Sarcopenia – The search for emerging biomarkers

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ABSTRACT
Sarcopenia, an age-related decline in skeletal muscle mass and function, dramatically affects the life quality of older people. In view of increasing life expectancy, sarcopenia renders a heavy burden on the health care system. However, although there is a consensus that sarcopenia is a multifactorial syndrome, its etiology, underlying mechanisms, and even definition remain poorly delineated, thus, preventing development of a precise treatment strategy. The main aim of our review is to critically analyze potential sarcopenia biomarkers in light of the molecular mechanisms of their involvement in sarcopenia pathogenesis. Normal muscle mass and function maintenance are proposed to be dependent on the dynamic balance between the positive regulators of muscle growth such as bone morphogenetic proteins (BMPs), brain-derived neurotrophic factor (BDNF), follistatin (FST) and irisin, and negative regulators including TGFβ, myostatin, activins A and B, and growth and differentiation factor-15 (GDF-15). We hypothesize that the shift in this balance to muscle growth inhibitors, along with increased expression of the C-terminal agrin fragment (CAF) associated with age-dependent neuromuscular junction (NMJ) dysfunction, as well as skeletal muscle-specific troponin T (sTnT), a key component of contractile machinery, is a main mechanism underlying sarcopenia pathogenesis. Thus, this review proposes and emphasizes that these molecules are the emerging sarcopenia biomarkers.

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1. Introduction

Lean body mass, in particular, skeletal muscular mass (SMM), represents one of the three major components of body composition, which also includes fat and bone mass. All components are highly important for normal physiology and metabolism, and deviations from normal values, in particular, related to age, are often associated with various pathological conditions. The health risks and complications caused by obesity and bone loss, as well as osteoporosis are well established, and the implications of age-related SMM loss have recently attracted increasing attention (Berger and Doherty, 2010).

Skeletal muscle is the largest organ in the human body and contributes up to 60% of the total body weight in young non-obese adults. After the age of 30, about 0.5–1% of SMM is lost per year, with a dramatic acceleration of the rate of decline after the age of 65 (Kyle et al., 2001; Melton et al., 2006). On average, 5–13% of older persons over 60 have low SMM, with the prevalence increasing to as high as 50% in persons over the age of 80 (Morley et al., 2014). Muscle functional capacity (strength and power) also decreases with advancing age, and even to a greater degree than SMM (Newman et al., 2003). This age-related reduction in SMM and function is referred to as “sarcopenia” (from Greek sarx “flesh” and penia “loss”). It is associated with limitations of physical performance in older people, leading to increased risk of falls, fractures, hospitalization, dependency, frailty, profound metabolic consequences, and mortality (Abellan van Kan, 2009; Janssen, 2011; Liu et al., 2014). Moreover, although sarcopenia is generally observed and diagnosed in older adults, it may be present in different clinical settings, including critical illness. Recent data also demonstrated that sarcopenia in an intensive care unit negatively impacts patients’ outcomes and may promote functional disability in the long-term after hospital discharge (Muscariello et al., 2013).

Sarcopenia has been reported to affect more than 40% of elderly individuals >70 years of age, approximately 50 million people worldwide. Taking into account the increase in life expectancy that has occurred during the last decades, this number is estimated to increase 10-fold in the year 2050 (Hida et al., 2013). However, not all the individuals display the same rate of SMM loss, and considerable variation in SMM exists among individuals of the same age (Fig. 1). An important open question is what are the major factors that cause this variation? It is clear that a variety of intrinsic and extrinsic sources are involved in this situation, including (but not limited to) a mixture of lifestyle as well as genetic and metabolic factors.

Currently the contribution of the genetic and familial factors to a variation of SMM is well established, and heritability estimates in studies reach 40% or even higher (Garatacha and Lucia, 2013; Korostishevsky et al., 2015; Nabulsi et al., 2013). However, replicated and confirmed specific genetic polymorphisms are rare and are able to explain only a minor part of this variation (Livshits et al., 2012; Rothhammer et al., 2014; Urano et al., 2014). As such, no solid evidence currently exists supporting an “unfavorable” genotype associated with accelerated sarcopenia or frailty, or a combination of molecular-genetic factors explaining the significant part of the inter-individual variation in SMM.

