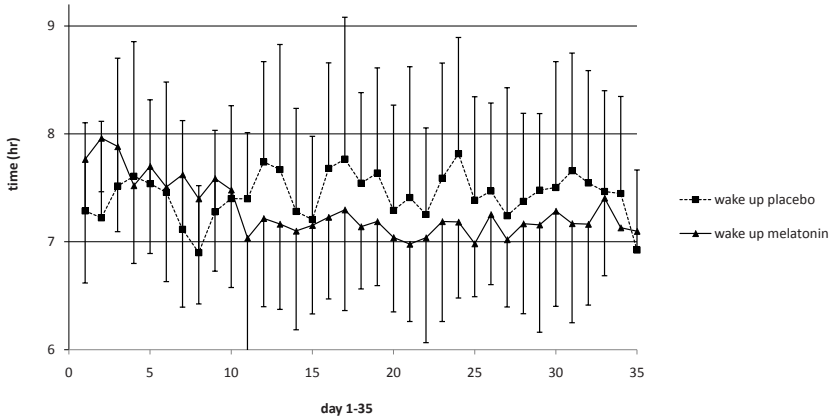


Figure 3. Mean wake up time in day 1-7 (i.e. baseline) and day 8-35 (i.e. intervention).

Figure 3 depicts wake up time over the course of treatment. Wake up time shows a phase advance which is most robust in the first treatment week and gradually stabilizes in the next treatment weeks. Values in placebo show a continuing irregular pattern.

Results of multivariate analyses of variance are summarized in Table 1. The results show that pretreatment to treatment changes of sleep onset, sleep latency, total sleep time lights out and wake up time are significantly different whilst melatonin treatment as compared to placebo treatment (i.e. week 1 vs later * treatment group). Further analysis shows that the difference in pretreatment to treatment changes of these sleep parameters are already significant after one week of treatment (i.e. week 1 vs week 2 * treatment group), except for get up time.

Main effects of week number and treatment group on total sleep time are significant where the interaction is not. This means that the melatonin group exceeds the placebo group on the average, but that the trend over the weeks does not differ between the two groups. Compared to placebo, melatonin resulted in a significantly larger decrease in children's perceived difficulties in falling asleep, from baseline to later weeks as well as from baseline to the second week. As compared to placebo, the melatonin group showed a significantly different change in feeling rested in the morning from baseline to the first treatment week. However, this effect did not sustain during the 4-week treatment period. No significant effects were found for mood during the evening, mood at daytime and perceived sleep quality (not shown).

Finally, table 2 shows the change in sleep latency, sleep onset time, and total sleep time from the final baseline night to the first treatment night within the melatonin treatment group. The results reveal significant improvements in all three parameters during this first treatment night. The Wilcoxon signed rank test revealed that for the 34 pairs of

Table 1. Results of multivariate analyses of variance for sleep parameters

Parameter / Type	'Exposure' variable[a]	*df*1	*df*2	*F*	*p*	form of the function
Sleep onset time						
Main	week number	4	44	25.226	< 0.001	advances
	week number*treatment group	4	44	16.000	< 0.001	advances
	treatment group	1	47	16.511	< 0.001	advances
Time contrasts	week 1 vs later*treatment group	1	47	39.311	< 0.001	advances
	week 1 vs week 2*treatment group	1	47	30.186	< 0.001	advances
Sleep latency						
Main	week number	4	44	9.011	< 0.001	decreases
	week number*treatment group	4	44	7.168	< 0.001	decreases
	treatment group	1	47	19.240	< 0.001	decreases
Time contrasts	week 1 vs later*treatment group	1	47	19.489	< 0.001	decreases
	week 1 vs week 2*treatment group	1	47	18.344	< 0.001	decreases
Total sleep time						
Main	week number	4	44	11.203	< 0.001	increases
	week number*treatment group	4	44	2.523	.054	
	treatment group	1	47	16.284	< 0.001	increases
Time contrasts	week 1 vs later*treatment group	1	47	7.375	.009	increases
	week 1 vs week 2*treatment group	1	47	8.076	.007	increases
Difficulty falling asleep						
Main	week number	4	44	20.095	< 0.001	decreases
	week number*treatment group	4	44	7.168	< 0.001	decreases
	treatment group	1	47	29.833	< 0.001	decreases
Time contrasts	week 1 vs later*treatment group	1	47	18.853	< 0.001	decreases
	week 1 vs week 2*treatment group	1	47	18.348	< 0.001	decreases
Feeling rested (morning)						
Main	week number	4	44	4.925	.002	increases
	week number*treatment group	4	44	3.226	.021	increases
	treatment group	1	47	7.108	.010	increases
Time contrasts	week 1 vs later*treatment group	1	47	.849	.362	
	week 1 vs week 2*treatment group	1	47	5.465	.024	increases

[a] = week number and week number*treatment group effects tested by Wilks' lambda;treatment group effect tested by type III between-subjects test

Table 2. Results for the change over from the final baseline night to the first treatment night in the melatonin treatment group, tested with the Wilcoxon signed rank test (α-level set at .00147)

Sleepparameter	*z*	*p*
Sleep latency		
Difference in means of 7th night - 8th night	-3.92	.00009
Sleep on set time		
Difference in means of 7th night - 8th night	-3.95	.00008
Total sleeptime		
Difference in means of 7th night - 8th night	-3.77	.00016

consecutive daily means for sleep latency, sleep onset time and total sleep time the difference between consecutive pairs of nights was only statistically significant (α-level set at .00147) for the change over the final baseline night to the first treatment night in the melatonin treatment group. Differences between consecutive night-pairs of the four parameters within the placebo group were all statistically non-significant.

DISCUSSION

This paper is a further analysis of data from our two previous publications, and we have now found that effects of melatonin on sleep latency, sleep onset time and total sleep time occur from the first night of treatment. These effects were stable during a 4-week intervention period. Melatonin initially also improved feeling rested in the morning, which however did not sustain during intervention.

Individual results for sleep onset latency, and using this result as a criterion for successful therapy, corroborate the mean study population results, since the variance of sleep measures (sleep onset and wake up time in particular) is large in a population with varying sleep needs (age 6 -12 years) and this variance is much smaller in the mean calculated measure SOL. What strikes in the responders results is that most responders in the melatonin group are consistent during all four treatment weeks (11), as in the placebo group this was only applicable for one patient.

In contrast to the melatonin group (which showed immediate decrease of SOL starting from treatment day 1), the placebo group showed decreasing SOL values from week 1 to week 4, although not statistically significant. One reason for this finding could be the conditioning effect of parents checking on the child every 10 minutes. If children are anxious, knowing and having a parent come in and check on them in set intervals may have a curing effect on their insomnia. It is a common finding in randomized clinical trials studying treatment effect in insomniacs that placebo produces significant changes on self reported sleep measures (Perlis et al. 2005). However, in our studies, sleep measures

did not significantly change during placebo treatment, with one exception. This might be explained by the fact that the sleep measures were not assessed by the patients themselves.

There are several limitations to this study; one of which is that we did not assess sleep objectively by means of polysomnography or actigraphy. However, parents of sleep-disturbed infants have shown to be accurate reporters of actigraphically assessed sleep onset and sleep duration (Sadeh. 1996).

We used the data of two studies, with different timing of melatonin administration, although the two subsets show very similar characteristics of sleep-wake rhythm and endogenous melatonin onset. Moreover, the effect of melatonin on sleep parameters did not differ between the two studies (Smits et al. 2001; Smits et al. 2003). Melatonin treatment for chronic insomnia in children has been criticized in the Netherlands. Jenni. (2005) stressed that cultural variation and developmental variation and biological variability of sleep behavior among normal healthy children should be taken into account. However, in our studies we did not include healthy children, but children with chronic complaints of insomnia who showed impaired general health and quality of life likely due to their insomnia problems (van der Heijden et al. 2005b).

To conclude, the results presented here provide proof that onset of melatonin treatment effect can be expected within a few days and this effect remains stable in the weeks after that, in addition to the earlier evidence (Smits et al. 2001; Smits et al. 2003; van der Heijden et al. 2005a) that melatonin is an effective treatment for sleep onset insomnia in children with late melatonin onset.

ACKNOWLEDGEMENTS

We thank Prof dr A.C.G. Egberts *(Utrecht Institute for Pharmaceutical Sciences [UIPS], Department of Pharmacoepidemiology and Pharmacotherapy, Faculty of Science, Utrecht University)* and Prof dr H. Vaarkamp *(Utrecht University, Faculty of Veterinary Medicine, Pharmacy Department)* for helpful discussions and critically reading the manuscript.

REFERENCES

Arendt J, Skene DJ (2005) Melatonin as a chronobiotic. Sleep Med Rev 9:25-39.

Ashrafi MR, Mohammadi M, Tafarroji J, Shabanian R, Salamati P, Zamani GR (2009) Melatonin versus chloral hydrate for recording sleep EEG. Eur J Paediatr Neurol doi:10.1016/j.ejpn.2009.06.010.

Carr R, Wasdell MB, Hamilton D, Weiss MD, Freeman RD, Tai J, Rietveld WJ, Jan JE (2007) Long-term effectiveness outcome of melatonin therapy in children with treatment-resistant circadian rhythm sleep disorders. J Pineal Res 43:351-9.

Hoebert M, van der Heijden KB, van Geijlswijk IM, Smits MG (2009) Long-term follow-up of melatonin treatment in children with ADHD and chronic sleep onset insomnia. J Pineal Res 47:1-7.

Jenni OG (2005) Sleep onset insomnia during childhood or poor fit between biology and culture: comment on van der Heijden et al. 'Prediction of melatonin efficacy by pre-treatment dim light melatonin onset in children with idiopathic chronic sleep onset insomnia'. J Sleep Res 14:195-7; discussion 197-9.

Perlis ML, McCall WV, Jungquist CR, Pigeon WR, Matteson SE (2005) Placebo effects in primary insomnia. Sleep Med Rev 9:381-9.

Sadeh A (1996) Evaluating night wakings in sleep-disturbed infants: a methodological study of parental reports and actigraphy. Sleep 19:757-62.

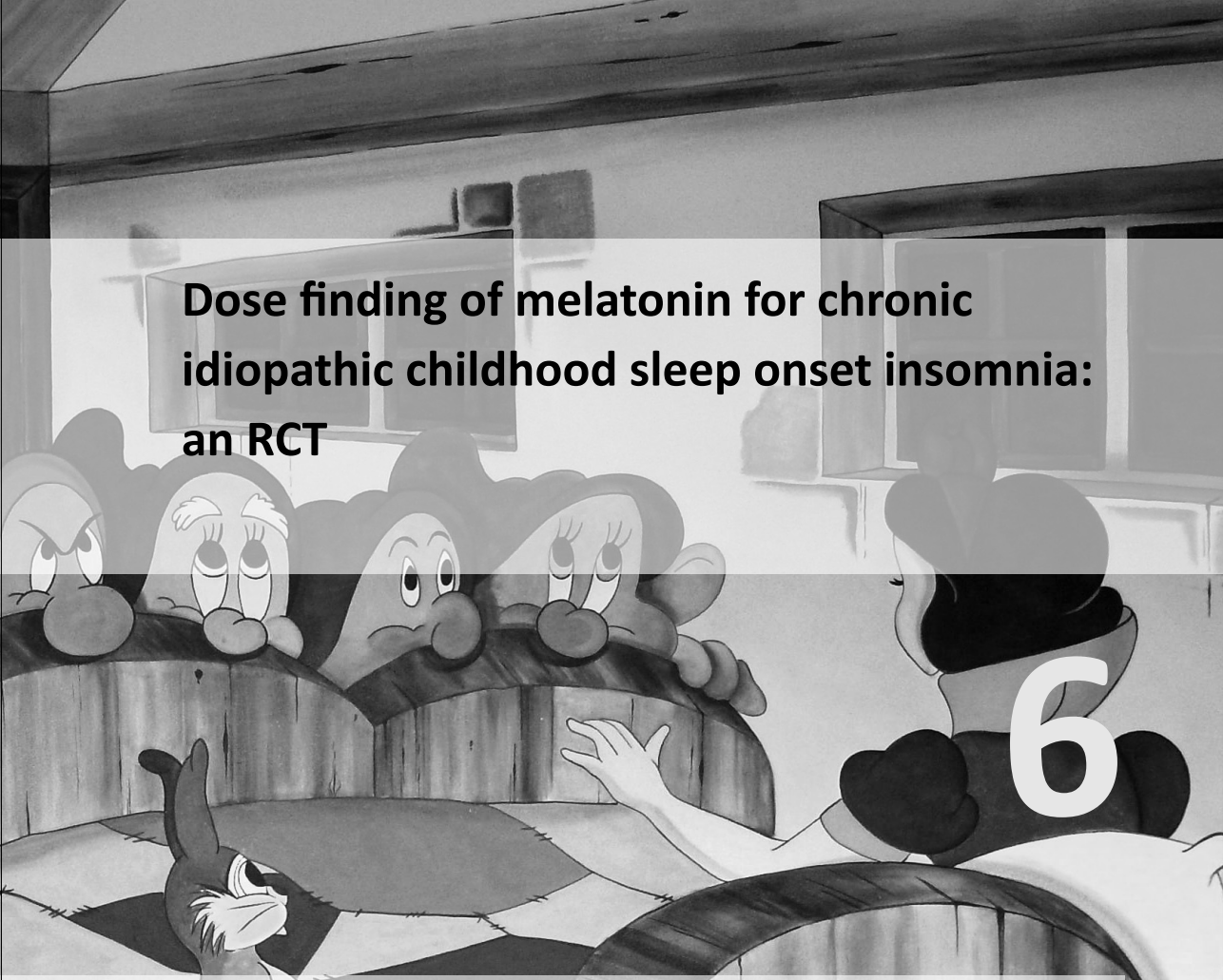
Smits MG, van Stel HF, van der Heijden K, Meijer AM, Coenen AM, Kerkhof GA (2003) Melatonin improves health status and sleep in children with idiopathic chronic sleep-onset insomnia: a randomized placebo-controlled trial. J Am Acad Child Adolesc Psychiatry 42:1286-93.

Smits MG, Nagtegaal EE, 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.

van der Heijden KB, Smits MG, van Someren EJ, Boudewijn Gunning W (2005a) Prediction of melatonin efficacy by pretreatment dim light melatonin onset in children with idiopathic chronic sleep onset insomnia. J Sleep Res 14:187-94.

van der Heijden KB, Smits MG, van Someren EJ, Boudewijn Gunning W (2005b) Reply to Jenni - Childhood chronic sleep onset insomnia and late sleep onset: What's the difference? J Sleep Res 14:197-199.

Wasdell MB, Jan JE, Bomben MM, Freeman RD, Rietveld WJ, Tai J, Hamilton D, Weiss MD (2008) A randomized, placebo-controlled trial of controlled release melatonin treatment of delayed sleep phase syndrome and impaired sleep maintenance in children with neurodevelopmental disabilities. J Pineal Res 44:57-64.



Dose finding of melatonin for chronic idiopathic childhood sleep onset insomnia: an RCT

6

Ingeborg M. van Geijlswijk [a,b]; Kristiaan B. van der Heijden [c]; Toine C.G. Egberts [b,d]; Hubert P.L.M. Korzilius [e]; Marcel G. Smits, MD, PhD [f]

[a] Utrecht University, Faculty of Veterinary Medicine, Pharmacy Department
[b] Utrecht Institute for Pharmaceutical Sciences (UIPS), Department of Pharmacoepidemiology and Clinical Pharmacology, Faculty of Science, Utrecht University
[c] Department of Clinical Child and Adolescent Studies, Faculty of Social Sciences, University of Leiden, Leiden, The Netherlands
[d] University Medical Centre Utrecht, Department of Clinical Pharmacy, Division of Laboratory and Pharmacy
[e] Institute for Management Research, Radboud University Nijmegen, Nijmegen, The Netherlands
[f] Gelderse Vallei Hospital Ede, Department of Sleep-wake disorders and Chronobiology

(Psychopharmacology (Berl) 2010; 212:379-391)
The definitive Open Access version is available at www.springerlink.com

ABSTRACT

Rationale

Pharmacokinetics of melatonin in children might differ from that in adults.

Objectives

This study aims to establish a dose–response relationship for melatonin in advancing dim light melatonin onset (DLMO), sleep onset time (SOT), and reducing sleep onset latency (SOL) in children between 6 and 12 years with chronic sleep onset insomnia (CSOI).

Methods

The method used for this study is the randomized, placebo-controlled double-blind trial. Children with CSOI (*n*=72) received either melatonin 0.05, 0.1, and 0.15 mg/kg or placebo during 1 week. Sleep was assessed with log and actigraphy during this week and the week before. Outcomes were the shifts in DLMO, SOT, and SOL.

Results

Treatment with melatonin significantly advanced SOT and DLMO by approximately 1 h and decreased SOL by 35 min. Within the three melatonin groups, effect size was not different, but the circadian time of administration (TOA) correlated significantly with treatment effect on DLMO (r_s=−0.33, p=0.022) and SOT (r_s=−0.38, p=0.004), whereas clock TOA was correlated with SOT shift (r=−0.35, p=0.006) and not with DLMO shift.

Conclusions

No dose–response relationship of melatonin with SOT, SOL, and DLMO is found within a dosage range of 0.05-0.15 mg/kg. The effect of exogenous melatonin on SOT, SOL, and DLMO increases with an earlier circadian TOA. The soporific effects of melatonin enhance the SOT shift. This study demonstrates that melatonin for treatment of CSOI in children is effective in a dosage of 0.05 mg/kg given at least 1 to 2 h before DLMO and before desired bedtime.

Keywords Melatonin treatment - Elementary school-aged children - Chronic sleep onset insomnia - Randomized placebo controlled - Dose finding

INTRODUCTION

Prevalence of chronic sleep onset insomnia in the nondisabled school-aged population is approximately 10% (Blader et al. 1997). A chronically reduced sleep due to insomnia may induce various cognitive and behavioral problems in children as well as more widespread difficulties within their families (Dahl. 1996; Blader et al. 1997; Ring et al. 1998). It has even been suggested that the current attention-deficit hyperactivity disorder (ADHD) epidemic might partly be attributable to delayed sleep phase disorder, due to a shared underlying pathophysiology or to misinterpretation of daytime consequences of insomnia as ADHD symptoms (Szeinberg et al. 2006).

Chronic sleep onset insomnia in children is often associated with a delayed time at which endogenous melatonin concentration starts to rise in dim light (DLMO) indicating that the biological clock rhythm in these children is set at a later clock time than desired(van der Heijden et al. 2005). The DLMO is a convenient parameter, as it can usually be obtained before—instead of during—sleep time and is more reliable than many other endocrine or temperature markers of the circadian pacemaker (Klerman et al. 2002). Administration of exogenous melatonin in children with insomnia shifts DLMO as well as sleep onset to an earlier time in the evening, thereby ameliorating the insomnia problems (Smits et al. 2001; Smits et al. 2003; van der Heijden et al. 2007). The direction, a phase advance or phase delay, and the magnitude of the response of the circadian pacemaker to exogenous melatonin depends on the timing of administration of melatonin relative to the rhythm phase of the pacemaker, the so-called phase response curve. The phase response curve illustrates that the largest phase-advancing therapeutic effects of melatonin can be expected when administration occurs approximately 5 to 6 h before the individual DLMO. Lewy et al. (2004) were the first to describe this in blind people with a free-running sleep–wake rhythm. Van der Heijden et al. (2005) demonstrated that the earlier (within a window of 3/4-6 h before DLMO) melatonin is administered in children with sleep onset insomnia and normal vision, the larger the phase advance of sleep onset is.

Since melatonin administration in the afternoon has the potential to cause undesired direct soporific effects, administration in children usually takes place in the early evening, preferably not earlier than 18 h. Most studies apply a melatonin dosage of 5 mg, although melatonin plasma concentrations in children are generally higher than in adults due to the fixed size of the pineal gland in humans during development, while the body volume increases (Waldhauser et al. 1988; Schmidt et al. 1995; Griefahn et al. 2003). Children metabolize melatonin, however, more quickly than adults (Cavallo and Dolan. 1996; Cavallo and Ritschel. 1996). Consequently, the dose–response relationship of melatonin in children may differ from that in adults.

Several small studies and case reports on the efficacy of melatonin for childhood insomnia have been published, with pharmacological doses of 2–12 mg. These studies showed that melatonin treatment is effective and safe in children with sleep onset disorders with or

without co-morbidity (Jan et al. 1994; McArthur and Budden. 1998; Jan et al. 2000; Jan. 2000; Smits et al. 2001; Smits et al. 2003; Coppola et al. 2004; Weiss et al. 2006; van der Heijden et al. 2007; Wasdell et al. 2008) The applied dosage of melatonin in these studies is very diverse, and—except in one study (van der Heijden et al. 2007) - not adjusted to age or bodyweight. This is at least exceptional, in comparison to other drug regimens in children. Most drugs are dosed in children in relation to their bodyweight.

Recently, several reviews concluded that melatonin is effective and safe in children irrespective of the dosage (Pandi-Perumal et al. 2007; Owens and Moturi. 2009; Bendz and Scates. 2010). So, a knowledge gap remains as to the dosage of melatonin in children. The aim of the present trial was to assess the dose–effect relationship of melatonin in advancing the sleep–wake rhythm in elementary school children aged 6–12 years suffering from chronic sleep onset insomnia and to find the most appropriate dosage with the largest effect and least adverse events.

With the results of a trial of short length and noninvasive measurements, we intend to contribute to evidence-based medicine and, therefore, to rational drug prescription in children (Sutcliffe and Wong. 2006; Vitiello. 2007) suffering from insomnia, finding the appropriate dosage of melatonin.

METHODS AND MATERIALS

Study design

The trial consisted of two consecutive periods: a 1-week qualification period and 1 week of treatment, in which participants were randomly and evenly allocated to one of the doses of melatonin or to placebo.

The trial was performed according to the 1997 European Guidelines for Good Clinical Research Practice in children and followed the 1983 revised provisions of the 1975 Declaration of Helsinki.

The protocol was approved by the institutional review board as a mono-center trial by the Central Committee on Research Involving Human Subjects and registered in the International Standard Randomized Controlled Trial Number Register (ISRCTN20033346).

Participants

Children who suffered from chronic sleep onset insomnia were referred by their general practitioner, pediatrician, or child psychiatrist to the Centre for Sleep-Wake Disorders and Chronobiology of the Hospital Gelderse Vallei Ede. Children were eligible if they were 6-12 years old, suffering from sleep onset insomnia more than four nights a week for more than 1 year, and insufficiently responded to sleep hygiene improving measures based on parental reports. Sleep onset insomnia was defined as sleep onset later than 8:30 p.m. in children aged 6 years and for older children 15 min later per year until age

12 (10:00 p.m.). Furthermore, the latency between lights-off time and sleep onset (sleep onset latency) had to be more than 30 min on average. Their sleep onset had not been advanced sufficiently with the usual sleep hygiene improving measures (Lam and Mason. 2007). Further inclusion criteria were normal sleep architecture as indicated by a normal hypnogram, performed within 2 months prior to participation, and written informed consent obtained from parents. Exclusion criteria were chronic sleep onset insomnia due to psychiatric or pedagogic problems, known intellectual disability, pervasive developmental disorder, chronic pain, known disturbed hepatic or renal function, epilepsy, prior use of melatonin, and use of stimulants, neuroleptics, benzodiazepines, clonidine, antidepressants, hypnotics, or beta-blockers within 4 weeks before enrollment. Finally, DLMO was determined by saliva measurements before inclusion as described elsewhere (Nagtegaal et al. 1998) to validate the diagnosis of DSPD.

