



Mechanisms of Sarcopenia and Regenerative Decline During Aging of Skeletal Muscle

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Abstract

Sarcopenia is the age-related loss of muscle mass and function, which directly contributes to physical disability, loss of independence, and mortality in the elderly. Aging triggers functional decline in many species, and the physiopathology of sarcopenia is largely conserved in rodents, pets, and humans. This paper will present the mechanisms of sarcopenia and age-related muscle decline in chronic and acute settings where regeneration and muscle stem-cell function are key for recovery from physical injury or surgery. In particular, the influence of lifestyle contributions such as nutrition and physical activity will be discussed, and the interplay between endocrine, metabolic, and cellular causes that lead to sarcopenia will be reviewed. This review will highlight that the causes of muscle aging and sarcopenia are multifactorial and will discuss the underlying proof-of-concept studies for therapeutic interventions.

Skeletal muscle is a highly dynamic tissue that represents 30 to 40% of body mass and directly influences energy metabolism and physical performance. Animal breeding selection has demonstrated that genetics play an important role in the development and maintenance of the musculature.¹ For example, mutations in the myostatin gene have direct monogenic association with hypermuscularity in a wide range of animal species from rodents to humans.²⁻⁵ Belgian blue cattle selected for high meat production are hypermuscular because of a homozygous loss-of-function mutation in myostatin, a gene of the TGF β family that acts as a negative regulator of muscle growth.² Interestingly, Whippet dogs selected for racing performance have a high prevalence of heterozygous myostatin mutations but become hypermuscular and bad performers when inbreeding leads to homozygous mutations of both myostatin alleles.⁴ Thus, genetics can directly drive muscle mass and performance, and unlike the myostatin example, the vast majority of genetic determinants of skeletal muscle mass, function, and physiology are complex multigenic interactions that

Glossary of Abbreviations

ATP: Adenosine Triphosphate

CRP: C-Reactive Protein

FOXO: Forkhead Box Transcription Factors Subgroup O

GRMD: Golden Retriever Muscular Dystrophy

mTOR: Mechanistic Target of Rapamycin

NAD: Nicotinamide Adenine Dinucleotide

Pax 7: Paired Box 7

SR: Sarcoplasmic Reticulum

also cross-talk with physical activity and nutrition.

Skeletal muscle is very plastic in health and disease, and multiple physiological and environmental cues are integrated and coordinated to ensure optimal muscle health and optimal performance and mobility. Physical activity and specialized exercise training programs are the major drivers that remodel the molecular, cellular, and structural characteristics of muscle tissue toward specialized contractile output. For example, resistance training leads to the growth of myofibers with the

accretion of additional contractile proteins to increase the strength of contraction, while endurance training will cause metabolic adaptations that are more favorable bioenergetically and reduce fatigability. In contrast, many diseases and medical conditions negatively influence muscle function, performance, and quality of life acutely or chronically. These conditions include muscle loss caused by trauma or immobilization that are transient and recoverable, but also chronic muscle wasting and reduced muscle strength caused by aging or diseases such as cancer, heart failure, or pulmonary and kidney disorders. Finally, rare genetic diseases also profoundly affect muscle function and survival through mutations of structural proteins or metabolic enzymes expressed in skeletal muscle, such as Golden Retriever muscular dystrophy (GRMD), which is caused by mutations of the muscle structural protein dystrophin.⁶

After giving a brief overview of the cellular architecture of skeletal muscle and how it contributes to strength and performance, this paper will focus on sarcopenia, the functional decline of skeletal muscle during aging. I will present the different factors contributing to the physiopathology of sarcopenia and will discuss the underlying mechanisms at the cellular, endocrine, and nutritional levels. The primary focus of this paper is human sarcopenia; however, many of the concepts presented also were studied in rodents at the mechanistic level, as many of the pathophysiological processes of skeletal muscle aging are conserved across mammalian species. Most of the discussion herein is therefore also relevant for pets.

What Are the Cellular Drivers of Muscle Function?