Likewise, although the term “sarcopenia” has become widespread, the criteria for its definition and clinical diagnosis are not yet clear-cut and vary among studies and experts. Nevertheless, there is a consensus that sarcopenia is a multifactorial syndrome (Berardi et al., 2014; Lauretani et al., 2014; Santilli et al., 2014) and correlates include endocrine dysfunction and inflammation (Lang et al., 2010; Malafarina et al., 2012). It also became obvious that beyond the basic action of mechanical contraction, the skeletal muscles are involved in endocrine and metabolic activities such as glucose, glycogen, and lipid metabolism (Pedersen and Febbraio, 2012) and perform certain immunogenetic functions (Nielsen and Pedersen, 2008).

Muscle-derived cytokines, myokines, such as myostatin, interleukin-6 (IL-6), irisin, and others were shown to exert autocrine, para- and endocrine actions (Pedersen, 2013). They mediate crosstalk between muscle and other tissues, mainly bone, fat, and liver. Their effects include regulation of systemic inflammation, immune function, energy metabolism, insulin sensitivity, cell growth, myogenesis, osteogenesis, and others (Demontis et al., 2013; Iizuka et al., 2014; Pedersen, 2013; Raschke and Eckel, 2013). In addition, a number of factors related to chronic diseases and aging such as oxidative stress, neurodegeneration, anorexia, insulin resistance, mitochondrial dysfunction, and DNA mutations may accelerate sarcopenia (Biolo et al., 2014; Sakuma et al., 2015).

These findings indicate that strategies aimed at diagnosing sarcopenia and counteracting its development and progression have to cover multiple and not yet well-defined factors, thus introducing challenges in searching for sarcopenia biomarkers. To date, although numerous biomarkers have been suggested to be associated with SMM and function, only a few are in fact solely muscle-specific biomarkers. Moreover, correlating their blood
levels to sarcopenia parameters is still a controversial issue (Cesari et al., 2014; Lippi et al., 2014).

In searching for potentially reliable sarcopenia biomarkers, we selected several molecules expressed by skeletal muscles and divided them into five clusters (Fig. 2). The rationale underlying this stratification is based on the supposed capability of the members of each cluster to significantly affect different aspects of muscle growth and function, finally leading to sarcopenia. This review was prompted by an analysis of these molecules’ mechanisms of action, their interrelationships in sarcopenia pathogenesis, and their role as emerging sarcopenia biomarkers. There are, of course, a variety of additional mechanisms potentially involved in sarcopenia pathogenesis such as mitochondria dysfunction, autophagy, apoptosis, endocrine factors, nutritional status, and immobility. These additional mechanisms have not been considered in the present paper but they have been comprehensively discussed by others (Cruz-Jentoft et al., 2014; Ichikawa et al., 2014; Kob et al., 2015; Malafarina et al., 2012; Marzetti et al., 2013; Prado and Heymsfield, 2014; Sakuma et al., 2015).

2. Definition of sarcopenia

As mentioned, sarcopenia remains a poorly defined phenomenon, even referring to it as a “describing rather than defining condition” (Alchim, 2014). This perhaps explains a huge variance in the estimates of sarcopenia prevalence, ranging from ≤10% (Dam et al., 2014) to ≥70% (Batis et al., 2014) in individuals older than 60 years, thus, again emphasizing the urgency in clearly defining the sarcopenia cutoff points.

The term sarcopenia was first introduced by Rosenberg (1989), referring exclusively to a decline in muscle mass with aging (2 SD below the young adult mean). However, later, muscle dysfunction has been linked to the definition of sarcopenia, thus making the term “sarcopenia with impaired mobility” more clinically applicable (Morley et al., 2001). In addition, European Working Group on Sarcopenia in Older People (EWGSOP) defined three stages for sarcopenia: presarcopenia (loss of muscle mass), sarcopenia (loss of muscle mass accompanied by either loss of strength or physical performance), and severe sarcopenia (all three aspects are present) (Cruz-Jentoft et al., 2010). More recently, the Foundation for the National Institutes of Health Sarcopenia Project (FNHI) used the data collected from nine international sources of community-dwelling older persons (26,625 participants). They extended the methodological approach to diagnostic criteria of sarcopenia by applying “Classification and Regression Tree Analysis” to epidemiologic and clinical trial data (McLean et al., 2014; Studenski et al., 2014). This is a two-step approach aimed at identifying criteria for clinically relevant low muscle strength (weakness) and low lean mass.

