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Metabolomic and transcriptomic effects of melatonin and gut microbiota through microbes and exosomes on muscle regeneration and enhancement of sports performance: a systematic review

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Abstract

Introduction: Sleep and recovery are essential for optimizing exercise performance. However, the effectiveness of melatonin supplementation in improving sleep quality and next-day physical performance remains uncertain. Research has demonstrated the ergogenic effect of melatonin (N-acetyl-5-methoxytryptamine) (MEL) in increasing exhaustive aerobic activity. Associated with the effects of MEL, adult tissue stem cells (mesenchymal stem cells) mediate homeostasis and regeneration of tissues and organs, integrating signaling cues and metabolic inputs with the release of exosomes and microRNAs to enhance athletic performance. **Objective:** It was demonstrated through a systematic review study the regulation of melatonin and gut microbiota by cellular and molecular metabolic pathways, such as microRNAs and exosomes, in the process of muscle regeneration and increased sports performance. **Methods:** The PRISMA Platform systematic review rules were followed. The research was carried out from July to August 2025 in the Scopus, Embase, PubMed, Science Direct, Scielo, and Google Scholar databases. The quality of the studies was based on the GRADE instrument and the risk of bias was analyzed according to the Cochrane instrument. **Results and Conclusion:** A total of 136 articles were found, and 55 articles were evaluated in full and 29 were included and developed in the present systematic review study. Considering the Cochrane tool for risk of bias, the overall assessment resulted in 25 studies with a high risk of bias and 12 studies that did not meet GRADE and AMSTAR-2. Most studies showed homogeneity in their results, with X^2=82.9%>50%. It was concluded that administering 6 mg of melatonin at night improved performance during high-intensity exercise the following day and enhanced perceived recovery up to 72 hours after exercise. Melatonin intake during training has beneficial effects on physical performance and protects tissues against the deleterious effects of reactive oxygen species and cellular damage.

Furthermore, nocturnal melatonin supplementation during an athlete's intense training session alleviated oxidative stress, leukocytosis, and cellular damage, and improved performance recovery. Melatonin plays important roles in regulating the regenerative activities of mesenchymal stem cells, which, along with nutrients, modulate the activities of exosomes and microRNAs in the muscle regeneration process.

Keywords: Sports performance. Gut microbiota. microRNAs. Exosomes. Muscle regeneration. Melatonin.

Introduction

Sleep and recovery are essential for optimizing exercise performance. However, the effectiveness of melatonin supplementation in improving sleep quality and physical performance the following day remains uncertain. Research has demonstrated the ergogenic effect of melatonin (N-acetyl-5-methoxytryptamine) (MEL) in increasing exhaustive aerobic activity. However, the role of MEL in muscle glycogen and triglyceride content a few hours after exercise has not yet been fully clarified [1,2].

Attention is focused on better understanding the administration of MEL in PGC-1α and NRF-1, which are considered representatives of aerobic energy metabolism, as well as identifying proteins related to aerobic activity and adaptations that could improve mitochondrial capacity and possibly modulate the content of energy substrates in muscle tissue after an exercise session [3].

Regarding PGC-1α and NRF-1 proteins, the γ receptor coactivator 1α (PGC-1α) is a coactivator that interacts with nuclear respiratory factors 1 and 2 (NRF-1 and NRF2) to stimulate mitochondrial biogenesis and functions [4]. In addition, PGC-1α appears to act on energy metabolism by increasing glycogen content and fatty acid oxidation, improving tolerance to exhaustive exercise [5].

In this context, research has advanced on the physiological role of MEL and its pharmacological analogues as therapeutic agents for the treatment of various pathologies [6]. Thus, solid experimental and some clinical evidence has accumulated in the last 20 years on the important role of MEL in the regulation of metabolism [7-9].

The sleep-wake cycle is critical for the secretion and physiological variations of several hormones, including MEL [1,8]. Indoleaminergic melatonin is a hormone produced primarily by the pineal gland, but also in the gastrointestinal tract, retina, lacrimal glands, skin, erythrocytes, platelets, lymphocytes, and bone marrow mononuclear cells, derived from noradrenergic stimulation of tryptophan and serotonin by α1 and β1 adrenoreceptors in postsynaptic pinealocytes [9].

Unlike other hormonal axes, MEL secretion is not regulated by feedback and, for this reason, its plasma concentrations do not depend on its production. The secretion of the pineal gland is controlled by the circadian cycle in the suprachiasmatic nucleus of the hypothalamus and, consequently, promotes the peak of MEL secretion during the night and decreases during the day due to exposure to light [7,8].

