

# The pathogenetic bases of sarcopenia

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## Summary

**Aging is accompanied by involuntary loss of skeletal muscle mass, strength and function, called sarcopenia. The mechanisms underlying the development of sarcopenia are not completely understood and most likely multi-factorial, but significant progress has been made over the past few years to identify some of the major contributors.**

**Besides life style-related factors, as diet and physical activity, sarcopenia seems to be also determined by hormonal dysregulation, chronic inflammatory status, ectopic adipose tissue accumulation, neurological and vascular changes associated with aging.**

**The present mini-review focused on the basic factors that primarily impact muscle homeostasis in older subjects.**

**A better understanding of cellular mechanism leading to sarcopenia is required to establish evidence-based intervention in order to prevent onset of symptoms associated with sarcopenia and to extend the time free from disability in older adults.**

*KEY WORDS: sarcopenia; aging; chronic inflammation; muscle lipotoxicity.*

## Introduction

Numerous clinical studies proved the presence of an involuntary loss of skeletal muscle mass and strength loss with aging, starting in the fifth decade of life (1). This progressive age-related muscle wasting process, called sarcopenia, is associated with an increased risk of falls and fractures (2), disability, loss of functionality and independence in older individuals (3), leading to increased morbidity and all-cause mortality (4). As the population over 60 years of age is expected to triple in the next fifty years (5), there is an in-

creased interest on the necessity of healthy ageing, not only to prevent many chronic diseases, but also to maintain elderly subjects free from disability. Although the term "sarcopenia" has been used over the last two decades, is in 2010 that the European working group on sarcopenia (EWGSOP) defined it as a *syndrome characterised by progressive and generalized loss of muscle mass and strength with the risk of adverse outcome, such as physical disability, poor quality of life and death* (6). Thus, the working definition requires the presence of both low muscle mass plus low muscle function (strength or physical performance) (Table 1). From a histopathological muscle-specific point of view, sarcopenia is characterised by the atrophy of type II fibers, necrosis and reduction of cross-bridging elements between fibers, smaller and fewer mitochondria (7). Even though there are still few data on the specific mechanism of human muscle aging, significant progress has been made over the past few years to identify some of the major contributors to this multifactorial process (Figure 1). A better understanding of the cellular mechanism driving these changes is required in order to establish evidence-based interventions to prevent the onset of symptoms associated with sarcopenia and to promote healthy ageing. Hence, this review focuses on the basic factors that primarily influence muscle homeostasis in older individuals.

## Inactivity and bed rest

Older adults are more likely to have prolonged periods of inactivity or bed rest, in particular related with hospitalization. Several studies showed that bed rest (with or without hospitalization) may contribute to functional impairment in older patients, as inactivity could compromise muscle metabolic homeostasis (8-10). Kortebein et al. reported that healthy older volunteers lost almost 1 kg of lean leg mass after only 10 days of bed rest, with an associated 16% decline in isokinetic knee extensor strength and a significant decrease in protein synthesis (8). This marked loss of muscle mass was greater than that observed in young healthy individuals after 28 days of bed rest, whereas the decline in protein synthesis and strength was similar to that of younger participants evaluated after 14 day of inactivity (11). Moreover, in another study, the same group found that 10 days of bed rest result-

Table 1 - EWGSOP criteria. Diagnosis is based on documentation of criterion 1 *plus* criterion 2 *or* criterion 3 (adapted from *Cruz-Jentoft AJ and Morley JE, Wiley-Blackwell Ed. 2012*).

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### Criteria for the diagnosis of SARCOPENIA