Since concerns remain as to the best terminology to embrace the evolving concepts of sarcopenia and age-related muscle dysfunction, the concept of “Skeletal Muscle Function Deficit” (SMFD) has been also introduced (Correa-de-Araujo and Hadley, 2014). It comprises the variety of muscular health-associated conditions that contribute to clinically important mobility impairments. “SMFD” attributable to diminished muscle mass has been termed “sarcopenic SMFD,” whereas impairments in muscle strength or power that contribute to SMFD independent of muscle mass (and even in the presence of normal muscle mass) has been termed “dysnepnic SMFD,” and mixed categories could also be defined. The authors believe that the concept of “SMFD”, which covers a variety of contributory etiologies, could provide a framework for developing diagnostic categories being useful for both clinical practice and research.

Overall, currently the standard diagnosis for sarcopenia includes low muscle mass (estimated by the ratio of appendicular lean mass over the height squared, ≤8.0 kg/ht² for men and ≤6.0 kg/ht² for women), accompanied by either low muscle strength (measured by grip strength ≤26–30 kg for men and ≤16–20 kg for women) or low physical performance (measured by gait speed ≤0.8 m/s).

An additional complexity in defining sarcopenia arose from introducing the term “sarcopenic obesity” (Roubenoff, 2000), defined as a higher fat mass relative to a fat-free mass. In recent years, sarcopenic obesity has received increased attention as a risk factor for the impairment of physical performance, leading to frequent fragility fractures and even being a predictor for worse clinical outcomes when compared with sarcopenia or obesity alone (Biolo et al., 2014; Binkley et al., 2013; Ichikawa et al., 2014; Kob et al., 2015; Prado et al., 2012; Stenholm et al., 2008; Zamboni et al., 2008). Moreover, it has been suggested that sarcopenia and osteoporosis are almost the same disease simply expressed in muscle and bone, respectively (Sjöblom et al., 2013). Indeed, a substantially higher correlation has been observed between the bone mineral density (BMD) and lean mass, as compared with the fat body mass (Korostishevsky et al., 2015). In attempting to combine the presence of sarcopenia, osteoporosis, and obesity in the context of impaired mobility, falls and fractures in older adults, the term “dysmobility syndrome” has been proposed (Binkley et al., 2013). Following this idea, a new syndrome “osteosarcopenic obesity” has been coined, which also explains the coexistence of deteriorated bone and muscle in the presence of obesity and is applicable not only to the elderly but also to a wider age range (Ichikawa et al., 2014; Ormsbee et al., 2014). Moreover, it has been suggested that “sarcopenia, sarcopenic obesity, osteosarcopenic obesity, or other combinations are the same disease at different points of evolvement and development” (Ichikawa et al., 2014). However, the physiological interconnections between these tissues are so complex that it is still unclear whether fat infiltration causes bone loss or fat simply fills the space where bone has retreated (Prado and Heymsfield, 2014; Rosen and Bousse, 2006).

Taken together, all current definitions of sarcopenia are incomplete and need to be extended to include additional, more relevant factors that make defining sarcopenia a more precise issue. Hence, identifying the reliable sarcopenia biomarkers will help to resolve these problems.

3. Sarcopenia biomarkers

Sarcopenia biomarkers should fit the acceptable biomarker definition as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (BDWG, 2001). Meeting such a requirement is not a simple task. Currently, it has become clear that muscle dysfunction, a main component of sarcopenia, along with muscle loss, embraces not only contractile impairment but also involves metabolic and endocrine abnormalities affecting the whole body metabolism, systemic inflammation, and the immune system, including chronic elevated low-grade systemic inflammation, “inflamm-aging” (Beyer et al., 2012; Franceschi et al., 2000; Ichikawa et al., 2014; Marzetti et al., 2013). Impaired regenerative capacity, reactive oxygen species production, altered mitochondrial function, modified innervation of muscles, attenuated ability to respond to stress, and the accompanying effects of inactivity were also proposed as key contributors to sarcopenia (Beyer et al., 2012; Ichikawa et al., 2014; Lightfoot et al., 2014; Marzetti et al., 2013; Sakuma et al., 2015).

