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Role of melatonin on immobilization induced effects in rat soleus muscle

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Keywords

Immobilization
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Skeletal muscle

Abstract

Objectives: Immobilization results in dramatic losses of skeletal muscle mass. Several reports have strongly implicated oxidative stress as partially causative of disuse atrophy. So, the aim of this study was to investigate the deleterious effects of immobilization upon rat skeletal muscle and to detect the possible protective role of melatonin on these effects. **Methods**: male Wister albino rats were subjected to immobilization procedure, then divided into (C) control group, and three treated groups with melatonin either once daily at morning (M1) group or at night (M2) group or twice daily (M3) group. Soleus muscle was used to detect myosin heavy chain (MHC) distribution, gene expression and histological examination. **Results:** The study demonstrated that treatment with melatonin once at night and twice per day prevented the deleterious effects of immobilization by increasing the total antioxidant capacity, decreasing the muscle atrophy F-box (MAFbx) and muscle-specific RING finger-1 (MuRF-1) and increasing in insulin-like growth factor 1 receptors (IGF-1R) gene expression. **Conclusion**s: The results of this study provide evidence that melatonin administration reduces the deleterious effects of immobilization on skeletal muscle. SO, exogenous melatonin may be a possible candidate for hormonal therapy in immobilization-induced muscle atrophy.

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Introduction

Skeletal muscle is a tissue that readily adapt in response to changes in loading pattern. In the case of increased load, the response is hypertrophy. In contrast, atrophy occurs with a reduction in load. The most obvious indication of atrophy is the reduction in muscle mass and cross-sectional area[1]. Muscle atrophy is the result of an imbalance between two opposing conditions: an imbalance in protein synthesis and degradation mechanisms[2]. Loss of skeletal muscle mass occurs with disuse or "hypokinesia"[3] as well as muscle wasting pathologies including sepsis, muscular dystrophy, heart failure, COPD, cancer, and AIDS [4-7]. Extreme disuse and muscle wasting occur with spaceflight and chronic bed rest [3].

Two models were commonly used to induce atrophy either cast immobilization or hindlimb unweighting (HLU)[1, 6]. With HLU, there is an initial reduction in recruitment, which is nearly absent by one week. However, with Cast immobilization, there is a reduction in recruitment that persists for the duration of immobilization[8]. Furthermore, when the soleus is immobilized in a shortened position, any fiber recruitment will result in very low-force contractions because the actomyosin complexes are in a near maximally shortened position. Although differing in their nature, HLU and immobilization both result in dramatic losses of skeletal muscle mass. This loss in mass appears to be primarily of type I fibers, with a minor contribution from type II fibers during HLU and both types I and II during immobilization[8]. Nevertheless, both models result in a soleus type II shift [9].

Several reports have strongly implicated oxidative stress as partially causative of disuse atrophy as both damage to lipids and proteins have been detected [1,10,11]. The increase in the cell reactive oxygen species (ROS) often involves the expression of number of genes encoding antioxidant proteins, DNA repair proteins, stress-regulated chaperones, and antiapoptotic proteins. In many cases, these induced genes are regulated by transcription factors[12]. Nuclear factor kappa B (NF-κB) is the first eukaryotic transcription factor which has been shown to respond directly to oxidative stress in skeletal muscle myoblasts and may be involved in disuse muscle atrophy[13]. Several studies have furthered these observations and utilized antioxidant supplementation in an effort to reduce oxidative damage and preserve muscle. **Appell et al.** [11] found larger muscle cross-sectional area in vitamin E-supplemented animals following immobilization. However in contrast, **Koesterer et al.** [14] found that an antioxidant cocktail containing vitamin E

failed to preserve muscle mass during unweighting.

It was established in numerous studies that melatonin, which is mainly secreted from the pineal gland in the body, reduced oxidative stress by its free radical eliminating and direct antioxidant effects [15–17]. Previous studies reported that melatonin administration inhibited lipid peroxidation stimulated by fenton in the thyroid gland [18], as well as ischemia-reperfusion injury in the liver and kidney [19]. In addition, Park et al. demonstrated that exogenous and endogenous melatonin has a prophylactic effect on muscle atrophy in the hindlimb in spinal cord-injured rats [16].

Thus, the first aim of this study was to investigate the deleterious effects of immobilization on rat skeletal muscle (soleus). The second aim was to detect the possible protective effects of melatonin supplementation on these deleterious effects.