Skeletal muscle tissue is primarily composed of myofibers, a long multinucleated cell type specialized in contraction-for-force generation. Myofibers are structurally organized in specialized units called sarcomeres, where contractile proteins slide against each other to shorten the cell and thereby contract the muscle. Muscle strength is, thus, directly linked to the amount of contractile proteins present in myofibers. Myofibers specialize in different types of contraction at the cellular level. Fast-twitch fibers generate high-intensity contraction over short durations using energy sources that can be rapidly mobilized, such as phosphocreatine and anaerobic glucose oxidation, and thereby primarily account for acute activities of daily living. In contrast, slow-twitch fibers contract with lower amplitude but are much more fatigue resistant as they generate energy through efficient metabolic coupling using aerobic glucose and fatty oxidation that generate adenosine triphosphate (ATP) in mitochondria. The regulation of muscle mass and strength relies on a tightly regulated balance between muscle protein synthesis (anabolism) and muscle protein breakdown (catabolism) in myofibers.⁷ At the molecular level, protein synthesis is primarily driven by the mTOR (mechanistic target of rapamycin) complex, which controls ribosomal activity and amino acid incorporation in new proteins,⁸ while protein degradation is orchestrated by FOXO (forkhead box transcription factors subgroup O) proteins, which control the expression of enzymes marking contractile proteins for proteasomal degradation.⁹ The detailed molecular pathways regulating anabolism and catabolism have been extensively studied^{10,11} and are beyond the scope of this paper. However, it is important to note that these pathways are directly controlled by endocrine cues to adapt muscle physiology to whole body metabolism and by nutritional cues to adapt muscle growth to the availability of amino acids. Recent breakthrough discoveries have identified the precise molecular sensors that respond to intracellular amino acid concentrations to trigger the appropriate molecular and physiological response on protein synthesis versus breakdown via mTOR.¹²⁻¹⁵ In addition, myofibers contain many more specialized structures and molecular mechanisms to orchestrate and fine-tune contraction and metabolism. Organelles such as mitochondria and sarcoplasmic reticulum (SR) indirectly regulate contraction. Mitochondria generate cellular energy required for contraction by converting nutritional substrates to ATP, and the SR stores calcium that is released upon stimulation by the nervous system to initiate contraction.

Many other cell types contribute to the homeostasis of skeletal muscle to ensure efficient contraction. Muscle strength acutely requires efficient coordination between the neuromuscular system to relay voluntary input from the brain and the cardiovascular and metabolic systems to cope with the energetic needs of contraction. Neuromuscular input is

driven by motoneurons, which project from the spinal cord and coordinate information from the brain for coordinated contraction. These motoneurons require myelination by Schwann cells to ensure efficient propagation of action potentials, as well as specialized terminal synapses called neuromuscular junctions, where the neurotransmitter acetylcholine is released upon neuronal excitation and binds nicotinic receptors to depolarize the muscle membrane and trigger calcium release and contraction. The frequency of motoneuron firing directly controls the type of contraction as well as myofiber specialization in slow or fast fibers. Endothelial cells and blood vessels also play a prominent role for muscle performance by ensuring adequate blood supply in skeletal muscle. The regulation of muscle perfusion from blood vessels integrates systemic neuroendocrine cues and local signals to adapt the delivery of oxygen and nutrients to the intensity of contraction and the underlying metabolic needs of myofibers.

Finally, skeletal muscle is a very plastic tissue that can efficiently heal when myofibers are injured. Muscle regeneration primarily relies on a population of muscle-resident stem cells called satellite cells, which are marked by the transcription factor Pax 7 (Paired Box 7).¹⁶ Muscle stem cells are quiescent in healthy muscles, but they sense that muscle is injured when they lose structural input from the muscle membrane and the extracellular matrix, and when they cross-talk through paracrine communication with immune cells that invade injured muscle. The complex spatiotemporal effect of immune cell infiltration during muscle regeneration allows initial clearing of cellular debris from injured cells by activating innate immunity and proinflammatory macrophages.^{17,18} In a second phase, inflammation is gradually resolved by switching to anti-inflammatory macrophages, thereby controlling muscle stem-cell expansion and myogenic differentiation for myofiber repair.

What Is Sarcopenia?

Muscle mass and performance reach a peak at adulthood and then decline progressively during aging (Figure 1A). Sarcopenia is the threshold at which this functional decline becomes debilitating, leading to functional and mobility deficits that negatively affect quality of life and longevity.¹⁹⁻²² The term sarcopenia historically has been proposed to define muscle wasting that occurs as an intrinsic consequence of aging,²³ but the term has mistakenly been used to describe the loss of muscle mass and function caused by disuse/immobilization or by chronic diseases such as cancer, heart failure, and kidney or pulmonary disorders. Muscle wasting secondary to these diseases is called cachexia as these diseases share common physiopathological mechanisms that primarily lead to amino acid mobilization from muscle pools because of systemic hypercatabolism.^{24,25} Importantly, the progression of sarcopenia involves distinct mechanisms, unlike cachexia, as sarcopenia does not associate with a major

hypercatabolic condition. Although aging associates with many co-morbidities that most likely contribute to sarcopenia in certain patients, this paper will focus on the intrinsic mechanisms of sarcopenia caused by aging.