Interventions

During the treatment week, all participants took medication on nights 1–6 between 5:30 p.m. and 7:30 p.m., placebo or melatonin 0.05 or 0.1 or 0.15 mg/kg (constituting four treatment groups). The children and their parents were instructed to administer the trial medication every day at the same time, depending on age and designated bedtime. For practical reasons, we aimed at 1.5–2 h before bedtime. This way, we ensured to be in the previously mentioned timeframe of preferred time of administration (TOA). The time of administration was recorded in the sleep diary every evening.

Participants were not allowed to have their co-medication changed. Both weeks had to be regular school weeks, at least 2 weeks after time-shift weeks (summertime/wintertime), and preferably without parties, school camps, holidays, etc.

Compliance of the medication was assessed by counting the number of capsules returned.

Outcomes

Sleep: sleep onset, sleep onset latency, wake-up time, and total sleep time

During the baseline and treatment periods, the parents recorded lights-off time, sleep onset, and wake up time daily in a sleep log (on paper or online in a specialized internet software application (Medsys/De Nieuwe Coster/2004)); additional information on mood and adverse events were also recorded.

During all 14 days of the trial, participants were instructed to wear an actigraph (Cambridge Neurotechnology) from the moment they went to bed until the moment they got up in the morning (get-up time). This motion-sensing device—the size of a normal wristwatch—was attached to the non-dominant wrist. Actigraphic monitoring measured movements in 30-s periods. It is a validated method to assess sleep patterns in children (Morgenthaler et al. 2007; Werner et al. 2008). Actigraphic data were converted into sleep parameters by the validated automatic Actiwatch scoring algorithm, combined with subsequent manual verification based on sleep log-derived bedtime and get-up time

(Kushida et al. 2001). Sleep onset time (SOT) and wake-up time, as derived from the wrist activity records, averaged over three to seven nights of each week and were estimated as described elsewhere (Littner et al. 2003). Sleep onset latency (SOL) and total sleep time (TST) were calculated (SOL=SOT-bed time and TST=wake up time-SOT). Sleep log data were used to validate the actigraphy data; in case of discrepancy, the actigraphy data prevailed.

Dim light melatonin onset

On the last nights of the baseline and the treatment week, five saliva samples were collected by chewing on a cotton plug during 1 min (Salivetten, Sarstedt Nümbrecht, Germany) at 7:00 p.m., 8:00 p.m., 9:00 p.m., 10:00 p.m., and 11:00 p.m.. In the treatment week, at this night, no trial medication was used. Salivary melatonin concentrations were measured as described elsewhere (Nagtegaal et al. 1998). To prevent suppression of melatonin secretion by bright light (Bojkowski et al. 1987) during the collection period, the children were instructed to stay in bed or in the living room, with closed curtains and only dim light allowed, 40 lx (Brainard et al. 2000) . DLMO was defined as the time at which salivary melatonin concentration reaches 4 pg/ml and was calculated by linear interpolation between the two samples just below and just above 4 pg/ml.

Sample size

Based on results of a previous study of melatonin in a similar population (Smits et al. 2001) sample size calculation with the SPSS Sample Power 2.0 program showed that 26 subjects in the melatonin-treatment group and 26 subjects in the placebo-treatment group are needed to find a significant (p<0.05; power 0.90; one tailed) advance (SD) sleep onset of 67 (85) min compared to an advance (SD) of 10 (46) min in the placebo group. When four subjects have to be excluded in each treatment group, 30 subjects can be considered to be enough to find a significant advance of sleep onset time. For four treatment groups, the planned sample size was 120 participants to be recruited within 3 years.

Randomization

For this trial, a specialized internet software application (Medsys/De Nieuwe Coster/2004) was developed for randomization of participants, for calculation of the assigned dose (based on body weight), and for collection of sleep log data.

Patients were randomized in blocks of eight to keep possible seasonal time effects to a minimum.

During a visit with the neurologist, eligible patients were invited to participate in Meldos and, if willing, added to the database Medsys. Afterwards, the hospital pharmacist made a telephone call to check willingness, to make an appointment, and to randomize participants in Medsys. For this appointment, the hospital pharmacy prepared the appropriate trial medication and programmed the actigraph. During the visit, the hospital

pharmacist handed over all materials (actigraph, salivettes, medication, and sleep log) and gave instructions.

Blinding

The assigned dose of melatonin was ad hoc prepared by one of the hospital pharmacy technicians in capsules, containing only microcrystalline cellulose (Bufa, Haarlem, The Netherlands) as placebo or containing melatonin (melatonin supplied by Pharma Nord, Denmark) in the appropriate calculated dosage and microcrystalline cellulose. The capsules were packed in unit dose strips, labeled with "Melatonine X mg" masked with an X to keep participants blind to the treatment allocation and subject number.

All participants, care providers, and investigators involved in the study were unaware of the treatment allocation.

Data analysis

The time measurements bed-, sleep onset, wake-up, and get-up time was expressed in 24 h/min.

The difference (shift) between baseline and treatment week for DLMO and mean sleep measures (SOT, SOL, and TST) was calculated for each participant individually.

This way, we assessed individual responses to one of the treatments. These shifts were expressed in hours: minutes or minutes alone and the means per treatment group (mean (±SD)) were compared.

Comparisons of demographic and clinical characteristics between treatment groups were conducted using independent samples Student's *t* test for continuous variables with a normal distribution and Mann–Whitney *U* test when distribution was not normal, using SPSS 15.0 for Windows (SPSS Inc. 2006).

We wanted to differentiate between dosing effects and timing effect in the observed baseline-treatment week shifts of DLMO, SOT, and SOL. Second-degree polynomial trend line estimation in Microsoft Office Excel 2003 (Microsoft Inc 1985–2003) and quadratic curve fit and two-tailed correlation analysis (Pearson and Spearman's r_s; SPSS 15.0) were used to assess timing effect. We studied all shifts as function of clock TOA and as function of circadian TOA. The circadian TOA is determined by defining DLMO as CT14 (Lewy et al. 1999); a clock TOA 2 h before DLMO means a circadian TOA of CT12.

Additionally, shifts of DLMO, SOT, and SOL were studied in relation to the baseline individual circadian alignment, characterized by the phase angle difference (PAD). PAD reflects the time distance between baseline DLMO and baseline mid-time of sleep measured by actigraphy (Lewy et al. 2006).

We first analyzed the effect of melatonin treatment (different dosages) compared to placebo. Then, we analyzed the differences between the different melatonin dosages. The latter analyses required exclusion of the placebo group as a considerable part of the

correlation between dosage and outcome parameters is due to the difference between placebo and melatonin and not to differences between the different dosages of melatonin. Wake-up time and total sleep time data are not analyzed since those data were found to be strongly influenced by fixed wake-up time.

RESULTS

Baseline demographic and clinical characteristics

Initially, 88 children were found eligible to participate in this study. Due to several reasons (logistic problems due to shortage of actigraphs, holidays, social activities, attending high school, winter/summertime shift, unexpected family circumstances, and not allowed-co medication), 16 children were excluded before randomization (Fig. 1).

Based on the results of interim analyses of data of the 72 included children during a period of almost 3 years, the decision was made to finish recruitment instead of extending the trial over a longer period of time. The trial was conducted between May 2004 and February 2007.

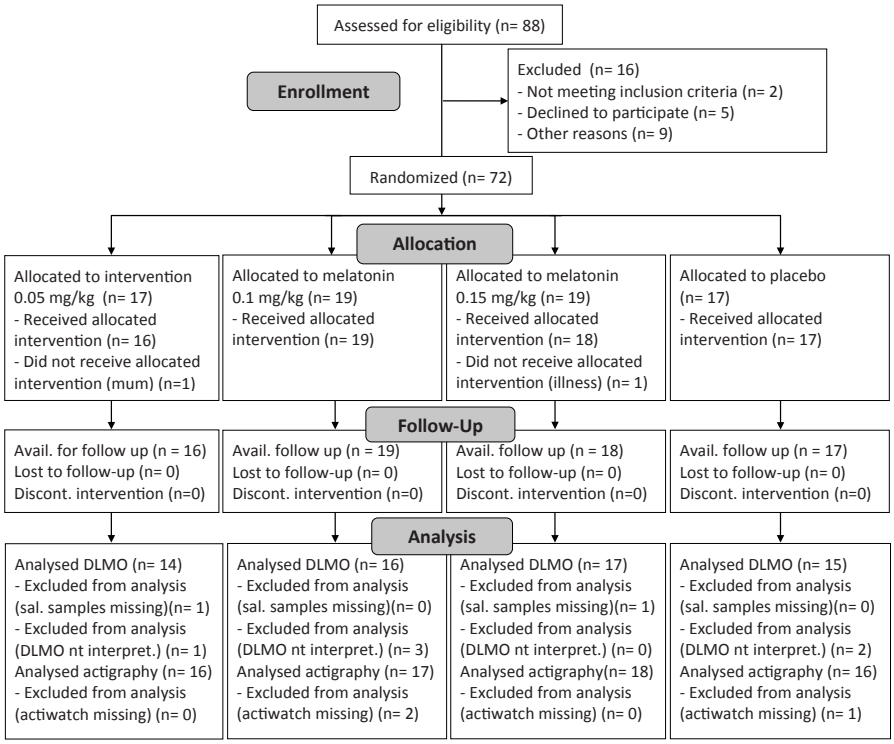


Figure 1. Randomization scheme and justification of obtained outcome data (per group actigraphy and DLMO data obtained within the same group of included participants).

Table 1 shows demographic characteristics of participants per treatment group, including bed and medication times; the participants were encouraged not to change bedtime and get-up time during the 2 weeks.

The mean (±SD) bedtime, measured by actigraphy, averaged over the four treatment groups was 8:41 p.m. (±0:41) in the baseline week and 8:33 p.m. (±0:33) in the treatment week. Mean get-up time was 07:40 a.m. (±0:23) at baseline and 07:39 a.m. (±0:25) in the treatment week. Both weeks are comparable in events (ordinary [school] weeks, no special days or activities); observed effects on sleep parameters can, therefore, be attributed to the melatonin administration. Get-up time was -for most days and most children- clearly restricted due to school times and, therefore, excluded from evaluation as a treatment result. At baseline, there were no significant between-group differences in demographic variables.

Seventy-two children were randomized to one of the four treatment arms. Actigraphic data were collected from 67 participants, and DLMO was determined in 62 participants. Two children ended participation after randomization but before the start with the trial medication: one boy because his mother was concerned that he would see the actigraph as a challenge to stay awake as long as possible, the second child because of diagnosis of mononucleosis infectiosa, just prior to starting.

Three children forgot to wear the actigraph the second week and were, for that reason, excluded from actigraphic data analysis.

The analysis was based on 5.3 (±0.87) nights (mean (±SD)).

Two children forgot to collect saliva samples and were, for that reason, excluded from DLMO analysis. Additionally, in six children, the DLMO could not be calculated because the first salivary level at 7:00 p.m. was already higher than 4 pg/ml (DLMO reached), resulting in blank values. The collected saliva samples were not suitable for determination of individual thresholds like Voultsios et al. (1997) and Burgess et al. (2008) did as the timing was aimed at determination of DLMO. For this reason, we adhered to the traditional definition of DLMO (saliva 4 pg/ml).

The parents of 25 (35%) children did report most results online; the other parents filled in the print out. These data were added to the database afterwards.

Seventy-two children received seven capsules each. Two children returned the medication unused. One child returned two capsules because his mother decided to advance the second DLMO test due to bedwetting (three nights in a row). Eight children returned zero capsules: five because of postponing the final DLMO test for social reasons, three because the remaining capsule was used afterwards because its effect was much appreciated.

TOA, as daily recorded in the sleep diary, was related to age and varied between 5:58 p.m. and 8:42 p.m. (mean 7:08 p.m. ± 0:34 (SD); Table 1).

Table 1. Demographic characteristics of participants

Group		1	2	3	4
Dose (mg/kg)		*0.05*	*0.1*	*0.15*	*0*
n		*16*	*19*	*18*	*17*
Bodyweight	*Mean*	*32*	*31*	*29*	*27*
	Min	*18*	*16*	*16*	*20*
	Max	*45*	*49*	*42*	*35*
	SD	*8*	*8*	*7*	*4*
Dose	Mean	1.60	2.91	4.39	0
	Min	0.9	1.4	2.4	0
	Max	2.2	4.9	6.3	0
	SD	0.39	0.91	0.98	0
Age	Mean	9.5	8.9	8.7	8.7
	Min	6.9	6.5	6.0	6.2
	Max	11.7	11.6	11.3	11.8
	SD	1.8	1.4	1.4	2.8
Boys (%)		9 (56%)	5 (26%)	10 (56%)	6 (35%)
Bedtime week 1	Mean	8:52 pm	8:48 pm	8:38 pm	8:27 pm
	Min	7:03 pm	7:49 pm	7:56 pm	7:25 pm
	Max	10:40 pm	10:06 pm	9:26 pm	9:40 pm
	SD	0:59 am	0:34	0:28	0:34
Bedtime week 2	Mean	8:44 pm	8:35 pm	8:26 pm	8:25 pm
	Min	7:13 pm	7:58 pm	7:25 pm	7:35 pm
	Max	9:34 pm	9:21 pm	9:14 pm	9:02 pm
	SD	0:50	0:26	0:26	0:24
Clock TOA	Mean	7:15 pm	7:08 pm	7:11 pm	6:59 pm
	Min	5:58 pm	6:15 pm	6:30 pm	6:08 pm
	Max	8:17 pm	8:42 pm	8:20 pm	8:00 pm
	SD	0:41	0:36	0:32	0:25
Get-up time week 1	Mean	7:41 am	7:41 am	7:41 am	7:38 am
	Min	7:14 am	7:11 am	7:14 am	7:17 am
	Max	9:18 am	8:55 am	8:24 am	8:09 am
	SD	0:32	0:25	0:18	0:16
Get-up time week 2	Mean	7:39 am	7:41 am	7:32 am	7:48 am
	Min	7:01 am	7:11 am	6:53 am	7:05 am
	Max	8:36 am	8:47 am	8:31 am	8:33 am
	SD	0:25	0:26	0:26	0:24

TOA time of administration

Co-medication

Ten participants reported use of co-medication during the trial, two in groups 1 and 4 and three in groups 2 and 3. Four participants used anti-histaminics: desloratadine, ketotifen, levocetirizine, hydroxyzine; five participants used methylphenidate. One participant used fluticason and salbutamol by inhalation, and one participant used valproic acid, trimethoprim, and lactitol.

DMLO, SOT, and SOL results

DLMO was delayed by 16 min in the placebo group and was advanced by 50–90 min in the melatonin treatment groups. SOT was advanced by 9 min in the placebo group and 51–66 min in the melatonin treatment groups. SOL was reduced by 12 min in the placebo group and by 43–54 min in the melatonin treatment groups.

Table 2 shows the comparison of three melatonin treatments (0.05, 0.1, and 0.15 mg/kg) with placebo.

The DLMO advance in the 0.1 and 0.15 mg/kg treatment group was significantly (p<0.001) different from placebo; the 0.05 mg/kg group did not reach significance (p=0.053).

Table 2 Comparison of DLMO and sleep measures sleep onset and sleep onset latency between the three melatonin dosage groups and placebo

Dose	Mean difference in comparison to placebo group[a]	Standard error of the difference	95% Confidence interval of the difference		*df*	*p* Value
mg/kg	h:m	h:m	Lower	Upper		
0.05						
DLMO shift	1:05	0:32	−0:01	2:12	22.6	0.053
SOT shift	0:42	0:10	0:20	1:03	29.6	<0.001
SOL shift	0:31	0:10	0:09	0:54	29.6	0.007
0.1						
DLMO shift	1:45	0:26	0:53	2:38	29.0	<0.001
SOT shift	0:50	0:11	0:27	1:13	29.6	<0.001
SOL shift	0:36	0:10	0:15	0:57	30.7	0.001
0.15						
DLMO shift	1:31	0:24	0:41	2:21	30.7	<0.001
SOT shift	0:56	0:10	0:34	1:18	29.7	<0.001
SOL shift	0:42	0:09	0:22	1:02	31.5	<0.001

Equal variances not assumed
DLMO dim light melatonin onset, SOT sleep onset time, SOL sleep onset latency
[a]Positive value=phase advance, negative value=phase delay

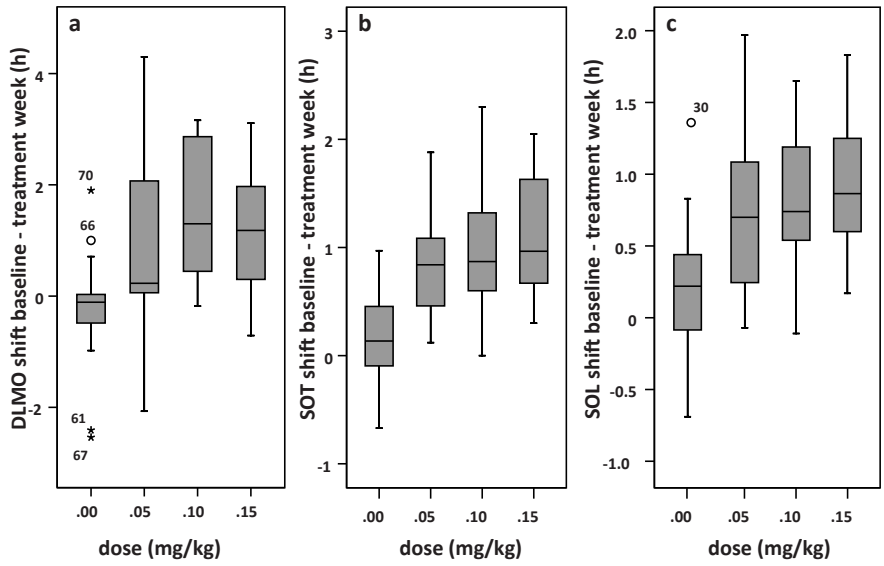


Fig. 2 a DLMO (threshold=4 pg/ml) advance (individual differences between baseline and treatment week) in the four treatment groups.
b SOT shift (individual differences between baseline and treatment week) in the four treatment groups.
c SOL reduction (individual differences baseline and treatment week) in the four treatment groups. Solid box upper and lower quartiles, box length contains the middle 50% of the data (IQR); line median, lines extending from box (whiskers) the distance to the largest and smallest observations that are less than one quartile range from the box, dots O outliers (>1.5xIQR); x extremes (>3xIQR). DLMO dim light melatonin onset, SOT sleep onset, SOL sleep onset latency.

SOT advanced in all three melatonin groups compared to placebo; the SOT shift difference between melatonin treatment and placebo treatment is 42–56 min, which is significant (p<0.001) for all melatonin groups.

SOL was reduced in all three melatonin groups compared to placebo. The difference between placebo treatment and melatonin treatment for SOL shift was 31–42 min, and the reduction of SOL differed significantly in all three treatment groups from placebo (p=0.007, p=0.001, and p<0.001).

Because no clear dose–response relationship was detected in all groups, the individual time of administration of melatonin relative to baseline DLMO were calculated (circadian TOA). Shifts of DLMO, SOT, and SOL in the three groups with melatonin were plotted as function of clock TOA (Fig. 3a) and as function of circadian TOA (Fig. 3b). These figures demonstrate the relationship of the DLMO shift with circadian TOA and not with clock TOA. On the contrary, for SOT and SOL shifts, the TOA relationship does not show distinct differences between clock TOA and circadian TOA.

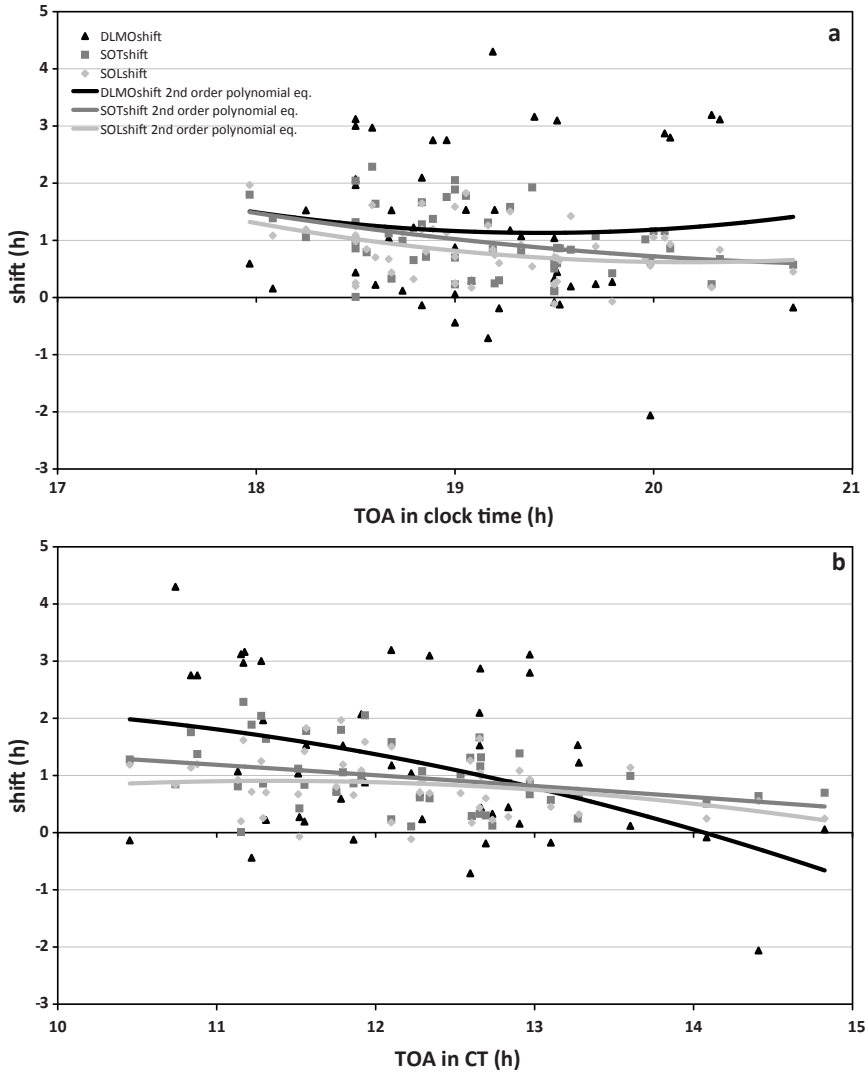


Fig. 3 a DLMO, SOT, and SOL shifts with clock TOA in the three melatonin-treatment groups. b DLMO, SOT, and SOL shifts with circadian TOA in the three melatonin-treatment groups.

Dose–response relationship versus time–response relationship

The shifts of DLMO, SOT, and SOL are visualized in Fig. 2a–c.

PAD was significantly correlated to the DLMO shift, but not to the SOT and SOL shift (Fig. 4).

Curve fitting of DLMO shift (of the three melatonin groups) with TOA expressed in relative Circadian Time, with DLMO (CT14) as reference point=0 according to (Burgess et al. 2008) resulted in the small part of the expected PRC of melatonin (R^2=0.175, p=0.015; Fig. 5a).

