INFLAMMAGING IN MUSCLE: THE MISSING LINK BETWEEN SARCOPENIA AND IDIOPATHIC INFLAMMATORY MYOPATHIES

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Abstract
Ageing is associated with a progressive decline in muscle mass and quality. Inflammaging, chronic low-grade inflammation is a major causative as well as maintenance factor in age-related disorders. Idiopathic inflammatory myopathies or myositis also exhibit a chronic stage of inflammation caused by various immune and non-immune-mediated processes. This review draws parallels between the mechanisms of inflamaging, sarcopenia, and myositis, and their possible interconnection.

We searched literature on information about myositis, sarcopenia, ageing, inflamaging, and senescence to draw parallels between the mechanisms linking myositis, sarcopenia, and inflamaging. Further, we discuss the evidence base to support that the process of senescence is hastened in an inflamed muscle [1].

Keywords: Muscle, ageing, senescence, metabolism, myositis, sarcopenia
Introduction
Sarcopenia or muscle failure is a generalized and progressive muscle disorder causing accelerated loss of muscle strength, muscle mass, and muscle function leading to increased risk of falls, fractures, morbidity, and mortality [2]. Multifactorial causation is associated with sarcopenia including lack of exercise, hormonal and cytokine changes as well as chronic low-grade inflammation [3,4].

Idiopathic inflammatory myopathies (IIMs) are chronic systemic autoimmune conditions characterized by muscle weakness, myalgia and organ involvements like skin, heart, lungs, and joints. A fraction of this muscle weakness in IIMs is imputable to active inflammation whereas atrophy and damage are responsible for the remaining part of the muscle weakness. The precise pathophysiology behind the atrophy and damage is not clear. Fibrosis is one aspect of this mechanism. Accelerated ageing is the other proposed cause. The presence of chronic muscle weakness and atrophy hints toward the presence of sarcopenia in the muscle. Although standalone myositis and myositis with sarcopenia are difficult to distinguish, when present together or in a sequence of myositis leading to increased senescence in the same muscle, it will further add to disability and morbidity caused by each condition. This would lead to poorer quality of life, and potentially even an increased number of falls and accidents as a result of accelerated ageing in inflamed muscles. The harsh fact encountered in the clinics is that muscle strength lost in myositis is often not fully regained after controlling the disease because of irreversible damage and atrophy caused by fibrosis and inflammation. A comprehensive approach for the treatment of IIMs can be designed considering sarcopenia-related treatment options.

Conversely, sarcopenia has also been recently explained using inflammaging pathways which brings the case for the possibility of intricate links between the two conditions. Because both IIMs and sarcopenia have a coexisting role of inflammation and similar symptoms on presentation, it creates a basic premise to explore if accelerated ageing and sarcopenia have a role to play in the disease pathophysiology of IIMs. This article aims to assess the similarities in the mechanism of sarcopenia and IIMs and highlight the presence of accelerated ageing in IIMs.

Sarcopenia
According to the European Working Group on Sarcopenia in Older People (EWGSOP) definition, sarcopenia is muscle failure associated with reduced muscle strength and is graded based on 3 variables: muscle strength, muscle quality or quantity, and physical performance [5]. Although sarcopenia is generally associated with ageing there have been studies relating sarcopenia with birth weight, strength peak in adult life, and the number of primary and secondary muscle fibers [6]. The main triggers for sarcopenia are malnutrition, decreased physical activity, age-associated reduced levels of anabolic hormones like growth hormone, insulin-like growth factor (IGF) and sex hormones (testosterone and estrogen), derangement of muscle growth regulators like myostatin, activins, and bone morphogenic proteins (BMP) age-related neuromuscular junction degeneration [7].

Mechanisms of Sarcopenia
The imbalance between muscle protein anabolic and catabolic pathways leads to a gross loss of skeletal muscle with age. Multiple mechanisms of action for sarcopenia have been proposed (Figure 1 and 2): cellular senescence, mitochondrial dysfunction, hormonal changes, satellite cell dysfunction, apoptosis, disuse atrophy, microvascular changes, inflammation, motor neuron loss, and oxidative stress play an important role in the loss of muscle mass [2]. Similarly, an imbalance in the main cellular degradation pathways, the ubiquitin/proteasome, and the autophagy/lysosome systems, as well as the impairment of protein degradation pathways also accelerate sarcopenia [8].

