# Mechanisms of muscle wasting in sarcopenia

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## ABSTRACT

Approximately 66% of the patients with rheumatoid arthritis (RA) have significant loss of cell mass (rheumatoid cachexia), mainly of skeletal muscle (rheumatoid sarcopenia). Sarcopenia is defined as muscle wasting associated with functional impairment. Patients with RA possess significant reduction in muscle strength, caused by muscle protein wasting, and loss of functionality. Various conditions leading to muscle wasting involve different pathways of intracellular signaling that trigger: (i) programmed cell death (apoptosis); (ii) increased protein degradation through autophagy, calcium-dependent proteases (calpains and caspases), and proteasome system; (iii) decreased satellite cell activation, responsible for muscle regeneration. This article aimed at reviewing these general mechanisms of sarcopenia and their involvement in RA. Greater knowledge of these mechanisms may lead to the development of innovative therapies to this important comorbidity.

**Keywords:** muscular atrophy, inflammation, regeneration, rheumatoid arthritis.

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## INTRODUCTION

Rheumatoid arthritis (RA) is a systemic inflammatory disease of unknown etiology with autoimmune manifestations, characterized by symmetrical and erosive chronic synovitis, preferentially affecting peripheral joints.[1] In most patients, the rheumatoid factor can be detected. The prevalence of RA is approximately 0.46% in the Brazilian population[2] and 1% in the world population.[3] The disease affects mainly women aged between 30 and 60 years.

In addition to articular manifestations, RA has several systemic manifestations that significantly influence its morbidity and mortality. Rheumatoid cachexia[4] occurs in approximately 66% of patients with RA, being characterized by cell mass loss, predominantly in the skeletal muscle (rheumatoid sarcopenia), accompanied by maintenance or slight elevation of the fat mass (total of adipose tissue), which results in limited or no weight loss (total mass). The etiology of rheumatoid cachexia is multifactorial, including the increased production of pro-inflammatory cytokines, mainly TNF-α and IL-1β, hormonal changes, and physical inactivity. So far, no standardized therapy has been proposed aiming specifically at that aspect of RA, and the effects of the current treatments have not been well studied.

This study aimed at reviewing the molecular mechanisms involved in sarcopenia, more specifically in rheumatoid sarcopenia. For reviewing the clinical aspects of rheumatoid sarcopenia, the article by Rocha et al.[4] is recommended.

## SARCOPENIA

Sarcopenia is muscle wasting associated with functional impairment. It results from several factors, such as innervation disorders, physical activity reduction, ageing, metabolic abnormalities (especially in proteins, carbohydrates, and lipids), in addition to changes in the activation of satellite cells.[4,5] In RA, the following are believed to play a role in the development of sarcopenia: the action of pro-inflammatory cytokines; the reduction in protein synthesis in myocytes; physical activity limitation; insulin resistance; and inadequate protein ingestion.[6,7]

The diagnosis of sarcopenia can be performed by use of several methods, such as nuclear magnetic resonance, computed

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tomography, bioimpedance, ultrasonography, total body bone densitometry, and anthropometric measures. Densitometry is often used because it provides assessment of the body composition, bone mass, lean mass, and total fat mass.[8] The anthropometric measures proposed by Ashwell, including waist-to-hip ratio, have also been used to assess sarcopenia.[9]

## MOLECULAR MECHANISMS INVOLVED IN SARCOPENIA

The different conditions leading to muscle wasting involve different cell signaling pathways that might lead to programmed cell death (apoptosis), increased protein breakdown, or even decreased activation of the satellite cells responsible for muscle regeneration (Figure 1). Most of our knowledge about those mechanisms derives from studies with experimental models of atrophy, such as models of denervation, hind limb unloading, disuse, fasting,[10] *diabetes mellitus*, and cancer,[11] as well as from studies with muscle biopsies of volunteer patients.[12]

Such mechanisms and the current knowledge about their involvement in rheumatoid sarcopenia are discussed.

### Cell mass loss

Apoptosis is an important process that occurs in multicellular organisms, both during normal development and for maintaining tissue homeostasis.[13] However, the role of apoptosis in post-mitotic tissues, such as skeletal muscle, has not been clarified.