Associated with the effects of MEL, adult tissue stem cells (mesenchymal stem cells) mediate homeostasis and tissue and organ regeneration, making decisions about whether to remain quiescent, proliferate, or differentiate into mature cell types. These decisions are directly integrated with the organism's energy balance and nutritional status. Metabolic byproducts and substrates that regulate epigenetic and signaling pathways are considered to have an instructive, rather than observer, role in regulating cell fate decisions [4].

In this sense, it is suspected that the quiescent state of stem cells is characterized by an inherently glycolytic metabolism, followed by a transition to favor mitochondrial oxidative phosphorylation during differentiation [1-3]. However, growing evidence suggests that metabolism during quiescence, activation, and differentiation can vary between tissues, integrating signaling cues and metabolic inputs with the release of exosomes and microRNAs as important metabolic messengers in the body, with this process being strongly regulated by nutrients [4,9].

Therefore, the present study aimed to demonstrate, through a systematic review, the regulation of melatonin and the gut microbiota by cellular and molecular metabolic pathways, such as microRNAs and exosomes, in the process of muscle regeneration and increased athletic performance.

Methods

Study Design

This study followed an international model for systematic review, adhering to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. Available at: http://www.prisma-statement.org/?AspxAutoDetectCookieSupport=1. Accessed on: 08/16/2025. The methodological quality standards of AMSTAR-2 (Assessing the methodological quality of systematic reviews) were also followed. Available at: https://amstar.ca/. Accessed on: 08/16/2025.

Data Sources and Research Strategy

The literature search process was conducted from July to August 2025 and employed on Web of Science, Scopus, Embase, PubMed, Lilacs, Ebsco, Scielo, and

Google Scholar, covering scientific articles from various periods to the present day. The following descriptors were used (DeCS/MeSH Terms): *"Sports performance. Gut microbiota. microRNAs. Exosomes. Muscle regeneration. Melatonin"*, and using the Boolean operator "and" between MeSH terms and "or" between historical findings.

Study Quality and Risk of Bias

Quality was classified as high, moderate, low, or very low regarding the risk of bias, clarity of comparisons, precision, and consistency of analyses. The most evident highlight was for systematic review articles or meta-analyses of randomized clinical trials, followed by randomized clinical trials. Low-quality evidence was attributed to case reports, editorials, and brief communications, according to the GRADE instrument. The risk of bias was analyzed according to the Cochrane instrument through the analysis of the Funnel Plot (Sample size versus Effect size), using Cohen's d test.

Results and Discussion

Summary of Findings

A total of 144 articles were submitted to eligibility analysis, with 30 final studies selected to compose the results of this systematic review. The listed studies presented medium to high quality (Figure 1), considering the level of scientific evidence of studies such as meta-analyses, consensus, randomized clinical trials, and prospective and observational studies. Biases did not compromise the scientific basis of the studies. According to the GRADE instrument, most studies showed homogeneity in their results, with X^2=82.9%>50%. Considering the Cochrane tool for risk of bias, the overall evaluation resulted in 25 studies with a high risk of bias and 12 studies that did not meet the GRADE and AMSTAR-2 criteria.

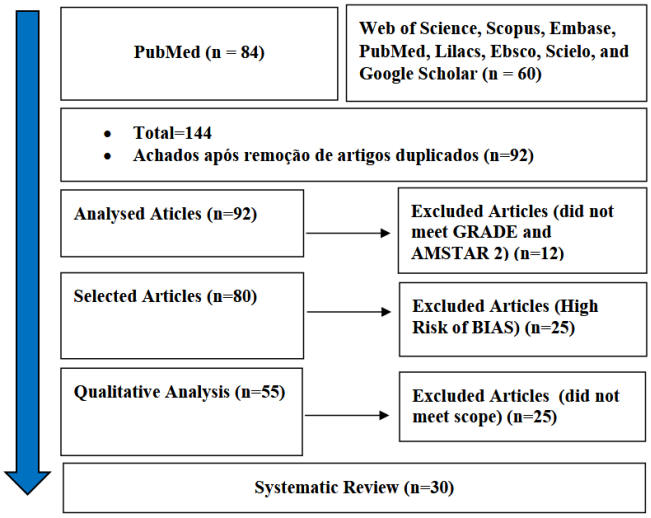

Figure 1. Flowchart showing the article selection process. Source: Own Authorship.

