Effects of melatonin ingestion on physical performance and biochemical responses following exhaustive running exercise in soccer players

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ABSTRACT: Antioxidant supplementation has become a common practice among athletes to boost sport achievement. Likewise, melatonin (MEL) has been ingested as an ergogenic aid to improve physical performance. To date, no study has checked whether the multiple beneficial effects of MEL have an outcome during a maximum running exercise until exhaustion. Therefore, the present study aimed to evaluate the effect of MEL ingestion on physical performance and biochemical responses (i.e., oxidative stress) during exhaustive exercise. In a double blind randomized study, thirteen professional soccer players [age: 17.5 ± 0.8 years, body mass: 70.3 ± 3.9 kg, body height: 1.80 ± 0.08 m; maximal aerobic speed (MAS): 16.85 ± 0.63 km/h; mean ± standard deviation], members of a first league squad, performed a running exercise until exhaustion at 100% of MAS, after either MEL or placebo ingestion. Physical performance was assessed, and blood samples were obtained at rest and following the exercise. Compared to placebo, MEL intake prevented the increase in oxidative stress markers (i.e., malondialdehyde), alleviated the alteration of antioxidant status (i.e., glutathione peroxidase, uric acid and total bilirubin) and decreased post-exercise biomarkers of muscle damage (i.e., creatine kinase and lactate dehydrogenase) (p < 0.05). However, physical performance was not affected by MEL ingestion (p > 0.05). In conclusion, acute MEL intake before a maximal running exercise protected athletes from oxidative stress and cellular damage but without an effect on physical performance.

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INTRODUCTION

Muscle fatigue is a complex physiological process. Several theories have been proposed to explain the underlying mechanisms of muscle fatigue after exercise. One of them suggested that high levels of reactive oxygen species (ROS) promote contractile dysfunction, resulting in muscle weakness and fatigue [1]. ROS affects muscle fatigue mainly through the oxidation of cell proteins such as the Na+–K+ pump, myofilaments, dihydropyridine and ryanodine receptors, leading to the inhibition of sarcoplasmic reticulum Ca2+ release and myofibrillar Ca2+ sensitivity [1].

Muscle fatigue is usually accompanied by impairment of physical performance [2]. Therefore, recovery strategies between training sessions or during competitive events are required in order to relieve fatigue and improve performance. Indeed, several studies have reported that antioxidant supplementation reduces muscle damage and oxidative stress [3], which consecutively relieves muscle fatigue, enhances performance and expedites recovery [3].

Numerous studies have referred to the potential role of melatonin (N-acetyl-5-methoxytryptamine) (MEL) as an antioxidant that can help athletes endure increased training loads and congested competitive schedules [4, 5]. This indole stimulates the secretion and activity of several antioxidant enzymes [4, 5]. It also reduces muscle damage [5] and has anti-inflammatory and immunomodulatory
actions [4]. In addition, MEL displayed tissue repair and skeletal muscle healing capacities [6] with improvement of muscle adaptation to training [7]. More recently, MEL was shown to improve short-term and aerobic performances during an increasing load exercise [8]. It interestingly has a protective effect on muscle and hepatic glycogen stores by inhibiting their depletion [9].

Nevertheless, the effect of acute MEL ingestion on aerobic performance in a continuous exercise until exhaustion has been previously investigated only during indoor exercises which are not specific to running activities (i.e., swimming, cycling) [10, 11, 12]. In addition, knowing that MEL could act on many fronts, it was deemed wise to analyse several biochemical parameters to identify the effect of this hormone on cellular damage during this type of exercise. Therefore, the aim of the present study was to assess the effect of MEL ingestion on physical performance and biochemical responses during an intensive running exercise until exhaustion in an ecological environment.

