Melatonin is an Ergogenic Aid for Exhaustive Aerobic Exercise only during the Wakefulness Period

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**Abstract**

This study tested the ergogenic effects of acute administration of melatonin on exhaustive exercise (tlim) at the anaerobic threshold intensity (iAnT) during periods of lower (L) and higher (H) spontaneous physical activity in swimming rats. Additionally, we evaluated the time of day effect on aerobic exercise tolerance. The periods of L and H were determined gravimetrically. All animals were subjected to an incremental test to determine the iAnT. Melatonin was administered (10 mg.kg⁻¹, intraperitoneal) and after 30 min, the rats were subjected to tlim during the L (LM) or H (HM) period. Control groups were called LC and HC. The criterion of significance was 5%. Melatonin enhanced tlim by 169% during H (HC = 72 min; HM = 194 min; P < 0.01; ES = 1.23) and by 90% during L (LC = 31 min vs. LM = 59 min; P = 0.39; ES = 1.18), demonstrating a significant effect on tlim (F = 10.35; P < 0.01) and a strong effect size (ES). Additionally, tlim was higher during H (F = 14.24; P < 0.01). Melatonin is a reasonable ergogenic aid, particularly during the wakefulness period, and the exercise tolerance is dependent on the time of day for swimming rats.

**Introduction**

Secretion of melatonin, an indoleamine pineal hormone, is inhibited by environmental light exposure [22, 33]. As a result, blood melatonin concentrations are higher at night than during daylight in entrained mammals [17, 43]. Because of its daily cycles, melatonin is classically associated with the synchronization of the circadian rhythms and synchronization of many physiological functions [4, 16, 29]. Similar to the cycles of melatonin [17], the spontaneous physical activity (SA) of nocturnal rats is high at night (wakefulness) and low during the day (resting/sleep) [25, 44]. The core body temperature follows a similar cycle [25, 40]. Anti-inflammatory [13] and antioxidant [35] properties are also attributed to melatonin. The intracellular activities of melatonin include inhibiting lipid peroxidation [12], increasing the levels of mitochondrial electron transport chain and Krebs cycle enzymes and maintaining liver and brain microsomal membrane stability [1]. As a result, melatonin is thought to have therapeutic potential for diseases such as insulin resistance [42] and obesity [16]. Melatonin also has pro-apoptotic activity in cancer cells [14].

In addition to the effects of melatonin on circadian rhythms, including reported improvements in exercise tolerance (i.e., core body temperature and spontaneous activity), some of the functions of melatonin may have direct and positive effects on performance. Exercise-induced inflammation [13] and oxidative stress [35] are inhibited when melatonin is administered in both humans [30] and rats [2]. Melatonin also preserves glycogen content, stimulating fatty acid oxidation during exercise [32] and protecting muscle from exercise-induced damage [23]. Nevertheless, the direct role of melatonin as an ergogenic aid for exercise performances is not clear in humans [4, 21, 45] and this topic has not been directly investigated in rats, despite the fact that this rodent model allows the researchers to control all environmental parameters, nutrition, drug administration and exercise conditions. Furthermore, it is not clear whether melatonin improves the swimming performance of nocturnal rats across the circadian periods. This is a critical issue because previous studies have demonstrated higher swimming performance during the natural wakefulness period for swimming nocturnal rats [7]. Therefore, the use of exogenous melatonin as an ergogenic aid to physical exercise is an interesting approach for improving exercise tolerance.
exercise remains unclear, particularly when it is administered at different times during the day and based on objective exercise parameters. The aim of this study was to investigate the ergogenic effects of the acute administration of melatonin on exhaustive exercise (t_{lim}) at the anaerobic threshold intensity (iAnT) during periods of lower and higher spontaneous physical activity in swimming rats. Additionally, we aimed to confirm reports in the literature suggesting that the time of day has a significant effect on aerobic exercise tolerance.

Methods

Animals

60 male Wistar rats were obtained from an institutional facility and maintained under a 12h light/dark cycle, with the lights switched on at 06:00h (zeitgeber time 0). We used a 100W lamp during the light period (Phillips® soft white light; 2700K; 565–590 nm; 60 lux). The rats were housed in polyethylene cages with free access to water and rodent chow. The relative humidity was kept at 45–55%, temperature at 22±2°C and noise levels below 85 decibels. The study was approved by the institutional Ethics Committee on the Use of Animals under process 2502–1 and followed the ethical standards of the International Journal of Sports Medicine as described in Harriss and Atkinson [24]. All procedures performed were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Experimental design

