Original Article (short paper)

Acute melatonin administration enhances aerobic tolerance: an analysis of biochemical and hematological parameters

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Abstract — Aims: This study is aimed at testing the acute melatonin administration (oral; 6 mg) on aerobic tolerance at cycloergometer and analyzing the consequences on biochemical and hematological parameters. **Methods:** The maximal aerobic capacity intensity (iMAC) at cycloergometer of eleven male healthy men (24.18±3.92 years-old; 87.07±12.48 kg; 1.82±0.05 m; 26.18±3.63 kg/m^2; and 16.28±5.77 % of fat) was individually determined and used to perform a time to exhaustion (*t*lim) trial of 30 minutes after melatonin or placebo administration. We observed 48-72h interval between tests, performed in a double-blind experiment design. In order to determine hematological and biochemical parameters we collected venous blood samples before and after *t*lim. Statistical significance was set at 5%. **Results:** The intensity and the lactatemia corresponding to the maximal aerobic capacity were 120.88±18.78 W and 3.32±1.03 mmol.L^{-1}, respectively. The *t*lim with placebo (33.94±15.26 min, confidence interval = 24.92 - 42.95) was significantly lower than the *t*lim with melatonin (41.94±17.22 min; CI = 31.76 - 52.12; p = 0.03; 19.06%; effect size = 0.49). All of the 21 analyzed blood physiological variables resulted in no significant variation after *t*lim when placebo was compared to melatonin, except for total sera cholesterol (lower after exercise with melatonin). **Conclusion:** Acute melatonin administration enhanced aerobic tolerance at iMAC in 19% at cycloergometer; however, the biochemical and hematological variables assessed were not significantly modulated.

Keywords: maximal aerobic capacity, time to exhaustion, N-acetyl-5-methoxytryptamine, ergogenic aid.

Introduction

Despite meaningful information regarding melatonin effects on circadian rhythm modulation, sleep disorders, jet lag treatment and health application, the effects of this indoleamine on exercise performance remains uncertain. After the remarkable review of Atkinson, Drust B, Reilly T, Waterhouse[1], Escames, et al.[2] produced an important compilation approaching the available information about melatonin and sport performance. Regardless of some molecular, cognitive, cardiovascular and metabolic modulations associated to positive effects, improvements for aerobic performance is not a concensus[1,2].

Observing the physiological effects of melatonin[3-12], our laboratory has studied the acute effects of melatonin on aerobic performance, once we interpreted the actions of this indoleamine allows advantage mainly for this kind of exercise instead of high intensity and short duration exercises. We firstly employed the animal model with swimming rats and found an over expected ergogenic effect at wakefulness period[13] even when not reproducing melatonin's protective effect described at literature, i.e., skeletal muscle inflammation, oxidative stress and tissue damage markers, which were also significantly increased when maximal aerobic tolerance was tested with melatonin[14]. These and other studies elicited us to hypothesize an inference to human model once most of aerobic assessment employed with rats showed similar results when compared to human[15]. The scientific literature has been systematically demonstrated that the blood lactate modulation during incremental exercise in rats mimics human behavior, allowing the standardization and use of reliable and reproducible physical assessment protocols for swimming rats[16,17,18,19].

In human studies regarding melatonin, we could find interventions on the sleep time to improve resting state (and then expect better performance)[20,21] or experiments to test effects of melatonin administration in the morning on exercise performance in the evening[22,23]. Nevertheless, oral melatonin administration elicits bioavailability in humans between 30 and 45 minutes[7] and its acute effect on aerobic performance of humans was never tested despite potentially important. So, this study was aimed at testing the acute melatonin administration on aerobic tolerance at cycloergometer and investigating consequences on biochemical and hematological parameters.

Material and Methods

Subjects

Eleven male volunteers (24.18±3.92 years-old; 87.07±12.48 kg; 1.82±0.05 m; 26.18±3.63 kg/m^2; and 16.28±5.77 % of fat) were assessed. The subjects were moderately active, recreationally involved in soccer, handball, basketball and cycling. All of the volunteers were informed about the risks and the benefits of the experiment, and signed a consent document of the institutional ethics committee prior to any assessment (protocol number 1.293.938/2015).