Figure 2 presents the results of the risk of bias of the studies using the Funnel Plot, showing the calculation of the Effect Size (Magnitude of the difference) using Cohen's Test (d). The precision (sample size) was determined indirectly by the inverse of the standard error (1/Standard Error). This graph had a symmetrical behavior, not suggesting a significant risk of bias, both between studies with small sample sizes (lower precision) that are shown at the bottom of the graph and in studies with large sample sizes that are presented at the top.

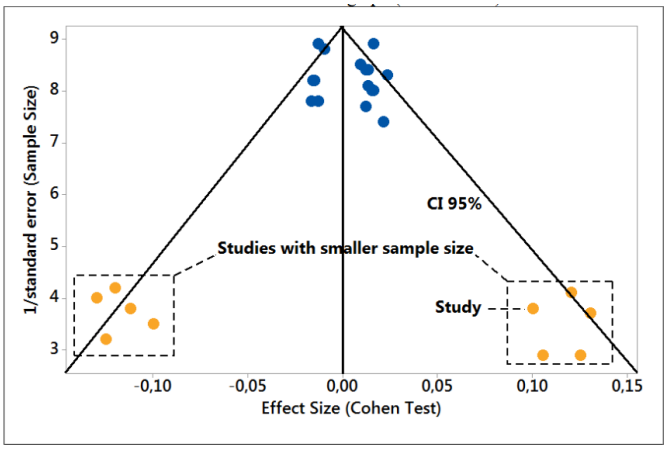

Figure 2. The symmetrical funnel plot does not suggest a risk of bias between the small sample size studies that are shown at the bottom of the graph. High confidence and high recommendation studies are shown above the graph (n=30 studies).

Melatonin and Sport Performance

A randomized controlled crossover clinical trial was conducted by the authors, Mahdi et al. (2025) [10], to examine the effects of melatonin intake on sleep and performance-related outcomes the following day in trained men. A total of 12 trained men (age: 21.92±2.84 years) ingested 6 mg of melatonin (MEL) or a placebo (PLA) the night before performing the 5 m shuttle run test (5mSRT). Compared to PLA, MEL did not modify any sleep parameters or blood markers (all with $p > 0.05$). However, MEL improved total distance, fatigue index, percentage decrease between sprints, and peak HR (all with $p < 0.05$) in the 5mSRT compared to PLA. Therefore, 6 mg of melatonin administered at night improved performance in high-intensity exercise the following day and improved perceived recovery up to 72 hours after exercise. Evidence has demonstrated the effect of MEL on tolerance to exhaustive exercise and its modulating role in muscle energy substrates at the end of exercise. PGC-1α and NRF-1 also appear to act on physical exercise tolerance and metabolic recovery after exercise.

Based on this, a study by Faria et al. 2022 [11] determined the effects of MEL administration on muscle

(PGC-1α and NRF-1), and its modulating role in glycogen and triglyceride contents subjected to exhaustive swimming exercise at an intensity corresponding to the anaerobic lactate threshold (iLAn). In a randomized controlled trial design, a total of 39 Wistar rats were allocated into four groups: control (GC = 10), rats treated with MEL (MG = 9), rats subjected to exercise (EXG = 10), and rats treated with MEL and subjected to exercise (MEXG = 10). Forty-eight hours after the graded exercise test, the animals received MEL (10 mg/kg) or vehicles 30 min before the time for the exhaustion test in the iLAn (tlim). Three hours after completion, the animals were euthanized, followed by the collection of muscles for specific analyses: soleus muscle for immunofluorescence, gluteus maximus, red and white gastrocnemius for evaluation of glycogen and triglyceride content, and liver for glycogen content measurement. MEXG swam 120.3% more than the vehicle-treated animals (EXG; $p < 0.01$). PGC-1α and NRF-1 were higher in MEXG compared to GC ($p < 0.05$); however, only PGC-1α was higher for MEXG when compared to EXG. MEL reduced triglyceride content in the gluteus maximus, red and white gastrocnemius ($F = 6.66$, $F = 4.51$ and $F = 6.02$, $p < 0.05$). Glycogen content in the red gastrocnemius was higher in MEXG than in GC ($p = 0.01$), but not in EXG ($p > 0.05$). Therefore, MEL was found to increase exercise tolerance, potentiate exercise-mediated increases in PGC-1α, decrease muscle triglyceride content, and increase muscle glycogen 3 h after exhaustive exercise, rapidly providing a better cellular metabolic environment for future efforts.

