

Influence of melatonin on fatigue severity in patients with chronic fatigue syndrome and late melatonin secretion

R. O. van Heukelom^a, J. B. Prins^b, M. G. Smits^a and G. Bleijenberg^c

^aDepartment of Neurology, Sleep-Wake Disorders and Chronobiology, Hospital De Gelderse Vallei, Ede; ^bDepartment of Medical Psychology, Radboud University Medical Centre Nijmegen, Nijmegen; and ^cExpert Centre Chronic Fatigue, Radboud University Medical Centre Nijmegen, The Netherlands

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The effect of melatonin, a chronobiotic drug, was explored in 29 patients with chronic fatigue syndrome (CFS) and Dim Light Melatonin onset (DLMO) later than 21.30 hours, reflective of delayed circadian rhythmicity. The patients took 5 mg of melatonin orally, 5 h before DLMO during 3 months. Their responses to the checklist individual strength (CIS), a reliable questionnaire measuring the severity of personally experienced fatigue, were assessed twice with a 6-week interval immediately before the treatment and once after 3 months treatment. In the pre-treatment period the fatigue sub-score improved significantly. After treatment, the total CIS score and the sub-scores for fatigue, concentration, motivation and activity improved significantly. The sub-score fatigue normalized in two of the 29 patients in the pre-treatment period and in eight of 27 patients during treatment. This change was significant. In the patients with DLMO later than 22.00 hours ($n = 21$) the total CIS score and the sub-scores for fatigue, concentration and activity improved significantly more than in the patients ($n = 8$) with DLMO earlier than 22.00 hours. Melatonin may be an effective treatment for patients with CFS and late DLMO, especially in those with DLMO later than 22.00 hours.

Introduction

The contribution of delayed melatonin secretion onset to the experience of fatigue is suggested by several studies which have demonstrated possible circadian rhythm disturbances in chronic fatigue patients [1–3]. Melatonin, a hormone produced by the pineal gland during the dark phase of the day–night cycle, plays a major role in the synchronization of circadian rhythms [4]. The dim light melatonin onset (DLMO) test, which measures the time at which melatonin secretion starts to increase in dim light, is an established and particularly convenient method for assessing circadian phase position [5]. In healthy subjects the DLMO usually occurs between 18.00 and 21.30 hours [6].

Melatonin, 5 mg, administered 5 h before DLMO, advances melatonin onset and sleep–wake rhythm in patients with delayed sleep–wake rhythm and late DLMO (delayed sleep phase syndrome). Furthermore it makes these patients feel more refreshed in the morning and increases quality of life, as assessed by the SF-36 health questionnaire [7,8].

Previous work has demonstrated that chronic fatigue syndrome (CFS) patients with late DLMO, who have received 3 months of melatonin treatment, experience

improved scores on the SF-36 questionnaire [9]. The influence of melatonin on fatigue severity has not yet been studied in these patients.

To guide the design of a future randomized placebo-controlled trial in CFS patients with delayed melatonin onset, we analyzed the data of CFS patients with late melatonin onset, who were treated with melatonin, 5 mg, administered 5 h before DLMO.

Methods

Study protocol

Patients referred to the specialist CFS outpatient clinic of the Gelderse Vallei hospital participated in the study. All patients with chronic fatigue studied fulfilled the following criteria of CFS: (1) persistent fatigue sufficient to impair daily activities for at least 1 year, and present for at least 50% of the day during the preceding 6 months; (2) no coexistent physical illness which could explain chronic fatigue; (3) no current or recent medication which could interfere with sleep, mood or circadian rhythmicity (e.g. hypnotics, antidepressants or steroids); (4) no disrupted sleep patterns with loss of restorative sleep.

When the patient reported disturbances in the sleep–wake rhythm at the first visit, an ambulatory polysomnography test was performed at the patient's

Correspondence: M. G. Smits, Hospital 'Gelderse Vallei', Box 9025, 6710 HN Ede, The Netherlands (tel.: + +31 318 435016; fax: + +31 318435025; e-mail: smitsm@zgv.nl).

home. This was done to exclude symptoms of disturbed sleep architecture which might have contributed to measurement artefacts: these included fragmented sleep, shortage or slow wave sleep and rapid eye movement sleep, sleep apnea syndrome, and restless legs/periodic leg movement disorder. Furthermore endogenous salivary melatonin was assessed by collecting saliva at the patient's home in dim light, hourly from 21.00 to 1.00 hours. Melatonin was measured in saliva as previously described [10]. DLMO, defined as the time at which salivary melatonin reaches 4 pg/ml, was calculated as the linearly interpolated time of the first sample above 4 pg/ml that was preceded by a lower value [10].