**MATERIALS AND METHODS**

**Participants**

Thirteen professional soccer players [age: 17.5 ± 0.8 years, body mass: 70.3 ± 3.9 kg, body height: 1.80 ± 0.08 m; maximal aerobic speed (MAS): 16.85 ± 0.63 km/h; mean ± standard deviation (SD)], members of a Tunisian first league squad, participated in the present study. All athletes were healthy, non-smokers and did not use medications or supplements or engaged in transmeridian travel previously investigated only during indoor exercises which are not specific to running activities (i.e., swimming, cycling) [10, 11, 12]. In addition, knowing that MEL could act on many fronts, it was deemed wise to analyse several biochemical parameters to identify the effect of this hormone on cellular damage during this type of exercise. Therefore, the aim of the present study was to assess the effect of MEL ingestion on physical performance and biochemical responses during an intensive running exercise until exhaustion in an ecological environment.

**Vigilance test (VT)**

This test was used to assess vigilance as described by Farjallah et al. [16]. It consisted of identifying a particular sign (a figure composed of three numbers) and circulating it as much as possible in a limited time (1 min), working line by line, from left to right, leaving aside all the other figures which were not composed of three numbers. The test paper contained 600 signs divided into 36 lines. The total circling number was considered to determine vigilance performance for each participant [16].

**Running exercise test (RET)**

All participants performed a running exercise to exhaustion with a constant maximal intensity (100% of the individual MAS). During the RET, cones were set at 40-m intervals along the 400-m track. The running pace was dictated by audio cue and the participants had to be within 2-m of the cones at each beep. The test was over when participants were unable to maintain the required pace and to reach the required cones on each audio cue (a 2-m shortfall was used as an objective criterion). Prior to the RET, the participants completed a 15 min warm-up, running at 60% MAS, followed by a 5 min recovery period [14].

**Blood sampling and variables**

Blood samples were collected from a forearm vein after 10 min of seated rest and 3 min after the RET. Two distinct blood collection tubes were used in the present study. A first EDTA tube was used to determine oxidative stress parameters such as malondialdehyde (MDA), advanced oxidation protein products (AOPP), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR). A heparinized tube was used to measure uric acid (UA) and total bilirubin (TBIL) as indirect biomarkers of antioxidant status and creatine kinase (CK) and lactate dehydrogenase (LDH) as biomarkers of muscle damage.
Samples were placed in an ice bath then they were centrifuged at 2500 × g and 4°C for 10 min. Aliquots of the resulting plasma were stored at -80°C until analysis. To eliminate inter-assay variance, all samples were analysed in the same assay run.

**Analysis of blood variables**

Lipid peroxidation was assessed by thiobarbituric acid reactive substances (TBARS) by measuring plasma levels of MDA, using the method described by Buege and Aust [17]. The AOPP levels were determined according to the method of Kayali et al. [18]. SOD activity was assayed in terms of its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) according to Beauchamp and Fridovich [19]. Plasma GR levels were measured as described by Weckbercker and Cory [20] and GPx activity was quantified by the procedure of Flohé and Günzler [21].

TBIL, UA, CK and LDH measurements were done as adapted for the autoanalyzer by Siemens ADVIA 1800 chemistry system (Erlangen, Germany). TBIL measurements were performed by a diazo reaction. The total analytical imprecision of the TBIL method was 1.4% at 18 μmol·L⁻¹ and 1.1% at 90 μmol·L⁻¹. UA was determined by an enzymatic method at 550 nm. The intra-assay coefficient of variation for UA was < 1.9%. CK was measured by the N-acetyl-L-cysteine method. The intra-assay coefficient of variation (CV) for this parameter kit was 1.85%. LDH activity was determined by the oxidation of lactate on pyruvate. The intra-assay CV for the lactate dehydrogenase kit was 0.2%.

**Statistical analysis**

Data were presented as mean ± SD. All statistical tests were processed using STATISTICA 10 software (StatSoft, Maisons-Alfort, France). The Shapiro–Wilk test revealed that the data were normally distributed.

Physical performance and RPE scores were analysed with paired samples Student’s t-test. Moreover, OT, VT and biochemical parameters were analysed using a two-way (condition × exercise) analysis of variance with repeated measures. Both factors were “within-participants” by definition and included two “levels” (MEL/PLA) in the condition factor and two “levels” (before/after) in the exercise factor. When appropriate, significant differences were tested using Tukey’s honestly significant difference post hoc test.

