Ageing Research Reviews 65 (2021) 101200

Contents lists available at ScienceDirect

Ageing Research Reviews

journal homepage: www.elsevier.com/locate/arr

Review

Sarcopenia – Molecular mechanisms and open questions

Petra Wiedmer [a], Tobias Jung [a], José Pedro Castro [a,1], Laura C.D. Pomatto [b,2], Patrick Y. Sun [b], Kelvin J.A. Davies [b,c,d], Tilman Grune [a,e,f,g,h,*]

[a] German Institute of Human Nutrition Potsdam-Rehbrücke, Nuthetal, Germany
[b] Leonard Davis School of Gerontology of the Ethel Percy Andrus Gerontology Center, the University of Southern California, Los Angeles, CA 90089-0191, USA
[c] Molecular and Computational Biology Program, Department of Biological Sciences of the Dornsife College of Letters, Arts & Sciences, the University of Southern California, Los Angeles, CA 90089-0191, USA
[d] Department of Biochemistry & Molecular Medicine, Keck School of Medicine of USC, the University of Southern California, Los Angeles, CA 90089-0191, USA
[e] German Center for Diabetes Research (DZD), 85764 München-Neuherberg, Germany
[f] German Center for Cardiovascular Research (DZHK), Partner Site Berlin, 13347 Berlin, Germany
[g] University of Potsdam, Institute of Nutritional Science, Nuthetal, Germany
[h] NutriAct-Competence Cluster Nutrition Research Berlin-Potsdam, Nuthetal, Germany

ARTICLE INFO

Keywords:
Molecular pathways
Proteostasis
Proteasome
Autophagy
Mitochondria, Muscle fibre composition

ABSTRACT

Sarcopenia represents a muscle-wasting syndrome characterized by progressive and generalized degenerative loss of skeletal muscle mass, quality, and strength occurring during normal aging. Sarcopenia patients are mainly suffering from the loss in muscle strength and are faced with mobility disorders reducing their quality of life and are, therefore, at higher risk for morbidity (falls, bone fracture, metabolic diseases) and mortality.

Several molecular mechanisms have been described as causes for sarcopenia that refer to very different levels of muscle physiology. These mechanisms cover e. g. function of hormones (e. g. IGF-1 and Insulin), muscle fiber composition and neuromuscular drive, myo-satellite cell potential to differentiate and proliferate, inflammatory pathways as well as intracellular mechanisms in the processes of proteostasis and mitochondrial function.

In this review, we describe sarcopenia as a muscle-wasting syndrome distinct from other atrophic diseases and summarize the current view on molecular causes of sarcopenia development as well as open questions provoking further research efforts for establishing efficient lifestyle and therapeutic interventions.

1. Introduction

Aging is one of the leading risk factors for physiological decline in organ function and overall health (Franceschi et al., 2018; Kennedy et al., 2014). At the molecular level, the aging 'phenotype' has been well-characterized by the presence of protein aggregates (Raynes et al., 2016), lipid peroxidation (Ayala et al., 2014), and DNA damage (Aunan et al., 2016). Though all organs show age-associated markers, post-mitotic cells are especially damage-prone, due to their inability to undergo cell division. As a result, many chronic diseases of the elderly disproportionally impact post-mitotic tissue, including skeletal muscle and brain.

Sarcopenia is highly prevalent in the elderly population. It is characterized by progressive and generalized degenerative loss of skeletal muscle mass, quality, and strength with normal aging (Argiles et al., 2015; Cruz-Jentoft et al., 2010; Gallagher et al., 2000) and is often accompanied by a progressive increase in body fat (Gallagher et al., 1996). As a result, older adults are at greater risk for morbidity (falls, bone fracture, institutionalization) and mortality (Batsis et al., 2014). Paradoxically, sarcopenia patients are also at increased risk for developing metabolic syndrome (e. g. Type II Diabetes) (Umegaki, 2015). Skeletal muscle comprises 40–50 % of mammalian tissue and is one of the most important sites for metabolic control (Sandri, 2010). Considering the ever-increasing life expectancy and the growing elderly population, the prevalence of sarcopenia will certainly escalate in the upcoming decades. This provides the rationale for careful investigation and dissection of the molecular causes of sarcopenia. Understanding the molecular mechanisms will build the basis for developing efficient

* Corresponding author.
E-mail address: tilman.grune@dife.de (T. Grune).
[1] Current address: Division of Genetics, Brigham and Women's Hospital, Harvard Medical School.
[2] Current address: National Institute of General Medical Sciences, 45 Center Drive MSC 6200, Bethesda, MD 20892, USA.

https://doi.org/10.1016/j.arr.2020.101200
Received 16 April 2020; Received in revised form 18 October 2020; Accepted 20 October 2020
Available online 29 October 2020

preventive and therapeutic treatment strategies.

Many pathways and mechanisms underlying sarcopenia have been characterized. Much effort has focused on identifying the role of hormones, e.g. through insulin signaling (Léger et al., 2008), inflammatory pathways (Schaap et al., 2006), and loss of proteostasis (Jiao and Demontis, 2017b) as causes for sarcopenia. However, we have a very limited understanding of sarcopenia development, due to lack of appropriate animal models and the difficulty in separating "pure sarcopenia" from other age-related comorbidities. To begin addressing this gap, the present review focuses on understanding the hallmarks of sarcopenia as an age-related symptom or phenomenon, mainly focusing on sarcopenia-related changes in muscle composition and underlying molecular changes such as loss of proteostasis and mitochondrial dysfunction as well as inflammatory pertubations.

2. Sarcopenia definition and prevalence

2.1. Differentiating sarcopenia from other muscle wasting syndromes

Sarcopenia represents a muscle-wasting syndrome characterized by progressive and generalized degenerative loss of skeletal muscle mass, quality, and strength occurring during normal aging (Argiles et al., 2015; Cruz-Jentoft et al., 2010; Gallagher et al., 2000). Patients experience a multitude of physically adverse effects such as mobility disorders, increased risk of falls and fractures, impaired ability to perform activities of daily living, disabilities, poor quality of life, loss of independence, and increased risk of death (Cruz-Jentoft et al., 2010). Therefore, the European Working Group on Sarcopenia in Older People (EWGSOP) promoted the recognition of sarcopenia as a geriatric syndrome with the definition of mainly two criteria for clinical diagnosis of sarcopenia: low muscle function (characterized by low muscle strength) and decreased muscle mass (Cruz-Jentoft et al., 2010). In 2019, EWGSOP updated their guidelines for sarcopenia diagnostics (EWGSOP2) and prioritized decreased muscle strength as the most important diagnostic parameter, since it mainly predicts adverse outcomes of the disease (Cruz-Jentoft et al., 2019). Therefore, impaired muscle strength pointing towards the presence of sarcopenia might be verified by additional determination of muscle mass. Concomitantly occurring problems in muscle performance indicate already severely established sarcopenia (Cruz-Jentoft et al., 2019). The molecular cause and triggers of sarcopenia are not well understood and as a result, the differentiation from other muscle wasting syndromes is complicated.

Depending on the cause, sarcopenia can be categorized into 'primary' or 'secondary' sarcopenia (Bauer et al., 2019; Cruz-Jentoft et al., 2019). In 'primary sarcopenia' aging is considered the only cause and it is therefore also called 'age-related sarcopenia'. Whereas 'secondary sarcopenia' results from the compounding occurrence of one or more other modifying conditions, such as lack of physical activity (activity-related sarcopenia), advanced organ failure (disease-related sarcopenia), or inadequate dietary intake of energy and/or protein (nutrition-related sarcopenia) (Bauer et al., 2019; Cruz-Jentoft et al., 2019). However, it can be difficult to differentiate between primary and secondary sarcopenia, since causes can overlap or intertwine particularly in aged individuals, further indicating sarcopenia is a multi-faceted geriatric syndrome (Cruz-Jentoft et al., 2010). Recently, sarcopenia was formally recognized as a muscle disease by receiving an ICD-10-MC Diagnosis Code (Cruz-Jentoft et al., 2019). For evaluating the severity of sarcopenia in clinical settings, the EWGSOP recommends 4 steps using the" F-A-C-S algorithm". The steps of this algorithm are "find" (cases), "assess" (grip strength), "confirm" (muscle quantity or quality), and "severity" (check by evaluating muscle performance), which should be consecutively completed (Cruz-Jentoft et al., 2019). The task force of the International Conference on Sarcopenia and Frailty Research (ICSFR) recommends a similar approach for diagnosing sarcopenia as a basis for appropriate treatment (Dent et al., 2018). Recently, a new categorization of the disease was introduced by EWGSOP according to the duration of the presence of sarcopenia. Acute sarcopenia, e. g. related to acute illness or injury, is considered to last no longer than 6 months. Chronic sarcopenia, mostly related to chronic and progressive conditions, is considered to last longer than 6 months (Cruz-Jentoft et al., 2019). This time-dependent classification implies the regular screening of individuals at risk for signs and status of sarcopenia to start therapeutic countermeasures as early as possible.

Age-related degradation in skeletal muscle mass is a continuous process. Defining the starting age of muscle reduction, however, appeared to be difficult. Some studies report a reduction in lean muscle mass by 3–8 % per decade starting at age 30 (Paddon-Jones and Rasmussen, 2009). Whereas, others report a broad-range, with muscle decline starting at either 27, 45, or 60 years depending on the method used for analyzing lean mass or muscle mass and on the study control population (Mitchell et al., 2012). On the other extreme, reports indicate skeletal muscle mass decline only after the age of 50 (Gallagher et al., 2000; Janssen et al., 2000). Due to the wide range of findings, most reports assume a relatively stable lean mass until the age of 40 and a slow but continuous loss in lean mass thereafter, which is accelerated by age 70 (Mitchell et al., 2012; Murton, 2015).

Dynapenia (Greek translation for poverty of strength, power, or force) is the age-associated loss of muscle strength that occurs independently from changes in muscle mass or due to neurologic or muscular diseases (Clark and Manini, 2008, 2012; Mitchell et al., 2012). This definition enables teasing apart the different disease stages. Thus, dynapenia, which is associated with changes in age-related muscle *strength*, is separated from sarcopenia, which exhibits also a decline in muscle strength but due to loss in muscle *mass* (Clark and Manini, 2008). Studies show that the regulation of both muscle mass and muscle strength is independent and that distinct pathways are responsible for each symptom (Clark and Manini, 2008, 2012; Mitchell et al., 2012).

As mentioned above, sarcopenia, besides the functional decline, represents a muscle-wasting syndrome. The difficulty is differentiating sarcopenia from other muscle-wasting conditions, such as cachexia, muscle disuse atrophy, and frailty (Nicolini et al., 2013). However, the causes of muscle wasting and clinical presentation differ, making these differences diagnostically useful in the clinical and research settings. Sarcopenia is mainly caused by factors inherent to skeletal muscle function such as a reduction in functional motor units, decreased anabolic hormone levels and protein synthesis (Argiles et al., 2015; Cade and Yarasheski, 2006; Morley et al., 2014). In contrast, cachexia may result from serious illness associated with systemic inflammatory impairments, e. g. cancer or organ failure, resulting in large energy imbalances by malnutrition (e. g. anorexia) and a hypermetabolic state (Argiles et al., 2015; Morley et al., 2014). In a progressive state, this leads to strong reductions in body weight, associated with both reduced lean muscle and fat mass (Argiles et al., 2015; Cruz-Jentoft et al., 2010; Drescher et al., 2016). As a consequence, most cachectic patients are also sarcopenic (secondary sarcopenia), but most sarcopenic patients cannot be considered cachectic (Cruz-Jentoft et al., 2010). Therefore, sarcopenia represents a feature or a consequence of cachexia wasting syndrome (Argiles et al., 2015). Apart from different causes, both diseases lead to a decline in muscle mass and muscle strength, suggesting these conditions may show similar drug-treatment responses (Morley et al., 2014).

Different from cachexia, sarcopenia-related loss in skeletal mass is not necessarily accompanied by decreased body weight. Instead, sarcopenia typically shows no change in overall body weight, but the ratio of fat to muscle increases. This so-called 'obesity-related sarcopenia' or 'sarcopenic obesity' shows alterations in muscle composition, e.g. 'marbling', resulting from fat infiltration into muscle (Cruz-Jentoft et al., 2010; Visser et al., 2002). In turn, fat 'marbling' decreases muscle quality and performance (Cruz-Jentoft et al., 2010; Visser et al., 2002). This trend is further exacerbated with age, as fat deposition shifts away from subcutaneous fat, while intramuscular and visceral fat depots increase (Cruz-Jentoft et al., 2010). This change in muscle mass quality

impairs muscle function and elevates the patient's risk of mortality (e. g. bone fracture) (Cruz-Jentoft et al., 2010; Gallagher et al., 2000; Prado et al., 2008).

These changes in fat deposition can promote metabolic complications since visceral and intramuscular fat accumulation is associated with a higher risk for cardiovascular diseases (Neeland et al., 2015), insulin resistance, and diabetes (Heilbronn et al., 2004). In the 1999–2004 National Health and Nutrition Examination Survey about 25 % of adults ≥ 60 years old were diagnosed with sarcopenic obesity (Batsis et al., 2016

Features of sarcopenic obesity are also observed in recipients of organ transplants (Schütz et al., 2012) and in cancer patients, where it represents an adverse prognostic factor and may considerably influence chemotherapy tolerance and toxicity (Anandavadivelan et al., 2016; Carneiro et al., 2016; Prado et al., 2008; Yip et al., 2015).

Sarcopenia also overlaps with frailty (Cruz-Jentoft et al., 2019). Frailty is a geriatric syndrome, while sarcopenia is a disease. Frailty is characterized by age-related cumulative declines across multiple organ systems resulting in impaired homeostatic reserves and reduced capacity of the organism to withstand stress. Therefore, frailty increases the individual's vulnerability to adverse health outcomes such as falls, hospitalization, institutionalization, and mortality (Cruz-Jentoft et al., 2010). Most frail people are sarcopenic, and conversely, some older people with sarcopenia are also frail. As in sarcopenia, low physical performance is a hallmark of frailty. However, the frailty syndrome includes additional impairments, such as unintended weight loss, exhaustion, and weakness and largely impacts cognitive status, social involvement, and other environmental circumstances (Cruz-Jentoft et al., 2019).

2.2. Prevalence of sarcopenia

The prevalence of sarcopenia is highly dependent on the parameters used for clinical diagnosis which makes it a rather subjective measure. In a meta-analysis, using EWGSOP criteria for evaluating sarcopenia prevalence only studies accounting for both muscle mass and function were considered. The study showed sarcopenia prevalence ranging from 1 to 29 % in community-dwelling older patients living on their own (Cruz-Jentoft et al., 2014). Prevalence rates were higher in long-term care facilities (14–33 %), especially in male residents (68 %), while only 10 % of acute care patients in the hospital setting met the criteria (Cruz-Jentoft et al., 2014). In another study analyzing chronically ill patients, prevalence rates were evaluated by muscle mass only (based on CT scans) without considering muscle function. Prevalence rates were higher, with 15–50 % in cancer patients, 30–45 % in patients with liver failure, and 60–70 % in critically ill patients in intensive care units (Peterson and Braunschweig, 2016). In the 1999–2004 National Health and Nutrition Examination Survey about 23–30 % of adults ≥60 years old were diagnosed with sarcopenia based on the measurement of appendicular lean mass by dual X-ray absorptiometry (Batsis et al., 2016). This agrees with the Berlin aging study II (BASE-II) that showed sarcopenia prevalence of about 24 % based on the measurement of appendicular lean mass (Spira et al., 2016). However, considering EWGSOP criteria, sarcopenia was only present in 2–4 % of the participants. It appeared that reduced muscle function (estimated by either reduced grip strength or limited mobility) was not always accompanied by reduced skeletal muscle mass (Spira et al., 2016). Importantly, reduced muscle function was associated with a greater impairment of physical performance than reduced muscle mass alone. Based on this observation, EWGSOP prioritized muscle functional measures before measurement of muscle mass for diagnosing sarcopenia (s. above). Hence, for sarcopenia diagnosis, measures of muscle function such as grip strength (i.e. muscle strength) and a measure of mobility (e. g. the "Timed Up and Go Test") are needed in addition to determining skeletal muscle mass.

It is generally suggested that the prevalence of sarcopenia increases with age although age specifications are not consistently reported or categorized into age groups (Cruz-Jentoft et al., 2014; Dodds et al., 2015). Indeed, evidence in the BASE-II study showed sarcopenia prevalence was higher in the oldest age group (70–84 years) when EWGSOP criteria were applied (Spira et al., 2016). However, when only reduced appendicular lean mass was considered, disease prevalence did not differ between younger (60–70 years) and older subjects (>70 years). Sarcopenia as an age-dependent disorder is mainly investigated and reported in older age groups. Prevalence rates at younger ages are only rarely reported. Kim and colleagues determined sarcopenia prevalence in a control group aged 40–59 years within the Korean sarcopenic obesity study only based on skeletal mass (appendicular skeletal mass (ASM)/height^2 below two standard deviations) (Kim et al., 2009). Under these conditions, sarcopenia prevalence was 2.8 % in men and 2.5 % in women. However, rates were doubeld in subjects aged 60 years and above (Kim et al., 2009).

In most studies, gender was not associated with sarcopenia prevalence (Cruz-Jentoft et al., 2014). Yet, in other studies either men (Kim et al., 2016) or women (Diz et al., 2016) showed higher prevalence rates.

The pathogenesis of sarcopenia is complex and several mechanisms are believed to contribute to the phenotype (Cade and Yarasheski, 2006) (s. also Fig. 1). As previously discussed, differentiating sarcopenia from other muscle atrophy syndromes is difficult. Therefore, on the molecular level, only general muscle atrophy mechanisms are reported here, which may not only be solely a consequence of sarcopenia.