At the second visit, 6 weeks after the first visit, the results of these investigations were discussed with the patient. When sleep architecture was normal, DLMO occurred later than 21.30 hours and when there were no contra indications for melatonin treatment, including epilepsy and use of oral anticoagulants [11], patients were told that they possibly suffered from a Delayed Sleep Phase Syndrome-like disorder. And that we usually treat this disorder with melatonin, administered 5 h before individual DLMO. We use this high pharmacological dose as this dose was used in the placebo-controlled studies on melatonin in Delayed Sleep Phase Syndrome [7,12]. The patients were given oral and written information about melatonin. Melatonin is a not-licensed drug in Europe. In the United States it has the status of a food supplement, which is over the counter available. Severe adverse effects have not yet been reported [13]. The patients who wished to be treated received melatonin, 5 mg, administered 5 h before their individual DLMO. Three months later the results were evaluated at the third visit.

Checklist individual strength

The checklist individual strength (CIS) [14] is a reliable and valid questionnaire for measuring fatigue [14–19]. It measures four aspects, namely fatigue severity (eight items), concentration (five items), motivation (four items) and activity (three items). Each item is scored on a 7-point scale. A maximum score (140) indicates the most severe clinical symptoms. The CIS has been tested thoroughly in clinical settings among patients with chronic fatigue, other chronic diseases and healthy controls [14–17,19,20–23] In healthy controls the sub-score fatigue is ≤ 35 .

To assess changes in fatigue, all patients who visit the specialist CFS outpatient clinic complete a CIS questionnaire at every visit. We analyzed the questionnaires, completed at the first, second and third visit.

Statistics

The results were analyzed using the SPSS 11.5 package (SPSS Inc., Chicago, IL, USA). The Wilcoxon rank-sum test was used to test the differences of the CIS scores between visit 1 and 2 (baseline) and between 2 and 3 (before and after 3 months melatonin treatment) and to test whether there were differences between the CIS scores of the patients at the first and second visit and those of a control population ($n = 38$) described earlier [17]. Relations between DLMO and changes of CIS scores were analyzed by computing Spearman's rho correlations.

To see if patients with relatively early DLMO (between 21.30 and 22.00 hours) responded differently from patients with late DLMO (later than 22.00 hours) the difference in change in the CIS scores during 3 months melatonin treatment were analyzed between these two groups of patients, using general linear model repeated measures procedure with DLMO (early versus late) as between-subjects factor and measurement (second visit versus third visit) as within subjects factor.

To assess the number of patients, who reached normality as to the sub-score fatigue, we calculated its frequencies. The Mc Nemar test was used to test the differences between the three visits.

Results

Forty CFS patients with feelings of insomnia and normal polysomnography were seen successively at the outpatient clinic. In seven patients DLMO could not be determined because salivary melatonin concentrations remained lower than 4.0 pg/ml. Four patients could not be treated with melatonin because they wanted to be pregnant during the treatment period. Twenty-nine patients, 24 women and five men, completed the CIS questionnaire at the three visits. There were no drop-outs. Mean (SD) age was 33.2 (11.2) years. Mean (SD) DLMO occurred at 22.52 (1.04) hours. In eight patients DLMO occurred between 21.30 and 22.00 hours (relatively early DLMO). Mean (SD) interval between first and second visit (baseline period) was 6.4 (0.7) weeks. Mean (SD) interval between second and third visit (treatment period) was 12.9 (0.9) weeks. The CIS scores of the patients with relatively early DLMO did not differ significantly from those with late DLMO.

The results of the CIS scores are recorded in Table 1. The scores of the chronic fatigue patients at the first and second visit were significantly higher than those of the control population ($P < 0.05$). From visit 1 to 2 the sub-score fatigue improved significantly. During melatonin treatment (visit 2–3) total CIS score and all sub-scores improved significantly.

