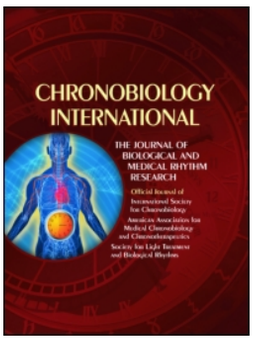

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ORIGINAL ARTICLE

The benefits of four weeks of melatonin treatment on circadian patterns in resistance-trained athletes

Roberto C. Leonardo-Mendonça[1,2,3], Antonio Martinez-Nicolas[4], Carlos de Teresa Galván[5,6], Javier Ocaña-Wilhelmi[7], Iryna Rusanova[1,2], Eduardo Guerra-Hernández[3], Germaine Escames[1,2], and Darío Acuña-Castroviejo[1,2,8]

[1]Instituto de Biotecnología, Centro de Investigación Biomédica, Parque Tecnológico de Ciencias de la Salud, Universidad de Granada, Granada, Spain, [2]Departamento de Fisiología, Facultad de Medicina, Universidad de Granada, Granada, Spain, [3]Departamento de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Granada, Granada, Spain, [4]Laboratorio de Cronobiología, Departamento de Fisiología, Facultad de Biología, Universidad de Murcia, IMIB-Arrixaca, Murcia, Spain, [5]Centro Andaluz de Medicina del Deporte, Granada, Spain, [6]Hospital San Juan de Dios, Granada, Spain, [7]Facultad de Ciencias del Deporte, Universidad de Granada, Spain, and [8]Unidad de Gestión Clínica de Laboratorios, Hospital Universitario San Cecilio, Granada, Spain

Exercise can induce circadian phase shifts depending on the duration, intensity and frequency. These modifications are of special meaning in athletes during training and competition. Melatonin, which is produced by the pineal gland in a circadian manner, behaves as an endogenous rhythms synchronizer, and it is used as a supplement to promote resynchronization of altered circadian rhythms. In this study, we tested the effect of melatonin administration on the circadian system in athletes. Two groups of athletes were treated with 100 mg day^{-1} of melatonin or placebo 30 min before bed for four weeks. Daily rhythm of salivary melatonin was measured before and after melatonin administration. Moreover, circadian variables, including wrist temperature (WT), motor activity and body position rhythmicity, were recorded during seven days before and seven days after melatonin or placebo treatment with the aid of specific sensors placed in the wrist and arm of each athlete. Before treatment, the athletes showed a phase-shift delay of the melatonin circadian rhythm, with an acrophase at 05:00 h. Exercise induced a phase advance of the melatonin rhythm, restoring its acrophase accordingly to the chronotype of the athletes. Melatonin, but not placebo treatment, changed daily waveforms of WT, activity and position. These changes included a one-hour phase advance in the WT rhythm before bedtime, with a longer nocturnal steady state and a smaller reduction when arising at morning than the placebo group. Melatonin, but not placebo, also reduced the nocturnal activity and the activity and position during lunch/nap time. Together, these data reflect the beneficial effect of melatonin to modulate the circadian components of the sleep–wake cycle, improving sleep efficiency.

Keywords: Activity, ambulatory circadian monitoring, circadian rhythms, melatonin, sleep, temperature

INTRODUCTION

The main circadian pacemaker is located in the suprachiasmatic nuclei (SCN) of the hypothalamus, where each individual neuron can independently generate a self-sustained circadian rhythm. The central pacemaker and the peripheral oscillators need regular periodic inputs from environmental time cues to maintain their circadian rhythms entrained to 24 h cycles. Among these inputs, the 24 h light–dark cycle is the main *zeitgeber* for the SCN (Welsh et al., 2010). The synchronized activity of SCN cells communicates circadian phase information through neural and humoral signals to every cell in the organism. The autonomic nervous system, melatonin and core body temperature rhythms are well-known output signals from SCN to generate circadian rhythms in most physiological and behavioral variables. Melatonin plays a key role in the circadian system. Its synthesis is controlled by the SCN clock through a sympathetic pathway which releases norepinephrine on the pinealocytes during the night, thereby allowing the entrainment of the circadian rhythms of several biological functions (Alonso-Vale et al., 2008). Humans, however, may modify voluntarily some of these output rhythms

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Correspondence: Darío Acuña-Castroviejo, Centro de Investigación Biomédica, Parque Tecnológico de Ciencias de la Salud, Avda. del Conocimiento s/n, 18100-Armilla, Granada, Spain. Tel: +34 958 241000, ext. 20169. Fax: +34 958 819132. E-mail: dacuna@ugr.es

through physical exercise, sleep, mealtime, and social lifestyle, and these outputs can also act as input signals that, in turn, modify the activity of the central and peripheral clocks (Escames et al., 2012).

It has been shown that exercise can induce significant circadian phase shifts, although the effects depend on duration, intensity and frequency. Since athletes are likely to be engaging in intense physical exercise as a part of their general training, appropriate scheduling could potentially allow them to take advantage of any synchronizing action of exercise and avoid detrimental effects (Redlin & Mrosovsky, 1997). Nevertheless, athletes are particularly susceptible to desynchronization of their circadian clock, because they travel frequently for long distances to compete at their maximal performance at times that are very different to their internal body clock timings. Optimal performance also requires coordination of many physiological systems that are sensitive to chronodisruption, as they are regulated by the circadian clock (Forbes-Robertson et al., 2012). Melatonin is classified as an excellent and safe chronobiotic; therefore, melatonin has a potential use in circadian disorders. In these regards, the American Academy of Sleep Medicine recommends the timed use of melatonin supplements to promote the circadian rhythm synchronization (Herxheimer & Petrie, 2002).