At 45 days of age, rats were evaluated for spontaneous physical activity (SA) using gravimetry [11]. The means of the hourly activity during a 24-h period were used to identify the periods of lowest and highest SA and to randomly divided the 60 rats into 2 groups: rats to be assessed at the higher (H, n = 30); and rats to be assessed at the lower (L, n = 30) SA. All of the subsequent procedures were performed at the time of day corresponding to the specific group. Special environmental illumination was employed for animals assessed during the dark period (>600 nm; <15 lux), as described elsewhere [8]. From 76 to 89 days of age, all the rats were subjected to a water environment and swimming adaptation to avoid stress due to novelty during the exercise tests. The individual swimming ergometer consisted of a cylindrical PVC tank with a diameter of 30 cm filled to a depth of 100 cm with clean water at 31±1°C. At 90 days of age, all the animals were subjected to an incremental test (IT) to determine the anaerobic threshold intensity (iAnT), according to the time of day of each group. At 92 days of age, animals that received melatonin 30min prior to exhaustive exercise at iAnT (t_{lim}) during L were designated as the LM group; animals that received melatonin 30min prior to exhaustive exercise at iAnT (t_{lim}) during H were designated as the HM group. Control groups for the effect of melatonin were included for both times of day (LC, n = 15; HC, n = 15).

Melatonin (SIGMA-ALDRICH, Co., St. Louis, MO, USA; Reference M5250, ≥ 98%, Lot # SLBC7539V) was dissolved in ethanol (<0.1%) and diluted in saline solution (NaCl 0.9%) to be administered via intraperitoneal at 10 mg.kg$^{-1}$, corresponding to a non-physiological dosage. The control animals received an equal volume of vehicle alone.

Blood samples were collected before and after t_{lim} to determine blood lactate concentration levels. The time of exhaustion at iAnT (t_{lim}) was recorded as the performance parameter. The experimental design is shown in Fig. 1.

Gravimetric apparatus

The signal acquisition system consisted of a primary sensor element (load cell – PLA30Kg, Líder Balanças®, Araçatuba, São Paulo, Brazil) involving in 2 hard iron platforms located under the animals’ cage. Due to the sensitive nature of the apparatus, minimal movements were noted. After amplification (MKTC5–10®, MK control and instrumentation™, São Paulo, São Paulo, Brazil) and processing (USB-6008® signal-conditioning module, National Instruments, Austin, Texas, USA), the collected data were transmitted to a software program (LabView Signal Express® 2009, National Instruments™, Austin, Texas, USA) and

![Fig. 1 Experimental design of the study.](https://example.com/supplementary/fig1.png)
continuously recorded at 30Hz over 24h. The apparatus was previously calibrated using 14 known loads from 17.12 to 6,395.98 g. This procedure provided a linear relationship regression ($R^2 = 0.99$), employing a unit conversion from millivolts (mv) to kilograms (kg). Next, the digital signals were treated as described elsewhere [11], using MatLab® 7.0 (MathWorks™). The SA scores were adjusted by the rat’s body mass (kg.g⁻¹) and statistically processed. The apparatus is illustrated in Fig. 2.

**Incremental swimming test**

Exposing animals to proportional incremental loads over time leads to disproportional increases in blood lactate levels at a given moment [48]. The IT employed in this experiment consisted of 5-min stages with overloads corresponding to 3, 3.5, 4, 4.5, 5, 5.5, 6 and 6.5% of the body mass (%bm), and tail vein blood samples were collected after each stage from the rat’s distal part of the tail to determine the lactate levels. The lead overloads were inserted in the chest of the rats using an elastic strap. The blood lactate concentration relative to the exercise intensity was graphically plotted. Using these data, the alteration of the proportional blood lactate concentration increases was defined by visual inspection by 2 experienced researchers, as described by Matsumoto, Araki, Tsuda, Odajima, Nishima, Higaki, Tanaka, Tanaka and Shindo [31]. Next, 2 linear regressions were constructed following the described break point, defined as the intersection of the linear regressions interpolated to the X-axis [48]. The intensity corresponding to this interpolation was defined as the iAnT (Fig. 3). This procedure was recently tested to determine its validity and reliability [6], eliciting individual assessment of the swimming intensity for rats.

**Analytical procedures for biological specimens**

Blood samples (25µL) were collected using micro heparinized glass capillaries, and immediately transferred to 1.5 mL plastic tubes containing 400 µL of trichloroacetic acid [4%]. The samples were shaken and centrifuged to use the supernatant, which was added to a reactive solution (Glycine/EDTA; Hydrazine Hydrate; Beta-nicotinamide dinucleotide; L-Lactic dehydrogenase bovine heart; adjusted to a pH of 8.85). After an adequate period of incubation with controlled temperature, the blood lactate concentrations were determined using the enzymatic method [19], being the processed samples analyzed by spectrophotometry at 340 nm and compared to a calibration curve.

