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SARCOPENIA IS MORE THAN A MUSCULAR DEFICIT

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INTRODUCTION

Sarcopenia is a term coined by Irwin Rosenberg in 1988, in order to define the loss of mass and muscular function with age. It can be identified with the metabolic situation when the muscle, which is one of the most important energy consumers of the body, gradually loses, from about 45 years of age its ability to produce and to consume energy at the same rate as before.

MUSCLE AGEING

Although it is true that sarcopenia begins to appear around the age of 40-50 years, it accelerates around the age of 75. This functional state debuts and develops faster in inactive subjects, since early physical activity is an element of protection, albeit relative, capable of slowing down both its insurgence and its develop. Sarcopenia is believed to be the result of both intrinsic factors involving changes at the molecular and cellular levels, and extrinsic or environmental factors such as nutrition and exercise (1). There are many changes at the cellular level that contribute to the sarcopenia related loss of mass: a reduction in the number of motor units coupled with an increase in motor unit size, progressive denervation, loss of muscle fibres, decreased synthesis of myofibrillar components, atrophy due to disuse, accumulation of connective tissue, etc. While sarcopenia is directly responsible for the profound decrease in skeletal muscle mass (approximately 25% between the ages of 20 and 80), it is also indirectly accountable for a reduction in both muscle strength and overall performance. It is important to make the distinction between the involuntary muscle mass loss seen in patients suffering from starvation, advanced cancer, or acquired immunodeficiency syndrome as a result of inadequate intake and that seen with sarcopenia. Sarcopenia also differs from cachexia, a cytokine-driven loss of lean body mass seen in patients with rheumatoid arthritis, congestive heart failure, or renal failure that occurs despite maintenance of weight (10).

Is sarcopenia only a special mechanism of a general atrophic state of the muscle, or does it have a specific genesis and development of its own? An observation that supports this apparently rhetorical question can be found in the mechanisms of com-

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munication that are formed inside the motor units, between the axonal terminals and innervation of muscular fibers. Because of the different neurodegenerative developments, as a consequence of the lack of trophic stimulus connected to the synaptic activity, a process is started that leads to the atrophy of muscular fibers (anterograde effect). On the other hand, in sarcopenia, it is the muscle which, without evident causes, sends the "remodelling" information to the motor terminal (retrograde effect). With age, indeed, the number of working motor units decrease in an irreversible way, and even if their numbers do not decrease drastically from an anatomic point of view, the number of active terminals is much lower because of the functional alterations which reduce the numbers of synapses in the motor terminal and make nervous stimuli uneffective. Notwithstanding, as a consequence of retrograde effects and/or neurodegenerative processes with a nerve loss, leading to a secondary denervation and consequent atrophic muscular state. One fact is however remarkable, sarcopenia represents a unique event of functional preservation inside an almost completely degenerative state: the myofilaments and the cycle of the cross-sectional bridges, do not seem to undergo appreciable alterations in connection with age.

OXIDATIVE DAMAGE IN HUMAN MUSCLE

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 (Fig. 1). 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 (7). 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 (3). 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 (4). However, the decreased Cat activity can determine H_2O_2 accumulation that represents the main ROS source during the muscle contraction.

OXIDATIVE STRESS AND SARCOPENIA

It seems quite obvious, however that Sarcopenia cannot be considered as a simple process connected with a cause-effect relation and having a specific etiologic

factor; rather, it is necessary to consider it as a multifactorial event. Under this point of view, we may possibly follow a more correct line of investigation, running the risk of confusing the effects (alteration of the electron transport system, hormonal alterations and/or of the growth factors that lead to an alteration of protein synthesis, loss of the repair ability of the satellite cells etc.) and the causes (biological clock, accumulation of free radicals, anterograde remodelling etc.). The problem is that we are very far from defining the causes and the specificity of the process, and it is a fair guess that several more years must pass before we can start to solve this question. Obviously, taking into account both the human cost and the serious situation that connects substantial levels of sarcopenia with states of partial or total social inability (cost for the health system), every effort must be undertaken in order that at least the most alarming symptomatology (lowering of the pain threshold, preinflammatory states, depression etc.) may be in some way controlled. What can be done? Ageing is associated with several modifications of the hormonal levels, including the drastic modification of the plasmatic GH levels (above all in relation with the variations induced from physical exercise) and the less evident modification of testosterone (T) and IGF-I.

