Melatonin Increases Brown Adipose Tissue Volume and Activity in Patients With Melatonin Deficiency: A Proof-of-Concept Study

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Melatonin, a pineal hormone synthesized at night, is critical for the synchronization of circadian and seasonal rhythms, being a key regulator of energy metabolism in many animal species. Although studies in humans are lacking, several reports, mainly on hibernating animals, demonstrated that melatonin supplementation and a short photoperiod increase brown adipose tissue (BAT) mass. The present proof-of-concept study is the first, to our knowledge, to evaluate BAT in patients with melatonin deficiency (radiotherapy or surgical removal of pineal gland) before and after daily melatonin (3 mg) replacement for 3 months. All four studied patients presented increased BAT volume and activity measured by positron emission tomography-MRI. We also found an improvement in total cholesterol and triglyceride blood levels without significant effects on body weight, liver fat, and HDL and LDL levels. Albeit not statistically significant, fasting insulin levels and HOMA of insulin resistance decreased in all four patients. The present results show that oral melatonin replacement increases BAT volume and activity and improves blood lipid levels in patients with melatonin deficiency, suggesting that melatonin is a possible BAT activator. Future studies are warranted because hypomelatoninemia is usually present in aging and appears as a result of light-at-night exposure and/or the use of β-blocker drugs.

Melatonin, a pineal hormone synthesized and released at night that plays a critical role in the synchronization of circadian and seasonal rhythms, has been studied as a key regulator of energy metabolism in many animal species for a long time (1,2). Pinealectomized rats show increased body weight gain and metabolic disturbances that are prevented by daily melatonin supplementation at night, without decreasing energy intake (1–5). This suggests that melatonin also regulates energy metabolism by its action on energy expenditure, possibly as related to the activation of brown adipose tissue (BAT). Indeed, melatonin has been shown to increase BAT recruitment, measured as increased BAT mass, in experimental models, such as hibernating animals (3). BAT has long been recognized as a thermogenic tissue in mammals, but its significance in humans was considered to be minor and limited to newborns. Recently, positron emission tomography (PET) with 2-deoxy-2-[fluorine-18] fluoro-D-glucose (FDG) showed that human adults present active BAT, especially after cold exposure (6,7). This finding quickly led to an exponential increase in BAT research, since activation of BAT leads to increased energy expenditure that could, at least theoretically, be a possible tool for the treatment of obesity and type 2 diabetes (8). Many compounds, including melatonin, have been studied to determine their ability of BAT recruitment and activation, although none of these studies have been carried out in humans (3,9). Melatonin has been used in the treatment of several pathologies, including sleep disturbances, neurodegenerative diseases, cardiovascular diseases, and cancer (2). As far as human endocrine

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and metabolic diseases are concerned, the importance of regular melatonin daily secretion in determining adequate energy balance and glucose metabolism is seen in several articles that showed a negative correlation between the levels of nocturnal melatonin secretion and BMI, insulin resistance (IR), and type 2 diabetes incidence (10,11). In addition, a few articles demonstrated that chronic therapeutic daily melatonin administration can induce a reduction of fat mass and an increase of lean mass besides being able to counteract antipsychotic drug–induced metabolic adverse effects, including overweight (12–14). However, the exact mechanism by which melatonin exerts its metabolic effects in humans is not known.

In the present study, we sought to determine whether melatonin therapy for patients with melatonin deficiency as a result of pinealectomy could increase BAT activation evaluated by FDG-PET after cold exposure as a proof of concept of a potential influence of melatonin in BAT in humans. Since pineal tumors are very rare, only four patients could be selected. Metabolic parameters were also measured, since a recent meta-analysis suggested a role of melatonin in improving total cholesterol and triglyceride levels (15). Given that high exposure to light at night (LAN) is associated with a reduction in or even suppression of melatonin levels, commonly leading to individuals having functional melatonin deficiency, an investigation in this particular population would also be relevant (16). However, the evaluation of melatonin replacement in patients after pinealectomy is very important, given that despite being widely accepted in classical endocrinology that absolute hormone deficiency should be treated with hormonal replacement, data about melatonin replacement in these patients are scarce.

RESEARCH DESIGN AND METHODS

Ethical Statement

This protocol has been approved by the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo ethical committee and is registered in the Brazilian Unified Research Platform (Plataforma Brasil) under the number 30460114.5.0000.0068. All patients signed an informed consent approved by the ethical committee.