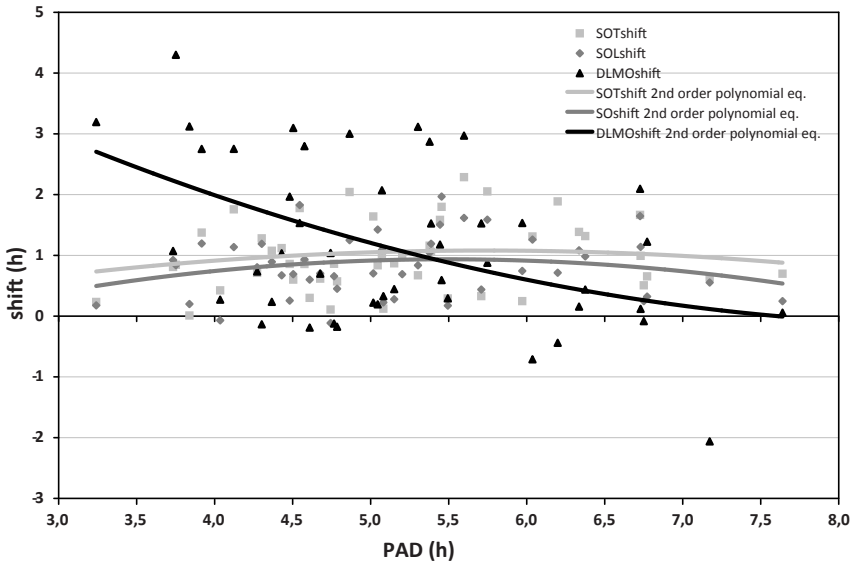


Fig. 4 DLMO, SOT, and SOL shifts with PAD in the three melatonin-treatment groups.

Dosage differentiation did not result in distinct curves due to the small number of subjects per dose (*n*=16–19).

Curve fitting of SOT shift versus TOA in relation to baseline DLMO resulted in distinct curves for all groups (Fig. 5b). For the higher doses, a bigger shift was noted with an early TOA; for the TOA closer to the DLMO, this dose relationship disappeared.

In the bivariate correlation analysis, the dose was significantly correlated with all outcome parameters (DLMO, SOT, and SOL shift), as was the circadian TOA, when tested in all treatment groups.

DLMO shift was correlated with SOT shift and SOL shift as well (Spearman correlation r_s=0.38, p=0.003 and r_s=0.36, p=0.05). None of the sleep outcome parameters appeared to be significantly related to clock TOA, in contrast to the circadian TOA.

After exclusion of the placebo group from analysis, correlation of all sleep parameters with dosage disappeared, as did the previous association of the DLMO shift with SOT shift and SOL shift (Table 3). After exclusion of placebo, correlation between SOT shift and clock TOA became significant, in addition to the relation with circadian TOA. For SOL shift, exclusion of the placebo group resulted in an additional correlation with clock TOA and in disappearance of the correlation with the circadian TOA. All correlations with TOA are negative, indicating a larger shift when medication is taken earlier.

DLMO shift was significantly correlated to PAD and circadian TOA, and not to clock TOA (Table 3).

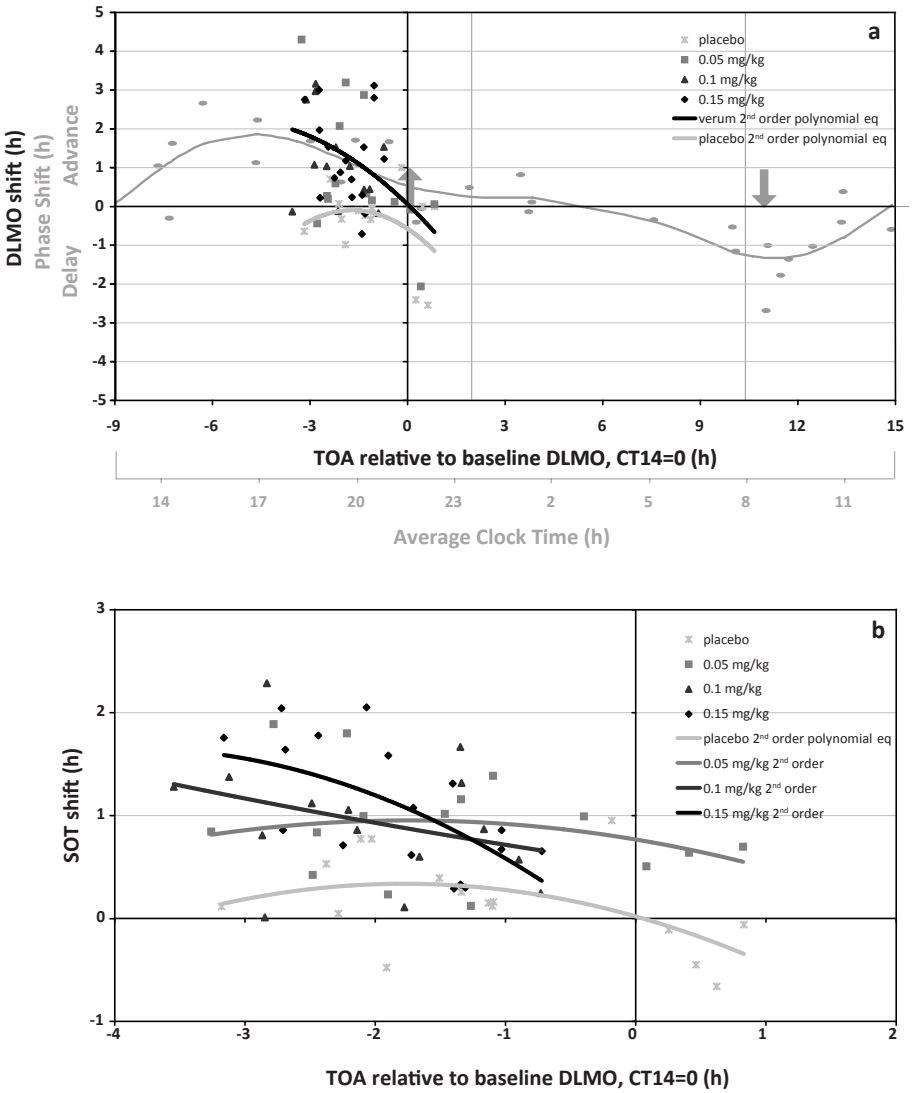


Fig. 5 a DLMO shift (individual differences between baseline and treatment week) with TOA related to baseline DLMO, for all groups, plotted on top of a 24-h phase response curve adapted from Burgess et al. (2008).
b SOT shift (individual differences between baseline and treatment week) with TOA related to baseline DLMO in all treatment groups.

Adverse effects

The most common adverse events were red cheeks, red earlobes, and red eyes and yawning within an hour after administration (*n*=15); pale looks, dizziness, and cold feelings (eight); headache (two); nausea and stomachache (one); and dizziness and nausea (one). Most of the adverse events wore off during the treatment week. Headache

Table 3 Results of bivariate correlation analysis for dosage, PAD, TOA in clock time and in circadian time, and shifts of DLMO, SOT, and SOL, tested for melatonin treatment groups 1–3 (n=46–53)

	Dosage	PAD	Clock TOA	Circadian TOA	DLMO shift	SOT shift
PAD	0.16					
	p=0.13					
Clock TOA	−0.05	−0.18				
	p=0.37	*p*=0.11				
Circadian TOA	−0.10	−0.65	−0.32			
	p=0.26	*p*<0.001	*p*=0.012			
DLMO shift	0.15	0.37	−0.09	−0.33		
	p=0.16	*p*=0.005	*p*=0.28	*p*=0.022		
SOT shift	0.17	0.17	−0.35	−0.38	0.03	
	p=0.12	*p*=0.13	*p*=0.006	*p*=0.004	*p*=0.42	
SOL shift	0.15	0.12	−0.32	−0.29	0.15	0.75
	p=0.15	*p*=0.21	*p*=0.011	*p*=0.024	*p*=0.17	*p*<0.001

Correlations with DLMO measures (DLMO shift and Circadian TOA) are Spearman correlations; the others are Pearson correlations
PAD phase alignment difference, DLMO dim light melatonin onset, TOA time of administration, SOT sleep onset time, SOL sleep onset latency

and stomachache were reported in the placebo group, not in the melatonin-treatment groups. The sleep-related adverse events (red cheeks or rather pale looks, cold feelings) and dizziness were reported in the three melatonin groups; the frequency was related to dosage (0.15:0.1:0.05=5:4:3). One participant ended the treatment period early due to bedwetting, attributed to the medication by his mother (0.05 mg/kg). Two other participants reported enhanced urination during the evening and night (0.1 and 0.15 mg/kg).

DISCUSSION

In children with chronic sleep onset insomnia, 1-week treatment with melatonin significantly advanced sleep onset and dim light melatonin onset by approximately 1 h and reduced sleep onset latency by approximately 35 min, compared to placebo. Surprisingly, there was, within the dosage range of 0.05–0.15 mg/kg, no dose–response relationship of melatonin and shifting of the sleep parameters or DLMO.

It is unlikely that the treatment duration of 1 week was too short to show differences in the efficacy between dosages. Data from earlier studies of melatonin effects on sleep parameters with duration of 5 weeks showed that as early as after the first treatment night, robust treatment effects emerged and that the effects remained stable during the following weeks (van Geijlswijk et al. 2010).

There may be a point of diminishing returns at a dosage lower than the tested lowest dosage of 0.05 mg/kg. Hence, each additional increase in dosage beyond this dosage yields less and less additional response, until reaching a "ceiling effect," like the upper right part of a traditional dose–response curve. Another possibility is that the dose–response relationship reflects an "all-or-nothing" principle. That is, all dosages above a certain threshold dose induce similar magnitudes of responses, like the acetylcholine receptor-mediated innervations of motor cells (Ruff. 1998). The absence of a dose–response relationship in this study is in line with findings in a sleep–EEG study where melatonin was administered at 6:00 p.m. in the dose range of 0.5-10 mg in six healthy adults (Stone et al. 2000).

In contrast, the timing of drug administration seems to have substantial influence on the treatment effect. TOA was recorded daily; the naturalistic design of this study allowed for some flexibility on this aspect. As a result of this, the average TOA on Friday and Saturday was later than the TOA on weekdays. When correcting for circadian TOA, the differences between the 0.05 mg/kg group and the other two dosing groups for mean DLMO shift disappear. This is at least partly due to the considerably wider range of DLMO–TOA interval values in the 0.05 mg/kg group that can be attributed to the extreme minimum and maximum results of the DLMO shift ([−2.04]–4.18) within this group.

From a pharmacokinetic point of view, one could argue that the lower the dosage, the shorter the interval between TOA and DLMO should be since melatonin has a very short elimination half-life in most individuals (between 35 and 45 min). Recently, the association between time of administration and dosage in relation to endogenous melatonin onset is made (Burgess et al. 2008). It's plausible that very low dosages (0.5 mg or less) given early (5 h before DLMO) are already cleared to below physiological levels before endogenous melatonin onset occurs, and we expect that no shift of DLMO will be observed (Burgess et al. 2008). This phenomenon might also have contributed to the nonsignificant DLMO shift observed in the 0.05 mg/kg group, since the maximum of DLMO–TOA interval was similar (low dosage to high dosage 3.27, 3.55, and 3.17 h) in all melatonin groups.

SOT was significantly advanced by administration of exogenous melatonin. The magnitude of effect was not predicted by dosage but was significantly related to clock TOA and to circadian TOA. Especially, the correlation with clock TOA could imply that the effects on SOT and SOL that we measured were induced by the direct soporific effects of melatonin rather than by a chronobiotic effect, comparable to the way traditional sedatives act.

There is a methodological difference between measuring the DLMO shift and measuring SOT and SOL shifts. Post-treatment DLMOs are determined after a period of melatonin

administration; but on the night of melatonin measurements, no exogenous melatonin is administrated. The DLMO shift is, therefore, not influenced by direct effects of administrated melatonin. This is in contrast to the effects on SOT shift and SOL shift, which are influenced by melatonin administration on the measurement nights. This might explain the relationship of PAD with DLMO shift, and not with SOT and SOL shift, the DLMO shift reflecting exclusively chronobiotic effects. The soporific effect of melatonin improves SOT and reduces SOL, which is why individuals with a PAD ≥6 still experience a SOT and SOL shift, without a DLMO shift.

The children included all had late DLMOs. A long TOA–DLMO interval in this population will result in a large response to melatonin therapy, in DLMO shift, which is a demonstration of the chronobiotic mechanism, and in SOT and SOL shift. In addition to the chronobiotic effect, soporific effects of melatonin will add to the size effect on SOT and SOL. The effect of the same dosage of exogenous melatonin on SOT in a normal population can be completely different since this DLMO–TOA interval will be shorter when taken at the same clock TOA. Melatonin administration at the TOA of traditional hypnotics confers risk for the TOA being later than DLMO, thus minimizing the potential for phase advancing the rhythm (Fig. 5a). This may be the mechanism behind the inefficacy of melatonin as an ordinary hypnotic. When timing is correct, the magnitude of effect on SOT, SOL, and DLMO is not related to the dose in the threefold dose range we have studied. This supports earlier findings stressing the importance of measuring DLMO before starting melatonin treatment (Hoebert et al. 2009).

A number of potential limitations need to be noted. First, this trial assessed the effects of only 1-week treatment with melatonin. In children with sleep onset insomnia using melatonin, drug-holiday breaks during 1 week result in return of the former sleep pattern in more than 90% of the users (Hoebert et al. 2009). This implies that the chronobiotic effect can only be sustained with chronic treatment, although in children, the need for advancing sleep onset disappeared in 8% of the children after 4 years of treatment (Hoebert et al. 2009). For the report of adverse events, long-term studies need to be done. In fact, we did readdress the participants of this trial 1.5–4.6 years after inclusion and evaluated their experiences with prolonged therapy. We will report on this soon.

Second, the groups are small, 16–19 observations per group. Additionally, third, we should interpret all outcomes after correction for the multiple statistical comparisons of DLMO, SOT, and SOL with the standard Bonferroni procedure. This procedure is under discussion for its usefulness and limitations, especially in small-numbered studies like this (Nakagawa. 2004). This is why we finally decided to report all *p* values instead of reporting significance categories. Fourth, TOA in this study neither depended on applied dosage nor DLMO; TOA was determined in a naturalistic way instead. This caused a wide range of DLMO–TOA intervals, which might have hampered the effects, especially of the lowest dosage. Due to the double-blind dose assigning, a dose-related TOA was not

even possible. In future study design, this relationship should be taken into account; for instance with lower doses, a smaller DLMO–TOA interval is strived for.

Strength of the present study is that we studied individual responses. This differs from most melatonin trials, where response consisted of the shift of means of the different treatment groups. We applied a naturalistic design for timed melatonin administration, related to desired bedtime, but with focus on maximizing the DLMO–TOA interval.

The current finding, that the effects of melatonin treatment on sleep–wake rhythm are not related to the dosage in the pharmacologic dosing range (>0.05 mg/kg) but rather to the time of administration relative to the endogenous melatonin rhythm, is highly suggestive of melatonin's chronobiotic properties instead of primarily hypnotic pharmacological properties.

In conclusion, we do not expect that dosages higher than 0.15 mg/kg will exert larger shifting effects (based on the present data and our clinical experience). On the contrary, we recommend that dosages higher than 0.05 mg/kg for children with chronic insomnia are not necessary and probably should be avoided. Whether clinically effective dosages should be expected in the range achieving physiological serum levels or at least in dosages lower than 0.05 mg/kg cannot be inferred from the present data. Further dose–response studies should be performed in order to find the lowest possible dosage of melatonin in children, in combination with the most appropriate time of administration. The issue of bioavailability should be taken into account in further studies, with the sublingual tablet with ultralow dosages as an interesting candidate. Furthermore, additional long-term studies are needed to verify the safety of melatonin in children in the long run.

This study demonstrates that melatonin for treatment of chronic sleep onset insomnia in children is effective in a dosage of 0.05 mg/kg given 1–2 h before DLMO and before desired bedtime, resulting in 1-h shifts of DLMO and SOT and a SOL reduction by 35 min.

ACKNOWLEDGMENTS

We thank Y.G. van der Meer, Pharm D, and technicians *(Gelderse Vallei Hospital, dep Pharmacy*) for facilitating the trial, F. van Dijk *(De Nieuwe Coster BV)* for developing and supporting Medsys, B.C.G Dujardin and analysts (G*elderse Vallei Hospital, dep Laboratory of Clinical Chemistry and Haematology*) for the analysis of all saliva samples, and Prof dr H. Vaarkamp (*Utrecht University, Faculty of Veterinary Medicine, Pharmacy Department*) for helpful discussions and critically reading the manuscript.

Disclosure statement

Pharma Nord, Denmark supplied the melatonin to prepare the individual medication.

REFERENCES

Bendz LM, Scates AC (2010) Melatonin treatment for insomnia in pediatric patients with attention-deficit/hyperactivity disorder. Ann Pharmacother 44:185-91.

Blader JC, Koplewicz HS, Abikoff H, Foley C (1997) Sleep problems of elementary school children. A community survey. Arch Pediatr Adolesc Med 151:473-80.

Bojkowski CJ, Aldhous ME, English J, Franey C, Poulton AL, Skene DJ, Arendt J (1987) Suppression of nocturnal plasma melatonin and 6-sulphatoxymelatonin by bright and dim light in man. Horm Metab Res 19:437-40.

Brainard GC, Rollag MD, Hanifin JP, van den Beld G, Sanford B (2000) The effect of polarized versus nonpolarized light on melatonin regulation in humans. Photochem Photobiol 71:766-770.

Burgess HJ, Revell VL, Eastman CI (2008) A three pulse phase response curve to three milligrams of melatonin in humans. J Physiol 586:639-47.

Cavallo A, Dolan LM (1996) 6-Hydroxymelatonin sulfate excretion in human puberty. J Pineal Res 21:225-30.

Cavallo A, Ritschel WA (1996) Pharmacokinetics of melatonin in human sexual maturation. J Clin Endocrinol Metab 81:1882-6.

Coppola G, Iervolino G, Mastrosimone M, La Torre G, Ruiu F, Pascotto A (2004) Melatonin in wake-sleep disorders in children, adolescents and young adults with mental retardation with or without epilepsy: a double-blind, cross-over, placebo-controlled trial. Brain Dev 26:373-6.

Dahl RE (1996) The impact of inadequate sleep on children's daytime cognitive function. Semin Pediatr Neurol 3:44-50.

Griefahn B, Brode P, Blaszkewicz M, Remer T (2003) Melatonin production during childhood and adolescence: a longitudinal study on the excretion of urinary 6-hydroxymelatonin sulfate. J Pineal Res 34:26-31.

Hoebert M, van der Heijden KB, van Geijlswijk IM, Smits MG (2009) Long-term follow-up of melatonin treatment in children with ADHD and chronic sleep onset insomnia. J Pineal Res 47:1-7.

Jan JE, Hamilton D, Seward N, Fast DK, Freeman RD, Laudon M (2000) Clinical trials of controlled-release melatonin in children with sleep-wake cycle disorders. J Pineal Res 29:34-9.

Jan JE, Espezel H, Appleton RE (1994) The treatment of sleep disorders with melatonin. Dev Med Child Neurol 36:97-107.

Jan MM (2000) Melatonin for the treatment of handicapped children with severe sleep disorders. Pediatr Neurol 23:229-32.

Klerman EB, Gershengorn HB, Duffy JF, Kronauer RE (2002) Comparisons of the variability of three markers of the human circadian pacemaker. J Biol Rhythms 17:181-93.

Kushida CA, Chang A, Gadkary C, Guilleminault C, Carrillo O, Dement WC (2001) Comparison of actigraphic, polysomnographic, and subjective assessment of sleep parameters in sleep-disordered patients. Sleep Med 2:389-96.

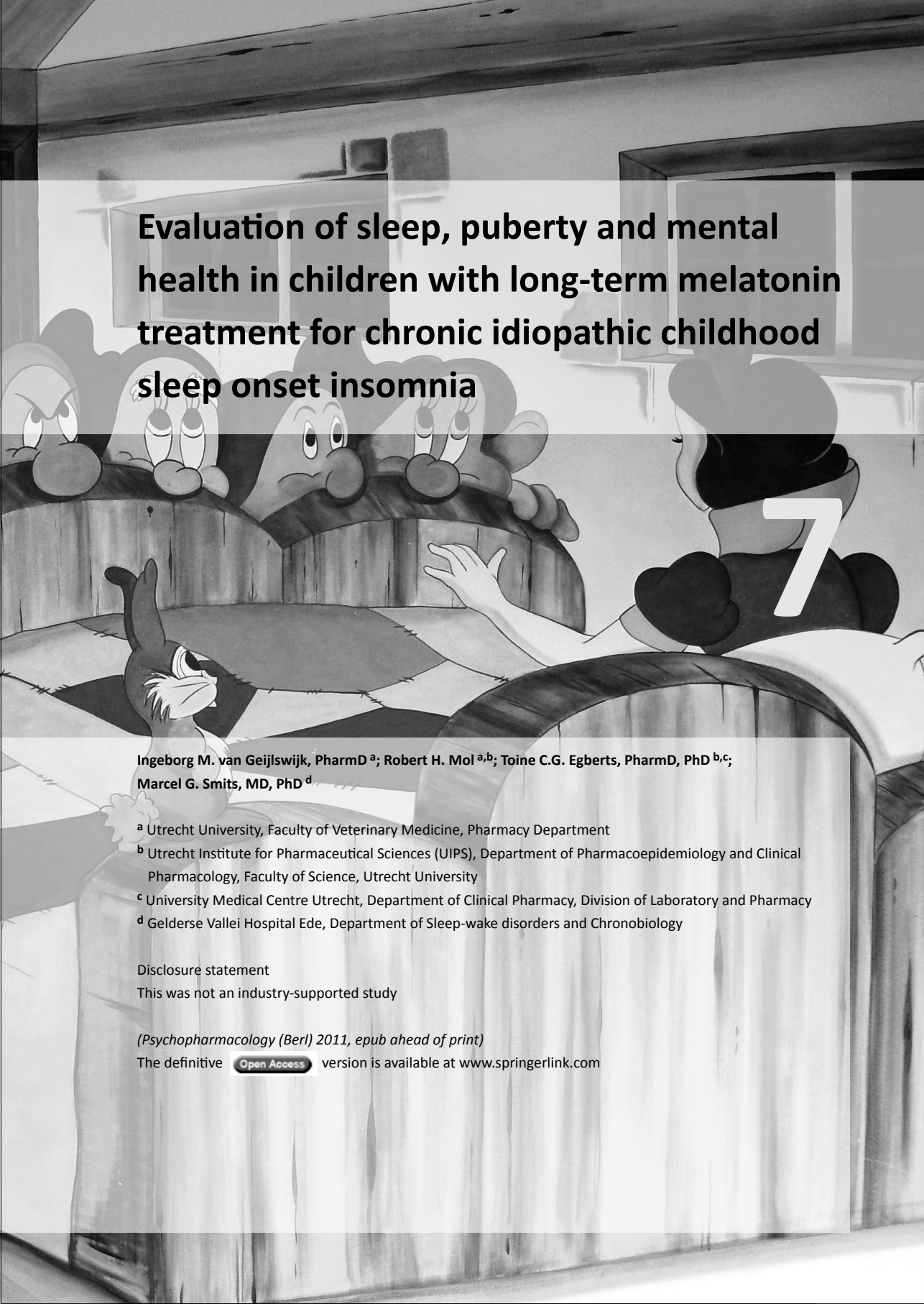
Lam JC, Mason TB (2007) Treatment of sleep disorders in children. Curr Treat Options Neurol 9:404-13.

