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Regulation of melatonin and nutrients in the process of bone and cartilage regeneration with exosomes and microRNAs: a systematic review

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Abstract

Introduction: Bone diseases comprise a large group of common diseases, including fractures, osteoporosis, and osteoarthritis that affect a large number of individuals. Without intervention, the prevalence of osteopenia is projected to increase to 64.3 million Americans and that of osteoporosis to 11.9 million by the year 2030. Melatonin exerts numerous physiological effects, including the induction of anti-inflammatory and antioxidants, resetting circadian rhythms, and promoting wound healing and tissue regeneration, participating in the maintenance and regenerative processes of bones and cartilage. **Objective:** A systematic review was carried out to present the state of the art of melatonin regulation, mesenchymal stem cells, exosomes, microRNAs, and nutrients in the bone regeneration process. **Methods:** The systematic review rules (PRISMA) were followed. The search was carried out from January to March 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 138 articles were found. A total of 64 articles were fully evaluated and 32 were included in this systematic review. Considering the Cochrane tool for risk of bias, the overall assessment resulted in 9 studies at high risk of bias and 24 studies that did not meet the GRADE. Most studies showed homogeneity in their results, with I^2 =92.8%>50%. The symmetrical funnel plot does not suggest a risk of bias between small sample-size studies. Based on the results, melatonin has important functions in regulating the regenerative activities of mesenchymal stem cells that modulate, together with nutrients, the activities of exosomes and microRNAs in the bone and cartilage regeneration process.

Keywords: Bone diseases. Bone regeneration. Cartilage regeneration. Melatonin. Nutrients. Exosomes. MicroRNAs.

Introduction

Bone diseases comprise a large group of common conditions, including fractures, osteoporosis, and osteoarthritis, that affect a large number of individuals, particularly the elderly. Without intervention, the prevalence of osteopenia is projected to increase to 64.3 million Americans and that of osteoporosis to 11.9 million by the year 2030 **[1]**.

With existing prevention and treatment methods, the incidence and mortality of bone diseases are still gradually increasing, creating a significant financial burden on societies worldwide. To prevent the occurrence of bone diseases, slow their progression, or reverse the damage they cause, new alternatives or complementary treatments need to be developed. Thus, melatonin exerts numerous physiological effects, including inducing anti-inflammatory and antioxidant functions, resetting circadian rhythms, and promoting wound healing and tissue regeneration. Melatonin also

participates in the maintenance and regenerative processes of bone and cartilage [2].

In this context, research has advanced on the physiological role of melatonin (MEL) and its pharmacological analogues as therapeutic agents for the treatment of various pathologies. Thus, over the last 20 years, solid experimental and some clinical evidence has accumulated on the important role of MEL in regulating metabolism [3,4]. The sleep-wake cycle is critical for the secretion and physiological variations of several hormones, including MEL [5]. Melatonin (N-acetyl-5-methoxytryptamide) is an indoleaminergic 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 the noradrenergic stimulation of tryptophan and serotonin by α1 and β1 adrenoreceptors in postsynaptic pinealocytes [6].

Unlike other hormonal axes, MEL secretion is not regulated by feedback, and therefore, its plasma concentrations do not depend on its production. Pineal gland secretion is controlled by the circadian rhythm in the suprachiasmatic nucleus of the hypothalamus, consequently promoting peak MEL secretion at night and decreasing during the day with light exposure [7].

Furthermore, MEL has endocrine and paracrine actions and binds to three receptors, central and peripheral, in various locations throughout the body [8]. The highaffinity receptors MT1 and MT2, or MTNR1A and MTNR1B, belong to the family of membrane-bound receptors with G-protein activation by PKC and reduced cyclic GMP monophosphate (cGMP), respectively. MT3, a recently discovered nuclear receptor of the retinoic acid family (RZR/ROR), has a quinone reductase-like structure with a function that is not yet fully understood [9].

In this sense, MEL secretion decreases with aging and the presence of various diseases [9]. Sleep patterns change, and this has a significant impact with advancing age and the development of certain diseases such as osteoporosis and osteoarthritis [10]. Associated with the effects of MEL, adult tissue stem cells (mesenchymal stem cells) mediate homeostasis and regeneration of tissues and organs, making decisions about whether to remain quiescent, proliferate, or differentiate into mature cell types. These decisions are directly integrated with the body'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 [11].