Skeletal muscle is composed of multiple types of cells including myocytes (muscle fibers), fibroblasts, pericytes, adipocytes, motoneurons, and connective tissues. Among these skeletal...
muscle–constituting cells, not only myocytes but also pericytes including satellite cells [muscle stem cells] were reported to trigger signal interactions with surrounding cells that relate to bioactive factor secretion (Cappellari and Costi, 2013), in particular, muscle derived myokines.

The term “myokine”, introduced by Pedersen’s group (Pedersen and Fischer, 2007), refers to factors secreted by contracting skeletal myocytes whose increase in the circulating blood stream is detected in response to enhanced muscle contraction (Pedersen, 2013). However, many of the contraction–regulated myokines such as IL-6, myostatin, irisin, and others have been found to be secreted also by adipocytes, and therefore, they have been renamed “adipomyokines” (Raschke and Eckel, 2013).

3.1. IL-6 as a prototype of sarcopenia biomarkers

The well-known pro-inflammatory cytokine, IL-6, was recognized as a myokine because of the finding that following physical exercise, its basal plasma concentration may transiently increase up to 100-fold, although less dramatic increases are more common (Catoire and Kersten, 2015; Pedersen, 2013; Pedersen and Febrario, 2012). IL-6 production by both types of I and II fibers, as well as its secretion by primary human skeletal muscle cells in vitro, all increased by contraction, have also been observed (Lambernd et al., 2012; Raschke and Eckel, 2013).

These breakthrough observations in the field resulted in identifying skeletal muscle as an endocrine organ. Of note, the increase of IL-6 in the circulation occurs during exercise without any sign of muscle damage (Fischer, 2006). This is not preceded by an increase in other mediators involved in inflammation, mainly TNFα. Remarkably, in contrast, exercise elevates circulating levels of classical anti-inflammatory cytokines, such as IL-1 receptor antagonist (IL-1ra), IL-10, and soluble TNFR, a naturally occurring inhibitor of TNFα (Pedersen, 2013), suggesting that elevated exercise-induced IL-6 levels could display anti-inflammatory effects. On the other hand, elevated circulating levels of IL-6 are known to be associated with a reduction in anti-inflammatory factors such as IL-10 (Michaud et al., 2013), thus, leading to inflamming–aging often occurring in the elderly. In resolving this apparent paradox, it is worth noting that the contractile-induced beneficial effects of IL-6 are normally associated with its transient production and short-term action. Conversely, persistent inflammatory conditions, some types of cancer, and other chronic disease states are associated with long-lasting elevated systemic IL-6 levels. In the latter situations, IL-6 has shown to be associated with muscle disability (Barbieri et al., 2003), acting in combination with other molecules or functioning indirectly to promote muscle atrophy (Catoire and Kersten, 2015; Lippi et al., 2014).

It was also noted that patients with insulin resistance, obesity, and type-2 diabetes, especially in the elderly, because of a sedentary lifestyle or physical inactivity leading to sarcopenic obesity, display chronically elevated serum levels of pro-inflammatory cytokines including IL-6, thus, linking metabolic disorders with inflammation (Sell et al., 2012). In contrast, regular physical exercise has been shown to reduce basal levels of inflammatory markers (Christiansen et al., 2010), thus, protecting against a number of chronic diseases that are characterized by inflammation (Gleeson, 2007; Pedersen and Fischer, 2007). This suggests that physical activity, by stimulating IL-6 production, may counteract systemic inflammation and modulate glucose and lipid metabolism. If confirmed, such IL-6 activity may have some beneficial effects in aged individuals suffering from inflamm-aging and metabolic disorders. However, the contradictory situation “how can elevated IL-6 lead to the development of insulin resistance, and yet also lead to increased insulin sensitivity?” (Sarvss et al., 2013) also remains an unsolved issue. Moreover, with regard to sarcopenia and sarcopenic obesity, some open questions remain to be elucidated, for example, whether inflammaging (accompanied by elevated IL-6 levels) is involved in the loss of SMM and whether insulin resistance is driven by primary changes in a chronically activated immune system, by adipokines/adipo-myokines secreted by rising fat mass, by reduced physical activity, and muscle loss, or by all or none of these factors.
3.2. Negative regulators of muscle growth