The decline in muscle mass and physical performance is gradual during aging but accelerates at a very old age when it becomes debilitating and puts activities of daily living and physical autonomy at risk (Figure 1A). While preventive strategies, especially through physical training and exercise, are beneficial throughout the life span, one major challenge is defining inter-individual cutoffs for disability. In humans, a consensus has been reached on the most relevant clinical tests to diagnose sarcopenia.²⁶ These involve the coincidence of low-muscle mass measured by dual-energy X-ray absorptiometry (DXA) or magnetic resonance imaging (MRI) and low-muscle function measured by low-grip strength or low-gait speed. Taken individually, these clinical parameters have been shown to predict disability and mortality.²⁷⁻²⁹ Consequently, sarcopenia has been recognized as an official human disease in 2016 with an associated ICD-10 code.^{30,31} Nevertheless, there is no international consensus on the cutoff values to define disability. Several operational definitions of sarcopenia have been proposed,^{27,32,33} and the disability thresholds are still being debated and tested in various epidemiological studies.

Although we can foresee that there will be worldwide consensus on the precise criteria to define sarcopenia as a disease in the near future, one should not forget the challenges of population-based statistics. The disability thresholds set for the entire population rely on an average maximal peak of

physical performance and an average rate of physical decline. In reality, these parameters can be independent, and disability thresholds are likely to differ among individuals (Figure 1B-C). The ideal clinical management of sarcopenia would, therefore, involve longitudinal follow-up to diagnose the rate of physical decline in each individual over decades. While the medical costs of imaging to measure muscle mass and the emerging medical recognition of sarcopenia today preclude this long-term longitudinal diagnosis, the advent of personalized medicine and digital health will hopefully in the future provide better health monitoring of physical decline to anticipate intervention and prevent loss of autonomy.

Mobility Declines in Different Phases During Aging

Mobility declines gradually in community-dwelling individuals who maintain physical autonomy and independence. In this context, aging generally leads to a chronic vicious cycle between muscle weakness and inactivity (Figure 2), where lack of physical exercise can initially prime for the decline of muscle mass and function, but muscle weakness then subsequently leads to low physical activity through rapid exhaustion, loss of stamina, and ultimately lack of self-confidence and fear of falling. The mechanisms of this chronic decline involve the wasting of contractile proteins leading to low muscle mass, as well as inefficient contraction and coordination leading to decline in strength.

In contrast, acute events such as falls, muscle injuries, or surgeries lead to an acute accelerated vicious cycle (Figure 2). These acute events lead to injury of myofibers