**Statistical methods**

The results are presented as the mean ± standard deviation (SD). The Kolmogorov-Smirnov and Liliefors tests confirmed the normality of all the variables; we therefore used parametrical statistics. The SA data were expressed as the 60-min means over 24h to determine the moments of higher and lower spontaneous activity. Percentage analysis and t-tests for dependent samples were conducted to analyze the differences in SA between a period of total daylight and a period of total darkness. The t-test for independent samples was used to analyze the differences between the iAnT and blood lactate levels with the iAnT determined during the H and L periods. 2-way analysis of variance was used for the tlim variables (tlim and blood lactate levels before and after tlim) to test for a i) the main effect of the drug [2, melatonin and placebo] and ii) the main effect of SA [2, higher and lower] and a possible interaction. It was also calculated and included the effect size (ES) for the studied variables. The Newman-Keuls post hoc was used when appropriated. The criterion for significance was $P<0.05$. All statistical procedures were performed using MatLab® 7.0 (MathWorks™).
Results

The spontaneous activity during periods of total daylight was significantly lower when compared to that during periods of total dark \( P < 0.01 \) (ES = 0.87). This result is not surprising, given that 75.24% of the rat's entire daily activity is performed at night. The lowest SA values were observed at 12:00 h (mean values of activity from zeitgeber time 6–7), and the highest values were observed at 20:00 h (zeitgeber time 14–15), as illustrated in ‹ Fig. 4. These results were used to determine the time of day to begin all of the assessments for the L and H groups, respectively.

The iAnT values were 4.86±0.43 % bm for the LC group, 4.74±0.35 % bm for the LM group, 5.49±0.41 % bm for the HC group and 5.19±0.43 % bm for the HM group, with significant differences between the groups. The iAnT of the HC group was higher than that of the LM group \( P < 0.01 \); higher than that of the HM group \( P < 0.01 \); and the iAnT of the HC group was higher than that of the LM group \( P < 0.01 \); \( P = 0.90 \) on iAnT; however, the time of day significantly influenced iAnT \( F = 23.77 \); \( P < 0.01 \), and there was no interaction between effects \( F = 0.61 \); \( P = 0.44 \). Neither the time of day \( F = 0.43 \); \( P = 0.51 \) nor melatonin \( F = 0.01 \); \( P = 0.90 \) influenced the blood lactate concentration at iAnT.

Descriptive results from exhaustive exercise at anaerobic threshold intensity (tlim) are shown in ‹ Fig. 5. In summary, the tlim of the HM group was 169 % higher than the HC group \( (P < 0.01) \), and the tlim for the LM group was 90 % higher than the LC group \( (P = 0.39) \). For tlim, the effects of melatonin were significant \( F = 10.35 \); \( P < 0.01 \); Melatonin > Vehicle, as were the effects of the time of day \( F = 14.24 \); \( P = 0.01 \); higher spontaneous activity > lower spontaneous activity activity, resulting in a significant interaction between the effects \( F = 4.05 \); \( P = 0.04 \). The blood lactate concentrations collected at rest just before tlim were 0.92±0.06 mM in the LC group, 1.29±0.08 mM in the LM group, 1.47±0.03 mM in the HC group and 2.02±0.18 mM in the HM group. The administration of melatonin \( F = 17.31 \); \( P < 0.01 \) and assessment during higher spontaneous activity \( F = 3.328 \); \( P < 0.01 \) were associated with significantly increased blood lactate concentration, with no interaction between the effects \( F = 0.72 \); \( P = 0.39 \). The blood lactate concentrations after exercise were 7.01±0.48 mM for the LC group, 6.75±0.31 mM for the LM group, 6.93±0.46 mM for the HC group and 6.28±0.60 mM for the HM group. There were no significant effects of melatonin \( F = 0.80 \); \( P = 0.37 \) or time of day \( F = 0.29 \); \( P = 0.59 \) and there was no interaction between the effects \( F = 0.14 \); \( P = 0.70 \).

Discussion

The main finding of the present study was the significant performance-enhancing effect of melatonin on the tested exercise. The time to exhaustion at the anaerobic threshold intensity was higher in animals that received melatonin than in control animals, which is a novel result. However, it is important and interesting to note that the melatonin was significantly effective only when it was administered during the period of higher spontaneous activity \( H \). Additionally, performances were significantly better during \( H \) compared with \( L \), which corroborates a previous study [7].

Spontaneous physical activity

The SA data were used to objectively determine the periods of higher and lower activity throughout the day, as initially proposed. We confirmed the nocturnal activity of albino Wistar rats that has been reported in the literature [37], as 75.24% of the total daily spontaneous physical activity occurred during the dark period, with a peak at 20:00 h (zeitgeber time 14). Our data are consistent with those reported by Ikeda, Sagara and Inoue [25], who showed that approximately 70% of daily activity occurs during the dark period.