Sleep has been recognized as an essential component for athlete preparation and was suggested to be the single best recovery strategy available for athletes (Juliff et al., 2014). Despite the importance of sleep for athletic performance, data on elite athletes are limited. Anecdotal reports suggest athletes often sleep worse around competition periods, particularly the night(s) prior to an important competition (Juliff et al., 2014). Sleep deprivation studies in athletes following 24 h of reduced sleep, reduced both aerobic and anaerobic performances (Oliver et al., 2009; Skein et al., 2011). While it may be uncommon that athletes experience a total sleep deprivation prior to competition, acute partial sleep deprivation may exist. As many sports rely on fine motor movements and the ability to make fast accurate decisions, reduced sleep in athletes is a genuine concern.

To evaluate the most overt rhythms controlled by the circadian clock, melatonin, core body temperature and motor activity are the most widely used rhythms. Melatonin and core body temperature are mostly under endogenous control, while motor activity is modify voluntarily, but it has also an endogenous component (Ortiz-Tudela et al., 2010). In addition, body position influences above-mentioned rhythms and it is related to sleep pattern (Blazquez et al., 2012). The combination of these rhythmic variables converts their measurements in a suitable tool to evaluate the status of the human circadian system (Ortiz-Tudela et al., 2010). The purpose of this study was to investigate the effects of four weeks of melatonin treatment on circadian rhythms of resistance-trained subjects under habitual training conditions.

SUBJECTS AND METHODS

Subjects

Twenty-four male resistance-trained volunteer students from the Faculty of Sport Sciences of the University of Granada were enrolled in the study. All volunteers were healthy subjects without a significant previous medical history, non-smokers, they were not taking any medicine or supplementation, non-shift-workers and they did not do any transmeridian travel in the last month. In order to take in consideration light–dark exposure all the subjects had lived in the city of Granada, Spain (37.18° N, 3.60° W), during the experimental period among the months of October and November. All participants received appropriate information about the study characteristics and signed an informed consent form prior to their inclusion in the study (Portaluppi et al., 2010). The study was performed in accordance with the bioethical principles set out by the Declaration of Helsinki. Data from the volunteers were included in a database and were protected according to the Spanish Law 15/1999 of 13 September.

Study design

The study was carried out as a randomized double-blind design. Subjects were randomized to experimental group (melatonin; $n = 12$) or control group (placebo; $n = 12$). The experimental group was treated with 100 mg day^{-1} of melatonin during four weeks, administered orally in capsules 30–60 min before bed time; control group was equally treated with placebo.

The study was conducted through five weeks (Figure 1). All subjects were monitored to assess the circadian system status during the first week (days 0–7), prior to melatonin treatment and during last week (days 28–35) within melatonin treatment. Melatonin treatment started at day 7 until the end of the study (day 35). Saliva samples were collected overnight previous (S1) and during the last night (S2) of melatonin treatment. To ensure that subjects were following the treatment, saliva samples were taken in three different time points along the study to control saliva melatonin levels (C1, C2 and C3).

The research was planned so that it did not interfere with the regular training program of participants. The physical training during the study time consisted of eight sessions per week lasting approximately 1–1.5 h each (10 h week^{-1}), comprising resistance training (five sessions), weight training (two sessions) and aerobic running (one session).

Body composition and anthropometry

Subjects' standing height and weight (lightly dressed and barefooted) were measured using a calibrated Seca stadiometer and electronic scale (model 701, UK), with a

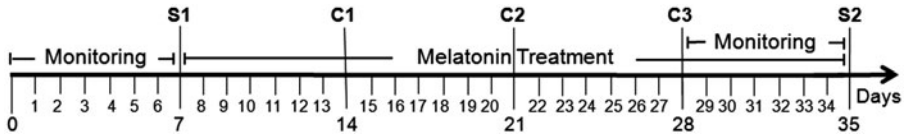

FIGURE 1. Chronological schedule of the study.

precision of 0.5 cm and 100 g, respectively. Thereafter, body mass index (BMI) was calculated.

Dietary intake

To estimate the average energy and nutritional intake, participants recorded their dietary intake during three consecutive days (one being a weekend day), prior and during the last week of treatment. A trained nutritionist gave detailed verbal and written instructions about proper dietary recording. A full description of foods and fluids consumed was requested, including the brand names of packaged food, cooking or processing methods, and food items and ingredients added during preparation. Participants estimated the amount of food or fluids consumed by referring to the weight or volume information provided on food package or by using standardized household measures. Participants were instructed to continue their habitual dietary intake during this period, and to avoid the omission and replacement of foods that are hard to register or that they feel that should not be eating. Dietary-record information was converted to energy and nutrients with the software DIAL (Alce Ingenieria, Madrid, Spain). This program was supplemented with information for composite dishes, commercial foods and sports foods whenever reliable nutritional composition data could be obtained. Nutritional intake was compared taking into account the dietary reference intakes (Otten et al., 2006).

Assessment of the circadian system status

To evaluate the chronotype, subjects completed the Horne and Östberg questionnaire to assess the morningness–eveningness (Horne & Östberg, 1976). This questionnaire establishes five behavioral categories: definitively morning types (score = 70–86), moderately morning types (score = 59–69), neither types (score = 42–58), moderately evening types (score = 31–41) and definitively evening types (score = 16–30).

Wrist temperature (WT) rhythm was assessed continuously for seven days, prior and during the seven last days of treatment, using a temperature sensor (Thermochron iButton DS1921H; Maxim Integrated Products, Sunnyvale, CA) with a sensitivity of 0.125 °C and programmed to sample every 10 min during the data collection periods. It was attached to a double-sided cotton sport wrist band, and the sensor surface was placed over the inside of the wrist on the radial artery of the non-dominant hand (Martinez-Nicolas et al., 2013). The information stored in the iButton was transferred through a Blue Dot™ Receptor (DS1402D-DR8; Maxim Integrated Products, Sunnyvale, CA) to a computer using iButton Viewer v. 3.22 (software provided by the manufacturer).