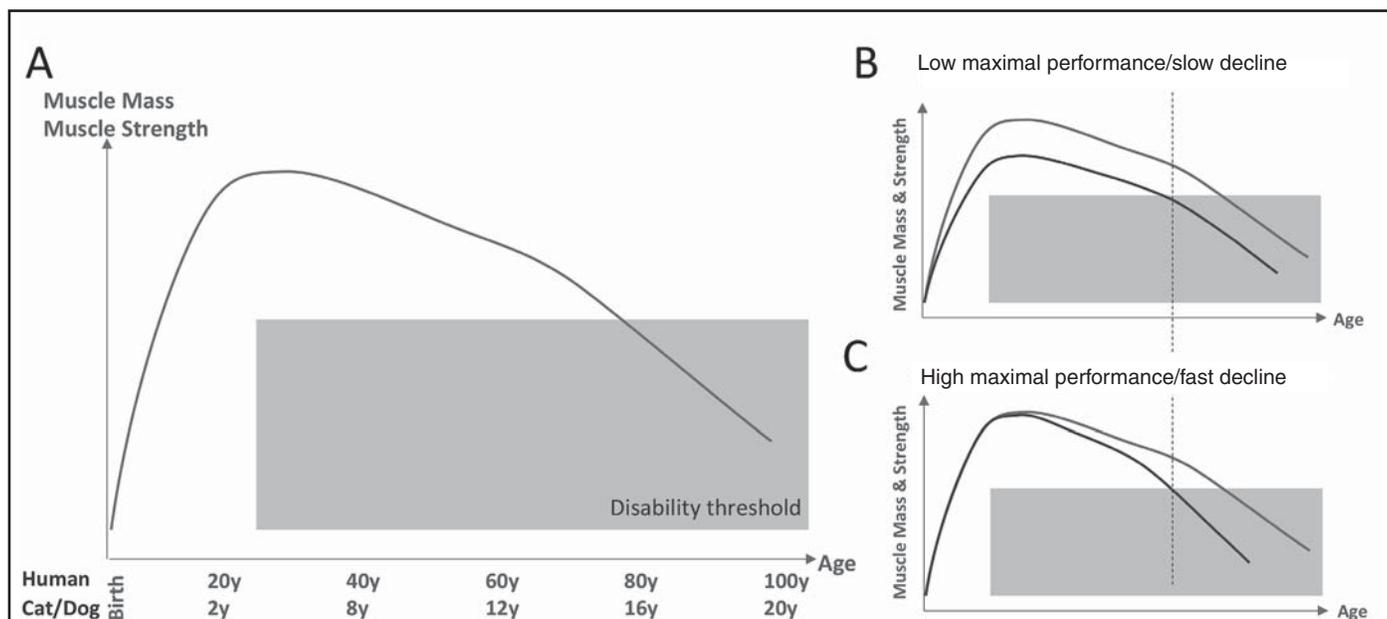


Figure 1. Different profiles of the decline of muscle mass and physical function during aging. Sarcopenia is diagnosed when muscle mass and function fall below a disability threshold. Both the maximal performance peak and the rate of decline vary among individuals and can influence the diagnosis of sarcopenia. (A) Average physical decline. (B) Example of physical decline of an individual with low maximal performance and slow decline. (C) Example of physical decline of an individual with high maximal performance and fast decline. Note that cases B and C result in the diagnosis of sarcopenia at the same age, though disease progression after diagnosis will be faster in case C.