Recruitment and Selection of Patients

We searched for patients referred to surgical removal of the pineal gland or to radiotherapy in the pineal area because of pineal tumors at Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, a reference center for complex and rare diseases in Brazil. We were able to find records of 18 patients in the past 12 years. Five patients could not be contacted because of a lack of an updated address and phone number, three patients died, four patients refused to participate, one did not comply with the protocol, and one was excluded because of a sympathetic lesion caused by an infiltrating tumor. The remaining four patients were studied, and all were male. The characteristics of the patients are described in the Supplementary Data. We had some concern about patient 3 because of the presence of panhypopituitarism that could bias the analysis, as it is well known that cortisol levels present a circadian rhythm that could influence BAT (17). Despite this, we did not exclude the patient because of the low number of subjects. Before melatonin treatment, all subjects had salivary melatonin measured every 3 h on a 27-h schedule (from 7 P.M. until 10 P.M. the next day), and the absence or very low levels of melatonin were confirmed by ELISA (IBL International, Hamburg, Germany). Salivary melatonin was also collected just before the end of melatonin treatment.

Melatonin Replacement

Patients received 108 tablets of melatonin 3 mg (Aché, Guarulhos, Brazil) and were advised to take one tablet 30 min before sleep every day for 3 months. Blister packs were brought back to evaluate adherence.

FDG-PET/MRI

After overnight fasting, the patients were admitted to the Nuclear Medicine Institute. They were exposed to a cold, air-conditioned room (18°C) wearing light clothes and a cooling vest (Polar Products, Stow, OH) for 2 h. After 1 h of cold exposure, the FDG radioisotope was injected, and PET-MRI was performed 1 h later in accordance with protocols used in other studies in the field (18,19).

BAT volume (mL) and activity (volume × mean standardized uptake value [SUV]) with an SUV threshold of 1.5 and 2.0 were assessed by PET-MRI, and liver fat was analyzed by MRI. The software AMIDE (Amide's a Medical Imaging Data Examiner; http://amide.sourceforge.net/packages.html) was used to analyze BAT volume and SUV by a nuclear medicine specialist. Adipose tissue was automatically segmented by function approximation techniques magnetic resonance attenuation correction (FAT-MRAC) or liver imaging with volume acceleration-flexible (LAVA-Flex) sequence using an SUV signal over 1.5 and 2.0 as the threshold for detection of adipose tissue. The activity of the renal pelvis was then subtracted after manually defining the area of interest to exclude urinary activity in regions of possibly erroneously identified adipose tissue. Volumes and activities inside the adipose tissue segment were then calculated. The above procedure was done before and after melatonin replacement.

Blood Test Analysis

We collected blood samples immediately before the PET-MRI protocol both before and after melatonin replacement. Total cholesterol, HDL cholesterol, LDL cholesterol, fasting triglycerides, fasting insulin, and fasting glucose levels were determined and analyzed in the Central Laboratory, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo. A difference in any of them was then considered a secondary end point.

Statistical Analysis

Data were analyzed by paired t test with one-tailed distribution since an increase in BAT was expected as well as

a decrease in triglyceride and total cholesterol levels. For small sample sizes, normality tests have little power to reject the null hypothesis, and, therefore, small samples most often pass normality tests.

End Points

The primary end points were an increase in BAT volume and/or activity by PET-MRI. The secondary end points were differences in the blood levels of any of the metabolites studied.

RESULTS

All four patients completed the protocol, with an adherence of >90%. The study protocol also analyzed sleep patterns, quality of life, and dysautonomia tests, but those data will not be presented here since there are several end points still to be measured with a longer duration of the treatment.

The individual patterns of BAT volume and activity are shown in Fig. 1*A* and *B*, respectively, considering an SUV threshold of 2.0. Both BAT volume and BAT activity statistically increased in all patients after melatonin replacement (P = 0.0179 and 0.0139, respectively). BAT volume and activity considering an SUV threshold of 1.5 are presented in the Supplementary Data and have a very similar pattern of increase (P = 0.0199 and 0.0159, respectively).

Patient 1 had a notably clear pattern of response (Fig. 2) with volume and activity increase of 2.7 and 2.39 times, respectively, after melatonin replacement. Nevertheless, the results could be potentially affected by external factors, such as outdoor temperature and photoperiod, but a correlation between outdoor temperature and BAT volume and/or activity was not found (Supplementary Data).

