Melatonin increases brown adipose tissue volume and activity in melatonin deficient patients: a proof-of-concept study

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Abstract

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, demonstrate that melatonin supplementation and short photoperiod increase brown adipose tissue (BAT) mass. The present proof-of-concept study is the first, to our knowledge, to evaluate BAT in melatonin non-proficient patients (radiotherapy or surgical removal of pineal gland) before and after daily melatonin (3 mg) replacement for three months. All four studied patients presented increased BAT volume and activity measured by PET-MRI. We also found an improvement in total cholesterol and triglycerides blood levels, without significant effects on body weight, liver fat, HDL and LDL levels. Albeit not statistically significant, fasting insulin levels and HOMA-IR decreased in all four patients. In conclusion, the present results show that oral melatonin replacement increases BAT volume and activity and improves blood lipids levels in melatonin non-proficient patients, being suggested as a possible BAT activator. Future studies are warranted as hypomelatoninemia is usually present in aging and appears as a result of light-at-night exposure and/or the use of beta blocker drugs.

Introduction

Melatonin, a pineal hormone synthesized and released at night with 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). That suggests that melatonin regulates energy metabolism also by its action on energy expenditure, possibly 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 with 2-deoxy-2¬[fluorine-18] fluoro-D-glucose (FDG-PET) 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 its activation 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 in order 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 and metabolic diseases are concerned, the importance of regular melatonin daily secretion determining adequate energy balance and glucose metabolism is seen in several papers showing that there is a negative correlation between the levels of nocturnal melatonin secretion and body mass index, insulin resistance, and type 2 diabetes incidence (10, 11). In addition, a few papers 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 the antipsychotic drugs-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 ought to determine if melatonin therapy for pinealectomized melatonin non-proficient patients 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, 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 reduction or even suppression of melatonin levels, commonly leading to functional melatonin deficient individuals, an investigation in this particular population would also be relevant (16). However, the evaluation of melatonin replacement in pinealectomized patients is very important, given that, despite of being widely accepted in classical endocrinology that absolute hormone deficiency should be treated with hormonal replacement, data about melatonin replacement in pinealectomized patients are very scarce.

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 have agreed and signed an informed consent approved by the Ethical Committee.

Recruitment and selection of patients

We searched for patients submitted to surgical removal of the pineal gland or to radiotherapy in the pineal area due to pineal tumors at Hospital das Clínicas, University of São Paulo, a reference center for complex and rare diseases in Brazil. We were able to find records of 18 patients in the last twelve years. Five patients could not be contacted due to lack of updated address and phone number, three patients had passed away, four patients refused to participate, one did not comply with the protocol and one was excluded due to sympathetic lesion caused by an infiltrating tumor. The remaining four patients were studied and all of them were male. The characteristics of the patients are described in the supplementary appendix. We had some concern regarding patient 3 due to the presence of panhypopituitarism that could bias the analysis, since it is well known that cortisol levels present a circadian rhythm that could influence BAT (17). Despite this, we did not exclude the patient due to the low number of subjects. Before melatonin treatment, all subjects had salivary melatonin measured every three hours on a 27-hour schedule (beginning at 7PM until 10PM in the next day) and 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 Pharmaceutics, Brazil) and were advised to take one tablet 30 minutes before sleep every day for three months. Blisters were brought back to evaluate adherence.

FDG-PET/MRI

After an overnight fasting, the patients were admitted at the Nuclear Medicine Institute. Patients were exposed to a cold air-conditioned room (18oC) wearing light clothes and also a cold-vest (Polar, USA) for two hours. After one hour of cold exposure, the FDG radioisotope was injected and PET-MRI was performed one hour later, in accordance to protocols used in other studies in the field (18,19).

BAT volume (ml) and activity (volume x mean Standard Uptake Value [SUV]) with a SUV threshold of 1.5 and 2.0 were assessed by PET-MRI and liver fat was analyzed by MRI. The software AMIDE (Amide Medical Imaging Data Examiner, http://amide.sourceforge.net/packages.html) was employed to analyze BAT volume and SUV by a nuclear medicine specialist. Adipose tissue was automatically segmented by “FAT MRAC” or “lavaflex sequence” using a 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, in order 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 tests analysis

We collected blood samples immediately before PET-MRI protocol, both before and after melatonin replacement. Total cholesterol, HDL-c, LDL-c, fasting triglyceride, fasting insulin and fasting glucose levels were determined and analyzed in the same laboratory (Central Laboratory, Hospital das Clínicas University of São Paulo). A difference in any of them was then considered a secondary endpoint.

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.

Endpoints

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

Results

All four patients completed the protocol, with an adherence of more than 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 endpoints still to be measured with a longer duration of the treatment.