Effect sizes were calculated as partial eta-squared ($\eta^2_p$) for the ANOVA to estimate the meaningfulness of significant findings. $\eta^2_p$ values of 0.01, 0.06, and 0.13 represent small, moderate, and large effect sizes, respectively. Cohen’s d effect size (d) in each measured variable is defined as the difference between the means divided by the common standard deviation [22]. Threshold values for the interpretation of “d” were 0 to < 0.20 (trivial), 0.20 to < 0.50 (small), 0.50 to < 0.80 (medium) and $\geq$ 0.80 (large). A probability level of 0.05 was selected as the criterion for statistical significance.

**TABLE 1.** Physical performance (PP), maximal heart rate (HR max) and rating of perceived exertion (RPE) values following melatonin (MEL) or placebo (PLA) ingestion.

|       | PLA               | MEL               | p-value |
|-------|-------------------|-------------------|---------|
| PP    | 362.46 ± 42.06    | 374.54 ± 57.97    | 0.19    |
| HR max| 190.54 ± 2.93     | 188.92 ± 4.70     | 0.22    |
| RPE   | 8.85 ± 0.69       | 8.38 ± 0.77       | 0.26    |

**TABLE 2.** Oral temperature (OT) and vigilance (VT) values measured before (BRET) and after (ARET) the running exercise test (RET) following melatonin (MEL) or placebo (PLA) ingestion.

|       | BRET     | ARET     | BRET     | ARET     | Condition | Exercise | Interaction |
|-------|----------|----------|----------|----------|-----------|----------|-------------|
| OT (°C)| 36.61    | 37.80    | 36.51    | 37.58    | $F_{(1,12)} = 6.51$; $p < 0.05$; $\eta^2_p = 0.35$ | $F_{(1,12)} = 731.48$; $p < 0.001$; $\eta^2_p = 0.98$ | $F_{(1,12)} = 2.4$; $p > 0.05$; $\eta^2_p = 0.17$ |
|       | ± 0.19   | ± 0.24a  | ± 0.35   | ± 0.19ab |           |          |             |
| VT (AU)| 67.38    | 71.92    | 64.85    | 66.54    | $F_{(1,12)} = 9.78$; $p < 0.05$; $\eta^2_p = 0.45$ | $F_{(1,12)} = 2.99$; $p > 0.05$; $\eta^2_p = 0.20$ | $F_{(1,12)} = 0.49$; $p > 0.05$; $\eta^2_p = 0.04$ |
|       | ± 11.43  | ± 10.06  | ± 9.97   | ± 11.63  |           |          |             |

*a significant difference in comparison with values measured before the RET. b significant difference in comparison with values measured in PLA condition.
RESULTS

Physical performance, HR max and RPE
The mean ± SD values for physical performance, HR max and RPE recorded during the two conditions are presented in Table 1. Paired t-test showed no significant difference between MEL and PLA conditions regarding these parameters.

Psychocognitive performance
Data of psychocognitive performance are presented in Table 2. Statistical analysis revealed a significant MEL effect for the VT. However, the post hoc test showed no significant difference between MEL and PLA conditions.

TABLE 3. Oxidative stress and muscle damage parameters measured before (BRET) and after (ARET) the running exercise test (RET) following melatonin (MEL) or placebo (PLA) ingestion.

