



Age-related biochemical and histological changes in the soleus muscle of male mice

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KEY WORDS

Aging, Antioxidants,
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ABSTRACT

During aging, skeletal muscle is subjected to progressive loss of muscle mass and strength (sarcopenia) and a decline in the functional ability. The reduction in the antioxidative capacity of the skeletal muscle resulting in an abnormal accumulation of the reactive oxygen species (ROS) that is one of the possible causes of the decline in muscle performance. In this study the histological changes in the soleus muscles of male mice at the age of 1st and 15th old were examined and the activities of the antioxidant enzymes, CAT and SOD, non- enzymatic antioxidant GPH and MDA level were measured to clarify the age-related changes in healthy subjects. Results showed that there is a significant reduction ($P \leq 0.05$) in the antioxidants SOD, CAT and GPH and there is a significant increase ($P \leq 0.05$) in MDA level at the age of 15th month when compared to 1st month old. We concluded that the activities of the antioxidants were affected by aging. Moreover, there is age- related lipid peroxidation.

1. Introduction

Aging is characterized by a decrease in the physical function of the daily activities resulting in reduced quality of life. The decrease of motor activity is related to the weakness and the atrophy of the muscle (Miljkovic et al., 2015). Sarcopenia is one of the main characteristics of aging and, is characterized by loss of skeletal muscle mass and strength (Edstrom et al., 2007; Frontera and Ochala, 2015; Musumeci

et al., 2015). The decline of the muscle strength was due to the progressive increase in the catabolism with a decrease in the anabolism, as well as the reduced regeneration capacity of the muscle (Musumeci et al., 2015). The muscle reduced in size progressively, and muscle fibers were replaced by fat and fibrous tissue. This causes an increase in the oxidative stress, changes in the muscle metabolism, and degeneration of the

neuromuscular junction, resulting in progressive loss of muscle function and frailty (Tsochatzis et al., 2014). Sarcopenia increased by 14% in people those aged between 65 and 70 years and 53% after 80 years (Santilli et al., 2014).

The skeletal muscle constitutes of 40% of the body weight and contains 50%–75% of the body's proteins. The principal functions of the skeletal muscle are maintaining body structure and posture, controlling motor movement and storing energy (Fong and Tapscott, 2013). Skeletal muscle is considered as an organ for the muscular system which consists of the tissues of skeletal muscle, connective, nerve and blood or vascular to serve multitude of functions. The good maintenance of skeletal muscle health is important for the prevention of various diseases and the psychological stress due to the disability (Bentzinger et al., 2012). Skeletal muscle is made up of thousands of cylindrical, multinucleated muscle fibers (myofibers) composing an array of stacked myofibrils running the entire length of the cell. Myofibrils consist of thick and thin filaments which are organized into a contractile unit called a sarcomere and surrounded by a basal lamina (Scime et al., 2009). Adult skeletal muscle undergoes changes in its size and metabolic activity in response to extracellular and intracellular effects (Sandri, 2008).

Oxidative stress is characterized by increased levels of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS). Oxidative stress can be caused by decreased antioxidant capacity due to low concentrations of the antioxidants and reduced antioxidant enzymes activity, and/or by increased ROS production (Altenhofer et al., 2015). Skeletal muscles consume large quantities of oxygen and can generate a great amount of ROS and (RNS) (Choi et al., 2016). The antioxidant system includes endogenous and exogenous molecules neutralizes the oxidative stress. The main antioxidant enzymes are superoxide

dismutase, catalase, and glutathione peroxidase (Bouزيد et al., 2015). These enzymes can be altered by exercise, nutrition, and aging (Franzke et al., 2015). The antioxidants maintain the muscle redox status and decrease ROS-induced intracellular changes (Baumann et al., 2016). Due to the increased oxidative stress levels observed in aged muscle, ROS accumulation has been suggested as playing a role in muscle changes and sarcopenia. Harman (1956) was the first to propose the free-radical theory of aging. Therefore, the present study will examine the biochemical and histological changes that may appear in aged male mice that had 15month old.

Materials and Methods

Animals

Twenty-eight male mice of 1 and 15 months old were used in the present study. The mice were housed in steel mesh cages in the research lab at the Faculty of Science, Kafrelsheikh University, Kafrelsheikh, Egypt. They were maintained under standard conditions of temperature, humidity, and 12 h light/dark cycle and were provided with standard diet and excess tap water. The procedures of the experiment adhered to the guidelines of the ethical committee of Kafrelsheikh University, Kafrelsheikh, Egypt.