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1. Low Muscle Mass
  2. Low Muscle Strength
  3. Low Physical Performance
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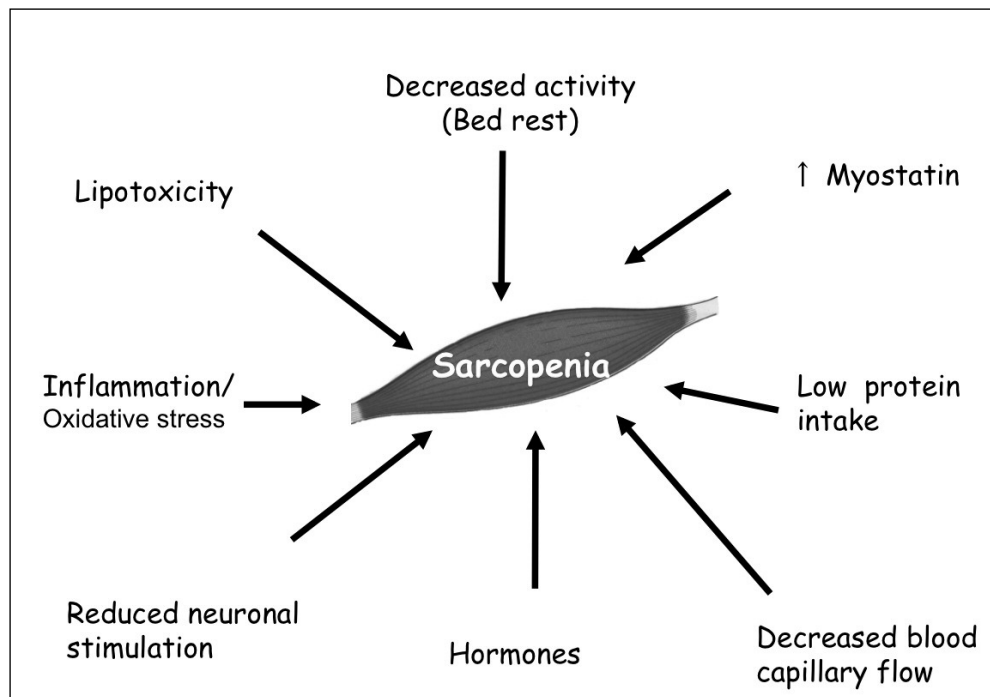


Figure 1 - Main mechanisms involved in sarcopenia. The etiology of sarcopenia is multifactorial; the control of overall muscle mass is practically due to an imbalance between the rates of muscle protein synthesis and breakdown.

ed in a substantial loss of lower extremity strength, power and aerobic capacity with a reduction in physical activity, with no effect on physical performance (12). Additionally, Drummond et al. demonstrated a significant bed rest-induced reduction in total lean mass in a group of 6 healthy volunteers (mean age 67.2 years) after 7 days of bed rest (10). Moreover, this study links this result to the impaired response to amino-acids supplementation, as bed rest seemed to attenuated the amino acid-induced increase in muscle protein synthesis with a mechanism involving reduced mTORC1 signalling and amino acid transporter protein content. Nevertheless, it has been demonstrated that short-term bed rest in older overweight volunteers determined a significant decrease in hepatic and peripheral insulin sensitivity, which could further negatively influence muscular homeostasis (9). Additionally, muscle levels of TLR4 protein expression and IL6, nuclear factor-kB1, IL10 and IL15 mRNA expression were increased after short-time bed rest in healthy older adults, while serum samples of IFN-gamma and macrophage inflammatory protein – 1 beta (MIP-1β) were elevated (13). This increased expression of local pro-inflammatory mediators, may be an important factor predisposing to muscle catabolism response triggered by concomitant acute disease and bed rest inactivity in older adults. In fact, in a recent study, Puthuchery et al. (14) observed a significant muscle mass loss among 63 critically ill patients (mean age 55 years) admitted to an intensive care unit. This wasting process was early and rapid during the first week of critical illness and was more severe among subjects with multi-organ failure and higher inflammatory status. Additionally, the net leg protein balance was catabolic, despite the administration of enteral nutrition (14).

### Nutrition

Older people need to make up for age-related changes in

protein metabolism, such as high splanchnic extraction and declining anabolic response to ingested proteins. Additionally, aging is characterized by the reduction of nutrients intake, known as “anorexia of aging”, representing a physiological feature of old age associated with decreased energy expenditure and loss of muscle mass (15). They also need more protein in order to counteract inflammatory and catabolic conditions associated with chronic and acute diseases. All these aspects contribute to the development of sarcopenia, as observed in the Health ABC study population. In this study, the participants that had the highest intake of protein presented approximately 40% less lean mass waste after the 3 years of observation. For this reason, the PROT-AGE study group recommended average daily intake in the range of 1.0 to 1.2 g protein/kg body weight/day, and higher protein intake (i.e., ≥ 1.2 g/kg body weight/day) was advised for subjects with high physical activity level (16). Moreover, protein quality, timing of ingestion, and intake of other nutritional supplements may be relevant, but evidence is not yet sufficient to support specific recommendations. To this date, there are a few studies regarding the effect of protein intake on muscle mass and function, most of them using a combined intervention, including protein supplementation and resistance exercise. So far, evidence from long-term intervention studies shows no clear benefit of dietary protein supplementation on skeletal muscle mass in elderly people. Yet, Tieland et al. showed that 24 weeks of protein supplementation in frail older adults improved physical performance, that might be attributable to improvements in neuromuscular action or muscle quality (17). On the other hand, Daly et al. (18) showed that 1.3 g protein/kg body weight/day supplementation was effective in enhancing the effects of resistance exercise training on muscle mass and strength in 100 elderly women (60 to 90 years). Other studies investigated the role of essential amino acids (EAA) supplementation on muscle homeostasis, combined or not with exercise training. Dillon et al. (19) proved that EAA improved lean body mass

and basal muscle protein synthesis in older individuals, while Kim et al. (20) suggested that EAA and exercise together may be effective in enhancing not only muscle strength, but also physical performance as evaluated by walking speed in a population of sarcopenic women. Further studies are needed in order to evaluate long-term benefits determined by long term protein supplementation.