The body position and rest-activity rhythms were assessed over the same time using a HOBO Pendant G Acceleration Data Logger UA-004-64 (Onset Computer, Bourne, MA) placed on the non-dominant arm by means of a sports band, with its *x* axis parallel to the humerus bone. The sensor was programmed to record data every 30 s. The information stored in the actimeter was transferred through an optical USB Base Station (MAN-BASE-U-4, HOBO; Onset Computer, Bourne, MA) to a computer using the software HOBO 2.2. From the information provided by the actimeter, we define two variables: activity and position. Activity was calculated as degrees of change in *x*-, *y*- and *z*-axes per minute. Position was calculated as the angle between *x* axis of the actimeter and the horizontal plane, being its value 0° when the arm is in a horizontal position and 90° when it is vertically aligned (Ortiz-Tudela et al., 2010).

Over the monitoring time of WT, activity and position, all subjects were instructed to complete a sleep and food diary designed by the Chronobiology Laboratory at the University of Murcia (Murcia, Spain), and from these diaries we obtained the habitual sleep and food intake time recording (Azevedo et al., 2012).

Saliva samples

Saliva samples (2 mL) were collected using a Salivette (SARSTEDT, Nümbrecht, Germany) before brushing teeth and no earlier than 30 min after eating or drinking. Saliva was kept at 4 °C and protect from light until centrifuged at 3000*g* for 10 min and frozen at −80 °C. Saliva samples to assess melatonin rhythm were collected overnight, at 20:00, 23:00, 02:00, 05:00 and 08:00 h, prior (S1) and during the last night (S2) of melatonin treatment. Saliva samples to ensure that subjects follow the treatment were taken at 8:00 h in three different time points of the treatment period (C1, C2 and C3; Figure 1) for melatonin determination.

Melatonin determination

The levels of saliva melatonin were analyzed using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Melatonin direct Saliva ELISA; IBL International, Hamburg, Germany). Melatonin levels were expressed in $pg\,mL^{-1}$ with a sensitivity of $0.3\,pg\,mL^{-1}$.

Statistical Analysis

Data are expressed as means ± standard deviations. WT, activity and position data were initially filtered using

TABLE 1. Anthropometric and nutritional intake features of the subjects enrolled in the study.

	Placebo		Melatonin	
	Pre	Post	Pre	Post
Anthropometry				
Age (years)	20.3 ± 0.71		19.2 ± 0.33	
Height (cm)	176.7 ± 1.83		176.7 ± 1.71	
Weight (kg)	74.7 ± 3.22	74.8 ± 3.31	74.1 ± 3.24	74.21 ± 3.12
BMI	23.9 ± 0.47	24.0 ± 0.48	23.7 ± 0.44	23.8 ± 0.40
Waist (cm)	82.4 ± 2.90	81.9 ± 2.72	82.3 ± 2.41	81.4 ± 2.01
Nutritional intake				
Energy intake (kcal)	3114 ± 160.6	3176 ± 153.4	3145 ± 168.1	3130 ± 164.0
Proteins (g)	144.3 ± 7.21	147.1 ± 6.88	145.0 ± 7.55	144.1 ± 7.43
Proteins (% of EI)	18.5 ± 0.78	18.9 ± 0.79	18.7 ± 0.87	18.6 ± 0.87
Carbohydrates (g)	397.1 ± 30.60	405.3 ± 29.11	401.0 ± 32.14	399.4 ± 31.20
Carbohydrates (% of EI)	50.9 ± 1.78	51.9 ± 1.73	51.4 ± 1.89	51.2 ± 1.85
Fibre (g)	26.1 ± 2.14	26.7 ± 2.02	26.3 ± 2.14	26.2 ± 2.14
Fat (g)	106.8 ± 5.66	109.0 ± 5.42	107.9 ± 6.03	107.3 ± 5.77
Fat (% of EI)	30.9 ± 1.33	31.5 ± 1.31	31.2 ± 1.42	31.0 ± 1.35
Saturated fat (g)	36.6 ± 11.31	37.3 ± 10.65	36.9 ± 11.81	36.7 ± 11.50
Monounsaturated fat (g)	41.6 ± 13.01	42.4 ± 12.33	42.1 ± 13.60	41.7 ± 13.21
Polyunsaturated fat (g)	16.3 ± 5.14	16.7 ± 4.86	16.4 ± 5.35	16.3 ± 5.23

Data are the means ± SD. Pre and post correspond to the measures obtained in the placebo and melatonin groups before and after treatments, respectively. EI, energy intake.

automatic rejection criteria to remove artifacts, such as those produced when the sensors were removed for bathing. All data deviated by more than three times the standard deviation of the mean were deleted.

The interdaily stability of the rhythm (IS) quantifies the similarity between the different 24 h cycles, that is, the day-by-day regularity of the circadian patterns. The intra-daily variability (IV) quantifies the fragmentation of the rhythm, that is, the frequency and extension of transitions between periods of high and low values of the variables. The relative amplitude (RA) was calculated by subtracting the average between the 5 consecutive hours of maximum values (M5) from the 10 consecutive hours of minimum values (L10) for skin temperature, as well as the difference between the 10 h of maximum values (M10) and the 5 h of minimum values (L5) for activity(Martinez-Nicolas et al., 2014; Van Someren et al., 1999).

The circadian function index (CFI) was used to assess the circadian function status, as described by Ortiz-Tudela et al. (2010). The CFI integrates the IV, IS and RA parameters, oscillating between 0, absence of circadian rhythmicity, and 1, robust circadian rhythmicity (Ortiz-Tudela et al., 2010).