Table 1 Results of CIS total score and sub-scores

	Healthy people (<i>n</i> = 38) ^b [mean (SD)]	Visit 1 (<i>n</i> = 29) [mean (SD)]	Visit 2 (<i>n</i> = 29) [mean (SD)]	Visit 3 (<i>n</i> = 29) [mean (SD)]	Dif. visit 1–2 (<i>P</i> ^a)	Dif. visit 2–3 (<i>P</i> ^a)
Total score	47.3 (19.8)	111.0 (14.4)	105.3 (23.9)	92.4 (27.2)	0.284	0.006
Fatigue	21.9 (11.4)	52.4 (4.2)	48.3 (11.2)	43.2 (13.2)	0.026	0.017
Concentration	9.9 (5.4)	25.1 (7.5)	25.6 (8.4)	22.3 (8.9)	0.465	0.031
Motivation	9.9 (5.1)	16.9 (6.9)	16.7 (6.4)	14.0 (6.3)	0.909	0.010
Activity	5.6 (3.0)	16.7 (4.6)	14.8 (6.2)	12.9 (6.3)	0.056	0.008

Dif, difference; CIS, checklist individual strength.

^aAnalyzed with the Wilcoxon rank-sum test. In bold statistically significant differences.

^bRef.: Vercoulen *et al.* [15].

During melatonin treatment total CIS score and the sub-scores for fatigue, concentration and activity improved significantly more in the patients with late DLMO than in those with relatively early DLMO (Table 2).

In the pre-treatment period the sub-score fatigue normalized in two of the 29 patients. During treatment this score normalized in eight of 27 patients. This change was significant ($P = 0,031$). DLMO did not correlate significantly with change of the CIS scales except for the sub-score physical activity between visit 1 and 2 ($R = -0,369$; $P = 0,049$).

Discussion

This open label study showed that in CFS patients with DLMO later than 21.30 hours the sub-score fatigue normalized during the melatonin treatment period in significantly more patients than during pre-treatment. Total CIS scores and the sub-scores fatigue, concentration, motivation and activity improved during treatment, while in the pre-treatment period only the sub-score fatigue improved.

The decrease of fatigue during melatonin treatment, assessed with the CIS, corresponds with findings of an earlier study [9], showing improved quality of life in CFS patients with late melatonin onset during melatonin treatment.

The improvement of the CIS 'fatigue' sub-score in the 6-week pre-treatment period corresponds with the improvement of fatigue severity in CFS patients seen in the CFS outpatient clinic in the period between the first visit and the next visit several weeks later (J.B. Prins, personal communication).

The improvement during the melatonin treatment can be due to a placebo effect or to the effects of melatonin. When the improvement should be due to a placebo effect, one should expect that patients with relatively early DLMO respond the same to the treatment as patients with late DLMO. However we found that these latter patients improved much more than patients with relatively early DLMO. Furthermore, the relatively long period between starting treatment and completing the outcome measure CIS [24], makes it unlikely that the effects of melatonin treatment were because of a placebo response.

Using an arbitrary cut-off of early and late DLMO at 22.00 hours our findings suggested circadian problems being related to fatigue, concentration, and activity. However we did not find a significant correlation between DLMO and change in different CIS scales.

Results of observational studies should not be used for defining evidence based medical care [25]. However when observational studies are well designed, they do not systematically overestimate the magnitude of the

Table 2 Checklist individual strength scores differences between relatively early [dim light melatonin onset (DLMO) between 21.30 and 22.00 hours] and late DLMO (DLMO \geq 22.00 hours) treatment groups in the change during melatonin treatment (from visit 2 to visit 3), analyzed with general linear model repeated measures procedure

	Early DLMO (<i>n</i> = 8)		Late DLMO (<i>n</i> = 21)		Difference early – late DLMO	
	Visit 2 [mean (SD)]	Visit 3 [mean (SD)]	Visit 2 [mean (SD)]	Visit 3 [mean (SD)]	<i>F</i>	<i>P</i>
Total score	100.25 (19.97)	102.25 (17.32)	107.29 (25.40)	88.67 (29.65)	6.612	0.016
Fatigue	47.63 (8.54)	48.38 (8.90)	48.52 (12.25)	41.19 (14.24)	4.206	0.050
Concentration	25.13 (9.08)	27.38 (5.34)	25.71 (8.31)	20.38 (9.29)	7.821	0.009
Motivation	16.00 (4.84)	14.13 (5.28)	17.00 (6.99)	14.13 (5.28)	0.298	0.590
Activity	11.50 (7.4)	12.38 (7.19)	16.05 (5.29)	13.14 (6.08)	5.549	0.026

effects of treatment as compared with those in randomized controlled trials [26,27].