One of the factors that could play a key role in triggering sarcopenia is the accumulation of reactive oxygen species (ROS) because a correlation exists between aerobic metabolism, cumulative oxidative damage, and senescence. The ROS are by-products found in practically all tissues and are usually generated in the mitochondrial respiratory chain. Such reactive elements are often quite harmful, resulting in oxidative stress that can damage other cellular components such as DNA, proteins, lipids, etc. which in turn results in subsequent damage to the cells and tissues. Mammalian cells respond to oxidative stress by variations in the rate of cell growth, changes in cell cycle length, and increasing their defensive mechanisms (i.e. antioxi-

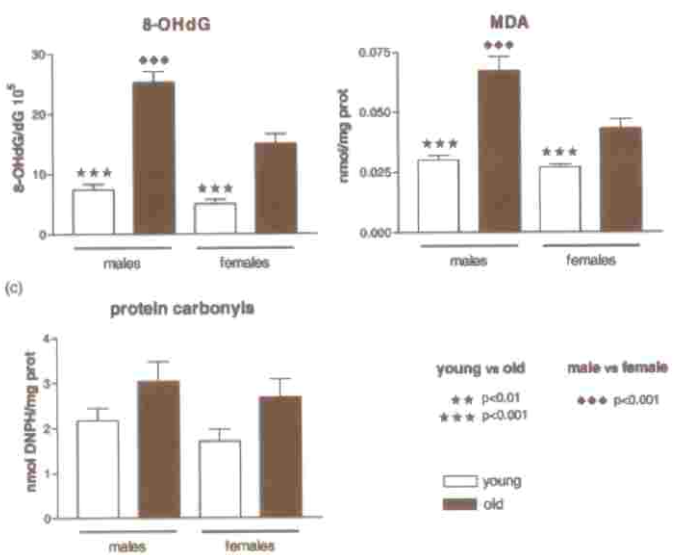

Fig. 1. - *Oxidative damage markers present in various macromolecules (8-OHdG in DNA; MDA in lipids; protein carbonyls in proteins).*

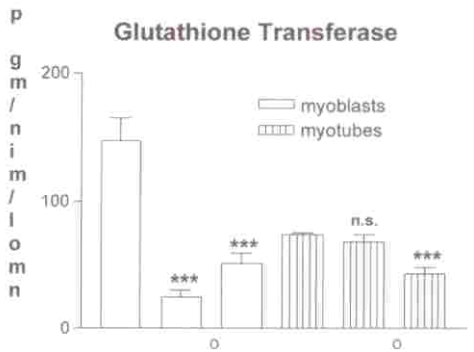

Fig. 2. - *Specific activity of Gluathione transferase enzyme in myoblasts and myotubes derived from skeletal muscle biopsies of young and old subjects.*

dant defence system). Free radicals cause severe damage if they are not promptly eliminated by the action of anti-oxidant agents.

ROS production increases drastically during aging. This increase is most likely due to two factors: (1) an altered function of the respiratory chain and (2) an insufficient functioning of the antioxidant cellular defence mechanisms. Currently, it is generally accepted that free radicals or ROS play a primary role in the ageing process, especially in those tissues in which the generation of free radicals is more pronounced, such as skeletal muscle. This is a consequence of the high level of oxygen consumption, seen in skeletal muscle compared to other tissues, which results in higher concentrations of ROS.

SATELLITE CELLS

The meaning of this phenomenon and its possible agreement with the data inferred from the oxidative damage of the components, especially the lipids of the cellular membranes, it still in need of explanation. In opposition to notions that seemed consolidated until not many years ago, the muscle tissue is certainly not a post mitotic tissue, i.e., unable of self-remodelling in the presence of different stimuli. There is a group of cells, inside the muscle tissue itself, which may be considered as adult staminal cells: quiescent cells able to assume the muscular phenotype, known as satellite cells. If they are present, suitable stimuli, such as autocrine and/or paracrine muscular increasing factors, among these mIGF-I as the more relevant representative, are able to proliferate and differentiate, first as myoblasts and after the fusion, in myotubes.