All the rhythmic parameters were obtained by using an integrated package for temporal series analysis ''Circadianware'' (Chronobiology Laboratory, University of Murcia, 2010). Statistical analyses were performed with the software SPSS 16.0 (Chicago, IL). Group data were expressed as mean ± standard error. All data were assessed for normal distribution with the Shapiro–Wilk test. All data were analyzed using the mixed model repeated-measures General Linear Model, with treatment (melatonin versus placebo) as the between-subject factor and time (pre-treatment versus post-treatment) as the within-subject factor. Effects of each factor and combined factors (interaction) on each parameter were analyzed. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Twenty-four out of 30 resistance-trained subjects volunteered fulfilled the acceptance criteria to participate in the study. Six subjects were excluded given the fact they were taking regular medication or supplementation or were regular smokers. There were no adverse events attributable to melatonin treatment. No subject reported headache, nausea, vomiting, diarrhea, abdominal pain or any other discomfort at any time during the study or the one month follow-up period.

Some of the subjects' relevant characteristics about anthropometric, energy and nutritional intake are shown in Table 1. All athletes were on a similar range of age and anthropometric parameters. The estimated energy and nutritional intake of participants had not changed during the study period and no significant differences were found among groups.

Figure 2 shows the overnight salivary melatonin rhythm of both groups pre- and post-melatonin treatment. All subjects have shown a similar behavior in the salivary melatonin rhythm both before and after treatment. Before treatment, salivary melatonin levels were very low during the daytime, beginning to rise in the evening just prior to usual sleep time and holding this rising until 02:00 h, with the peak around 05:00 h, and falling back to baseline levels shortly after usual wake time. After treatment, melatonin levels were significantly higher in melatonin group in all saliva time points, especially at night since melatonin intake was

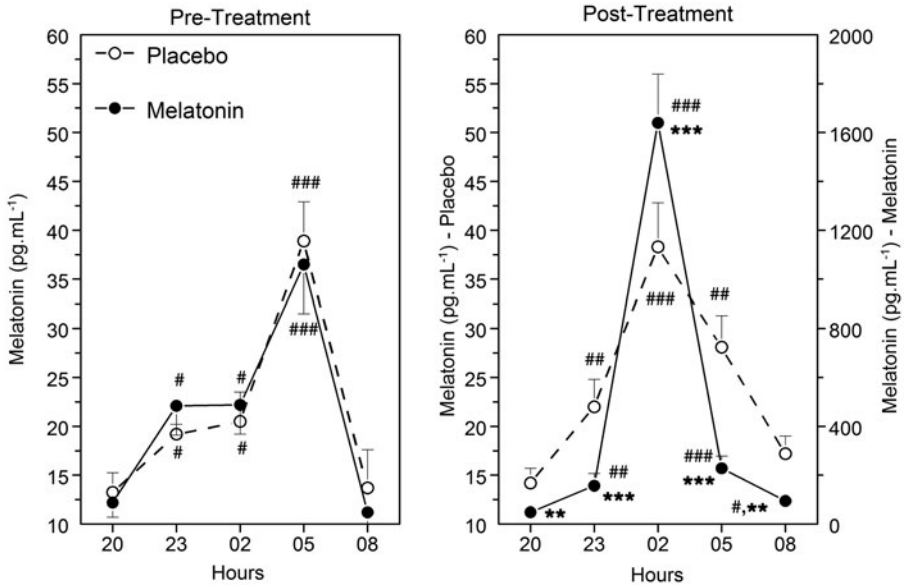

FIGURE 2. Overnight rhythm of melatonin in saliva. Left, melatonin rhythms before placebo or melatonin treatment (pre-treatment); right, melatonin rhythms after placebo or melatonin treatment (post-treatment). Open circles, placebo; black circles, melatonin. Melatonin levels in the right figure correspond to the right *y*-axis. #$p<0.05$ and ##$p<0.01$ versus 20 and 08 h; **$p<0.01$ and ***$p<0.001$ respective time-points between melatonin versus placebo ###$p<0.001$.

TABLE 2. Salivary melatonin levels along the treatment period collected at 08:00 h of placebo and melatonin group.

	Treatment period			
	Placebo		Melatonin	
C1 – Day 14 (pg mL^{-1})	16.1	2.15	91.5	19.10***
C2 – Day 21 (pg mL^{-1})	10.8	1.77	102.9	22.87***
C3 – Day 28 (pg mL^{-1})	14.6	2.60	96.4	20.14***

Data are the means ± SD. Pre and post correspond to the measures obtained in the placebo and melatonin groups before and after treatments, respectively. C, control sample.
***$p<0.001$ versus placebo.

30–60 min before sleep time. Interestingly, both groups have shown a phase advance of nocturnal melatonin peak to an earlier time, around 02:00 h, after the treatment period. The saliva melatonin levels measured along the study period (Table 2), in three different time points at 08:00 h, were significantly higher in melatonin group with no significant changes along the study period.

All subjects presented the same chronotype along the study, neither types (score: 42–58), as assessed by the Horne and Östberg questionnaire (Horne & Östberg, 1976). Placebo group had a chronotype score (mean ± standard error) of 51.3 ± 3.3 and 51.4 ± 3.0 in pre- and post-treatment period and melatonin group had a chronotype score of 49.8 ± 2.1 and 49.8 ± 2.2 in pre- and post-treatment period, respectively.

Daily mean waveforms in both melatonin and placebo groups recorded over an eight-day pre- and post-treatment period are represented in Figure 3(A)–(F) for WT, activity and position. As expected during the pre-treatment week, both groups exhibited similar daily patterns of WT, activity and position without any significant difference. WT was characterized by an increase before the time of lights-off at bedtime (23:30–01:30 h), a nocturnal steady state with high temperatures (01:30–08:30 h) and a pronounced drop after arising at morning (08:30–14:00 h). There were some secondary faint peaks around afternoon (14:00–17:00 h), a period associated with naps, and held until the nocturnal increase (Figure 3A). A roughly inverse pattern was observed for activity and position, which displayed higher values during the day and lower values at night, when the subjects were resting (Figure 3B and C, respectively).