A study analyzed the effect of MEL supplementation during a period of intensive training on the decline in physical performance, oxidative stress, and cellular damage status. The investigation was conducted on 20 soccer players who participated in a six-day exhaustive training program associated with daily intake of 5 mg of oral MEL or placebo. Resting blood samples and physical performance were measured before and after the training period. A two-way mixed ANOVA (group x training field) showed that, compared to placebo, MEL intake prevented an increase in advanced oxidation protein products ($p>0.05$) and increased antioxidant enzyme activity (i.e., superoxide dismutase; $p<0.001$). Furthermore, MEL prevented an increase in biomarkers of renal function (e.g., creatinine; $p>0.05$) and biomarkers of muscle damage (e.g., creatine kinase; $p>0.05$) and liver damage (e.g., gamma-glutamyltransferase; $p>0.05$). Furthermore, MEL alleviated the deterioration of physical performance (countermovement jump, five-jump test, and 20 m sprint; $p>0.05$) [12].

The authors Baptista et al. 2022 [13] analyzed the effects of using blue lenses on MEL levels in physical and cognitive performance. A total of 15 youth volleyball athletes (15.0±1.5 years) attended the laboratory at 3 times (48h interval): on the 1st visit, they were familiarized with the study procedures, and on the 2nd and 3rd visits, they underwent the test protocol using glasses with clear lenses (control) or blue lenses in a counterbalanced crossover design. The protocol consisted of 10 min in "total darkness", 30 min of light stimulation (using blue or clear lenses), followed by an attention test and a T-test of agility (without the use of glasses). Saliva samples (to determine MEL concentration) were obtained pre- and post-exposure (30 min) to artificial light, using the lenses. Sleepiness, alertness, attention, mood, perceived recovery status, and performance variables (reaction time and T-test) were assessed after lens exposure. MEL levels did not differ within or between groups (blue lenses, pre: 0.79±0.73 and post: 1.19±1.374 pg/dl, $p=0.252$, effect size (ES)=0.38; control, pre: 0.97±1.00 and post: 0.67±0.71 pg/dl, $p=0.305$, ES=-0.35). However, MEL differences were significantly correlated with physical sedation for blue lens glasses ($r=-0.526$; $p=0.04$). No other variable differed ($p>0.05$) between protocols, including performance on the T-test ($p=0.07$; ES=0.41). Therefore, blue lenses did not influence MEL levels, cognitive/physical performance, and mood state in amateur youth volleyball players.

In this context, jet lag has potentially serious deleterious effects on the performance of athletes after transmeridian travel, where time zones are crossed east or west; as such, travel causes specific effects related to the desynchronization of the athlete's internal biological clock or circadian clock. Athletes are particularly sensitive to the effects of jet lag, as many intrinsic aspects of sports performance show a circadian rhythm, and the best competitive results require all aspects of the athlete's mind and body to work together at their maximum efficiency. International competition often requires transmeridian travel, and competition schedules cannot be adjusted to accommodate individual athletes. Therefore, it is in the interest of the individual athlete and the team to understand the effects of jet lag and the possible adaptation strategies that can be adopted [1,2].

A study developed by Forbes-Robertson et al. 2012 [14] described the underlying genetic and physiological mechanisms that control the circadian clock and its inherent ability to adapt to external conditions daily. Then, the fundamentals of various adaptive stimuli, such as light, chronobiotics (e.g., MEL), exercise, diet, and meal timing, were examined, with particular emphasis on their suitability as strategies for competitive athletes on the international circuit. These

stimuli can be artificially manipulated to produce phase shifts in the circadian rhythm to promote adaptation in the optimal direction, but care must be taken to apply them at the correct time and dose, as the effects produced on the circadian rhythm follow a phase curve response, with pronounced changes in direction at different times. Light is the strongest realignment stimulus, and careful timing of light exposure and avoidance can promote adjustment. Chronobiotics, such as MEL, can also be used to realign the circadian clock, but in addition to timing and dosage issues, there are also concerns about their legal status in different countries and with the World Anti-Doping Agency.