MATERIALS AND METHODS

30 adult male Wistar rats aged 4-5 months, weighing 200–220 g, were obtained from the Experimental Animal House of Mansoura University. The experimental animals were housed individually in metabolic cages and were fed with standard rat pellet and tap water and kept under 12h/12h dark/light conditions (switch on light at 6 am and switch off light at18:00 o'clock). The temperature and humidity were controlled at 21°C and 50 ± 5% respectively. Then the animals were divided into four groups each consisted of 6 rats, all groups were subjected to immobilization procedure: **(C)** control group in which immobilized rats were supplemented with saline, **(M1)** group with rats supplemented with melatonin once daily at 7:00 **(M2)** group with rats supplemented with melatonin once daily at 19:00 **(M3)** group with rats supplemented with melatonin twice daily at 7:00 and 19:00. Melatonin was purchased from (Sigma-Aldrich, St Louis, MO, USA) and it was dissolved in a mixture of ethanol and 0.9% physiological saline (final concentration, 5%). Fresh melatonin solution was prepared in a dark room with switch on the red lamp. Animals were injected subcutaneously (10 mg/kg)[20] for 7 days of immobilization. In order to ensure that the contra-lateral leg was an adequate control reference, a fifth group of animals, non-immobilized age-matched controls **(NI)** was studied.

The experimental protocol was approved by the Institutional Animal Ethics Committee and care of the animals was carried out as per the guidelines of the Committee. All sacrificed animals were disposed in a safety cabinet in the Medical Experimental Research Centre in Mansoura University.

Hind limb immobilization procedure

The animals were anesthetized by intra-peritoneal injection of a mixture of ketamine

(30 g / kg body weight) and xilazine (10 g / kg body weight) before a mono-lateral hind limb immobilization was performed in all rats, with the exception of the **NI** group. The left limb of each animal was immobilized (I) with a cast on the total plantar extension soleus muscles remained in shortened positions for 7 days as previously described by **Ansved,** [21]. The contra-lateral leg that remained loose was utilized as a control for the immobilized leg of each animal and it was expressed as **(C-CL), (M1-CL)**, **(M2-CL)** and **(M3-CL).** The immobilized leg in groups was expressed as **(C-I)**, **(M1-I), (M2-I)** and **(M3-I)**. Fine-meshed steel net involved the cast to avoid chewing. Great care was taken to make sure that the cast did not cause ischemia. At the end of the experimental period, all rats were fasted overnight, weighed, anaesthetized with intraperitoneal thiopental sodium (40 mg/Kg BW) and the cast was removed. Then the Soleus muscle was surgically removed from both hind limbs, trimmed of excess fat, tendon, and nerve, then blotted dry, weighed, and immediately frozen in liquid nitrogen and stored at -70°C until further processing.

Myosin heavy chain (MHC) protein analysis

Muscles samples preparation to electrophoretic separation was conducted as described before by **Talmagde and Roy** [22]. After electrophoresis (275 V) for 120 min, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and Stacking gels were stained for 60 min in Coomassie Brilliant Blue and destained with 20% methanol and 5% acetic acid. MHC bands were scanned and analyzed by the Kodak Digital Sciencet ™ 1D Image Analyses Software using BioRad apparatus.

Total antioxidant capacity (TAC)

The total antioxidant capacity was measured by means of the Trolox equivalent antioxidant capacity (TAC) assay, as described by **Berg van den et al.** [23] samples were deproteinated with trichloro-acetic acid. The absorbance of the radical solution was 0.7±0.02. Then the decrease in absorbance, caused by antioxidant capacity in the sample, was measured at 734 nm using an UV spectrophotometer (T80+UV/VIS Spectrometer PG Instruments Ltd, UK) and related to that of trolox standards.

Lipid peroxidation

Peroxidative damage to cellular lipid constituents was determined by measuring malondialdehyde (MDA). Tissue MDA were measured in butanol extracts, using an UV spectrophotometer according to **Urchiyama and Mihara** method (thiobarbituric acid method) [24].

Protein oxidation

Muscle protein oxidation was evaluated by measuring carbonyl formation using 2, 4-dinitrophenylhydrazine (DNPH) as a reagent, using an UV spectrophotometer according to Levine et al. [25].

Histological analysis

Specimens were mounted on cryostat blocks, cut into transverse sections (5±6 um thick) at ®20 °C, mounted on glass slides, air dried and stained with haematoxylin and eosin.

Gene analysis

The fresh skeletal muscle tissues were homogenized with 1ml of Tri-reagent (Sigma-Aldrich, St. Louis, MO, USA) to prepare a total RNA samples. The RNA was reverse transcribed with oligo d (T) 12~18 using reverse transcriptase #18064-014 (Invitrogen, Carlsbad, CA, USA) and this reaction mixture served as a template for polymerase chain reaction (PCR). To identify gene transcription, a reaction mixture (50 μL) for PCR was made up of 2.0 μL of cDNA synthesis mixture, 40 nM dNTPs, 10 pM of sense and antisense primer, and 1.25 U of GoTaq® DNA polymerase (Promega, Medison, WI, USA). PCR were performed with denaturation at 95oC for 30 sec, annealing at 60oC for 1 min, and extension at 72oC for 1min in each cycle, followed by a final 10 min extension at 72oC using Px2 Thermal cycler HBPX2220 (Thermo electron corporation, Waltham, MA, USA) Complementary DNA, corresponding to 40 ng of total RNA, from the RT reaction was used as a template in the subsequent real-time PCR, performed in an ABI-Prism 7000 (Applied Biosystems). Primer sets are listed in Table 1.