Despite the positive responses observed in BAT, the patients' body weight did not change (Table 1) after melatonin replacement therapy. Although significant differences in weight in such a small sample were not expected, three patients presented a small weight gain. Maybe a larger sample and/or a longer supplementation could lead to a different result in body weight.

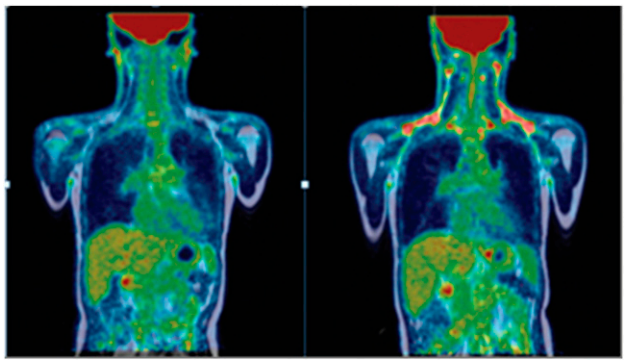

Figure 2—PET-MRI after cold exposure of patient 1 before and after melatonin supplementation. Note the increase in FDG uptake in the supraclavicular and cervical areas.

Although such a small sample was analyzed, melatonin replacement caused a significant decrease in total cholesterol (P = 0.0204) and triglyceride (P = 0.0104) levels (Table 1). With regard to glycemic parameters, no differences were observed; however, fasting insulin and HOMA-IR decreased in all subjects after melatonin replacement (Table 1). Hepatic fat was also evaluated by MRI, and no difference was found (Supplementary Data).

DISCUSSION

The present study shows a significant increase in BAT volume and activity after melatonin replacement therapy

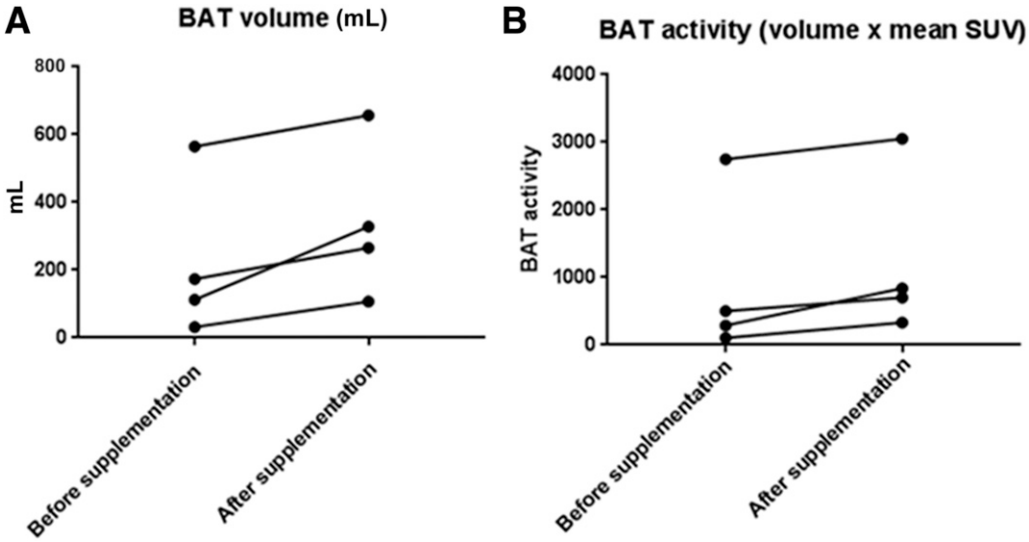

Figure 1—BAT volume (*A*) and activity (*B*) as measured by PET-MRI after cold exposure before and after melatonin supplementation. Significance by Student *t* test in panels *A* and *B* were P = 0.0179 and P = 0.0139, respectively.

