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Melatonin role in skeletal muscle disorders

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Abstract. – OBJECTIVE: This review discusses the impact of the neuro-hormone melatonin on skeletal muscle disorders based on recent literature data with the aim to clarify the utility of the melatonin therapy in patients affected by muscle diseases.

MATERIALS AND METHODS: It has been pointed out the possible role of melatonin as a food supplement to cure muscular disorders characterized by muscle wasting. Oxidative damage has been proposed as one of the major contributors of the skeletal muscle decline occurring both in physiological and pathological conditions. It is known that excessive oxidant levels lead to mitochondrial damage, and in turn, contribute to apoptotic signaling activation and autophagic impairment. This condition is common in a variety of skeletal muscle disorders.

RESULTS: The scientific evidence enhances the antioxidant effect of melatonin, that has been demonstrated by several studies both in vitro and in vivo. This effect counteracts mitochondrial impairments and reduces oxidative stress and autophagic alterations in muscle fibers. Its beneficial role in restoring muscle decline, takes place mainly in atrophic conditions correlated to muscle aging.

CONCLUSIONS: The findings of the research suggest that melatonin may be considered as a valid dietary supplement, useful to prevent muscle wasting, in particular, in sarcopenia-associated diseases.

Key Words:
Melatonin, Skeletal muscle, Muscle disorders.

Introduction

An analysis of the morpho-functional aspect of the skeletal muscle and of the melatonin molecule has been carried out, based on recent evidence derived from the literature. The objective was to better understand the possible role of the melatonin as preventive and curative agent of some skeletal muscle disorders. Muscle dystrophy and atrophy conditions were therefore correlated with published data on the use of melatonin use in these pathologies.

Material and Methods

Skeletal Muscle

Skeletal muscular tissue, essential for voluntary movements and postural maintenance, plays a crucial role in controlling thermal regulation, nutritional balance, glucose uptake, and endocrine activity too[1-3]. Therefore, loss of skeletal muscle mass has been associated with impaired whole body glucose homeostasis, falls, fractures, disability and chronic diseases[4-6].

Skeletal muscle tissue (Figure 1) is characterized by a well-organized arrangement of multinucleated and post-mitotic muscle fibers and associated connective tissue. In addition, satellite cells can be found in skeletal muscle, located between the sarcolemma and the basal lamina, with the aim to contribute to muscle growth, repair, and regeneration[7].

Skeletal muscle is a tissue formed by multiple types of fibers. Briefly, type I fibers are slow contracting and use an oxidative metabolism. Differently, type II fibers are fast contracting and mainly glycolytic. Muscle mass reduction, typical of sarcopenia and aging, is primarily due to a loss of muscle fibers particularly characterized by a preferential atrophy of type II fibers[8]. At the same time, a conversion of fast type II muscle fibers into slow type I fibers, with loss in muscle power and decline in protein synthesis (in particular for myosin heavy chains) has been described[9,10]. Overall, these changes lead to a smaller, slower contracting muscle with resulting reduced capacity to adequately perform activities of daily living. These anatomical modifications have been, at

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least partly, attributed to the age-related increase of oxidative stress damage. In fact, the skeletal muscle is the largest consumer of oxygen in the body with muscle fibers continuously generating ROS (especially during the contractile activity). Studies adopting muscle biopsies have confirmed that markers of oxidative stress are particularly and locally elevated in skeletal muscle of older adults and in patients affected by muscular dystrophies[11-16]. On the other hand, the inadequacy of the antioxidant system, in particular catalase, glutathione transferase, and superoxide dismutase which appears downregulated in muscle atrophy, is not able to prevent damages[17,18].