In addition, some beneficial effects of exercise are attributed to the endocrine state. A study conducted by the authors Kocahan et al. 2021 [15] evaluated the effect of eight weeks of basketball training on MEL, serotonin, and hematological parameters in basketball players. The experimental group was selected from 34 healthy boys, aged between 13 and 16 years. Participants were randomly assigned to the control group (n=17) and the exercise group (n=17). The exercise program consisted of 2 h/day of aerobic basketball training activity on 5 days per week for 8 weeks. Serotonin and MEL levels increased significantly in the post-exercise group compared to the other groups ($p<0.05$). Exercise caused an increase in WBC, RBC, HCT, and Hb levels ($p<0.05$), but did not alter PLT, MCH, and PCT levels ($p>0.05$). Regular aerobic exercise for eight weeks increased MEL and serotonin levels, and also altered some hematological parameters. Therefore, the improvement in serotonin levels, MEL, and hematological parameters after eight weeks of regular training of basketball players can be attributed to the beneficial effects of exercise.

The authors Farjallah et al. 2020 [16] evaluated the effect of oral supplementation of 5 mg of MEL on repeated sprint recovery (RSA) performance and biochemical responses (i.e., oxidative stress, cell damage by leukocytosis) after an intensive training camp (TC). A total of 20 soccer players performed an RSA test before and after a six-day intensive TC associated with nightly intake of MEL (n=10) or placebo (n=10). Resting and post-RSA test blood samples were obtained before and after the TC. Compared to placebo, MEL intake decreased resting oxidative stress markers (i.e., advanced oxidation protein products), leukocytosis (i.e., white blood cells (WBC), neutrophils (NE)), and biomarkers of cellular damage (i.e., creatine kinase (CK)). It also reduced post-exercise leukocytosis (i.e., WBC, NE, lymphocytes (LY), monocytes (MO)) and biomarkers of cellular damage (i.e., CK, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT)) and increased the activity of key antioxidant enzymes (i.e., glutathione peroxidase (GPx), glutathione reductase (GR)). Compared to placebo, MEL reduced the deterioration of peak and total time during the RSA test after the TC. Therefore, nighttime MEL supplementation during an intensive CT relieved oxidative stress, leukocytosis, and cell damage and improved RSA performance recovery in soccer players.

In 2018, authors Farjallah et al. [17] assessed whether MEL intake could improve recovery in athletes after an intermittent training session (ITS). A total of 15 elite athletes (17.4 ± 0.4 years, 76.4 ± 5.6 kg, 1.76 ± 0.04 m; mean ± standard deviation) participated in two test campaigns. During each period, they performed a battery of physical and cognitive tests before and after an ITS, as well as after ingesting a MEL (6 mg tablet) or placebo in a randomized design. The ITS comprised the modified T-test of agility, squat jump, countermovement jump, maximum speed ball throw test, and 20 m sprint. Oral temperature (OT) and alertness were assessed before and after the ITS. Rating of perceived exertion (RPE), blood lactate (La), and glucose (Gl) were recorded after each ITS. Short-term performance, physical performance recovery, and OT were not affected by MEL ingestion after the ITS. Furthermore, MEL did not affect cognitive performance or RPE scores after the ITS. However, La and Gl ($p < 0.05$ for both) decreased after MEL ingestion.

Melatonin - Metabolomic and Regenerative Process

In the context of endocrine physiology, due to its amphiphilic molecule characteristics, MEL is able to cross cells, organelles, and nuclear membranes and directly interact with intracellular molecules in what are called non-receptor-mediated actions [18,19]. MEL is a well-known and effective antioxidant, as it is both a proficient scavenger of direct free radicals and an activator of a series of elimination mechanisms, such as stimulating the transcription and activity of antioxidant enzymes and binding to transition metals that inhibit the formation of hydroxyls. In addition, MEL protects lipids, proteins, and DNA against oxidative damage, being highly concentrated in mitochondria [1,9].

The antioxidant properties of MEL are of crucial importance for mitochondrial functions, playing critical roles in mitochondrial function beyond antioxidant protection, such as regulating the activities of respiratory complexes I and IV and protecting mitochondrial DNA against chromosomal/chromatid alterations and mutations [6-8].

Thus, some of the effects mentioned above are generally a consequence of the direct MEL-protein interaction. It is also noteworthy that MEL plays a role in regulating the ubiquitin-proteasome system that

ultimately controls protein degradation [1,4,8].