Lewy AJ, Emens J, Jackman A, Yuhas K (2006) Circadian uses of melatonin in humans. Chronobiol Int 23:403-12.

Lewy AJ, Emens JS, Bernert RA, Lefler BJ (2004) Eventual entrainment of the human circadian pacemaker by melatonin is independent of the circadian phase of treatment initiation: clinical implications. J Biol Rhythms 19:68-75.

Lewy AJ, Cutler NL, Sack RL (1999) The endogenous melatonin profile as a marker for circadian phase position. J Biol Rhythms 14:227-36.

Littner M, Kushida CA, Anderson WM, Bailey D, Berry RB, Davila DG, Hirshkowitz M, Kapen S, Kramer M, Loube D, Wise M, Johnson SF (2003) Practice parameters for the role of actigraphy in the study of sleep and circadian rhythms: an update for 2002. Sleep 26:337-41.
McArthur AJ, Budden SS (1998) Sleep dysfunction in Rett syndrome: a trial of exogenous melatonin treatment. Dev Med Child Neurol 40:186-92.
Morgenthaler TI, Lee-Chiong T, Alessi C, Friedman L, Aurora RN, Boehlecke B, Brown T, Chesson AL, Jr., Kapur V, Maganti R, Owens J, Pancer J, Swick TJ, Zak R (2007) Practice parameters for the clinical evaluation and treatment of circadian rhythm sleep disorders. An American Academy of Sleep Medicine report. Sleep 30:1445-59.
Nagtegaal E, Peeters T, Swart W, Smits M, Kerkhof G, van der Meer G (1998) Correlation between concentrations of melatonin in saliva and serum in patients with delayed sleep phase syndrome. Ther Drug Monit 20:181-3.
Nakagawa S (2004) A farewell to Bonferroni: the problems of low statistical power and publication bias. Behav Ecology 15:2.
Owens JA, Moturi S (2009) Pharmacologic treatment of pediatric insomnia. Child Adolesc Psychiatr Clin N Am 18:1001-1016. DOI: 10.1016/j.chc.2009.04.009.
Pandi-Perumal SR, Srinivasan V, Spence DW, Cardinali DP (2007) Role of the melatonin system in the control of sleep: therapeutic implications. CNS Drugs 21:995-1018.
Ring A, Stein D, Barak Y, Teicher A, Hadjez J, Elizur A, Weizman A (1998) Sleep disturbances in children with attention-deficit/hyperactivity disorder: a comparative study with healthy siblings. J Learn Disabil 31:572-8.
Ruff RL (1998) Electrophysiology of postsynaptic activation. Ann N Y Acad Sci 841:57-70.
Schmidt F, Penka B, Trauner M, Reinsperger L, Ranner G, Ebner F, Waldhauser F (1995) Lack of pineal growth during childhood. J Clin Endocrinol Metab 80:1221-5.
Smits MG, van Stel HF, van der Heijden K, Meijer AM, Coenen AM, Kerkhof GA (2003) Melatonin improves health status and sleep in children with idiopathic chronic sleep-onset insomnia: a randomized placebo-controlled trial. J Am Acad Child Adolesc Psychiatry 42:1286-93.
Smits MG, Nagtegaal EE, 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.
Stone BM, Turner C, Mills SL, Nicholson AN (2000) Hypnotic activity of melatonin. Sleep 23:663-9.
Sutcliffe AG, Wong IC (2006) Rational prescribing for children. BMJ 332:1464-5.
Szeinberg A, Borodkin K, Dagan Y (2006) Melatonin treatment in adolescents with delayed sleep phase syndrome. Clin Pediatr 45:809-18.
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 46:233-41.
van der Heijden KB, Smits MG, van Someren EJ, Boudewijn Gunning W (2005) Prediction of melatonin efficacy by pretreatment dim light melatonin onset in children with idiopathic chronic sleep onset insomnia. J Sleep Res 14:187-94.
van Geijlswijk IM, Didden R, van der Heijden KB, Smits MG (2010) Onset and stability of melatonin treatment effect in childhood sleep onset insomnia. Sleep Sci 3:16-21.
Vitiello B (2007) Research in child and adolescent psychopharmacology: recent accomplishments and new challenges. Psychopharmacol 191:5-13.
Voultsios A, Kennaway DJ, Dawson D (1997) Salivary melatonin as a circadian phase marker: validation and comparison to plasma melatonin. J Biol Rhythms 12:457-66.
Waldhauser F, Weiszenbacher G, Tatzer E, Gisinger B, Waldhauser M, Schemper M, Frisch H (1988) Alterations in nocturnal serum melatonin levels in humans with growth and aging. J Clin Endocrinol Metab 66:648-52.
Wasdell MB, Jan JE, Bomben MM, Freeman RD, Rietveld WJ, Tai J, Hamilton D, Weiss MD (2008) A randomized, placebo-controlled trial of controlled release melatonin treatment of delayed sleep phase syndrome and impaired sleep maintenance in children with neurodevelopmental disabilities. J Pineal Res 44:57-64.
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 45:512-9.
Werner H, Molinari L, Guyer C, Jenni OG (2008) Agreement rates between actigraphy, diary, and questionnaire for children's sleep patterns. Arch Pediatr Adolesc Med 162:350-8.



Evaluation of sleep, puberty and mental health in children with long-term melatonin treatment for chronic idiopathic childhood sleep onset insomnia

7

Ingeborg M. van Geijlswijk, PharmD [a]; Robert H. Mol [a,b]; Toine C.G. Egberts, PharmD, PhD [b,c]; Marcel G. Smits, MD, PhD [d]

[a] Utrecht University, Faculty of Veterinary Medicine, Pharmacy Department
[b] Utrecht Institute for Pharmaceutical Sciences (UIPS), Department of Pharmacoepidemiology and Clinical Pharmacology, Faculty of Science, Utrecht University
[c] University Medical Centre Utrecht, Department of Clinical Pharmacy, Division of Laboratory and Pharmacy
[d] Gelderse Vallei Hospital Ede, Department of Sleep-wake disorders and Chronobiology

Disclosure statement
This was not an industry-supported study

(Psychopharmacology (Berl) 2011, epub ahead of print)
The definitive Open Access version is available at www.springerlink.com

ABSTRACT

Objectives

To establish whether long-term use of melatonin influences pubertal development, sleep quality and mental health development in children as compared with the normal Dutch population of the same age.

Methods

This follow-up research study was conducted in children included in a previous melatonin dose-finding trial. Outcomes were measured using questionnaires Strength and Difficulties Questionnaire (SDQ), Children Sleep Habits Questionnaire (CSHQ and Tanner Stages) adopted for Dutch children. Mean duration of therapy, persistence of effect, adverse events and (other) reasons leading to cessation of therapy were additional objectives of this study.

Results

Mean years of usage (*n*=51) was 3.1 years (min 1.0 year, max 4.6 years), mean dose 2.69 mg (min 0.3 mg, max 10 mg). Mean SDQ score, mean CSHQ score and Tanner Stages standard deviation scores did not differ in a statistically significant way from published scores of the general Dutch population of the same age and sex.

Conclusions

This follow-up study demonstrates that melatonin treatment in children can be sustained over a long period of time without substantial deviation of the development of children with respect to sleep quality, puberty development and mental health scores, as compared with the general Dutch population.

Key Words: Melatonin treatment, puberty, long-term effect, CSHQ, SDQ, Tanner

INTRODUCTION

The prevalence of chronic sleep onset insomnia (CSOI) is approximately 10% among the non-disabled school-aged population (Blader et al. 1997). CSOI is the inability to fall asleep at the desired sleep time. A stable sleep schedule that is substantially later than the conventional or desired time is one of the main symptoms of delayed sleep phase disorder (DSPD) (Sack et al. 2007). CSOI, combined with the finding of (an age relative) late dim light melatonin onset (DLMO), is suggestive for DSPD. Late melatonin onset in children between 6 and 12 years old is defined as a DLMO later than the mean DLMO in children without CSOI, being 7:45 p.m. ± 60 min in children of 8.2 y ± 2.1 y (Smits et al. 2003). In the Netherlands, melatonin is increasingly used for the treatment of children with idiopathic CSOI and late melatonin onset . In the second half of 2008, melatonin capsules took the fifth rank of extemporaneous mixtures compounded in Dutch pharmacies (Anonymous 2009).

A recent meta-analysis showed the short-term efficacy and safety of melatonin in adults as well as in children with a mean reported DLMO between 8:37 p.m. and 9:06 p.m. (van Geijlswijk et al. 2010a). Also in children with attention deficit hyperactivity disorder (ADHD) (van der Heijden et al. 2007; Weiss and Salpekar 2010) or autistic spectrum disorder (ASD) (Cortesi et al. 2010), melatonin effectively improved quality of sleep.

However, treatment of children with melatonin has been controversial, because of its effects on reproduction in animals (Arendt 1997; Szeinberg et al. 2006).

Schertbarth *et al.* concluded that the normal variation in melatonin levels resulted in different results of gonadotrophic effect and reproduction, depending on the animal species involved. Melatonin is solely the mediator for the environmental cue that activates the seasonal breeder organism in a species appropriate way to the seasonal changes (Scherbarth and Steinlechner 2010). Even though humans are no seasonal breeders, there is still concern that enduring high nocturnal levels due to exogenous melatonin uptake might postpone puberty onset (Srinivasan et al. 2009).

Case reports of precocious puberty (Waldhauser et al. 1991) and delayed puberty development associated with a disturbed melatonin rhythm (Verhoeven and Massa 2005) have led to different hypotheses about puberty and lowered nocturnal melatonin levels (Waldhauser et al. 1991; Debruyne 2006; Srinivasan et al. 2009; Silman 2010). The lower nocturnal levels of melatonin in children after onset of puberty and adults might stem from growth (Silman 2010). However, in children with precocity ,also lower nocturnal melatonin levels are found in comparison with preadolescent children of the same age and stature (Waldhauser et al. 1991). This suggests that lower levels of melatonin are not a result of a larger volume of distribution due to growth. Moreover, after suppression of the pituitary-gonadal axis resulting in a normalized gonadotropin and sex steroid levels these lower melatonin levels do not normalize to age-related preadolescent levels. This implies melatonin levels are not determined by hormonal status.

Long-term data on efficacy and safety are scarce and especially with respect to pubertal development needed. A recently published long-term study (Hoebert et al. 2009) suggested that long-term use of melatonin is safe. However, these authors did not study the influence on pubertal development.

The objective of this study was to evaluate the long-term efficacy and the long-term safety of melatonin therapy in pre-pubertal children. This is to our knowledge, the first study evaluating pubertal development in children using melatonin for a long period of time in pre-puberty as compared with pubertal development in the general Dutch population (controls).

METHODS AND MATERIALS

Study Design

For this follow-up study, all participants of the Meldos trial, a melatonin dose-finding investigation in children with CSOI were invited (van Geijlswijk et al. 2010b). The study consisted of a written interview with inventory questions about demographic data and melatonin use and three international questionnaires about sleep habits, mental health and pubertal development, adapted to the population of Dutch children. The children, having used melatonin for 6 months or longer, were asked to complete the full questionnaire. The Meldos protocol was approved by the institutional review board, as a mono-centre trial by the Central Committee on Research Involving Human Subjects, and registered in the International Standard Randomized Controlled Trial Number Register (ISRCTN20033346). The protocol included the possibility to assess health several years after finishing the placebo-controlled part of the study.

Participants

All children that participated in the Meldos trial between May 2004 and February 2007 (van Geijlswijk et al. 2010b) were eligible to participate in this follow-up study. After finishing the Meldos trial, the participants were prescribed melatonin; the dose was determined by the subjective results of the last trial week. In the previous Meldos trial eligible participants were children aged between 6 and 12 years who were in good general health, otherwise than suffering from sleep onset insomnia more than four nights a week for more than 1 year, based on parental reports. Sleep onset insomnia was defined as sleep onset later than 8:30 P.M. in children aged 6 years and for older children, 15 min later per year until age 12 (10:00 P.M.). Furthermore, the latency between lights-off time and sleep onset (sleep onset latency) had to be more than 30 min on average. Sleep hygiene interventions did not result in better sleep. Exclusion criteria were CSOI due to psychiatric or pedagogic problems, known intellectual disability, pervasive developmental disorder, chronic pain, known disturbed hepatic or renal function, epilepsy, prior use of melatonin, and use of

stimulants, neuroleptics, benzodiazepines, clonidine, antidepressants, hypnotics, or beta-blockers within 4 weeks before enrolment.
In December 2008, questionnaires were sent to all 69 children who completed the placebo-controlled part of the Meldos trial.

Outcome measures

The questionnaire consisted of four distinct parts.

1. Demographic data and melatonin use.
   Part I were 24 multiple choice, open and scaled questions about continuance of melatonin usage, the way melatonin prescription was obtained, the applied dose(s), therapy compliance, drug-holidays, other medication, length and weight, school, sports, reading activities, gaming and watching TV, occurrence and severity of headache.
2. Mental health
   In part II, 25 questions about mental health, to assess social development were asked. Mental health was assessed by means of the self-administered Dutch version of the Strengths and Difficulties Questionnaire (SDQ) for adolescents and children. The SDQ is a questionnaire that is suitable as an index of therapy outcome (Garralda et al. 2000; Goodman 2001; Muris et al. 2003).
   It includes 25 symptom items and measures both negative and positive behavioural and emotional attributes of the child or adolescent. There are five sub-scales: emotional symptoms, conduct problems, hyperactivity–inattention, peer relationship problems and pro-social behaviour. Every item has three categories: 'not true' (0), 'somewhat true' (1) or 'certainly true' (2). The scores were summed for each scale. A total difficulties score was calculated by summing the scores of all the items, except those of the pro-social behaviour scale. This Dutch version is validated as a parents questionnaire in the Dutch population (Muris et al. 2003) (Muris et al. 2004), and it is also used as a self-administered version in 13 and 14-year-old children (Havas et al. 2010). The SDQ in this study addressed the parents and children, irrespective of age.
3. Sleep habits
   Part III consisted of the Children's Sleep Habits Questionnaire (CSHQ), which is a retrospective, 45-item parent questionnaire that has been used in a number of studies to examine sleep behaviour in young children. The CSHQ includes sleep complaints in this age group: bedtime behaviour and sleep onset; sleep duration; anxiety around sleep; behaviour occurring during sleep and night wakings; sleep-disordered breathing; parasomnias; and morning waking/daytime sleepiness. Parents are asked to recall sleep behaviours occurring over a 'typical' recent week. Items are rated on a three-point scale: 'usually' if the sleep behaviour occurred five to seven times/week; 'sometimes' for two to four times/week; and 'rarely' for zero to one time/week (Owens et al. 2000). The Dutch version was recently validated in the Dutch population (Waumans et al. 2010). The tool to objectify sleep in the Meldos study was dim light

melatonin onset (DLMO) and Sleep Onset and Sleep Onset Latency, obtained by actigraphy. Additionally, sleep hygiene measures in Meldos were evaluated by means of a questionnaire based on the Sleep Disturbance Scale of Children (SDCS) (Bruni et al. 1996). Since these parameters change with age, especially in puberty, repeating these measurements for this study seemed of less value than comparing the outcomes of this questionnaire to outcomes in controls.

4. Pubertal development
   Pubertal development was assessed by three Tanner score questions and one additional question for girls (mothers age at menarche) and two additional questions for boys (oigarche age [the age at first ejaculation (Laron et al. 1980; Carlier and Steeno 1985)] of him and his father).

The Tanner scores consist of three scores for boys and three for girls, describing size of genitals, testicles and growth of pubic hair in boys, and breasts, pubic hair and menarche in girls (Marshall and Tanner 1969; Marshall and Tanner 1970). The Tanner scores were self-reported, based on photographs and sketches of testicle volumes added to the questionnaire (Vlaamse Groeicurven 2004). Results in our population were compared with the general Dutch population (Mul et al. 2001) to assess pubertal development.
Timing of pubertal development is influenced by genetic factors. Comparison of pubertal timing between generations is difficult because of the absence of a distinct criterion apart from menarche in girls (Carskadon and Acebo 1993; Sedlmeyer et al. 2002; Wehkalampi et al. 2008). The age of oigarche was added as a menarche equivalent for boys for its distinct value, if attainable. The parents' ages at menarche and oigarche were retrieved as indicators for genetic predisposition for early or late puberty onset.
A successful pilot of the questionnaire was done in five children.

Data Analysis

Primary outcomes are SDQ score, CSHQ score and Tanner scores of children under melatonin treatment for more than 6 months. Secondary outcomes are percentage of persistent use of melatonin in this group of children, mean effective dose, reported adverse events, menarche/oigarche related to parental menarche/oigarche.
For the analysis of the SDQ and CSHQ scores, one sample *t* test was applied to compare scores obtained in (subgroups of) this population with previously published scores of the general Dutch population of the same age and/or sex (controls). Additionally, the CSHQ score was compared with the score in a subpopulation identified as without sleeping problems, and with the score in a subpopulation with sleeping problems.
Tanner scores were analyzed using the web application (http://vps.stefvanbuuren.nl/puberty) (van Buuren and Ooms. 2009). This tool calculates standard deviation scores (SDS) of individual observations of Tanner scores, and additionally plots those scores in a stage line diagram. The traditional way to evaluate an individual's score to a population is

the application of a nomogram, which calculates the relative position of the individual as compared with his/hers age-peers. This is suitable for continuous parameters like length or weight, the standard deviation of the mean determining the relative position between 0 and 100, p0 meaning that 0% of the general population is smaller/weighing less, p100 that 0% of the general population is taller/weighing more. But it is not very convenient for processes that occur in a limited period of time, like breast growth and are characterized by discrete measures (stages) instead of a continued parameter. The determination of the deviation of a distinct stage at a specified age in relationship to the distribution (expressed in SDS) of this stage over ages in controls (data of 1997 (Mul et al. 2001)) moderates the evaluation of puberty development in a more continuous way. The stage line diagram is created by modelling the probabilities of successive category transitions in the reference data (general population) as functions that are smooth in age. Then, the mid-*p* value for each category is calculated and transformed into the *Z* scale by a probit transformation (van Buuren and Ooms 2009). The resulting scores can be plotted against age to produce the stage line diagram. A first stage, like B1 (breasts-1, meaning no sign of growth yet) is present in 100% (mid-*P* value) of the general population of 8-year-old girls, but in 12-year old-girls this stage occurs only in 10% of the girls (late), SDS=1,645. For the most mature breasts stage, B5, the opposite is true; for 13-year-olds this stage occurs only in 10% of the girls (early), whereas the mid-*P* value of 100% is reached in nearly all 20-year-olds.

RESULTS

Demographics and melatonin use

The questionnaire was returned by 59 of the 69 children (Fig.1). Two children continued melatonin use for less than 6 months, and refrained from completing the questionnaire. The mean age of the remaining 57 children was 12.0 (min 8.6, max 15.7 years). Nine children discontinued melatonin therapy after more than 6 months and did complete the questionnaire. Melatonin was still used by 48 children at the time of questioning, mean duration of use was 3.1 years (min 1.0, max 4.6 years) and mean dose was 2.7 mg (min 0.3, max 10 mg) (Table 1).

Effect of cessation

Eleven children stopped therapy. In one case because the family physician decided that 6 months of therapy was enough, one boy because even 10 mg of melatonin did not have a sustained effect, one girl because of the adverse event of apathy combined with weight gain, and eight children because the need of early sleep disappeared. Of those eight, one child indicated to have adopted a delayed sleeping pattern and one child accepted increased sleep onset latency. The remaining six children indicated the sleep onset insomnia had disappeared.

Drug-free intervals

Melatonin therapy was interrupted during holidays by 31 children. Reasons for interruption were the advice of the prescribing physician, a delayed rhythm during holidays and checking the continuing need for melatonin to advance sleep rhythm. Four children skip

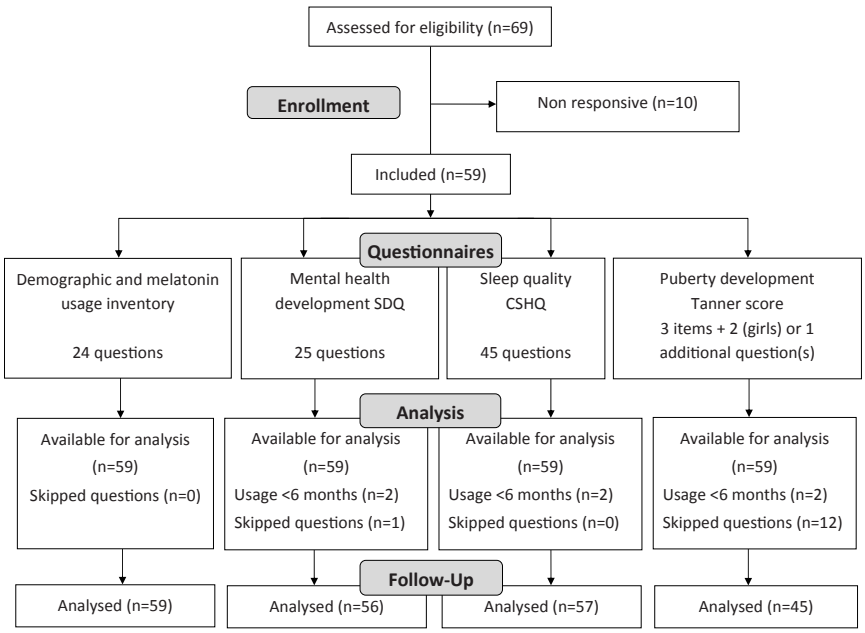


Fig. 1. Justification of obtained outcome data

Table 1 . Demographic characteristics of participants

	No.	Mean	SD	Min	Max	Median
Age (year)	57	12.0	1.73	8.6	15.7	12.0
Dose (mg)	54	2.7	1.74	.3	10.0	2.5
Period of use (year)	51	3.11	.87	1.00	4.61	3.11
TOA-bedtime interval (h)	56	1.05	.61	0	2.50	1.00
Sports (h/week)	56	3.80	2.18	0	10	3
Reading (h/week)	55	4.4	3.74	0	15	3
Gaming and watching tv (h/week)	53	13.3	8.34	0	35	14
BMI (kg/m^2)	49	17.8	2.19	13.6	24.6	18.0
Bedtime (hh:mm)	57	8:56 p.m.	0:44	7:30 p.m.	10:30 p.m.	9:00 p.m.
Sleep time (h/night)	55	9.6	.85	8	11	10

(ped) medication on a regular basis every week, for instance in the weekends, or one or three days a week. Eighteen children never skip(ped) medication (unless forgotten).

Adverse events

Several adverse events were reported: nausea at start (one child), apathy combined with weight gain (one child), weight gain (one child), nocturnal diuresis (three children), short temper during high-dose therapy (10 mg) (one child).

We explicitly asked for the occurrence of headache. Twenty one (38%) children reported suffering from headache regularly (once a week-once a month), 11 (20%) seldom and 23 (42%) never experienced headache.

Mental health

Mean SDQ score was 9.75 (min 2, max 29, SD= 5.72). This score and the subscores of age-related subgroups were compared with the one sample *t* test with previously published scores in controls (Muris et al. 2004; Havas et al. 2010). Muris et al. (2004) studied the validity of the self-report SDQ in 1,111 Dutch, non clinical children, mean age 10.6 years, Havas et al. (2010) studied mental health problems of Dutch adolescents aged 13 and 14 years. Nineteen children (33.3%) of this study population were 13 years or above.