3.2.1. Myostatin and TGFβ

In the pioneering study by Lee's group (McPherron et al., 1997), hypermuscularity was observed in mice carrying a targeted inactivation of growth and differentiation factor 8 (GDF-8), termed myostatin due to its ability to inhibit muscle differentiation and growth (myogenesis inhibitor). Myostatin belongs to the TGFβ family, is highly evolutionarily conserved, and is secreted mainly by skeletal muscle fibers. Myostatin physiological function was repeatedly confirmed in experiments with laboratory animals. Knockout mice consistently showed significant skeletal muscle hypertrophy, whereas myostatin over-expression or systemic administration resulted in dramatic muscle atrophy (Lee, 2004; Lee and McPherron, 2001). Since its secretion has also been recently determined by cultured human adipocytes, it was categorized as belonging to the group of adipomyokines (Raschke and Eckel, 2013).

Like most TGFβ family members, myostatin is produced within the cell as the precursor molecule that after cleavage by furin protein convertase is transformed to an N-terminal fragment (myostatin prodomain) and a biologically active C-terminal fragment (myostatin ligand) (Breitbart et al., 2011; Lee, 2004). Then, both fragments are secreted by the cell as an inactive multi-protein complex bound to the pro-domain and/or other inhibitory proteins such as FST, which constitutes the main form of myostatin found in serum (Hill et al., 2002). Full activation of myostatin occurs when the prodomain is cleaved by members of the tolloid family of metalloproteinases, leading to the release of the myostatin ligand from the complex (Wolfman et al., 2003). Free myostatin then binds to a combination of activin type II receptors A and B (ActRIIA/B) on the cell membrane with higher affinity for ActRIIB, and forms a heterodimer with activin-like kinase 4 (ALK4) or ALK5. The intracellular serine/threonine kinase domain of ALK4 and ALK5 phosphorlates Smad2 and Smad3, which form a complex with Smad4, thus utilizing the TGFβ signaling pathway (Fig. 3). This complex translocates to the nucleus to regulate the transcription of genes involved in the proliferation and differentiation of skeletal muscle precursor cells and protein degradation pathways in mature myofibers (Langley et al., 2002; Sartori et al., 2014). The activation of Smad2/3 by myostatin also inhibits the Akt/mammalian target of rapamycin (mTOR) pathway and therefore suppresses protein synthesis (Trendelenburg et al., 2009). In contrast, Smad2/3 inhibition promotes muscle hypertrophy in a manner partially dependent on mTOR signaling (Sartori et al., 2014). In addition, myostatin-mediated signaling decreases AKT phosphorylation and signals through forkhead box O (FOXO) transcription factors to induce atrophy, but the mechanisms through which myostatin regulates the proteolytic systems need to be further investigated (Rodriguez et al., 2014; Sakuma et al., 2015).

Regardless of the signaling pathway, because of the strong capacity of myostatin to inhibit skeletal muscle growth, it is associated with sarcopenia. Interestingly, elevated serum and mRNA myostatin levels were observed in old individuals as compared with their young counterparts (Léger et al., 2008; Yarasheski et al., 2002). However, other human reports failed to show age-related differences in either circulating protein or skeletal muscle myostatin mRNA levels (Ratkevicus et al., 2011; Welle et al., 2002). Moreover, animal studies have also yielded conflicting results, demonstrating no changes in myostatin mRNA expression in mouse sarcopenic muscles (Carlson et al., 2008) and even lower expression in aged rats compared with younger animals (Haddad and Adams, 2006). These controversial findings suggest that myostatin may not be a primary trigger of sarcopenia. In addition, there are difficulties related to myostatin availability such as a disbalance between myostatin circulating levels and its activity, blocking of myostatin activity by endogenous inhibitors (e.g., by FST, see below), and lack of reliable myostatin detection methods (Breitbart et al., 2013; Sakuma et al., 2015; White and LeBrasseur, 2014).