In this sense, MEL is considered a potent cytoprotective agent, not just a hormone [20,21]. MEL can synchronize the circadian clock in peripheral tissues, maintain the synchronization of bone metabolism with the Light/Dark cycles, and participate in numerous important physiological processes, such as anti-inflammatory, antitumor, and antioxidant processes, in addition to regulating circadian and endocrine rhythms, regulating immunity, and promoting wound healing and tissue regeneration [22-24]. Along with this, MEL's properties of regulating tissue regeneration are related to the functions of adult stem cells, such as mesenchymal stem cells (MSCs), pointing to them as an alternative for cell therapy and human tissue engineering, since they have been found to have a high degree of plasticity, with the capacity for self-renewal and differentiation into specialized progenitors [25].

In this respect, MSCs are primordial mesodermal cells present in all tissues and are capable of differentiating in vitro and in vivo into different cell types. Their therapeutic potential is mainly explained by the production of bioactive molecules, which provide a regenerative microenvironment in injured tissues [25]. MSCs secrete a cascade of cytokines and growth factors with paracrine, autocrine, and endocrine activities, such as IL-6, IL-7, IL-8, IL-11, IL-12, IL-14, IL-15, macrophage colony-stimulating factor (M-CSF), Flt-3 ligand and Stem Cell Factor (SCF), leukemia inhibitory factor (LIF), granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF). When conjugated, these factors can produce a series of responses from the local immune system, stimulating angiogenesis and inducing the proliferation and differentiation of mesenchymal stem cells in the desired tissue [26].

Also, MSCs induce the expression of junction proteins and increase microvascular integrity and nitric oxide (NO) production by macrophages. The vascular stromal fraction (VSF) derived from MSCs is a heterogeneous mixture of cells, including fibroblasts, pericytes, endothelial cells, blood cells, and adipose tissue-derived mesenchymal stem cells (AMSCs) [27].

Besides, exosomes stand out along with AMSCs. Exosomes are extracellular vesicles with a diameter of 40-100 nm and a density of 1.13-1.19 g/mL, containing proteins, mRNAs, miRNAs, and DNAs. Exosomes change the biochemical characteristics of recipient cells by delivering biomolecules and play a role in cell communication. These vesicles are produced from body fluids and different cell types. Evidence suggests that Adipose-Derived Stem Cell (AMSC)-derived exosomes (AMSC-EXO) exhibit functions similar to AMSCs with low immunogenicity and without tumorization [27].

The composition of exosomes differs based on their sources. The protein and lipid content of exosomes was measured by several methods, such as fluorescence-activated cell sorting, Western blotting, mass spectrometry, and immunoelectron microscopy. In this aspect, Rabs and Annexin, including Annexin I, II, V, and VI, are cytosolic proteins present in exosomes that contribute to exosome docking formation, membrane fusion, and kinetic regulation of cytoskeletal membranes. In addition, adhesion molecules such as intercellular adhesion molecule-1, CD11a, CD11b, CD11c, CD18, CD9, adipose tissue globule, EGF-factor VIII (MFG-E8), CD58, CD146, and CD166 have also been identified in exosomes [28]. Exosomes also contain heat shock proteins (Hsp70 and Hsp90), which facilitate the loading of peptides into MHC I and II [29].

In addition, exosomes contain non-coding RNAs or fragments, including overlapping RNA transcripts, protein-coding regions, structural RNAs, transfer RNA fragments, YRNAs, short hairpin RNAs, small interfering RNAs (siRNAs), microRNA (miRNA), messenger RNA (mRNA), and DNA [29]. Regarding miRNA, exosomes have miR-1, miR-15, miR-16, miR-17, miR-18, miR-181, and miR-375. Thus, several cytokines, such as Tumor Necrosis Factor-α (TNF-α), Granulocyte Macrophage Colony-Stimulating Factor (GMCSF), Interleukin (IL)-2, IL-6, IL-8, IL-10, IL-15, and IL-1β, are expressed in exosomes [28-30].

Conclusion

It was concluded that 6 mg of melatonin administered at night improved performance in high-intensity exercise the following day and improved perceived recovery up to 72 hours after exercise. Melatonin intake during the training period exerts beneficial effects on physical performance and protects tissues against the deleterious effects of reactive oxygen species and cell damage. Furthermore, nighttime melatonin supplementation during an athlete's intense training relieved oxidative stress, leukocytosis, and cell damage, and improved performance recovery. Melatonin plays important roles in regulating the regenerative activities of mesenchymal stem cells, which, along with nutrients, modulate the activities of exosomes and microRNAs in the muscle regeneration process.

CRediT

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