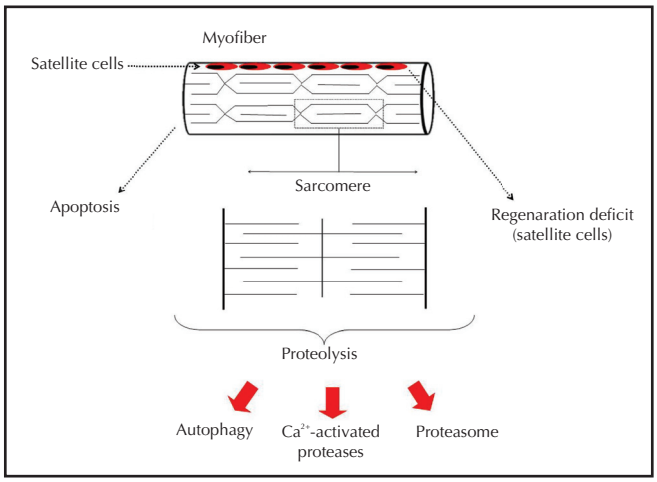


**Figure 1**
Molecular mechanisms involved in sarcopenia. Muscle wasting can occur through distinct mechanisms, such as a deficiency in regeneration due to inactivity of satellite cells, apoptosis, and protein degradation pathways, such as calcium-activated proteases, proteasome, and autophagy.

The initial stage of apoptosis involves the induction of signals of cell death, which cause unbalance in the regulation of free calcium and alteration in the composition of some protein families.[14] After that stage, cell surface receptors or mitochondrial pathways are activated, triggering cytoplasmic and nuclear events that lead to cell death.[15] Caspases are the major enzymes involved in the beginning and development of apoptosis. They account for proteolytic cleavage of a wide range of cell targets,[16] although they do not exclusively initiate that process.[17]

Regarding the potential participation of apoptosis in sarcopenia, even in a model of marked atrophy, such as that of muscle denervation in mice, evidence of significant apoptosis has only been observed after two months, indicating a limited role of that mechanism in the initial stages of atrophy.[18]

### Muscle proteolysis

Sarcopenia is the result of unbalance between protein degradation and synthesis, although apparently the exact contribution to each of those factors varies according to the model studied.

Some proteolytic systems have been described as participating in muscle degradation, and the following are examples: autophagy; calcium-activated proteases, such as calpain and caspases; and the ubiquitin-proteasome system (Figure 1).[19,20]

In *in vivo* experimental models and in humans, there is no consensus about the relative importance of the different protein degradation pathways. Purintrapiban et al.[20] have studied the role of these different mechanisms of proteolysis in muscle cell culture. The inhibition of the calpain, proteasome, and lysosome enzymatic systems caused a 20%, 62%, and 40% reduction in total protein degradation, respectively. However, the participation of each of those pathways varies significantly, depending on the clinical situation involved, such as denervation, immobilization, malignant cachexia, and chronic inflammation.[19–21]

#### Autophagy

Autophagy is a very old mechanism of cell survival that allows cell self-consumption during periods of extreme nutritional deprivation.[22] Such process occurs with the consumption of cytoplasmic components, such as cytosol and cell organelles, and is lysosome dependent. During autophagy, double-membrane vesicles (the autophagosomes) form around large part of the cytoplasm or whole organelles, sequestrating the protein substrates in the vacuolar system. Then, the autophagosome fuses with the lysosome, forming an autolysosome, and then, the substrates are hydrolyzed by lysosomal hydrolases[23]

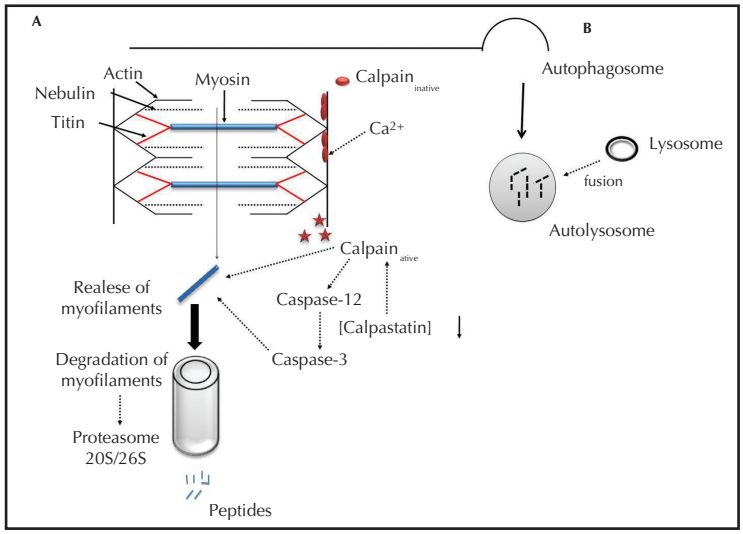


**Figure 2**
Muscle proteolysis pathways. A: activation of calcium-activated proteases. Calpains cleave proteins that anchor the actin-myosin complex, releasing those proteins to be degraded by another cell proteolysis system (proteasome). B: the autophagy system, in which the cytoplasmic constituents are isolated and degraded in an autolysosome.