Experimental design

Four visits to the laboratory were conducted. Firstly, the subjects were accordingly informed of all experiment steps,

were subjected to the anthropometric assessments and performed a familiarization session at the cycloergometer used for all the exercise trials. At the second visit we individually determined the maximal aerobic capacity intensity (iMAC) through incremental test. During the third and the fourth days we collected venous blood samples at rest, administered melatonin or placebo and then performed the time to exhaustion exercise at iMAC (*t*lim), respecting the 48 to 72 hours of interval between these visits. For both days venous blood sample was also collected immediately after *t*lim. The experiment was conducted in a double-blind design, being only one of the scientists responsible for randomly decide whether to use melatonin or placebo and counterbalancing within the subjects at the fourth visit. The blood collection before *t*lim at both trials was used to identify if the biochemical and hematological parameters were similar when the subjects started the second *t*lim. This analysis gave information if the interval of 48-72 h was enough to the individual's recovery.

In order to compare data, the samples collected before *t*lim (at rest) at the day when the subject received placebo was called PR (placebo, rest); the after *t*lim with placebo was called PEx; the before *t*lim, when melatonin was employed, was called MR (melatonin, rest); and the after *t*lim with melatonin, was called MEx. All of the procedures were conducted between 18:00 and 21:00h at 22±1°C, 45-55% of relative air humidity and fluorescent lights between 250 and 300 lux.

Incremental test

We conducted the incremental test to determine the maximal aerobic capacity intensity, corresponding to stable blood lactate concentration. We employed the BIOTEC 2100 cycloergometer (CEFISE, SP, Brazil) with initial workloads of 75 W and increments of 15 W each 3 minute. The subjects had to maintain a 60 RPM with the minor variation possible. At the end of each stage 25 µL of blood samples from ear lobe were collected for lactataemia determination. The incremental test was performed until voluntary exhaustion or when achieved the maximal predicted heart rate (220 – age).

After lactatemia determination, we plotted it against workload to identify the disruption in the proportional lactatemia increases on proportional workload increases, determined by two experienced researchers through a visual inspection according to Matsumoto et al.[24]. Based on such break point we inserted two linear regressions whose intersection was elicited to identify the intensity (x axis; iAnT) and lactatemia (y axis) corresponding to the maximal aerobic capacity intensity[25].

Melatonin administration

Placebo or melatonin was double-blind administered 30 minutes before the *t*lim. Melatonin consists of commercial tablets containing 3 mg each, with 72 mg calcium and 55 mg phosphorus (Optimum Nutrition, Inc, IL, USA). The melatonin dosage was 6 mg[7] and the placebo was made under the same conditions at the laboratory of pharmacology, however, with no melatonin.

Time to exhaustion at maximal aerobic capacity (tlim)

This test consisted of recording the time to exhaustion of an exercise performed at 100% of the iMAC under the researcher's verbal stimulus. We interrupted the test when we noted a voluntary exhaustion or when the subject showed inability to keep the 60 RPM after two warnings with ten seconds of interval.

Biological material analysis

We collected 25 µL of blood during the incremental test and immediately transferred to a plastic tube containing 50 µL of fluoride sodium (1 % NaF). Then, the 25 µL of the homogenate was inserted into an electrochemical lactate analyzer (YSI 2300 Sport, Yellow Springs, OH, USA) frequently calibrated according to the manufacturer instructions. We confirmed the calibration reading standard lactate at 1, 2.5, 5 and 10 $mmol.L^{-1}$.

Venous blood samples collected before and after the *t*lim were separated in two aliquots. One aliquot of 3 mL was immediately transferred to a polyethylene tube containing anticoagulant K3EDTA and gently mixed to avoid hemolysis but agitating enough to assure no sample coagulation. We used the LH780 Beckman Coulter Inc. to analyze samples by hemochromocytometric tests, eliciting counts of total white blood cells (WBC), neutrophils (NEUTR), lymphocytes (LYMP), monocytes (MONO), red blood cells (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular height (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW) and platelets (PLT). The second aliquot was transferred to an empty glass tube, rested for 15 minutes at room temperature and centrifuged at 3000 RPM during 15 minutes. The supernatant (sera) was separated and stored at -80°C in many aliquots to avoid thaw cycles. These samples were used to determine sera concentrations of uric acid (UA), glucose (GLUC), total protein (TP), total cholesterol (CHOL), triglycerides (TG), creatinine (CREAT) and skeletal muscle creatine kinase (CK-NAK) using commercial kit from Laborlab Ltda (SP, Brazil) and Urea (UREA) and lactate dehydrogenase (LDH) by Labclin Bioliquid Ltda (PR, Brazil). We used the spectrophotometric method (Biochrom Asys, Expert Plus UV, MA, USA) for biochemical analysis, following the kits manufacturer´s instructions.