Table 1—Metabolic parameters of the patients before and after melatonin supplementation

Patient	Metabolic parameters													
	HDL-C (mg/dL)		LDL-C (mg/dL)		Total cholesterol (mg/dL)		Triglyceride (mg/dL)		Fasting glucose (mg/dL)		Fasting insulin (μU/mL)		HOMA-IR		Body weight (kg)	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	28	42	69	54	120	109	153	54	91	54	16.1	4.0	3.6	0.5	54.2	51.2
2	32	45	113	92	162	155	94	90	78	77	13.4	10.6	2.6	2.0	55.7	56.7
3	29	26	91	108	223	193	552	479	76	81	22.2	19.8	4.2	4.0	76.0	79.2
4	37	38	111	72	177	143	338	249	88	98	24.4	8.6	5.2	2.1	98.5	99.0
Mean	31.5	37.5	96	81.5	170	150	284	218	83.25	77.5	19.0	10.75	3.85	2.15	71.1	71.5
P value (paired *t* test, one-tailed)	0.11		0.15		0.0279*		0.0268*		0.31		0.05		0.058		0.76	

HDL-C, HDL cholesterol; LCL-C, LDL cholesterol. *Statistically significant.

as measured by PET-MRI in four patients with melatonin deficiency as a result of pinealectomy. Despite the very small sample size, the increase was observed in all patients, suggesting that melatonin may be a potential BAT recruiter that should be taken into consideration in further studies in humans, at least in situations in which a reduction in melatonin production occurs. Many experimental studies associated melatonin treatment with an increase in BAT mass as well as melatonin deficiency with a decrease in BAT mass, but these data were restricted to animal studies, mainly hibernating animals (3), and were obtained before the identification of active BAT in human adults (6–8). Today, BAT is being studied in view of its possible relation with obesity, type 2 diabetes, and other metabolic diseases, and potential BAT activators and recruiters have been proposed (20) as adjuvant treatment of these diseases. Some authors hypothesized that an increase in BAT not only would lead to increased energy expenditure after cold exposure but also could be related to diet-induced thermogenesis (21,22).

The present observed increase in volume suggests that melatonin replacement is able to recruit BAT, with a greater activation in response to the acute cold challenge. Melatonin replacement to pinealectomized animals decreases body weight gain with a very small decrease in food intake, with the increased energy expenditure being the most plausible mechanism for the final body weight reduction (5). Taking these experimental data and the present results into account, it is possible to speculate that the same may be true in humans (1), even though energy expenditure was not addressed.

Because several clinical and social conditions that present hypomelatoninemia, such as LAN, diabetes, and neurological disorders (2), are associated with increased body fat, a reduction in BAT as a result of the hypomelatoninemic condition could be a possible explanation for this correlation (23), but we do not have any direct evidence of that. Importantly, we do not believe that melatonin could be an independent antiobesity drug, but rather, a deficiency in melatonin could lead to weight gain in the long-term (5), and its replacement or supplementation could prevent weight gain in some obesogenic situations, such as reduced BAT activity after the use of antipsychotic drugs (13,14).

It is interesting to note that patient 2 had a high BAT activity even before melatonin replacement, suggesting that BAT recruitment and/or activation can occur in the absence of melatonin. However, the further increase in activity after replacement in this patient suggests an important role played by melatonin even when BAT is already reasonably recruited.

The observed reduction in triglyceride and total cholesterol levels is in agreement with the conclusion of a recent meta-analysis (15). However, none of the studies included in the meta-analysis evaluated patients with melatonin deficiency, and the present results with such a small sample suggest that the clinical benefit to lipid levels would be much higher in patients with melatonin

deficiency. Such a suggested positive correlation between the therapeutic effect of melatonin and the previous endogenous amount of melatonin production was already demonstrated in sleep and daily activity/rest rhythm clinical studies (24). Notably, two of our four patients had an increase in HDL cholesterol of ~10 mg/dL, with clinical significance. Differently from other studies, liver fat evaluated by MRI was not affected by melatonin supplementation (25,26). Although no difference was observed in the glycemic parameters (which is somehow expected because of the small number of patients), fasting insulin and HOMA-IR were reduced in all four patients after melatonin replacement (despite the weight gain in three of them), with a clinically significant reduction in patients 1 and 4 (Table 1). In our opinion, it is possible to speculate that a reduction in IR could likely be observed in a larger sample and can possibly be related to an increase in BAT activity and recruitment after melatonin treatment because BAT activation is known to improve whole-body glucose homeostasis in humans (18).