Table (1) Oligonucleotide primers used for RT-PCR

Gene	Primer sequence (5' to 3')	Product length (bp)	GenBank accession No.
MAFbx	F: GCAAAACATAAGACTCATACG R: GTAGAGTGGTCTCCATTCG	83	NM_133521
MuRF1	F: AGGTGAAGGAGGAACTGAG R: AACTGCTCTCGGTACTGG	86	NM_080903
IGF-1R	F: TCCCAAGCTGTGTGTCTCTG R: GTGCCACGTTATGATGATGC	178	NM_052807
GAPDH	F:GTATGACTCCACTCACGGCAAA R:GGTCTCGCCTCCTGGAAGATG	100	BC094037

Statistical analysis

Data were collected from repeated experiments and are presented as mean ± SD. A one-way analysis of variance (ANOVA) followed by Tukey's post hoc test were used for statistical analysis. Differences were deemed statistically significant when $P<0.05$. While values < 0.05 were significant ($*^1$), values < 0.01 were considered highly significant ($*^2$), and values < 0.001 were extremely significant ($*^3$). Analysis was performed using the SPSS software (ver. 19.0, IBM, Chicago,IL, USA).

RESULTS

Mass parameters

Body weight after immobilization suffered a mild (but not statistically different) decrease until the 2nd day, after which it started to increase at the same rate as that of the NI group. Furthermore, no differences were observed in the weight of the SOL muscle from the NI group, as compared to the contra-lateral leg (C-CL). The immobilization markedly decreased weight of SOL muscle in control (immobilized) and M1 groups (P<0.001) (Table1 and fig 1). While M2-I and M3-I legs in M2 and M3 groups show significant increase in SOL weight as compared to the C-I leg in control group.

Table (2) Soleus muscle weight (mg) and relative muscle weight (muscle weight–body weight ratio).

	SOL weight (mg)	Relative muscle weight (%)
NI	110±5.2	0.46±0.03
C-CL	112±7.7	0.48±0.01
C-I	65±5.6*^{3a}	0.26±0.02*^{3a}
M1-CL	114±10	0.49±0.02
M1-I	69±6.3*^{3b}	0.27±0.01*^{2b,}
M2-CL	112±9.5	0.49±0.02
M2-I	78±6.7*^{3c,}*^{1e}	0.32±0.01*^{3c,}*^{1e}
M3-CL	114±9	0.49±0.03
M3-I	103±7.8*^{3e}	0.43±0.03*^{3e}

Values are expressed as mean ±SD. Non-immobilized leg (NI; n =6), contra-lateral leg of the control group (C-CL; n=6), immobilized leg of the control group (C-I; n=6), contra-lateral leg of the M1group (M1-CL; n=6), immobilized leg of the M1 group (M1-I; n=6), contra-lateral leg of M2 group (M2-CL; n=6) and immobilized leg of M2 group (M2-I; n=6), (M3-CL; n=6), contra-lateral leg of M3 group (M3-I; n=6) immobilized leg of the M3 group. *: means statistically significant *^{1}<0.05, *^{2}<0.01,*^{3}<0.001. . a; Different from C-CL, b; different from M1-CL , c; different from M2-CL , d; different from M3-CL, e; different from C-I.

No statistical difference was observed between M1-I and C-I relative mass, however, the relative muscle mass of the SOL of M2-I and M3-I were 20% (P<0.05) and 58.5% (p<0.001) greater than C-I, respectively.

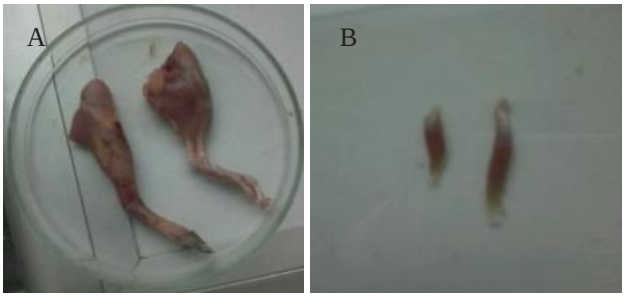

Figure (1) Effect of immobilization on hindlimb (A) and soleus muscle (B) size

MHC distribution

The decrease in type MHC I induced by immobilization was observed in SOL muscles of immobilized legs (Figures 2 and 3, P<0.001), indicating a main effect of immobilization. While the C-CL, M1-CL, M2-CL and M3-CL displayed more than 90% of MHC I expression, C-I, M1-I, M2- I and M3- I expressed approximately 60%, 62%, 70%, and84% MHC I expression, respectively. These changes were also accompanied by an corresponding changes in MHC IIa (Figures 2 and 3) led to a shift in MHC (slow-to-fast) in the postural SOL muscle in immobilized legs which was corrected by about16.7% in M2-I of M2 group and by 40% in M3-I of M3 group .