Their ability to remodel, however, does not remain constant in time. On the contrary, their intervention seems to be led not only by the functional state of the muscle from which they derive, but also by its age. This seems slightly paradoxical for cells capable of being in a "quiescent" state which, according the current meaning of the term, should not resent the effect of possible functional dynamics connected with muscular activity. On the other hand, it seems that accumulation of the damage caused from free radicals, a quick but effective way to represent "the wrinkles" both of age and of activity of the muscle, may produce deep changes also in the ability of satellite cells to repair the damages endured from the fibers.

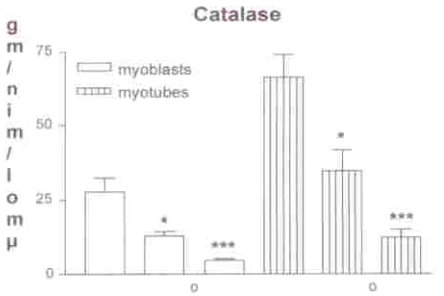

Fig. 3. - *Specific activity of Catalase enzyme in myoblasts and myotubes derived from skeletal muscle biopsies of young and old subjects.*

REPAIR CAPACITY AND OXIDATIVE STRESS

Although different adult stem cell types are present in postmitotic muscle, the satellite cell pool undoubtedly represents the cell typology more directly involved in fiber repair and/or substitution. Thus, it is possible that the decrease in fiber number due to ageing depends, at least in part, on impairment of satellite cell capacity to intervene. In fact, it has been shown that the percentage of this cell population decreases with age from 4-6% in the young to 1-2% in old muscle (8, 9), and that there is a substantial impairment in their proliferative potential and mobility (6).

The potential division number of satellite cells in the elderly does not change, but the rate of growth slows down, as is evident from the decreased number of cells that incorporate bromodeoxyuridine (2). As a result, these "old" cells are still able to form complete myotubes, but at different times and modalities, compared to young satellite cells. Since oxidative damage affects the functional capacity of skeletal muscle (4, 5), we decided to investigate whether the antioxidant capacity of satellite cells, which are located close to the adult skeletal muscle fibres and may be at least indirectly involved in sarcopenia, also undergoes alterations in response to this damage or not. Our results show that at least two enzyme scavenger activities (Cat and GST) decrease with age, similar to findings in adult myofibers *in situ* (Figs. 2 and 3).

CONCLUSION

Our data indicate that the destabilizing oxidative damage observed in skeletal muscle ageing is also applicable to satellite cells that spend their life both anatomically that functionally, close related to differentiated cells. A decrease in the antioxidative capacity of main scavenger enzymes (Catalase and Glutathione-S-Transferase) may be the factor, or at least one of the factors, causing this status. It is possible to speculate that this could be one of the reasons that causes the decrease in muscle repair capacity of satellite cells that is evident during genesis and maintenance of sarcopenia.

SUMMARY

Sarcopenia is a complex process that appears in aged muscle associated with a decrease in mass, strength, and velocity of contraction. This process is the result of

many molecular, cellular and functional alterations. It has been suggested that sarcopenia may be triggered by reactive oxygen species (ROS) that have accumulated throughout one's lifetime.

We found a significant increase in oxidation of DNA and lipids in the elderly muscle, more evident in males, and a reduction in catalase and glutathione transferase activities. Experiments on Ca^{2+} transport showed an abnormal functional response of aged muscle after exposure to caffeine, which increases the opening of Ca^{2+} channels, as well a reduced activity of the Ca^{2+} pump in elderly males. From these results we concluded that oxidative stress play an important role in muscle aging and that oxidative damage is much more evident in elderly males, suggesting a gender difference may be related to hormonal factors.

The progression of sarcopenia is directly related to a significant reduction of the regenerative potential of muscle normally due to a type of adult stem cells, known as *satellite cells*, which lie outside the sarcolemma and remain quiescent until external stimuli trigger as growth factors (IGF-1 or mIGF-1) their re-entry into the cell cycle. One possibility is that the anti oxidative capacity of satellite cells could also be altered and this, in turn, determines the decrease of their regenerative capacity. Data concerning this hypothesis are discussed

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