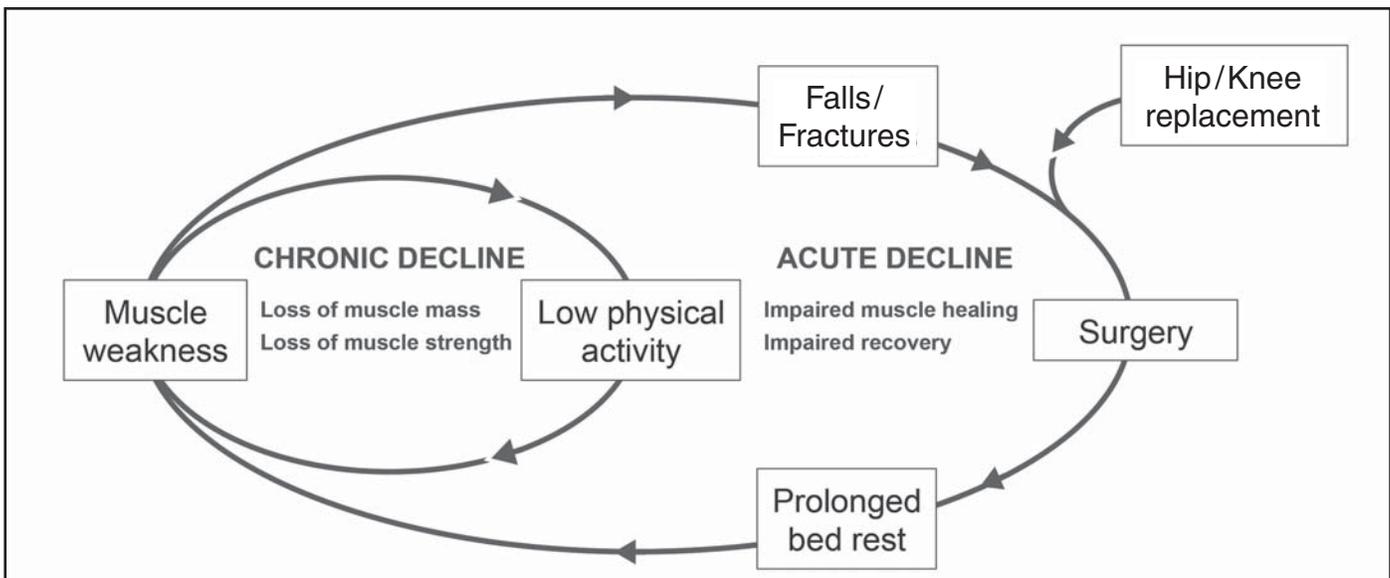


Figure 2. Chronic and acute vicious cycles of mobility decline in the elderly. Chronic physical decline happens over decades in community-dwelling individuals and largely depends on loss of muscle mass and strength. Acute physical decline happens after trauma or surgery, generally in a hospital setting, and largely depends on impaired muscle healing because of poor regeneration and altered muscle stem-cell function.

as a result of trauma or because locomotor muscles are damaged during surgical procedures such as hip or knee surgery. Recovery from these acute injuries primarily relies on muscle stem-cell function and myogenic repair, which are impaired in old muscle and lead to slow and inefficient muscle healing. In addition, these conditions require hospital stays and prolonged bedrest, which also contribute to functional decline of nonaffected muscles and thereby indirectly contribute to the vicious cycle of muscle weakness and inactivity.

Mechanisms of Sarcopenia

The major challenge in understanding the causes of sarcopenia to develop preventive or therapeutic interventions is that they are multifactorial. This cross-talk between multiple factors happens at different levels within the population, within an individual, and even within different muscle types of the same individual. In this paper, I will present various mechanisms that have been associated with sarcopenia and will discuss the evidence currently available to infer causality at the level of the population.

The factors that contribute to sarcopenia can be stratified to lifestyle causes that are primarily linked to physical activity and nutrition, to systemic endocrine and metabolic cues through which whole-body homeostasis influences muscle performance and finally to cellular causes in skeletal muscle, as well as the supporting cell types required for efficient contraction (Figure 3). Muscle mass is strongly influenced by genetic factors,³⁴ and it is very likely that genetics also modulate the susceptibility to sarcopenia. However, genomewide association studies of sarcopenia are only emerging, and future large-scale studies will be

required to identify and replicate genetic variants associated with sarcopenia.

Exercise and regular physical activity are the most dominant protective factors against sarcopenia.^{35,36} Indeed, lack of regular contraction leads to both systemic and cellular defects that trigger poor performance through an imbalanced protein synthesis/degradation, altered bioenergetics, and degradation of neuromuscular transmission. Many epidemiological and intervention studies have exemplified the beneficial effects of acute and chronic exercise on muscle function in elderly individuals.³⁷ Resistance and endurance aerobic training seem beneficial in the elderly³⁸ likely by triggering different physiological adaptations. Resistance training favors anabolism and accretion of contractile proteins, while endurance training triggers metabolic adaptations with better bioenergetic coupling and resistance to fatigue. Consequently, current exercise recommendations for elderly patients involve balanced holistic programs targeting strength, fatigue, and balance/coordination. Exercise programs have been shown to be effective at restoring muscle function in a few weeks but also at preventing chronic functional decline.³⁵ The most recent and robust evidence for the beneficial effects of exercise and physical training chronically is the LIFE study in which 1,635 participants underwent a randomized physical activity program or health education during two and a half years to demonstrate that physical activity chronically reduces major mobility disability.³⁹ Interestingly, exercise also is indirectly beneficial for other age-related conditions, including metabolic diseases and cognitive decline.^{36,40,41}

Nutrition plays a direct role in the progression of sarcopenia.³⁵ Elderly individuals are often at risk of malnutrition, mainly because taste and appetite decline with age but also because