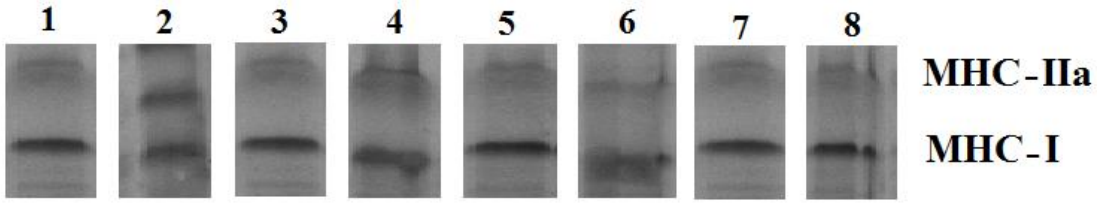

Figure (2) Changes of Myosin heavy chain, MHC-IIa and MHC-I, in immobilization-induced muscle atrophy in the soleus. Lane 1, C-CL leg; Lane 2, C-I leg; Lane 3, M1-CL leg; Lane 4, M1-I leg; Lane 5, M2-CL leg; Lane 6, M2-I leg; Lane 7, M3-CL leg and Lane 8, M3-I leg.

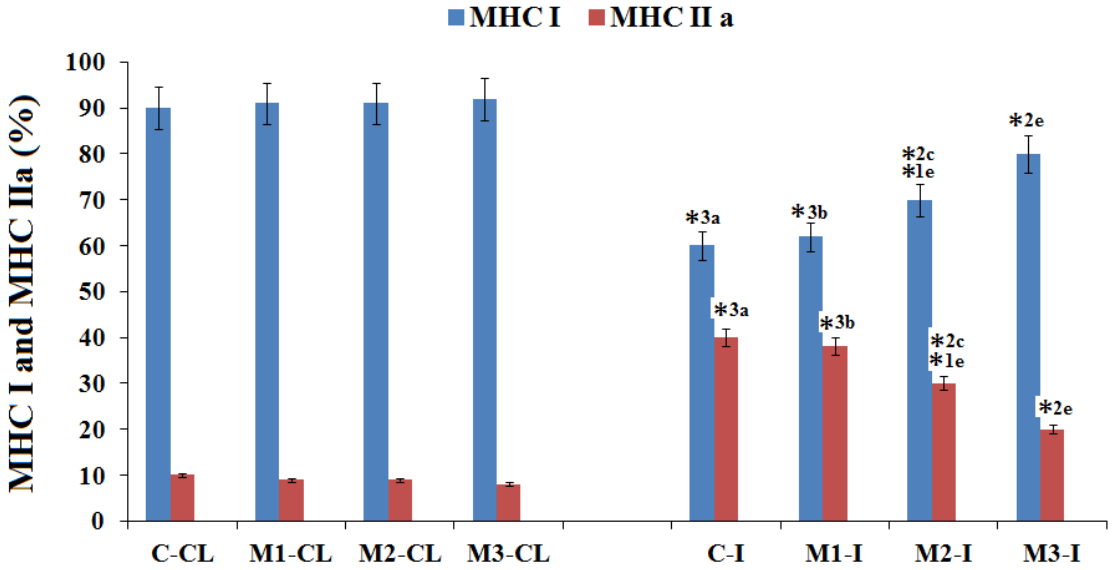

Figure (3) Myosin heavy chain I and Myosin heavy chain IIa distribution of soleus muscle from C-CL leg (n=6), M1-CL leg (n=6), M2-CL leg (n=6), M3-CL leg (n=6), C-I leg (n=6), M1-I leg (n=6), M2-I leg (n=6) and M3-I leg (n=6). Values are expressed as mean ±SD. *: means statistically significant $*^{1}$<0.05, $*^{2}$<0.01,$*^{3}$<0.001. a; Different from C-CL, b; different from M1-CL , c; different from M2-CL , d; different from M3-CL, e; different from C-I.