Nevertheless the results of our study cannot be used to justify treatment with melatonin in CFS patients. But they can be used to calculate the power for a randomized placebo controlled study. In this study the weak points of the present study should be addressed. Especially co-morbidity, co-medication, sleep-wake parameters, duration and probable cause of the circadian rhythm disorder should be assessed. Furthermore, studies in patients who complete both CIS and SF-36 questionnaires will show whether changes of fatigue correspond with changes in quality of life.

Melatonin has both a hypnotic and a chronobiotic effect [28] The hypnotic effect occurs within one hour after administration and decreases one hour later. The chronobiotic effect, which advances sleep onset and offset times, occurs maximally when melatonin is administered 5 h before DLMO [29]. Probably the positive effect of melatonin in our patients is because of this chronobiotic effect. We did not measure DLMO after melatonin treatment because several placebo-controlled studies evidently showed that melatonin advances DLMO in patients with late DLMO [7,30–33]. Williams *et al.* [34] administered melatonin 2 h before desired bed time in CFS patients. They could not demonstrate effectiveness. This is probably because they did not assess circadian rhythmicity and because they administered melatonin at a time at which chronobiotic effects do not occur. Another explanation could be the cross-over design of their study. Consequently some patients received melatonin during the first part of the study. This could normalize their circadian rhythmicity. After stopping the melatonin treatment the rhythmicity could remain normal during the following study period.

We found a late DLMO in our patients. This late DLMO can be due to a defect of clock genes [35], enzymes involved in the melatonin synthesis [7], or neural connections between the retina and pineal gland [36]. The late DLMO in our patients suggests that delayed circadian rhythmicity might be related to CFS symptomatology. However, the CIS scores of the patients with early DLMO did not differ significantly from those with late DLMO.

Chronic fatigue syndrome is often preceded by viral infections. These viral infections could have damaged the neural connections between retina and pineal gland, resulting in late melatonin onset and disturbed circadian rhythm. Just as these connections can be damaged by a whiplash trauma, resulting in a chronic whiplash syndrome with delayed sleep phase syndrome [33,36]. Another possibility is that the late melatonin onset is because of the shift of the sleep-wake rhythm of the chronic fatigue patient.

According to several reports known physical causes for chronic fatigue have to be excluded before diagnosing CFS [37–42]. In our chronic fatigue patients, circadian rhythmicity seemed to be disturbed, as suggested by the late melatonin onset. Consequently they could be considered as CFS patients with overlapping DSPS, or DSPS patients formerly misdiagnosed as CFS.

The insomnia complaints of our patients could not be confirmed polysomnographically. The pathophysiology of this insomnia subtype, sleep state misperception, is still unclear [43].

The present study warrants to be aware of delayed rhythmicity in CFS patients. As long as the effectiveness of melatonin has not been established in randomized placebo-controlled studies, special attention should be given to other generally accepted treatments for delayed circadian rhythmicity. Consequently special attention should be given to strengthen time cues and the influence of early morning light [44].

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References

1. Moldofsky H. Sleep, neuroimmune and neuroendocrine functions in fibromyalgia and chronic fatigue syndrome. *Advances in Neuroimmunology* 1995; **5**: 39–56.
2. Parker AJ, Wessely S, Cleare AJ. The neuroendocrinology of chronic fatigue syndrome and fibromyalgia. *Psychological Medicine* 2001; **31**: 1331–1345.
3. Racciatti D, Guagnano MT, Vecchiet J, *et al.* Chronic fatigue syndrome: circadian rhythm and hypothalamic-pituitary-adrenal (HPA) axis impairment. *International journal of immunopathology and pharmacology* 2001; **14**: 11–15.
4. Arendt J, Deacon S, English J, Hampton S, Morgan L. Melatonin and adjustment to phase shift. *Journal of sleep research* 1995; **4**: 74–79.
5. Lewy AJ, Cutler NL, Sack RL. The endogenous melatonin profile as a marker for circadian phase position. *Journal of Biological Rhythms* 1999; **14**: 227–236.
6. Zaidan R, Geoffriau M, Brun J, *et al.* Melatonin is able to influence its secretion in humans: description of a phase-response curve. *Neuroendocrinology* 1994; **60**: 105–112.
7. Nagtegaal JE, Kerkhof GA, Smits MG, Swart AC, van der Meer YG. 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. *Journal of sleep research* 1998; **7**: 135–143.
8. Nagtegaal JE, Laurant MW, Kerkhof GA, Smits MG, van der Meer YG, Coenen AM. Effects of melatonin on the quality of life in patients with delayed sleep phase syndrome. *Journal of Psychosomatic Research* 2000; **48**: 45–50.