|       | PLA   | MEL   | Condition   | Exercise | Interaction |
|-------|-------|-------|-------------|----------|-------------|
| MDA (µmol/l) | BRET  | ARET  | BRET  | ARET  | F(1,12) = 1.82; p > 0.05; η² = 0.13 | F(1,12) = 33.69; p < 0.001; η² = 0.74 | F(1,12) = 4.97; p < 0.05; η² = 0.29 |
|       | ± 0.70| ± 0.77 | ± 0.77 | ± 1.3  |             |             |             |
| AOPP (nmol/mg protein) | BRET  | ARET  | BRET  | ARET  | F(1,12) = 4.93; p < 0.05; η² = 0.29 | F(1,12) = 0.74; p > 0.05; η² = 0.06 | F(1,12) = 0.26; p > 0.05; η² = 0.02 |
|       | ± 0.05| ± 0.05 | ± 0.05 | ± 0.05 |             |             |             |
| SOD (U/g) | BRET  | ARET  | BRET  | ARET  | F(1,12) = 0.04; p > 0.05; η² = 0.00 | F(1,12) = 32.27; p < 0.001; η² = 0.73 | F(1,12) = 5.05; p < 0.05; η² = 0.30 |
|       | ± 35.26| ± 49 a | ± 50.88| ± 43.77 |             |             |             |
| GPx (mU/mg) | BRET  | ARET  | BRET  | ARET  | F(1,12) = 0.71; p > 0.05; η² = 0.06 | F(1,12) = 8.21; p < 0.001; η² = 0.41 | F(1,12) = 4.97; p < 0.05; η² = 0.29 |
|       | ± 10.82| ± 8.57 a | ± 7.41 | ± 5.23  |             |             |             |
| GR (nmol/mg protein) | BRET  | ARET  | BRET  | ARET  | F(1,12) = 0.02; p > 0.05; η² = 0.00 | F(1,12) = 3.97; p < 0.05; η² = 0.25 | F(1,12) = 0.01; p > 0.05; η² = 0.00 |
|       | ± 3.83| ± 1.33 | ± 1.13 | ± 0.63  |             |             |             |
| UA (µmol/L) | BRET  | ARET  | BRET  | ARET  | F(1,12) = 0.11; p > 0.05; η² = 0.01 | F(1,12) = 7.88; p < 0.001; η² = 0.40 | F(1,12) = 0.58; p > 0.05; η² = 0.05 |
|       | ± 24.78| ± 27.81 a | ± 34.44| ± 38.70 |             |             |             |
| TBIL (µmol/L) | BRET  | ARET  | BRET  | ARET  | F(1,12) = 0.37; p > 0.05; η² = 0.003 | F(1,12) = 8.14; p < 0.001; η² = 0.40 | F(1,12) = 1.95; p > 0.05; η² = 0.05 |
|       | ± 16.20| ± 2.03 a | ± 3.64 | ± 3.01  |             |             |             |
| CK (IU/L) | BRET  | ARET  | BRET  | ARET  | F(1,12) = 0.74; p > 0.05; η² = 0.06 | F(1,12) = 38.4; p < 0.001; η² = 0.76 | F(1,12) = 6.49; p < 0.001; η² = 0.35 |
|       | ± 99.61| ± 124.60 a | ± 130.30| ± 125.33 b |             |             |             |
| LDH (IU/L) | BRET  | ARET  | BRET  | ARET  | F(1,12) = 0.68; p > 0.05; η² = 0.05 | F(1,12) = 81.18; p < 0.001; η² = 0.87 | F(1,12) = 12.38; p < 0.001; η² = 0.05 |
|       | ± 38.61| ± 51.42 a | ± 45.41| ± 62.72 ab |             |             |             |

MDA: malondialdehyde; AOPP: advanced oxidation protein products; SOD: superoxide dismutase; GPx: glutathione peroxidase; GR: reduced glutathione; UA: uric acid; TBIL: total bilirubin; CK: creatine kinase; LDH: lactate dehydrogenase. a significant difference in comparison with values measured before the RET. b significant difference in comparison with values measured in PLA condition.

Oral temperature (OT)
There was a significant effect of MEL and exercise for OT (Table 2). The post hoc test showed a reduced OT after exercise under MEL condition (p < 0.05, d = 4.01) (Table 2).

Biomarkers of oxidative stress and muscle damage
The mean ± SD values of oxidative stress and muscle damage biomarkers investigated during the different conditions are presented in Table 3.

Concerning MDA, statistical analysis showed a significant effect of exercise and interaction condition × exercise (Table 3). The post hoc test showed a significant increase of MDA level after the RET only in the PLA condition (p < 0.05, d = 1.83).
Moreover, a significant condition effect was recorded for AOPP (Table 3). However, the post hoc test did not show any significant effect of MEL or PLA on AOPP level.