The SA was recorded using a device that was undetectable to the rats and under identical the same environmental conditions, thus avoiding potential influence from handling during the SA assessments. These measurements estimate the natural resting (or sleep period) and wakefulness periods at times when physiological [25, 28, 29, 40] and behavioral [15, 27] modifications are systematically observed under laboratory conditions in entrained animals.
Swimming exhaustive bouts at iAnT (tlim): the SA effect

The effects of circadian rhythm on athletic performance are currently under investigation, and there is considerable controversy related to the variability of environmental factors and differences among the types of exercise [18,20,36,38,47]. According to Drust, Waterhouse, Atkinson, Edwards and Reilly [18], both field and laboratory studies are necessary to appropriately understand the effects of biological rhythms on exercise, and the control of environmental factors is crucial for study validity. Our experiment showed that the aerobic exercise tolerance was improved when the rats were evaluated during the period of high spontaneous physical activity. Other experiments compared exercise during both circadian periods and systematically found that rats performed better at night [5,7]. In addition to the effect of the time of day, it is important to note that the environmental light employed for the exercise testing during the dark period (higher spontaneous physical activity, wakefulness period of nocturnal rats) can also influence the performance [8]. Because the focus of this experiment was to investigate the effects of melatonin on performance, we will briefly refer the reader to published work discussing the time-of-day effect on tlim [7].

Swimming exhaustive bouts at iAnT (tlim): the melatonin effect

According to the data shown in \( \text{Fig. 5} \), the animals that received melatonin and were evaluated during the wakefulness period (HM) presented the highest exercise tolerance \( (P<0.01) \). The time to exhaustion of HM was 169% higher than that of the HC group \( (P<0.01) \). The tlim of the LM group, although not statistically significant, was 90% higher than that of the LC group \( (P=0.39) \). These results demonstrate that the ergogenic effect of melatonin was dependent on the time of day for swimming rats subjected to the proposed exercise and testing periods described in this study. This interesting result suggests that there is an additive effect of SA and melatonin on performance. Additionally, the importance of melatonin and the time of day effect on such result was confirmed by the interaction between the effects \( (F=4.05; P=0.04) \). Although some studies have been performed in humans, the literature on the effects of melatonin in exercised rats is scarce, and no experiments have directly investigated melatonin as an ergogenic intervention in these animals. Mapeza, Cuevas, Col-lado and Gonzalez-Gallego [32] elegantly analyzed the metabolic effects of melatonin on energy substrates and postulated that melatonin preserves glycogen by increasing the use of lipids as energy substrates during exercise. However, the exercise parameters were not individualized, and the exercise outcomes were not shown. Other studies have investigated the effects of melatonin on energy metabolism in rats during exercise using different assays [10,26,34]. However, no studies have directly investigated the ergogenic effects of melatonin, and several studies failed to control the exercise intensity or subjected the animals to swimming trials individually. According to the American Physiological Society, these are important limitations in studies measuring exercise swimming in rats [3].

Regarding the drug concentration, other studies have used melatonin dosages of 10 mg kg\(^{-1}\) for rats [9,41,49] and intraperitoneal administration is appropriate because of the rapid bioavailability [46]. There are many possible mechanisms that could explain the ergogenic effects of melatonin [21,39,45]. This hormone acts by reducing exercise-induced inflammation and oxidative stress [2,30], and alters energy availability during exercise [10,26,32]. Therefore, it is plausible to suggest that melatonin will have the most robust ergogenic effects in situations where inflammation, oxidative stress and a lack of available energy are important limitations. Due to the circadian cycling of physiological, cognitive and behavioral parameters, it also seems also plausible that exercise tolerance might be improved during the normal wakefulness period, compared to the resting or sleep period, for both humans and rats. Nevertheless, from the viewpoint of comparative physiology, the level of endogenous melatonin is typically increased during wakefulness for entrained nocturnal rats, but this is not the case for humans. It is possible that the combination of endogenous and exogenous melatonin is required for the ergogenic effect observed in our experiment. These effects may differ for humans, who frequently possess low endogenous melatonin concentrations during the wakefulness period.

It is important to note that despite our methodological attempts to objectively determine the SA, drug administration and exercise parameters, our study has limitations. Because this is the first study to show the ergogenic effects of melatonin on exercise tolerance in rats, new experimental designs are needed to confirm the consistency of this phenomenon and to understand the role of melatonin in acute exercise tolerance (i.e., anti-inflammatory effects, antioxidant effects, modulation of energy substrates, alertness, core body core temperature and circadian rhythms). Such studies should use different dosages of melatonin and exercise intensities/durations.

Conclusion

Finally, our results strongly suggested that melatonin is a reasonable ergogenic aid for swimming rats, particularly during the wakefulness period. This work also supports the assertion that exercise tolerance is dependent on time of day for swimming rats assessed under individualized exercise intensity regimens with strict control of the laboratory’s environmental conditions.

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