We compared the subgroup of children <13 years with the primary school controls (Muris et al. 2004) and ≥ 13 years with the adolescent controls (Havas et al. 2010). No significant differences on SDQ (sub) scores were found (Table 2).

Muris et al. (2004) reported boys and girls scores separately. The conduct problem score of the girls subgroup aged 8-13 in the Meldos population is statistically significantly lower than the primary school controls, as is their SDQ total score. Other subscores of the girls and all (sub) scores of the boys did not deviate from controls (Table 3).

Table 2. Mental health development in comparison with Dutch primary school children and with Dutch adolescents

	this study boys and girls aged 8-12 (37)		Muris boys and girls aged 8-13 (1111)			this study boys and girls aged 13-15 (19)		Havas boys and girls aged 13-14 (1741-1845)		
	Mean	SD	Mean	SD	t value	Mean	SD	Mean	SD	t value
SDQ_tot	10.05	6.05	10.4	5.4	-.35	9.16	5.10	8.28	4.89	.75
Emotional	2.68	2.20	2.6	2.1	.21	2.37	2.22	2.09	1.97	.55
Conduct	1.86	1.78	2.2	1.6	-1.14	1.16	1.21	1.45	1.34	-1.05
Hyperactivity	3.81	3.00	3.7	2.3	.22	3.53	2.39	3.70	2.45	-.32
Peer	1.65	1.80	2.0	1.7	-1.19	2.11	2.23	1.07	1.31	2.02
Prosocial	7.38	2.06	7.4	1.7	-.06	7.89	1.59	8.00	1.65	-.29

Means of two age subpopulations of this study compared with mean of Muris et al. (2004) and mean of Havas et al. (2010)

Table 3. Mental health development of boys and girls in comparison with a Dutch primary school population

	this study boys aged 8-13 (22)		Muris boys aged 8-13 (549)			this study girls aged 8-13 (25)		Muris girls aged 8-13 (558)		
	Mean	SD	Mean	SD	t value	Mean	SD	Mean	SD	t value
SDQ_tot	12.05	6.87	10.2	5.3	1.26	8.20	4.58	10.7	5.5	-2.73*
Emotional	2.82	1.97	2.2	1.9	1.47	2.40	2.38	3.1	2.2	-1.47
Conduct	2.09	2.04	2.3	1.7	-.48	1.40	1.38	2.0	1.5	-2.17*
Hyperactivity	4.64	3.20	3.7	2.3	1.37	2.88	2.33	3.6	2.3	-1.54
Peer	2.41	2.32	2.0	1.7	.83	1.52	1.58	2.0	1.7	-1.87
Prosocial	7.22	2.26	7.0	1.8	.47	7.92	1.68	7.7	1.6	.65

*=<.05 comparing the mean of a age-related subgroup of boys and girls in this study to the mean of boys and girls of Muris et al.(2004)

Sleep habits

The CSHQ score of our study population was 42.9 (min 34, max 62) (Table 4). This score and the subscores were compared with previously published Dutch population scores (van Litsenburg et al. 2010) (Table 4). Two subscores-sleep onset delay and daytime sleepiness- and the CSHQ total score were significantly higher than in the controls, indicative for worse sleep. Sleep-disordered breathing was significantly lower. In the general population a subpopulation of problem sleepers (PS) was defined based on (subjective) parental report endorsing at least one of the CSHQ items as a problem (van Litsenburg et al. 2010). In comparison with these PS (23%) in the general population, the participants of this study had significantly deviant scores on all eight subscores (indicating better sleep). When compared to the 77% non-problem sleepers (NPS) of this study showed, in addition to the three deviant subscores mentioned earlier, higher subscores for sleep duration, sleep anxiety and night wakings. In conclusion, five out of eight subscores indicated statistically significant worse sleep in this group in comparison with the NPS in the controls; one score indicated better sleep (Table 4).

In accordance with findings in controls, girls in general have higher scores than boys. In contrast to that, in this study population, boys' scores for sleep onset delay were significantly higher in comparison with controls (1.62 vs 1.25, *t* value=2.08), and even higher than the girls' scores (1.53, general population 1.34. *t* value=1.26, NS).

The questionnaire was developed for parental report on sleep behaviour of children between 4 and 10 years old. For this reason, we compared the subgroup of children <11 years with the controls. In this group of 17 children, 8 to 10 years old, no significant diversion of CSHQ scores from the controls was detected (Table 5). The complimentary group of children between 11 and 15 years old showed three statistically significant higher

Table 4. Sleep habit results compared with problem sleepers and non problem sleepers in a Dutch primary school population

	this study Total (57)		Van Litsenburg Total (1282-1507)		Van Litsenburg PS (44-265)		Van Litsenburg NPS (888-1424)	
	Mean	SD	Mean	t value	Mean	t value	Mean	t value
Bedtime resistance	6.79	1.54	6.68	.535	8.81	-9.88***	6.54	1.22
Sleep Onset Delay	1.57	.83	1.30	2.45*	2.11	-4.87***	1.25	2.90**
Sleep Duration	3.79	1.35	3.50	1.62	4.88	-6.12***	3.42	2.07*
Sleep Anxiety	5.18	1.64	4.86	1.45	7.07	-8.73***	4.70	2.19*
Night Wakings	3.86	1.08	3.62	1.68	5.74	-13.19***	3.55	2.17*
Parasomnias	8.33	1.65	8.57	-1.08	10.85	-11.51***	8.40	-.30
Sleep-disordered Breathing	3.12	0.54	3.30	-2.49*	5.08	-27.52***	3.28	-2.21*
Daytime Sleepiness	12.53	3.24	11.16	3.19**	13.77	-2.90**	10.94	3.70***
CSHQ_total score	42.91	5.94	40.50	3.06**	44.72	-2.30*	39.25	4.65***

*=<.05, ** = <.01, *** =<.001 comparing mean of this study to mean of all, mean of Problem Sleepers (PS) and mean of Non Problem Sleepers (NPS) of Van Litsenburg et al. (2010)

Table 5. Sleep habit results of two age sub groups compared with a Dutch primary school population

	this study 7-10 (17)	Van Litsenburg 7-10 (554-632)		this study 11-15 (40)	Van Litsenburg 10-14 (412-507)	
	Mean	Mean	*t* value	Mean	Mean	*t* value
Bedtime resistance	6.76	6.74	.093	6.80	6.48	1.22
Sleep Onset Delay	1.59	1.29	1.41	1.56	1.43	1.02
Sleep Duration	3.29	3.49	-1.18	4.00	3.57	1.81
Sleep Anxiety	5.35	4.96	.98	5.10	4.56	2.08*
Night Wakings	3.47	3.59	-.56	4.02	3.44	3.30**
Parasomnias	8.24	8.64	-.83	8.38	8.23	.61
Sleep-disordered Breathing	3.24	3.29	-.40	3.08	3.24	-1.98
Daytime Sleepiness	11.94	11.02	.93	12.78	11.52	2.82**
CSHQ_total score	41.47	40.57	.67	43.52	40.16	3.50***

*=<.05, ** = <.01, *** =<.001 comparing means of this study to mean of boys, mean of girls, mean of aged 7-10 and mean of aged 10-14 of Van Litsenburg et al.(2010)

subscores and the total score that differed significantly with the age-related controls, indicating worse sleep.

Pubertal development

Tanner Stages standard deviation scores could be determined for 16 boys and 30 girls. Female breasts/pubic hair/menarche SDS were 0.003 (min -1.9, max +1.5)/0.013 (min -1.0, max +1.4)/0.143 (min -0.87 max +2.47) (Fig.2. for all individual female scores). Male genital development/pubic hai/testis volume SDS were 0.038 (min -2.1, max +2.8)/0 .171 (min -1.8, max +2.55)/0.299 (min -1.83, max 2.67) (Fig.3. for all individual male scores). Two boys had all three SDS outside the 80% percentile: one boy in the 1-5 percentile early development, one boy in the 5-10 percentile late development.

Comparison of maternal menarche (median 13 years) with the menarche of the girls (median 12 years) revealed an earlier menarche, in accordance with Mul et al. (2001) who reported a 0.25 year reduction of menarche in the period between 1965 and 1997 in Dutch girls. Oigarche data in the population of this study and in the general population are too scarce to draw conclusions.

DISCUSSION

Six of the 11 children that stopped therapy indicated that the delayed sleep onset had disappeared. This suggests that successful cessation of therapy is attainable after a longer period of melatonin usage without rebound phenomena as demonstrated by typical hypnotics. Adverse events occurred infrequently and were acceptable in most cases, leading to cessation of melatonin use in 1.6%. Upon explicit inquiry, though, most children (58%) reported headache. The finding of regular headaches in 38% of the children was disturbing at first glance. However, recently, Arruda et al. (2010) reported a prevalence of low-frequency episodic headaches (suffering from headaches in the past year but less than 5 days of headache in the past month) of 38.9% in preadolescent children from the general population.

The social development of the 57 former participants of Meldos as measured by SDQ did not deviate from the children without sleep problems of the general population (controls), thanks to or in spite of long-term melatonin use.

The sleep habit questionnaire indicated that the sleep patterns of these long-term melatonin users were not as good as the sleep patterns of healthy sleepers, but better than the patterns of 23% of the controls that were categorized as PS. In fact, all melatonin users were still satisfied with the results of the therapy.

The CSHQ was recently validated in Dutch children (Waumans et al. 2010). It was concluded that this questionnaire is appropriate for ages 4-10, but not for older children. This outcome might reduce our results to 17 valid scores that, however, did not deviate from the controls.

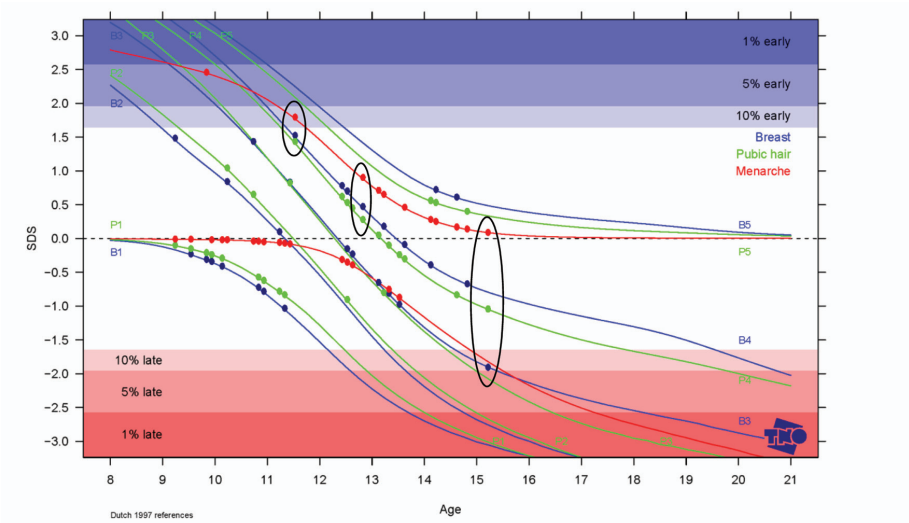


Fig.2. Pubertal development: Stage line diagram with SDS plot for girls (*n*=30)
This figure depicts the SDS values for development of breast (blue lines, five stages), pubic hair (green lines, five stages) and menarche (red lines, yes or no) of 30 girls. The black circles depict the three SDS values for three individual girls, the first circle represents a girl with an early menarche and nearly p90 values for breast and pubic hair, the middle circle represents a girl with p50 development, and the third circle represents a girl with normal menarche but late breast development.

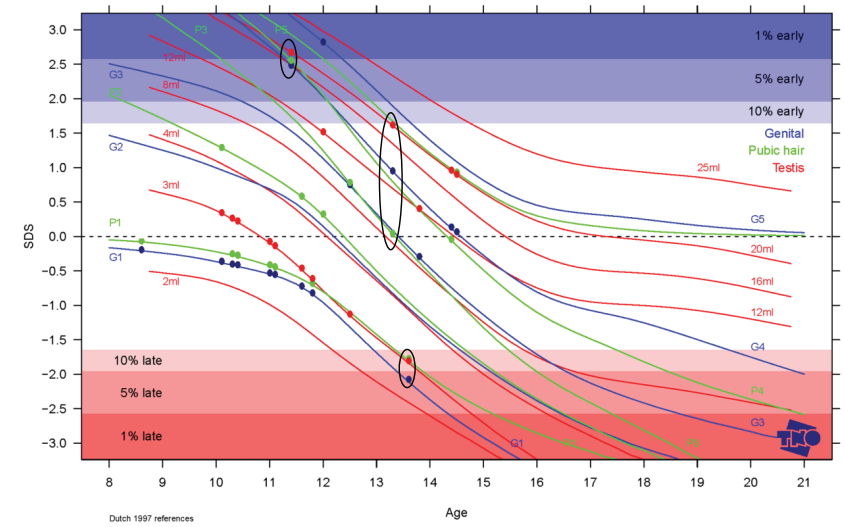


Fig. 3. Pubertal development; Stage line diagram with SDS plot for boys (*n*=15)
This figure depicts the SDS values for development of genitals (blue lines, five stages), pubic hair (green lines, five stages) and testis (red lines, eight volumes) of 15 boys. The black circles depict the three SDS values for three individual boys, the first circle represents a boy with an early development, almost p99 values for all parameters, the middle circle represents a boy with p50 development, and the third circle represents a boy with late development of all parameters (p5).

Nineteen children (33.3%) in our population reached the age of 13 during the follow-up period, which allowed us to evaluate the effects of melatonin therapy on timing of puberty development. The Tanner results indicated undisturbed puberty onset, as did the comparisons of menarche of the girls and their mothers. However, only 62% of the boys and 91% of the girls answered the Tanner score questions. Fourteen children (3 girls and 11 boys) did not answer this part of the interview. . The reluctance to answer the Tanner questions could be caused by (real or perceived) deviated pubertal development, but might also emerge from the strong religious background of some of the children, as for instance, is demonstrated by the absence of TV watching or gaming in some of the children (concerning only boys, not shown) (Table 1). The self-assessment of the scores instead of an intrusive physical examination by a physician might have augmented the response rate at the expense of the objectivity of the results.

The phenomenon of repetitive loss of response after initial good response and dose escalation in response to the effect, wearing off was described in three case reports of (Braam et al. 2010). The loss of response was associated with CYP1A2 poor metabolism, resulting in the loss of rhythmicity of melatonin levels due to saturation kinetics. The incidence of slow CYP1A2 metabolism ranges from 12% to 14% (Butler et al. 1992; Nakajima et al. 1994; Zhou et al. 2009). The boy in who even 10 mg melatonin failed might be a slow metaboliser for CYP1A2.

Some other limitations of our study need to be addressed. The children in our study came from one sleep clinic, and the sample with long-term results is small, 57 children. This population might not be representative for otherwise healthy Dutch children with CSOI. A recently published long-term study (Hoebert et al. 2009) studied the efficacy and safety in a larger population (99). In this study, only 8% of the children stopped therapy after 4 years of treatment. However, most of these children suffered from other health problems, remained under supervision of a specialist and used other medication as well. For evaluation of long-term effects and results of any intervention, one would ideally apply the same measurements in due course. The Meldos study applied a sleep hygiene questionnaire that was deemed inappropriate for the older children of the present evaluation. Even more, even the applied CSHQ was recently invalidated. So, the CSHQ scores may only be valid for 17 children.

In conclusion, we found that melatonin was still used by 81% of children, after a mean term of usage of 3.1 years. Six (10%) children stopped therapy successfully, two others adopted a delayed sleep pattern after cessation. One girl quit melatonin therapy because of apathy and weight gain, one boy quit because of loss of response. One girl was forced to stop therapy by her GP after 6 months. The CSHQ results indicate that the sleep habits in melatonin users are better than in PS without medication, but worse than in NPS. Social development assessed by SDQ indicates a normal development. Puberty onset,

as assessed by Tanner scores, seems to be undisturbed after 3.1 years of exogenous melatonin usage in this limited population.

ACKNOWLEDGEMENTS

We thank all children and their parents for their contribution to this study. This research would not have been possible without the support of the management of the Faculty of Veterinary Medicine Utrecht University. We thank Prof . H. Vaarkamp for his encouragement and his comments during the conduct of the trial and the preparation of this manuscript.

Conflict of interest
All authors state they have no conflicts of interest to declare.

REFERENCES

Anonymous (2009) [Composition of extemporaneous mixtures]. PW 144:11-12. DOI: http://www.sfk.nl/publicaties/farmacie_in_cijfers/2009/2009-11.html.

Arendt J (1997) Safety of melatonin in long-term use (?). J Biol Rhythms 12:673-81.

Arruda MA, Guidetti V, Galli F, Albuquerque RC, Bigal ME (2010) Frequent headaches in the preadolescent pediatric population: a population-based study. Neurology 74:903-908. DOI: 10.1212/WNL.0b013e3181d561a2.

Blader JC, Koplewicz HS, Abikoff H, Foley C (1997) Sleep problems of elementary school children. A community survey. Arch Pediatr Adolesc Med 151:473-80.

Braam W, van Geijlswijk I, Keijzer H, Smits MG, Didden R, Curfs LM (2010) Loss of response to melatonin treatment is associated with slow melatonin metabolism. J Intellect Disabil Res 54:547-555. DOI: 10.1111/j.1365-2788.2010.01283.x.

Bruni O, Ottaviano S, Guidetti V, Romoli M, Innocenzi M, Cortesi F, Giannotti F (1996) The Sleep Disturbance Scale for Children (SDSC). Construction and validation of an instrument to evaluate sleep disturbances in childhood and adolescence. J Sleep Res 5:251-261.

Butler MA, Lang NP, Young JF, Caporaso NE, Vineis P, Hayes RB, Teitel CH, Massengill JP, Lawsen MF, Kadlubar FF (1992) Determination of CYP1A2 and NAT2 phenotypes in human populations by analysis of caffeine urinary metabolites. Pharmacogenetics 2:116-127.

Carlier JG, Steeno OP (1985) Oigarche: the age at first ejaculation. Andrologia 17:104-6.

Carskadon MA, Acebo C (1993) A self-administered rating scale for pubertal development. J Adolesc Health 14:190-195.

Cortesi F, Giannotti F, Ivanenko A, Johnson K (2010) Sleep in children with autistic spectrum disorder. Sleep Med 11:659-664. DOI: 10.1016/j.sleep.2010.01.010.

Debruyne C (2006) Melatonin. Neurobiological background and neuropsychiatric application in children. Tijdschr Psychiatr 48:107-117.

Garralda ME, Yates P, Higginson I (2000) Child and adolescent mental health service use. HoNOSCA as an outcome measure. Br J Psychiatr 177:52-58.

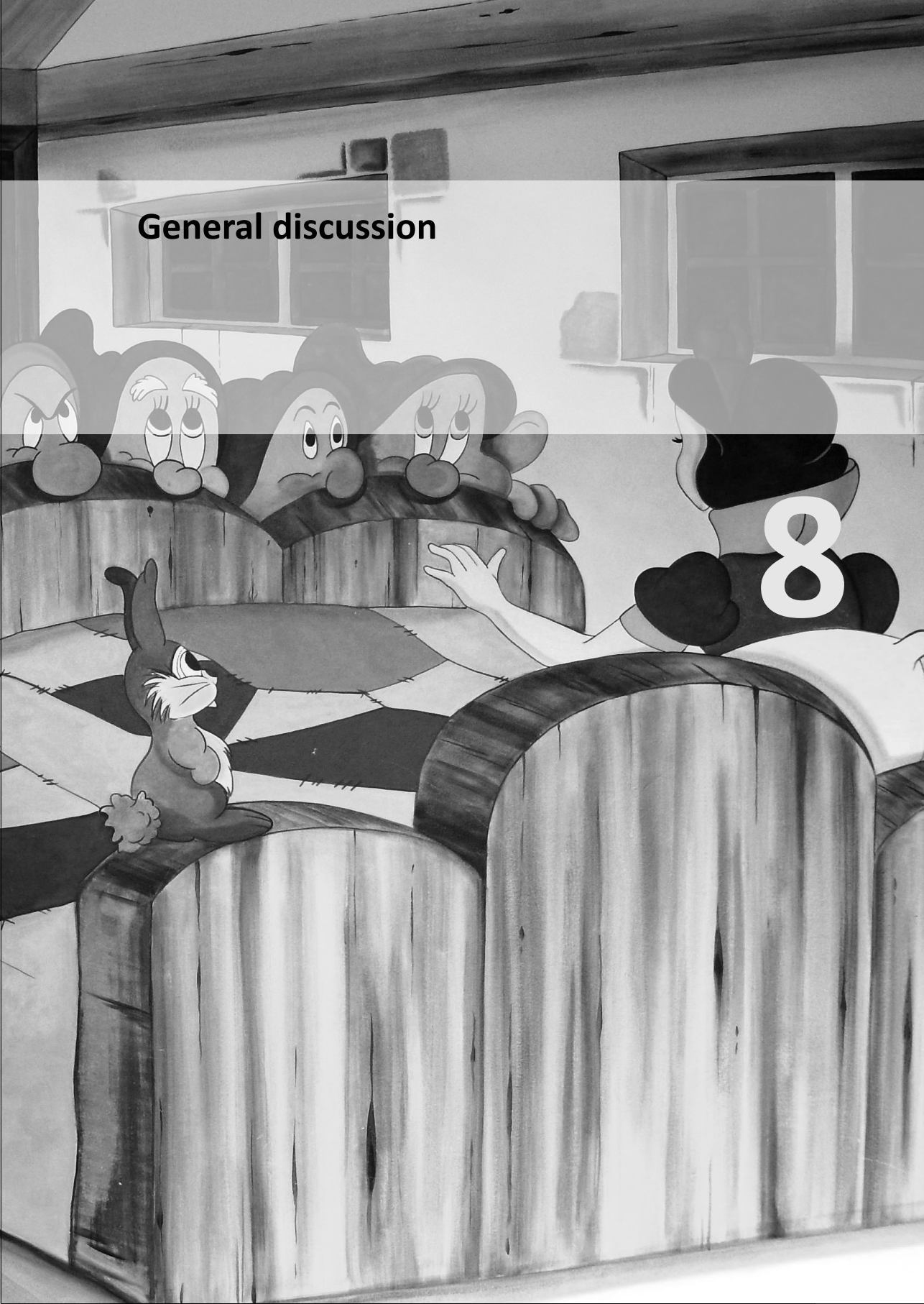
Goodman R (2001) Psychometric properties of the strengths and difficulties questionnaire. J Am Acad Child Adolesc Psychiatry 40:1337-1345. DOI: 10.1097/00004583-200111000-00015.

Havas J, Bosma H, Spreeuwenberg C, Feron FJ (2010) Mental health problems of Dutch adolescents: the association with adolescents' and their parents' educational level. Eur J Public Health 20:258-264.