Nevertheless, an obvious negative role of myostatin in muscle growth has been utilized in developing some specific inhibitors of myostatin signaling shown to stimulate muscle growth or to prevent muscle loss. These include neutralizing antibodies to myostatin (Whittemore et al., 2003), a modified myostatin pro-peptide, which blocks myostatin action (Rogdanovich et al., 2005), and various forms of a soluble ActRIIB receptor (Busquets et al., 2012; Goncalves et al., 2010; Koncarevic et al., 2010; Lee et al., 2005). For example, administering soluble ActRIIB led to muscle hypertrophy in normal and myostatin-knockout mice, confirming the notion that other ligands, in addition to myostatin, normally function to limit muscle growth (Lee et al., 2005). These data also suggest that the capacity for modulating muscle growth by perturbing the signaling pathway at the ActR level is much greater than that achieved by blocking myostatin only. Accordingly, a novel, human anti-ActRII antibody (bimagrumab, or BYM338) dramatically increased SMM upon its administration to mice. In addition, bimagrumab enhanced the differentiation of primary human skeletal myoblasts and counteracted the inhibition of differentiation induced by myostatin, and also prevented myostatin-induced atrophy through inhibition of Smad2/3 phosphorylation (Lach-Triffelieff et al., 2014). Moreover, bimagrumab induced enhanced muscle hypertrophy in myostatin mutant mice, further confirming its beneficial effect on muscle growth beyond myostatin inhibition alone by blocking ActRII ligands (Lach-Triffelieff et al., 2014). These data highlight the compelling therapeutic potential of bimagrumab for treating skeletal muscle atrophy and weakness in multiple settings. In a recent pilot study of persons suffering from a rare muscle-wasting disease, sporadic inclusion body myositis (sIBM), characterized by enhanced muscle Smad2/3 phosphorylation, the bimagrumab-treated patients had a significantly increased thigh muscle volume and lean body mass as well as an improved 6-min walking distance (Amato et al., 2014). These findings seem promising with regard to their application to other muscle-wasting disorders including sarcopenia.

In this context, there is growing evidence of the complexity of myostatin involvement in sarcopenia pathogenesis. These include findings that myostatin levels in skeletal muscle may be significantly modulated by the content of adipose tissue, especially in patients suffering from sarcopenic obesity. For example, increased myostatin expression and secretion have been found in skeletal muscle and adipose tissue samples derived from obese and patients suffering from sarcopenic obesity. For example, increased myostatin expression and secretion have been found in skeletal muscle and adipose tissue samples derived from obese and extremely obese middle-aged women, in whom circulating concentrations of myostatin were found to be correlated with insulin resistance (Hittel et al., 2009). Accordingly, high muscular expression of myostatin mRNA is associated with impaired metabolism, systemic inflammation, obesity, and a poor fitness level in healthy subjects, and these associations are disrupted in patients with type-2 diabetes (Brandt et al., 2012). Moreover, physical exercises in humans and animals reduced myostatin expression, and myostatin inhibition attenuated the adverse metabolic consequences of obesity primarily through its effects on muscle and not through changes in adipose growth, thus increasing the skeletal muscle metabolic platform available for glucose and fatty acid uptake and utilization (Allen et al., 2011; Bernardo et al., 2010). However, the mechanisms underlying myostatin involvement in metabolic disorders as well as in sarcopenia remain poorly understood.