9. Smits MG, van Rooij R, Nagtegaal JE. Influence of melatonin on quality of life in patients with chronic fatigue and late melatonin onset. *JCFS* 2000; **48**: 45–50.
10. Nagtegaal E, Peeters T, Swart W, Smits M, Kerkhof G, van der MG. Correlation between concentrations of melatonin in saliva and serum in patients with delayed sleep phase syndrome. *Therapeutic Drug Monitoring* 1998; **20**: 181–183.
11. Herxheimer A, Waterhouse J. The prevention and treatment of jet lag. *BMJ* 2003; **326**: 296–297.
12. Dahlitz M, Alvarez B, Vignau J, English J, Arendt J, Parkes JD. Delayed sleep phase syndrome response to melatonin. *Lancet* 1991; **337**: 1121–1124.
13. Nagtegaal JE, Smits MG, van der Meer YG, Fischer-Steenvoorden MGJ. Melatonin a survey of suspected adverse drug reactions. *Sleep-Wake Res Netherl* 1996; **7**: 115–118.
14. Vercoulen JH, Alberts M, Bleijenberg G. De checklist individual strength (CIS). *Gedragstherapie* 1999; **32**: 131–136.
15. Vercoulen JH, Swanink CM, Fennis JF, Galama JM, van der Meer JW, Bleijenberg G. Dimensional assessment of chronic fatigue syndrome. *Journal of Psychosomatic Research* 1994; **38**: 383–392.
16. Swanink CM, Vercoulen JH, Bleijenberg G, Fennis JF, Galama JM, van der Meer JW. Chronic fatigue syndrome: a clinical and laboratory study with a well matched control group. *Journal of Internal Medicine* 1995; **237**: 499–506.
17. Beurskens AJ, Bultmann U, Kant I, Vercoulen JH, Bleijenberg G, Swaen GM. Fatigue among working people: validity of a questionnaire measure. *Occupational and Environmental Medicine* 2000; **57**: 353–357.
18. Brouwers FM, Van Der WS, Bleijenberg G, Van Der ZL, van der Meer JW. The effect of a polynutrient supplement on fatigue and physical activity of patients with chronic fatigue syndrome: a double-blind randomized controlled trial. *Monthly journal of the Association of Physicians* 2002; **95**: 677–683.
19. Bultmann U, Kant I, Kasl SV, Beurskens AJ, van den Brandt PA. Fatigue and psychological distress in the working population: psychometrics, prevalence, and correlates. *Journal of Psychosomatic Research* 2002; **52**: 445–452.
20. Vercoulen JH, Hommes OR, Swanink CM, et al. The measurement of fatigue in patients with multiple sclerosis. A multidimensional comparison with patients with chronic fatigue syndrome and healthy subjects. *Archives of Neurology* 1996; **53**: 642–649.
21. Vercoulen JH, Swanink CM, Fennis JF, Galama JM, van der Meer JW, Bleijenberg G. Prognosis in chronic fatigue syndrome: a prospective study on the natural course. *Journal of Neurology, Neurosurgery and Psychiatry* 1996; **60**: 489–494.
22. Vercoulen JH, Swanink CM, Zitman FG, et al. Randomised, double-blind, placebo-controlled study of fluoxetine in chronic fatigue syndrome. *Lancet* 1996; **347**: 858–861.
23. Prins JB, Bleijenberg G, Bazelmans E, et al. Cognitive behaviour therapy for chronic fatigue syndrome: a multicentre randomised controlled trial. *Lancet* 2001; **357**: 841–847.
24. Peck C, Coleman G. Implications of placebo theory for clinical research and practice in pain management. *Theoretical Medicine* 1991; **12**: 247–270.
25. Sackett DL, Wennberg JE. Choosing the best research design for each question. *BMJ* 1997; **315**: 1636.
26. Benson K, Hartz AJ. A comparison of observational studies and randomized, controlled trials. *New England Journal of Medicine* 2000; **342**: 1878–1886.