In this sense, it is suspected that the quiescent state of stem cells is characterized by an inherently glycolytic metabolism, which then transitions to favor mitochondrial oxidative phosphorylation during differentiation [12-15]. However, growing evidence suggests that metabolism during quiescence, activation, and differentiation may vary between tissues, integrating signaling cues and metabolic inputs with the release of exosomes and microRNAs as important metabolic messengers in the organism, with this process being strongly regulated by nutrients.

Nutrient-mediated metabolomics provides insight into cellular pathways, observing metabolic substrates and products through different pathways [16,17]. Combined with transcriptomic and proteomic analysis, it has been observed that metabolism can affect cell fate (and vice versa) [18].

Therefore, the present study aimed to conduct a systematic review to present the state-of-the-art in the regulation of melatonin, mesenchymal stem cells, exosomes, microRNAs, and nutrients in the bone and cartilage regeneration process.

Methods

Study Design

This study followed the international systematic review model, following the PRISMA (preferred reporting items for systematic reviews and meta-analysis) guidelines. Available at: http://www.prisma-statement.org/?AspxAutoDetectCookieSupport=1. Accessed on: January 16, 2025. The AMSTAR-2 (Assessing the methodological quality of systematic reviews) methodological quality standards were also followed. Available at: https://amstar.ca/. Accessed on: January 16, 2025.

Data Sources and Search Strategy

The bibliographic search process was conducted from January to March 2025 and developed based on the Scopus, Embase, PubMed, Lilacs, Ebsco, Scielo, and Google Scholar databases, covering scientific articles from various periods up to the present day. The following descriptors (DeCS/MeSH Terms) were used: "Bone diseases. Bone regeneration. Cartilage regeneration. Melatonin. Nutrients. Exosomes. MicroRNAs", using the Boolean "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 based on the risk of bias, clarity of comparisons, precision, and consistency of analyses. The most evident emphasis was on systematic review articles or meta-analyses of randomized controlled trials, followed by randomized clinical trials. Low-

quality evidence was assigned to case reports, editorials, and brief communications, according to the GRADE instrument. Risk of bias was analyzed according to the Cochrane instrument by analyzing the funnel plot (sample size versus effect size) using Cohen's d test.

Results and Discussion

Summary of Findings

As a corollary to the literature search system, 126 studies were analyzed and submitted to eligibility analysis, and 46 of the final 59 studies were subsequently selected for this systematic review. The selected studies were of medium to high quality (Figure 1), considering, first, the level of scientific evidence of studies of meta-analysis, consensus, randomized clinical, prospective, and observational types. Biases did not compromise the scientific basis of the studies. According to the GRADE instrument, most studies presented homogeneous results, with I^2 = 92.8% >50%.

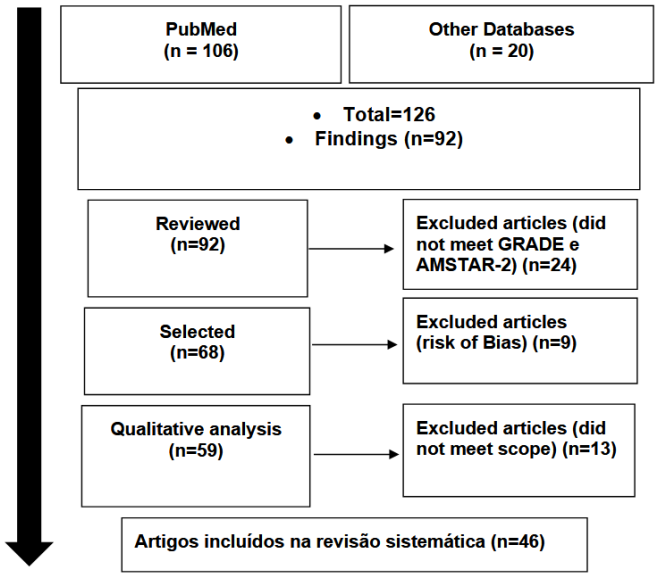

Figure 1. Flowchart showing the article selection process. Source: Own Authorship.