3. Sarcopenia along the hallmarks of aging

Sarcopenia is an age-related disorder. Therefore, one may attempt to describe it using the hallmarks of aging as characterized by Lopez-Otin et al. in 2013 (Lopez-Otin et al., 2013).

The **primary hallmarks of aging** consider damage on the cellular level, namely genomic instability, telomere attrition, epigenetic alteration and loss of proteostasis. So far, the evidence of a genetic basis as a cause of sarcopenia is still low, but it is emerging. As mentioned above, functional muscle strength tests are essential for clear diagnosis of sarcopenia. However, a large body of studies investigating genetic influences on sarcopenia have been performedbased on skeletal muscle mass or lean body mass only . Additionally, investigations have been performed in knock-out animals showing characteristics of sarcopenia. In this context, *age-related DNA damage* especially in satellite cells and their capacity for DNA repair might play a significant role in muscle aging (Goljanek-Whysall et al., 2016). Furthermore, it was demonstrated that telomere attrition takes place in satellite cells (Tichy et al., 2017) leading to diminished regenerative capacity (Barberi et al., 2013; Renault et al., 2002). More specifically, a loss of the circadian clock gene Bmal1 (Vitale et al., 2019) as well as impaired function of the antioxidant enzyme peroxiredoxin 6 (Prdx6, (Pacifici et al., 2020) have been discussed as factors that could lead to sarcopenia or muscle atrophy. *Telomere attrition* has been considered as a possible cause of sarcopenia, however, reported results are inconclusive. Mostly, telomere length in human studies was analyzed in peripheral blood mononuclear cells, which may have little relevance or association with telomere length in skeletal muscle cells (Lorenzi et al., 2018). Lorenzi and colleaugues reported either no association between telomere length of leukocytes and sarcopenia or an association of telomere length with muscle or lean mass, but not with muscle strength (Lorenzi et al., 2018). However, in Chinese older persons longer telomers were associated with a slower decline in grip strength (Woo et al., 2014). Furthermore, the absence of Prdx6 reduced telomere length in skeletal muscle of knock-out mice, which was associated with increased proteolytic drive, muscle atrophy and decreased muscle strength (Pacifici et al., 2020) pointing towards a partial role of telomere length in the development of muscle atrophy. However, telomere shortening in the absence of Prdx6 might actually be a consequence of the loss in myocellular antioxidant capacity and of the consequent proteostatic disbalance (Pacifici et al., 2020) and, therefore,

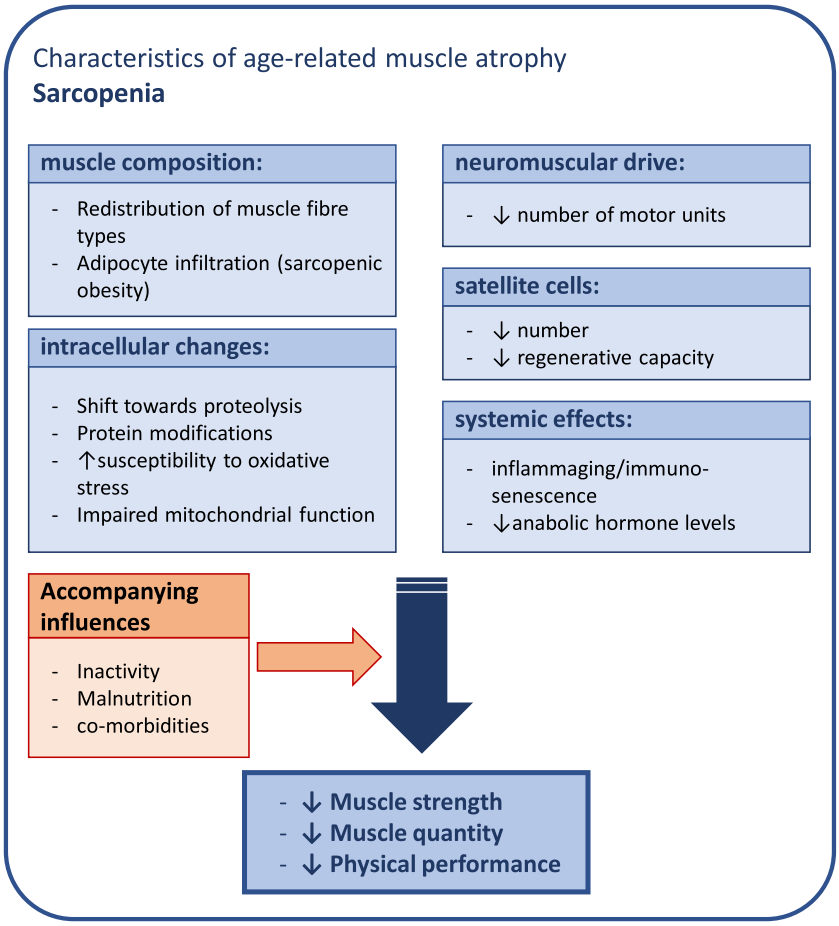

Fig. 1. Major characteristics of sarcopenia comprise several components from the cellular level to neuromuscular innervation and systemic effects. This complex mixture of components might, in combination with other accompanying situations, lead to the major features of sarcopenia.

not the main cause of muscle atrophy. *Epigenetic alterations* in the development of sarcopenia seem to play a significant role. In several studies the methylation pattern (Gensous et al., 2019; He et al., 2019) and microRNA species (Brown and Goljanek-Whysall, 2015; Goljanek-Whysall et al., 2016) could be associated with age-related skeletal muscle dysfunction including sarcopenia. Interestingly, a therapeutical potential of relevant microRNA species has been discussed for ameliorating age-related loss of muscle mass and function (Goljanek-Whysall et al., 2016). Besides the emerging role of epigenetic alterations, *loss of proteostasis* plays a pivotal role in the development of sarcopenia. This primary hallmark of aging skeletal muscles has been studied extensively and, as we will show below, proteostatic impairments in skeletal muscles contribute significantly to the atrophic drive that is evident in sarcopenic muscles. Intracellular mechanisms of protein homeostasis are suggested to shift towards proteolysis. This proteolytic imbalance is induced by intracellular protein modifications leading to enzymatic dysfunction and increased susceptibility to oxidative stress (Cade and Yarasheski, 2006)

So called **antagonistic hallmarks** of aging comprise metabolic responses to age-related cellular damage (*i.e.* primary hallmarks of aging) and includes deregulated nutrient sensing, mitochondrial dysfunction and cellular senescence (Lopez-Otin et al., 2013). *Deregulated nutrient sensing* mainly refers to impaired function of the growth hormone (GH)/Insulin-like factor-1 (IGF-1)-axis. It represents the main anabolic signal for muscle protein synthesis (s. Fig. 2). Serum levels of GH, IGF-1 and mechanical growth factor (MGF) are lower in older individuals with sarcopenia compared to non-sarcopenic individuals (Bian et al., 2020). In addition, IGF-1 and MGF were independently associated with the reduction of skeletal muscle mass (Bian et al., 2020) und lower IGF-1 serum levels were associated with lower handgrip strength and diminished physical performance (van Nieuwpoort et al., 2018) pointing to a crucial role for the GH/IGF-1 axis in skeletal muscle maintainance. *Mitochondrial function* is also adversely affected in the aged skeletal muscle leading to compromised energy supply and elevated intracellular oxidative stress (Cade and Yarasheski, 2006; Romanello and Sandri, 2015). One key element in mitochondrial physiology associated with muscle atrophy is dysfunctional mitochondrial quality control usually provided by mitophagy, the unfolded protein response, shedding of vesicles, proteolysis, and degradation by the ubiquitin-proteasome system (Pickles et al., 2018). These elements will be described in more detail below. *Cellular senescence* has been defined as a stable arrest of the cell cycle coupled to stereotypical phenotypic changes (Lopez-Otin et al., 2013). However, evidence suggest that cellular senescence plays only a minor role in skeletal muscle (Lopez-Otin et al., 2013).

Integrative hallmarks represent the physiologic consequences of primary and antagonistic hallmarks of aging and finally provide the functional decline associated with aging (Lopez-Otin et al., 2013). This is mainly characterized by stem cell exhaustion and altered intracellular communication. In skeletal muscle, satellite cells represent the *stem cell niche* playing an important role for its proliferative and regenerative capacity (Cade and Yarasheski, 2006; Domingues-Faria et al., 2015). With age, both the number and activity of satellite cells, e.g. their proliferative and regenerative capacity, are reduced further promoting muscle atrophy (Verdijk et al., 2014). A more in-depth discussion of this point is presented below. *Intracellular communication* involves endocrine, neuroendocrine, or neuronal signaling with adjacent cells as well as with cells in distant tissues (Lopez-Otin et al., 2013). During aging, intracellular communication is mainly influenced by inflammatory events.

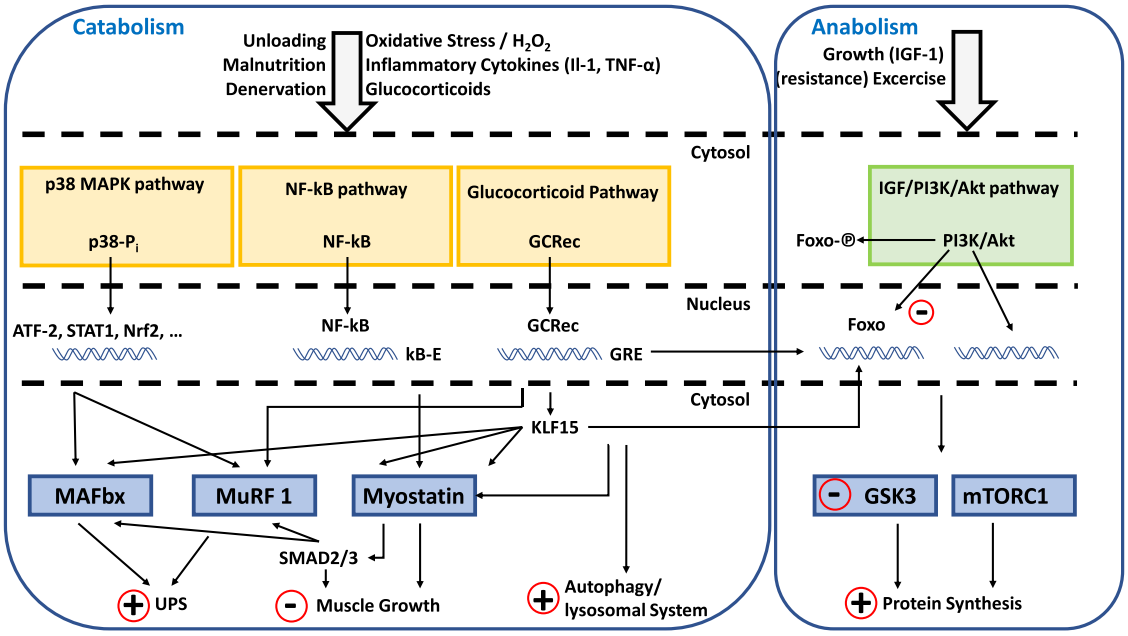

Fig. 2. Major signaling pathways influencing synthesis and breakdown of muscle proteins . (Akt: protein kinase B; ATF-2: activating transcription factor 2; FOXO: forkhead box proteins; GCRec: glucocorticoid receptor; GSK3: glycogene synthase kinase 3; GRE: glucocorticoid responsive element; IGF-1: insulin-like growth factor 1; IL-1: Interleukin 1 ; kB-E: kB responsive element; KLF15: Krüppel-like factor 15; MAFbx: muscle atrophy F-Box; MAPK: mitogen-activated protein kinase; mTORC1: mammalian target of rapamycin (mTOR) complex 1; MuRF 1: muscle RING-finger protein-1 ; NF-kB: nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2: Nuclear factor E2-related factor 2; p38-P_i: phosphorylated p38 mitogen-activated protein kinase; PI3K: phosphatidylinositol 3-kinase; TNF-α: tumor necrosis factor alpha; SMAD2/3: SMAD family transcription factors 2 and 3; STAT1: Signal transducer and activator of transcription 1; UPS: ubiquitin-proteasomal system).

Low-grade inflammation is observed in a considerable percentage of the elderly (also termed 'inflammaging' or 'immunosenescence') (Franceschi and Campisi, 2014), which may further exacerbate the pathogenesis of muscle atrophy (Cade and Yarasheski, 2006). Additionally, reactive oxygene species (ROS), which are increasingly present in the aged organism due, for example, to mitochondrial dysfunction, are suggested to promote 'contagious aging' from one cell to adjacent cells (Lopez-Otin et al., 2013). As further described below, both inflammaging and increased presence of ROS affect skeletal muscle function during aging and contribute to atrophic changes in the muscle.

The above mentioned age-related alterations consequently result in the phenotype of sarcopenia with the main characteristics of the loss in muscle mass and strength accompanied by changes in muscle composition and impaired neuromuscular drive. (Cade and Yarasheski, 2006). These phenomena are mainly provided by redistribution of muscle fiber subtypes, decreased capacity of myo-satellite cells to differentiate and proliferate (especially following muscle injury), and a decrease in functional motor units, all of which considerably contribute to the loss in muscle mass and strength (Cade and Yarasheski, 2006). We will now discuss sarcopenia-related changes in muscle composition and underlying molecular changes such as loss of proteostasis and mitochondrial dysfunction as well as inflammatory events in further detail.

4. Age-related changes in skeletal muscle composition

A major feature of sarcopenia is a decrease in lean muscle mass, which is the primary source of protein in the body. This decrease is mainly caused by a decline in the number of muscle fibers, especially type II fibers (Cade and Yarasheski, 2006; Cho et al., 2016; Domingues-Faria et al., 2015; Phu et al., 2015). Lean muscle comprises type I and II fibers. Type I fibers (slow-twitch) possess greater oxidative capacity due to their higher density of mitochondria and capillaries and mainly comprise the contractile protein myosin heavy chain (MHC) 1 (Cade and Yarasheski, 2006). Type II fibers (fast-twitch) possess higher glycolytic capacity and can be subdivided into type IIA and type IIB fibers, which are mainly made up of MCH2a and MHC2x, respectively (Cade and Yarasheski, 2006). The expression of MHC2a and MHC2x mRNA reportedly declines with advanced age and may be responsible for the specific loss in type II muscle fibers (Cade and Yarasheski, 2006).

The loss in muscle strength associated with sarcopenia might also be a consequence of the preferential atrophy of type II fibers (Cade and Yarasheski, 2006). Also, a decrease in the size of some type II fibers has been reported, while other type II fibers were of normal size as compared to muscles from young individuals (Andersen, 2003). Several studies, however, also report a slight decrease in the amount of type I fibers (Andersen, 2003) and considerable phenotypic changes in these slow-twitch fibers (e. g. a shift in adult MHC expression) in rats at higher age leading to considerable functional impairments (Carter et al., 2010).

Interestingly, a detailed examination of fiber type composition of single fibers from very old subjects revealed a switch in the fiber type along the length of the muscle fiber (based on ATPase staining). Close to one-third of the fibers in the fiber pool at very old age were neither strictly type I nor strictly type II fibers but exhibited fibers co-expressing both MHC I and IIA (Andersen, 2003). This phenomenon of mixed fibers was not observed in young muscle fibers and may partly explain the conflicting results of the loss in muscle mass being due to either type I or type II muscle fibers. Also, in the cross-section of muscle tissue of very old subjects there are areas with muscle fiber type clustering, while there is a random distribution of type I and type II fibers in young muscle tissue (Andersen, 2003). These phenomena may additionally contribute to reduced functional efficiency of skeletal muscles with increasing age.

Advanced age is associated with a diminished number of motor units, defined as a motor neuron with its innervated muscle fibers (Cade and Yarasheski, 2006). Axonal cell body size and the number of motor neurons decreases with age (Kawamura et al., 1977), which may contribute to compromised neuronal activation of skeletal muscle (Kido et al., 2004) and thereby further promoting age-related muscle dystrophy. The cause of age-related motor unit loss has not been well characterized yet. Decreased capacity for re-innervation after motor neuron loss was suggested to play a role (Cade and Yarasheski, 2006). A period

of muscle unloading in old rats, leading to hindlimb muscle atrophy was accompanied by a diminished capacity for muscle remodeling and for regaining initial muscle strength during reloading. This was suggested to be partly due to neuromuscular junction instability or denervation (Baehr et al., 2016). Intriguingly, lack of restoration in muscle strength with mass reload affected inactivity and/or denervation more in *tibialis anterior* muscle than in the *soleus* muscle and applied to both type I and type II muscle fibers. This suggests an age-related deficit in neuromuscular transmission, which is muscle-type specific and may become further aggravated by age-related immobility. Therfore, regular exercise even at old age (e. g. above age 70) has the potential of slowing down age-related muscle dystrophy (Cho et al., 2016).

Satellite cells of skeletal muscles represent the stem cell niche of this tissue and are involved in muscle growth and regeneration after injury and disease (Domingues-Faria et al., 2015). When leaving their quiescent state, satellite cells can proliferate, differentiate, and fuse to augment existing muscle fibers or to form new fibers. With aging, the number of satellite cells per myo-fiber decreases along with its regenerative capacity (Cade and Yarasheski, 2006; Domingues-Faria et al., 2015). In humans, this decrease was specifically observed for satellite cells associated with type II myo-fibers, which correlates with the pronounced type II fiber loss seen in aging (Domingues-Faria et al., 2015). Most likely, this is associated with a decreased proliferation and growth rate due to several age-related phenomena such as prolonged doubling time of satellite cells, reduced responsiveness to proliferating stimuli, and a reduction in telomere length throughout the proliferation cycles (Cade and Yarasheski, 2006; Domingues-Faria et al., 2015). The decreased proliferative capacity of muscle stem cells with age is further promoted by increased expression of cell cycle inhibitors. Aged satellite cells constitutively produce fibroblast growth factor-2 (FGF2), even in the absence of injury, leading to a disrupted stem cell quiescence and impaired self-renewal capacity (Domingues-Faria et al., 2015). Apart from proliferation, differentiation capacity of satellite cells is also diminished with age (Domingues-Faria et al., 2015), and is associated with a reduced number of differentiating cells and a blunted expression of differentiation markers such as Myf-5, MyoD, Myogenin and MRF4/Myf6 (Domingues-Faria et al., 2015). Consequently, a reduced rate of satellite cell differentiation into myo-tubes and a decreased expression of the final differentiation marker, myosin, were reported in aged rat muscle (Domingues-Faria et al., 2015).