This pro-oxidant status results in the alteration of mitochondrial DNA and abnormalities in the electron transport system, leading to reduced calcium uptake by the sarcoplasmic reticulum, irreversible damage of the cell, and its consequent death [19-21]. In the healthy muscle, proteins and aminoacids are ideally balanced between synthesis and breakdown. During immobilization and denervation, this equilibrium is disrupted with an increased breakdown rate of myofibrillar and mitochondrial proteins. As a consequence, mitochondria undergo a series of detrimental changes characterized by downregulation of PGC-1α and antioxidant defense, increased ROS generation, activated FoxO, NFκB, and inflammation, enhanced ubiquitination, with mitophagy and finally apoptotic cascades[20-26].

The progressive reduction of mitochondrial number and efficiency, represents a mechanism capable of inducing muscle atrophy and it seems involved in muscular dystrophy[27-30]. A relevant consequence of mitochondrial dysfunction is the activation of apoptosis, a mechanism believed to represent a final common pathway of several muscle disorders[31]. In particular, aberrantly enhanced apoptosis has been reported in muscle atrophy due to immobilization[32,33] as well as both in muscles from patients with muscle dystrophy and in mouse models of muscle dystrophy[34,35].

In skeletal muscle biology, apoptosis has been described as a normal developmental event, both in proliferating myoblasts and in post-mitotic muscle fibers[36,37]. Muscle atrophy conditions, which occur in neuromuscular diseases, muscle disuse, sarcopenia and aging, have been all associated to an increase of apoptosis which affected skeletal muscles mass.

Muscle wasting and weakness take places in physiological or pathological conditions, at least in part, due to an imbalance between apoptosis