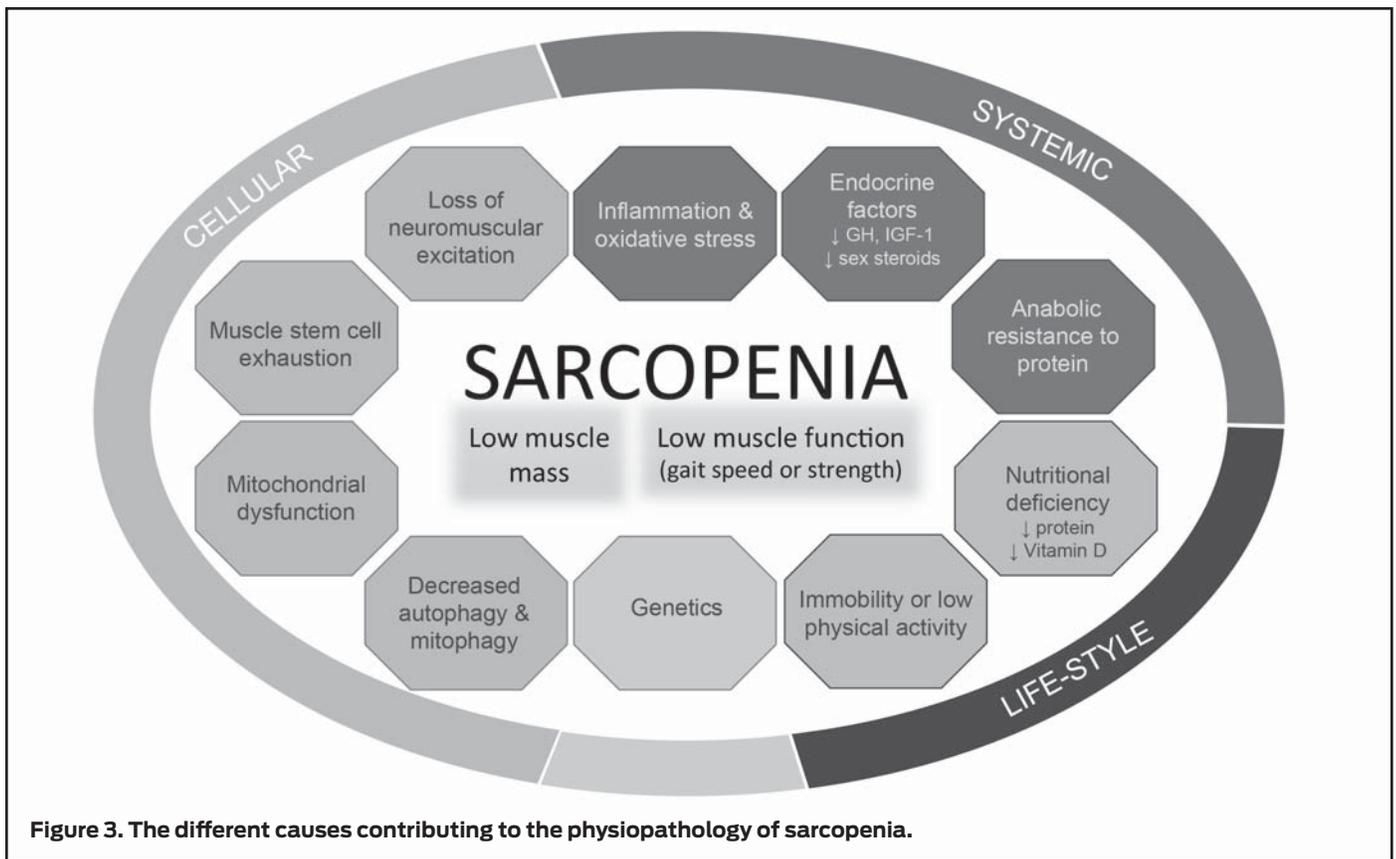


Figure 3. The different causes contributing to the pathophysiology of sarcopenia.

the biological processes of macronutrient and micronutrient absorption and metabolism are impaired during aging. The role of dietary proteins has been widely studied in the context of sarcopenia and age-related physical dysfunction,^{42,43} as amino acids are the building blocks for contractile protein anabolism in skeletal muscle and branched-chain amino acids directly stimulate muscle-protein synthesis through Sestrin/Rag/mTOR signaling.^{14,44,45} Protein intake is generally low in the elderly, and aging leads to distinct protein requirements,^{43,46} which directly contribute to sarcopenia. Protein supplementation is, therefore, the standard of care to maintain muscle mass and mobility during aging. Interestingly, work in model organisms has demonstrated that lifelong supplementation with high protein is detrimental to longevity,^{47,48} and both human and animal studies have demonstrated that a critical physiological shift happens at middle age leading to beneficial effects of high protein in the elderly but detrimental effects in young adults.⁴⁹ Vitamin deficiencies also can contribute to sarcopenia. Low vitamin D levels have widely been associated with a higher prevalence of physical frailty and sarcopenia.^{35,50-52} Vitamin D supplementation has beneficial effects on physical function in some but not all elderly populations,^{35,53} and vitamin D is a widely prescribed supplement for the nutritional management of sarcopenia. However, the mechanisms of action through which vitamin D exert beneficial effects in skeletal muscle remains elusive and further mechanistic understanding could allow better definition of combinations of vitamin D with

other interventions to further enhance its efficacy. Other nutrients such as vitamin B12 have also been reported to decline in sarcopenia,⁴⁶ but intervention studies supplementing these nutrients will be required to establish causality and determine whether they could become therapeutic options.