Hoebert M, van der Heijden KB, van Geijlswijk IM, Smits MG (2009) Long-term follow-up of melatonin treatment in children with ADHD and chronic sleep onset insomnia. J Pineal Res 47:1-7.
Laron Z, Arad J, Gurewitz R, Grunebaum M, Dickerman Z (1980) Age at first conscious ejaculation: a milestone in male puberty. Helv Paediatr Acta 35:13-20.
Marshall WA, Tanner JM (1970) Variations in the pattern of pubertal changes in boys. Arch Dis Child 45:13-23.
Marshall WA, Tanner JM (1969) Variations in pattern of pubertal changes in girls. Arch Dis Child 44:291-303.
Mul D, Fredriks AM, van Buuren S, Oostdijk W, Verloove-Vanhorick SP, Wit JM (2001) Pubertal development in The Netherlands 1965-1997. Pediatr Res 50:479-86.
Muris P, Meesters C, Eijkelenboom A, Vincken M (2004) The self-report version of the Strengths and Difficulties Questionnaire: its psychometric properties in 8- to 13-year-old non-clinical children. Br J Clin Psychol 43:437-448. DOI: 10.1348/0144665042388982.
Muris P, Meesters C, van den Berg F (2003) The Strengths and Difficulties Questionnaire (SDQ)--further evidence for its reliability and validity in a community sample of Dutch children and adolescents. Eur Child Adolesc Psychiatry 12:1-8.
Nakajima M, Yokoi T, Mizutani M, Shin S, Kadlubar FF, Kamataki T (1994) Phenotyping of CYP1A2 in Japanese population by analysis of caffeine urinary metabolites: absence of mutation prescribing the phenotype in the CYP1A2 gene. Canc Epidemiol Biomarkers Prev 3:413-421.
Owens JA, Spirito A, McGuinn M (2000) The Children's Sleep Habits Questionnaire (CSHQ): psychometric properties of a survey instrument for school-aged children. Sleep 23:1043-51.
Sack RL, Auckley D, Auger RR, Carskadon MA, Wright KP, Jr., Vitiello MV, Zhdanova IV (2007) Circadian rhythm sleep disorders: part II, advanced sleep phase disorder, delayed sleep phase disorder, free-running disorder, and irregular sleep-wake rhythm. An American Academy of Sleep Medicine review. Sleep 30:1484-501.
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. DOI: 10.1007/s00360-010-0498-2.
Sedlmeyer IL, Hirschhorn JN, Palmert MR (2002) Pedigree analysis of constitutional delay of growth and maturation: determination of familial aggregation and inheritance patterns. J Clin Endocrinol Metab 87:5581-5586.
Silman R (2010) Catastrophe and homeostasis. Crim Behav Ment Health 20:177-189. DOI: 10.1002/cbm.765.
Smits MG, van Stel HF, van der Heijden K, Meijer AM, Coenen AM, Kerkhof GA (2003) Melatonin improves health status and sleep in children with idiopathic chronic sleep onset insomnia: a randomized placebo-controlled trial. J Am Acad Child Adolesc Psychiatr 42:1286-93.
Srinivasan V, Spence WD, Pandi-Perumal S, Zakharia R, Bhatnagar KP, Brzezinski A (2009) Melatonin and human reproduction: Shedding light on the darkness hormone. Gynecological Endocrinology 25:779-785.
Szeinberg A, Borodkin K, Dagan Y (2006) Melatonin treatment in adolescents with delayed sleep phase syndrome. Clin Pediatr 45:809-18.
van Buuren S, Ooms JC (2009) Stage line diagram: an age-conditional reference diagram for tracking development. Stat Med 28:1569-79.
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 Psychiatr 46:233-41.
van Geijlswijk IM, Korzilius HPLM, Smits MG (2010a) Theuse of exogenous melatonin in delayedsleep phase disorder: a meta-analysis. Sleep 33:1605-1614
van Geijlswijk IM, van der Heijden KB, Egberts AC, Korzilius HP, Smits MG (2010b) Dose-finding of melatonin for chronic idiopathic childhood sleep onset insomnia: an RCT. Psychopharmacology (Berl) 212:379-391. DOI: 10.1007/s00213-010-1962-0.
van Litsenburg RR, Waumans RC, van den Berg G, Gemke RJ (2010) Sleep habits and sleep disturbances in Dutch children: a population-based study. Eur J Pediatr 169:1009-1015. DOI: 10.1007/s00431-010-1169-8.
Verhoeven A, Massa G (2005) Impaired melatonin secretion in a girl with delayed puberty: cause or consequence? Tijdschr Kindergeneesk 73:227-230.

Waldhauser F, Boepple PA, Schemper M, Mansfield MJ, Crowley WF,Jr (1991) Serum melatonin in central precocious puberty is lower than in age-matched prepubertal children. J Clin Endocrinol Metab 73:793-796.
Waumans RC, Terwee CB, Van den Berg G, Knol DL, Van Litsenburg RR, Gemke RJ (2010) Sleep and sleep disturbance in children: Reliability and validity of the Dutch version of the Child Sleep Habits Questionnaire. Sleep 33:841-845.
Wehkalampi K, Silventoinen K, Kaprio J, Dick DM, Rose RJ, Pulkkinen L, Dunkel L (2008) Genetic and environmental influences on pubertal timing assessed by height growth. Am J Hum Biol 20:417-423. DOI: 10.1002/ajhb.20748.
Weiss MD, Salpekar J (2010) Sleep problems in the child with attention-deficit hyperactivity disorder: defining aetiology and appropriate treatments. CNS Drugs 24:811-828. DOI: 10.2165/11538990-000000000-00000.
Zhou SF, Liu JP, Chowbay B (2009) Polymorphism of human cytochrome P450 enzymes and its clinical impact. Drug Metab Rev 41:89-295.

General discussion



8

Melatonin has gained increasingly interest during the past twenty years. After the recognition of its special function as endogenous *Zeitgeber,* influencing anticipation and adaptation of the individual to the environment, the results from research concerning its role in human sleep patterns and daily behaviour are expanding every day. Additionally, several other disciplines (oncology amongst others) became interested in melatonin.

In search of the best dose of the drug melatonin to treat children suffering from chronic sleep onset insomnia we were faced with the special properties of melatonin as a chronobiotic, that did not comply with normal pharmacological dose-response-curves. This demonstrated strongly its potential to assist a specific group of children with a late melatonin rhythm (or eveningness) in their adaptation to our rigid society.

In recent literature three distinct research strategies concerning chronotype, social capabilities and related disorders can be recognized.

One is the epidemiological / sociological approach, measuring sleep habits, psychological and habitual development, that refrains from classical disorder typecasting and emphasizes the lack of concordance of individual and social environment. They consider the extreme eveningness chronotypes as part of the normal distribution curve of chronotypes. The disability of the eveningness chronotype to adjust to societal norms is qualified by the group of Roenneberg (Wittmann et al. 2006) as the social jetlag, the misalignment of biological and social time. They introduced the measure mid time sleep on free days, corrected for sleep restoring (MSFsc), and they have correlated this measure with social jetlag and depressed mood. Additionally they found correlations of MSFsc with the consumption of caffeinated drinks (Energy®), alcohol and smoking (Wittmann et al. 2006). Several other groups also connected the definite eveningness type to the increased risk for developing depressions. 14% of the children has the definite eveningness chronotype (Werner et al. 2009). These children may develop a shortage of sleep, leading to increased daytime sleepiness with emotional, attentional and behavioural problems. (Werner et al. 2009)

The second research strategy is a more biochemical and disorder oriented approach. It comprises the measurements of melatonin rhythm, qualifying circadian time, sleep phase and often also other biochemical parameters (like cortisol) are measured. What is striking in most studies of melatonin for children with sleep onset insomnia is that Attention Deficit Hyperactivity Disorder (ADHD) was co-diagnosed in 25%-50%, while overall ADHD is diagnosed 6-9% in childhood (Dopheide and Pliszka. 2009); in the Meldos study 5 children (7%) used methylphenidate. This particular presence of ADHD in Sleep Onset Insomnia (SOI) studies is consistent with the finding that 78% of adults with ADHD (van Veen et al. 2010) and 73% of children with ADHD (van der Heijden et al. 2005) have SOI probably related to Delayed Sleep Phase Disorder (DSPD). If 73% of children with ADHD have symptoms of DSPD, it is likely that most children with ADHD are definite eveningness chronotypes. Their internal clock rhythm is characterised by misalignment of biological and social time and reinstitution of a socially accepted rhythm might contribute to an improvement of daily life, including amelioration of the ADHD symptoms. Adjusted sleep

phase rhythm in children with DSPD can effectively be accomplished by administration of well dosed well timed melatonin (this thesis). Nagtegaal et al. (2000) and Smits et al. (2001) stated that QOL is improved by melatonin, although this could not be confirmed by others (van der Heijden et al. 2007).
As the definite eveningness chronotypes are prone to overuse of caffeinated drinks (Wittmann et al. 2006), and ADHD is treated with stimulating amphetamine-like drugs, one may suggest that the Energy® drink consumption of the definite eveningness chronotypes amongst adolescents can be considered as sort of auto therapy.
The third research approach emphasises the influence of the external *Zeitgebers* of which food and light are the most powerful. When those two *Zeitgebers* are not synchronized, it may result in a seemingly disorganized endogenous melatonin production. We have observed an erratic endogenous melatonin production in domesticated pigs when sampling every hour during 24 hours (Chapter 2). Under normal and experimental circumstances only 5 time samples in the children with SOI are measured to determine a (late) DLMO; we do not study complete curves, therefore we are unaware of additional irregularities, for instance related to daily food intake or artificial light. Whether conflicting *Zeitgebers* are the inducing factor for a late Dim Light Melatonin Onset (DLMO) and the way of eating is an additional determinative factor is not clarified yet. In animals a relationship with feeding is found: 14 h after restricted feeding in rats (Feillet et al. 2008) and mice (Mendoza et al. 2005) a melatonin rise is observed. The circadian rhythm in humans is relative to wake up time: 14 h after getting up we normally observe the DLMO (in the original definition of the clock time when serum melatonin concentration rises > 4 pg/mL) in humans (Lewy et al. 1999).

Pezuk et al. (2010) concluded that circadian organization is governed by extra SCN pacemakers, for instance the food entrainable oscillator (FEO).

Jenni. (2005) stated that cultural variation and developmental variation and biological variability of sleep behaviour among normal healthy children should be taken into account when analysing sleep deviation in a population. He doubted whether deviation always should lead to medication. When we combine the three approaches described above and look outside boundaries of diagnosed disorders, the ability of an individual to anticipate and adapt in order to obtain harmony with its physical and social environment is the central issue. Every individual is striving for harmony while eating and sleeping and with melatonin we may have a relatively simple tool at our disposition to help individuals with anticipation or adaptation problems like DSPD or ADHD.

EXOGENOUS MELATONIN AS A MODULATOR: DOSING AND TIMING ASPECTS

At this moment endogenous DLMO is used as an individual phase marker and exogenous melatonin is applied as a modulator.

As is described in chapter 2, we have not found a distinct melatonin rhythm in domestic pigs which we for now attribute to too many conflicting *Zeitgebers*: light and food oscillator? Or have our domesticated meat swine lost their endogenous melatonin rhythm in due course?

In arctic reindeer a direct response to the scotophase with a melatonin rise is observed, but a spontaneous circadian melatonin rhythm in continuous dark or light (as occurs in the arctic environment) is absent (Lu et al. 2010). Both examples illustrate that we still are unaware of a lot of the mechanisms underlying melatonin rhythm.

In 2005 Arendt recommended to have the appropriate melatonin dose determined (Arendt and Skene. 2005). Also it becomes more and more clear that timing and dosing are closely related (Burgess et al. 2008). Initiating melatonin therapy in children with SOI despite an early DLMO confers the risk of delaying DLMO, inducing DSPD rather than curing the original SOI. A melatonin dose too high given too late may result in persisting high melatonin levels in the morning (spill over), especially in combination with poor metabolism, which may result in a further delayed DLMO. This is in concordance with earlier observations of for instance (Lewy et al. 2002; Wise. 2006): with a dose too low no concrete effects occur, too high the chronobiological effects may be lost.

High levels of melatonin may even be harmful, for instance resulting in what Zhdanova. (2005) thought to be desensitizing melatonin receptors. Melatonin displays an extreme first pass effect, which might be as big as 80% (Zhou et al. 2009) and saturation kinetics (Lin et al. 2001; Miller and Guengerich. 2001). These complex pharmacokinetics of melatonin lead to extreme differences in individual levels after exogenous administration (Fourtillan et al. 2000). In chapter 3 we present three patients (Braam et al. 2010) in whom we have demonstrated that decreasing the dose instead of increasing dose is necessary to preserve effect. We ascribe this phenomenon of loss of response to poor melatonin metabolism. Other reasons for disappearing effectiveness of melatonin therapy, like desensitisation, tolerance, tachyphylaxis or wearing off phenomenon are less likely with this effective strategy of dose lowering.

In chapter 5 we have demonstrated the sleep effects of melatonin in Dutch children, suffering from chronic sleep onset insomnia (CSOI), which is defined as sleep onset later than 8:30 p.m. in children aged 6 years and for older children 15 min later per year until age 12 (10:00 p.m.). Furthermore, the latency between lights-off time and sleep onset (sleep onset latency) had to be more than 30 min on average. Solid effects are detectable as from night 1, are stable at night 7, and remain constant thereafter in eleven of 14 (of 25 verum patients) responders (effect > 25 min reduction of SOL). The SOL of 53 minutes

is reduced by 36 min to 16-18 minutes. In the dose-finding study in the same population (Chapter 6) we also found that SOL was reduced by 31-42 minutes, SOT was advanced by 42-56 minutes and DLMO was advanced by 65-105 minutes.

Timing and dosing of melatonin are intimately connected. For every individual the distance between the mid time of sleep on free days (MSF) (Roenneberg et al. 2007), which is the mid time between SOT en WUT, and DLMO should be evaluated before melatonin therapy for sleep onset insomnia is considered. This time interval is called the Phase Angle Difference (PAD), which predicts the probability of DSPD (Lewy et al. 2006). Chapter 6 demonstrates that SOT and SOL effects of melatonin are induced by two characteristics of melatonin: firstly the soporific effects as suggested by the observed relationship with clock- time of administration (clock-TOA), secondly the chronobiotic effects, as proven by the DLMO shift which is still measurable on a medication free night and by the fact that the effect of exogenous melatonin on SOT, SOL, and DLMO increases with an earlier circadian TOA. The direct soporific effects might take account for the positive results in children with a PAD≥6 (which implies that the DLMO is 6 hours or more before mid sleep and is considered normal), although hypnotic effects of melatonin can only be expected when endogenous melatonin is still low (Dollins et al. 1994; Nave et al. 1995; Zhdanova et al. 1995). In contrast to the found time-response relationship, no dose–response relationship of melatonin with SOT, SOL, and DLMO is found within a dosage range of 0.05-0.15 mg/kg.

As it has been demonstrated that in order to sustain the desired effect melatonin therapy needs to be continued for a longer period of time and it is applied as (in most countries) off label therapy in young, preadolescent children, long-term effects need to be known.

Long-term efficacy reports are available since 2007 (Carr et al. 2007; Wasdell et al. 2008; Hoebert et al. 2009), but long term therapy effects are difficult to evaluate. How can we differentiate between changes caused by the intervention and changes due to circumstantial influences? In children this is even more difficult. Validated questionnaires are only applicable within small age groups, for this reason is repeating questionnaires before therapy, after initiating therapy and after long term use not possible. This reflects of course the enormous development of children in a time span of a few years, therefore changes in development can't be ascribed to the intervention alone.

Comparison of some characteristics in a group of individuals subject to an intervention with recent data collected in the general population of the same age and the same cultural background overcomes the individual's circumstantial developmental deviations and is therefore the only way to detect (long term) deviations or normalisations induced by the intervention in children.

In chapter 7 the follow-up research study in the children previously included in the Meldos trial is described, after mean 3.1 years of melatonin therapy, mean dose 2.69 mg. Outcomes were measured using questionnaires (Strength and Difficulties Questionnaire SDQ, children sleep habits questionnaire CSHQ and Tanner Stages) adopted for Dutch

children. Mean duration of therapy, persistence of effect, adverse events and (other) reasons leading to cessation of therapy were additional objectives of this study. This follow up study demonstrates that melatonin treatment in children can be sustained over a long period of time without substantial deviation of the development of children with respect to sleep quality, puberty development and mental health scores, as compared with the general Dutch population.

IMPLICATIONS FOR THERAPY

- Individual therapy management is the key stone of melatonin therapy evaluation. Melatonin therapy optimisation is an excellent example of personalized medicine: the tailored approach of a patient with SOI takes into account 1) the genetic variation of CYP 450 1A2 predisposing for reduced first pass effects and prolonged elimination half life and saturation kinetics in order to assess the optimal dose 2) the individual DLMO in order to assess the optimal time of administration 3) evaluation during therapy to further advance DLMO by shifting TOA if necessary.
- Melatonin therapy might be one of the few sleep therapies that are easily sustained for a long period of time without addiction issues.
- Melatonin in children to treat distinct sleep disorders has grown to be EBM and should be positioned as such by professionals, government and insurance companies.
- Melatonin is a hormone by pharmacological definition, but should better be referred to as a neurotransmitter.

IMPLICATIONS FOR FUTURE RESEARCH

- Pharmaceutical formulations should be developed to reduce variability due to first pass mechanisms and accounting for the T_{max}, as this feature is most important in assisting proper timing of melatonin.
- Establishment of the lowest melatonin dose in relation to optimal circadian time
- Additional long term studies should be performed in a more representative population especially with regard to the pubertal development, to confirm or to reject our preliminary results.
- In pigs, the contradicting results of melatonin rhythm should be further examined in an extended trial in 10 pigs, taking samples hourly during several days, comparing unrestricted feeding and outside housing versus restricted feeding twice a day, 40 Lx light during daytime and dark during nights.

- The relationship between bodyweight, eating habits, endogenous melatonin rhythm, sleep disorders and melatonin efficacy should be examined bearing chronotypes and social jetlag in mind.

CONCLUSION

In conclusion, the inability of an individual to adequately anticipate and adapt in order to obtain harmony with its rigid social environment may be a main provoking factor for sleep disorders. Melatonin is helpful as an endogenous marker and as an exogenous modulator. For the treatment of chronic sleep onset insomnia in children exogenous melatonin is effective in a dosage of 0.05 mg/kg given 1–2 h before DLMO and before desired bedtime, resulting in 1 h shifts of DLMO and SOT and a SOL reduction by 35 min. For most efficient and effective therapy, before therapy initiation DLMO determination and genotyping or phenotyping of CYP 450 1A2 metabolism should be performed. Melatonin retains its efficacy during several years of use and social and pubertal development seems undisturbed.

REFERENCES

Arendt J, Skene DJ (2005) Melatonin as a chronobiotic. Sleep Med Rev 9:25-39.

Braam W, van Geijlswijk I, Keijzer H, Smits MG, Didden R, Curfs LM (2010) Loss of response to melatonin treatment is associated with slow melatonin metabolism. J Intellect Disabil Res 54:547-555. DOI: 10.1111/j.1365-2788.2010.01283.x.

Burgess HJ, Revell VL, Eastman CI (2008) A three pulse phase response curve to three milligrams of melatonin in humans. J Physiol 586:639-47.

Carr R, Wasdell MB, Hamilton D, Weiss MD, Freeman RD, Tai J, Rietveld WJ, Jan JE (2007) Long-term effectiveness outcome of melatonin therapy in children with treatment-resistant circadian rhythm sleep disorders. J Pineal Res 43:351-9.

Dollins AB, Zhdanova IV, Wurtman RJ, Lynch HJ, Deng MH (1994) Effect of inducing nocturnal serum melatonin concentrations in daytime on sleep, mood, body temperature, and performance. Proc Natl Acad Sci U S A 91:1824-8.

Dopheide JA, Pliszka SR (2009) Attention-deficit-hyperactivity disorder: an update. Pharmacotherapy 29:656-679. DOI: 10.1592/phco.29.6.656.

Feillet CA, Mendoza J, Pevet P, Challet E (2008) Restricted feeding restores rhythmicity in the pineal gland of arrhythmic suprachiasmatic-lesioned rats. Eur J Neurosci 28:2451-2458. DOI: 10.1111/j.1460-9568.2008.06538.x.

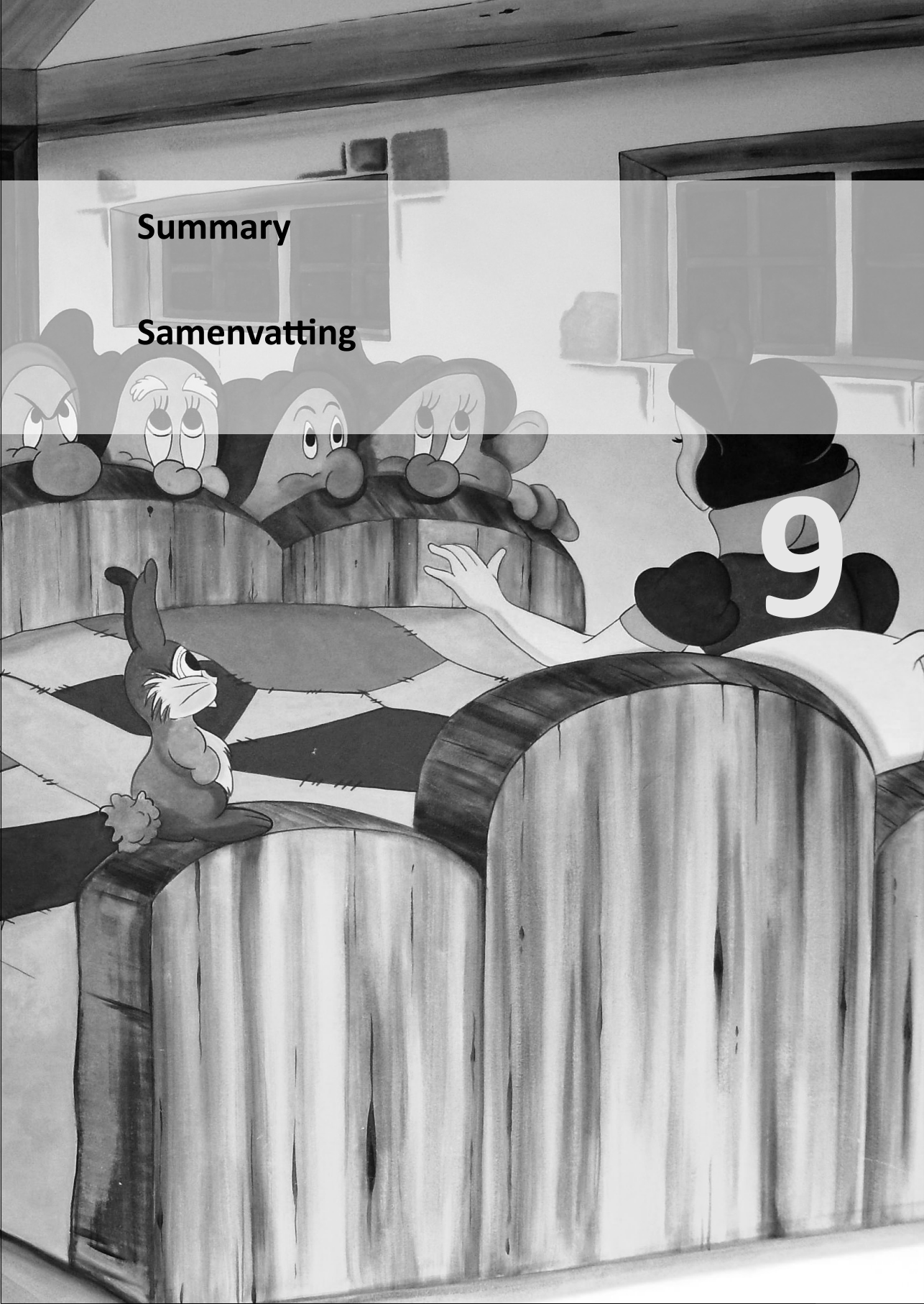
Fourtillan JB, Brisson AM, Gobin P, Ingrand I, Decourt JP, Girault J (2000) Bioavailability of melatonin in humans after day-time administration of D(7) melatonin. Biopharm Drug Dispos 21:15-22.