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 d Test. Precision (sample size) was determined indirectly by the inverse of the standard error (1/Standard Error). This graph showed symmetrical behavior, suggesting no significant risk of bias, either among studies with small sample sizes (lower precision), which are shown at the bottom of the graph, or among studies with large sample sizes, which are presented in the upper region.

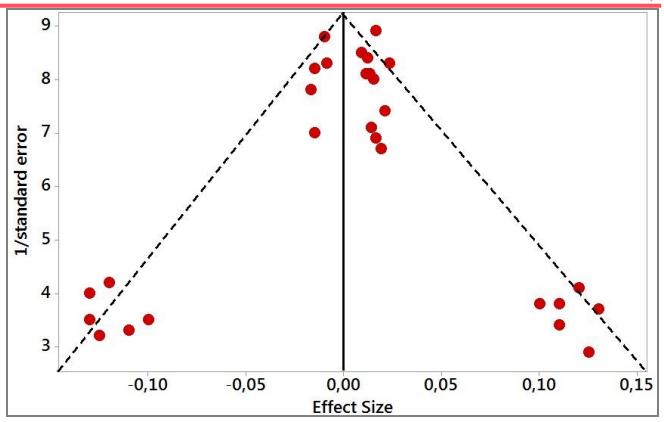

Figure 2. The symmetrical funnel plot suggests no risk of bias among the small-samplesize studies shown at the bottom of the graph. High-confidence and high-recommendation studies are shown above the graph (n=46 clinical studies). Source: Own Authorship.

Melatonin - Metabolomics and Bone/Chondrogenic Regeneration

In endocrine physiology, due to its amphiphilic nature, MEL is capable of crossing cells, organelles, and nuclear membranes and directly interacting with intracellular molecules in so-called non-receptor-mediated actions **[3]**. MEL is a well-known, effective antioxidant, as it is both a proficient scavenger of direct free radicals and an activator of a series of scavenging mechanisms, such as stimulating transcription and antioxidant enzyme activity and binding to transition metals, inhibiting hydroxyl formation. Furthermore, MEL protects lipids, proteins, and DNA against oxidative damage, being highly concentrated in mitochondria **[4]**.

In this context, the antioxidant properties of MEL are crucial for mitochondrial function, 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 **[5]**. Thus, some of the aforementioned effects are generally a consequence of direct MEL-protein interaction. It is also notable that MEL plays a role in regulating the ubiquitin-proteasome system, which ultimately controls protein degradation **[6]**.

MEL has been reported to inhibit Ca2+/calmodulin-dependent protein kinase II activity and autophosphorylation by a direct interaction with Ca2+-activated calmodulin, acting as an antagonist. It has also been suggested that MEL influences the expression of circadian rhythm genes **[19]**. The MEL receptors MT1 and MT2, formerly termed MEL1a and MEL1b, are specific high-affinity G-protein-coupled receptors encoded by the MTNR1A and MTNR1B

genes, which have been found in several areas of the CNS, including the CNS, mediobasal hypothalamus, thalamus, temporal, parietal and frontal cortex, hippocampus, preoptic basal ganglia, area postrema, retina, cerebellum, and pars tuberalis region, as well as in adipose tissue, kidney, pancreas, islets, parotid glands, adrenal glands, liver, bone, skin, reproductive tract, immune cells, and cardiovascular system **[20]**.

In this sense, the MEL receptors MT1 and MT2 are heterotrimetric G-proteincoupled receptors (Gi/Go and Gq) that interact with messengers such as adenylyl cyclase, phospholipase A, phospholipase C, and calcium and potassium channels, generally decreasing cAMP and cGMP production and/or activating phospholipase C. Thus, MT1 and MT2 generally dimerize, forming homodimers or heterodimers that maintain both functional MEL binding sites and the corresponding selectivity **[20]**. Furthermore, GPR61/62 and GPR135 are other G-protein-coupled receptors that can dimerize to MT, reducing their affinity for MEL and its agonists, representing a potential regulatory step in the signaling mechanism. MT signaling pathways involve, for example, activation of potassium ion channels (K), which mediate the inhibition of neuronal firing in the CNS. Modulation of protein kinase C (PKC) and phospholipase A1 **[21]**.