### Chronic inflammation

It has been demonstrated that inflammation, along with oxidative stress, increase with aging both are considered significant contributors to age-related muscle wasting process (21, 22). Hence, some studies reported that high IL6 and C-Reactive Protein (CRP) levels are associated with increased risk of muscle mass and strength loss (23, 24). Nevertheless, only few studies evaluated loss of muscle strength in relation with inflammatory status in hospitalized elderly patients (25, 26). For example, in a study with 620 patients (mean age 56.4 years), Norman et al. (25) showed that CRP, as an indicator of acute inflammation, was an independent predictor of grip strength even after adjustment for relevant confounders, such as age, gender and body composition. Moreover, Bautmans et al. (26) proved that geriatric hospitalized patients with inflammation showed significantly worse muscle function, which did not improve during hospitalization despite adequate treatment of the primary disease. Reduced strength and fatigue resistance were significantly related with circulating CRP, IL6 and fibrinogen levels. Moreover, some studies proved that elevated levels of tumour necrosis factor-alpha (TNF-alpha) can increase muscle catabolism by suppressing the Akt/mTOR pathway (28). In addition, it seems that cytokines may antagonize the anabolic effect of Insulin Growth Factor-1 (IGF-1), because of the development of growth hormone resistance, which decreases both circulating and muscle IGF-I (27, 28). The relation between inflammation, muscle strength and muscle mass seems to have a pathogenetic explanation based on the effect of inflammation on the balance between protein synthesis and protein catabolism, at the muscle level associated with the presence of CD68+ macrophage infiltration. Moreover, skeletal muscle aging is associated with myofibrosis and myosteatosis and to gradual fibrous replacement in the muscle (29). In addition, recent histological studies have shown that high values of serum CRP seem to be related with reduced protein synthesis and increased protein catabolism (30).

### Lipotoxicity

With aging fat mass increase is associated with ectopic adipose tissue deposition and lipid deposition is modified with increased ectopic adipose tissue deposition, including muscle (31). This age-associated fat depots seems to act synergically with sarcopenia and the ageing process should be considered as a physiological degenerative process potentially accelerated by concomitant lipotoxic insults (32). Some studies proved that the increased accumulation of ectopic fat inside the muscle in older persons is independently associated with metabolic abnormalities as insulin resistance, and with reduced strength and performance (33). Moreover, Bollheimer et al. (34) showed a strong negative correlation be-

tween muscle volume and myosteatosis with proves of a blunted muscular protein biosynthesis in old high-fat diet fed rats. In addition, in a recent *in vivo* study, Tardif et al. (35) registered lipid redistribution and ectopic lipid accumulation into the muscle of diet-induced obese old rats. The same study showed that insulin resistance observed in these animals was independently related to intramuscular fat accumulation, and that it could be induced by high ceramide synthesis, stimulated by adipocyte-derived cytokines, including TNF-alpha and that decreases the ability to upregulate muscle insulin pathway, in old high-fat diet fed rats. Furthermore, Zhou et al. (36) showed that high fat fed mice presented increased muscle protein catabolism and this was associated with an increase in plasma free fatty acid and a decrease of adiponectin plasma levels. In fact, free fatty acids increased protein degradation of C2C12 skeletal muscle cells and this process was blocked by adiponectin over-secretion, mainly by attenuating the E3 ubiquitin ligase activation by increasing both insulin receptor substrate 1 tyrosine phosphorylation. Hence, this study suggested that the balance between free fatty acids and adiponectin impacts muscle proteolysis in insulin-resistant condition. Additionally, ectopic fat accumulation into the muscle determines mitochondrial changes determining a reduction of fatty acids oxidation capacity. Free fatty acids negatively influence protein anabolism, which becomes less sensitive to nutritional state in old high-fat fed animals (35).

### Other mechanism involved in sarcopenia

Nevertheless, muscle homeostasis relies also on the status of motor units, neuromuscular junctions, circulation factors and hormones.