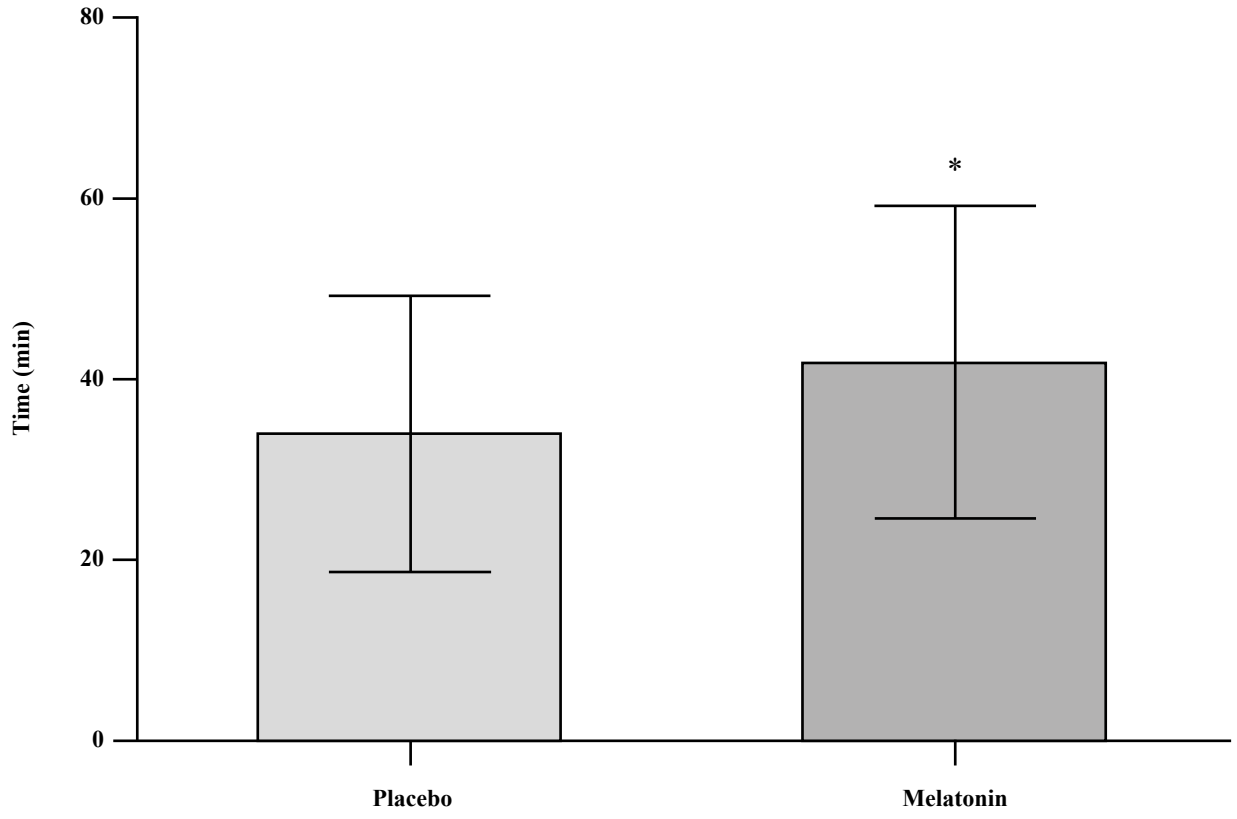
Statistical analysis

Data was expressed at mean ± standard deviation. Percentage analysis and T-test for dependent samples were performed to compare only the time to exhaustion (*t*lim) between placebo and melatonin. For such comparison we also determined the effect size (ES) according to Cohen[26] and confidence intervals according to Hopkins[27] for 95%. We performed factorial ANOVA with two main effects: Exercise (pre versus post *t*lim), Treatment (placebo versus melatonin), and their interaction. When appropriated, Newman-Keuls post hoc test was used to compare groups. Statistical significance was set at 5%.

Results

The intensity and lactatemia corresponding to the maximal aerobic capacity intensity was 120.88±18.78 W and 3.32±1.03 mmol.kg^{-1}, respectively. When the subjects performed the *t*lim, they achieved 33.94±15.26 min (CI = 24.92 - 42.95) with placebo and 41.94±17.22 min (CI = 31.76 - 52.12) under melatonin effect (p = 0.03; 19.06%; ES = 0.49; figure 1).

Figure 1. Time to exhaustion at maximal aerobic capacity intensity performed with placebo or melatonin. * p = 0.03 in relation to placebo.