MT3 is a third binding site for mammalian MEL, which is a form of quinone reductase, a detoxifying enzyme, and has been reported to be involved in enhancing chemotherapy-induced cytotoxicity and MEL-derived apoptosis in tumor cell lines. Furthermore, MEL can also interact with nuclear receptors of the retinoic acid-related receptor (ROR) group Z retinoid receptor **[22]**.

Despite all these findings on the physiological functions of MEL, the metabolic pathways involved in human sleep have yet to be investigated using a metabolomics approach. Therefore, a study performed targeted liquid chromatography (LC)/MS metabolomics to examine the effect of acute sleep deprivation on plasma metabolite rhythms. Twelve healthy young male subjects remained under controlled laboratory conditions regarding ambient light, sleep, meals, and posture during a 24-hour sleep/wake cycle, followed by 24 hours of wakefulness. Two-hour plasma samples collected during the 48-hour period were analyzed by LC/MS. Principal component analysis revealed a clear time-of-day variation with a significant cosine adjustment during the sleep/wake cycle and during 24 hours of wakefulness in both untargeted and targeted analyses. Of the 171 metabolites quantified, daily rhythms were observed in the majority (n = 109), with 78 of them maintaining their rhythmicity during 24 hours of wakefulness, most with reduced amplitude (n=66). During sleep deprivation, 27 metabolites (tryptophan, serotonin, taurine, 8 acylcarnitines, 13 glycerophospholipids, and 3 sphingolipids) exhibited significantly increased levels compared to sleep. The increased levels of serotonin, tryptophan, and taurine may explain the antidepressant effect of acute sleep deprivation **[19]**.

In this context, MEL is considered a potent cytoprotective agent, not just a hormone **[21,22]**. MEL can synchronize the circadian clock in peripheral tissues, maintain the synchronization of bone metabolism with light/dark cycles, and participate in numerous important physiological processes, such as anti-inflammatory, antitumor, and antioxidant effects. MEL also regulates circadian and endocrine rhythms, regulates immunity, and promotes wound healing and tissue regeneration **[23,24]**. MEL also plays an important role in bone-related diseases. Although there are several physical and pharmacological treatments for bone diseases, MEL has the advantage over other medications of low cost, a wide safety margin, broad tissue impact, and virtually no side effects, suggesting its potential as a primary or complementary treatment strategy for a wide variety of bone diseases **[24]**.

MEL is involved in regulating bone mass accrual and loss. Egermann et al. **[25]** confirmed that bone mass decreases significantly after pinealectomy. Decreased MEL secretion is associated with menopause and is one of the most important causes of osteoporosis **[26]**. MEL production declines with age, which may lead to greater bone loss in the elderly **[27]**. Furthermore, the expression of melatonin receptor 1A (MTNR1A) on the surface of human osteoblasts decreases with age, more frequently in women **[28]**. Exogenous melatonin supplementation is effective and safe, resulting in more osteoblasts and fewer osteoclasts. Melatonin application can reduce elevated levels of the NLRP3 inflammasome in individuals suffering from estrogen deficiency. Melatonin also attenuates osteoblast autophagy in patients with diabetes mellitus, which is considered beneficial in reducing bone loss. Furthermore, melatonin regulates calcium metabolism and prevents osteoporosis **[28]**.

Inflammatory processes play a crucial role in the pathogenesis of osteoarthritis (OA), as mild and chronic inflammation has been shown to contribute to OA symptoms and progression **[29,30]**. Cartilage's self-repair capacity is limited, with the cell-based repair capacity of articular cartilage in inflamed joints being even lower. Thus, melatonin intervention can partially restore the chondrogenic differentiation capacity of mesenchymal stem cells affected by IL-1β-induced inflammation **[31,32]**. The effect of long-term

intervention (21 days) is significant. Melatonin can also reduce the phosphorylation of p65 and IκBα, thus inhibiting the activation of the downstream NF-κB signaling pathway, which plays a key role in metabolism, inflammation, and apoptosis **[33]**.