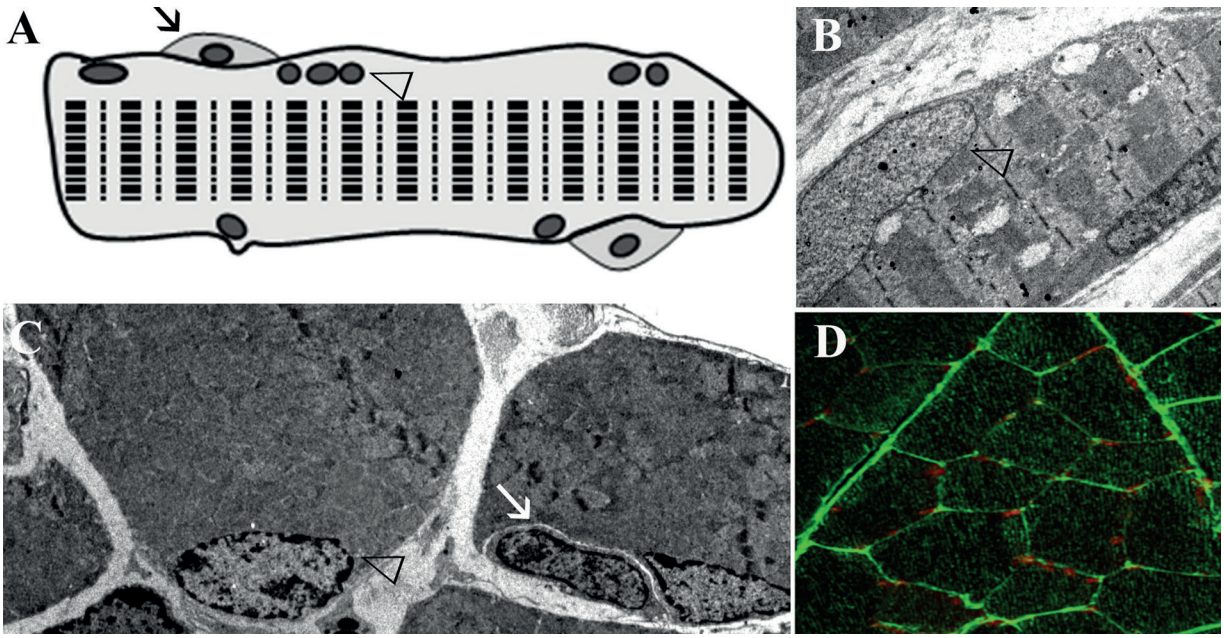

Figure 1. Schematic illustration of a skeletal muscle fiber (**A**). It is possible to distinguish myonuclei (arrowheads) and satellite cells (arrows) localized beneath the surrounding basal lamina and outside the myofiber plasma membrane. These two cell types clearly appear in the longitudinal (**B**) and transversal (**C**) sections, obtained by means of transmission electron microscopy (TEM). In D, a confocal microscopy image shows transversal section of muscle in which the myofibers (in green, stained with talin, a structural protein of basal lamina) and myonuclei (in red, stained with propidium iodide) can be observed. Bars: 2 μm (**B, C**); 10 μm (**D**).