On the molecular level, the imbalanced activity of two signaling pathways, the Notch and the Wnt signaling pathways, contribute to the age-related decrease in muscle regenerative capacity (Domingues-Faria et al., 2015). Normally, the Notch pathway is activated following muscle lesion, thereby inducing satellite cell proliferation and self-renewal. Inhibition of this pathway by Wnt signaling allows these cells to differentiate. During aging, however, the balance between the Notch and Wnt pathways required for appropriate satellite cell proliferation and differentiation is impaired. Aging is associated with decreased activity of Notch signaling, and conversely, hyperactivity of Wnt signaling, blocking differentiation and promoting fibrinogenic signaling (Domingues-Faria et al., 2015).

Age-related loss of muscle mass was reported to account for up to 42 % of muscle mass between the ages of 30 and 80 years with a rapid decline after 50 years of age (Cade and Yarasheski, 2006). This implies that relevant interventions to remediate and prevent age-related muscle atrophy, especially exercising and diet modification, should start much earlier than age 50 when the most dramatic decline occurs.

5. Age-related changes of molecular function in skeletal muscle

5.1. Muscle protein homeostasis

Skeletal muscle represents the largest reservoir of amino acids in the body. The regulated balance between protein synthesis and breakdown (proteolysis), also known as protein homeostasis, maintains a balance between proteins and free amino acids (Fig. 2).

In general, aging is associated with a decreased rate of proteolysis, which results in the formation of toxic protein aggregates (Breusing et al., 2009; Grune et al., 2004; Hohn et al., 2017; Pomatto and Davies, 2017; Raynes et al., 2016; Sitte et al., 2000a,b). However, it was suggested that protein turnover in muscle is mainly determined by the declining rate of protein synthesis (rather than degradation), which may negatively impact muscle regeneration (Cade and Yarasheski, 2006). Protein synthesis rate is reduced by 30 % mainly due to changes in the synthesis of mixed muscle proteins (myofibrillar, mitochondrial and sarcoplasmic) (Cade and Yarasheski, 2006). As an example, the rate of MHC synthesis is reduced in middle-aged men (approximately age 55) and further reduced in older individuals (approximately age 77), which may considerably contribute to the age-associated loss in muscle strength (Balagopal et al., 2001). Additionally, mitochondrial protein synthesis is decreased with age (Cade and Yarasheski, 2006) and together, with decreased MHC synthesis, contributes considerably to decreased muscle strength in advanced age. While the total synthesis rate of sarcoplasmic proteins is normal, individual sarcoplasmic protein synthesis decreases with age. Protein turnover rates of calcium regulatory proteins such as the Ca^{2+}-ATPase transporter, and the ryanodine receptor, however, declined with age (Cade and Yarasheski, 2006).

At the molecular level, protein synthesis and protein degradation, are regulated by different pathways. ***Protein synthesis*** is mainly induced during normal growth (e. g. by growth factors and growth hormone) or by conditions such as (resistance) exercise. The main anabolic signal in skeletal muscle is insulin-like growth factor 1 (IGF-1). IGF-1 binding to its receptor, a trans-membrane tyrosine kinase receptor, on the plasma membrane of myocytes causes intracellular trans-phosphorylation of the receptor and the formation of a docking site for insulin receptor substrate 1 (IRS-1). This receptor activation induces IRS-1 phosphorylation activating the phosphatidylinositide 3-kinases (PI3K)/protein kinase B (Akt) pathway. This pathway activation leads to increased protein synthesis and muscle hypertrophy by:

- Inhibition of glycogen synthase kinase-3 (GSK3),
- Activation of mammalian target of rapamycin complex-1 (mTORC-1),
- mTORC-1-mediated phosphorylation of p70S6 kinase and IF-4E-binding protein (4EBP) inhibition (Fig. 2).

In addition, PI3K/Akt activation also prevents protein degradation in skeletal muscle by phosphorylating and thereby inactivating O-type fork head box (FOXO) transcription factor. This mechanism is crucial in cell cycle regulation, apoptosis, and metabolism. Of the FOXO transcription factors FOXO1, FOXO3a, and FOXO4 are all expressed in skeletal muscle. Upon phosphorylation, FOXO transcription factors are unable to translocate into the nucleus, preventing the upregulation of proteolysis-related genes, such as the E3 ligases muscle RING-finger protein-1 (MuRF-1) and muscle atrophy F-Box (MAFbx) (see below). Therefore, IGF-1 stimulates both protein synthesis and the proliferation of satellite cells, while suppressing protein degradation (Philippou et al., 2007)

Insulin is another anabolic hormone acting on myocytes (Tokarz et al., 2018). Aging is characterized by decreased sensitivity of myocytes to insulin, which may additionally contribute to diminished muscle mass. The decreased sensitivity to insulin found in elderly individuals may be due to increased ectopic lipid storage (e. g. of ceramides) in muscle (Slawik and Vidal-Puig, 2007; Tardif et al., 2014), accompanied by impaired mitochondrial activity and muscle protein synthesis (Tardif et al., 2014). Additionally, reduced endothelial function may contribute to decreased insulin sensitivity, but it can be restored by co-administration of sodium nitroprusside (a vasodilator) with a protein-rich meal (Muller et al., 2014). Other data suggest that reduced PPAR gamma coactivator 1-alpha (PGC-1α) signaling (involved in the regulation of mitochondrial biogenesis in skeletal muscle) results from reduced insulin sensitivity and decreased Akt- and mTOR-expression.

Together, this highlights the importance of anabolic function in the pathology of sarcopenia.

Protein degradation in skeletal muscle is induced by various hormonal and metabolic stimuli, such as glucocorticoids, oxidative stress and inflammatory cytokines occurring during several pathogenic conditions (denervation, malnutrition, and muscle unloading, Fig. 2). Myocytes are particularly susceptible to oxidative damage since they are post-mitotic and are, therefore, especially predisposed to accumulate oxidatively damaged molecules (Rom and Reznick, 2016). Also, skeletal muscle accounts for a large share of total oxygen consumption, increasing the inherent risk of elevating mitochondria-derived reactive oxygen species (ROS), such as hydrogen peroxide (Rom and Reznick, 2016). Inflammatory cytokine production and circulation are induced by oxidative stress, and aging ("inflammaging"), and during acute or chronic pathogenic conditions. As an example, tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) and -6 (IL-6) are relevant inflammatory cytokines promoting protein degradation in muscle (Rom and Reznick, 2016). Glucocorticoids are important players in stimulating immediate sources of cellular energy, including the initiation of skeletal muscle proteolysis. Therefore, glucocorticoid levels are increased in some pathogenic conditions, which require higher energy supply (e. g. sepsis, cachexia, starvation, stress and insulinopenia) (Hoppeler, 2016).

Protein degradation is tightly regulated in coordination with anabolic hormones like insulin and IGF-1 and their intracellular signaling pathways. All of the stimulators of protein degradation act on common intracellular pathways.

In general, intracellular protein degradation or proteolysis is mediated mainly by four mechanisms:

(1) the ubiquitin proteasomal system (UPS), the major regulatory mechanism of skeletal muscle atrophy,
(2) the lysosomal proteolytic system with cathepsins as major lysosomal proteases,
(3) the calcium-dependent calpains, non-lysosomal proteases, that mediate cleavage of specific substrates,
(4) the caspases, the cysteine-dependent aspartate-specific proteases (Rom and Reznick, 2016).

In skeletal muscle, catabolic stimuli increase protein degradation by the ubiquitin-proteasome system (UPS) and autophagy. Calpains initiate the breakdown of large proteins, some of them are further degraded by the UPS (Pedrozo et al., 2010; Shenkman et al., 2015). Therefore, myofibrillar components are mostly turned over by the UPS (loss of contractile force), while mitochondria and some soluble proteins are preferentially degraded by autophagy (loss of endurance capacity) (Hoppeler, 2016).

The degradation process is activated by two principal pathways: the p38 mitogen activated protein kinase (MAPK) pathway and the nuclear factor-kappa B (NF-κB) pathway (Fig. 2). Additionally, glucocorticoids can directly affect gene expression via binding of dimerized ligand-activated glucocorticoid receptors (GR) on glucocorticoid receptor responsive elements (GRE) on target promotor regions.

MAPK p38 is responsible for activating the expression of the muscle-specific E3 ligases MuRF-1 and MAFbx (Rom and Reznick, 2016). Increased activity of MAPK p38β in myotubes, for example, induces phosphorylation and activation of the CCAAT/enhancer-binding protein-β (C/EBPβ), a transcription factor capable of binding to and activating the MAFbx promotor (Zhang and Li, 2012).

Alternatively, induction of the canonical NF-κB pathway by oxidative stress or inflammatory cytokines may lead to MuRF-1 and MAFbx activation. In general, genes carrying kappaB elements are known to be involved in immune function, growth regulation, inflammation, carcinogenesis and apoptosis (Rom and Reznick, 2016). Among those genes is MuRF-1, its activation representing a key step in NF-κB-induced atrophy (Rom and Reznick, 2016). NF-κB also binds to kappaB elements on the myostatin gene, a small secreted protein derived from skeletal muscle and a central negative regulator of muscle growth (Braun and Marks, 2015; Hoppeler, 2016). Myostatin exerts activation of protein degradation through interaction with several pathways. It inhibits phosphorylation and thereby activation of Akt leading to instability of FOXO phosphorylation. Dephosphorylated FOXO transcription factors, especially FOXO1 and FOXO3a, show increased expression during several forms of atrophy and are prominent activators of MuRF-1 and MAFbx expression by binding to FOXO-responsive elements in their promoter region (Bodine et al., 2001; Hoppeler, 2016). Interestingly, FOXO transcription factors not only control the transcription of the E3-ligase enzymes MuRF-1 and MAFbx inducing UPS proteasomal degradation but also control the transcription of core components of autophagosomes and lysosomes such as Bnip3, which promotes the autophagy-lysosomal system (Sandri, 2010). Therefore, both degradation systems are interacting and are carefully regulated.

Additionally, myostatin by binding to activin type two receptors (ActRIIA/B) phosphorylates and thereby activates the transcription factors SMAD2 and SMAD3 (Braun and Marks, 2015). Through activation of the SMAD transcription factors, myostatin down-regulates genes involved in myogenic differentiation such as MyoD, myogenin, and myf5 (Braun and Marks, 2015), thereby compromising muscle regenerative capacity. Also, SMAD2 and SMAD3 activation induce MuRF-1 and MAFbx expression. The gene expression of MuRF-1 induced by SMAD transcription factors was observed to be especially induced in cooperation with FOXO transcription factors since multiple conserved FOXO-responsive elements adjacent to SMAD-binding elements were identified in the MuRF-1 promoter (Bollinger et al., 2014). Co-expression of SMAD3 and FOXO3a increased expression of both, MuRF-1 as well as MAFbx genes, and this transcriptional activity was higher than with FOXO expression alone (Bollinger et al., 2014). It is believed that myostatin causes increased muscle protein degradation mainly by direct activation and increased activity of FOXO, through the suppression of the Akt/mTOR axis (Rodriguez et al., 2014). Furthermore, myostatin-mediated degradation is suggested to be facilitated by the activity of the ubiquitin-proteasome and autophagy pathways (Hoppeler, 2016).

Myostatin is a member of the TGF-β-family and a negative regulator of muscle growth (Sakuma et al., 2015). Ectopic overexpression of myostatin in rodents led to lower muscle mass (Amirouche et al., 2009; Durieux et al., 2007), and increased myostatin expression is assumed to contribute to muscle wasting under pathologic conditions like HIV infection, sarcopenia, or muscle disuse-induced atrophy (Sakuma et al., 2015). Genetic inhibition of myostatin production (shown in mdx mice, a model of Duchenne Muscular Dystrophy) causes both recovery of muscle mass and muscle force production compared to wild-type mice (Lu-Nguyen et al., 2017). Myostatin reverses the IGF-1/PI3K/AKT hypertrophy pathway by inhibition of AKT-phosphorylation thereby increasing FOXO1-expression and inducing atrophy-related genes (McFarlane et al., 2006; Sakuma et al., 2015). Following myostatin treatment, the ubiquitin associated proteins MuRF-1 and MAFbx were upregulated, followed by a 60 % increase in the level of ubiquitin-conjugated proteins, especially prevalent in proteins of 70 kDa or smaller (McFarlane et al., 2006) thereby promoting proteolysis. Myostatin levels increased in muscle atrophy induced by muscle unloading (Sakuma et al., 2015). On the contrary, inhibition of myostatin activity was able to increase muscle mass and size (Sakuma et al., 2015). However, an age-dependent increase of myostatin levels (either as circulating protein or as mRNA residing in skeletal muscle) as a potential cause of sarcopenia could not be consistently proven so far (Sakuma et al., 2015). Intriguingly, it is suggested that myostatin levels increase with obesity (Sakuma et al., 2015) possibly as a consequence of ectopic lipid storage in skeletal muscle. For example, myostatin levels were found to be increased in plasma and myotubes of extremely obese middle-aged women, which correlated with insulin resistance and BMI (Hittel et al., 2009). The potential role of myostatin in sarcopenic

obesity, which is also accompanied by ectopic lipid storage in skeletal muscle, has not been elucidated so far.

As stated above, glucocorticoids represent prominent catabolic players in skeletal muscle homeostasis. Among the genes containing a glucocorticoid receptor response element (GRE) is MuRF-1. While there is no GRE on MAFbx promotor, MAFbx expression is still increased with glucocorticoid action indicating that indirect activation through interaction with other transcription factors may occur (Braun and Marks, 2015). Indeed, GRE have been identified in the promoter regions of the transcription factors FOXO3a and Krüppel-like factor 15 (KLF15). While the activity of the GRE on FOXO3a promotor still needs to be verified, GRE activation on KLF15 promotor leads to increased KLF15 expression (Braun and Marks, 2015). KLF15 subsequently interacts with KLF15-responsive elements in MuRF-1 and MAFbx promotor regions and induces their expression. Overexpression of both transcription factors KLF15 and ligand-activated clucocorticoid receptor resulted in additive increases in MAFbx and MuRF-1 promoter activity and expression (Braun and Marks, 2015). Furthermore, KLF15 indirectly induces MAFbx and MuRF-1 expression via stimulation of FOXO1 and FOXO3a expression. Therefore, FOXO transcription factors and KLF15 act cooperatively with GRE activation to induce expression of MuRF-1 and MAFbx. It is the synergistic interplay between ligand-bound glucocorticoid receptors and several transcription factors that partly activate each other and constitute a transcription factor network regulating protein degradation in the myocyte by activating the E3 ligases MAFbx and MuRF-1 (Braun and Marks, 2015). Intriguingly, glucocorticoid-related induction of muscle atrophy is more prominent in fast-twitch skeletal muscle that in slow-twitch muscle. This is obviously due to much higher expression of GR in these muscle types, further explaining the muscle fibre-specific proneness to atrophy (Braun and Marks, 2015). Glucocorticoids also induce myostatin transcription and myostatin stability by regulating posttranslational modifications, thereby further pronouncing protein degradation (Hoppeler, 2016). As mentioned above already, expression of MuRF-1 and MAFbx is regulated by direct binding of several transcription factors such as NF-κB (including p65, c-Rel, RelB, p52, p50), the CCAAT-enhancer-binding proteins (or C/EBPs), and SMAD3 opening the probability of several transcription factor combinations acting to induce MuRF-1 and MAFbx gene expression (Bodine and Baehr, 2014b).

Growth differentiation factor 11 (GDF11) appears to be another relevant player in the induction of muscle atrophy (Hoppeler, 2016). Although it shares about 90 % homology with myostatin in the active regions of the mature protein sequence, expression patterns differ. Myostatin is expressed mainly in developing and adult skeletal muscle, whereas GDF11 expression is highly tissue-specific (e. g. dental and neural tissues) (Nakashima et al., 1999). Myostatin (which is also called GDF8) and GDF11 belong to the transforming growth factor β (TGF-β) superfamily and share similar signaling pathways; both bind activin type II receptors and activate the intracellular mediator SMAD 2/3 pathway. Intriguingly, GDF11 expression was shown to increase with age in mice and humans and the highest GDF11 levels coincided with muscle pathology (Egerman et al., 2015). Therefore, it seems reasonable to suggest that GDF11 may also be involved in age-dependent protein degradation in skeletal muscle. However, the precise function of GDF11 for sarcopenia-related muscle loss still needs to be unraveled.

Myostatin has been identified as a potent inducer of producing reactive oxygen species (ROS) in myotubes (Sriram et al., 2011). Increased myostatin levels (as occurring during aging) induce TNF-α production via stimulation of NF-κB signaling, which increases the generation of ROS by NAPDH oxidase. Increased ROS levels induce a "feed-forward loop" further increasing myostatin levels via TNF-α and NF-κB signaling. This mechanism, therefore, maintains sustained ROS production in situations of elevated myostatin levels and may worsen skeletal muscle maintenance under these conditions (Sriram et al., 2011).