In addition, multiple microRNAs (miRNAs/miRs) are involved in OA **[34]**. For example, miR-140-5p has been shown to be expressed in cartilage and plays an important role in chondrocyte differentiation and cartilage degeneration **[35]**. Cartilage changes associated with OA occur in mice lacking miR-140 **[36]**, while overexpression of miR140 has been shown to inhibit matrix catabolic enzyme synthesis **[37]**. Elevated levels of proinflammatory cytokines in cartilage can reduce miR-140 expression **[38]**.

MEL plays a protective role in OA-induced cartilage degradation by upregulating miR-140 and activating SMAD signaling pathways **[32]**, which can inhibit NF-κB pathways in articular cartilage **[39]**. Furthermore, other miRNAs involved in cartilage protection, such as miR-526b-3p and miR-590-5p, can be upregulated by melatonin, improving the chondrogenic differentiation of mesenchymal stem cells **[40]**.

Main Cellular and Molecular Processes of Bone Regeneration

In this scenario, adult stem cells, such as mesenchymal stem cells (MSCs), are emerging as an alternative for cell therapy and human tissue engineering, as they have been shown to exhibit a high degree of plasticity, with the capacity for self-renewal and differentiation into specialized progenitors **[41]**. In this regard, 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 primarily explained by the production of bioactive molecules, which provide a regenerative microenvironment in injured tissues **[42]**.

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 granulocytemacrophage colony-stimulating factor (GM-CSF). These factors, when combined, can produce a series of local immune system responses, stimulating angiogenesis and inducing the proliferation and differentiation of mesenchymal stem cells in the desired tissue **[43]**.

In addition, MSCs induce the expression of junction proteins and increase microvascular integrity and nitric oxide (NO) production by macrophages **[42]**. The stromal vascular fraction (SVF) from MSCs is a heterogeneous mixture of cells, including fibroblasts, pericytes, endothelial cells, blood cells, and adipose-derived mesenchymal stem cells (ADSCs).

Exosomes stand out along with ADSCs. Exosomes are extracellular vesicles measuring 40–100 nm in diameter and with a density of 1.13–1.19 g/mL, containing proteins, mRNAs, miRNAs, and DNAs. Exosomes alter the biochemical characteristics of recipient cells through the delivery of biomolecules and play a role in cellular communication. These vesicles are produced from bodily fluids and different cell types. Evidence suggests that ADSCs-derived exosomes (ADSCs-EXO) exhibit similar functions to CTMA with low immunogenicity and no tumorigenesis **[44]**.

The composition of exosomes differs based on their source. The protein and lipid content of exosomes has been measured by various methods, such as fluorescence-activated cell sorting, Western blotting, mass spectrometry, and immunoelectron microscopy. In this regard, Rabs and Annexin, including Annexin I, II, V, and VI, are cytosolic proteins present in exosomes that contribute to the formation of exosome docking, membrane fusion, and kinetic regulation of cytoskeletal membranes. Furthermore, 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 **[45]**.

Exosomes also contain heat shock proteins (Hsp70 and Hsp90), which facilitate the loading of peptides onto MHC I and II **[46,47]**. Furthermore, 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 **[48]**. Regarding miRNA, exosomes present miR-1, miR-15, miR-16, miR-17, miR-18, miR-181, and miR-375 **[49]**. Furthermore, 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, IL-1β, are expressed in exosomes **[50]**.

Based on this, normal bone formation and tissue repair involve a coordinated interaction between bone-forming cells and biological signals. The main driving force in this process is osteoblasts and their precursors **[51]**. Osteoblasts can produce new bone along with biomaterials and can initiate the release of biological signals that guide bone formation and remodeling. These biological signals attract bone-forming cells to the recipient site. Growth factors and other proteins are some biological signals that may be involved in

new bone formation and tissue remodeling. Furthermore, through chemotaxis, bone-forming cells migrate to the application site, as cell migration is stimulated in response to chemical stimuli **[52]**.

Monocytes, macrophages, and endothelial cells contribute to bone remodeling, either through contact with osteogenic cells or through the release of soluble factors such as cytokines and GF **[52]**. In the skeletal system, TNF-α stimulates bone and cartilage resorption and inhibits collagen and proteoglycan synthesis. IL-1 induces the expression of a wide variety of cytokines. LIF and IL-6 are two such molecules known to stimulate the differentiation of mesenchymal progenitor cells into the osteoblast lineage and are also potent antiapoptotic agents for osteoblasts. In bone, the main sources of IL-6 are osteoblasts, not osteoclasts. Prostaglandin E2 (PGE2) is also directly related to the expression of the cytokine IL-6 **[53,54]**.