Table (3) the levels of malondialdehyde (nmol/g.w.w.), protein carbonyl (nmol/mg protein), and total antioxidant capacity (nmol Trolox Eq/mg protein) in the soleus muscle.

n=6	C-CL	C-I	M1-CL	M1-I	M2-CL	M2-I	M3-CL	M3-I
MDA	108±4.8	153±6.1$*^{3a}$	108±4.99	143±6.2$*^{3b,}$ $*^{1e}$	106±5.2	119±3.4$*^{1c,}$ $*^{3e}$	110±4.5	113±2.4$*^{3e}$
Carbonyl	2.4±0.1	4.43±0.4 $*^{3a}$	2.45±0.1	4.13±0.23$*^{2b}$	2.53±0.2	3.28±0.2$*^{1c}$ $*^{1e}$	2.55±0.1	2.68±0.12$*^{3e}$
TEAC	46±8.8	30±5.4$*^{2a}$	46±7.3	31±6.4$*^{2b}$	47±5.8	36±5.4$*^{1c}*^{1e}$	47±6.1	40±5.4$*^{2e}$

Values are expressed as mean ±SD. Non-immobilized leg (NI; n =6), contra-lateral leg of the control group (C-CL; n=6), immobilized leg of the control group (C-I; n=6), contra-lateral leg of the M1 group (M1-CL; n=6), immobilized leg of the M1 group (M1-I; n=6), contra-lateral leg of M2 group (M2-CL; n=6) and immobilized leg of M2 group (M2-I; n=6), contra-lateral leg of M3 group (M3-CL; n=6) and immobilized leg of M3 group (M3-I; n=6), malondialdehyde(MDA), and total antioxidants (TAO). *: means statistically significant $*^{1}$<0.05, $*^{2}$<0.01,$*^{3}$<0.001. a; Different from C-CL, b; different from M1-CL , c; different from M2-CL , d; different from M3-CL e; different from C-I.