Protein breakdown occurs by activation of the ubiquitin/proteasome pathway and caspases under transcriptional control of the transcription factors forkhead O (Fox-O) and nuclear factor (NF)-κB. The myostatin pathway is also an important contribution to atrophy.
Inflammaging in muscle: the missing link between sarcopenia and idiopathic inflammatory myopathies

Figure 1. Mechanism of Inflammaging

Figure 2. Mechanism of Sarcopenia

1) Cellular senescence

A permanent cell cycle arrest or cellular senescence is driven by a variety of stressors like DNA damage, telomere shortening, mitochondrial dysfunction, and oxidative stress. These stimuli regulate the cell cycle through CIP/KIP and INK4A pathways by hyperphosphorylation of the Retinoblastoma gene and cell cycle exit. In sarcopenia, satellite cell senescence results in cell cycle arrest [9].

2) Inflammaging and Senescence-associated secretory phenotypes (SASPs)

Inflammaging is known to contribute to many age-related disorders [10]. Ageing is associated with both quantitative and qualitative loss of muscle tissue. The enhancement of the proinflammatory state with age is responsible for deficits in maximum strength and functional capacity [11]. Chronically elevated inflammatory cytokines have been shown to predispose sarcopenia through increased activation of the ubiquitin-protease pathway [12,13]. The ubiquitin-proteasome system degrades myofibrillar proteins, but the precise role of these cytokines in sarcopenia is not known [14]. The Longitudinal Aging Study of Amsterdam showed that raised levels of interleukin-6 (IL-6) and C-reactive protein (CRP) were correlated with increased risk of loss of muscle strength [15]. Multiple other cross-sectional and longitudinal studies have revealed that inflammatory markers, such as IL-6, TNF-α, and CRP, increase during ageing and correlate with disability, mortality, and the decline in muscle mass, muscle strength, physical performance, and physical function [15-17].

Senescent cells develop an altered secretory activity which induces changes in the tissue microenvironment. These cells secrete various chemokines, inflammatory cytokines, and matrix remodeling proteins which disrupt the tissue structure and function. Different SASP proteins include IL-6, IL-8, IL-1, TNF-α, matrix metalloproteinases (MMP-1 and 3), monocyte chemotactic proteins (MCP), IGF binding proteins, plasminogen activator inhibitor-1, granulocyte-macrophage colony-stimulating factor (GM-CSF), growth regulated oncogene (GRO)α, and C-reactive protein (CRP). These cytokines not only predispose
to sarcopenia but also play an important role in the induction and maintenance of inflammaging [18].

Drugs targeting senescent cells are known as senolytic drugs. These drugs have been shown to reduce inflammaging and SASPs. Dasatinib and quercetin have been proven to decrease senescent cell burden in humans [19].

3) Mitochondrial Dysfunction

In sarcopenia, imbalance in redox homeostasis causes the oxidation of cellular contents like protein, lipids, and DNA. Mitochondrial turnover is maintained by fusion, fission, mitophagy, and mitochondrial biogenesis. Reduced mitophagy and biogenesis along with unbalanced fusion and fission induce the accumulation of dysfunctional atypically large mitochondria [20]. Mitochondrial dysfunction is characterized by abnormal calcium homeostasis, increased ROS, and decreased ATP production. This makes the muscle more susceptible to apoptotic loss of muscle cells [9].

4) Satellite cell dysfunction

Skeletal muscle cells have the potential to regenerate by activation of skeletal muscle stem cells, the satellite cells. These cells rest in the quiescent stage unless activated by a stimulus in mature muscle. Loss of satellite cells or their function impairs muscle fiber regeneration leading to sarcopenia. Satellite cells play a role only in muscle cell regeneration and muscle mass maintenance following muscle injury because depletion of satellite cells in adult mice did not result in muscle atrophy [21]. Skeletal muscles go through multiple injuries or stressors and hence satellite cells play a crucial role in muscle mass maintenance.

5) Myostatin Pathway

Myostatin, a member of the transforming growth factor β (TGFβ) family is secreted by muscle cells, circulates in the blood, and acts locally as a negative muscle mass regulator by downregulating the Akt/mTOR pathway and by decreasing the number of satellite cells [16]. Although multiple recent studies reported either no change or even reduced myostatin levels in the elderly, myostatin inhibitors have proven to promote muscle growth in the elderly [16].

GDF 11, another TGFβ member, can also potentially cause sarcopenia. GDF11 and myostatin have a lot of structural similarities and hence often have cross-reactions with detecting antibodies. GDF 11 levels are reported to increase with ageing [22].