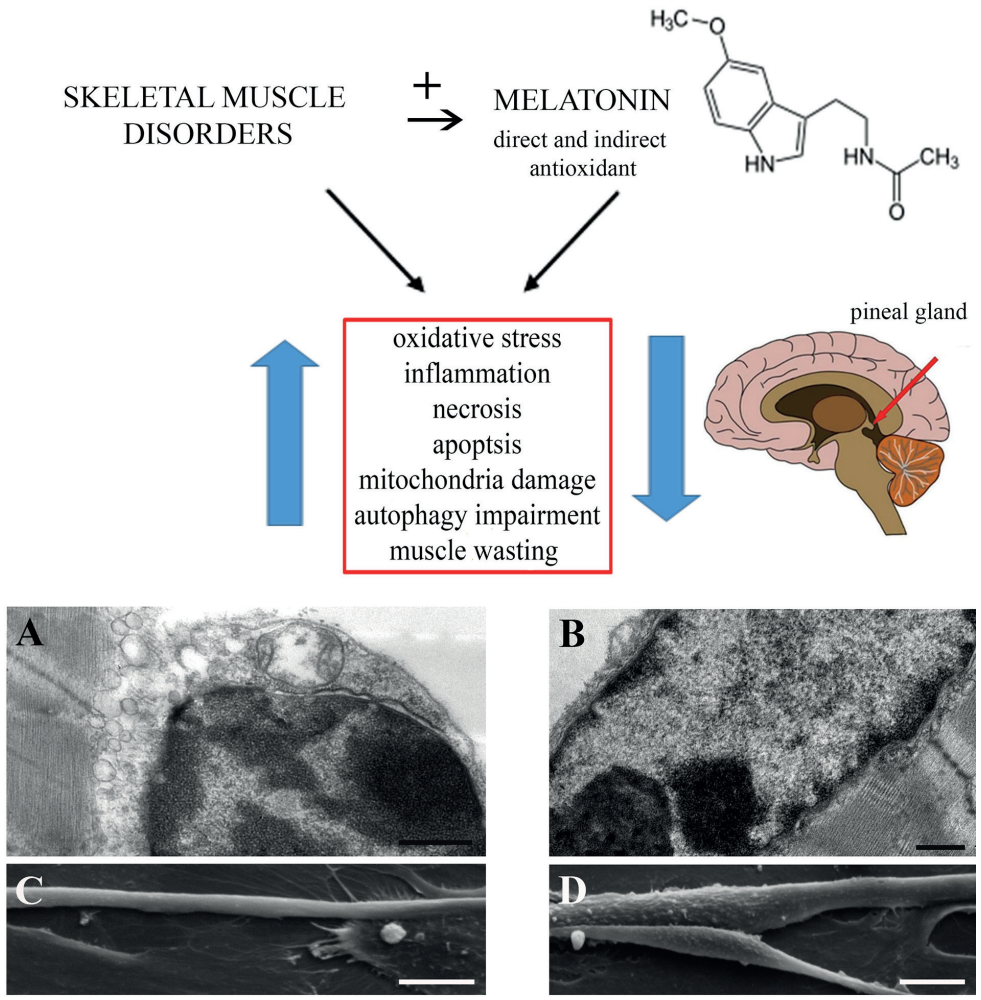

Figure 2. The figure describes melatonin possible action on muscle diseases. The images, obtained at transmission (TEM; A, B) and scanning (SEM; C, D) electron microscopy, show, the effect of the neurohomone in aged mouse skeletal muscle fibers and in a murine skeletal muscle cell line. TEM observations show a necrotic muscle fiber and altered mitochondria in aged mouse (**A**). This damage appears reduced after melatonin treatment (**B**). A thin C2C12 myotube (**C**) can be observed after treatment with a chemoterapic drug which causes loss of muscle mass. Melatonin treatment before drug is able to prevent muscle wasting (**D**)[28]. Bars: 0.5 µm (**A, B**); 10 µm (**C, D**)

and autophagy[38]. It should be noted that inhibition of autophagy and the consequent accumulation of dysfunctional organelles characterize several muscle disorders. Autophagy is an important mechanism for cell survival and for the clearance of damaged proteins and altered organelles[30]. In particular, it exerts a critical role in myofiber integrity and muscle mass preservation. Its inhibition or alteration can contribute to myofiber degeneration and weakness in muscle diseases, characterized by accumulation of abnormal mitochondria and inclusions[39,40].

In this review, the effect of melatonin in preventing mitochondria dysfunctions leading to cell death and autophagy impairment, which occur in skeletal muscle disorders, has been discussed.

Melatonin

Melatonin (N-acetyl-5-methoxytryptamine), the hormone produced by the pineal gland, has significantly broad actions including oncostatic effects[41,42], immune system stimulation[43,44] and anti-inflammatory functions[45-48].

Moreover, melatonin has been identified as a direct free radical scavenger[49] and an indirect antioxidant[50-52]. Its function consists in the reduction of oxidative stress, i.e. molecular damage produced by reactive oxygen and nitrogen spe-

cies[53,54]. So the ability of melatonin to scavenge free radicals is undoubtedly an important property in its protection against oxidative stress.

In addition, melatonin improves the intramitochondrial antioxidative defence by enhancing, for example, reduced glutathione levels. An additional action concerns in the inhibition of cardiolipin peroxidation and in maintaining the integrity of the mitochondrial membrane[53,55].

Some studies[56,57] showed that melatonin anti-oxidant and anti-apoptotic effects may be explained by a direct interaction with the mitochondria transition pore. The recent discovery that mitochondria are a target for melatonin opened a new perspective to understand the mechanism of action of this neuro-hormone. In particular, it exerts a direct role in mitochondrial homeostasis[28,58], which may explain its protective effect in several disorders such as Parkinson's disease, Alzheimer's disease, epilepsy, aging, ischemia–reperfusion and sepsis and, recently, also in muscular diseases[3,59].

The exact structure of mitochondrial transition pore is not clearly established; however, it has been proposed to consist of a large complex located at the contact sites between mitochondrial inner and outer membranes. This complex controls the voltage-dependent anion channel, the adenine nucleotide translocator and some other proteins[60].

Opening of the permeability transition pore, a phenomenon described as mitochondrial permeability transition, causes a sudden increase in the permeability of the inner mitochondrial membrane. This event leads to dissipation of the mitochondrial potential, uncoupling of mitochondria pumps, swelling of the mitochondrial matrix and rupture of the outer mitochondrial membrane[61]. As a consequence, the release of pro-apoptotic factors (i.e., cytochrome c) from the intermembrane space to the cytoplasm, induces activation of caspase cascade responsible for apoptosis.

In the following sections the role of melatonin vs muscle disorders has been discussed. In particular, the action of melatonin on dystrophies as well as in muscle atrophy following aging or sarcopenia has been described.

Results

Melatonin Effect on Muscle Dystrophy

Progressive muscle weakness is a typical feature of dystrophic patients[3,62]. Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are muscular wasting disorders that affect both skeletal and cardiac muscle as result of mutations in the dystrophin gene[63]. A progressive muscle degeneration affects dystrophic patients which lose ambulation. If the disease is untreated, patients die from cardiac or respiratory insufficiency at the average age of 19[64].