After melatonin treatment, daily waveforms for WT, activity and position changed in both groups, especially in WT (Figure 3D–F). When we analyzed potential differences in the mean waveforms of melatonin and placebo subjects, we observed that melatonin caused an advance in the increase in WT before bedtime (22:00–01:00 h), while placebo had a much slower increase in WT until reach high WT 1 h later (22:00 – 02:00 h). The nocturnal steady state with high WT was longer for melatonin group (01:00–09:20 h) with a smaller decrease after arising in the morning (09:20–14:00 h), whereas placebo had a first temperature decrease at 06:30 and had the pronounced drop 2 h earlier (08:20 h) than melatonin. Melatonin group showed a clear secondary WT peak around afternoon, at nap time (14:00–17:00 h), followed by a dip in the usual afternoon training time (17:00–19:00 h) and during the period already known as the ''wake maintenance zone'' (20:00–22:00 h); however, placebo group had a late and faint secondary peak after nap time and an early WT dip at the ''wake maintenance zone'' (Figure 3D). Some significant differences were also obtained in activity and position at some time points post-melatonin treatment. Melatonin has slightly reduced activity at night between 03:00 and 04:00 h, as well activity and position during lunch/nap time

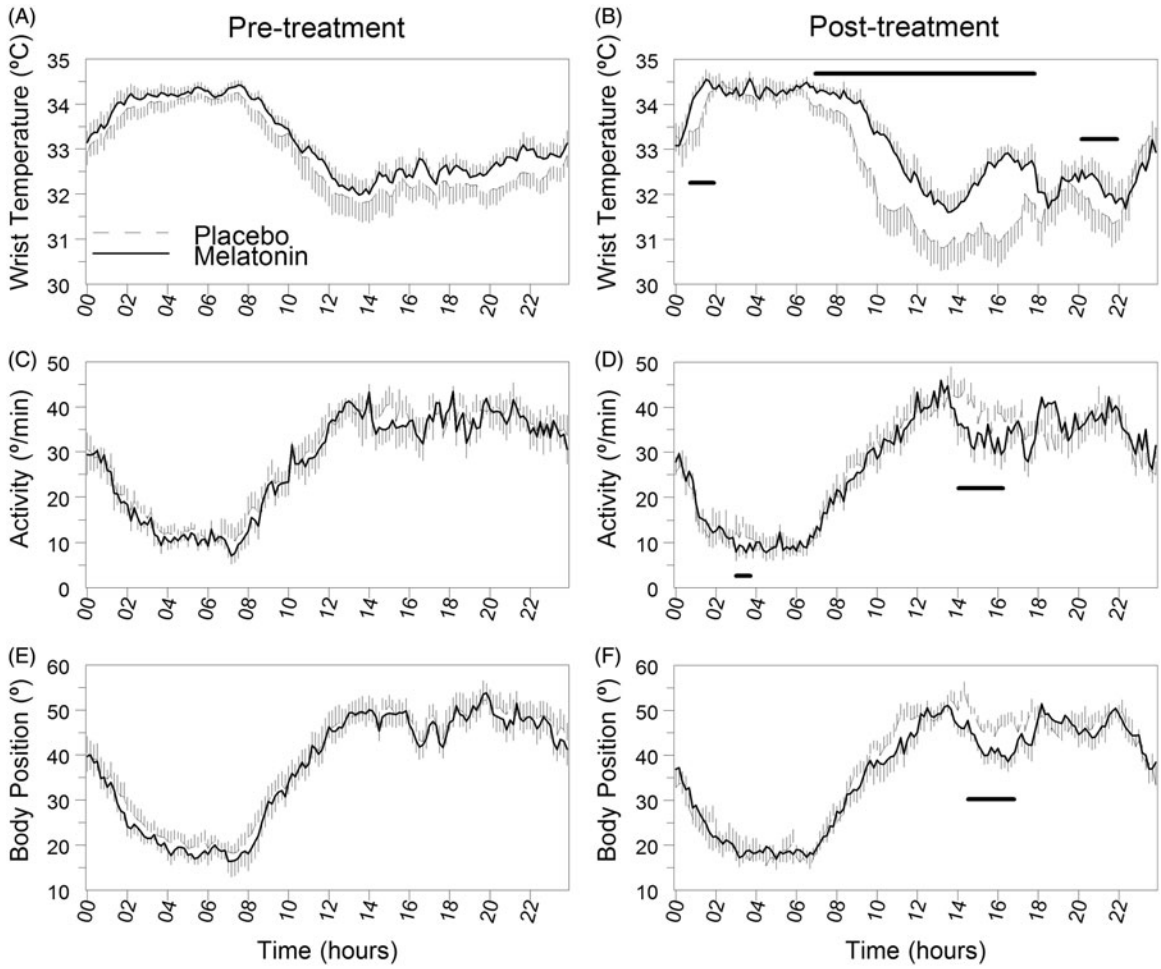

FIGURE 3. Daily mean waveforms of WT, motor activity and body position recorded over an eight-day pre and post-treatment period in both melatonin and placebo groups. Black bars inside the figures indicate significant differences ($p < 0.05$) between placebo and melatonin treatment.

(13:30 – 16:00 h) in comparison with placebo (Figure 3E and F, respectively).

In order to show the functional relevance of melatonin treatment in circadian regulation, we assessed its influence on WT, activity and position rhythmicity parameters in Table 3. There were no significant differences among groups in pre-treatment week. After treatment, melatonin group had particularly relevant differences in WT. Melatonin increased mean WT, maintaining its IV higher and its RA lower than placebo; also temperature values during the active period (L10) were higher. Significant differences were not found for activity and position parameters among both groups. After treatment, the CFI of WT improved in both group, being better in placebo treatment as consequence of lower daytime value (L10), which increased RA value.