At the physiological level, sarcopenia is a condition in which anabolic signals are lost systemically and within skeletal muscle, leading to an imbalance where protein breakdown overrides synthesis and results in a net loss of muscle contractile proteins. The loss of systemic anabolism results from a decline in growth hormone and insulin growth factor,⁵⁴ as well lower levels of the anabolic sex hormone testosterone.⁵⁵ Importantly, sarcopenia does not associate with loss of fat from adipose tissue, and sarcopenia can actually overlap with states of overweight or obesity that are prevalent in elderly populations.⁵⁶ The complex metabolic interplay between fat redistribution in metabolic tissues and perturbed insulin signaling leads to insulin resistance, including in skeletal muscle.^{57,58} Muscle insulin resistance is detrimental for glucose uptake and utilization for contraction but also for the anabolic effects of insulin in the regulation of muscle mass.⁵⁹ Aging and sarcopenia also associate with a state of anabolic resistance to protein where the ability of dietary amino acids to stimulate muscle protein synthesis is impaired.^{38,50,61} Thus, on top of the low-protein consumption that has been mentioned, dietary proteins are not as efficient in aged individuals. Consequently, elderly subjects have higher protein requirements per amount of

body weight to maintain adequate muscle protein synthesis and prevent muscle wasting.^{43,62} Another systemic contributor to sarcopenia is the chronic low-grade inflammation that associates with aging.⁶³ The most robust evidence for this contribution comes from elevated levels of C-reactive protein (CRP).⁶⁴ Cytokines, such as TNF α and IL-6, also have been suggested to be involved in the pathophysiology of sarcopenia.⁶⁴ However, it remains unclear if this cytokine production is a cause or consequence of sarcopenia, and the role of immune cells in innate and adaptive immunity linked to sarcopenia remains poorly studied.⁶⁵

Many cellular mechanisms are perturbed in skeletal muscle and contribute to sarcopenia. Mitochondrial dysfunction is a prominent feature of aging in many tissues including skeletal muscle, where perturbations in cellular bioenergetics in mitochondria alter the ATP generation that is required for efficient and sustained contraction.^{34,66,67} Sarcopenic muscles have a strong downregulation of genes and proteins from the electron transfer chain that control oxidative phosphorylation to generate ATP.⁶⁸ A decline of nicotinamide adenine dinucleotide (NAD) levels has recently been proposed as a causal factor for age-related mitochondrial dysfunction in muscle and an entry point for intervention with NAD-boosting therapies.⁶⁹⁻⁷² In addition, the biogenesis of mitochondria, their fusion, and fission dynamics, as well as the ability to clear damaged mitochondria through mitophagy, are altered in aged muscle and can contribute to muscle wasting on top of bioenergetic defects.⁷³ Interestingly, this latter mechanism recently has been demonstrated to be amenable to nutritional intervention with urolithin A supplementation, which enhanced muscle strength by enhancing mitophagy and mitochondrial respiration.⁷⁴ Mitochondria also are a site of very active radical oxygen species generation, which contribute to enhanced oxidative stress during aging, largely because the mechanisms to detoxify oxidative stress become inefficient during aging.^{75,76} Age-related oxidative stress directly impacts the mechanisms of muscle contraction, in particular by creating calcium leaks in the SR of aged myofibers, which limit the amplitude of calcium release and contraction.^{77,78}

Another major cellular contribution to the progression of sarcopenia is the decline of neuromuscular input, which directly participates to loss of muscle strength in the elderly.^{79,80} Neuromuscular junctions are fragmented and denervated in aged skeletal muscle,^{68,81} leading to the inability of nerve terminals to contact postsynaptic acetylcholine receptors to efficiently transduce motor excitation. The origin of these deficits is not fully elucidated and probably results from direct molecular perturbations in muscle fibers such as mitochondrial dysfunction and oxidative stress, but also from alterations of the peripheral nervous system and possibly from central neurodegeneration.⁸² Peripheral nerve myelination by Schwann cells declines during aging and leads to altered propagation of neuronal signals along the motoneuron

axons.^{81,83} In addition, the repair mechanisms through which motoneuron sprouting allows reestablishment of functional connections with denervated fibers also are impaired by aging and sarcopenia.⁸⁴ Interestingly, neuromuscular decline tightly correlates with the amplitude of sarcopenia in different muscles of a single individual in rodents and in humans.⁸¹ In addition, neuromuscular decline is reversible as both exercise and caloric restriction have been shown to prevent synaptic fragmentation.⁸⁵