A study by Liang et al. (2022) **[55]** showed that mesenchymal stem cell-derived exosomes (MSC-Exosomes) perform stem cell regulatory functions by transporting proteins, nucleic acids, and lipids. Intervertebral disc degeneration (IDD) is a leading cause of low back pain and is characterized by a decrease in the number of nucleus pulposus cells, decomposition of the extracellular matrix, aging of the annulus fibrosus, and calcification of the cartilage endplate. Furthermore, nutrient transport and structural repair of intervertebral discs depend on bone and cartilage and are closely related to bone health. Trauma, disease, and aging can all cause bone damage. Recent fine-tuning of MSC-Exosomes has led to significant progress in the treatment of IDD and in bone repair and regeneration.

Regenerative Processes and Nutrology

In the context of regenerative processes, endogenous metabolites and dietary nutrients can directly influence epigenetic enzymes. Epigenetic modifications to DNA and histone proteins alter cell fate by controlling chromatin accessibility and downstream gene expression patterns **[56]**. Thus, many substrates and cofactors for chromatinmodifying enzymes are derived from metabolic pathways involving the tricarboxylic acid cycle, the methionine cycle, the folate cycle, glycolysis, β-oxidation, and the hexosamine pathway. These metabolites can serve as activators or inhibitors of epigenetic writers, such as Jumonji C (JmjC) domain-containing proteins, DNA methyltransferases (DNMTs), histone acetyltransferases (HATs), ten-eleven translocase DNA demethylases (TETs), and histone deacetylases (HDACs). In this sense, metabolites can influence nutrient-sensing signaling pathways **[56]**.

Thus, the mechanistic target of rapamycin complex 1 (mTORC1) can be activated by growth factor-induced signaling only when the amino acids arginine and leucine, as well as the cofactor S-adenosyl methionine (SAM), are detected within the cell. Furthermore, the energy balance communicated through the cellular AMP/ADP-ATP ratio can be sensed by AMP-activated protein kinase (AMPK). Furthermore, transcription factors can be directly regulated by metabolites. For example, the tryptophan metabolite kynurenine is an endogenous agonist of the aryl hydrocarbon receptor, and alpha-ketoglutarate (α-KJ) binds to and activates IKKβ and initiates NF-κβ signaling **[56]**.

Epigenetic signaling pathways and transcription are affected by changing nutrient levels. Furthermore, a focus of the literature on stem cell metabolism centers on central carbon metabolism and the balance between glycolysis and oxidative phosphorylation in regulating cell fate **[57]**. Therefore, future research defining the dietary and metabolic control of cell fate decisions in muscle tissues will be of great importance in the fields of metabolism and regenerative medicine.

Over the past decade, several flavonoids have been reported to have osteogenic-angiogenic potential in bone regeneration due to their excellent bioactivity, low cost, availability, and minimal in vivo toxicity. During new bone formation, the osteoinductive nature of certain flavonoids is involved in the regulation of multiple signaling pathways that contribute to osteogenic-angiogenic coupling **[58]**.

A meta-analysis identified micronutrients from the "European Union (EU) Register of Nutrition and Health Claims Made on Foods" that are related to bone health. Nineteen studies were identified that demonstrated the importance of vitamin D, magnesium, resveratrol, vitamin C, a mixture of calcium, magnesium, zinc, and vitamin D, and synthetic bone mineral in bone formation and maintenance **[59]**.

Conclusion

It was concluded that melatonin plays important roles in regulating the regenerative activities of mesenchymal stem cells, modulating, along with nutrients, the activities of exosomes and microRNAs in the process of bone and cartilage regeneration.

CRediT

Author contributions: **Conceptualization**; **Data curation**; **Formal Analysis**; **Investigation**; **Methodology**; **Project administration**; **Supervision**; **Writing - original draft**; **Writing-review & editing-** Yuri de Freitas Tobias, João Paulo Rodrigues Pacheco and João Paulo Ramos de Morais.

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Not applicable.

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