Subject's night sleep, naps characteristics and food intake time during pre- and post-treatments are shown in Table 4. During the pre-treatment week no differences were found during night sleep characteristics among both groups and after treatment period placebo group increase the number of awakenings and the time that they spent awake after sleep onset (WASO). Melatonin treatment reduced the number of awakenings during sleep as well the WASO, improving significantly the sleep efficiency in comparison with placebo. Melatonin did not affect significantly the total time on bed, the total sleep time, the naps duration and the percentage of subject who took naps. No differences were found in food intake time, all subjects had the same breakfast, lunch and dinner schedule.

DISCUSSION

The main aim of this study was to investigate the effects of four weeks of melatonin treatment on saliva melatonin rhythm and circadian system of resistance-trained subjects. To our knowledge, this study represents the first evaluation of circadian rhythms of melatonin treatment ($100\,mg\,day^{-1}$) in healthy subjects under habitual resistance-training conditions. In the present study, melatonin treatment (30–60 min before bedtime) modulated the circadian components of the sleep–wake cycle improving sleep efficiency.

TABLE 3. Effect of melatonin treatment on WT, motor activity and body position rhythmicity variables of subjects.

	Placebo		Melatonin	
	Pre	Post	Pre	Post
WT				
Mean (°C)	32.92 ± 0.611	32.54 ± 0.718	33.23 ± 0.501	33.14 ± 0.550*
IS	0.38 ± 0.142	0.49 ± 0.143#	0.34 ± 0.111	0.41 ± 0.055#
IV	0.15 ± 0.049	0.12 ± 0.047	0.18 ± 0.066	0.17 ± 0.068*
RA	0.038 ± 0.0167	0.050 ± 0.0222#	0.031 ± 0.0076	0.034 ± 0.0088*
TL10 (hh:mm)	17:28 ± 02:50	16:06 ± 02:13#	16:13 ± 01:21	16:28 ± 01:20
TM5 (hh:mm)	05:49 ± 01:56	04:25 ± 01:53#	05:30 ± 01:32	04:09 ± 01:17#
L10 (°C)	32.0 ± 1.01	31.1 ± 1.16#	32.4 ± 0.55	32.2 ± 0.77*
M5 (°C)	34.2 ± 0.31	34.4 ± 0.44	34.4 ± 0.44	34.5 ± 0.40
CFI	0.45 ± 0.059	0.49 ± 0.055#	0.43 ± 0.041	0.45 ± 0.031#,*
Activity				
Mean (°.min^{-1})	29.7 ± 2.86	27.6 ± 4.13	27.5 ± 4.6	27.4 ± 4.7
IS	0.35 ± 0.087	0.37 ± 0.099	0.35 ± 0.05	0.35 ± 0.06
IV	0.62 ± 0.047	0.61 ± 0.107	0.67 ± 0.08	0.63 ± 0.08
RA	0.44 ± 0.110	0.48 ± 0.106	0.47 ± 0.09	0.45 ± 0.056
TL5 (hh:mm)	05:34 ± 01:20	04:26 ± 01:03	05:42 ± 01:01	04:41 ± 01:00
TM10 (hh:mm)	17:42 ± 02:34	16:01 ± 01:44	17:31 ± 01:59	16:19 ± 01:39
L5 (°/min)	11.8 ± 5.20	9.5 ± 3.40	10.2 ± 3.7	8.7 ± 1.8
M10 (°/min)	40.5 ± 3.21	38.6 ± 6.20	38.2 ± 5.3	37.5 ± 6.5
CFI	0.49 ± 0.069	0.52 ± 0.066	0.50 ± 0.05	0.50 ± 0.04
Position				
Mean (°)	39.3 ± 2.56	37.0 ± 4.15	37.0 ± 4.01	36.2 ± 2.59
IS	0.39 ± 0.157	0.46 ± 0.170	0.43 ± 0.069	0.41 ± 0.117
IV	0.34 ± 0.133	0.35 ± 0.141	0.33 ± 0.078	0.35 ± 0.108
RA	0.34 ± 0.101	0.39 ± 0.107	0.39 ± 0.060	0.36 ± 0.101
TL5 (hh:mm)	05:36 ± 01:35	05:04 ± 01:29	05:31 ± 01:07	04:47 ± 01:09
TM10 (hh:mm)	17:47 ± 02:06	16:32 ± 02:30	17:11 ± 01:55	16:24 ± 02:03
L5 (°)	20.0 ± 6.11	17.1 ± 5.33	17.8 ± 4.01	18.0 ± 4.30
M10 (°)	50.2 ± 4.68	49.0 ± 7.10	49.0 ± 4.54	46.7 ± 4.41
CFI	0.52 ± 0.090	0.56 ± 0.106	0.55 ± 0.041	0.53 ± 0.079

Data are the means ± SD. Pre and post correspond to the measures obtained in the placebo and melatonin groups before and after treatments, respectively.IS, intradaily stability; IV, intra-daily variability; RA, relative amplitude; TL10, time of hourly average during 10 consecutive hours of minimum values; TL5, time of hourly average during 5 consecutive hours of minimum values; TM10, time of hourly average during 10 consecutive hours of maximum values; TM5, time of hourly average during 5 consecutive hours of maximum values; L10, hourly average during 10 consecutive hours of minimum values; L5, hourly average during 5 consecutive hours of minimum values; M10, hourly average during 10 consecutive hours of maximum values; M5, hourly average during 5 consecutive hours of maximum values; CFI, circadian function index.

*$p < 0.05$ versus placebo; #$p < 0.05$ versus pre-treatment.