The individual patterns of BAT volume and activity were shown, respectively, in Figures 1a and 1b considering a SUV threshold of 2.0. Both BAT volume and activity statistically increased in all patients after melatonin replacement (p value=0.0179 and 0.0139, respectively). BAT volume and activity considering a SUV threshold of 1.5 are presented in the supplementary appendix, and have a very similar pattern of increase (p value=0.0199 and 0.0159, respectively).

Patient 1 had a notably clear pattern of response (Figure 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 (data on supplementary appendix).

Despite the positive responses observed in BAT, the patients' body weight did not change (table 2) 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 on body weight.

Albeit analyzing such a small sample, melatonin replacement caused a significant decrease in total cholesterol (p= 0.0204) and triglyceride levels (p=0.0104) (Table 1). In regard to glycemic parameters, no differences were observed; however, fasting insulin and HOMA-IR decreased in all subjects after melatonin replacement (table 2). Hepatic fat was also evaluated by MRI and no difference was found (data on supplementary appendix).

Discussion

The present study shows a significant increase in BAT volume and activity, measured by PET¬-MRI, in four melatonin-deficient pinealectomized patients after melatonin replacement therapy. 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 prior to the identification of active BAT in human adults (6-8). Nowadays, 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) to adjuvants for the treatment of these diseases. Some authors hypothesized that an increase in BAT would not only lead to increased energy expenditure after cold exposure, but could, as well, be related to diet-induced thermogenesis (DIT) (21,22).

The present observed increase in volume suggests that melatonin replacement was 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, being the increased energy expenditure (EE) the most plausible mechanism for the final body weight reduction (5). Taking these experimental data into account and the present results, it is possible to speculate that the same may be true in humans (1), even though EE was not addressed.

As several clinical and social conditions that present hypomelatoninemia, as LAN, diabetes, neurological disorders, etc (2) are associated with increased body fat, a reduction in BAT due to the hypomelatoninemic condition could be a possible explanation for this correlation (23), although we do not have any direct evidence of that. Importantly, we do not think 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 one patient (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 melatonin non-proficient patients and the present results with such a small sample suggest that the clinical benefit to lipid levels would be much higher in melatonin¬deficient subjects. Such 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 rhythms clinical studies (24). Notably, two out of our four patients had an increase in HDL-cholesterol around 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 due to the small number of patients), fasting insulin and HOMA-IR reduced in all four patients after melatonin replacement (despite the weight gain in three of them), with a clinical significant reduction in patients 1 and 4 (table 1). In our opinion, it is possible to speculate that a reduction in insulin resistance could likely be observed in a larger sample and can possibly be related to an increase in BAT activity and recruitment after melatonin treatment, as 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 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 EE 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 pinealectomized patients with

melatonin deficiency, individuals that 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, insulin resistance and decreased thermoregulatory responses, as well as remarkable effects of melatonin replacement in reversing these alterations (1,2). However, no data is available in humans, neither evaluating baseline metabolic characteristics of pinealectomized patients or the effects of melatonin replacement therapy. Besides that, we decided to study pinealectomized patients to mirror the evidences 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, evaluating sleep, cardiovascular and energy metabolism in a larger sample of melatonin-deficient patients, potentially indicating to 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, beta-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 if melatonin supplementation does have an impact in body weight and metabolic parameters also in these patients. We decided not studying control subjects since they are regular producers of melatonin and, in addition, the use of melatonin for 3 months in healthy individuals could have low adherence, due to putative side effects such as headache, drowsiness or daytime somnolence (31).

Conclusion

In this small sample of pinealectomized patients 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 potential for future therapeutic use. Further research on melatonin supplementation in a larger sample of pinealectomized patients, and other clinical situations associated with melatonin reduction is warranted, since the paucity of data in the literature is evident.

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Author contributions:

BH, MCM and JCN designed the experiments. BH conducted all steps of research. BH, MCM and JCN analyzed data. BH wrote the manuscript. MCM and JCN reviewed the manuscript and gave opinions and ideas. CB and IPB recruited the patients and helped with the collection of melatonin salivary samples. MSL, CGC, MTS and CAB were responsible for the PET-MRIs procedures and analysis. FGA analyzed salivary melatonin samples and gave important opinion and ideas about the procedures. MEM, BH and MCM performed statistical analysis of all data. JCN is responsible for the Thematic Project in which this trial belongs and received funding.

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294 Dr. Jose Cipolla Neto is the guarantor of this work and, as such, had full access to all the data in
295 the study and takes responsibility for the integrity of the data and the accuracy of the data
296 analysis.