Concerning SOD, a significant effect of exercise and interaction condition × exercise was noted (Table 3). Post hoc analysis showed a significant decrease of SOD level following the RET in MEL (p < 0.05, d = 0.74) and PLA conditions (p < 0.001, d = 1.63).

For GPx, a significant effect of exercise and interaction condition × exercise were observed (Table 3). The post hoc analysis demonstrated a significant decrease in GPx activity following the RET only in PLA session (p < 0.001, d = 0.93).

Furthermore, statistical analysis did not reveal a significant effect of exercise or condition for GR values.

In addition, exercise had a significant effect on UA and TBIL (Table 3). The post hoc test showed a significant increase of UA and TBIL levels following the RET only in PLA condition (p < 0.05, d = 0.57; p < 0.05, d = 0.45 respectively).

Concerning CK, statistical analysis showed a significant effect of exercise and interaction condition × exercise (Table 3). The post hoc test showed a significant increase of CK level after the RET only in the PLA condition (p < 0.001, d = 0.76). In addition, in MEL condition, CK level after exercise was lower compared to PLA condition (p < 0.001, d = 0.53).

Moreover, the statistical analysis showed a significant effect of exercise and condition × exercise interaction for LDH (Table 3). The post hoc analysis showed a significant increase in LDH level in MEL (p < 0.05, d = 0.69) and PLA (p < 0.001, d = 2.06) conditions. In addition, after MEL ingestion, post-exercise LDH level was lower compared to the PLA condition (p < 0.05, d = 0.63).

**DISCUSSION**

The aim of this study was to verify whether acute MEL ingestion had a positive effect on aerobic performance in football players. In other words, we check the contribution of the multiple beneficial effects of MEL in the decrease of athletes’ fatigue and improvement of their performance in a running exercise until exhaustion.

The main findings of this study proved that MEL does not affect physical performance. Despite meaningful information regarding effects of MEL on health benefits [5, 8], the effect of MEL on physical performance remains uncertain and previous research obtained conflicting results. In line with the present findings, some previous studies concluded that MEL ingestion had no effect on endurance (“aerobic”) performance [23, 24]. However, a more recent study [8] demonstrated that the administration of a single 10-mg dose of MEL after strenuous late-evening exercise improved aerobic performance and decreased fatigue perception on the following day. These findings have been attributed to the improvement in sleep quality and quantity after MEL ingestion. Moreover, Beck et al. [11] found that MEL ameliorated aerobic performance during a cycling exercise until exhaustion. The difference between the present study and that of Beck et al. [11] may be due to the difference in the exercise type used, since it has been shown that physical performance during a limited time test is superior during a cycling exercise than a running one [25].

In addition, other studies conducted on rats [10, 12] found that MEL ameliorates aerobic performance during a swimming exercise until exhaustion. This ergogenic effect appeared especially at night, the preferred activity period in rats. These authors used a very high dose of MEL (10 mg/kg) at a time when the level of MEL was already high (i.e., evening). However, in the present study, we used a lower dose (6 mg) in the afternoon, which is a time when fatigue [26] and the markers of oxidative stress and muscle damage [27] are at their maximum.

This study showed a significant effect of MEL on OT and is in accordance with Marrin et al. [28], who reported, in a meta-analysis, a logarithmic dose–response relationship between MEL and its hypothermic effect. MEL doses of 2–5 mg lowered core temperature by ~0.2°C and higher doses did not substantially increase this hypothermic effect. In addition, and in accordance with our previous research [16], VT showed a slight but non-significant decrease after exercise. The lack of MEL effect on VT in this study is perhaps due to the insignificant interaction (condition × exercise) for OT. Furthermore, the RPE score did not show any difference between MEL and PLA conditions. This is in agreement with a previous study that provided evidence that MEL, ingested at the same time of day as the present study, has no inherent psychological impact on physical performance [16].