Skeletal muscle sampling:

Soleus muscles of mice were collected from the mice in each group under strict hygienic conditions to minimize the contamination or autolysis of the samples. Then, some samples were quickly preserved in 10% neutral buffered formalin solution for 12 hours for histological study and the other samples were frozen in liquid nitrogen before storing at -80°C to be used for biochemical analysis.

Biochemical investigations:

Catalase activity was estimated according to the method of **Xu et al. (1997)**. SOD was estimated according to the method of **Kakkar et al. (1984)**. Glutathione level in cell lysates was estimated by the method of **Ellman (1959)**. Lipid peroxidation was estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS). The method is based on the determination of malondialdehyde (MDA) an end product of lipid peroxidation, which can react with thiobarbituric acid to yield a pink colored complex exhibiting a maximum absorption at 532nm (**Yoshioka et al., 1979**).

Histological investigations:

The preserved soleus muscles in 10% formalin were washed with running water overnight then samples were dehydrated by passing through a graded series of ethanol and then were embedded in paraffin. Sections of 5µm were cut, deparaffinized, hydrated and were stained with hematoxylin and eosin. The samples were processed for examination under light microscope and were photographed under standard procedures (**Bancroft et al., 1994**).

Statistics

All data are the means of 4 replicates. One-way analysis of variance (ANOVA), was used. If there a significant difference between means, Tukey post hoc comparisons were performed. For all statistical tests P values ≤ 0.05 was considered to be statistically significant. Means that do not share a letter are significantly different.

Results

Biochemical assay:

Results showed a significant reduction ($p \leq 0.05$) in the activity of the SOD, CAT, and GPH in male mice at the

15th month when compared with the control group at the 1st month. The value of SOD enzyme activity was 31.5625 U/g and 10.175 U/g at the 1st month the 15th month, respectively. The value of CAT enzyme activity was 88.35 U/g and 46.375 U/g at the 1st month the 15th month, respectively. The GSH concentration was 888.0 mg/g tissue at the 1st month and 114.7 mg/g tissue at the 15th month. However, a significant increase ($p \leq 0.05$) in the MDA content in mice at the age of 15th month were recorded when compared with the control group at the 1st month. The MDA concentration was 66.925 n mol /g tissue at the 1st month and the concentration was 285.75 n mol /g tissue at the 15th month (Table 1).

Table 1. The effect of aging on the SOD, CAT and GPH activities and MDA level, $n= 4$, Means that do not share a letter are significantly different ($p \leq 0.05$).

Age	SOD (U/g)	CAT (U/g)	GPH (mg/g)	MDA (nmol/g)
1 month	31.5 625± 2.52 ^a	88.35 ± 2.69 ^a	888.0± 129.05 ^a	66.93 ± 4.70 ^c
15 months	10.1 75 ± 2.04 ^d	46.38 ± 2.66 ^c	114.7± 50.60 ^d	285.75± 17.84 ^a

Histological observation:

Observation of the soleus muscles of the mice that had 1- month age showed normal architecture of the muscle fibers with bundles of nonbranching cylindrical shaped myofibers, acidophilic sarcoplasm and multiple elongated nuclei that stained dark purple and peripherally located beneath the sarcolemma. The muscle fibers of 15- month old were degenerated.

Dark nuclei, linear nuclei, clumping of nuclei, myolysis and spacing, uncontinuous sarcoplasm and wavy sarcoplasm were observed (**Figure 1**).