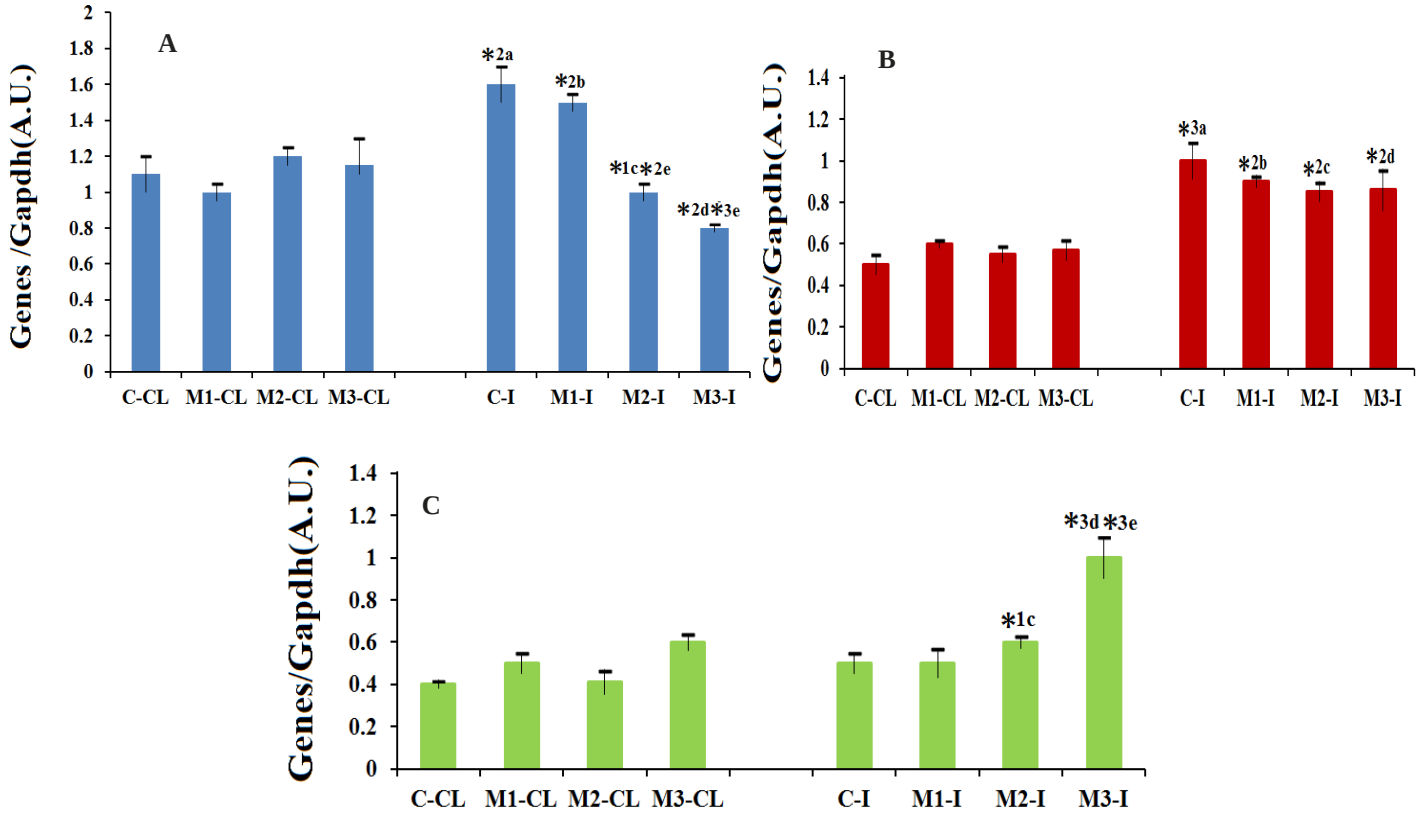

Figure (4) Changes of muscle atrophy- and hypertrophy-related gene expression on immobilization-induced muscle atrophy in the soleus. A= MAFbx mRNA expression, B = MuRF1 mRNA expression, and C= IGF-1R mRNA expression. Values are expressed as mean ±SD. Contra-lateral leg of the control group (C-CL; n=6), immobilized leg of the control group (C-I; n=6), contra-lateral leg of the M1 group (M1-CL; n=6), immobilized leg of the M1 group (M1-I; n=6), contra-lateral leg of M2 group (M2-CL; n=6) and immobilized leg of M2 group (M2-I; n=6), contra-lateral leg of M3 group (M3-CL; n=6) and immobilized leg of M3 group (M3-I; n=6). *: means statistically significant $*^1$<0.05, $*^2$<0.01,$*^3$<0.001. a; Different from C-CL, b; different from M1-CL , c; different from M2-CL , d; different from M3-CL e; different from C-I.

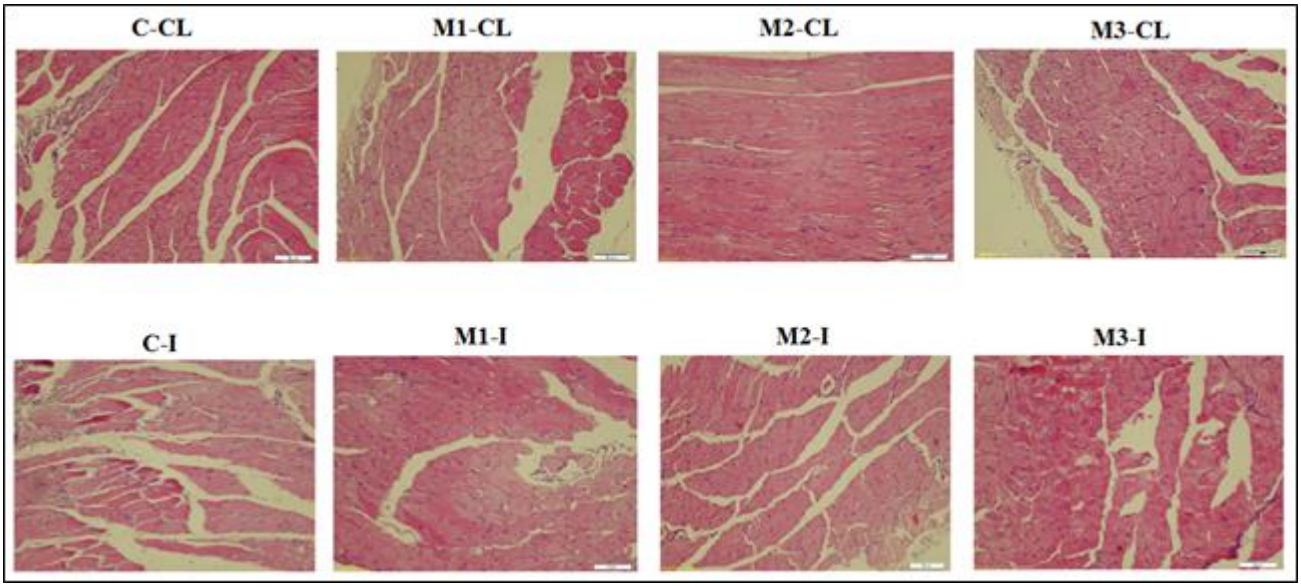

Figure (5) Morphology of soleus muscle following melatonin after hindlimb immobilization for 7 days: C-CL; contra-lateral leg of the control group, C-I; immobilized leg of the control group, M1-CL; contra-lateral leg of the M1 group, M1-I; immobilized leg of the M1 group, M2-CL; contra-lateral leg of M2 group, M2-I; immobilized leg of M2 group, M3-CL; contra-lateral leg of M3 group and M3-I; immobilized leg of M3 group (H&E, 100x).