Even if DMD and BMD are genetic diseases, they are characterized by muscular frailty of the elderly, abnormal calcium homeostasis, mitochondria dysfunctions and strong oxidative damage. All these conditions contribute to the onset of the muscle degeneration.

Dystrophic muscle fibers showed some typical alterations such as, the presence of necrotic events followed by regeneration, release of pro-inflammatory cytokines and oxidative stress mechanism activation[65,66]. Therefore, inflammation and oxidative stress represent the pathogenic pathways targeted by nutraceutical therapies in dystrophy pathology.

In particular, the sources of oxidative stress in DMD are related to inflammatory cells, NAD(P)H oxidase, altered mitochondrial function or to ROS producing enzymes. Given that dystrophic disease is exacerbated, at least in part, by oxidative stress, melatonin could have some therapeutic benefit. First of all, thanks to its lipophilicity, it can easily pass-through cell membranes and the blood-brain barrier, becoming an effective antioxidant. In skeletal muscle, melatonin preserves mitochondrial function[28,52] and regulates calcium homeostasis during muscle contraction[67,68]. Some studies highlight the role of melatonin in preventing muscle wasting in dystrophic patients. In 2010, Chahbouni et al[69] demonstrated a significant increase of lipid peroxidation, nitrites and cytokine levels in plasma of DMD patients compared with controls. These plasma values appeared down-regulate in DMD patients treated with melatonin, suggesting that melatonin administration significantly reduced the hyperoxidative and inflammatory process in DMD subjects, by slowing down the muscle degenerative process[69].

Then, in 2011, Hibaoui et al[70] described melatonin action in a DMD mouse model in which the therapy with the neuro-hormone decreased plasma creatine kinase activity, a fundamental pattern involved in muscle injury. In addition, in DMD mouse, melatonin increased total glutathione content and lowered the oxidized/reduced glutathione ratio. These data enhanced melatonin ability in restoring the redox status in dystrophic muscle.

Despite some studies showed a benefit of mela-

tonin in DMD pathologies, no recent data describe its role as therapeutic agent to cure these diseases.

On the other hand, previously we discussed the autophagy impairment, a harmful condition which leads to muscle damage and which is strictly correlated to the pathogenesis of muscle dystrophy[71]. It is known that excessive stimulation of autophagy in a normal muscle is detrimental because it causes depletion of proteins and organelles that are functional and necessary. This despite the fact that an adequate rate of autophagy is essential for the correct homeostasis of skeletal muscles[30,72].

Pharmacological treatments, with the capabilities of recovery both autophagy/mitophagy and mitochondrial function, represent an intriguing tool to reactivate autophagy in dystrophic muscles.

However, the role of melatonin in rescuing authophagy impairment in DMD phenotypes has been not described.

Melatonin Effect on Muscle Atrophy Conditions (Aging, Sarcopenia)

During healthy aging there is a progressive loss of muscle mass and consequently of skeletal muscle contractile strength that causes a decline in muscle function with a consequent important effect on the quality of life[73]. This phenomenon has been widely observed during mammalian aging and can lead to increased morbidity and mortality. In this regard, about 40% of the human muscle is wasted from 20 to 80 years of age, associated with muscle strength decline.

There are likely several factors that contribute to muscle loss in aging. These include but may not be limited to: reduced protein synthesis[74,75], declines in neural function[76-78], hormonal deficits[79], chronic inflammation[80-82], oxidative stress[83-87], loss of mitochondrial function[40,86-89], inappropriate signaling in muscle, due at least in part, to inadequate nutrition[90-93], nuclear apoptosis[94-100], and reduced satellite cell function[22,101,102].

Sarcopenia is a multidimensional phenomenon of aging and represents a powerful risk factor for the development of negative health related events in the elderly. In fact, the relationships of sarcopenia with impaired physical performance, frailty, loss of functional independence, and increased risk of falls are all well established in the literature[103-105].

