The Relationship between oxidative stress and the functional capacity of skeletal muscle

Stefania Fulle, Tiziana Pietrangelo, Rosa Bellomo[(1)], Diane Sagnella, Silvia Belia

Istituto Interuniversitario di Miologia, Ce.SI Centro Studi Sull'Invecchiamento, (1) Centro Universitario di Medicina dello Sport – Università "G.d'Annunzio" Chieti-Pescara

Abstract

The sarcopenia, charaterised by a decrease in muscle mass associated with reduced physical activity, is a direct cause of the age-related loss of muscle strength. Studies previously performed in our laboratory show, in human *vastus lateralis* muscle, a direct correlation between age and oxidative damage to biological molecules. The reactive oxygen species are involved not only in muscle damage but are also able to modulate skeletal muscle contraction. In fact, the Sarcoplasmic Reticulum (SR) Ca^{2+} channel may be in an high oxidation status, due to oxidative stress, that decreases the channel opening status. Furthermore, the CFS patients usually suffering by fatigue and muscle pain, symptoms that are common to muscle aging. In the skeletal muscles of these patients, which may be identified with an old muscle in the young subject and take as model of ageing muscle, we have shown that exists a correlation between oxidative damage and alteration of SR membranes. We hypothesise that the oxidative damage that accumulates in the muscle affects different structures and is also located in the SR membranes and that all its alterations can affect the functional capacity of skeletal muscle.

Key Words: Sarcopenia, Oxidative Stress, ROS.

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Ageing has been the subject of scientific research for several years; however, its primary cause and a unitary theory describing its molecular basis have proven to be elusive. In the 1950's, D.Harman proposed a correlation between aerobic metabolism, the oxidative damage due to the accumulation reactive oxygen species (ROS), and ageing [13]. A possible cause may be the utilising oxygen enzyme activities, as those that contain iron. This theory pertains mainly to the tissues of skeletal and nervous systems, in which ROS generation is very important.

Senescence is associated with a loss of skeletal muscle mass, also known as *sarcopenia* [4]. Sarcopenia, therefore, is a consequence of normal ageing and is not a result of any specific pathology; however, the presence of sarcopenia does make the body more vulnerable to possible disease. The onset of sarcopenia may begin as early as 40-50 years of ages with a 20-30% loss in muscle mass by the age of 80. The decrease in muscle mass associated with reduced physical activity is a direct cause of the age-related loss of muscle strength. For this reason, inactivity in the elderly is the primary cause of increased invalidity. However, a loss of muscle mass can only explain part of this decline, as it is possible that alterations also occur in the quality of the muscle tissue [23]. The considerable variability of features found in fiber architecture, fiber type, and, in the distribution and size of motor units, reflects the difference between the various skeletal muscles. These differences are a result of the different contractile quality and quantity of each muscle during its lifetime [8]. Reduction in activity, immobility, or lack of use rapidly leads to atrophy as a result of disuse, thus expressing a strong dependence on daily activity for the structural preservation of muscle [1]. On the other hand the ability of skeletal muscle to produce strength and to make movement can also be compromised by incomplete activity of the motor units (*central motor drive*), periferic nerve dysfunction, loss of response to trophic and/or hormonal factors, changes of excitation-contraction coupling mechanism, or by alteration of contractile elements [25]. Furthermore, ageing may also involve the satellite cells which play a primary role in muscle tissue repair. For this reason, physical activity and exercise have several beneficial effects on the physical and mental health of both young and old subjects. The balance between beneficial and potentially dangerous effects could fundamentally be

important in the elderly, in which nutritional deficiency, sedentary life style, and the synergism of these factors contribute to the depletion of the antioxidant supplies and to increased oversensitivity to oxidative stress [14].

In reality, the reactive oxygen species (ROS) are involved not only in muscle damage but are also able to modulate skeletal muscle contraction. Contraction is activated by a cascade of events, named excitation-contraction coupling (e-c), that originate from electric signal transduction at the muscle surface membrane and, through Ca^{2+} release from the Sarcoplasmic Reticulum (SR), causes the formation of cross-bridges. The e-c coupling process is bound to specific structures in adult skeletal muscle known as calcium release units (CRU) or triads. These units are formed by a protein system complex that is able to modulate the Ca^{2+} release from SR [7]. Among these proteins, dihydropiridine receptors (DHPR), L-type Ca^{2+} voltage-operate channels found in the external membrane and Ryanodine receptors (RyR) that in the skeletal muscle are mainly present as the RyR1 isoform, are the most important. The RyRs are considered ion channels whose activity is dependent upon several factors, in addition to the redox state of the channel. In particular RyR1 contains a considerable amount of free thiols: approx. 50 for every 100 cysteines on each subunit [3]. Channel activity is altered by changes in the redox state of these "critical thiols", in fact a considerable increase of thiol oxidation decreases the opening channel probability [24].