(Figure 2). Such hydrolases are physically isolated from the cytoplasmic constituents by the lysosomal membrane, and, thus, have greater capacity to degrade cytoplasmic components, as compared with myofibrillar components.[24]

*In vitro*[25] and *in vivo*[26] studies have evidenced the presence of autophagosomes in muscle fibers of myotube culture and in mice. An *in vitro* study with myocyte culture under amino acid restriction has shown that the acceleration of protein catabolism was mainly due to autophagy induction.[25] In an *in vivo* study, Mizushima et al.[26] have shown, through the observation of overexpression of microtubule-associated protein 1 light chain 3 (LC3), the activation of the autophagy system in skeletal muscle of fasting mice. LC3 is essential to maintain membrane integrity and cell growth, and is overexpressed, along with other genes involved with autophagy and muscle wasting, in different models of atrophy,[27,28] in addition to being an indicator of autophagic activity.[29]

Despite the existence of distinct mechanisms of sarcopenia, the pathways that activate the autophagy and ubiquitin-proteasome systems are common. Both pathways involve the forkhead box O3 (FOXO3) transcription factor and the nuclear transcription factor kappa-B (NF-κB). FOXO3 is translocated to the nucleus in the absence of stimuli of protein synthesis,[30] while NF-κB is translocated in the presence of inflammation.[31] FOXO3 has been identified as a critical factor for controlling muscle autophagy,[32] and several genes of autophagy are regulated by that transcription factor.[30]

## Calcium-activated proteases: calpain and caspases

The calpain system is a protein-degradation pathway of eukaryotic cells composed of two enzymes (calpains) and calpastatin. Such proteases are calcium-dependent, non-lysosomal cysteine proteases,[33] and have an endogenous inhibitor, calpastatin, which regulates their activity[21] (Figure 2).

Calpains cannot degrade proteins into amino acids or small peptides and do not catalyze the degradation of the complex of sarcoplasmic proteins. Although they do not directly degrade muscle contractile proteins, calpains cleave the proteins that anchor the actin-myosin complex, releasing the protein components of the sarcomere to be degraded by another cell proteolysis system.[20,34] The substrates of calpain include titin, nebulin, desmin and filamin, proteins that anchor the sarcomere,[33,35] and also troponin and tropomyosin of the sarcomere,[33,36] which release the actin-myosin complex.

Activation of the calpain system has already been shown in several situations of muscle atrophy, such as during prolonged periods of inactivity,[33] ageing, dystrophies, and other pathologies that accompany muscle wasting.[24]

Caspases are non-calcium-dependent cytoplasmic cysteine-proteases that can cleave other proteins after an aspartic acid residue, an uncommon specificity among proteases.[24]

Caspase-3 seems to be able to degrade the actin-myosin complex. Du et al.[37] have shown that purified and activated caspase-3 can cleave actin, breaking the muscle actin-myosin complex, releasing those proteins to be degraded by other proteolytic complexes[16] (Figure 2). Although activated in muscle wasting, the real role of those caspases is still controversial.

## Proteasome

Another proteolytic system related to sarcopenia and currently considered one of the most important is the ubiquitin-proteasome system. This highly conserved system is the major

machinery of non-lysosomal protein degradation in eukaryotic cells[38] (Figure 2).

The ubiquitin-proteasome system is responsible for processing and degrading cell proteins essential for the regulation of development, differentiation, proliferation, apoptosis, signal transduction, and immune and inflammatory response, governing, thus, basic cell processes.[39,40]

Cell proteins destined for degradation by proteasome should be properly labeled with a covalent bond of multiple ubiquitin monomers, peptides composed of 76 amino acids. Ubiquitin can be conjugated with specific protein substrates, a process that requires three enzymes (Figure 3): E1, an ubiquitin-activating enzyme; E2, an ubiquitin-conjugating enzyme; and E3, an ubiquitin ligase. Initially, E1 is activated and, in an energy-dependent reaction, transfers, through E2, ubiquitin to E3, which catalyzes ubiquitin binding to the protein, labeling it for degradation.[41] This process of degradation of polyubiquitinated proteins occurs in the proteasome (20S or 26S), which is a complex composed of one or three large enzymes with the function of degrading unnecessary or damaged cell proteins.[19]