Hoebert M, van der Heijden KB, van Geijlswijk IM, Smits MG (2009) Long-term follow-up of melatonin treatment in children with ADHD and chronic sleep onset insomnia. J Pineal Res 47:1-7.

Jenni OG (2005) Sleep onset insomnia during childhood or poor fit between biology and culture: comment on van der Heijden et al. 'Prediction of melatonin efficacy by pre-treatment dim light melatonin onset in children with idiopathic chronic sleep onset insomnia'. J Sleep Res 14:195-7; discussion 197-9.

Lewy AJ, Emens J, Jackman A, Yuhas K (2006) Circadian uses of melatonin in humans. Chronobiol Int 23:403-12.

Lewy AJ, Emens JS, Sack RL, Hasler BP, Bernert RA (2002) Low, but not high, doses of melatonin entrained a free-running blind person with a long circadian period. Chronobiol Int 19:649-58.
Lewy AJ, Cutler NL, Sack RL (1999) The endogenous melatonin profile as a marker for circadian phase position. J Biol Rhythms 14:227-36.
Lin Y, Lu P, Tang C, Mei Q, Sandig G, Rodrigues AD, Rushmore TH, Shou M (2001) Substrate inhibition kinetics for cytochrome P450-catalyzed reactions. Drug Metab Dispos 29:368-374.
Lu W, Meng QJ, Tyler NJ, Stokkan KA, Loudon AS (2010) A circadian clock is not required in an arctic mammal. Curr Biol 20:533-537. DOI: 10.1016/j.cub.2010.01.042.
Mendoza J, Graff C, Dardente H, Pevet P, Challet E (2005) Feeding cues alter clock gene oscillations and photic responses in the suprachiasmatic nuclei of mice exposed to a light/dark cycle. J Neurosci 25:1514-1522. DOI: 10.1523/JNEUROSCI.4397-04.2005.
Miller GP, Guengerich FP (2001) Binding and oxidation of alkyl 4-nitrophenyl ethers by rabbit cytochrome P450 1A2: evidence for two binding sites. Biochemistry 40:7262-7272.
Nagtegaal JE, Laurant MW, Kerkhof GA, Smits MG, van der Meer YG, Coenen AM (2000) Effects of melatonin on the quality of life in patients with delayed sleep phase syndrome. J Psychosom Res 48:45-50.
Nave R, Peled R, Lavie P (1995) Melatonin improves evening napping. Eur J Pharmacol 275:213-216.
Pezuk P, Mohawk JA, Yoshikawa T, Sellix MT, Menaker M (2010) Circadian organization is governed by extra SCN pacemakers. J Biol Rhythms 25:432-441. DOI: 10.1177/0748730410385204.
Roenneberg T, Kuehnle T, Juda M, Kantermann T, Allebrandt K, Gordijn M, Merrow M (2007) Epidemiology of the human circadian clock. Sleep Med Rev 11:429-438. DOI: 10.1016/j.smrv.2007.07.005.
Smits MG, Nagtegaal EE, 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.
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 46:233-41.
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 22:559-70.
van Veen MM, Kooij JJ, Boonstra AM, Gordijn MC, Van Someren EJ (2010) Delayed circadian rhythm in adults with attention-deficit/hyperactivity disorder and chronic sleep-onset insomnia. Biol Psychiatry 67:1091-1096. DOI: 10.1016/j.biopsych.2009.12.032.
Wasdell MB, Jan JE, Bomben MM, Freeman RD, Rietveld WJ, Tai J, Hamilton D, Weiss MD (2008) A randomized, placebo-controlled trial of controlled release melatonin treatment of delayed sleep phase syndrome and impaired sleep maintenance in children with neurodevelopmental disabilities. J Pineal Res 44:57-64.
Werner H, Lebourgeois MK, Geiger A, Jenni OG (2009) Assessment of chronotype in four- to eleven-year-old children: reliability and validity of the Children's Chronotype Questionnaire (CCTQ). Chronobiol Int 26:992-1014. DOI: 10.1080/07420520903044505.
Wise ME (2006) Does melatonin improve sleep? Muddles with melatonin. BMJ 332:550.
Wittmann M, Dinich J, Merrow M, Roenneberg T (2006) Social jetlag: misalignment of biological and social time. Chronobiol Int 23:497-509. DOI: 10.1080/07420520500545979.
Zhdanova IV (2005) Melatonin as a hypnotic: pro. Sleep Med Rev 9:51-65.
Zhdanova IV, Wurtman RJ, Lynch HJ, Ives JR, Dollins AB, Morabito C, Matheson JK, Schomer DL (1995) Sleep-inducing effects of low doses of melatonin ingested in the evening. Clin Pharmacol Ther 57:552-8.
Zhou SF, Yang LP, Zhou ZW, Liu YH, Chan E (2009) Insights into the substrate specificity, inhibitors, regulation, and polymorphisms and the clinical impact of human cytochrome P450 1A2. The AAPS journal 11:481-94.



9

Summary

Samenvatting

SUMMARY

Melatonin has gained increasingly interest during the past twenty years, as an endogenous hormone and as an exogenous drug. Every living organism has an endogenous rhythm, the biological clock. This endogenous rhythm is synchronized (entrained) by exogenous impulses like daylight, length of day, temperature and feeding to adapt the organism to the environment. The necessity of this adaptation to the outside world varies with animal species, age and gender, but often concerns physiologic processes, behaviour and hormone regulation. The normal variation in melatonin levels results in different outcomes of gonadotrophic effect and reproduction, depending on the animal species involved. Melatonin is the mediator for the environmental cue that activates the seasonal breeder organism in a species appropriate way to the seasonal changes. After the recognition of its special function as endogenous *Zeitgeber,* influencing anticipation and adaptation of the individual to the environment, the results from research concerning its role in human sleep patterns and daily behaviour are expanding every day. Inappropriately applied bright light disturbs 24 hour melatonin rhythm and consequently desynchronizes biological rhythms, resulting in decrease of human and animal well-being. Some large swine producers apply artificial light, for instance three times a day for three hours. Consequently, the 24 hour melatonin rhythm of these pigs might differ from pigs living in farms with natural light schemes and if so, might contribute to difference in well-being. To contribute to the discussion whether or not large swine production units decrease well being of pigs, 24 hour melatonin rhythm of pigs in a large swine production unit (Farm B) was compared with pigs in a farm with natural light schemes (Farm A) (**chapter two**). Salivary samples were collected hourly during 24 hours at both farms. Mean scotophase and mean photophase MEL levels were not statistically significant different, neither on Farm A nor on Farm B. No statistically significant differences were found comparing mean MEL levels of Farm A and Farm B during scotophase and photophase, the MEL levels one hour after lights off and one hour after lights on, MEL levels just before and after feeding. The controversy in existing literature about domestic pigs having an entrained melatonin rhythm or not, is not adjourned by our study. Whether or not the lack of an entrained melatonin rhythm is an indicator for quality of life of the domestic pig has not been elucidated by our results.

In some patients with intellectual disability (ID) and sleep problems, the initial good response to melatonin disappeared within a few weeks after starting treatment, while the good response returned after considerable dose reduction. The cause for this loss of response to melatonin is yet unknown. We hypothesize that this loss of response is associated with slow metabolisation of melatonin. In the pilot study (**chapter three**) we determined melatonin clearance in two female (aged 61 and 6 years) and one male (aged 3 years) patients who had chronic insomnia, late melatonin onset and mild ID, and whose

sleep quality worsened a few weeks after initial good response to melatonin treatment, suggesting melatonin tolerance. After a three week washout period, patients received melatonin 1.0, 0.5 or 0.1 mg respectively at 11 a.m. Salivary melatonin levels were measured just before melatonin administration, and two and four hours thereafter. After this melatonin clearance test, melatonin treatment was resumed with a considerably lower dose.

In all patients melatonin concentrations remained >50 pg/ml at two and four hours after melatonin administration. After resuming melatonin treatment sleep problems disappeared. The same procedure has been followed in three patients who did not show loss of response to melatonin after six months of treatment. In all patients of this control group melatonin concentrations decreased between two and four hours after melatonin administration with a mean of 76%.

We hypothesize that loss of response to melatonin treatment can be caused by slow metabolisation of exogenous melatonin. As melatonin is metabolised in the liver almost exclusively by cytochrome P450 enzyme CYP1A2, this slow metabolisation of melatonin is probably due to decreased activity/inducibility of CYP1A2. In patients with loss of response to melatonin, a melatonin clearance test should be considered and a considerably dose reduction is advised.

In **chapter four** the meta-analysis of the efficacy and safety of exogenous melatonin in advancing sleep-wake rhythm in patients with delayed sleep phase disorder is described. We used papers indexed for PubMed, Embase, and the abstracts of sleep and chronobiologic societies (1990-2009) that described data of randomized controlled trials involving individuals with delayed sleep phase disorder which have been published in English, compared melatonin with placebo, and reported one or more of the following: endogenous melatonin onset, clock hour of sleep onset, wake-up time, sleep-onset latency, and total sleep time.

The five trials including 91 adults and four trials including 226 children showed that melatonin treatment advanced mean endogenous melatonin onset by 1.18 hours (95% confidence interval [CI]: 0.89-1.48 h) and clock hour of sleep onset by 0.67 hours (95% CI: 0.45-0.89 h). Melatonin decreased sleep-onset latency by 23.27 minutes (95% CI: 4.83 -41.72 min). The wake-up time and total sleep time did not change significantly. The conclusion of the meta analysis is that melatonin is effective in advancing sleep-wake rhythm and endogenous melatonin rhythm in delayed sleep phase disorder.

Chapter five describes the post hoc retrospective analysis of unpublished data obtained in two previously published randomized, placebo-controlled, double-blind trials on melatonin efficacy for childhood insomnia in children with chronic sleep onset insomnia, age 6-12 yrs, n =49 to assess onset and stability of therapeutic effect of 4-weeks melatonin treatment. The intervention consisted of placebo (n=25) or melatonin 5 mg

(n=24) administered at 6:00 p.m. (n=9) or 7:00 p.m. (n=40) during 4 weeks. Collected data were lights out time, sleep onset latency, sleep onset, total sleep time, wake up time, and subjective sleep measures recorded in a diary.
After one week of melatonin treatment a phase-advance of lights out of [time (SD)] 9:15 p.m. (1.05) to 8:28 p.m. (1.07) hrs. was shown; sleep onset advanced from 10:05 p.m. (0.93) to 8:45 p.m. (1.09) hrs. and sleep latency decreased from 53 (39) min to 18 (16) min. After the 4-week trial period these values were 8:44 p.m. (1.27) hrs., 9:09 p.m. (1.33) hrs., 25 (39) min.
Melatonin advances sleep latency and sleep onset and increases total sleep time starting right from the first treatment night in children with chronic sleep onset insomnia. Evidence is provided that the onset of melatonin treatment effect can be expected within a few days after commencement and remains stable after that.

As pharmacokinetics of melatonin in children might differ from that in adults, the Meldos study (**chapter six**) aimed to establish a dose–response relationship for melatonin in advancing dim light melatonin onset (DLMO), sleep onset time (SOT), and reducing sleep onset latency (SOL) in children between six and twelve years with chronic sleep onset insomnia (CSOI). The method used for this study is the randomized, placebo-controlled double-blind trial. Children with CSOI (n=72) received either melatonin 0.05, 0.1, and 0.15 mg/kg or placebo during 1 week. Sleep was assessed with log and actigraphy during this week and the week before. Outcomes were the shifts in DLMO, SOT, and SOL.
Treatment with melatonin significantly advanced SOT and DLMO by approximately 1 hour and decreased SOL by 35 min. Within the three melatonin groups, effect size was not different, but the circadian time of administration (TOA) correlated significantly with treatment effect on DLMO (r_s=−0.33, p=0.022) and SOT (r_s=−0.38, p=0.004), whereas clock TOA was correlated with SOT shift (r=−0.35, p=0.006) and not with DLMO shift.
No dose–response relationship of melatonin with SOT, SOL, and DLMO was found within a dosage range of 0.05–0.15 mg/kg. The effect of exogenous melatonin on SOT, SOL, and DLMO increased with an earlier circadian TOA. The soporific effects of melatonin enhanced the SOT shift. This study demonstrated that melatonin for treatment of CSOI in children is effective in a dosage of 0.05 mg/kg given at least 1 to 2 h before DLMO and before desired bedtime.
After the Meldos study we aimed to establish whether long-term use of melatonin influences pubertal development, sleep quality and mental health development in children as compared with the normal Dutch population of the same age. This follow-up research study, described in **chapter seven**, was conducted in children included in the melatonin dose finding trial. Outcomes were measured using questionnaires (Strength and Difficulties Questionnaire SDQ, children sleep habits questionnaire CSHQ and Tanner Stages) adopted for Dutch children. Mean duration of therapy, persistence of effect,

adverse events and (other) reasons leading to cessation of therapy were additional objectives of this study.
Mean years of usage (n=51) was 3.1 year (min 1.0 yr, max 4.6 yr), mean dose 2.69 mg (min 0.3 mg, max 10 mg). Mean SDQ score, mean CSHQ score and Tanner Stages standard deviation scores did not differ in a statistically significant way from published scores of the general Dutch population of the same age and sex.
This follow up study demonstrated that melatonin treatment in children may be sustained over a long period of time without substantial deviation of the development of children with respect to sleep quality, puberty development and mental health scores, as compared with the general Dutch population.
In conclusion, the inability of an individual to adequately anticipate and adapt in order to obtain harmony with its rigid social environment may be a main provoking factor for sleep disorders. Melatonin is useful as an endogenous marker and as an exogenous modulator. For the treatment of chronic sleep onset insomnia in children exogenous melatonin is effective in a dosage of 0.05 mg/kg given at least 1–2 h before DLMO and before desired bedtime, resulting in 1 h shifts of DLMO and SOT and a SOL reduction of 35 min. For most efficient and effective therapy, before therapy initiation DLMO determination and genotyping or phenotyping of CYP 450 1A2 metabolism should be performed.
Melatonin retains its efficacy during several years of use and social and pubertal development seem undisturbed.

Keywords: melatonin rhythm, pigs, delayed sleep phase disorder, chronic sleep onset insomnia in (human) children, melatonin treatment, poor CYP1A2 metaboliser, metaanalysis, randomized placebo controlled dose finding trial, long term effect, puberty

SAMENVATTING

Melatonine heeft de afgelopen twintig jaar als hormoon en als geneesmiddel steeds meer belangstelling gekregen. Ieder levend organisme kent een eigen ritme dat bepaald wordt door de biologische klok. Dit eigen ritme wordt in fase gebracht met de omgeving door prikkels van buiten zoals daglicht, daglengte, temperatuur en eten. Hoe noodzakelijk deze aanpassing is hangt af van het organisme, zoals soort (zoogdier, vis of mens), leeftijd (in de vruchtbare leeftijd of niet) en geslacht, maar het betreft meestal fysiologische processen zoals slaap-waak ritme en hormonale processen waaronder vruchtbaarheid bij zoogdieren met een seizoensgebonden voortplanting en gedrag. De normale variatie in melatonine bloedspiegels resulteert dus bij verschillende diersoorten in verschillende effecten: de hoge spiegel 's nachts resulteert bijvoorbeeld in slaap bij dagdieren en waakzaamheid bij nachtdieren, verschuiving van lange naar korte dagen veroorzaakt bij sommige dieren (met een korte draagtijd) voortplantingsactiviteit, terwijl bij andere dieren (met een lange draagtijd) juist de verschuiving van korte naar lange dagen voortplantingsactiviteit veroorzaakt. Melatonine is in al deze gevallen de interne *Zeitgeber* die zorgt dat het individu adequaat omgaat met de omgevingsfactoren. Ondertussen nemen de onderzoeksresultaten naar melatonine in relatie tot slaappatronen en dagelijks gedrag iedere dag toe. Fel licht op verkeerde momenten verstoort het 24 uurs melatonine ritme en daarmee de biologische klok ritmes, waardoor zowel mens als dier aangetast zouden kunnen worden in hun welbevinden. Toch worden door verschillende grote varkensproducenten kunstmatige lichtritmes toegepast, bijvoorbeeld driemaal daags drie uur licht, tijdens het voederen. Het melatonine ritme van de varkens in deze houderijen zou wel eens sterk kunnen afwijken van het melatonine ritme van varkens uit biologische houderijen, waarmee misschien een verschil in welbevinden kan worden aangetoond. Mede ingegeven door het maatschappelijke debat over dierenwelzijn in megastallen hebben we het melatonine ritme van varkens gehuisvest in een stal met uitsluitend kunstmatig licht (boerderij B) vergeleken met varkens in een stal met natuurlijk licht (boerderij A) (**hoofdstuk 2**). Dit deden we door speekselmonsters te verzamelen op beide boerderijen gedurende 24 uur. Op beide boerderijen verschilden gemiddelde melatoninespiegels in de donkere periodes niet van de gemiddelde spiegels in de lichte periodes. Ook onderlinge vergelijking van boerderij A en B in gemiddelde spiegels tijdens donker en licht, 1 uur na een donker/licht wisseling of een licht/donker wisseling, en de spiegels net voor en na voederen waren niet significant verschillend. In de literatuur worden tegenstrijdige resultaten gevonden voor het melatonine ritme bij gedomesticeerde varkens: wij kunnen met onze studieresultaten uitsluitend concluderen dat we eigenlijk geen ritme vinden, onafhankelijk van het type huisvesting. Ook kunnen we geen uitspraak doen over of het melatonine ritme een indicator zou kunnen zijn voor dierenwelzijn.

Patiënten met een verstandelijke beperking en slaapstoornissen die aanvankelijk goed reageren op melatonine als slaapmiddel, kunnen na een aantal weken ineens weer terugvallen in de oude slaapproblemen. Ondertussen hebben we de ervaring dat een aanzienlijke dosisverlaging de werkzaamheid van melatonine weer kan herstellen, zonder dat we precies weten waarom. Het vermoeden is dat het verdwijnen van de effectiviteit te maken heeft met een trage afbraak van melatonine in het lichaam.

In **hoofdstuk drie** beschrijven we een *pilot* studie naar de melatonineklaring van twee vrouwelijke (61 en 6 jaar oud) en een mannelijke (3 jaar oud) patiënt, allen mild verstandelijk beperkt en met chronische slaapproblemen gecombineerd met laat op gang komen van de endogene melatonineproductie. Ze werden behandeld met melatonine voor de slaapproblemen. Hoewel de behandeling aanvankelijk zeer succesvol leek, kwamen na een aantal weken de slaapproblemen weer terug, ondanks voortzetten van de medicatie. Het leek of tolerantie voor de effecten van melatonine was ontwikkeld. Na drie weken staken van de behandeling kregen deze patiënten daarna een lage dosis (respectievelijk 1, 0,5 en 0,1 mg) melatonine om 11 uur 's ochtends. In speekselmonsters werd melatonine bepaald: direct voor toediening, en twee en vier uur na toediening. Vervolgens werd melatoninetherapie weer bij alle drie hervat in een veel lagere dosering. Bij de drie patiënten bleek de melatonineconcentratie twee en vier uur na toediening boven de 50 pg/ml te zijn. De slaapproblemen bleken vervolgens met de lage dosis langdurig opgelost te zijn.

Dezelfde procedure werd uitgevoerd bij drie patiënten zonder verlies van effectiviteit na 6 maanden melatoninegebruik. Bij deze drie patiënten was de melatonine spiegel 2 en 4 uur na toediening sterk afgenomen met gemiddeld 76%.

We denken dus dat het verdwijnen van melatonine effect veroorzaakt kan worden door langzame afbraak van melatonine. Aangezien melatonine voornamelijk in de lever wordt afgebroken door cytochroom P450 iso-enzym CYP1A2, zal de trage afbraak waarschijnlijk door afgenomen activiteit of verminderde induceerbaarheid van dit enzym worden veroorzaakt. Bij patiënten met aanvankelijk goed effect van melatonine dat in tweede instantie lijkt te verdwijnen, kan een melatonine klaringstest worden overwogen en moet in ieder geval een aanzienlijke dosisreductie worden geadviseerd.

In **hoofdstuk vier** wordt de meta-analyse (combinatie van de gegevens van eerder gerapporteerde onderzoeken) naar effectiviteit en veiligheid van melatonine therapie om het slaap-waak ritme te vervroegen bij patiënten met het vertraagde slaapfase syndroom (verlaat slaapritme) beschreven. Artikelen uit de belangrijkste literatuurdatabases PubMed en Embase en samenvattingen uit *meetings* van verenigingen van slaap-waak onderzoekers zijn doorzocht op Engelstalige publicaties tussen 1990 en 2009 over het vertraagde slaapfase syndroom die een behandeling met melatonine vergeleken met een behandeling met een placebo. In het artikel moest gerapporteerd worden over op gang

komen van endogene melatonine productie (DLMO), kloktijd van inslaap vallen (inslaaptijdstip), ontwaken, periode van inslaapvallen (inslaapduur) en totale slaaptijd.
Uiteindelijk lieten de resultaten uit vijf *trials* met 91 volwassen en vier trials met 226 kinderen zien dat melatoninebehandeling resulteert in de vervroeging van de endogene melatonine productie met 1,19 uur (95% betrouwbaarheidsinterval [BI]: 0,89-1,48 u) en het inslaaptijdstip met 0,67 uur (95% BI: 0,45-0,89 uur). Melatonine verkortte de inslaapduur met 23,27 minuten (95% BI: 4,83-41,72 minuten). Ontwaaktijdstip en totale slaaptijd veranderden niet significant. De conclusie die aan de meta-analyse kan worden verbonden is dat melatonine effectief het slaap-waak ritme en de endogene melatonine productie kan vervroegen bij patiënten met het vertraagde slaapfase syndroom.

Hoofdstuk vijf beschrijft de retrospectieve analyse van niet eerder gepubliceerde resultaten uit twee eerder verrichte gerandomiseerde dubbelblinde onderzoeken met melatonine bij 49 kinderen tussen de 6 en 12 jaar oud met chronische inslaapstoornissen. We wilden vaststellen na hoeveel tijd het effect van de behandeling zichtbaar was, en hoe constant het effect bleef over de onderzoeksperiode van 4 weken. Gedurende de behandeling met placebo (25 patiënten) of melatonine 5 mg (24 patiënten), ingenomen om 6 (n=9) of 7 (n=49) uur 's avonds gedurende 4 weken werden gemeten: tijdstip van licht uit, inslaapduur, inslaaptijdstip, totale slaaptijd, ontwaaktijdstip en subjectieve beleving van de slaap zoals vastgelegd in een dagboekje.
Na 1 week melatonine behandeling was het tijdstip van "licht uit" verschoven van 21:15 uur (sd=1,05) naar 20:28 uur (sd=1,07), inslaaptijdstip verschoof van 22:05 uur (0,93) naar 20:45 uur (1,09), en inslaapduur verkortte van 53 (39) minuten naar 18 (16) minuten. Na 4 weken behandeling waren deze waarden 20:44 uur (1,27), 21:09 uur (1,33) en 25 (39) minuten. Melatonine verkort de inslaapduur en vervroegt het inslaaptijdstip en verlengt de totale slaaptijd vanaf de eerste nacht van gebruik bij kinderen met een chronische inslaapstoornis.
De resultaten tonen aan dat het effect van melatoninebehandeling binnen een aantal dagen zichtbaar is en daarna constant blijft.