297 The authors have declared no conflict of interest.

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299 Bruno Halpern - The author has declared no conflict of interest.

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301 Clarissa Bueno - The author has declared no conflict of interest

302 Isabella P. Barcelos - The author has declared no conflict of interest

303 Maria Edna de Melo - The author has declared no conflict of interest

304 Marcos S. Lima - The author has declared no conflict of interest

305 Camila G. Carneiro - The author has declared no conflict of interest

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307 Carlos Alberto Buchpiguel - The author has declared no conflict of interest

308 Fernanda Gaspar do Amaral - The author has declared no conflict of interest

309 José Cipolla-Neto - The author has declared no conflict of interest

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425 **Table 1 – Metabolic parameters of the patients, before and after melatonin supplementation**

426 *Statistically significant

	HDL-c (mg/dL)		LDL-c (mg/dL)		Total cholesterol (mg/dL)		Triglyceride (mg/dL)		Fasting glucose (mg/dL)		Fasting insulin		HOMA-IR		Body weight (kg)	
Melatonin replacement	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Patient 1	28	42	69	54	120	109	153	54	91	54	16.1	4.0	3.6	0.5	54.2	51.2
Patient 2	32	45	113	92	162	155	94	90	78	77	13.4	10.6	2.6	2.0	55.7	56.7
Patient 3	29	26	91	108	223	193	552	479	76	81	22.2	19.8	4.2	4.0	76.0	79.2
Patient 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	

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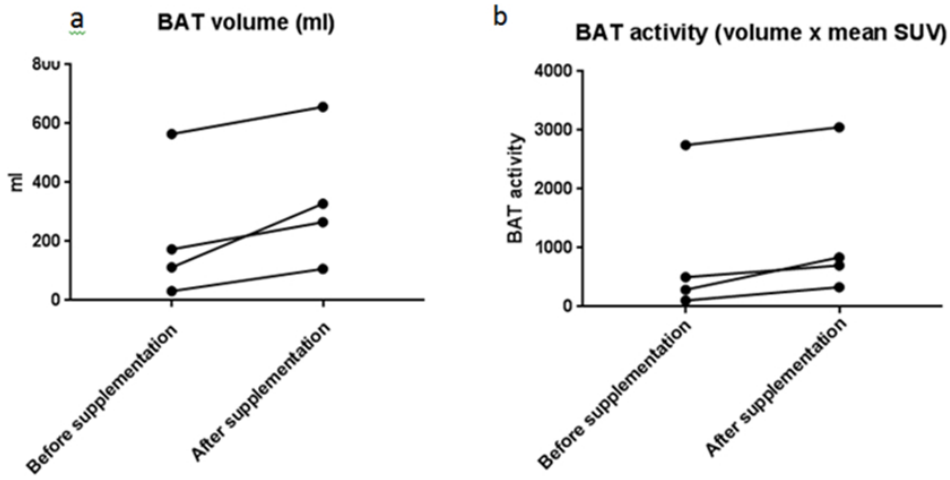

Figure 1 - BAT volume (a) and activity (b), measured by PET-MRI after cold exposure, before and after melatonin supplementation (PET-MRI). Student t-test
a) p= 0.0179 b) p= 0.0139

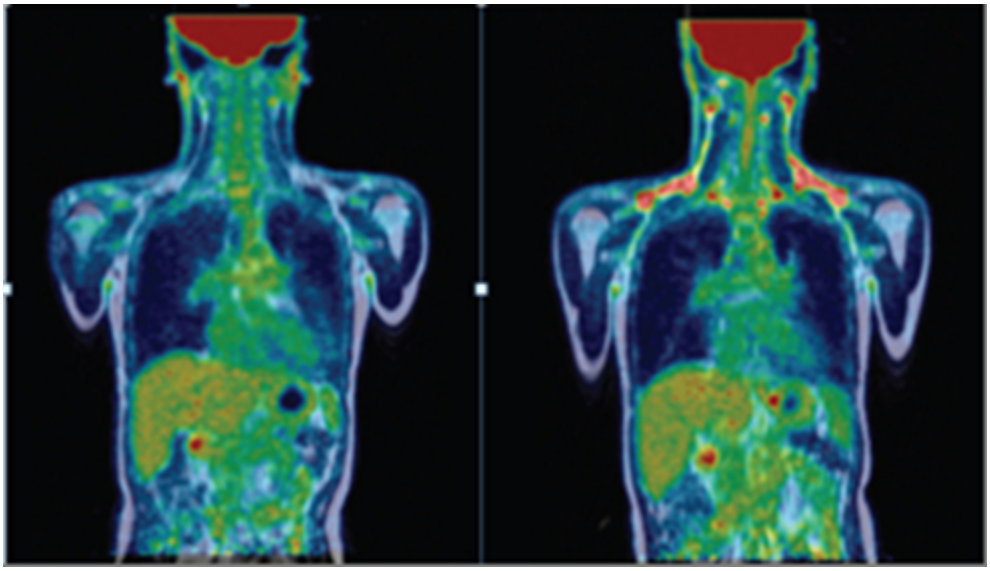

Figure 2 PET-MRI after cold exposure of patient 1, before and after melatonin supplementation. Note the increase in FDG uptake supraclavicular and cervical areas