Studies previously performed in our laboratory show that, in human *vastus lateralis* muscle, a direct correlation exists between age and oxidative damage to biological molecules. The molecules found to be most affected were DNA and lipids. This is more evident in male subjects, in which a correlation between age and an increase in oxidative damage has been observed (ca. four-fold in the DNA), as opposed to female subjects [16]. During a woman's reproductive years when estrogen levels are highest, there is likely less accumulation of oxidative damage thus establishing a more favourable condition in the ageing process. Therefore, this difference between male and female subjects may be due to the higher estrogen levels found in females, since this hormone may contribute to the reinforcement of the antioxidant defences in female muscle. In fact, it has been demonstrated that the estrogens have direct antioxidant effects and can protect against experimental oxidative stress conditions [11]. Also other causes, such as a different muscle activity based on the sexes (males > females) can have a role. The GSH-dependent antioxidant enzymatic pathway (except a decreased activity of glutathione transferase – GST) doesn't seem to undergo age-related modifications. On the other hand, the GSH-independent enzymatic system, constituted by Catalase (Cat) and superoxide dismutase (SOD), seems to be only depress the Cat activity, while the SOD activity doesn't show significant differences related to age or sex [5]. The last result is apparently conflicting with recent observations obtained from Pansarasa et al. [18] that, in human muscles have found a little but significant SOD activity increase. However, in more recent work, these researchers found that in subjects with loss of type II fibres, the SOD activity is lower [19]. The differences in these results may derive from different analysed subjects with different activity and training. However, the decreased Cat activity can determine H_2O_2 accumulation that represents the main ROS source during the muscle contraction.

In humans and other animals ageing is associated with striking morphological and physiological alterations. These modifications include the loss of muscle mass, strength and a decrease in the contraction velocity with the consequent shortening of twitch duration. This fact seems to be a general phenomenon, but there also exist qualitative and quantitative differences between fast and slow motor units. In theory, at least three different causes may work together to decide twitch duration: excitation-Ca^{2+} release uncoupling, changes in the number and/or gating properties of Ca^{2+} channels, and alterations of the Ca^{2+} transport system [21]. However, this possibility is not generally accepted since there exist contrasting results in the Ca^{2+} channels activity and the active transport of this ion in skeletal muscle, due to differences in muscle type, age and species [15,17]. The ryanodine binding decrease seen in elderly muscle does not necessarily indicate a decrease in the number of receptors present in individual skeletal muscle fibres. Studies performed by Renganathan et al. [22] on young and old mice show that the level of RyR1 doesn't change significantly with age; consequently the cause of a decrease in binding could be in the modification of the opening status of these channels due to the oxidation of the thiols.

The ROS increase due to changes in antioxidant enzyme activity may also be at the root of the observed modifications in membrane fluidity [2] and may affect the functional capacity of muscle by determining fatigue and weakness [5]. It should be kept in mind that patients suffering from Chronic Fatigue Syndrome (CFS) also show an increase in oxidative damage and alterations of the anti-oxidant system and membrane fluidity [10], such as that seen in elderly muscle. It is feasible that those modifications could cause alterations of the Ca^{2+} movement "in and out" of the sarcoplasmic reticulum (SR) [9]. In fact, the results obtained in our laboratory also show that in these muscles there are ryanodine binding and Ca^{2+}-ATPase activity decreases despite it doesn't seem to exist a difference in the expression of RyR1 between the muscle sample of CFS patients and controls. Furthermore, the CFS patients usually suffer from fatigue and muscle pain, symptoms that are common to muscle aging [6]. For these reasons this syndrome may be identified with an old muscle in

the young subject and considered as model of ageing muscle. In these muscles we have also noted that Na^+-ATPase activity is increased; this fact could be due to an alteration of redox status of K^+ channels that could be in an opening status by releasing more amount of this ion [20]. On the basis of this information, we hypothesise that the oxidative damage that accumulates in the muscle affects different structures and is also located in the SR membranes. This important structure is deputed to transfer the electric signal that is conducted along the sarcolemma, to transversal bridges determining their functional mobilisation; therefore, all its alterations can affect the functional capacity of skeletal muscle.

Address correspondence to:

Prof. Stefania Fulle, Istituto Interuniversitario di Miologia – Università "G. d'Annunzio", Via dei Vestini, 29, 66013 Chieti, Italy, Tel: +39 0871-3554036
Email: s.fulle@unich.it

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