Protein turnover is also regulated on the level of microRNAs (miR) (Braun and Marks, 2015). For instance, increased amounts of miR-1 lead to lower amounts of heat shock protein70 (HSP70), prevent the stabilization of phosphorylated Akt (pAkt), and enable the nuclear translocation and activation of FOXO target genes such as MURF-1 and MAFbx, which upon upregulation, promote protein degradation (Braun and Marks, 2015). On the contrary, miR-23a, miR-27a and miR-27b inhibit atrophy by either directly blocking MuRF-1 and MAFbx expression (miR-23a) or by decreasing stability and half-life of myostatin mRNA (miR-27a and miR-27b) (Braun and Marks, 2015).

5.2. The role of the UPS in skeletal muscle protein turnover

The UPS and the lysosomal proteolytic system are the most prominent executers of intracellular proteolysis (70 %–90 % of misfolded or damaged proteins are degraded via the UPS (Jung et al., 2009)).

The UPS is the main cellular system to mediate cytosolic protein degradation. It does so, by recognizing and removing damaged, misfolded, and dysfunctional proteins to prevent their accumulation in cytotoxic aggregates. This occurs in a highly regulated manner through the interaction with co-factors (Jung et al., 2009). A precondition for one way of UPS-mediated degradation is a polyubiquitin chain tagged to proteins. Polyubiquitinated substrates carrying at least 4 covalently-bound ubiquitin proteins, are recognized by the 19S regulator of the UPS, which subsequently unfolds the substrate in an ATP-dependent manner, releases the polyubiquitin tail to be recycled, and shuttles the target protein into the proteolytic core for degradation. The UPS degradation system is, therefore, a highly energy-intensive process (Benaroudj et al., 2003)

Polyubiquitination is not a one-way road to degradation, as 100 of the so-called deubiquitinating enzymes (DUBs) are reportedly involved in the fine-tuned regulation of cellular proteostasis. However, little is known regarding the role of these identified DUBs in sarcopenia. One of the rare studies revealed that the ubiquitin-specific protease 19 (USP19) deubiquitinating enzyme, that is induced in skeletal muscle under many catabolic conditions, was also involved in muscle wasting. USP19 knockout mice lost less muscle mass than the wild-type mice responding to glucocorticoids (common in systemic muscle atrophy) and denervation (model of disuse atrophy) (Bedard et al., 2015). They were characterized by more strength and less myofiber atrophy (referring to both slow type I and fast type IIb fibers). Synthesis rates of muscle protein were comparable between the wild-type and knock-out mice, thus pointing to a reduced rate of proteolysis. Expression of MuRF-1 and MAFbx were decreased in knock-out mice as well as several genes involved in autophagy (in this case Atg4 and Bnip3). Furthermore, in patients suffering from lung or gastrointestinal cancer, the expression of USP19 correlated positively with the expression of MuRF-1 and MAFbx.

5.3. The role of the E3 ubiquitin-ligases MuRF-1 and MAFbx in skeletal muscle atrophy

The activity of specific E3 ligases determines the protein substrate specificity of UPS-mediated proteolysis. MuRF-1and MAFbx are E3 ligases, initially identified for their specific expression in skeletal muscle and heart of atrophic rat models that underwent hindlimb suspension, immobilization, and denervation (Bodine et al., 2001; Gomes et al., 2001; Rom and Reznick, 2016). Additionally, increased MuRF-1 and MAFbx expression in muscle tissue is also observed with fasting, diabetes, cancer, inflammation, metabolic stress, and glucocorticoids, all representing conditions associated with muscle atrophy (Bodine and Baehr, 2014a; Gomes et al., 2001; Rom and Reznick, 2016). However, due to the lack of available antibodies, the dynamics of protein expression of these genes are not known (Bodine and Baehr, 2014a), so observations reported have been based on mRNA expression levels only. Additionally, while several studies report an increase in mRNA of both MuRF-1 and MAFbx under atrophy inducing conditions (Bodine and Baehr, 2014b), others report no increase or even a decrease (Bowen

et al., 2015). So the exact role of MuRF-1 and MAFbx on the protein level during muscle protein degradation conditions remains elusive (Fig. 3).

Overexpression of MAFbx leads to atrophy in cultured myotubes, while genetic knock-out of either MAFbx or MuRF-1 leads to reduced atrophy in mice in response to denervation. MAFbx and MuRF-1 knock-out is associated with protection against muscle loss, of as much as 56 % and 36 %, respectively (Bodine et al., 2001). Due to the specific tissue expression and the atrophy-specific induction of MAFbx and MuRF-1, these genes are considered key markers of muscle atrophy (Bodine and Baehr, 2014a). However, MuRF-1 and MAFbx expression may also play a role in skeletal muscle remodeling. Their expression levels are reported to be transiently induced at the onset of reloading following disuse atrophy and following eccentric contractions (Bodine and Baehr, 2014a). Therefore, these factors might not be pure atrogenes (i.e. a set of genes that are sensitive markers of the atrophic processes).

The identification of protein substrates targeted by MAFbx and MuRF-1 mediated ubiquitination is still a field of high interest (Bodine and Baehr, 2014a; Rom and Reznick, 2016). Until now, only two major MuRF-1 targets have been identified: proteins involved in ATP-generation and myofibrillar proteins (Witt et al., 2005). Though a number of myofibrillar proteins that interact with MuRF-1 (nebulin, titin, MLC-2, and cTNI) were determined not to be primary targets of MuRF-1-mediated polyubiquitination, those proteins were still found at similar amounts in both wildtype and MuRF-1-knockout mice (Witt et al., 2005). Also, overexpression of MuRF-1 did not suggest myofibrillar proteins to be the primary target because transgenic mice did not exhibit muscle atrophy or decreased amounts of myofibrillar proteins compared to the wildtype (Hirner et al., 2008; Mayans and Labeit, 2012). As mentioned above, some protein substrates of MuRF-1 (other than MAFbx) were found to be involved in ATP-generation (especially in glycolysis), pointing to a role of MuRF-1 in metabolic regulation (Witt et al., 2005). However, MuRF-1 plays a role in polyubiquitination of the myosin heavy chain (MHC), but not of actin or other thin filament proteins (Cohen et al., 2009). Although myofibrillar proteins can be polyubiquitinated by MuRF-1 *in vitro* (Bodine and Baehr, 2014b), the involved pathways still have to be determined. MAFbx targets are MyoD (a myogenic differentiaton factor found in skeletal muscle) and the eukaryotic translation initiation factor 3 subunit f (eIF3-f), while other potential targets are MHC and several sarcomeric factors like the intermediate filament proteins vimentin and desmin (Lokireddy et al., 2011, 2012). Table 1 summarizes the protein substrates of MAFbx- and MuRF-1-mediated ubiquitination proposed so far (Bodine and Baehr, 2014a; Rom and Reznick, 2016).

Table 1
Proposed protein substrates for the E3 ligases MAFbx and MuRF-1 (Bodine and Baehr, 2014b; Witt et al., 2005).

MAFbx[a]	MuRF-1
regulatory proteins: -myogenic factor MyoD1 -myogenin -eukaryotic translation initiation factor 3-subunit F (eIF3-f) other potential targets: -myosin heavy chain -vimentin -desmin	proteins involved in ATP generation mainly those involved in glycolysis and glycogen metabolism, e.g. mitochondrial ATP synthase and cytoplasmic creatine kinase. Structural/myofibrillar proteins[b]: -titin -troponin1, troponin-T -myosin heavy and light chains -myosin-binding protein C -nebulin, nebulin-related protein, -myotilin -T-cap

[a] all identified targets rely on *in vitro* experiments, validation *in vivo* still required (Bodine and Baehr, 2014a).
[b] interaction is likely indirect and involves other E3 ligases (Bodine and Baehr, 2014a).

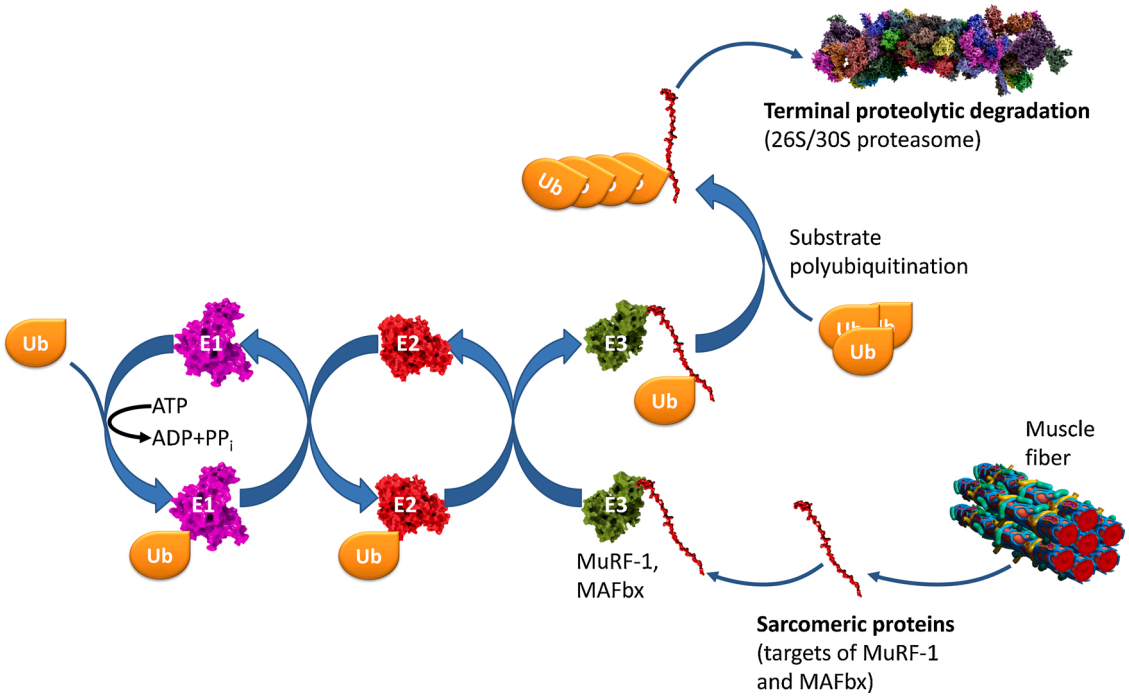

Fig. 3. Degradation of muscle fiber proteins by the ubiquitin-proteasomal system. The muscle fibers are dissolved to sarcomeric proteins, which can then be recognized by the activated E3 ubiquitin ligases (MuRF-1 or MAFbx). Activation of E3 ubiquitin ligases takes place by binding of ubiquitin (Ub) in a step-wise process via E1 (ubiquitin activating enzyme) and E2 (ubiquitin conjugating enzyme) with the concomitant consumption of ATP. Several molecules of ubiquitin are bound to the substrates in a K48-poly-ubiquitin chain. This poly-ubiquitin chain then binds to the 19S regulator of the 26S/30S proteasome, where the poly-ubiquitin chain is split-off and the substrate protein is 'fed' into the 20S core proteasome where it is degraded, by successive peptide bond cleavages, to short peptides and amino acids.

5.4. Conflicting evidence for the role of MuRF-1 and MAFbx in protein turnover

The relevance of the role(s) of MuRF-1 and MAFbx in the regulation of proteostasis has yet to be fully characterized. Muscle hypertrophy, for instance, is associated with both increased protein synthesis and degradation (Baehr et al., 2014a).

The atrophy-induced induction of MAFbx and MuRF-1 expression is also observed in humans (Bodine and Baehr, 2014a). However, there are still contradicting observations and some studies reported no increased expression in atrophy (Rom and Reznick, 2016). This discrepancy might be explained by the cause of atrophy (e. g. either immobilization, denervation, or severe illness) and/or by the time point of muscle biopsy relative to the triggering time point for the atrophic condition. Rodent studies revealed time-dependent dynamics of MAFbx and MuRF-1 expression with a rapid and strong increase within the first 48 h following the atrophic trigger, followed by a persistent elevation for 7–10 days, finally followed by a gradual decrease to baseline by 14 days (Bodine and Baehr, 2014a). However, a rigorous time course study in humans has yet to be done.

Another study investigating proteasomal activity and muscle hypertrophy after functional overload (FO) revealed a short increase in MuRF-1 and MAFbx expression (after the first 24 h) that was followed by significant reductions of their expression (after 7 and 14 days of functional overload) (Baehr et al., 2014b). FO of the plantaris muscles of mice was induced by surgical removal of the entire soleus and over half of the medial and lateral gastrocnemius, resulting in hypertrophy/increase of mass via removal of synergistic muscles. The primary increase was also accompanied by a significant increase in 20S and 26S proteasomal activity (beta5-subunit, starting after 24 h with a peak after 7 days). Intriguingly, proteasomal activity remained enhanced throughout the 14 days of functional overload, even though MuRF-1 and MAFbx expression had already returned to normal or decreased 3 days after FO. Expression of FOXO1 and FOXO3a increased only from 3 to 7 days and declined to baseline levels by 14 days after FO. FO was also accompanied by an increase in markers of ER-stress (the enzymes BiP, PDI, and CHOP).

In female MAFbx knock-out mice, the muscle mass did not increase after FO, while in male MAFbx knock-out mice muscle mass increased significantly (Baehr et al., 2014). The mice showed increased protein synthesis, that matched the increase in muscle mass. Enhanced expression of the endoplasmic reticulum (ER) chaperones BiP and PDI was also found, both enhancing the protein-folding and quality control of the ER (Baehr et al., 2014). This study concludes, that the amounts of MuRF-1 and MAFbx mRNA may not always be good markers for the actual proteasomal proteolysis as described above, since acute alcohol intoxication (Vary et al., 2008) and glucocorticoid treatment (Baehr et al., 2011) can induce MuRF-1 and MAFbx expression, without causing a corresponding increase in skeletal muscle proteolysis (alcoholic intoxication) or increase of proteasomal activity (glucocorticoid treatment). Others have questioned the assumed role of FOXO in MuRF-1 and MAFbx expression, since FOXO1 and -3a do not increase until after MuRF-1 and MAFbx return to baseline levels (Baehr et al., 2014). Finally, even induction of hypertrophy elevates both degradation and synthesis of muscle proteins and thus the individual roles of both MuRF-1 and MAFbx have to be better understood, including exact identifications of their substrates.

The large inconsistencies in experimental results may even suggest a major role of one of the other mentioned proteolytic pathways bringing the UPS slightly out of focus. Specifically, interpretation by Bowen and colleagues points to the particular importance of both the calpain and autophagy pathways in sarcopenia. There are, however, seemingly contradictory results, with some studies indicating that proteasome activity declines with age (Fernando et al., 2019; Ferrington et al., 2005), while others reported a significant increase of proteasomal protein concentration and activity (especially 26S) in aged sarcopenic muscle (Altun et al., 2010). These differing accounts may be due to the type of muscle investigated and the assay type used (Strucksberg et al., 2010).

5.5. The role of the lysosomal degradation in skeletal muscle protein turnover

As mentioned above already, lysosomal proteolysis plays an important role in intracellular protein degradation complementary to UPS-mediated degradation. Lysosomes are cytoplasmic organelles having a unique internal acidic pH environment coupled with different hydrolases and proteases (cathepsins). This equipment enables efficient degradation of misfolded and aggregated proteins not degradable by the UPS (Jackson and Hewitt, 2016). Usually, lysosomes are responsible for degrading soluble proteins that are long-lived and resist unfolding, which is a prerequisite for protein degradation by the UPS (Jackson and Hewitt, 2016; Mizushima et al., 2008). Lysosomes are able to degrade exogenous proteins (targeted by receptor-mediated endocytosis and pinocytosis) as well as endogenous proteins and organelles (targeted by microautophagy and macroautophagy, respectively) (Ciechanover, 2005). Affected proteins reach the lysosome either by lysosome fusion with an autophagosome or by chaperone-mediated autophagy (CMA) with a lysosome (Fig. 4).

Generally, lysosomal function, macroautophagy, and CMA decrease with age, which contributes to sarcopenia (Carnio et al., 2014; Jiao and Demontis, 2017a; Mizushima et al., 2008; Sakuma et al., 2015). Impaired function of micro- and macroautophagy leads to diminished clearance rate and, therefore, the accumulation of damaged cell components and mis-folded proteins. Among those are lipofuscin, chaperones such as Hsp27, and other polyubiquitinylated proteins such as p62/SQSTM1 (Jiao and Demontis, 2017a) that serve as markers for autophagic activity. These non-degraded proteins aggregate intracellularly, which further impairs the generation of autophagolysosomes and inhibits lysosomal activity (Mizushima et al., 2008). Concomitantly, age-related changes in lysosomal membrane structure lead to instability and decreased levels of LAMP-2A, which contributes to low CMA activity with increasing age (Kon and Cuervo, 2010; Mizushima et al., 2008). Decreased LAMP-2A levels are suggested to be the result of increased degradation rather than decreased synthesis (Kon and Cuervo, 2010). This molecular disarrangement causes a deteriorated cellular stress response and reduced cell viability, and may lead to muscle atrophy and loss (Jiao and Demontis, 2017a; Mizushima et al., 2008). The age-related decline of the autophagy/lysosomal system may be caused by decreased expression of autophagy-related genes, lower levels of autophagy core components, and sustained mTORC1 signaling, which is an established inhibitor of autophagy (Jiao and Demontis, 2017a).