Supplemental appendix

Individual description of the four patients:

Patient 1 - A 16-year-old man diagnosed with a pineal lesion in 2010 whose stereotaxic biopsy revealed a dysgerminoma. Chemotherapy and radiotherapy were performed with good response and no medication was in use recently. His BMI was 19.3 kg/m² and the highest salivary melatonin level was 1 pmol/L at 22:00.

Patient 2 – A 19-year-old man diagnosed with a pineal tumor in 2013 was submitted to two surgical procedures and two cycles of chemotherapy and radiotherapy and to four autologous bone marrow transplantations. The pathological examination revealed a pyneoblastoma grade IV. He did not use any medication and his BMI was 22.03 kg/m². The salivary melatonin levels were below the limit of detection.

Patient 3 – A 20–year-old man diagnosed with a pineal lesion after a ventriculoperitoneal shunt procedure due to hydrocephalus had 25 radiotherapy and nine chemotherapy sessions performed resulting in panhypopituitarism, diabetes insipidus and depression, taking hydrocortisone, phenytoin and desmopressin, testosterone cypionate injections, levothyroxine and amytriptilin. His BMI was 28.6 kg/m² and salivary melatonin levels were below the limit of detection.

Patient 4 – A 32-year-old man with a pineal tumor diagnosed in 2007 was submitted to a surgical procedure with pathological examination showing a germinoma and to one radiosurgery and 20 sessions of radiotherapy. The only medication used was levothyroxine due to primary hypothyroidism. The BMI was 30.4 kg/m² and the highest salivary melatonin level was 1.36 pmol/L at 1PM.

Table SA1 – Peak melatonin levels and time of each individual patient during an overnight stay in Hospital before and after melatonin replacement (BLD – below level of detection)

	Before intervention	After intervention
Patient 1	1 pg/mL (22:00)	22.83 pg/mL (22:00)
Patient 2	BLD	16.28 pg/mL (04:00)
Patient 3	BLD	7.48 pg/mL (01:00)
Patient 4	1.36 pmol/l (13:00)	Not performed

Figure SA1 – BAT volume (ml) before and after melatonin supplementation, considering a SUV threshold of 1.5 (p= 0.0159)

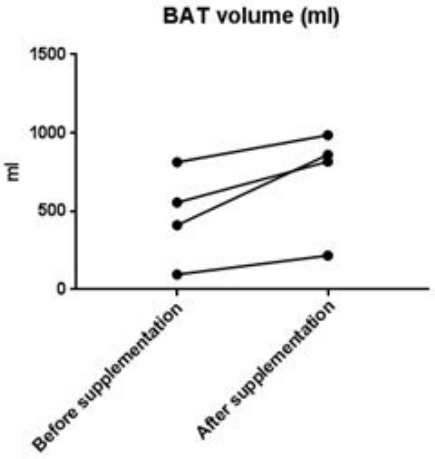

Figure SA2 – BAT activity (ml x mean SUV) before and after melatonin supplementation, considering a SUV threshold of 1.5 (p=0.0199)

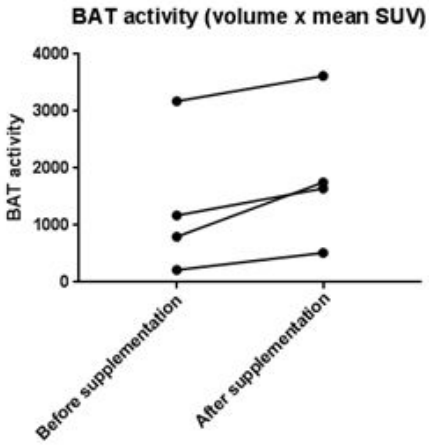

Table SA2 - Mean outdoor temperature in the day of each exam (source: timeanddate.com)

	Before melatonin supplementation	After melatonin supplementation
Patient 1	19.5 °C	12 °C
Patient 2	17 °C	18 °C

Patient 3	17.5 °C	26 °C
Patient 4	16.5 °C	24.5 °C

Figure SA3 – BAT volume by outdoor temperature – p=0.26