The dose of melatonin needed for its antioxidant action is thought to be considerably higher than the one given for modulation of the circadian cycle, but the actual dose required in humans is unclear, particularly because there are practically no studies using large oral doses of melatonin in humans. It has been found that large oral doses of melatonin (20–100 mg day^{-1}) in healthy volunteers were very well tolerated with no safety concerns and no clinically relevant changes in any physiological or biochemical measures (Galley et al., 2014). In this regard, we expected that the administration of 100 mg per day of melatonin would produce beneficial effects on the athlete's circadian system status.

The energy profile of the dietary intake of all subjects enrolled in this study was close to that recommended by the dietary reference intakes (Otten et al., 2006) for males between 19 and 30 years old, and similar to that reported elsewhere in resistance-trained subjects (Concepcion-Huertas et al., 2013), in cyclists (Serrano et al., 2010) and between Spanish university students who practice sports regularly (Leonardo Mendonça et al., 2012). Following the recommendation of the American College of Sports and Medicine (2009), athletes do not need a diet substantially different from the one recommended in the Dietary Guidelines for the general population. The lipid profile was similar to those reported in other studies in athletes (Teixeira et al., 2009) and university students (Leonardo Mendonça et al., 2012). No differences in the nutritional intake and anthropometric characteristics were found between melatonin and placebo group, ensuring that we had a homogenous sample.

The circadian system consists of a set of structures involved on the generation of circadian rhythms in behavioral, physiological and biochemical variables, as

TABLE 4. Food time, night sleep and naps characteristics of subjects.

	Placebo		Melatonin	
	Pre	Post	Pre	Post
Night sleep characteristics				
Bedtime (hh:mm)	01:09 ± 00:08	00:41 ± 00:07	00:40 ± 00:05	00:54 ± 00:05
Get up (hh:mm)	09:10 ± 00:05	09:13 ± 00:06	08:59 ± 00:05	09:11 ± 00:04
Total time on bed (hh:mm)	08:01 ± 00:05	08:32 ± 00:05	08:18 ± 00:06	08:17 ± 00:03
Sleep onset (hh:mm)	01:29 ± 00:08	01:08 ± 00:07	00:58 ± 00:05	01:15 ± 00:06
Sleep end (hh:mm)	09:00 ± 00:05	09:05 ± 00:06	08:52 ± 00:05	09:04 ± 00:04
Night-time sleep (hh:mm)	07:16 ± 00:14	07:36 ± 00:04	07:38 ± 00:05	07:41 ± 00:02
Sleep latency (hh:mm)	00:20 ± 00:02	00:27 ± 00:01	00:18 ± 00:01	00:21 ± 00:01
Awakenings (times)	2.60 ± 0.079	3.30 ± 0.140##	2.40 ± 0.069	1.20 ± 0.056###,***
WASO (hh:mm)	00:15 ± 00:01	00:20 ± 00:01##	00:14 ± 00:01	00:07 ± 00:01###,***
Sleep efficiency (%)	90.83 ± 0.285	89.19 ± 0.321	91.94 ± 0.117	92.71 ± 0.139***
Naps characteristics				
Indicial time (hh:mm)	14:53 ± 00:33	14:59 ± 00:39	15:04 ± 00:41	14:57 ± 00:36
Final time (hh:mm)	15:55 ± 00:37	16:12 ± 00:40	16:09 ± 00:33	15:49 ± 00:37
Naps duration (hh:mm)	01:05 ± 00:37	01:20 ± 00:28	01:07 ± 00:35	00:41 ± 00:20
Subjects who take naps (%)	33	25	41.7	25
Food time				
Breakfast time (hh:mm)	10:25 ± 01:10	09:28 ± 00:58	10:14 ± 01:02	09:41 ± 00:55
Lunch time (hh:mm)	14:22 ± 00:24	14:25 ± 00:28	14:27 ± 00:35	14:23 ± 00:20
Dinner time (hh:mm)	22:22 ± 00:40	22:04 ± 00:38	22:35 ± 00:43	22:20 ± 00:36

Data are the means ± SD. Pre and post correspond to the measures obtained in the placebo and melatonin groups before and after treatments, respectively. WASO, wake after sleep onset.
***$p < 0.001$ versus placebo; ##$p < 0.01$, ###$p < 0.001$ versus pre-treatment.

well as in the external and internal synchronizations of these variables to environmental cues and to each other. The most widely used rhythms controlled by the circadian clock are melatonin, core body temperature and activity patterns (Mormont et al., 2002; Van Someren, 2000).

Melatonin secretion under dim light conditions is the most accurate marker for assessing the circadian pacemaker. Serial sampling of melatonin measured in saliva can be used to assess circadian timing by determining the dim light melatonin onset (DLMO). Measuring melatonin rhythm in saliva is a less invasive method than using blood samples, although the level of melatonin present in saliva is about one-third of the one in plasma (Voultsios et al., 1997). Interestingly, the phase advance of nocturnal melatonin peak to an earlier time after treatment, in all subjects of this study, shows that regular physical exercise can induce circadian system synchronization. This mechanism is not completely understood; exercise causes a marked increase in the activity of the sympathetic nervous system and catecholamine secretion that could potentially modulate melatonin secretion causing a net phase-shifting effect, via the pineal gland, by acting directly on SCN cells, which express receptors for melatonin (Escames et al., 2012). This stress-mediated effect of exercise on the pineal gland could be responsible for the entraining effect of physical exercise on the SCN and, thus, on the 24 h melatonin rhythm (Redlin & Mrosovsky, 1997). Despite the similar behavior on salivary melatonin rhythm, melatonin levels were higher in melatonin group during all day, especially at night after melatonin intake. The rise of melatonin levels at DLMO (between 20 and 23 h) was steeper in melatonin group, around 100 pg mL^{-1} more than in the placebo group, who had an increase of 15 pg mL^{-1}. The timing of DLMO in the evening is strongly associated with the timing of sleep in normal individuals and a useful diagnostic tool for diagnosing circadian rhythm sleep disorders (Keijzer et al., 2014).