Data regarding hematological and biochemical outcomes are shown in tables I and II, respectively. No influence of the first *t*lim on the second was found for any of the hematological or biochemical variables, however, we found significant effects of exercise for many of those variables, as shown in tables I and II. Despite the melatonin effect on exercise tolerance showed at *t*-test (19.06%), there was no significant effect of melatonin for the most of hematological or biochemical variables after exercise (post hoc test for PEx vs MEx), except for cholesterol (p = 0.03).

Table I. Results from hematological parameters at rest and after time to exhaustion at maximal aerobic capacity intensity after placebo or melatonin.

	PR	PEx	MR	MEx	TREATMENT	EXERCISE	INTERACTION
WBC (10^9/L)	7.46±1.97	11.03±2.79*a*	7.51±2.13	11.31±2.90*b*	F = 0.04 p = 0.83	F = 23.77 p < 0.01	F = 0.02 p = 0.88
NEUTR (10^9/L)	4.19±1.55	5.71±2.34	4.24±1.63	5.77±2.15	F = 0.03 p = 0.85	F = 7.05 p = 0.01	F < 0.01 p = 0.93
LYMP (10^9/L)	2.42±0.84	4.23±1.38*a*	2.50±0.68	4.40±1.11*b*	F = 0.07 p = 0.79	F = 7.06 p = 0.01	F < 0.01 p = 0.93
MONO (10^9/L)	0.62±0.22	0.81±0.24*a*	0.60±0.19	0.86±0.28	F = 0.01 p = 0.92	F = 10.05 p < 0.01	F = 0.02 p = 0.88
RBC (10^{12}/L)	5.08±0.29	5.45±0.34*a*	5.06±0.36	5.39±0.43	F = 0.33 p = 0.56	F = 10.37 p < 0.01	F = 0.16 p = 0.69
HB (g/dL)	14.64±1.02	15.68±1.07	14.44±0.97	15.43±1.04	F = 0.45 p = 0.50	F = 10.08 p < 0.01	F < 0.01 p = 0.96

HCT (%)	44.62±2.13	48.13±2.19*a*	44.60±2.11	47.64±2.54*b*	F = 0.26 p = 0.61	F = 22.58 p < 0.01	F = 0.23 p = 0.63
MCV (fL)	87.95±3.02	88.39±3.14	88.30±2.91	88.43±2.76	F = 0.25 p = 0.62	F = 0.11 p = 0.74	F = 0.01 p = 0.91
MCH (pg)	28.82±1.31	28.78±1.39	28.59±1.28	28.63±1.17	F < 0.01 p = 0.94	F < 0.01 p = 0.99	F = 0.26 p = 0.61
MCHC (g/dL)	32.74±1.19	32.55±1.17	32.37±0.97	32.38±0.88	F = 0.30 p = 0.58	F = 0.09 p = 0.76	F = 0.36 p = 0.55
RDW (%)	13.44±0.69	13.29±0.75	13.31±0.69	13.22±0.70	F = 0.25 p = 0.62	F = 0.34 p = 0.56	F = 0.01 p = 0.89
PLT (10^9/L)	213.18±39.03	261.10±40.06*a*	199.00±41.09	256.37±35.33*b*	F = 0.37 p = 0.55	F = 19.51 p < 0.01	F = 0.34 p = 0.56

PR: placebo, at rest; PEx: placebo, exercised; MR: melatonin, at rest; MEx: Melatonin, exercised. WBC: White blood cells count; NEUTR: neutrophils; LYMP: lymphocytes; MONO: monocytes; RBC: red blood cells count; HB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular height; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; PTL: platelets. a: $p < 0.05$ in relation to PR; b: $p < 0.05$ in relation to MR.

Table II. Results from biochemical parameters at rest and after time to exhaustion at maximal aerobic capacity intensity after placebo or melatonin.