Intriguingly, in adult skeletal muscle, lysosomal protease activities are very sparse, further aggravating the age-related decreased autophagic activity and its consequences in aged muscle (Bechet et al., 2005). However, autophagy in skeletal muscle is still essential for proper functioning. It has been shown in a genetic mouse model of skeletal muscle-specific Atg7 knock-out that autophagy is important for myofiber integrity, for the appropriate interplay between muscle and nerve, for the quality control of mitochondria, and for the preservation of muscle mass (Carnio et al., 2014; Masiero et al., 2009). Muscle-specific Atg7 knock-out mice possessed unstable neuromuscular junctions leading to muscle denervation. Furthermore, Atg7 deficiency blocked removal of dysfunctional mitochondria, which induced oxidative stress through increased ROS production and protein oxidation, leading to decreased muscle strength (Carnio et al., 2014). In a similar mouse model for Atg7 muscle-specific knock-out, Masiero and colleagues reported profound muscle atrophy and age-dependent loss in force (Masiero et al., 2009). As described before, Atg7 null muscles showed considerable disarrangements in cellular quality control such as accumulation of abnormal mitochondria, sarcoplasmic reticulum distension, disorganization of sarcomeres, and formation of aberrant concentric membranous structures (Masiero et al., 2009). Intriguingly, autophagy

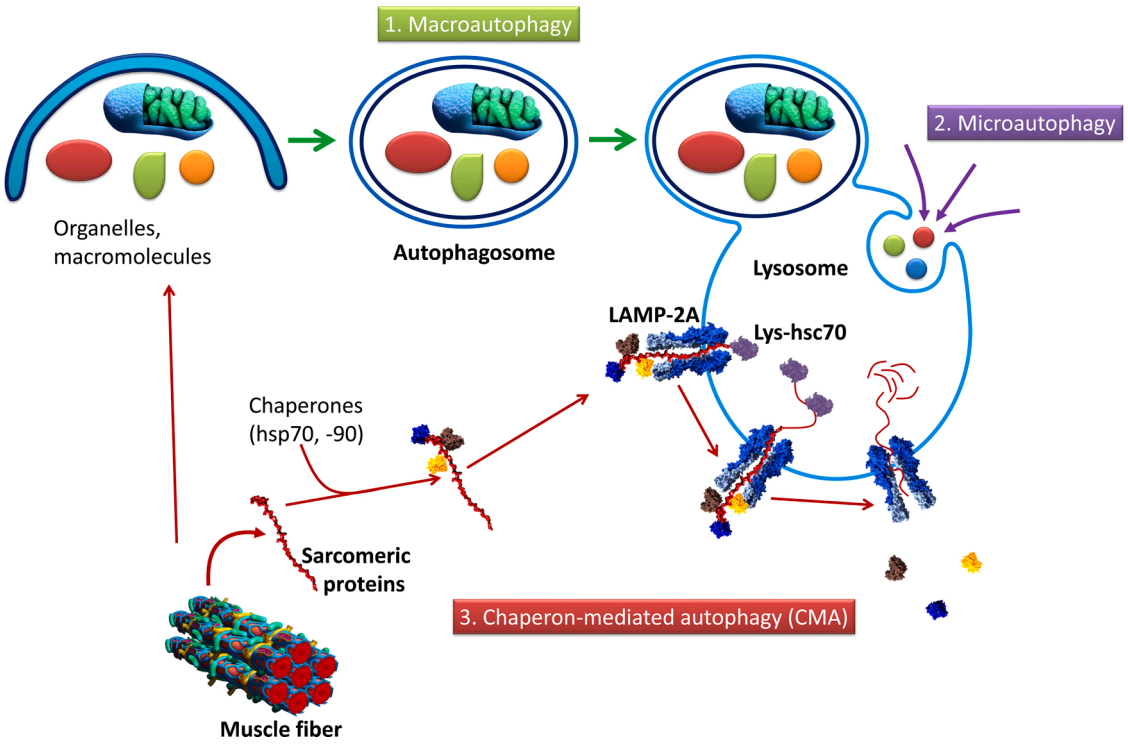

Fig. 4. Degradation of organells and soluble proteins in muscle fibers by the autophagy-lysosomal system. There are three types of autophagy known: macroautophagy, microautophagy, and chaperon-mediated autophagy. The macroautophagy pathway might encapsulate organelles or proteins forming an autophagosome. A complex protein machinery is required to form the membrane of the autophagosome and to target substrates towards the membrane that is simultaneously being formed. By fusion of the membrane of the autophagosome with the lysosomal membrane, the substrates are exposed to the inner lysosomal proteases (called cathepsins). Chaperone-mediated autophagy is a process where, with the help of several chaperones such as hsp70 and hsp90, substrates (here sarcomeric proteins) are unfolded, stabilized, transported toward the lysosome, and transported via the LAMP2A protein into the lumen of the lysosome. Here the lysosomal hsp70 (Lys-hsp70) is shown assisting the transport.

inhibition in Atg7 knockout mice induced upregulation of UPS components such as MAFbx and MuRF-1 accompanied by a 40 % loss of myofiber size (Masiero et al., 2009). This implies a compensatory increase in protein degradation by the UPS that may cause myofiber atrophy and muscle mass loss at old age (Jiao and Demontis, 2017a). The blunted lysosomal activity at advanced age, together with potentially increased protein degradation by the UPS, may partly explain sarcopenic atrophy of the skeletal muscle.

In addition, autophagy appears to be essential for keeping muscle satellite stem cells in their quiescent state (García-Prat et al., 2016). The age-related decline of autophagy in satellite cells is accompanied by their entry into the senescent state due to loss of protein degradation and increased oxidative stress. This results in impaired muscle regenerative capacity due to a decline in both the number and the function of satellite cells, as observed in aged mice (García-Prat et al., 2016).

Glycogen synthase kinase 3 (GSK-3α) was suggested a significant intracellular regulator of skeletal muscle autophagy and myocyte function (Sakuma et al., 2015). GSK-3α null mice are characterized by considerable activation of mTORC1, associated with suppression of several autophagy proteins and with sarcopenia in skeletal and cardiac muscle (Zhou et al., 2013). Under physiologic conditions and especially in young muscle, GSK-3α modulates mTORC1 activity by enhancing TSC2, thereby inhibiting mTORC1 and facilitating the synthesis of autophagy proteins such as Atg12, Bnip3 and LC3. In sarcopenic muscle, however, a lower GSK-3α level has less impact on TSC2 activation and therefore hyperactivates mTORC1 resulting in the inhibition of autophagy-dependent protein degradation (Sakuma et al., 2015). Therefore, balanced constitutive autophagic activity is essential for myocyte viability and for the prevention of sarcopenia development.

In summary, lysosomal protein degradation is an important contributor to myocyte function and muscle fiber integrity. Both reduced and excessive autophagy have been associated with age-related sarcopenia (Jiao and Demontis, 2017a; Wohlgemuth et al., 2010). To prevent sarcopenia, it is important to maintain autophagy flux for organelle recycling and to prevent the accumulation of dysfunctional proteins, mitochondria, and ER membranes, as well as to block excessive protein breakdown (Masiero et al., 2009).

6. Mitochondrial dysfunction during muscle aging

During aging, several tissues exhibit mitochondrial dysfunction and ROS leakage. These observations contributed to the postulation of “the mitochondrial free radical theory of aging” (Miquel, 1998). Progression of mitochondrial dysfunction over time has also been reported for muscle tissues, but it is unclear if mitochondrial dysfunction is a cause or a consequence (Hipkiss, 2010). Nevertheless, the bioenergetic organelle is certainly a central player in overall muscle aging.

Mitochondria are widely believed to be the main cellular source of ROS, especially through complex I and III electron leakage (Nakamura et al., 2009), which generates superoxide anions after incomplete reduction of oxygen. During aging, the leakage is intensified and the risk for cellular damage increases. For example, due to its localization, mitochondrial DNA (mtDNA) is one of the primary ROS targets, resulting in the generation of faulty proteins and overall dysfunction (Chocron et al., 2019). Generally, ROS generation is elevated in dysfunctional mitochondria of aged muscles (Chabi et al., 2008). To understand the origin of this functional impairment, skeletal muscle contractile properties, as well as mitochondrial biogenesis and function, were examined, in addition to apoptotic susceptibility in young *versus* senescent rats (Chabi et al., 2008). Muscle mass and maximal force production was lower in the older animal group and could be partially explained by a 30 % mitochondrial content decrease in fast-twitch muscle from the aged

animals. In addition, the peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α), an important factor for mitochondrial biogenesis, was also found to be decreased. Furthermore, mitochondrial ROS production during state 3 respiration was approximately 1.7-fold greater in mitochondria isolated from old animals compared to young animals and was accompanied by a 1.8-fold increase in the DNA repair enzyme 8-oxoguanine glycosylase 1 in fast-twitch muscle (Chabi et al., 2008). Increased mitochondrial protein modifications such as carbonylation and nitration were also seen. Mitochondrial enzyme proteomic profiling has revealed that during skeletal muscle aging, nitration of complex II and carbonylation of complex I, complex V, and isocitrate dehydrogenase occurs (Staunton et al., 2011) and may result in less ATP production (Mansouri et al., 2006; Yarian et al., 2005) and energy balance disruption.

Moreover, Demontis and colleagues have examined several studies indicating that mitochondrial dysfunction is relevant in skeletal muscle during aging (Demontis et al., 2013). Studies of isolated mitochondria from both aged mice and humans showed changes in multiple mitochondrial processes and phenotypes. These included shifts in mitochondrial enzyme concentrations (Staunton et al., 2011), diminished mitochondrial protein synthesis (Rooyackers et al., 1996), impaired mitochondrial permeability transition pore function (Seo et al., 2008), mitochondrial enlargement (Terman and Brunk, 2004), increased mtDNA mutations (Lee et al., 1997; McKenzie et al., 2002; Melov et al., 1995; Wanagat et al., 2001), and increasedgeneration of ROS (Mansouri et al., 2006). Accordingly, protein oxidation (i.e. carbonylation) of skeletal muscle proteins increase with age and mainly affect proteins involved in key myocyte functions such as cellular morphology, cellular transport, muscle contraction (e. g. myosin-7, troponin T, myosin-binding protein C), and energy metabolism (e. g. creatine kinase M-type, glycogene phosphorylase) (Lourenco dos Santos et al., 2015). In mice, DNA mutations are the highest in muscle compared to any other tissue (Wang et al., 2001), presumably because of the high metabolic rate. Oxidative stress is also prominently detected in aged muscle mitochondria, at rates higher than reported in the liver, kidney or heart (Szczesny et al., 2010).

In other models of muscle aging, such as *Drosophila melanogaster,* muscle tissue seems to display more accumulation of age-related damage compared to other tissues, such as the brain or adipose tissue (Zheng et al., 2005). For example, mitochondrial and nuclear damage are particularly important features in *Drosophila* muscle impairment. If PGC-1α is overexpressed specifically in the muscle, the flies are protected from sarcopenia and age-related metabolic complications during aging (Wenz et al., 2009). In contrast, mice with muscle-specific PGC-1α knock-out show exercise inability, myopathy, and glucose homeostasis disruption (Handschin et al., 2007a, b). Together, these studies show a deep connection between muscle aging and mitochondrial loss of function.

Mitochondria are often implicated in the apoptosis process, and a caspase-independent mechanism has been extensively described in skeletal muscles (Marzetti et al., 2010; Marzetti and Leeuwenburgh, 2006; Park et al., 2010; Whitman et al., 2005), suggesting that mitochondrial dysfunction might be the trigger for apoptosis-mediated cell death. Chabi and colleagues also found that the basal rates of cytochrome c and endonuclease G release in mitochondria were 3.5- to 7-fold higher from senescent animals when compared to young mice (Chabi et al., 2008). They concluded that sarcopenia is associated with increased mitochondrial apoptotic susceptibility combined with a reduced transcriptional drive for mitochondrial synthesis. Therefore, the muscle mass loss observed in sarcopenia might be explained by activation of the apoptosis cell-death program (Hiona and Leeuwenburgh, 2008; Wanagat et al., 2001). Supporting this statement, Whitman and colleagues reported increased apoptotic events in myocytes of older humans as evidenced by TUNEL assay (Whitman et al., 2005).

In summary, age-related sarcopenia seems to be intimately linked to increased ROS production, increased mitochondrial apoptotic susceptibility, and reduced mitochondrial biogenesis driven by the action of PGC-1α.

7. Influence of inflammation on protein degradation in skeletal muscle

Inflammation is considered to be the driving force of muscle wasting (Argiles, 2017). It may result from tumor cells releasing cytokines (e. g. in cancer cachexia) or from pro-oxidant conditions. In addition, increased permeability of the gut, resulting in invasion of intestinal microbiota into the blood stream, triggering an inflammatory response by release of lipopolysaccharides and bacterial toxins, may also be a contributing factor. This inflammatory response may contribute to further mucosal damage and gut permeability and increased systemic inflammation. While inflammatory cytokines are known to induce muscle protein degradation via the NF-kB pathways (s. above), their role in age-related sarcopenia is uncertain. Since there is a chronic low-grade inflammation present in aged subjects (’inflammaging’) it might be reasonable to assume that low-grade inflammation may also play a role in sarcopenic muscle protein degradation (Dalle et al., 2017). However, the extent of the inflammatory response in sarcopenia might be much lower than in cachexia induced by cancer or other serious diseases. In addition, recent evidence suggests that these age-dependent changes in immune function may result from physiologic remodeling, which may beneficially contribute to longevity (Fulop et al., 2017)

Both TNF-α and INF-γ are known to induce the immunoproteasome, however, much less is understood about the role of the immunoproteasome or the 11S regulator in muscle atrophy (Agarwal et al., 2010). In patients suffering from the autosomal-recessive auto-inflammatory JMP-syndrome, characterized by joint contractures, muscle atrophy, microcytic anemia, as well as panniculitis-induced lipodystrophy, the immunoproteasome was investigated. Those patients showed a mutation in the $i\beta_5$ subunit (homozygous missense mutation c.224C > T(p. Thr75Met)), causing decreased subunit activity and potentially detrimental impact on MHC class I antigen processing ability causing the JMP-syndrome (Agarwal et al., 2010). In recent experiments using the $i\beta_5$-specific inhibitor PR-957, a unique role of the immunoproteasome in the production of cytokines and in mediating the inflammatory processes was revealed. PR-957 blocked IL-23 formation by about 80 % and both IL-6 and TNF-α formation by about 50 %, massively ameliorating inflammation in mouse models of arthritis (Muchamuel et al., 2009).

Inflammatory cytokines and tumor-derived factors contribute to the activation of proteolysis. TNF-α, TNF-like weak inducer of apoptosis (TWEK), tumor necrosis factor receptor (TNFR), tumor necrosis factor receptor-associated factor (TRAF), IL-6, IFN-γ, and leukemia inhibitory factor (LIF) mediate their action through two different intracellular pathways: the NF-κB- and p38 MAP kinase pathways (Argiles, 2017), both known to induce MuRF-1 and MAFbx. In rodents, inhibitors of NF-κB limited limited muscle loss in tumor-bearing animals through inhibition of MuRF-1 upregulation (Moore-Carrasco et al., 2007). Both pathways induce $^{\bullet}$NO-synthase (iNOS), resulting in large amounts of nitric oxide that can react with superoxide ($O_2^{\bullet -}$). They also increase inflammatory processes, in turn forming peroxynitrite (ONOO-), the main cause of nitrosative stress. iNOS induction and cytokine formation have also been shown to be suppressed by proteasomal inhibitors (Qureshi et al., 2011). In contrast, several cytokines like IL-4 and IL-10 revealed an anti-cachectic effect (Argiles and Lopez-Soriano, 1999) or anti-proteolytic/anti-apoptotic effects in skeletal muscles of tumor-bearing animals (Busquets et al., 2005; Figueras et al., 2004).

An early anti-inflammatory strategy may be an approach to reduce muscle mass loss in sarcopenia. Interestingly, obesity-related inflammation induces the same cytokine “secretome” that also results in a loss of muscle mass. Here, hypertrophic adipocytes release free fatty acids and adipo-cytokines, triggering cytokine release by macrophage activation. Loss of obesity-induced muscle mass (sarcopenic obesity) is (probably and partially) mediated by increased proteasomal

degradation of muscle protein. This steady-state between synthesis and degradation is massively impacted by insulin resistance (Srikanthan et al., 2010). A plant-extract of *Artemisia daracunnunculus* L., termed as PMI5011 was able to reduce muscle loss in sarcopenic obesity, by changing polyubiquitination patterns, inhibiting the proteasome and non-proteasomal proteolysis, and decreasing MAFbx and MuRF-1 expression in a mouse model of obesity-related type 2 diabetes ($KK\text{-}A^{y}$) (Kirk-Ballard et al., 2014).

8. Preventive and interventional strategies for sarcopenia

Preventing sarcopenia or delaying its development would have a great impact on the quality of life for elderly persons. Current strategies are mainly focused on exercise, nutrition and medication. *Exercise* improves muscle mass and function in older adults (Bowen et al., 2015; Phu et al., 2015). It augments muscle IGF-1 expression which leads to increases in muscle protein synthesis and mass in senescent muscles (Cade and Yarasheski, 2006) Exercise also exerts beneficial effects by counteracting several of the mechanisms that cause sarcopenia. For example, exercise can cause a reduction of inflammation, an increase in satellite cells, and reduced fat infiltration. Interestingly, the amount of type-II fibers increase together with the number of satellite cells in type-II muscle fibers (Verdijk et al., 2014). Resistance exercise training is more effective in increasing muscle mass and strength, while endurance exercises improves muscle performance and helps to prevent future disability (Phu et al., 2015). Structural improvements of exercise-induced muscle growth are based on the addition of sarcomeres in the muscle that follows the direction dictated by the contraction mode (either eccentric or concentric) (Narici et al., 2016).