The wrist skin temperature rhythm is inversely related to the core body temperature and it results, at least partly, of an alternating balance between parasympathetic and sympathetic actions on peripheral skin vessels, driven by the suprachiasmatic nucleus. It is the result of internal and external influences and provides integral information about the master pacemaker function and internal and external synchronizers. High WT is closely linked to sleepiness, probably through parasympathetic activation and skin blood vessels vasodilatation, while it drops during arousal periods, associated with sympathetic activation and vasoconstriction (Krauchi, 2007). Melatonin treatment phase advanced the WT during the start of the nocturnal melatonin surge, known as DLMO, reaching the nocturnal high WT 1 h earlier and keeping it high for 2 h longer than placebo. Melatonin treatment 30–60 min before sleep seems to anticipate parasympathetic activation and keep it longer until awakening time. However, melatonin did not increase the maximum values of WT during five consecutive hours (M5), that is, maximum WT was not higher at night in melatonin group than in the placebo one. Several studies observed this relationship between exogenous melatonin and body temperature suppression (WT increase), where small concentrations of melatonin are capable of exerting a maximal

thermoregulatory effect, suggesting an effect related to a physiological threshold rather than a dose-response effect (Atkinson et al., 2003). On the other hand, during daytime the hypothermic effects of melatonin are shown clearly by the higher mean and minimum values along 10 consecutive hours (L10) of WT. Thereby, we can suggest that exogenous melatonin administration had clear hypothermic effect, as it is supported by the literature (Cagnacci et al., 1997), especially under resting conditions during the day as it can be seen at lunch/nap time of melatonin group. This finding agrees with the known effect of melatonin in promoting thermogenesis through the activation of the brown adipose tissue activation, and, thus, heat production at peripheral levels, whereas the hormone can reduce the core body temperature acting on either the thermoregulatory centers located in the hypothalamus and on the processes through which thermoregulatory centers regulate body temperature such as heat production and heat loss (Cagnacci, 1997; Lin & Chuang, 2002). A number of researchers have also showed changes in heat production including the heat dissipation by the increased peripheral temperature, reduction in heart rate and alteration in the endocrine responses. These endocrine responses are primarily responsible for non-shivering heat production following melatonin administration to humans (Cagnacci, 1996).

The rest-activity cycle can be obtained by suitable analysis of the activity record and it has been proposed as a methodology to evaluate circadian system status in humans (Carvalho et al., 2003). Furthermore, position has been used to assess human circadian functionality (Ortiz-Tudela et al., 2010), mainly to differentiate when the subject is in or out of bed. These two variables, activity and position, are less dependent on the endogenous component of the circadian system and they show a high correlation with the subject's rest-activity. As previously mentioned in our results, both variables respond to rest-activity cycle and their values vary dramatically at the exact moment of waking up and lying down. All subjects shown similar rest-activity variation along the day; this variation can be better appreciated observing the minimum values obtained during 5 consecutive hours (L5) and the maximum values during 10 consecutive hours (M10) of activity and position at night and daytime, respectively. The activity during sleep was slightly reduced (between 03:00 and 04:00 h) by melatonin treatment, as it can be seen too by the significant decrease in number of awakenings and WASO. For this reason, melatonin group had better night sleep efficiency than the placebo one, which had a significant increase in the number of awakenings and WASO after treatment. The activity and position values decreased during lunch/nap time in the melatonin group that, together with the WT increase, could suggest that melatonin group would have more propensity to sleep at lunch/nap time. However, subjects with melatonin treatment did not increase their nap's length or the percentage of subjects who took naps. Furthermore, none of the subjects report fatigue, reduced vigilance or decreased vigor during daytime. As a chronobiotic, melatonin administration 30–60 min before bedtime influences the circadian components of the sleep–wake cycle and promotes sleep onset and continuity in a ''hypnotic'' function by increasing the homeostatic drive to sleep. Surely, rapid eye movement (REM) sleep is the stage of sleep most strongly regulated or modulated by the circadian system. The timing and amount of REM sleep within the sleep cycle and the quality of REM sleep all depend on the proper functioning of the circadian system (Kunz et al., 2004). Melatonin suppressing with β-blockers is accompanied by specific changes in REM sleep in healthy subjects; these changes, however, can be reversed by simultaneously administration of evening melatonin (Van Den Heuvel et al., 1997). Similarly, the reduced individual capacity of the pineal gland to produce melatonin is associated with similar changes in REM sleep, which can be regulated by the adequately timed administration of melatonin (Kunz, 2013).

In our subjects, the administration of exogenous melatonin 30–60 min before bedtime performed as input of the SCN clock, causing a phase advance on the WT rhythm. Regarding to the chronobiotic effects of melatonin, several lines of evidence support the view that the SCN are the primary sites of action. Melatonin receptors are present in the SCN; therefore, circulating melatonin is able to feedback onto the SCN clock. The SCN is affected by exogenous melatonin *in vivo* and *in vitro*, as shown by phase-shifting effects on the firing rate of SCN neurons (Pevet et al., 2002). The effect of melatonin on sleep is believed to be a consequence of a dual mechanism, the increase in sleep propensity by enhancing the amplitude of circadian clock oscillations via MT1 receptors, and the synchronization of the circadian clock via MT2 receptors (Touitou & Bogdan, 2007).

In conclusion, the results of this study support the beneficial effects of the supplementation with the chronobiotic melatonin not only to adjust the circadian clock, but also to improve the sleep–wake cycle in athletes.

DECLARATION OF INTEREST

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