	PR	**PEx**	**MR**	**MEx**	**TREATMENT**	**EXERCISE**	**INTERACTION**
UA (mg/dL)	7.41±0.95	8.09±1.71	7.80±1.27	8.44±0.77	F = 0.94 p = 0.34	F = 3.04 p = 0.09	F < 0.01 p = 0.95
GLUC (mg/dL)	93.18±12.72	84.02±12.19	85.13±14.16	92.26±20.62	F < 0.01 p = 0.98	F = 0.05 p =0.83	F = 3.12 p = 0.85
TP (g/dL)	6.77±1.12	7.61±1.24	7.58±1.93	7.65±1.72	F = 0.74 p =0.39	F = 0.85 p = 0.36	F = 0.63 p =0.43
CHOL (mg/dL)	251.97±43.77	303.38±51.36	296.54±50.85	239.55±39.35*bc*	F = 0.38 p = 0.54	F = 0.03 p =0.86	F = 11.91 p < 0.01
TG (mg/dL)	125.24±26.20	123.08±27.69	140.54±35.66	138.32±21.73	F = 2.71 p =0.11	F = 0.05 p =0.81	F < 0.01 p = 0.99
CREAT (mg/dL)	1.03±0.51	1.38±0.53	1.77±1.13	1.77±0.90	F = 3.27 p = 0.08	F = 0.31 p = 0.58	F = 0.29 p = 0.59
UREA (mg/dL)	54.90±2.64	56.12±4.52	56.14±3.54	56.31±3.27	F = 0.44 p = 0.51	F = 0.42 p = 0.52	F = 0.24 p = 0.63
CK-NAK (U/L)	99.39±49.37	154.76±22.70	86.32±13.06	145.27±77.43	F = 0.14 p = 0.71	F = 3.60 p = 0.08	F < 0.01 p = 0.95
LDH (U/L)	131.32±28.67	136.88±25.08	135.71±35.27	155.44±28.63	F = 0.93 p = 0.34	F = 1.13 p = 0.29	F = 0.35 p = 0.56

PR: placebo, at rest; PEx: placebo, exercised; MR: melatonin, at rest; MEx: Melatonin, exercised. UA: uric acid; GLUC: glucose; TP: total protein; CHOL: total cholesterol; TG: triglycerides; CREAT: creatinine;CK-NAK: skeletal muscle creatine kinase; UREA: Urea; LDH: lactate dehydrogenase. b: $p < 0.05$ in relation to MR; c: $p < 0.05$ in relation to PEx.

Discussion

The main finding of this study was the novel and significant acute ergogenic effect of melatonin on aerobic tolerance in cicloergometer, nevertheless, the biochemical and hematological parameters assessed were not different when *t*lim was performed with melatonin or placebo (tables I and II).

Initially it is important to note that the experimental design elicited complete recovery for subjects between placebo and melatonin trials, once no significant difference between PR and MR could be found, meaning that before starting both *t*lim the subjects showed the same physiological (biochemical and hematological) condition.

Our study found that a single 6 mg dose of oral melatonin ingestion, 30 minutes before the exercise, improved 19% of the aerobic exercise tolerance, with significant statistical difference and confidence interval of medium effect according to Cohen[26], being very close to be considered a large effect (0.50). Bosman, Dormehl, Hugo, Redelinghuys, Threon[28] postulated that melatonin is able to increase the left ventricular ejection

fraction and cardiac output, likely increasing aerobic tolerance. Melatonin also increases the production of the grown factor and inhibits muscle oxidative stress and inflammation[29], optimizes mitochondrial metabolism[3] and conserves glycogen content when modulates the metabolism to use lipids instead of glucose during exercise[9], all possibly contributing to our findings. Moreover, we interpreted that melatonin is an interesting hormone specifically for long duration exercises once it reduces the catecholamine levels[10], reduces alert state and acts in thermoregulation[8,30,31] possibly delaying the pain perception and the onset of high muscle temperature, which impairs exercise tolerance, respectively.

Knowing that melatonin could act in many fronts, we analyzed several biochemical and hematological parameters trying to identify the effect of this hormone after exercise. Regarding these parameters, we found significant exercise effect, as expected, but no melatonin effect. The only variable with significant variation was total sera cholesterol, which showed significant smaller values when subjects performed *t*lim with melatonin in relation to the *t*lim with no melatonin or under rest conditions. This is the only variable addressed to Mazepa, Cuevas, Collado, Gonzalez-Gallego[9] or Acuña-Castroviejo et al.[3] experiments, who postulated better metabolic conditions to use lipids during exercise with melatonin.

Even with careful conduction, our experiment is not out of criticism. Once the assessed subjects were non-athletes, it is necessary to analyse if these data are reliable for trained subjects, who likely maintain exercise at higher intensity and longer periods of time. However, this was the first study to identify the ergogenic effect of melatonin in humans at individually determined aerobic exercise's intensity performed until exhaustion and, certainly, more investigations regarding involved physiological mechanisms for such phenomena are needed.

Conclusion

Acute melatonin administration enhanced aerobic tolerance at maximal aerobic capacity intensity in 19% at cycloergometer, but with no significant effect on the biochemical and hematological assessed variables.

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