Nutritional intervention in sarcopenia focusses on increased protein content in the diet. The application of essential amino acids, especially of leucine in the diet or as a supplement (also containing β-hydroxy β-methylbutyric acid (HMB)) have been reported as being beneficial (Argiles et al., 2015) (Domingues-Faria et al., 2015). It appears that resistance training in combination with amino acid-supplemented nutrition is the best candidate to attenuate age-related muscle atrophy (Argiles et al., 2015). To ideally support the exercise-induced increase in protein-synthesis rates that occur after the exercise, protein intake should be provided within 60 min of exercise (Phu et al., 2015). Furthermore, n-3 polyunsaturated fatty acids, polyphenols, and vitamin D supplementation have been reported to diminish inflammation, improve metabolic parameters and, therefore, support skeletal muscle function (Domingues-Faria et al., 2015). Caloric restriction is known to induce longevity in many experimental model systems. In rats it attenuates the age-related impairment of autophagy in skeletal muscle, which might be one of the mechanisms by which calorie restriction attenuates age-related cellular damage and cell death in skeletal muscle *in vivo* (Wohlgemuth et al., 2010). Caloric restriction may also act by lowering insulin levels since insulin is an inhibitor of autophagy (Mizushima et al., 2008). However, considering the beneficial effect of increased amino acid content, calorie restriction should probably be used while still maintaining a sufficient protein supply. In summary, the number of studies investigating the impact of nutrition on muscle repair in the context of aging is quite small and further research is needed to provide effective, efficient and reasonable strategies for preventing age-related muscle atrophy (Domingues-Faria et al., 2015).

Several molecular pathways were suggested with a potential for *drug development* for preventing or curing sarcopenia. Among those are factors for inhibiting TNFα, SHIP-2, GSK3β or proteasome activity (Cade and Yarasheski, 2006). In addition, pharmacologic interventions to activate Akt, mTOR or $p70^{S6K}$ have been discussed (Cade and Yarasheski, 2006). Only recently, also restoring normal miRNA levels as a potential therapeutic against muscle aging has been introduced (Brown and Goljanek-Whysall, 2015). However, most of these strategies still need to be tested for efficacy and lack of serious side effects.

9. Conclusions and open questions

The molecular mechanisms of sarcopenia are very similar to those of other diseases of muscle atrophy, which can be differentiated mainly by the disease cause. Apart from other genetically-induced atrophies or disease-related atrophies, the main risk factor for sarcopenic muscle atrophy is aging. Aging-associated co-morbidities, and conditions that impair muscle function, make it difficult to accurately diagnose sarcopenia and to distinguish it from other atrophic diseases. Therefore, the concept of secondary sarcopenia was introduced as an instrument for facilitating diagnosis. To further obviate diagnostic difficulties, EWGSOP updated their diagnostic guidelines in 2018 and focused on the most relevant parameter for predicting adverse outcomes of sarcopenia: decreased muscle strength (prioritized before lower muscle mass) (Cruz-Jentoft et al., 2019). However, careful studies with well-defined cohorts are still needed to improve sarcopenia diagnosis

Many molecular pathways and players within intracellular regulation of muscle atrophy have been identified already. The picture is becoming very complex due to the strong interaction of anabolic protein synthesis and catabolic protein degradation pathways; the balance between these two being shifted towards increased protein degradation during aging promotes net loss of muscle mass.

It seems that in applying the hallmarks of aging to sarcopenia, the most important mechanisms currently understood to be involved in its development are loss of proteostasis, mitochondrial dysfunction and inflammatory disturbances, which have been decribed and discussed in more detail. However, results are limited, especially from human studies, and quite often subjects have not been clearly diagnosed with sarcopenia using the EWGSOP guidelines (including functional tests) but only based on diminished muscle mass. Furthermore, taking muscle biopsies from sarcopenic patients, which would be essential for elucidating the molecular and physiologic interrelations in humans, raises serious ethical considerations. However, as the population in many affluent countries is aging, there will surely be more patients in the future suffering from age-related disorders such as sarcopenia, making it of increased importance to society at large. Hence, there is ample and increasing demand for further elucidating the cause(s) of the disease and finding appropriate therapeutic options on the basis of carefully designed human studies.

Considering the available data on the mechanisms of sarcopenia development it seems that further studies are urgently needed. One aspect focusses on the relevance of fat infiltration into muscle tissue during aging and sarcopenia development, respectively. It has been clearly shown, that this phenomenon compromises muscle function and increases the individual risk of mortality, for example from bone fractures or cardiometabolic diseases like type 2 diabetes. However, the molecular effects of intramuscular fat depots on the metabolism of muscle cells in atrophic conditions have not been elucidated yet as well as have been fat cell – myofiber signaling. The expression of Perilipin 2 (Plin-2), a protein associated with the metabolism of intracellular lipid-droplets, increases with age in skeletal muscle, which is associated with decreased muscle strength and thickness in patients with limited lower limb mobility (Conte et al., 2015, 2013). This coincides with increased expression of factors related to muscle atrophy, such as MuRF-1, Mafbx and p53 (Conte et al., 2015). Also, intramuscular lipids and their derivatives were reported to impair mitochondrial function and enhanced secretion of pro-inflammatory myokines might be responsible for inducing muscle dysfunction and for producing a vicious cycle that maintains adipose tissue and skeletal muscle inflammation (Kalinkovich and Livshits, 2017). Intramuscular fat-derived factors as well as mitochondrial damage, seem to be crucial for explaining lipotoxic effects on skeletal muscle. But the underlying mechanisms still need elucidation.

The other unresolved aspect of molecular mechanisms in sarcopenia relates to the specific regulation inside the two muscle fibers, type I and II. Why are type II fibers more affected by sarcopenia although type I fibers should be more prone to oxidative protein damage due to their

higher oxidative load? Are there differences in antioxidant defenses between these fibers? Some experiments in mice indicate that in adaptive non-sarcopenic conditions the fast-twitch EDL muscle reduces protein oxidation by an increase in antioxidant capacity (Fernando et al., 2019). Further studies will be required in order to demystify the muscle fiber-specific handling of atrophic triggers.

Intensifying the investigations of molecular pathways underlying sarcopenia development also demands further technical improvements. Generating antibodies for the E3-ligases MuRF-1 and MaFbx would help in recognizing the real impact of these proteins in muscular degradation. Also, when considering the alternative protein degradation route though autophagy, static measurement of autophagy proteins and genes is not sufficient to evaluate degradation capacity. Here monitoring autophagy flux would provide a more realistic and useful picture. However, monitoring the autophagic flux *in vivo* is notoriously difficult.

Furthermore, data on the impact of nutrition, in particular, amino acid composition of the diet, are still limited as are the effects of diet and activity on the amino acid composition of blood and plasma. The same is true for brain and mental activities and the resulting neuromuscular effects.

By further elucidating relevant mechanisms involved in sarcopenia development and exacerbation, we hope to gradually get closer to being able to identify efficient lifestyle and therapeutic interventions for preventing age-related loss of muscle mass and function.

Acknowledgements

KJAD was supported by grant # ES003598 from the National Institute of Environmental Health Sciences of the US National Institutes of Health, and by grant # AG052374 from the National Institute on Aging of the US National Institutes of Health.

TG was supported by the Deutsche Forschungsgemeinschaft (DFG, GR1240/20-1 and GR 1240/22-1) and is member oft he FP7 EU consortium FRAILOMIC.

References

Agarwal, A.K., Xing, C., DeMartino, G.N., Mizrachi, D., Hernandez, M.D., Sousa, A.B., Martinez de Villarreal, L., dos Santos, H.G., Garg, A., 2010. PSMB8 encoding the beta5i proteasome subunit is mutated in joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy syndrome. Am. J. Hum. Genet. 87, 866–872.

Altun, M., Besche, H.C., Overkleeft, H.S., Piccirillo, R., Edelmann, M.J., Kessler, B.M., Goldberg, A.L., Ulfhake, B., 2010. Muscle wasting in aged, sarcopenic rats is associated with enhanced activity of the ubiquitin proteasome pathway. J. Biol. Chem. 285, 39597–39608.

Amirouche, A., Durieux, A.C., Banzet, S., Koulmann, N., Bonnefoy, R., Mouret, C., Bigard, X., Peinnequin, A., Freyssenet, D., 2009. Down-regulation of Akt/mammalian target of rapamycin signaling pathway in response to myostatin overexpression in skeletal muscle. Endocrinology 150, 286–294.

Anandavadivelan, P., Brismar, T.B., Nilsson, M., Johar, A.M., Martin, L., 2016. Sarcopenic obesity: a probable risk factor for dose limiting toxicity during neoadjuvant chemotherapy in oesophageal cancer patients. Clin. Nutr. 35, 724–730.

Andersen, J.L., 2003. Muscle fibre type adaptation in the elderly human muscle. Scand. J. Med. Sci. Sports 13, 40–47.

Argiles, J.M., Lopez-Soriano, F.J., 1999. The role of cytokines in cancer cachexia. Med. Res. Rev. 19, 223–248.

Argiles, J.M., 2017. The 2015 ESPEN Sir David Cuthbertson lecture: inflammation as the driving force of muscle wasting in cancer. Clin. Nutr. 36 (3), 798–803.

Argiles, J.M., Busquets, S., Stemmler, B., Lopez-Soriano, F.J., 2015. Cachexia and sarcopenia: mechanisms and potential targets for intervention. Curr. Opin. Pharmacol. 22, 100–106.

Aunan, J.R., Watson, M.M., Hagland, H.R., Soreide, K., 2016. Molecular and biological hallmarks of ageing. Br. J. Surg. 103, e29–46.

Ayala, A., Muñoz, M.F., Argüelles, S., 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid. Med. Cell. Longev. 2014, 360438.

Baehr, L.M., Furlow, J.D., Bodine, S.C., 2011. Muscle sparing in muscle RING finger 1 null mice: response to synthetic glucocorticoids. J. Physiol. 589, 4759–4776.

Baehr, L., Tunzi, M., Bodine, S., 2014a. Muscle hypertrophy is associated with increases in proteasome activity that is independent of MuRF1 and MAFbx expression. Front. Physiol. 5 (69).

Baehr, L.M., Tunzi, M., Bodine, S.C., 2014b. Muscle hypertrophy is associated with increases in proteasome activity that is independent of MuRF1 and MAFbx expression. Front. Physiol. 5 (69).

Baehr, L.M., West, D.W., Marcotte, G., Marshall, A.G., De Sousa, L.G., Baar, K., Bodine, S. C., 2016. Age-related deficits in skeletal muscle recovery following disuse are associated with neuromuscular junction instability and ER stress, not impaired protein synthesis. Aging (Albany NY) 8, 127–146.

Balagopal, P., Schimke, J.C., Ades, P., Adey, D., Nair, K.S., 2001. Age effect on transcript levels and synthesis rate of muscle MHC and response to resistance exercise. Am. J. Physiol. Endocrinol. Metab. 280, E203–E208.

Barberi, L., Scicchitano, B.M., De Rossi, M., Bigot, A., Duguez, S., Wielgosik, A., Stewart, C., McPhee, J., Conte, M., Narici, M., Franceschi, C., Mouly, V., Butler-Browne, G., Musaro, A., 2013. Age-dependent alteration in muscle regeneration: the critical role of tissue niche. Biogerontology 14, 273–292.

Batsis, J., Mackenzie, T., Barre, L., Lopez-Jimenez, F., Bartels, S., 2014. Sarcopenia, sarcopenic obesity and mortality in older adults: results from the National Health and Nutrition Examination Survey III. Eur. J. Clin. Nutr. 68, 1001.

Batsis, J.A., Mackenzie, T.A., Jones, J.D., Lopez-Jimenez, F., Bartels, S.J., 2016. Sarcopenia, Sarcopenic Obesity and Inflammation: Results From the 1999–2004 National Health and Nutrition Examination Survey. Clinical Nutrition 35, 1472–1483. https://doi.org/10.1016/j.clnu.2016.03.028.

Bauer, J., Morley, J.E., Schols, A., Ferrucci, L., Cruz-Jentoft, A.J., Dent, E., Baracos, V.E., Crawford, J.A., Doehner, W., Heymsfield, S.B., Jatoi, A., Kalantar-Zadeh, K., Lainscak, M., Landi, F., Laviano, A., Mancuso, M., Muscaritoli, M., Prado, C.M., Strasser, F., von Haehling, S., Coats, A.J.S., Anker, S.D., 2019. Sarcopenia: a time for action. An SCWD position paper. J. Cachexia Sarcopenia Muscle 10, 956–961.

Bechet, D., Tassa, A., Taillandier, D., Combaret, L., Attaix, D., 2005. Lysosomal proteolysis in skeletal muscle. Int. J. Biochem. Cell Biol. 37, 2098–2114.

Bedard, N., Jammoul, S., Moore, T., Wykes, L., Hallauer, P.L., Hastings, K.E., Stretch, C., Baracos, V., Chevalier, S., Plourde, M., Coyne, E., Wing, S.S., 2015. Inactivation of the ubiquitin-specific protease 19 deubiquitinating enzyme protects against muscle wasting. FASEB J. 29, 3889–3898.

Benaroudj, N., Zwickl, P., Seemuller, E., Baumeister, W., Goldberg, A.L., 2003. ATP hydrolysis by the proteasome regulatory complex PAN serves multiple functions in protein degradation. Mol. Cell 11, 69–78.

Bian, A., Ma, Y., Zhou, X., Guo, Y., Wang, W., Zhang, Y., Wang, X., 2020. Association between sarcopenia and levels of growth hormone and insulin-like growth factor-1 in the elderly. BMC Musculoskelet. Disord. 21, 214.

Bodine, S.C., Baehr, L.M., 2014a. Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1. Am. J. Physiol. Endocrinol. Metab. 307, E469–E484.

Bodine, S.C., Baehr, L.M., 2014b. Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1. Am. J. Physiol. Endocrinol. Metab. 307, E469–484.

Bodine, S.C., Latres, E., Baumhueter, S., Lai, V.K.-M., Nunez, L., Clarke, B.A., Poueymirou, W.T., Panaro, F.J., Na, E., Dharmarajan, K., Pan, Z.-Q., Valenzuela, D. M., DeChiara, T.M., Stitt, T.N., Yancopoulos, G.D., Glass, D.J., 2001. Identification of ubiquitin ligases required for skeletal muscle atrophy. Science 294, 1704–1708.

Bollinger, L.M., Witczak, C.A., Houmard, J.A., Brault, J.J., 2014. SMAD3 augments FoxO3-induced MuRF-1 promoter activity in a DNA-binding-dependent manner. Am. J. Physiol. Cell Physiol. 307, C278–C287.

Bowen, T.S., Schuler, G., Adams, V., 2015. Skeletal muscle wasting in cachexia and sarcopenia: molecular pathophysiology and impact of exercise training. J. Cachexia Sarcopenia Muscle 6, 197–207.

Braun, T.P., Marks, D.L., 2015. The regulation of muscle mass by endogenous glucocorticoids. Front. Physiol. 6, 12.

Breusing, N., Arndt, J., Voss, P., Bresgen, N., Wiswedel, I., Gardemann, A., Siems, W., Grune, T., 2009. Inverse correlation of protein oxidation and proteasome activity in liver and lung. Mech. Ageing Dev. 130, 748–753.

Brown, D.M., Goljanek-Whysall, K., 2015. microRNAs: modulators of the underlying pathophysiology of sarcopenia? Ageing Res. Rev. 24, 263–273.

Busquets, S., Figueras, M.T., Meijsing, S., Carbo, N., Quinn, L.S., Almendro, V., Argiles, J. M., Lopez-Soriano, F.J., 2005. Interleukin-15 decreases proteolysis in skeletal muscle: a direct effect. Int. J. Mol. Med. 16, 471–476.

Cade, W.T., Yarasheski, K.E., 2006. Metabolic and molecular aspects of sarcopenia. In: Runge, M.S., Patterson, C. (Eds.), Principles of Molecular Medicine. Humana Press, Totowa, NJ, pp. 529–534.

Carneiro, I.P., Mazurak, V.C., Prado, C.M., 2016. Clinical implications of sarcopenic obesity in Cancer. Curr. Oncol. Rep. 18, 1–13.

Carnio, S., LoVerso, F., Baraibar, M.A., Longa, E., Khan, M.M., Maffei, M., Reischl, M., Canepari, M., Loefler, S., Kern, H., Blaauw, B., Friguet, B., Bottinelli, R., Rudolf, R., Sandri, M., 2014. Autophagy impairment in muscle induces neuromuscular junction degeneration and precocious aging. Cell Rep. 8, 1509–1521.

Carter, E.E., Thomas, M.M., Murynka, T., Rowan, S.L., Wright, K.J., Huba, E., Hepple, R. T., 2010. Slow twitch soleus muscle is not protected from sarcopenia in senescent rats. Exp. Gerontol. 45, 662–670.

Chabi, B., Ljubicic, V., Menzies, K.J., Huang, J.H., Saleem, A., Hood, D.A., 2008. Mitochondrial function and apoptotic susceptibility in aging skeletal muscle. Aging Cell 7, 2–12.

Cho, S.H., Kim, J.H., Song, W., 2016. In vivo rodent models of skeletal muscle adaptation to decreased use. Endocrinol. Metab. (Seoul) 31, 31–37.

Chocron, E.S., Munkacsy, E., Pickering, A.M., 2019. Cause or casualty: the role of mitochondrial DNA in aging and age-associated disease. Biochim. Biophys. Acta Mol. Basis Dis. 1865, 285–297.

Ciechanover, A., 2005. Proteolysis: from the lysosome to ubiquitin and the proteasome. Nat. Rev. Mol. Cell Biol. 6, 79.

Clark, B.C., Manini, T.M., 2008. Sarcopenia ≠ dynapenia. J. Gerontol. A Biol. Sci. Med. Sci. 63, 829–834.

Clark, B.C., Manini, T.M., 2012. What is dynapenia? Nutrition 28, 495–503.