Recent studies have demonstrated that cellular quality control plays an important role in removing damaged proteins and organelles from skeletal muscle, especially in the context of aging where cellular stress increases. This is particularly true for autophagy, a cellular recycling system through which damaged cellular material is targeted for lysosomal degradation. Autophagy is required to maintain muscle mass, and aberrant autophagy leads to severe muscle dysfunction that recapitulates many of the cellular dysfunctions of sarcopenia.⁸⁶⁻⁸⁹ The perturbations of autophagy in aged muscle can be reversed by exercise or AMP-kinase activation and prevent at least some of the cellular phenotypes of sarcopenia.^{88,90,91}

The inability of muscle stem cells to efficiently repair damaged muscle fibers also contributes to the functional decline of elderly individuals through several distinct mechanisms. The number of muscle stem cells available for repair and their ability to expand upon injury gradually decline with age.^{92,93} In addition, changes in cytokines, growth factors, and metabolic cues in aged skeletal muscle lead to a break in quiescence of muscle stem cells and inhibits their activation, commitment, and self-renewal upon myofiber injury.⁹⁴ Thus, the recovery from muscle injuries caused by falls or other traumatic events is considerably slowed down in aged individuals. A recent study reporting that stem-cell ablation in rodents does not contribute to age-related muscle wasting in the absence of injury has created controversy on the causal role of muscle stem-cell dysfunction for sarcopenia.⁹⁵ However, this study has been performed in a fully controlled and protected environment in the absence of any traumatic event. When muscle injury was stimulated experimentally, the study confirmed the absolute requirement of muscle stem cells for efficient muscle repair after injury. In addition, the authors demonstrated that muscle stem cells are required to prevent fibrosis during muscle repair. Thus, there is no doubt that muscle stem-cell decline contributes to age-related regenerative failure of skeletal muscle in real life and that this indirectly contributes to sarcopenia through the indirect acute vicious cycle explained in Figure 2. Age-related regenerative failure of skeletal muscle recently has been demonstrated to be reversed by several pharmacological strategies,^{96,97} but also through nutritional intervention with a vitamin B3 derivative that boosts muscle stem-cell function by rescuing mitochondrial dysfunction in these stem cells.⁷² It is, therefore, promising that options for acute and chronic management of age-related

muscle dysfunction are emerging and future studies addressing how these interventions can synergize will be of the highest importance.

Future Challenges

The medical recognition of physical decline among gerontologists has greatly increased in the past decade and led to the recent approval of an ICD-10 code for sarcopenia in humans. Nevertheless, the upcoming years will be of prime importance to increase the general medical awareness of sarcopenia, especially because the idea that age-related physical decline is a normal and inevitable process largely prevails among general practitioners. To this end, demonstrating efficacious therapeutic interventions with drugs and nutrition will be absolutely key. However, one of the major challenges toward that goal is that the sarcopenic population is heterogeneous and the mechanisms of age-related physical decline are multifactorial. Preclinical and clinical studies in the next few years will have to disentangle the causal mechanisms of sarcopenia from those that are secondary or adaptive. In particular, it will be key to demonstrate which mechanisms of sarcopenia are dominant and constitute upstream hubs for intervention with the potential to indirectly reverse more downstream perturbations. Similarly, characterizing efficient combination strategies among pharmaceuticals, nutrition, and exercise that target the different causes of sarcopenia will also be of utmost importance.

Given the complexity of aging phenotypes, there is no doubt that individual patients could benefit from personalized interventions targeting specifically the most prominent contributing factor(s) to their functional decline. However, the characterization of biomarkers of sarcopenia are in the early premises and primarily focus on ways to measure muscle mass and function accurately and cost-effectively.⁹⁸ Personalized interventions in sarcopenia are not foreseeable in the near future, and developing molecular biomarkers for the causal mechanisms of sarcopenia will be required long term for personalized sarcopenia interventions.

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