The E3 enzymes provide specificity to the target protein for degradation. Hundreds of different E3 have already been identified, and each one seems to modulate the ubiquitination of a group of protein substrates.[41] In the skeletal muscle, two

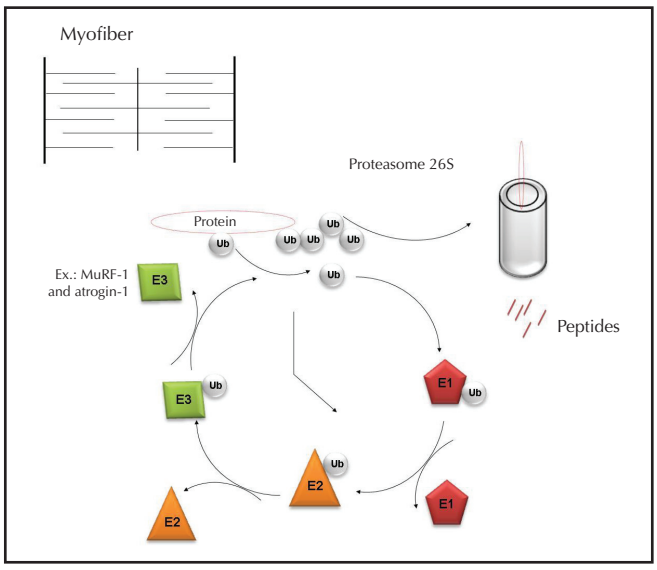


**Figure 3**
Proteasome system of muscle degradation. Ubiquitin-proteasome system is a cytoplasmic multiprotein complex that degrades proteins labeled with ubiquitin. That degradation requires the participation of three distinct proteins (E1, E2 and E3). The E3 proteins provide specificity to the proteins that will be degraded. In muscle atrophy, some E3 enzymes, such as MuRF-1 and atrogin-1, have been described.

specific E3 related to the atrophy process – MAF-bx (muscle atrophy F-box) or atrogin-1, and MuRF-1 (Muscle Ring Finger-1) – were identified.[42] A third E3 ubiquitin ligase, NEDD-4, has been reported, and it seems to facilitate muscle atrophy in denervation and hind limb unloading models.[43]

MuRF-1 is an E3 ubiquitin ligase recognized as a marker of the muscle atrophy process in several experimental models.[44] This protein can bind to titin of the M line,[45] the third in amount among muscle proteins (10%).[46]

Some studies have reported the increased expression of subunits of the proteasome and ubiquitinating enzymes during muscle atrophy,[47] as well as the increase in the expression of E3 ligases in models of denervation, immobilization, food restriction, *diabetes mellitus*, and uremia.[44] Such studies have suggested that muscle wasting is related with the activity of MuRF-1 and atrogin-1 E3 ligases.[44] In murine models, proteasome inhibition can reduce protein degradation during atrophy,[48] indicating an important role of the ubiquitin-proteasome pathway in sarcopenia. Such results, however, cannot be extrapolated to humans.[49] Biological and synthetic inhibitors of proteasome can inhibit the cell cycle and induce apoptosis, preferentially in neoplastic cells.[50]

The role of that pathway in human muscle wasting has been reviewed by Murton et al.,[12] who have suggested that the activation of ubiquitin-ligases, MAF-bx/atrogin-1 and MuRF-1, occurs mainly during inflammatory processes.

Muscle atrophy due to the overexpression of the ubiquitin-proteasome system seems to involve different pathways. Some studies have shown that signaling through the NF-κB pathway, which induces the expression of genes related to the sarcopenia process, such as MuRF-1 and MAF-bx, in addition to pro-inflammatory cytokines. The activation of the NF-κB pathway is involved in the muscle atrophy caused by disuse and cachexia, although its mechanisms have not been completely clarified.[51] There is evidence of the involvement of oxidative stress in such activation.[52] In addition to the NF-κB pathway, the increased expression of MuRF-1 and MAF-bx[31] can also occur via FOXO3[30] and myogenin.[53]

## Satellite cells

In addition to protein degradation, deficiencies in the process of muscle regeneration might also be involved in sarcopenia (Figure 1).