The etiology of the sarcopenia is still unclear, but several mechanisms depending on muscle atrophy and fiber loss have been proposed[106]. The main cause of these muscle changes is the accumulation of genetic damages and mutations in aged mitochondrial DNA[107].

Although the etiology of sarcopenia is not clearly defined, numerous mechanisms involved in this phenomenon have been suggested. These events include denervation of the skeletal muscle fiber, increased levels of nuclear apoptosis and oxidative stress, alteration of the hormonal environment and increased inflammation[108-110].

Several papers described melatonin beneficial action in attenuating, reducing or preventing muscle damage during sarcopenia. Sayen et al[111], demonstrated that melatonin administration was able to prevent age-dependent mitochondrial changes in aged mice. Later[112], it has been demonstrated that melatonin administration had the ability to maintain muscle fiber numbers as well as muscle mass and activity in older animals[5]. In fact, melatonin reduced the percentage of interstitial spaces and the infiltration of collagen tissue, as well as the percentage of apoptotic nuclei in elderly muscles, reflecting the anti-inflammatory and antioxidant properties of melatonin. In addition, in sarcopenic subjects, melatonin is also able to maintain the normal architecture of mitochondria and to restore a normal autophagy process, thus avoiding age-mediated damage to muscle fibers, mitochondrial changes and apoptosis. These results confirmed the protective effect of melatonin on the prevention of mitochondrial impairment, on the reduction of oxidative stress, on autophagic alterations and on low chronic inflammation, which may explain the reduction of sarcopenic changes in patients[6].

Since aging is characterized by an increase in oxidative stress that occurs during the development of sarcopenia, melatonin represents a valid candidate able to counteract free radical species increase.

Melatonin ability to improve the muscle function by reducing oxidative stress and inflammation in aged muscle has been summarized as follow:

- Melatonin and its metabolites serve as powerful antioxidants that protect the electron transport chain and mitochondrial DNA from oxidative damage more efficiently than other conventional antioxidants. This respiratory chain protection allows melatonin to increase ATP production in the mitochondria and thus to limit mitochondrial dysfunctions which affect sarcopenic muscles[113,114].
- It has been demonstrated that melatonin could induce or reduce autophagy. In relation to muscle, melatonin is a highly versatile

molecule and induces autophagy or inhibits it, depending on the pathological processes involved, since oxidative stress has a close relationship with autophagy[7].

- Melatonin can reduce endoplasmic reticulum stress in skeletal muscle by increasing the expression of different proteins as well as mRNA levels. This process improves protein synthesis. Furthermore, melatonin is an important regulator of the proteasome and of lysosomal mechanisms, thus improving the quality of cellular activity.
- Melatonin also increases satellite cells following muscle injury in rats[8] by reducing apoptotic processes, by modulating signalling pathways which causes significant muscle regeneration in these animals. Melatonin has a different antiapoptotic role in different cell lines. In normal skeletal muscle, melatonin prevents apoptosis and limits oxidative stress that causes the permeability of mitochondria to transition and subsequent death[8]. Melatonin significantly reduces or counteracts the various physiopathological processes specifically associated with sarcopenia[10,11].

Conclusions

Through the careful analysis of the literature, supported by previous experimental evidence of our group, we can conclude that melatonin, an ancient molecule produced by the pineal gland, could be used as a food supplement to cure muscular disorders characterized by muscle wasting. The scientific evidence enhances that melatonin counteracts mitochondrial impairments, reduces oxidative stress and autophagic alterations in muscle fibers (Figure 2). Its beneficial effect in restoring muscle decline takes place mainly in atrophic conditions correlated to muscle aging. Therefore, these findings suggest that melatonin may be considered as a safe dietary supplement useful to prevent or treat muscle wasting, in particular, in sarcopenia-associated diseases.

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