It has been shown that the loss of muscle strength is associated with age-related changes in motor units, mainly due to the age-dependent reduction in motor axon conduction velocity and the number of myelinated axons (37). Moreover, aging is associated with a reduction in motor unit reinnervation after denervation, specially in type II muscular fibers, and when denervation outpaces reinnervation, it triggers muscle loss by an increase in apoptotic potential of myocytes due to the loss of trophic factors. In particular, Kulakowski et al. (38) showed, using an animal model, that TrkB might play a role in synaptic stabilization and synaptic potentiating of the neuromuscular junction. Thus, reduced TrkB expression resulted in precocious neuromuscular aging of the predominantly slow-twitch soleus muscle, while methods to maintain TrkB-mediated signaling may be a potential treatment for sarcopenia.

Aging determines changes in microcirculation and ultrastructure of the vascular endothelial function, mainly due to an age-related reduction in vasodilatory capacity and capillarization (39) that leads to a reduction in oxygen, energy sources and metabolites exchanges in muscle. This may explain the decline in exercise induced blood flow observed in elderly patients (39). Recently, several studies highlight the potential contribution of inflammation and oxidative stress to an impaired endothelial responsiveness (40). TNF-alpha, in particular may reduce endothelial-dependent dilatation by disrupting intercellular communication (41).

Hormones and growth factors represent other important contributors of the maintenance of proper cellular functions, in particular, they are associated with the trophic state of my-

ocytes. It has been demonstrated that aging is associated with a decline in sex hormones, as androgen and oestrogen, in both male and female (42). Nevertheless, to this date, there are controversial results regarding the beneficial result of hormonal replacement therapy in preventing muscle mass loss (43). A recent meta-analysis showed that estrogen-based hormonal therapy was beneficial to skeletal muscle strength in post-menopause women, as women on hormonal treatment were 5% stronger than those without treatment (44). Considering that usually, woman lose strength at a rate of 1% a year after menopause, this relatively small effect might be clinically meaningful. Additionally, the levels of growth hormone (GH) and their relevant regulators (Insulin-like Growth Factor- IGF family) are usually lower in the elderly subjects, and these situation could further explain skeletal muscle mass wasting. In a recent *in vivo* study, Briosche et al. (45) proved that GH supplementation in old mice determined an increase in lean body mass, with an elevated synthesis rate of skeletal muscle protein and mitochondrial biogenesis pathways. Moreover, they registered a lowering of age-associated oxidative damage, an increase of plasma and hepatic IGF-1 levels, and an induction of antioxidant enzymes in the skeletal muscle of the treated animals. Further clinical studies are needed in order to confirm this preliminary results.

During aging, it has been registered a decline in 25(OH)vitamin D3 serum levels associated with reduced muscle mass, strength and performance in older adults (46, 47). Moreover, a recent skeletal muscle gene expression study carried out in older adults (68-79 years) showed that lower expression of vitamin D receptor (VDR) was associated with higher lean mass (48). Additionally, Redzic et al. demonstrated that vitamin D status might be involved in intramyocellular lipid accumulation independent of body mass index and physical activity level (49). Furthermore, a recent meta-analysis, considering randomized controlled trials, reported a positive association between vitamin D supplementation and muscle strength, but further studies are needed to define optimal treatment modalities (50). Moreover it seems that vitamin D supplementation increases intramyonuclear VDR concentration by 30% and increases muscle fiber size by 10% in older, mobility-limited women (46). Further studies are needed in order to clarify the role of vitamin D in affecting muscle metabolism and function.

Recently, great interest has been shown towards a specific myokine, called myostatin, that seems to be a potential target to prevent sarcopenia development. Myostatin negatively regulates skeletal mass and might also be implicated in regulating hepatic production of IGF-1 (51). Moreover, myostatin gene expression is up-regulated in elderly compared to young subjects, and the age-related elevation of myostatin serum levels correlated with muscle mass (52). Recently, McKay et al. showed that there is an age-related impairment of muscle stem cell function that might be explained by the co-localization with myostatin in older subjects (53). Other studies need to be made but myostatin could potentially prevent muscular mass wasting.

## Conclusions

This review aimed to summarised the current data regarding the physiopathology of sarcopenia in older subjects. Lifestyle factors as diet and physical activity, together with age-relat-

ed changes in cytokines and hormones levels are important risk factors for muscular impairment and each of them contribute in a different manner. To this date, epidemiological and clinical studies showed that muscle mass and strength are differently influenced by various interventional treatments, but long-term supplementation studies are still needed in order to further investigate the role of healthy lifestyle habits in the prevention and treatment of sarcopenia. Moreover, recent data showed that high fat diet is an important risk factor for ectopic fat depots, in particular intramyocellular adipose tissue accumulation. This phenomenon has a direct negative influence on muscular homeostasis and function with unfavourable consequences on the development of sarcopenia.

For all these reasons, sarcopenia should have a multidimensional approach in order to understand its pathophysiology and to define the molecular targets for intervention and future successful treatment.

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