Satellite cells (SC) are quiescent myogenic precursors found in the adult muscle between the basal lamina and the sarcolemma, and have some properties of stem cells.[54] SC can be activated in response to stimuli of growth, remodeling, or

muscle injury.[55,56] When activated, they enter the cell cycle, divide, differentiate into myoblasts, and fuse to form myotubes, which then develop into a new fiber or fuse with already existing muscle fibers to repair damaged myofibers and/or to increase hypertrophy of the muscle fibers.[57]

When activated, SC can be identified by the expression of markers, such as MyoD and myogenin, indicators of SC proliferation and differentiation, respectively.[58]

Some studies have shown that coculture of muscle precursors with macrophages increase proliferation and differentiation of myoblasts, suggesting the involvement of inflammatory mediators in SC activation.[59] Among the inflammatory mediators, TNF-α is increased in muscle tissue after an injury, but also seems to be involved in muscle regeneration.[60,61]

Our group, studying acute inflammatory processes in an experimental model of muscle injury, has demonstrated the important involvement of the local production of nitric oxide in SC proliferation and differentiation.[56,62]

However, little is known about the pathway through which sarcopenia is activated and which initial stimulus triggers the activation of SC in the presence of chronic inflammatory process. There is an apparent contradiction between the increased activation of those regenerative cells and the final result, which is muscle atrophy. Further studies are required to clarify whether that SC activation, which probably occurs as an attempt to regenerate the atrophic muscle, is not sufficient to compensate protein loss, or whether myogenesis is not completed due to, for example, apoptosis.

## SARCOPENIA IN RHEUMATOID ARTHRITIS

Despite the progress in understanding the molecular mechanisms that lead to muscle atrophy in several situations, rheumatoid sarcopenia is still rarely studied. Functionally, patients with RA have a significant reduction in muscle strength, but the muscle contractile velocity and properties remain unaltered.[6] Such data demonstrate that the impact of the disease occurs through protein loss, affecting mainly in-parallel sarcomeres, preserving the number of in-series sarcomeres.

Data on muscle wasting pathways in RA, especially involving apoptosis, are scarce. So far, studies on individuals with RA or on animal models of chronic arthritis demonstrating the real role of apoptosis in muscle wasting are still lacking. Studies carried out in our laboratory have not shown apoptotic bodies or labeling with caspase-3 in the gastrocnemius muscle of mice with collagen-induced arthritis (CIA) (unpublished data), suggesting that such mechanism does not play a striking role in rheumatoid sarcopenia.

Similarly, studies on experimental models or patients with RA assessing the participation of mechanisms of autophagy, activation of calpains, and caspases are still lacking.

Regarding the proteasome pathway, the increase in E3 ubiquitin ligases associated with muscle proteolysis has already been identified in the skeletal muscle of murine arthritis models.[63,64] However, the other components of the ubiquitin-proteasome pathway, such as ubiquitin and proteasomal subunits, and the disease stage at which atrophy develops are yet to be studied. In the ubiquitin-proteasome pathway, an increase in the expression of MuRF-1 and MAF-bx[31] has been observed via NF-κB, FOXO3,[30] and myogenin,[53] but such data have not been confirmed in muscles of individuals with arthritis. Thus, although it is the most studied proteolytic pathway in general, its importance is yet to be confirmed in patients with chronic arthropathy.

Finally, in the atrophy of the gastrocnemius muscle in the model of Freund's adjuvant-induced arthritis (AIA), Castillero et al.[64] have shown the activation and proliferation of SC by use of their myogenin and MyoD markers. Such findings require confirmation in other experimental models, as well as in studies with patients.

## CONCLUSION

We have discussed how several intracellular pathways are involved, in an inter-related manner, with the process of muscle wasting. Such pathways, comprising mechanisms of cell apoptosis, proteolysis of myofibrils, and alteration in cell regeneration through SC, have been actively studied in several clinical and experimental conditions. Those mechanisms are not uniformly present in those conditions, and their relative importance varies significantly according to the clinical situation. Thus, the best preventive and therapeutic management might not also be the same for all situations of muscle atrophy.

Despite the significant muscle wasting that occurs in most patients with RA, with a significant socioeconomic and functional impact on that population, so far no standardized therapy has been proposed for that complication. There are few studies assessing the impact of current therapies on muscle wasting.[4] Similarly, as already discussed, studies on the participation of several pathways that lead to muscle atrophy and regeneration in either experimental models or in patients with chronic arthropathies are scarce. Further studies on those topics are required, because greater understanding about the mechanisms of modulation between muscle catabolism and anabolism will result in the development of innovative and more effective therapeutic strategies and better quality of life for the patients.

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