6) Protein Homeostasis

Imbalance in protein homeostasis occurs as a result of an abnormality in signaling pathways controlling protein synthesis and degradation.

Protein Synthesis- The IGF1/AKT/mTOR pathway is a major regulatory pathway controlling protein turnover in skeletal muscles. Along with protein synthesis, this pathway also suppresses protein breakdown by negative control of FOXO transcription factors which are required for ubiquitin-proteasome and autophagy systems. Although reduced IGF 1 levels were found in old rats, there was no change in levels of IGF1 in recent human studies indicating pathways independent of the mTOR/AKT are responsible for sarcopenia [8]. However, growth-promoting stimuli like exercise and nutrition in the elderly do not effectively stimulate the AKT/MTOR pathway resulting in the decreased recovery of muscle following micro-injuries [8].

Protein degradation occurs by ubiquitin-proteasome and autophagy pathways. Repeated ubiquitin conjugation at Lysine-48 or 63 positions results in the formation of a polyubiquitin chain on a target protein [23]. These polyubiquitinated proteins are then degraded by proteasomes. Proper functioning of this pathway and autophagy is required to maintain protein quality, thereby delaying sarcopenia. Multiple mechanisms converge down to inflammaging which is a key contributor to the development of sarcopenia [10,24,25].

Histopathology of Sarcopenia

In Sarcopenia there is a reduction in the size and quality of the muscle. Muscle fibers are replaced with fat, there is increased functioning of fibrosis pathways, changes in muscle metabolism due to oxidative stress, and degeneration of neuromuscular junction [3]. Sarcopenia majorly affects fast-twitch fibers (type 2) more than slow-twitch (type 1) [26]. There is a reduction in muscle fiber number as well as in their size. Factors such as lifestyle, hormones, inflammatory cytokines, and genetic factors are responsible for these histological changes [3].
Evidence of Sarcopenia in Autoimmune Disorders

In this regard, it should be mentioned that several rheumatic autoimmune diseases, such as primary Sjogren’s syndrome [27], systemic lupus erythematosus [28], systemic sclerosis, spondyloarthritis, rheumatoid arthritis and vasculitis [29], present with sarcopenia.

Such findings are directly related not only to fat distribution, but also to elevated serum IL-6 levels and displays a response to treatments targeting inflammatory cytokines [30]. In these patients, burdened by long-term, systemic diseases, sarcopenia represents a major concern, being associated with the development of osteoporosis and frailty fractures.

Pathophysiology of Idiopathic inflammatory myopathies

Although considered heterogeneous disorders, IIMs usually present a typical inflammatory muscle involvement. Dermatomyositis (DM), polymyositis (PM), anti-synthetase syndrome (ASS), necrotizing myopathy (NM), and inclusion body myositis (IBM) all present with muscle weakness. In DM, autoantibody-mediated early activation of the C5b-9 membrane attack complex (MAC) leads to necrosis and ischemia, which appear similar to microinfarcts [31]. The MAC also stimulates the release of proinflammatory cytokines, facilitating the migration of activated lymphocytes, like B cells, CD4 cells, and plasmacytoid dendritic cells, to the perimysial and endomysial spaces [31]. Conversely, in PM and IBM, CD8+ T cells surround and invade healthy-appearing, non-necrotic muscle fibers that aberrantly express MHC class I [31]. Although inflammatory myositis is considered an immune-mediated condition, recent literature suggests a major role of non-immune mechanisms in the pathophysiology of IIMs (Figure 3). Non-immune mechanisms include endoplasmic reticulum (ER) stress, autophagy, hypoxia mitochondrial dysfunction (ROS Pathway), and myostatin pathway link muscle inflammation to damage and atrophy [32].

1) Endoplasmic Reticulum (ER) stress

ER, in addition to being a reservoir of calcium for muscle contraction, controls protein maturation and folding. Disruption in ER homeostasis due to persistent cytokine exposure, viral infections, abnormal calcium metabolism, and a high-fat diet leads to ER stress [33]. It is initiated by overexpression of MHC 1 in muscle fibers. Overaccumulation of MHC-I molecules within the sarcoplasmic reticulum of myocytes triggers an ER stress response through the UPR (unfolded protein response) and the EOR (ER-overload response) mechanisms, leading to the upregulation of glucose-regulated proteins (Grps) through activation of NFκb pathway [32]. The prolonged ER stress further activates multiple apoptotic pathways through mediators like CHOP (C/EBP homologous protein) and caspase 12 [32].