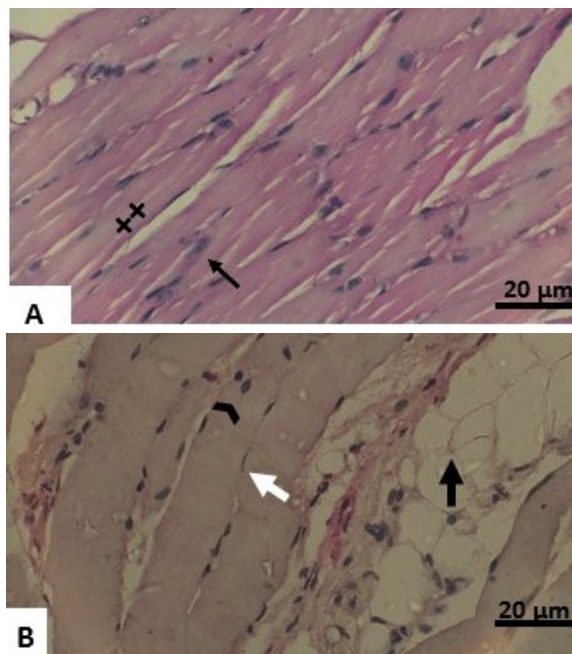


Fig. (1): Light photomicrograph of L.S of soleus muscle of the male mice stained with hematoxylin and eosin stain. **(A):** 1st month age (control group), showing bundles of cylindrical shaped myofibers with acidophilic sarcoplasm (++) and multiple elongated nuclei stained dark purple and peripherally located beneath the sarcolemma (black arrow). **(B):** 15th month age, showing dark nucleus (black arrow head), linear nuclei (white arrow) and vacuolation (black arrow).

Discussion and conclusion

The present study was designed to investigate the age related biochemical and histological changes occurring in the skeletal muscles of the male mice. The current results showed a significant decrease in the activities of GSH, SOD and CAT, and a significant increase in MDA level with aging. The present data agreed with **Gil et al. (2006)** since they found that plasma MDA levels increased

with age. Also, **Suresh et al. (2010)** and **Karolkiewicz (2011)** showed a decrease in the antioxidant enzymes activity such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), and the accumulation of reactive oxygen species with age.

Moreover, **Inal et al. (2001)** observed that SOD activities were decreased with aging in erythrocytes. They suggested that the decrease in SOD activity was related to its product, hydrogen peroxide, because exposure of intact erythrocytes to increased hydrogen peroxide caused the inactivation of endogenous SOD activity in the concentration-dependent manner **Salo et al. (1988)** suggested another explanation for the decrease in SOD activity since SOD includes copper and zinc. they suggested that the deficiency of zinc and copper led to a decrease in SOD activity. They also showed that MDA levels were increased with aging because of the increase of the oxidative damage.

Furthermore, the results of the current study showed differences from that of **Inal et al. (2001)** since they observed that CAT activities were increased. They suggested that the increase was because of the increase in Hydrogen peroxide (H_2O_2) formation since H_2O_2 is removed by catalase (**Alejandro et al., 1997**). Aging is a progressive accumulation of morphological and physiological changes and is responsible for an increasing susceptibility to disease (**Matsubara and Machado, 1991**).

Aging is associated with increased oxidative stress and many studies displayed the age-related increase of lipid peroxidation, protein oxidative modification, and DNA damage (**Gianni et al., 2004** and **Gunduz et al., 2004**). Skeletal muscles display a significant age-related increase in oxidative damage. So, aged skeletal muscles were susceptible to oxidative damage to DNA, lipids, and proteins (**Nabben et al., 2011**). In skeletal

muscle, increased exposure of mitochondrial reactive oxygen species (ROS) with aging reflects basic changes in redox signaling (**Muller et al., 2007**).

Similarly, the results of the current study suggested that the soleus muscles from aged mice (15-month old) had greater oxidative stress than muscles from the control mice of one-month age. Since our data displayed that MDA - an indicator of lipid peroxidation- increased with aging. In addition, the decreased activities of the antioxidant enzymes e.g. SOD and CAT, and the non-enzymatic GSH indicated the excessive production of free radical and reactive oxygen species which increase with aging. Current data agreed with the study of **Akila et al. (2007)** since they showed that lipid peroxidation was increased and antioxidants were decreased in normal elderly people.

Our results agreed with (**Sheard et al., 2012**) since they studied the age-related decline in muscle fiber number in the mouse and found that leg muscles showed significant fiber loss. Moreover, **Sakakima et al. (2004)** showed that body mass, muscle wet weight and the fiber size were decreased with aging. **Lexell (1995)** displayed that the Limb muscles from older men and women are 25-35% smaller and have more fat and connective tissue than limb muscles from younger individuals. **Reinking (1996)** showed that the recovery of muscle mass and force following muscular injury decreased with age. **Kragstrup et al. (2011)** showed that the skeletal muscles display structural and biochemical changes with aging. These changes in skeletal muscle contribute to the increased stiffness and impairment in force generated by the contracting muscle fibers seen with aging due to muscle mass loss.

In conclusion, our findings have displayed that the skeletal muscle histology and the activities of antioxidants

are affected by aging. Moreover, there is age-related lipid peroxidation. Therefore, free radical mediated peroxidative injury may have a critical role in the pathophysiological changes of aging.

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