Lipid peroxidation, protein oxidation and total antioxidant

Hindlimb immobilization resulted in significantly higher (+41.7%, P>0.001) soleus MDA levels when compared with controls (153±6.1 vs. 108±4.8 nmol/g.w.w.) (table 3). Also protein carbonyl showed significant increase in its level from 2.4±0.1 to 4.43±0.4 nmol/mg protein (+77%, P> 0.001) (table 3).While total antioxidant capacity (TEAC) of the soleus was significantly reduced with hindlimb immobilization. TAC was 34.8% (P>0.01) lower in C-I than C-CL (30±5.4 vs. 46±8.8 nmol Trolox Eq/mg protein) (table 3).

In M2 and M3 groups melatonin administration reduced the effects of hindlimb immobilization and resulted in significant decrease in MDA and carbonyl levels and significant increase in TEAC level in M2-I and M3-I legs when compared with C-I leg. However, melatonin administration in M1 group showed significant decrease in MDA level only and insignificant change in carbonyl and TEAC levels in M1-I leg when compared with C-I leg(table 3).

Gene expression

In the slow-twitch muscle, soleus, immobilization resulted in significant increase in the atrophy-related gene expression (MAFbx and MuRF1) P<0.01, P<0.001; Figures 4A and B. While IGF-1R expression, a hypertrophic signaling pathway, showed insignificant change with hindlimb immobilization (figure 4C). In M2 and M3 groups melatonin administration reduced the effects of hindlimb immobilization and resulted in significant decrease in MAFbx gene expression (P<0.01, P<0.001; Figure 4A) and significant increase in IGF-1R gene expression (P<0.05, P<0.001; Figure 4C) in M2-I and M3-I legs when compared with C-I leg. However, MuRF1 was unaltered. On the other hand melatonin administration in M1 group showed insignificant change in MAFbx, MuRF1, or IGF-1R gene expression in M1-I leg when compared with C-I leg.

Histopathological examination

Figure (5) demonstrated that control slide showed normal skeletal muscle cells with no evidence of fibrous connective tissue. Immobilization showed shrinkage or atrophy of skeletal muscle cells with increased intramuscular connective tissue. Melatonin administration reduces the effects of immobilization. In M1group melatonin resulted in partial reduction in fibrous connective tissue and increased skeletal muscle cells thickness with more reduction in fibrous connective tissue in M2 group. While melatonin administration in M3 group resulted in best normal skeletal muscle cells with scanty fibrous connective tissue.

DISCUSSION

In order to verify the role of melatonin supplementation in skeletal muscle plasticity during disuse, it was utilized in a model of hind limb immobilization induced hypokinesia. The lack of variation in weight gain and food intake indicated that the disuse model used in the present study did not interfere with developmental aspects of the animals. In order to refute the argument that the contralateral leg is not an adequate control reference, an age-matched group of animals that were not submitted to immobilization (NI) was utilized. Since no difference was observed in muscle mass between NI and C-CL, as also demonstrated in **Thomason et al.** [26], we proved that the contralateral legs could be considered as appropriate controls.

It was observed, a marked soleus muscle mass loss as a result of immobilization for 7 days (table 2) which agreed with Aokia et al. study [27]. In addition, we observed a marked decline in the cross sectional area of immobilized soleus cells with increased intramuscular connective tissue in comparison to C-CL. This result combined with the observation of a reduction in muscle weight, confirms that the hypokinesia model utilized was effective in promoting skeletal muscle atrophy.

As was described, prolonged periods of muscle disuse (limb immobilization, chronic bed rest, lack of physical activity, or spaceflight) resulted in a significant loss of muscle mass and strength [28]. Progress in the field of cellular and molecular biology has increased the amount of evidence pointing to oxidative stress as the essential regulator of the cell signaling pathways leading to muscle atrophy during periods of disuse. For instance, disturbances of the cell redox state resulting in the development of oxidant stress in skeletal muscle myotubes increase the expression of central components of the proteasome proteolytic system which plays an important role in protein breakdown in skeletal muscle during periods of inactivity [29].

This research observed that hindlimb immobilizations resulted in significantly higher soleus MDA and protein carbonyl levels and significantly lower antioxidant capacity (TEAC) when compared with controls. In M2 and M3 groups melatonin administration reduced the effects of immobilization and resulted in significant decrease in MDA and carbonyl levels and significant increase in TEAC level. However, melatonin administration in M1 group showed significant decrease in MDA level only and insignificant change in carbonyl and TEAC levels.