Similar to myostatin, TGFβ is also expressed in myocytes (Lafyatis et al., 1991) and adipocytes (Rahimi et al., 1998), and it also can inhibit myogenesis (Massagué et al., 1986). Its administration leads to marked muscle atrophy and reduced force generation...
by phosphorylation and activation of transcription factors Smad2/3 (Chen et al., 2014; Langley et al., 2002; Lee et al., 2005; Trendelenburg et al., 2009; Rodriguez et al., 2014; Sakuma et al., 2015; Sartori et al., 2014). GDF-15, a member of the TGF-β superfamily, is negatively regulated Smad2/3 and Smad1/5/8 signaling pathways both by using Smad4 – a key cofactor that is shared by different Smads (Ross and Hill, 2008). The whole heterocomplex translocates to the nucleus where it interacts directly with DNA to either activate or suppress several target genes responsible for muscle growth (Ross and Hill, 2008). We suggest that the final outcome of Smad4 activation depends on the balance between the depicted muscle growth inhibitors and promoters. It is likely that on the sarcopenic background (physical inactivity, inflamm-aging, and some other age-accompanying detrimental factors) up-regulation of the levels and/or activity of the illustrated muscle growth inhibitors occur. Accordingly, an increase in Smad2/3 activity along with decreased Smad1/5/8 activity would shift the balance to the primary activation of Smad4 by Smad2/3. In addition, BDNF activates the Smad1/5/8 pathway (Ji and Jaffrey, 2012), but its impaired activity may fail to trigger Akt/mTOR signaling. Overall, this results in inhibition of the Akt/mTOR/Wnt/β-catenin signaling pathway, and thus, in reduced muscle growth and function (sarcopenia). The arrows indicate an activating interaction. The broken lines and arrows indicate mechanisms that are currently unknown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. Hypothesized interrelationships between the main muscle growth inhibitors and promoters in sarcopenia pathogenesis. Similar to bone remodeling, where the dynamic equilibrium concerning the bone mass is maintained by the opposite forces of bone resorption (by osteoclasts) and bone formation (by osteoblasts) (Sims and Gooi, 2008), the present figure shows the opposite actions of muscle growth vs muscle promoter factors. The equilibria status between these forces supports healthy muscular mass. However, the one prevailing would lead to muscular mass growth (mostly at a young age) or loss (mostly at an older age), leading potentially to sarcopenia. Muscle growth inhibitors (shown in red) – activins A and B, myostatin, TGFβ, and potentially GDF-15. Muscle growth promoters (shown in green) – several bone morphogenetic protein (BMPs) such as BMP7, BMP13, and BMP14 as well as follistatin (FST), BDNF, and possibly irisin. Activins, myostatin, and TGFβ act via ActR/TGFβ receptors, followed by phosphorylation and activation of transcription factors Smad2/3 (Chen et al., 2014; Langley et al., 2002; Lee et al., 2005; Trendelenburg et al., 2009; Rodriguez et al., 2014; Sakuma et al., 2015; Sartori et al., 2014). GDF-15, a member of the TGFβ family, also triggers Smad2/3, probably via not yet known receptor (Bloch et al., 2015; Corre et al., 2013). BMPs act via Act/BMP receptors, followed by activation of Smad1/5/8 (Sartori et al., 2014). FST acts via suppression of activins, myostatin, and TGFβ (Gilson et al., 2009; Lee et al., 2005; Zhu et al., 2011), but also independently by recruiting the Wnt/β-catenin signaling pathway (Han et al., 2014). The mechanism of irisin action on muscle growth is suggested to act in concordance with FST (Vamvini et al., 2013) and by inhibition of Wnt/β-catenin signaling (Klangjareonchai et al., 2014). Smad6/7 negatively regulates Smad2/3 and Smad1/5/8 signaling pathways both by using Smad4 – a key cofactor that is shared by different Smads (Ross and Hill, 2008). The whole heterocomplex translocates to the nucleus where it interacts directly with DNA to either activate or suppress several target genes responsible for muscle growth (Ross and Hill, 2008). We suggest that the final outcome of Smad4 activation depends on the balance between the depicted muscle growth inhibitors and promoters. It is likely that on the sarcopenic background (physical inactivity, inflamm-aging, and some other age-accompanying detrimental factors) up-regulation of the levels and/or activity of the depicted muscle growth inhibitors, and down-regulation of the levels and/or activity of the illustrated muscle growth promoters occur. Accordingly, an increase in Smad2/3 activity along with decreased Smad1/5/8 activity would shift the balance to the primary activation of Smad4 by Smad2/3. In addition, BDNF activates the Smad1/5/8 pathway (Ji and Jaffrey, 2012), but its impaired activity may fail to trigger Akt/mTOR signaling. Overall, this results in inhibition of the Akt/mTOR/Wnt/β-catenin signaling pathway, and thus, in reduced muscle growth and function (sarcopenia). The arrows indicate an activating interaction. The broken lines and arrows indicate mechanisms that are currently unknown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