This small pilot study is innovative for several reasons. It is the first study in our knowledge to objectively analyze BAT activity after melatonin treatment in humans, despite a huge amount of data in animals. Although the clinical significance of this finding cannot be clearly defined (since energy expenditure was not addressed, the sample is small and the duration is shorter than required to evaluate body weight or glycemic responses), melatonin and its agonists could be considered potential BAT-recruiting agents for future research. Moreover, we studied patients with melatonin deficiency as a result of pinealectomy, individuals who have received very little attention in the previous literature. There is virtually no study (with the exception of a few individual case reports) that evaluated melatonin replacement in these patients (27). Since melatonin is a known chronobiotic in humans (2), we strongly expect that its replacement therapy could improve sleep and quality of life of our present patients. Many decades of research in pinealectomized animals have shown important metabolic derangements, such as weight gain, IR, and decreased thermoregulatory responses, as well as remarkable effects of melatonin replacement in reversing these alterations (1,2). However, no data are available in humans that evaluated either the baseline metabolic characteristics of patients who had undergone pinealectomy or the effects of melatonin replacement therapy. Besides that, we decided to study patients with pinealectomy to mirror the evidence obtained in animal models, in which the metabolic effect of melatonin replacement is clearly evident. The positive results of the present small study warrant a placebo-controlled study to evaluate sleep, cardiovascular disease, and energy metabolism in a larger sample of patients with melatonin deficiency, potentially indicating a systematic melatonin replacement treatment of those patients in the future. In addition, once the putative therapeutic effects of melatonin are shown in this melatonin-deficient population, future studies should also look at several clinical and social situations leading to the so-called hypomelatoninemia condition (2), such as shift work, excessive LAN exposure, β-blocker use, neurologic disorders, and aging (28–30). All these conditions are associated with weight gain and metabolic diseases, and it remains to be determined whether melatonin supplementation has an impact on body weight and metabolic parameters also in these patients. We decided not to study control subjects since they are regular producers of melatonin, and the use of melatonin for 3 months in healthy individuals could have low adherence because of putative side effects, such as headache, drowsiness, or daytime somnolence (31).

In conclusion, in this small sample of patients who had undergone pinealectomy with confirmed very low melatonin levels, replacement with 3 mg of melatonin increased BAT volume and activity analyzed by PET-MRI as well as reduced total cholesterol and triglyceride levels. Melatonin could be considered a possible BAT recruitment agent in humans with the potential for future therapeutic use. Further research on melatonin supplementation in a larger sample of patients with pinealectomy and other clinical situations associated with melatonin reduction is warranted since the paucity of data in the literature is evident.

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References

1. Cipolla-Neto J, Amaral FG, Afeche SC, Tan DX, Reiter RJ. Melatonin, energy metabolism, and obesity: a review. J Pineal Res 2014;56:371–381

2. Cipolla-Neto J, Amaral FGD. Melatonin as a hormone: new physiological and clinical insights. Endocr Rev 2018;39:990–1028

3. Tan DX, Manchester LC, Fuentes-Broto L, Paredes SD, Reiter RJ. Significance and application of melatonin in the regulation of brown adipose tissue metabolism: relation to human obesity. Obes Rev 2011;12:167–188

4. Wolden-Hanson T, Mitton DR, McCants RL, et al. Daily melatonin administration to middle-aged male rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat. Endocrinology 2000;141:487–497

5. Buonfiglio D, Parthimos R, Dantas R, et al. Melatonin absence leads to long-term leptin resistance and overweight in rats. Front Endocrinol (Lausanne) 2018;9:122

6. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, et al. Cold-activated brown adipose tissue in healthy men. N Engl J Med 2009;360:1500–1508
7. Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. Am J Physiol Endocrinol Metab 2007;293:E444–E452
8. Halpern B, Mancini MC, Halpern A. Brown adipose tissue: what have we learned since its recent identification in human adults. Arq Bras Endocrinol Metabol 2014;58:889–899
9. Jiménez-Aranda A, Fernández-Vázquez G, Campos D, et al. Melatonin induces browning of inguinal white adipose tissue in Zucker diabetic fatty rats. J Pineal Res 2013;55:416–423
10. McMullan CJ, Curhan GC, Schernhammer ES, Forman JP. Association of nocturnal melatonin secretion with insulin resistance in nondiabetic young women. Am J Epidemiol 2013;178:231–238
11. McMullan CJ, Schernhammer ES, Rimm EB, Hu FB, Forman JP. Melatonin secretion and the incidence of type 2 diabetes. JAMA 2013;309:1388–1396
12. Amstrup AK, Sikjaer T, Pedersen SB, Heickendorff L, Mosekilde L, Rejnmark L. Reduced fat mass and increased lean mass in response to 1 year of melatonin treatment in postmenopausal women: a randomized placebo-controlled trial. Clin Endocrinol (Oxf) 2016;84:342–347
13. Modabbernia A, Heidari P, Soleimani R, et al. Melatonin for prevention of metabolic side-effects of olanzapine in patients with first-episode schizophrenia: randomized double-blind placebo-controlled study. J Psychiatr Res 2014;53:133–140
14. Porfirio MC, Gomes de Almeida JP, Stornelli M, Giovinazzo S, Purper-Ouakil D, Masi G. Can melatonin prevent or improve metabolic side effects during antipsychotic treatments? Neuropsychiatr Dis Treat 2017;13:2167–2174
15. Mohammadi-Sartang M, Ghorbani M, Mazloom Z. Effects of melatonin supplementation on blood lipid concentrations: a systematic review and meta-analysis of randomized controlled trials. Clin Nutr 2018;37:1943–1954
16. Smolensky MH, Sackett-Lundeen LL, Portaluppi F. Nocturnal light pollution and underexposure to daytime sunlight: complementary mechanisms of circadian disruption and related diseases. Chronobiol Int 2015;32:1029–1048
17. Scotney H, Symonds ME, Law J, Budge H, Sharkey D, Manolopoulos KN. Glucocorticoids modulate human brown adipose tissue thermogenesis in vivo. Metabolism 2017;70:125–132
18. Chondronikola M, Volpi E, Børsheim E, et al. Brown adipose tissue improves whole-body glucose homeostasis and insulin sensitivity in humans. Diabetes 2014;63:4089–4099
19. Cypess AM, Chen YC, Sze C, et al. Cold but not sympathomimetics activates human brown adipose tissue in vivo. Proc Natl Acad Sci U S A 2012;109:10001–10005
20. Broeders E, Bouvy ND, van Marken Lichtenbelt WD. Endogenous ways to stimulate brown adipose tissue in humans. Ann Med 2015;47:123–132
21. Rothwell NJ, Stock MJ. A role for brown adipose tissue in diet-induced thermogenesis. Nature 1979;281:31–35
22. Wijers SL, Saris WH, van Marken Lichtenbelt WD. Individual thermogenic responses to mild cold and overfeeding are closely related. J Clin Endocrinol Metab 2007;92:4299–4305
23. Rybnikova NA, Haim A, Portnov BA. Does artificial light-at-night exposure contribute to the worldwide obesity pandemic? Int J Obes 2016;40:815–823
24. Laakso ML, Lindblom N, Leinonen L, Kaski M. Endogenous melatonin predicts efficacy of exogenous melatonin in consolidation of fragmented wrist-activity rhythm of adult patients with developmental brain disorders: a double-blind, placebo-controlled, crossover study. Sleep Med 2007;8:222–239
25. Celinski K, Konturek PC, Slomka M, et al. Effects of treatment with melatonin and tryptophan on liver enzymes, parameters of fat metabolism and plasma levels of cytokines in patients with non-alcoholic fatty liver disease–14 months follow up. J Physiol Pharmacol 2014;65:75–82
26. Pakravan H, Ahmadian M, Fani A, Aghaee D, Brumanad S, Pakzad B. The effects of melatonin in patients with nonalcoholic fatty liver disease: a randomized controlled trial. Adv Biomed Res 2017;6:40
27. Macchi MM, Bruce JN. Human pineal physiology and functional significance of melatonin. Front Neuroendocrinol 2004;25:177–195
28. Zhdanova IV, Wurtman RJ, Balcioglu A, Kartashov AI, Lynch HJ. Endogenous melatonin levels and the fate of exogenous melatonin: age effects. J Gerontol A Biol Sci Med Sci 1998;53:B293–B298
29. Leger D, Laudon M, Zisapel N. Nocturnal 6-sulfatoxymelatonin excretion in insomnia and its relation to the response to melatonin replacement therapy. Am J Med 2004;116:91–95
30. Scheer FA, Morris CJ, Garcia JI, et al. Repeated melatonin supplementation improves sleep in hypertensive patients treated with beta-blockers: a randomized controlled trial. Sleep (Basel) 2012;35:1395–1402
31. Costello RB, Lentino CV, Boyd CC, et al. The effectiveness of melatonin for promoting healthy sleep: a rapid evidence assessment of the literature. Nutr J 2014;13:106