27. Concato J, Shah N, Horwitz RI. Randomized, controlled trials, observational studies, and the hierarchy of research designs. *New England Journal of Medicine* 2000; **342**: 1887–1892.
28. Wirz-Justice A, Armstrong SM. Melatonin: nature's soporific? *Journal of sleep research* 1996; **5**: 137–141.
29. Lewy AJ, Ahmed S, Jackson JM, Sack RL. Melatonin shifts human circadian rhythms according to a phase-response curve. *Chronobiology International* 1992; **9**: 380–392.
30. Smits MG, Laurant M, Nagtegaal JE, Kerkhof GA, Coenen AML. Influence of melatonin on vigilance and cognitive functions in delayed sleep phase syndrome. *Chronobiology International* 1997; **14**: 159.
31. Smits MG, Nagtegaal JE, van der Heijden J, Coenen AM, Kerkhof GA. Melatonin for chronic sleep onset insomnia in children: a randomized placebo-controlled trial. *Journal of Child Neurology* 2001; **16**: 86–92.
32. Smits MG, van Stel HF, van der HK, Meijer AM, Coenen AM, Kerkhof GA. Melatonin improves health status and sleep in children with idiopathic chronic sleep-onset insomnia: a randomized placebo-controlled trial. *Journal of the American Academy of Child and Adolescent Psychiatry* 2003; **42**: 1286–1293.
33. Wieringen Sv, Jansen T, Smits MG, Nagtegaal JE, Coenen AML. Melatonin for chronic whiplash syndrome with delayed melatonin onset. Randomised, placebo-controlled trial. *Clin Drug Invest* 2001; **21**: 813–820.
34. Williams G, Waterhouse J, Mugarza J, Minors D, Hayden K. Therapy of circadian rhythm disorders in chronic fatigue syndrome: no symptomatic improvement with melatonin or phototherapy. *European Journal of Clinical Investigation* 2002; **32**: 831–837.
35. Archer SN, Robilliard DL, Skene DJ, et al. A length polymorphism in the circadian clock gene Per3 is linked to delayed sleep phase syndrome and extreme diurnal preference. *Sleep* 2003; **26**: 413–415.
36. Nagtegaal JE, Kerkhof GA, Smits MG, Swart AC, van der Meer YG. Traumatic brain injury-associated delayed sleep phase syndrome. *Functional Neurology* 1997; **12**: 345–348.
37. Holmes GP, Kaplan JE, Gantz NM, et al. Chronic fatigue syndrome: a working case definition. *Annals of Internal Medicine* 1988; **108**: 387–389.
38. Lloyd AR, Hickie I, Boughton CR, Spencer O, Wakefield D. Prevalence of chronic fatigue syndrome in an Australian population. *Medical Journal of Australia* 1990; **153**: 522–528.
39. Sharpe MC, Archard LC, Banatvala JE, et al. A report – chronic fatigue syndrome: guidelines for research. *Journal of the Royal Society of Medicine* 1991; **84**: 118–121.
40. Schluenderberg A, Straus SE, Peterson P, et al. NIH conference. Chronic fatigue syndrome research. Definition and medical outcome assessment. *Annals of Internal Medicine* 1992; **117**: 325–331.
41. Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. *Annals of Internal Medicine* 1994; **121**: 953–959.

42. Reeves WC, Lloyd A, Vernon SD, *et al.* Identification of ambiguities in the 1994 chronic fatigue syndrome research case definition and recommendations for resolution. *BMC health services research* 2003; **3**: 25.
43. Edinger JD, Krystal AD. Subtyping primary insomnia: is sleep state misperception a distinct clinical entity? *Sleep medicine reviews* 2003; **7**: 203–214.
44. Monk TH, Welsh DK. The role of chronobiology in sleep disorders medicine. *Sleep medicine reviews* 2003; **7**: 455–473.