Since 1993, melatonin has been known as a radical scavenger with the ability to remove reactive oxygen species (ROS) including singlet oxygen (1O2), superoxide anion radical ($O^{\bullet}_2$), hydroperoxide (H2O2),

hydroxyl radical (•OH) and the lipid peroxide radical (LOO•) [30,31]. Melatonin's ability to counteract ROS has special relevance as it crosses all morphophysiological barriers and it is widely distributed in tissues, cells and subcellular compartments, because of its distinct physical and chemical properties [32]. These allow its localization in cellular organelles and in the cytosol, cellular membranes and nucleus [33, 34]. Its widespread subcellular distribution guarantees its ability to interact with toxic molecules throughout the cell, thereby reducing oxidative damage to molecules in both the lipid and aqueous environments of the cells [35]. Melatonin also acts as an indirect antioxidant through the activation of the major antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [36,37]. This activation is a consequence of antioxidant enzyme mRNA synthesis and eventually enzyme stimulation. These processes probably involve melatonin receptors [38], although this remains unknown.

Also, ROS involves the activation of NF-κB [16] which is present in its inactive form in the cytoplasm due to an inhibitory subunit, I-κB. **Ram et al**. [39] reported that melatonin and the melatonin mt1 receptor agonist, AMMTC down regulating of I-κB gene which allow for NF-κB activation which leads to activation of antioxidant enzymes, including SOD, GPx and CAT.

Numerous studies have demonstrated that over expression of NF-κB in disuse conditions activates the machinery of MAFbx/MuRF1 signaling [40, 41]. These genes are expressed only in skeletal muscle and myocardium and have been found to be expressed at elevated levels under all conditions involving atrophy[42]. Over-expression of MuRF1 reduces the size of cultured muscle cells, and MAFbx targets the myosin heavy chain for degradation [2]. The present research observed that hindlimb immobilization resulted in significant increase in the atrophy-related gene expression (MAFbx and MuRF1) while IGF-1R expression, a hypertrophic signaling pathway, showed insignificant change when compared with controls (figure 4). In M2 and M3 groups melatonin administration reduced the effects of immobilization and resulted in significant decrease in MAFbx gene expression and significant increase in IGF-1R gene expression. However, MuRF1 was unaltered. On the other hand melatonin administration in M1 group showed insignificant change in MAFbx, MuRF1, or IGF-1R gene expression in M1-I leg when compared with C-I leg. Our results suggested that melatonin might attenuate immobilization-induced muscle atrophy by down regulating the MAFbx/MuRF1 signaling pathway and upregulating IGF-1R gene expression.

The present results demonstrated that exogenous melatonin could activate the transcription of IGF-1 mRNA. Drummond et al. [43] detected that IGF-1R is turned on in downstream signaling pathways, such as the PI3K/Akt/ mTOR signaling pathway. When Phosphatidylinositol 3-kinase (PI3K) is activated by IGF-1, it reduces the levels of tuberous sclerosis complex (TSC1/2) and forkhead box (FOXO) protein. TSC1/2 inhibits mammalian target of rapamycin (mTOR) signaling pathway. Consequently, when TSC1/2 is decreased, mTOR increases protein synthesis in ribosomes [44]. Through this pathway IGF-1 can increase muscle protein content and down-regulates protein breakdown.

Furthermore, our results revealed that exogenous melatonin had differential protective effects, depending on administration time. The increased effect of melatonin was detected in M2 and M3 groups and this could be explained by either greater sensitivity to melatonin at the time of administration due to an increased density of melatonin receptor [20], or by increasing the duration of high melatonin level as controlled photoperiod could change plasma levels of melatonin. Moreover, therapeutic effect of melatonin could be modulated via both photoperiod control and exogenous melatonin administration [16].

To characterize the effect of disuse and melatonin loading upon the MHC profile, we chose a postural muscle (soleus), which predominantly expresses the MHC I isoform. The present results showed that hypokinesia leads to a slow-to-fast MHC shift and that the proportion of MHC IIa isoform in soleus increases after hindlimb suspension. The results in the soleus were interesting and were in accordance with Lee et al. [45] results. Spontaneous restoration of MHC-I components in immobilization-induced muscle atrophy was observed (Figure 2), indicating that exogenous melatonin may have a protective effect against immobilization -induced muscle atrophy.

Since administration of melatonin during immobilization attenuates the muscle wasting, we suggest that melatonin supplementation might be a suitable strategy for individuals who will be submitted to hypokinesia, particularly athletes in the post-operative phase involved in osteo-articular injuries. In such conditions, to ensure surgery success, which is followed by a prolonged postoperative immobilization, an intense muscle reinforcement program should be initiated prior and after to surgery. Therefore, melatonin supplementation during the muscle reinforcement program might be a viable dietary manipulation to minimize muscle wasting during immobilization.

Conclusion

The results of this study provide evidence that melatonin administration reduces the deleterious effects of immobilization on skeletal muscle. SO, exogenous melatonin may be a possible candidate for hormonal therapy in immobilization-induced muscle atrophy. Moreover, melatonin administration time might be an important factor for physiological effect of melatonin in vivo.

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Author contribution: Abeer F. Mostafa and Shereen M. Samir designed and performed research; also, wrote the paper and analyzed data.

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