Cohen, S., Brault, J.J., Gygi, S.P., Glass, D.J., Valenzuela, D.M., Gartner, C., Latres, E., Goldberg, A.L., 2009. During muscle atrophy, thick, but not thin, filament

components are degraded by MuRF1-dependent ubiquitylation. J. Cell Biol. 185, 1083–1095.
Conte, M., Vasuri, F., Trisolino, G., Bellavista, E., Santoro, A., Degiovanni, A., Martucci, E., D'Errico-Grigioni, A., Caporossi, D., Capri, M., Maier, A.B., Seynnes, O., Barberi, L., Musaro, A., Narici, M.V., Franceschi, C., Salvioli, S., 2013. Increased Plin2 expression in human skeletal muscle is associated with sarcopenia and muscle weakness. PLoS One 8, e73709.
Conte, M., Vasuri, F., Bertaggia, E., Armani, A., Santoro, A., Bellavista, E., Degiovanni, A., D'Errico-Grigioni, A., Trisolino, G., Capri, M., Franchi, M.V., Narici, M.V., Sandri, M., Franceschi, C., Salvioli, S., 2015. Differential expression of perilipin 2 and 5 in human skeletal muscle during aging and their association with atrophy-related genes. Biogerontology 16, 329–340.
Cruz-Jentoft, A.J., Baeyens, J.P., Bauer, J.M., Boirie, Y., Cederholm, T., Landi, F., Martin, F.C., Michel, J.P., Rolland, Y., Schneider, S.M., Topinkova, E., Vandewoude, M., Zamboni, M., European Working Group on Sarcopenia in Older, P, 2010. Sarcopenia: european consensus on definition and diagnosis: report of the european working group on Sarcopenia in older people. Age Ageing 39, 412–423.
Cruz-Jentoft, A.J., Landi, F., Schneider, S.M., Zúñiga, C., Arai, H., Boirie, Y., Chen, L.-K., Fielding, R.A., Martin, F.C., Michel, J.-P., Sieber, C., Stout, J.R., Studenski, S.A., Vellas, B., Woo, J., Zamboni, M., Cederholm, T., 2014. Prevalence of and interventions for sarcopenia in ageing adults: a systematic review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS). Age Ageing 43, 748–759.
Cruz-Jentoft, A.J., Bahat, G., Bauer, J., Boirie, Y., Bruyere, O., Cederholm, T., Cooper, C., Landi, F., Rolland, Y., Sayer, A.A., Schneider, S.M., Sieber, C.C., Topinkova, E., Vandewoude, M., Visser, M., Zamboni, M., Writing Group for the European Working Group on Sarcopenia in Older, P, the Extended Group for, E, 2019. Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing 48, 16–31.
Dalle, S., Rossmeislova, L., Koppo, K., 2017. The role of inflammation in age-related sarcopenia. Front. Physiol. 8, 1045.
Demontis, F., Piccirillo, R., Goldberg, A.L., Perrimon, N., 2013. Mechanisms of skeletal muscle aging: insights from Drosophila and mammalian models. Dis. Model. Mech. 6, 1339–1352.
Dent, E., Morley, J.E., Cruz-Jentoft, A.J., Arai, H., Kritchevsky, S.B., Guralnik, J., Bauer, J.M., Pahor, M., Clark, B.C., Cesari, M., Ruiz, J., Sieber, C.C., Aubertin-Leheudre, M., Waters, D.L., Visvanathan, R., Landi, F., Villareal, D.T., Fielding, R., Won, C.W., Theou, O., Martin, F.C., Dong, B., Woo, J., Flicker, L., Ferrucci, L., Merchant, R.A., Cao, L., Cederholm, T., Ribeiro, S.M.L., Rodriguez-Manas, L., Anker, S.D., Lundy, J., Gutierrez Robledo, L.M., Bautmans, I., Aprahamian, I., Schols, J., Izquierdo, M., Vellas, B., 2018. International clinical practice guidelines for Sarcopenia (ICFSR): screening, diagnosis and management. J. Nutr. Health Aging 22, 1148–1161.
Diz, J.B.M., Leopoldino, A.A.O., Moreira, Bd.S., Henschke, N., Dias, R.C., Pereira, L.S.M., Oliveira, V.C., 2016. Prevalence of sarcopenia in older Brazilians: a systematic review and meta-analysis. Geriatr. Gerontol. Int. 17 (1), 5–16.
Dodds, R.M., Roberts, H.C., Cooper, C., Sayer, A.A., 2015. The epidemiology of sarcopenia. J. Clin. Densitom. 18, 461–466.
Domingues-Faria, C., Vasson, M.P., Goncalves-Mendes, N., Boirie, Y., Walrand, S., 2015. Skeletal muscle regeneration and impact of aging and nutrition. Ageing Res. Rev. 26, 22–36.
Drescher, C., Konishi, M., Ebner, N., Springer, J., 2016. Loss of muscle mass: current developments in cachexia and sarcopenia focused on biomarkers and treatment. Int. J. Cardiol. 202, 766–772.
Durieux, A.C., Amirouche, A., Banzet, S., Koulmann, N., Bonnefoy, R., Pasdeloup, M., Mouret, C., Bigard, X., Peinnequin, A., Freyssenet, D., 2007. Ectopic expression of myostatin induces atrophy of adult skeletal muscle by decreasing muscle gene expression. Endocrinology 148, 3140–3147.
Egerman, Marc A., Cadena, Samuel M., Gilbert, Jason A., Meyer, A., Nelson, Hallie N., Swalley, Susanne E., Mallozzi, C., Jacobi, C., Jennings, Lori L., Clay, I., Laurent, G., Ma, S., Brachat, S., Lach-Trifilieff, E., Shavlakadze, T., Trendelenburg, A.-U., Brack, Andrew S., Glass, David J., 2015. GDF11 increases with age and inhibits skeletal muscle regeneration. Cell Metab. 22, 164–174.
Fernando, R., Drescher, C., Deubel, S., Jung, T., Ost, M., Klaus, S., Grune, T., Castro, J.P., 2019. Low proteasomal activity in fast skeletal muscle fibers is not associated with increased age-related oxidative damage. Exp. Gerontol. 117, 45–52.
Ferrington, D.A., Husom, A.D., Thompson, L.V., 2005. Altered proteasome structure, function, and oxidation in aged muscle. FASEB J. 19, 644–646.
Figueras, M., Busquets, S., Carbo, N., Barreiro, E., Almendro, V., Argiles, J.M., Lopez-Soriano, F.J., 2004. Interleukin-15 is able to suppress the increased DNA fragmentation associated with muscle wasting in tumour-bearing rats. FEBS Lett. 569, 201–206.
Franceschi, C., Campisi, J., 2014. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J. Gerontol. Ser. A: Biomed. Sci. Med. Sci. 69, S4–S9.
Franceschi, C., Garagnani, P., Morsiani, C., Conte, M., Santoro, A., Grignolio, A., Monti, D., Capri, M., Salvioli, S., 2018. The continuum of aging and age-related diseases: common mechanisms but different rates. Front. Med. (Lausanne) 5, 61.
Fulop, T., Larbi, A., Dupuis, G., Le Page, A., Frost, E.H., Cohen, A.A., Witkowski, J.M., Franceschi, C., 2017. Immunosenescence and inflamm-aging as two sides of the same coin: friends or foes? Front. Immunol. 8, 1960.
Gallagher, D., Visser, M., Sepulveda, D., Pierson, R.N., Harris, T., Heymsfield, S.B., 1996. How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? Am. J. Epidemiol. 143, 228–239.
Gallagher, D., Ruts, E., Visser, M., Heshka, S., Baumgartner, R.N., Wang, J., Pierson, R.N., Pi-Sunyer, F.X., Heymsfield, S.B., 2000. Weight stability masks sarcopenia in elderly men and women. Am. J. Physiol. Endocrinol. Metab. 279, E366–E375.
García-Prat, L., Martínez-Vicente, M., Perdiguero, E., Ortet, L., Rodríguez-Ubreva, J., Rebollo, E., Ruiz-Bonilla, V., Gutarra, S., Ballestar, E., Serrano, A.L., Sandri, M., Muñoz-Cánoves, P., 2016. Autophagy maintains stemness by preventing senescence. Nature 529, 37.
Gensous, N., Bacalini, M.G., Franceschi, C., Meskers, C.G.M., Maier, A.B., Garagnani, P., 2019. Age-related DNA methylation changes: potential impact on skeletal muscle aging in humans. Front. Physiol. 10, 996.
Goljanek-Whysall, K., Iwanejko, L.A., Vasilaki, A., Pekovic-Vaughan, V., McDonagh, B., 2016. Ageing in relation to skeletal muscle dysfunction: redox homoeostasis to regulation of gene expression. Mamm. Genome 27, 341–357.
Gomes, M.D., Lecker, S.H., Jagoe, R.T., Navon, A., Goldberg, A.L., 2001. Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. Proc. Natl. Acad. Sci. 98, 14440–14445.
Grune, T., Jung, T., Merker, K., Davies, K.J., 2004. Decreased proteolysis caused by protein aggregates, inclusion bodies, plaques, lipofuscin, ceroid, and' aggresomes' during oxidative stress, aging, and disease. Int. J. Biochem. Cell Biol. 36, 2519–2530.
Handschin, C., Chin, S., Li, P., Liu, F., Maratos-Flier, E., Lebrasseur, N.K., Yan, Z., Spiegelman, B.M., 2007a. Skeletal muscle fiber-type switching, exercise intolerance, and myopathy in PGC-1alpha muscle-specific knock-out animals. J. Biol. Chem. 282, 30014–30021.
Handschin, C., Choi, C.S., Chin, S., Kim, S., Kawamori, D., Kurpad, A.J., Neubauer, N., Hu, J., Mootha, V.K., Kim, Y.B., Kulkarni, R.N., Shulman, G.I., Spiegelman, B.M., 2007b. Abnormal glucose homeostasis in skeletal muscle-specific PGC-1alpha knockout mice reveals skeletal muscle-pancreatic beta cell crosstalk. J. Clin. Invest. 117, 3463–3474.
He, L., Khanal, P., Morse, C.I., Williams, A., Thomis, M., 2019. Differentially methylated gene patterns between age-matched sarcopenic and non-sarcopenic women. J. Cachexia Sarcopenia Muscle 10, 1295–1306.
Heilbronn, L., Smith, S., Ravussin, E., 2004. Failure of fat cell proliferation, mitochondrial function and fat oxidation results in ectopic fat storage, insulin resistance and type II diabetes mellitus. Int. J. Obes. 28, S12.
Hiona, A., Leeuwenburgh, C., 2008. The role of mitochondrial DNA mutations in aging and sarcopenia: implications for the mitochondrial vicious cycle theory of aging. Exp. Gerontol. 43, 24–33.
Hipkiss, A.R., 2010. Mitochondrial dysfunction, proteotoxicity, and aging: causes or effects, and the possible impact of NAD+-controlled protein glycation. Adv. Clin. Chem. 50, 123–150.
Hirner, S., Krohne, C., Schuster, A., Hoffmann, S., Witt, S., Erber, R., Sticht, C., Gasch, A., Labeit, S., Labeit, D., 2008. MuRF1-dependent regulation of systemic carbohydrate metabolism as revealed from transgenic mouse studies. J. Mol. Biol. 379, 666–677.
Hittel, D.S., Berggren, J.R., Shearer, J., Boyle, K., Houmard, J.A., 2009. Increased secretion and expression of myostatin in skeletal muscle from extremely obese women. Diabetes 58, 30–38.
Hohn, A., Weber, D., Jung, T., Ott, C., Hugo, M., Kochlik, B., Kehm, R., Konig, J., Grune, T., Castro, J.P., 2017. Happily (n)ever after: Aging in the context of oxidative stress, proteostasis loss and cellular senescence. Redox Biol. 11, 482–501.
Hoppeler, H., 2016. Molecular networks in skeletal muscle plasticity. J. Exp. Biol. 219, 205–213.
Jackson, M.P., Hewitt, E.W., 2016. Cellular proteostasis: degradation of misfolded proteins by lysosomes. Essays Biochem. 60, 173–180.
Janssen, I., Heymsfield, S.B., Wang, Z., Ross, R., 2000. Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. J. Appl. Physiol. 89, 81–88.
Jiao, J., Demontis, F., 2017a. Skeletal muscle autophagy and its role in sarcopenia and organismal aging. Curr. Opin. Pharmacol. 34, 1–6.
Jiao, J., Demontis, F., 2017b. Skeletal muscle autophagy and its role in sarcopenia and organismal aging. Curr. Opin. Pharmacol. 34, 1–6.
Jung, T., Catalgol, B., Grune, T., 2009. The proteasomal system. Mol. Aspects Med. 30, 191–296.
Kalinkovich, A., Livshits, G., 2017. Sarcopenic obesity or obese sarcopenia: a cross talk between age-associated adipose tissue and skeletal muscle inflammation as a main mechanism of the pathogenesis. Ageing Res. Rev. 35, 200–221.
Kawamura, Y., O'Brien, P., Okazaki, H., Dyck, P.J., 1977. Lumbar Motoneurons of man II: the number and diameter distribution of large- and intermediate-diameter cytons in "Motoneuron columns" of spinal cord of man. J. Neuropathol. Exp. Neurol. 36, 861–870.
Kennedy, B.K., Berger, S.L., Brunet, A., Campisi, J., Cuervo, A.M., Epel, E.S., Franceschi, C., Lithgow, G.J., Morimoto, R.I., Pessin, J.E., 2014. Geroscience: linking aging to chronic disease. Cell 159, 709–713.
Kido, A., Tanaka, N., Stein, R.B., 2004. Spinal excitation and inhibition decrease as humans age. Can. J. Physiol. Pharmacol. 82, 238–248.
Kim, T.N., Yang, S.J., Yoo, H.J., Lim, K.I., Kang, H.J., Song, W., Seo, J.A., Kim, S.G., Kim, N.H., Baik, S.H., Choi, D.S., Choi, K.M., 2009. Prevalence of sarcopenia and sarcopenic obesity in Korean adults: the Korean sarcopenic obesity study. Int. J. Obes. (Lond.) 33, 885–892.
Kim, H., Hirano, H., Edahiro, A., Ohara, Y., Watanabe, Y., Kojima, N., Kim, M., Hosoi, E., Yoshida, Y., Yoshida, H., Shinkai, S., 2016. Sarcopenia: prevalence and associated factors based on different suggested definitions in community-dwelling older adults. Geriatr. Gerontol. Int. 16, 110–122.
Kirk-Ballard, H., Kilroy, G., Day, B.C., Wang, Z.Q., Ribnicky, D.M., Cefalu, W.T., Floyd, Z. E., 2014. An ethanolic extract of Artemisia dracunculus L. regulates gene expression of ubiquitin-proteasome system enzymes in skeletal muscle: potential role in the treatment of sarcopenic obesity. Nutrition 30, S21–25.
Kon, M., Cuervo, A.M., 2010. Chaperone-mediated autophagy in health and disease. FEBS Lett. 584, 1399–1404.
Lee, C.M., Weindruch, R., Aiken, J.M., 1997. Age-associated alterations of the mitochondrial genome. Free Radic. Biol. Med. 22, 1259–1269.