Otherwise, MEL ingestion before the RET prevented the increase of MDA responses. Likewise, previous research recommended MEL to alleviate exercise-induced oxidative stress [4, 5, 29]. This could be due to the extremely potent antioxidant effect of MEL since even its metabolites, formed when it scavenges toxic reactants, are themselves powerful detoxifiers of ROS [30]. In addition, the amphiphilic nature of this hormone allows it to cross all barriers between cells and exert its antioxidant effect in all milieus [31]. This greatly increases the effectiveness of MEL as a protector against ROS.

In addition, MEL ingestion alleviated the decrease in the activity of antioxidant enzymes. There would be less need of these enzymes given the radical scavenging and antioxidant effects of MEL. Accordingly, previous studies showed that MEL ingestion alleviated the decrease of antioxidant enzymes observed after strenuous exercise [5, 29]. This indole acts as an indirect antioxidant through the activation of the major antioxidant enzymes [4, 32]. Elevated activity of these enzymes has an important protective role in reducing lipid peroxidation and molecular damage [32]. The mechanisms involved in the regulation of the antioxidant enzymes by MEL have not yet been precisely explained. Multiple reports have proven that MEL upregulates the expression of genes coding for antioxidant enzymes and increases the synthesis of new proteins [32, 33]. MEL has also been shown to reduce the half-life of mRNAs coding for SOD and GPx, and in this case induces more abundant levels of mRNAs with short half-lives [32].
UA and TBIL rates did not increase in the MEL condition. This is consistent with the study of Ochoa et al. [29] showing a decrease in TBIL concentration after MEL ingestion and indicating a protective effect of MEL during strenuous exercise. UA and TBIL are natural antioxidants. Therefore, after MEL ingestion, there would be a lower need of them given the radical scavenger and antioxidant effects of MEL.

The results of the present study showed that CK and LDH levels increased after the RET, affirming an increase in muscle damage in the PLA condition. Similarly, previous studies [34, 35] demonstrated that the main markers of muscle damage are significantly altered after a running exercise until exhaustion. However, CK level did not increase after exercise in the MEL condition and CK and LDH levels after the RET were lower after MEL ingestion. These results revealed less muscle damage and may indicate a protective effect of MEL on skeletal muscles during an intensive running exercise. The decrease of exercise-induced cellular damage in the MEL condition is supported by the improved oxidative stress balance after RET exercise. Accordingly, our previous study showed similar trends for muscle damage and oxidative stress parameters after an intensive training camp [5]. This may help athletes to train better since it has been shown that increased levels of these enzymes were correlated with muscle cramps [36]. Accordingly, previous studies have found that MEL decreased markers of muscle injury [5, 37] and improved muscle function [38]. It also enhanced muscle healing and regeneration after skeletal muscle injury in rats [39]. MEL may be considered a possible therapy against trauma/sports-related muscle injury [40]. It inhibits NFkB, reduces cytokine expression, and increases Akt, which down regulates the ratio of MAFbx and MURF-1 in order to limit the extent of muscle injury and promote post-injury muscle recovery [40]. However, in a study performed by Beck et al. [10], the authors found that MEL decreased resting markers of muscle damage in rats but increased CK and LDH levels following a swimming exercise until exhaustion. These findings have been explained by the ergogenic effect of MEL. This hormone considerably increased the time to exhaustion and therefore led to greater muscle contraction, which could consequently induce more muscle damage.

**Limitations**

There are however some limitations of this study. We explored only the immediate response to the MEL vs. PLA intake and overlooked the delayed biochemical responses. Moreover, the findings of this study are specific to 6 mg of MEL dosed in the afternoon. Further studies are required to generalize the effects of MEL supplementation on physical performance in terms of the markers of exercise physiology we explored herein. Prospective investigations are needed to resolve the limitations of our study and to better understand the potential effect of MEL on athletic performance.

**CONCLUSIONS**

The results of our research indicate that MEL supplementation reduces lipid peroxidation, strengthens the defense mechanisms against oxidative stress and decreases muscle damage induced by a running exercise until exhaustion exercise. However, these protective effects have no significant effect on physical performance.

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**Declaration of interest**

There are no conflicts of interest of the authors with the information contained within the manuscript.

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