2) Autophagy

Autophagy is an intracellular catabolic process that clears unnecessary cellular materials with the help of lysosomes. Dysfunction leads to autophagic cell death. Overexpression of TLR 3 and 4 which are potent stimulators of autophagy is seen in IIMs [34]. The accumulation of amyloid by defective autophagy reduces muscular peak force and lowers calcium peak in mouse models of inclusion body myositis [34]. Lysosomal degradation defects are more pronounced in sIBM leading to increased and defective autophagy.

3) Hypoxia

Muscle histology shows a lesser number of capillaries in IIM patients indicating reduced blood supply and the presence of hypoxic conditions. Lower oxygen levels result in lesser ATP generation, thereby causing muscle weakness [32,34].

4) Mitochondrial dysfunction

ER stress can induce abnormal cellular respiration and mitochondrial dysfunction leading to the production of reactive oxygen species (ROS). Elevated ROS leads to oxidative damage and disruption of mitochondrial membrane potential impairing ATP generation. Mitochondrial dysfunction can also be induced by epigenetic modifications associated with coxsackievirus infection. These changes increase the expression of Harakiri (HRK) a pro-apoptotic mitochondrial gene. Increased levels of harakiri are responsible for impaired plasma membrane repair in the muscle which further causes the release of endogenous TLR agonists in the plasma. TLR 7 agonist is majorly responsible for high levels on HRK in IIMs [35].
5) Myostatin Pathway

The myostatin signaling pathway is upregulated in certain subtypes of IIMs like IBM and PM [36,37]. High myostatin levels in an active disease also stimulate the proliferation of muscle fibroblasts and the production of extracellular matrix proteins [38,39]. This is achieved by the production of TGF-b signal transducers Smad2 and Smad3 in response to myostatin. Smad 3 is further responsible for the proliferation of muscle fibroblasts [38,39]. A study systematically analyzed the maturation and secretion of myostatin precursor MstnPP and its metabolites in a human muscle cell line where they found that increased MstnPP protein levels induce ER stress. ER stress leads to increased production of ROS and ROS further promotes atrophy of skeletal muscle [40]. Reversing muscle atrophy, be it age-related or disease-mediated, is the most important unmet need. Bimagrumab, anti-ActRII antibody, although well tolerated failed to improve patients' functional profile and mobility in a 2 year long clinical trial [41]. On the other hand, Follistatin gene therapy trial of 6 patients showed marked improvement in clinical symptoms as well as histology of the muscle in terms of reduced fibrosis and increased muscle mass. Further studies on myostatin inhibitors like Follistatin and Decorin are required to assess and confirm their beneficial effects in myositis.

6) Other Mechanisms

It is noteworthy that muscle weakness and metabolic disturbances were detected prior to the appearance of infiltrating mononuclear cells in mice models of myositis. Muscle weakness corresponds to decreased AMP deaminase (AMPD1) expression. With the progression of myositis fast-twitch fibers are converted to slow-twitch fibers. Purine nucleotide pathway genes like AMPD1 and glycolysis genes are found to be suppressed in myositis [42]. Decreased phosphorus creatinine/Phosphate ratio has also been demonstrated in myositis muscle depicting defective muscle metabolism. Serum metabolomics profiles found considerable shift to anaerobic metabolism and increased muscle catabolism in IIM patients [43].

Histology of IIMs

The biopsy of inflamed muscle in PM includes muscle fiber invasion of CD8+ lymphocytes with degenerating and regenerating myofibers. DM shows perifascicular atrophy along with perivascular inflammation with CD4+ lymphocytes, B cells, and plasmacytoid dendritic cells. The presence of lined vacuoles and deposits of amyloid and ubiquitin is seen in IBM [44]. In advanced cases, biopsy findings are similar to sarcopenia, like intense atrophy and fibrosis. Muscle atrophy can also be appreciated on ultrasound as hyperechoic decreased muscle mass [44,45].

Links between Sarcopenia, Inflammaging and IIMs

Sarcopenia, Inflammaging, and IIMs are all related to each other. Multiple similarities in their mechanisms can be deduced from Figure 1,2 and 3 with inflammaging as a link between IIMs and sarcopenia (Figure 4 and 5). As there is increasing evidence of a prominent role of inflammation in the ageing muscle, an inflamed muscle can serve as a nidus for sarcopenia and atrophy. Multiple serum inflammatory markers, such as H2O2, TNF alpha, IL1 beta, hsCRP rise with ageing, while, conversely, several growth factors (IGF and PDGF) decrease, displaying an inverse correlation with IL 1 beta and TNF alpha [11].