Léger, B., Derave, W., De Bock, K., Hespel, P., Russell, A.P., 2008. Human sarcopenia reveals an increase in SOCS-3 and myostatin and a reduced efficiency of Akt phosphorylation. Rejuvenation Res. 11, 163B–175B.
Lokireddy, S., McFarlane, C., Ge, X., Zhang, H., Sze, S.K., Sharma, M., Kambadur, R., 2011. Myostatin induces degradation of sarcomeric proteins through a Smad3 signaling mechanism during skeletal muscle wasting. Mol. Endocrinol. 25, 1936–1949.
Lokireddy, S., Wijesoma, I.W., Sze, S.K., McFarlane, C., Kambadur, R., Sharma, M., 2012. Identification of atrogin-1-targeted proteins during the myostatin-induced skeletal muscle wasting. Am. J. Physiol., Cell Physiol. 303, C512–529.
Lopez-Otin, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., 2013. The hallmarks of aging. Cell 153, 1194–1217.
Lorenzi, M., Bonassi, S., Lorenzi, T., Giovannini, S., Bernabei, R., Onder, G., 2018. A review of telomere length in sarcopenia and frailty. Biogerontology 19, 209–221.
Lourenco dos Santos, S., Baraibar, M.A., Lundberg, S., Eeg-Olofsson, O., Larsson, L., Friguet, B., 2015. Oxidative proteome alterations during skeletal muscle ageing. Redox Biol. 5, 267–274.
Lu-Nguyen, N., Malerba, A., Popplewell, L., Schnell, F., Hanson, G., Dickson, G., 2017. Systemic antisense therapeutics for dystrophin and myostatin exon splice modulation improve muscle pathology of adult mdx mice. Mol. Ther. Nucleic Acids 6, 15–28.
Mansouri, A., Muller, F.L., Liu, Y., Ng, R., Faulkner, J., Hamilton, M., Richardson, A., Huang, T.T., Epstein, C.J., Van Remmen, H., 2006. Alterations in mitochondrial function, hydrogen peroxide release and oxidative damage in mouse hind-limb skeletal muscle during aging. Mech. Ageing Dev. 127, 298–306.
Marzetti, E., Leeuwenburgh, C., 2006. Skeletal muscle apoptosis, sarcopenia and frailty at old age. Exp. Gerontol. 41, 1234–1238.
Marzetti, E., Hwang, J.C., Lees, H.A., Wohlgemuth, S.E., Dupont-Versteegden, E.E., Carter, C.S., Bernabei, R., Leeuwenburgh, C., 2010. Mitochondrial death effectors: relevance to sarcopenia and disuse muscle atrophy. Biochim. Biophys. Acta 1800, 235–244.
Masiero, E., Agatea, L., Mammucari, C., Blaauw, B., Loro, E., Komatsu, M., Metzger, D., Reggiani, C., Schiaffino, S., Sandri, M., 2009. Autophagy is required to maintain muscle mass. Cell Metab. 10, 507–515.
Mayans, O., Labeit, S., 2012. MuRFs: specialized members of the TRIM/RBCC family with roles in the regulation of the trophic state of muscle and its metabolism. Adv. Exp. Med. Biol. 770, 119–129.
McFarlane, C., Plummer, E., Thomas, M., Hennebry, A., Ashby, M., Ling, N., Smith, H., Sharma, M., Kambadur, R., 2006. Myostatin induces cachexia by activating the ubiquitin proteolytic system through an NF-kappaB-independent, FoxO1-dependent mechanism. J. Cell. Physiol. 209, 501–514.
McKenzie, D., Bua, E., McKiernan, S., Cao, Z., Aiken, J.M., 2002. Mitochondrial DNA deletion mutations: a causal role in sarcopenia. Eur. J. Biochem. 269, 2010–2015.
Melov, S., Shoffner, J.M., Kaufman, A., Wallace, D.C., 1995. Marked increase in the number and variety of mitochondrial DNA rearrangements in aging human skeletal muscle. Nucleic Acids Res. 23, 4122–4126.
Miquel, J., 1998. An update on the oxygen stress-mitochondrial mutation theory of aging: genetic and evolutionary implications. Exp. Gerontol. 33, 113–126.
Mitchell, W., Atherton, P., Williams, J., Larvin, M., Lund, J., Narici, M., 2012. Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review. Front. Physiol. 3 (260).
Mizushima, N., Levine, B., Cuervo, A.M., Klionsky, D.J., 2008. Autophagy fights disease through cellular self-digestion. Nature 451, 1069–1075.
Moore-Carrasco, R., Busquets, S., Almendro, V., Palanki, M., Lopez-Soriano, F.J., Argiles, J.M., 2007. The AP-1/NF-kappaB double inhibitor SP100030 can revert muscle wasting during experimental cancer cachexia. Int. J. Oncol. 30, 1239–1245.
Morley, J.E., von Haehling, S., Anker, S.D., 2014. Are we closer to having drugs to treat muscle wasting disease? J. Cachexia Sarcopenia Muscle 5, 83–87.
Muchamuel, T., Basler, M., Aujay, M.A., Suzuki, E., Kalim, K.W., Lauer, C., Sylvain, C., Ring, E.R., Shields, J., Jiang, J., Shwonek, P., Parlati, F., Demo, S.D., Bennett, M.K., Kirk, C.J., Groettrup, M., 2009. A selective inhibitor of the immunoproteasome subunit LMP7 blocks cytokine production and attenuates progression of experimental arthritis. Nat. Med. 15, 781–787.
Muller, M.J., Geisler, C., Pourhassan, M., Gluer, C.C., Bosy-Westphal, A., 2014. Assessment and definition of lean body mass deficiency in the elderly. Eur. J. Clin. Nutr. 68, 1220–1227.
Murton, A.J., 2015. Muscle protein turnover in the elderly and its potential contribution to the development of sarcopenia. Proc. Nutr. Soc. 74, 387–396.
Nakamura, S., Takamura, T., Matsuzawa-Nagata, N., Takayama, H., Misu, H., Noda, H., Nabemoto, S., Kurita, S., Ota, T., Ando, H., Miyamoto, K.-i., Kaneko, S., 2009. Palmitate induces insulin resistance in H4IIEC3 hepatocytes through reactive oxygen species produced by mitochondria. J. Biol. Chem. 284, 14809–14818.
Nakashima, M., Toyono, T., Akamine, A., Joyner, A., 1999. Expression of growth/differentiation factor 11, a new member of the BMP/TGFβ superfamily during mouse embryogenesis. Mech. Dev. 80, 185–189.
Narici, M., Franchi, M., Maganaris, C., 2016. Muscle structural assembly and functional consequences. J. Exp. Biol. 219, 276–284.
Neeland, I.J., Turer, A.T., Ayers, C.R., Berry, J.D., Rohatgi, A., Das, S.R., Khera, A., Vega, G.L., McGuire, D.K., Grundy, S.M., 2015. Body fat distribution and incident cardiovascular disease in obese adults. J. Am. Coll. Cardiol. 65, 2150–2151.
Nicolini, A., Ferrari, P., Masoni, M.C., Fini, M., Pagani, S., Giampietro, O., Carpi, A., 2013. Malnutrition, anorexia and cachexia in cancer patients: a mini-review on pathogenesis and treatment. Biomed. Pharmacother. 67, 807–817.
Pacifici, F., Della-Morte, D., Piermarini, F., Arriga, R., Scioli, M.G., Capuani, B., Pastore, D., Coppola, A., Rea, S., Donadel, G., Andreadi, A., Abete, P., Sconocchia, G., Bellia, A., Orlandi, A., Lauro, D., 2020. Prdx6 plays a main role in the crosstalk between aging and metabolic sarcopenia. Antioxidants (Basel) 9 (4), 329.
Paddon-Jones, D., Rasmussen, B.B., 2009. Dietary protein recommendations and the prevention of sarcopenia. Curr. Opin. Clin. Nutr. Metab. Care 12, 86–90.
Park, S.Y., Kim, H.Y., Lee, J.H., Yoon, K.H., Chang, M.S., Park, S.K., 2010. The age-dependent induction of apoptosis-inducing factor (AIF) in the human semitendinosus skeletal muscle. Cell. Mol. Biol. Lett. 15, 1–12.
Pedrozo, Z., Sánchez, G., Torrealba, N., Valenzuela, R., Fernández, C., Hidalgo, C., Lavandero, S., Donoso, P., 2010. Calpains and proteasomes mediate degradation of ryanodine receptors in a model of cardiac ischemic reperfusion. Biochim. Biophys. Acta (BBA) – Mol. Basis Dis. 1802, 356–362.
Peterson, S.J., Braunschweig, C.A., 2016. Prevalence of Sarcopenia and associated outcomes in the clinical setting. Nutr. Clin. Pract. 31, 40–48.
Philippou, A., Halapas, A., Maridaki, M., Koutsilieris, M., 2007. Type I insulin-like growth factor receptor signaling in skeletal muscle regeneration and hypertrophy. J. Musculoskelet. Neuronal Interact. 7, 208–218.
Phu, S., Boersma, D., Duque, G., 2015. Exercise and Sarcopenia. J. Clin. Densitom. 18, 488–492.
Pickles, S., Vigie, P., Youle, R.J., 2018. Mitophagy and quality control mechanisms in mitochondrial maintenance. Curr. Biol. 28, R170–R185.
Pomatto, L.C., Davies, K.J., 2017. The role of declining adaptive homeostasis in ageing. J. Physiol. 595 (24), 7275–7309.
Prado, C.M.M., Lieffers, J.R., McCargar, L.J., Reiman, T., Sawyer, M.B., Martin, L., Baracos, V.E., 2008. Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: a population-based study. Lancet Oncol. 9, 629–635.
Qureshi, A.A., Tan, X., Reis, J.C., Badr, M.Z., Papasian, C.J., Morrison, D.C., Qureshi, N., 2011. Suppression of nitric oxide induction and pro-inflammatory cytokines by novel proteasome inhibitors in various experimental models. Lipids Health Dis. 10, 177.
Raynes, R., Pomatto, L.C., Davies, K.J., 2016. Degradation of oxidized proteins by the proteasome: distinguishing between the 20S, 26S, and immunoproteasome proteolytic pathways. Mol. Aspects Med. 50, 41–55.
Renault, V., Thornell, L.E., Eriksson, P.O., Butler-Browne, G., Mouly, V., 2002. Regenerative potential of human skeletal muscle during aging. Aging Cell 1, 132–139.
Rodriguez, J., Vernus, B., Chelh, I., Cassar-Malek, I., Gabillard, J.C., Hadj Sassi, A., Seiliez, I., Picard, B., Bonnieu, A., 2014. Myostatin and the skeletal muscle atrophy and hypertrophy signaling pathways. Cell. Mol. Life Sci. 71, 4361–4371.
Rom, O., Reznick, A.Z., 2016. The role of E3 ubiquitin-ligases MuRF-1 and MAFbx in loss of skeletal muscle mass. Free Radic. Biol. Med. 98, 218–230.
Romanello, V., Sandri, M., 2015. Mitochondrial quality control and muscle mass maintenance. Front. Physiol. 6, 422.
Rooyackers, O.E., Adey, D.B., Ades, P.A., Nair, K.S., 1996. Effect of age on in vivo rates of mitochondrial protein synthesis in human skeletal muscle. Proc. Natl. Acad. Sci. U.S.A. 93, 15364–15369.
Sakuma, K., Aoi, W., Yamaguchi, A., 2015. Current understanding of sarcopenia: possible candidates modulating muscle mass. Pflugers Arch. 467, 213–229.
Sandri, M., 2010. Autophagy in skeletal muscle. FEBS Lett. 584, 1411–1416.
Schaap, L.A., Pluijm, S.M., Deeg, D.J., Visser, M., 2006. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. Am. J. Med. 119 (526), e517 e529-526.
Schütz, T., Hudjetz, H., Roske, A.-E., Katzorke, C., Kreymann, G., Budde, K., Fritsche, L., Neumayer, H.-H., Lochs, H., Plauth, M., 2012. Weight gain in long-term survivors of kidney or liver transplantation—another paradigm of sarcopenic obesity? Nutrition 28, 378–383.
Seo, A.Y., Xu, J., Servais, S., Hofer, T., Marzetti, E., Wohlgemuth, S.E., Knutson, M.D., Chung, H.Y., Leeuwenburgh, C., 2008. Mitochondrial iron accumulation with age and functional consequences. Aging Cell 7, 706–716.
Shenkman, B.S., Belova, S.P., Lomonosova, Y.N., Kostrominova, T.Y., Nemirovskaya, T. L., 2015. Calpain-dependent regulation of the skeletal muscle atrophy following unloading. Arch. Biochem. Biophys. 584, 36–41.
Sitte, N., Merker, K., Von Zglinicki, T., Davies, K.J., Grune, T., 2000a. Protein oxidation and degradation during cellular senescence of human BJ fibroblasts: part II–aging of nondividing cells. FASEB J. 14, 2503–2510.
Sitte, N., Merker, K., Von Zglinicki, T., Grune, T., Davies, K.J., 2000b. Protein oxidation and degradation during cellular senescence of human BJ fibroblasts: part I–effects of proliferative senescence. FASEB J. 14, 2495–2502.
Slawik, M., Vidal-Puig, A.J., 2007. Adipose tissue expandability and the metabolic syndrome. Genes Nutr. 2, 41–45.
Spira, D., Norman, K., Nikolov, J., Demuth, I., Steinhagen-Thiessen, E., Eckardt, R., 2016. Prevalence and definition of sarcopenia in community dwelling older people. Data from the Berlin aging study II (BASE-II). Z. Gerontol. Geriatr. 49, 94–99.
Srikanthan, P., Hevener, A.L., Karlamangla, A.S., 2010. Sarcopenia exacerbates obesity-associated insulin resistance and dysglycemia: findings from the National Health and Nutrition Examination Survey III. PLoS One 5, e10805.
Sriram, S., Subramanian, S., Sathiakumar, D., Venkatesh, R., Salerno, M.S., McFarlane, C. D., Kambadur, R., Sharma, M., 2011. Modulation of reactive oxygen species in skeletal muscle by myostatin is mediated through NF-κB. Aging Cell 10, 931–948.
Staunton, L., O'Connell, K., Ohlendieck, K., 2011. Proteomic profiling of mitochondrial enzymes during skeletal muscle aging. J. Aging Res. 2011, 908035.
Strucksberg, K.H., Tangavelou, K., Schroder, R., Clemen, C.S., 2010. Proteasomal activity in skeletal muscle: a matter of assay design, muscle type, and age. Anal. Biochem. 399, 225–229.
Szczesny, B., Tann, A.W., Mitra, S., 2010. Age- and tissue-specific changes in mitochondrial and nuclear DNA base excision repair activity in mice: susceptibility of skeletal muscles to oxidative injury. Mech. Ageing Dev. 131, 330–337.

Tardif, N., Salles, J., Guillet, C., Tordjman, J., Reggio, S., Landrier, J.-F., Giraudet, C., Patrac, V., Bertrand-Michel, J., Migne, C., Collin, M.-L., Chardigny, J.-M., Boirie, Y., Walrand, S., 2014. Muscle ectopic fat deposition contributes to anabolic resistance in obese sarcopenic old rats through eIF2α activation. Aging Cell 13, 1001–1011.

Terman, A., Brunk, U.T., 2004. Myocyte aging and mitochondrial turnover. Exp. Gerontol. 39, 701–705.

Tichy, E.D., Sidibe, D.K., Tierney, M.T., Stec, M.J., Sharifi-Sanjani, M., Hosalkar, H., Mubarak, S., Johnson, F.B., Sacco, A., Mourkioti, F., 2017. Single stem cell imaging and analysis reveals telomere length differences in diseased human and mouse skeletal muscles. Stem Cell Reports 9, 1328–1341.

Tokarz, V.L., MacDonald, P.E., Klip, A., 2018. The cell biology of systemic insulin function. J. Cell Biol. 217, 2273–2289.

Umegaki, H., 2015. Sarcopenia and diabetes: hyperglycemia is a risk factor for age-associated muscle mass and functional reduction. J. Diabetes Investig. 6, 623–624.

van Nieuwpoort, I.C., Vlot, M.C., Schaap, L.A., Lips, P., Drent, M.L., 2018. The relationship between serum IGF-1, handgrip strength, physical performance and falls in elderly men and women. Eur. J. Endocrinol. 179, 73–84.

Vary, T.C., Frost, R.A., Lang, C.H., 2008. Acute alcohol intoxication increases atrogin-1 and MuRF1 mRNA without increasing proteolysis in skeletal muscle. Am. J. Physiol. Regul. Integr. Comp. Physiol. 294, R1777–1789.

Verdijk, L.B., Snijders, T., Drost, M., Delhaas, T., Kadi, F., van Loon, L.J., 2014. Satellite cells in human skeletal muscle; from birth to old age. Age (Dordr) 36, 545–547.

Visser, M., Kritchevsky, S.B., Goodpaster, B.H., Newman, A.B., Nevitt, M., Stamm, E., Harris, T.B., 2002. Leg muscle mass and composition in relation to lower extremity performance in men and women aged 70 to 79: the health, aging and body composition study. J. Am. Geriatr. Soc. 50, 897–904.

Vitale, J.A., Bonato, M., La Torre, A., Banfi, G., 2019. The role of the molecular clock in promoting skeletal muscle growth and protecting against Sarcopenia. Int. J. Mol. Sci. 20 (17), 4318.

Wanagat, J., Cao, Z., Pathare, P., Aiken, J.M., 2001. Mitochondrial DNA deletion mutations colocalize with segmental electron transport system abnormalities, muscle fiber atrophy, fiber splitting, and oxidative damage in sarcopenia. FASEB J. 15, 322–332.

Wang, Y., Michikawa, Y., Mallidis, C., Bai, Y., Woodhouse, L., Yarasheski, K.E., Miller, C. A., Askanas, V., Engel, W.K., Bhasin, S., Attardi, G., 2001. Muscle-specific mutations accumulate with aging in critical human mtDNA control sites for replication. Proc. Natl. Acad. Sci. U.S.A. 98, 4022–4027.

Wenz, T., Rossi, S.G., Rotundo, R.L., Spiegelman, B.M., Moraes, C.T., 2009. Increased muscle PGC-1alpha expression protects from sarcopenia and metabolic disease during aging. Proc. Natl. Acad. Sci. U.S.A. 106, 20405–20410.

Whitman, S.A., Wacker, M.J., Richmond, S.R., Godard, M.P., 2005. Contributions of the ubiquitin-proteasome pathway and apoptosis to human skeletal muscle wasting with age. Pflugers Arch. 450, 437–446.

Witt, S.H., Granzier, H., Witt, C.C., Labeit, S., 2005. MURF-1 and MURF-2 target a specific subset of myofibrillar proteins redundantly: towards understanding MURF-dependent muscle ubiquitination. J. Mol. Biol. 350, 713–722.

Wohlgemuth, S.E., Seo, A.Y., Marzetti, E., Lees, H.A., Leeuwenburgh, C., 2010. Skeletal muscle autophagy and apoptosis during aging: effects of calorie restriction and life-long exercise. Exp. Gerontol. 45, 138–148.

Woo, J., Yu, R., Tang, N., Leung, J., 2014. Telomere length is associated with decline in grip strength in older persons aged 65 years and over. Age (Dordr) 36, 9711.

Yarian, C.S., Rebrin, I., Sohal, R.S., 2005. Aconitase and ATP synthase are targets of malondialdehyde modification and undergo an age-related decrease in activity in mouse heart mitochondria. Biochem. Biophys. Res. Commun. 330, 151–156.

Yip, C., Dinkel, C., Mahajan, A., Siddique, M., Cook, G.J.R., Goh, V., 2015. Imaging body composition in cancer patients: visceral obesity, sarcopenia and sarcopenic obesity may impact on clinical outcome. Insights Imaging 6, 489–497.

Zhang, G., Li, Y.-P., 2012. p38β MAPK upregulates atrogin1/MAFbx by specific phosphorylation of C/EBPβ. Skelet. Muscle 2, 20.

Zheng, J., Edelman, S.W., Tharmarajah, G., Walker, D.W., Pletcher, S.D., Seroude, L., 2005. Differential patterns of apoptosis in response to aging in Drosophila. Proc. Natl. Acad. Sci. U.S.A. 102, 12083–12088.

Zhou, J., Freeman, T.A., Ahmad, F., Shang, X., Mangano, E., Gao, E., Farber, J., Wang, Y., Ma, X.-L., Woodgett, J., Vagnozzi, R.J., Lal, H., Force, T., 2013. GSK-3α is a central regulator of age-related pathologies in mice. J. Clin. Invest. 123, 1821–1832.