Omdat de farmacokinetiek van melatonine in kinderen anders zou kunnen zijn dan in volwassenen, is de Meldos studie gedaan, een gerandomiseerd, placebo gecontroleerd, dubbel blind onderzoek. Het doel van deze studie (beschreven in **hoofdstuk 6**) was om een dosis-respons relatie voor melatonine vast te stellen, voor het vervroegen van het op gang komen van de eigen melatonine productie bij schemerlicht (Dim Light Melatonin Onset = DLMO), het vervroegen van de inslaaptijdstip (Sleep Onset Time = SOT) en het verkorten van de inslaapduur (Sleep Onset Latency = SOL) bij kinderen tussen de 6 en 12 jaar oud met chronische inslaapproblemen. In het onderzoek werden gedurende 1 week de effecten van een nepmiddel (placebo) en drie doseringen melatonine, 0,05 mg/kg,

0,1 mg/kg en 0,15 mg/kg op de slaap gemeten met behulp van een bewegingsmeter en een dagboekje terwijl noch patiënten, noch onderzoekers wisten wie wat had gekregen. De resultaten werden vergeleken met de week voordat de onderzoeksmedicatie werd gegeven. De DLMO werd bepaald met een melatoninespeekseltest, na de eerste week zonder medicatie en na de week met medicatie (op de meet avond werd geen medicatie ingenomen).

Door de behandeling met melatonine werden DLMO en inslaaptijdstip 1 uur vervroegd, en werd de inslaapduur 35 minuten korter. Er was geen verschil tussen de drie doseringen, maar het tijdsverschil tussen het inname tijdstip en de oorspronkelijke DLMO bleek wel een duidelijke relatie op te leveren voor de verschuiving van de DLMO (r_s=−0.33, p=0.022) en het inslaaptijdstip (r_s=−0.38, p=0.004). Als de placebogroep niet werd meegenomen, bleek het tijdstip van inname in absolute tijd gerelateerd aan de verschuiving van het inslaaptijdstip (r=−0.35, p=0.006), maar niet aan de verschuiving van de DLMO.

Er werd geen relatie tussen dosis en mate van verschuiving cq verkorting van de DLMO, inslaaptijdstip en inslaapduur gevonden in het onderzochte doseringsgebied van 0,05-0,15 mg/kg. Wel nam het effect van melatonine op alle parameters toe bij een groter verschil tussen inname tijdstip en DLMO. Daarnaast versterkte het direct slaapverwekkende effect van melatonine de verschuiving van het inslaaptijdstip.

Deze studie heeft aangetoond dat melatonine in een dosering van 0,05 mg/kg effectief is voor de behandeling van chronische inslaapstoornissen bij kinderen als het tenminste 1 tot 2 uur voor de DLMO en het gewenste inslaaptijdstip wordt ingenomen.

In vervolg op de Meldos studie hebben we naar de effecten van langdurig gebruik van melatonine op puberteit ontwikkeling, slaap kwaliteit en algemene ontwikkeling (functioneren) van de kinderen gekeken, en vergeleken met onderzoeksresultaten in de algemene bevolking van dezelfde leeftijd. Dit vervolgonderzoek, beschreven in **hoofdstuk 7**, is gedaan onder de kinderen die ook hadden meegedaan aan het Meldos onderzoek. De uitkomsten waren de resultaten van drie voor Nederlandse kinderen aangepaste internationale vragenlijsten, de “sterke kanten en moeilijkheden” vragenlijst (Strength and Difficulties Questionnaire = SDQ), de “kinderen slaapgewoonten” vragenlijst (Children Sleep Habits Questionnaire = CSHQ), en de Tanner (puberteit ontwikkeling) vragenlijst. Verder werd de gemiddelde therapieduur, de duurzaamheid van het effect, de bijwerkingen en overige redenen om eventueel te stoppen met melatoninetherapie geïnventariseerd.

De gemiddelde therapieduur bij 51 kinderen was 3,1 jaar (minimum 1,0 en maximum 4,6 jaar) en de gemiddelde dosis was 2,68 mg (minimum 0,3 en maximum 10 mg). Gemiddelde vragenlijst scores verschilden niet statistisch significant van de bekende gepubliceerde scores in de gemiddelde Nederlandse bevolking van dezelfde leeftijd en geslacht.

Dit vervolgonderzoek toont aan dat melatoninebehandeling van kinderen langdurig kan worden voortgezet zonder dat meetbare afwijkingen worden gezien in de ontwikkeling,

van puberteit en in de algemene ontwikkeling, in vergelijking met de algemene Nederlandse bevolking.

Concluderend kan worden gesteld dat het onvermogen van een individu om zich adequaat voor te bereiden en aan te passen aan de strak georganiseerde sociale omgeving een belangrijke oorzaak kan zijn voor het ontstaan van slaapstoornissen. De meting van endogeen melatonine is een indicator (*biomarker*) voor de herkomst van de slaapproblemen en voor de te verwachten effectiviteit van het toedienen van exogeen melatonine (geneesmiddel) als modulator.

Voor de behandeling van chronische inslaapstoornissen bij kinderen is melatonine in een dosering van 0,05 mg/kg effectief als het tenminste 1 tot 2 uur voor de DLMO en het gewenste inslaaptijdstip wordt ingenomen, waarmee een verschuiving van DLMO en inslaaptijdstip van 1 uur en een inslaapduur reductie van 35 minuten wordt bewerkstelligd. Om zeker te zijn van optimale therapie is het aan te raden om voor het starten van de therapie de DLMO en de snelheid van melatonine afbraak door CYP450 1A2 (door fenotypering of genotypering) te bepalen. Melatonine behoudt de werkzaamheid gedurende meerdere jaren en algemene ontwikkeling en puberteitontwikkeling lijken er niet door te worden beïnvloed.

LIST OF ABBREVIATIONS

ADHD	attention deficit hyperactivity disorder
CSHQ	children sleep habits questionnaire
(C)SOI	(chronic) sleep onset insomnia
DLMO	dim light melatonin onset
DSPD	delayed sleep phase disorder
FEO	food entrainable oscillator
ID	intellectual disability
MEL	melatonin
MSF(sc)	mid time sleep on free days (corrected for sleep restoring)
NPS	non problematic sleeper
PAD	phase angle difference
PS	problematic sleeper
SDCS	sleep disturbance scale of children
SDS	standard deviation score
SDQ	social development questionnaire
SOL	sleep onset latency
SOT	sleep onset time
clock-TOA	clock time of administration
circadian-TOA	time of administration related to DLMO (circadian time)
TST	total sleep time
WU	wake up time

DANKWOORD

Na mijn bijvak/scriptie ervaring van ruim een jaar, dacht ik dat onderzoek doen niets voor mij was, en ben ik “toch maar apotheker” geworden. Het ziekenhuis was gelukkig echt helemaal mijn stiel, en in het ziekenhuis te Ede voelde ik mij in de kliniek als een vis in het water. Ook in de praktijk van alledag kwamen wel onderzoeksvragen boven, en juist die activiteiten vormden op den duur de krenten in de pap. In die lijn werd ook mijn onderzoek naar de beste kinderdosering voor melatonine in Ede gestart.
Hoewel mijn copromotor dr Marcel G. Smits, neuroloog in Ede, al snel de ambitie uitsprak om hiervan een promotietraject te maken, duurde het nog tot na mijn overstap naar de Universiteit Utrecht, en vereiste het “enige” aandrang van Marcel Smits, voordat ik hiervoor steun zocht bij hoogleraren van de faculteit Diergeneeskunde en het departement Farmaceutische Wetenschappen.

Dit boekje was er dus zeker nooit gekomen zonder mijn copromotor. Beste Marcel, jij bent een bevlogen onderzoeker, en bovenal gedreven om anderen tot onderzoek aan te zetten, een professor honoris causa. Ik denk dat dit het belangrijkste doel van promoveren is: ervoor zorgen dat goed en gedegen onderzoek gedaan blijft worden en de resultaten beschikbaar komen voor de maatschappij. Ik hoop dat ik de komende jaren naar jouw voorbeeld onderzoek zal blijven doen!

Mijn twee promotoren, Prof.dr Henk Vaarkamp en Prof.dr Toine C.G. Egberts, ben ik zeer erkentelijk voor hun enthousiasme vanaf het eerste moment dat ze betrokken raakten bij het onderzoek. Beste Toine, ik weet nog goed dat je zomer 2007 direct tijd wilde vrijmaken om eens te kijken naar de al verzamelde resultaten, en er zo positief over was dat ik de stap durfde te zetten om voor het eerst serieus na te denken over een promotie. Dank voor alle vertrouwen en motivatie. Beste Henk, samen met het managementteam (MT) van de apotheek reageerde je positief op het plan om het onderzoek uit Ede af te maken vanuit de faculteit Diergeneeskunde. Jij was er altijd als ik behoefte had aan een *sparrring partner*, en je hebt me veel *food for thought* gegeven, ik hoop dat we nog veel samen zullen optrekken!

Ik denk met veel plezier terug aan de regelmatige bijeenkomsten met de promotie-commissie, want waar kun je nu meer inspiratie vinden dan in zo’n multidisciplinair overleg met arts, apotheker en dierenarts?

The visit of Professor Alfred Lewy to Holland in May 2010 was very inspiring, and I’m very proud and grateful he agreed to join the assessment committee. Dear Al, your initiative to get to know melatonin researchers world wide meant a lot to me.

Ook de andere leden van de leescommissie, Prof.dr H.G.M. Leufkens, Prof.dr E. van Someren, Prof.dr D. Heederik en dr C. Rademaker, wil ik bedanken voor het beoordelen van mijn manuscript en voor de complimenten.

Departementsvoorzitter Paard, Prof.dr Ab Barneveld en manager bedrijfsvoering drs Anita Nieuwenhuis vormden destijds het MT, en hen ben ik veel dankbaarheid verschuldigd voor het faciliteren en daarna belangstellend volgen van mijn onderzoekswederwaardigheden. Ab, dank je wel voor je vertrouwen, en voor je adviezen in de afgelopen jaren. Anita, ik ben erg blij met jouw humor en flexibiliteit, waarmee je altijd weer problemen tot de juiste proporties wist terug te brengen….

Dr Monique de Vries, directeur Faculteit Diergeneeskunde Universiteit Utrecht, regelde het contract met de Faculteit Betawetenschappen. Monique, dank je wel!

De huidige manager bedrijfsvoering van de faculteit Diergeneeskunde, dr Rob Grift, heeft met zijn oog voor verhoudingen de afgelopen jaren mede gefaciliteerd dat de organisatie van de apotheek veel beter aansloot op alle behoeften, waardoor ook mijn onderzoek en het schrijven weer een nieuwe impuls hebben gekregen vorig jaar. Rob, dank je wel voor je vertrouwen en positieve inbreng in de positie van de apotheek.

Mijn eerste studievriendin en collega, mijn beste vriendin Rosiënne Franssen, fijn dat je mijn paranimf wilt zijn!

Mijn overige collega's uit Ede, Gert van der Meer, Mirian Kaal, Leonie Eppenga-Keder Susanne Marczinski, Els van der Wouden en Jeroen Brogtrop, wil ik danken voor de ruimte die ik kreeg om onderzoek te doen, en het constructief meedenken over allerlei praktische aspecten van de Meldos trial.

De apothekersassistenten van het trialteam, Hanna, Kirsten en Alie, ik dank jullie voor alle ondersteuning bij de uitvoering van de trial, inclusief het af en toe opvangen en instrueren van patiënten! De apothekersassistenten van de productie afdeling, Esther en Wilma, bedankt voor het maken van al die minibatches capsules!
De analisten van het klinisch farmaceutisch laboratorium, Ad, Ilja, Annemieke en José, vooral bedankt voor jullie begrip en support als ik het weer eens te druk had met andere dingen dan het lab….

Mijn dank gaat ook uit naar de BCWO/MEC, en dan met name Marjan Faassen als ambtelijk secretaris, van ziekenhuis Gelderse Vallei, voor het ondersteunen van de destijds niet gemakkelijke opgave om een kindertrial goedgekeurd te krijgen via de CCMO!

De afgelopen jaren waren er verschillende melatonine onderzoekers met wie ik op afstand heb mogen samenwerken. Dr Kristiaan van der Heijden wil ik bedanken voor het kritisch meelezen en meedenken bij diverse manuscripten en Dr Wiebe Braam wil ik bedanken voor het mij betrekken bij patiëntgebonden farmaceutische uitdagingen om zo mijn klinische blik te verbreden. Prof.dr Robert Didden en Dr Hubert Korzilius: bedankt voor alle statistiek ondersteuning! Prof.dr Stef van Buuren wil ik bedanken voor beantwoorden van alle vragen van "mijn" bijvak-student en het al eerder beschikbaar stellen van zijn alternatieve methode om puberteitsontwikkeling te meten.

Het secretariaat van de afdeling Neurologie, Marijke en Karin, dankzij jullie liep de logistiek vanaf het begin, en ook na mijn vertrek naar Utrecht gladjes. De medewerkers van de afdeling Klinische Neuro Fysiologie hebben mij geïnstrueerd over het programmeren en uitlezen van de actometers, en maakten altijd een computer vrij als ik weer eens binnenviel, bedankt!

Het onderzoek was niet mogelijk geweest zonder de medewerking van drs Ben Dujardin, klinisch chemicus en hoofd van het Klinisch Chemisch en Hematologisch Laboratorium waar alle patiënten speekselmonsters in het kader van onderzoek werden geanalyseerd, en later Gerard Jonker en dr Silvia Endenburg, die het speekselonderzoek bij varkens mogelijk maakten.

Pharma Nord leverde de melatonine grondstof waarvan we de patiënten capsules bereidden.

Mijn collega-apothekers van de faculteit Diergeneeskunde Sietske Mesu en Esther Winter wil ik bedanken voor hun vertrouwen, hun belangstelling en hun bereidheid telkens weer de honneurs van de apotheek waar te nemen als ik een schrijfweek reserveerde in mijn agenda. Alle andere apotheekmedewerkers wil ik bedanken voor hun meeleven!

Dr Arie van Nes, dierenarts/UHD van dep. Landbouwhuisdieren: dank voor de nachten in de varkensstallen die je met me wilde delen!

De Familie Zwijgers wil ik heel hartelijk bedanken voor hun gastvrijheid en de bereidheid om mee te werken aan mijn onderzoek.

De studenten die me hebben geassisteerd bij het data verzamelen, Marica Huijbregt-Smits tijdens haar apotheker-stage in het ziekenhuis, Robert Mol (onderzoeksstage farmacie) en Maaike Pannekoek (onderzoeksstage diergeneeskunde) wil ik bedanken voor hun inzet en hun gezelligheid.

Uiteraard ben ik ook veel dankbaarheid verschuldigd aan de kinderen met inslaapstoornissen en hun ouders die bereid waren mee te werken aan twee onderzoeken, en daar veel tijd in hebben gestoken.

Ik wil op deze plaats ook mijn ouders bedanken, die ooit de basis hebben gelegd voor wie ik nu ben. Mijn vader heeft mij altijd gestimuleerd om verder te gaan, ook al ging ik een aantal keer een andere kant op (eerst de farmacie, later de diergeneeskunde) dan hij had gehoopt. Hij zei iedere keer weer dat het toch goed was.

En tot slot een woordje tot mijn meest dierbare familieleden. Lieve Ed, jij bent al 28 jaar mijn grootste inspirator. We hebben samen met veel liefde fantastisch veel bereikt, en we hebben daarnaast elkaar de ruimte gegeven om ons eigen werk ook met veel toewijding te doen. De afgelopen maanden lag de balans meestal aan mijn kant, ik hoop dat het evenwicht de komende jaren weer veel vanzelfsprekender in het midden zal liggen. Het voelt superveilig dat je me wilt bijstaan als paranimf. Lieve Alex, Bas en Myrthe, ik ben zo trots op jullie; dankzij jullie zelfstandigheid kon ik zoveel tijd in mijn werk stoppen, ik hoop dat jullie ook trots op mij zijn!

CURRICULUM VITAE

Inge van Geijlswijk was born on may 7th, 1965 in Amsterdam, Netherlands. In 1983, she finished VWO at Werkplaats Kindergemeenschap, Bilthoven and studied Pharmacy in Utrecht. In 1991 she passed her pharmacist exam and immediately started as a junior pharmacist in Sint Lucas Hospital Amsterdam, more or less in the foot-steps of her father, who started as junior doctor in this same hospital after finishing his physician exam in 1969. After six months, in 1992, she started as a hospital pharmacist trainee at the Clinical Pharmacy Department of Erasmus Medical Centre in Rotterdam under supervision of Dr. J.W. Meilink and Drs. H. Graatsma. In 1996 she registered as a hospital pharmacist. In 1997 she was mainly involved as radio pharmacist at the Nuclear Medicine Department of Erasmus MC. In 1998 she started with a three months free lance job at the Clinical Pharmacy Department of Academic Medical Centre Amsterdam to update the hospital drug formulary and a six months period as interim hospital pharmacist in hospital Zevenaar (in affiliation with the Clinical Pharmacy Department of Rijnstate hospital Arnhem).
Eventually, she started in Gelderse Vallei Hospital, in 1998 in Bennekom, as from 2000 in Ede as hospital pharmacist (head: Drs. Y.G. van der Meer), with focus on clinical trial management, nuclear medicine (preparation of radiopharmaceuticals, local level 3 expert) and clinical-pharmaceutical laboratory. Soon she became involved in multiple melatonin trials of the sleep-wake specialist of this hospital, Dr M.G. Smits, neurologist. In 2003, the idea to initialize a children melatonin dose finding trial emerged, which eventually resulted in this thesis.
In 2007, she moved to the position of head of Pharmacy at the faculty of Veterinary Medicine of Utrecht University. Here, the focus was and still is primarily on development of the pharmacy department to a GMP facility and research concerning Dutch antibiotic medicine application in farm animals. In 2008, cooperation with the Clinical Pharmacy Department of University Medical Centre Utrecht under direction of Prof A.C.G. Egberts was established, which resulted in re-evaluation of obtained melatonin trial results and eventually in joining Utrecht Institute of Pharmaceutical Sciences (UIPS) to extend and finish the melatonin research.

Inge van Geijlswijk is married to Ed Moret, with whom she has three children, Alex (1996), Bas (1997) and Myrthe (2003).

PUBLICATIONS

Activity based costing: logistiek onder de loep. Van Geijlswijk IM *(Ziekenhuisfarmacie 1996; 12: 163-165)*

Site-specific intracoronary heparin delivery in humans after balloon angioplasty. A radioisotopic assessment of regional pharmacokinetics. Camenzind E, Bakker WH, Reijs A, van Geijlswijk IM, Boersma E, Kutryk MJ, Krenning EP, Roelandt JR, Serruys PW;. (*Circulation 1997; 96 (1): 154-165)*

Site-specific intravascular administration of drugs: history of a method applicable in humans Camenzind E, Bakker WH, Reijs A, van Geijlswijk IM, Foley D, Krenning EP, Roelandt JR, Serruys PW (*Cathet Cardiovasc Diagn 1997; 41(3): 342-347)*

Aripiprazol (Abilify). Geijlswijk IM van, Gudde TW, Croone H *(PW 2004; 139: 765-69)*

Site-Specific Intracoronary Delivery of Octreotide in Humans: A Pharmacokinetic Study to Determine Dose-Efficacy in Restenosis Prevention. Edoardo Camenzind, Willem H. Bakker, Ambroos Reijs, Alberto Righetti, Ingeborg M. van Geijlswijk, Eric Boersma, David Foley, Eric P. Krenning, Jos RTC. Roelandt, and Patrick W. Serruys *(J Cardiovasc Pharmacol 2004;43:133–139)*

De klok gelijkzetten Geijlswijk IM van, Dujardin BCG, Smits MG (*Medisch Contact 2005*; *60: 1259-61)*

Reliable New High-Performance Liquid Chromatographic Method for the Determination of Ciprofloxacin in Human Serum. Ingeborg M. van Geijlswijk, Arthur R. H. van Zanten and Y. Geert van der Meer *(Ther Drug Mon 2006; 28: 278-281)*

Coma with ECG abnormalities: consider tricyclic antidepressant intoxication **J**. Veris-van Dieren, L. Valk, I. van Geijlswijk, D. Tjan, A. van Zanten *(Neth J Med 2007; 63: 142-147)*

Ciprofloxacin pharmacokinetics in critically ill patients: a prospective cohort study. Arthur R. H. van Zanten, Kees H. Polderman, Ingeborg M. van Geijlswijk, Gert Y.G. van der Meer, Marinus A. Schouten, Armand R.J. Girbes *(J Crit Care 2008; 23: 422-430)*

Extracorporal elimination in acute valproate intoxication: a case report. Els A. van der Wouden, Angela Dekkers, Hannah M.E. Kruis, Ingeborg M. van Geijlswijk, David H.T. Tjan, and Geert W. Feith *(BMJ-case reports 2009 [doi:10.1136/bcr.06.2008.0337])*

Kwantificeren van veterinair antibioticagebruik, Inge M van Geijlswijk, Dik J Mevius, Linda F Puister-Jansen *(Tijdschrift voor Diergeneeskunde 2009; 134: 69-73)*

Exogenous melatonin for sleep problems in individuals with intellectual disability: a Meta-analysis. Wiebe Braam, Marcel G. Smits, Robert Didden, Hubert Korzilius, Ingeborg M. van Geijlswijk, Leopold M. G. Curfs *(Dev Med Child Neurol. 2009; 51:340-349)*

Long term follow up of melatonin treatment in children with ADHD and chronic sleep onset insomnia. Michel Hoebert, Kristiaan B. van der Heijden, Ingeborg M. van Geijlswijk, Marcel G. Smits *(J Pineal Res. 2009; 47:1-7)*

Transparante besluitvorming voor antimicrobiële therapie bij varkens. Van Leengoed L, Postma M, van Geijlswijk IM, Feitsma H, Meijer E, van Groenland GJ, Mevius D. *(Tijdschrift voor Diergeneeskunde 2010; 135: 282-288)*

Loss of response to melatonin treatment is associated with slow melatonin metabolism. Braam W, van Geijlswijk I, Keijzer H, Smits MG, Didden R, Curfs LMG *(J Intellectual Dis Res. 2010; 54: 547-555)*

Onset and stability of melatonin treatment effect in childhood sleep onset insomnia. van Geijlswijk IM, Didden R, van der Heijden KB, Smits MG *(Sleep Sci 2010;3:16-21)*

Dose finding of melatonin for chronic idiopathic childhood sleep onset insomnia: an RCT. van Geijlswijk IM, van der Heijden KB, Egberts AC, Korzilius HP, Smits MG *(Psychopharmacology (Berl) 2010; 212:379-391)*

The Use of Exogenous Melatonin in Delayed Sleep Phase Disorder: A Meta-analysis. van Geijlswijk IM, Korzilius HP, Smits MG *(Sleep 2010 33:1605-1614)*

Evaluation of Sleep, Puberty and Mental Health in Children with Long-Term Melatonin Treatment for Chronic Idiopathic Childhood Sleep Onset Insomnia. van Geijlswijk IM, Mol RH, Egberts AC, Smits MG *(Psychopharmacology (Berl) 2011)*

Universiteit Utrecht