Figure 3. Mechanism of Myositis
At the same time, inflammation occurring in several systemic disorders may lead to fibrosis and premature ageing, as well as early fibrosis, atrophy and muscle weakness, all features of sarcopenia. A study of the myostatin follistatin system has also evidenced that elevated levels of myostatin, present in active myositis, increase the hazard of early senescence in inflamed muscle. Furthermore, the serum myostatin: follistatin system reflects early senescence events in the muscle [37]. Even after recovery from an acute exacerbation of IIM, muscle failure continues. It is due to the presence of other risk factors for sarcopenia like decreased appetite and protein intake (as a result of dysphagia), reduced physical activity, and corticosteroid therapy.

![Figure 4. Relation between IIMs, Inflammaging and sarcopenia.](image)

Glucocorticoid-induced osteoporosis is the most common cause of secondary osteoporosis and up to 30% of patients treated with GCs [46] display a significant loss of bone mineral density. GCs impair both bone metabolism and bone mineralization by transrepression of osteocalcin and collagen I, but also cause steroid myopathy, which is an adverse event on muscle mass and muscle strength and increase itself fracture risk by enhancing the risk of falls. Corticosteroids produce oxidative stress which results in increased myosin degradation by ubiquitination of myosin [47]. Thus, corticosteroids which are used in the treatment of IIMs can themselves induce sarcopenia in the patients. As a result, residual weakness in patients with IIMs is commonly due to sarcopenia rather than active inflammation.

### Possible Therapeutic Strategies

Besides the mainstay treatment of IIM which includes glucocorticoids, DMARDs (methotrexate and azathioprine), and biologics like rituximab and anti TNF therapy [44], the myostatin signaling pathway (Figure 6) has recently emerged as a major target for symptomatic treatment of muscle atrophy [40].

Bimagrumab/ BYM338 (human anti-ActRII antibody) blocks the binding of myostatin to its activin 2 receptor [48]. Bimagrumab prevents activin A-induced atrophy through inhibition of Smad2/3 phosphorylation, sparing the degradation of the myosin heavy chain. Thus, Bimagrumab can be used to target the impaired regeneration potential in myositis, by inhibiting muscle atrophy and stimulating muscle hypertrophy [49].

Follistatin, a potent inhibitor of myostatin can be delivered by adenovirus-mediated gene therapy. A study showed promising results for Follistatin gene therapy for mild to moderately affected, ambulatory sporadic inclusion body myositis patients. Treatment with Follistatin caused decreased fibrosis and improved regeneration [50]. Follistatin can be boosted by intense exercise, whereas myostatin and other chemokines are required to be inhibited by blocking agents [36].

Although certain interventions like Vitamin D in older females and testosterone in older males showed improvement in muscle mass [51], no drug has yet been approved for sarcopenia, physical exercise along with appropriate nutrition remains the best strategy against sarcopenia. Older people should be recommended to engage in a balanced...
program of both endurance and resistance training, performed on a regular schedule. Both exercise regimens act on most signaling pathways involved in sarcopenia like Akt/mTOR, NfkB, and FoxOs [52]. As exercise has proven to be beneficial in patients with myositis also, it is the best therapeutic tool to improve patients having both, sarcopenia and myositis.

**Figure 6.** Therapeutic interventions in myostatin pathway. M- Myostatin, B -Bimagrumab/BYM338

Bimagrumab is a ACTIVIN 2 receptor blocker. Follistatin binds to myostatin, bound myostatin cannot activate the Activin receptor

**Conclusion**

Non-immune mechanisms similar to those seen in sarcopenia and inflammaging are related to the pathophysiology of IIMs. An inflamed muscle does serve as a nidus for early sarcopenic changes by increased functioning of the myostatin pathway accompanied by raised serum inflammation markers. Although the mechanisms of IIM, sarcopenia and inflammaging are not fully elucidated, both IIMs and sarcopenia share inflammatory pathways that lead to accelerated senescence, new treatment targets beyond immunosuppression are a promising field in IIMs. Currently, it is well established that physical exercises prevent inflammaging and are a common treatment strategy for both sarcopenia and IIMs, therefore they should be encouraged in these patients. Corticosteroids can potentially induce sarcopenia while treating IIMs. Ageing in a muscle can be slowed down effectively but requires further investigation in pathways of muscle degeneration.
Inflammaging in muscle: the missing link between sarcopenia and idiopathic inflammatory myopathies

and fibrosis and translational work to demonstrate in vitro and subsequently in vivo utility.

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