( Mendias et al., 2012). TGF-β is secreted into the extracellular matrix in an inactive form bound to a latency-associated peptide (LAP). The LAP is degraded by several types of proteases in the extracellular matrix (ECM). Many of these proteases become activated after skeletal muscle injury or remain persistently activated in several different types of skeletal muscle diseases. Once TGFβ is released from the LAP and becomes activated, it associates with its receptors TGFβRII and TGFβRI. The activated TGFβRII activates two signaling pathways, the canonical Smad2/3 pathway and the non-canonical TGFβ-activated kinase (TAK-1)-MAPK pathway, which mediate many of the intracellular actions of TGFβ including synthesis of ECM proteins, cell motility, and others (Ross and Hill, 2008; Sartori et al., 2014) (Fig. 3).

3.2.2. Activins A and B

Activins A and B are other members of the TGFβ family defined as negative regulators of muscle mass. Like myostatin, activins are synthesized as precursor molecules with the N-terminal prodomain mediating the folding and dimerization of the C-terminal mature domain. Activins are displaced from their pro-domains by the ActRIIA/IIB receptors, leading to the recruitment, phosphorylation, and activation of ALK4. Similarly to the myostatin pathway, activated ALK4 phosphorylates intracellular signaling molecules, Smad2/3, which in turn, form a complex with the coactivator Smad4 (Lee et al., 2005; Sartori et al., 2014). Recently it has been shown that the activin A-triggered ActRIIB signaling pathway includes decreased Akt/mTOR-mediated protein synthesis (Fig. 3), accompanied by muscle fibrosis and reduced muscle mass and function (Chen et al., 2014). Notably, genetic deletion of ActRIIB or Smad3 leads to a significant increase of skeletal muscle mass and function (Lee et al., 2005; Lee and McPherron, 2001). In models of cancer cachexia, inhibition of ActRIIB by administering soluble receptor-smant ActRIIB reversed muscle wasting and prolonged survival. In contrast, elevated expression of activins promoted muscle wasting and cachexia in mice (Chen et al., 2014). Noteworthy, in this study, both activin A and B were found to be 100-fold more potent in causing muscle wasting as compared with myostatin, making activins the strongest natural muscle growth inhibitors so far.

An interesting link between activins/ActR and inflammation in sarcopenia has also been documented (Trendelenburg et al., 2012). It was shown that the pro-inflammatory cytokines IL-1α and TNFα block the differentiation of human myoblasts into myotubes, and that this anti-differentiation effect requires activation of TAK-1. Surprisingly, this inhibitory effect of the cytokines required transcriptional upregulation of activin A, which in turn acted through its established ActRIIA/ALK4/Smad2/3 signaling pathway. Moreover, experiments in aged rats as a model of sarcopenia...
confirmed that this dual pathway is activated during aging. These findings link cytokine and activin signaling pathways as a new mechanism by which pro-inflammatory cytokines affect skeletal muscle, and establish the physiological relevance of this pathway in the impaired muscle regeneration seen in sarcopenia. The data also suggest that treatment of sarcopenia with agents capable of blocking the relevant cytokines that activate TAK-1 would provide an “upstream” inhibition of activin release, effectively shutting down two pathways that negatively perturb skeletal muscle in sarcopenia.

In concluding this section, it is important to stress that although the current findings clearly demonstrate that myostatin and activins are involved in muscle physiology and pathology, thus potentially making them genuine sarcopenia biomarkers, this assumption has been scarcely confirmed in the literature so far.

3.2.3. GDF-15

GDF-15, also termed macrophage inhibitory cytokine 1 (MIC-1), is another relevant member of the TGFβ superfamily. Under baseline conditions, it is weakly produced in most tissues with the exception of its very high expression in the placenta. However, in response to various types of poisoning, cancer, and adverse cardiovascular events, GDF-15 production is significantly increased in a variety of tissues (Corre et al., 2013; Lippi and Cervellin, 2014). In addition, elevated GDF-15 plasma levels have been recognized as a surrogate marker of the risk of mortality in heart failure (Kempf and Wollert, 2009) and all-cause mortality without cancer or cardiovascular diseases (Wilkund et al., 2010). With regard to muscle pathology, it was found that GDF-15 transgenic mice are resistant to induced cardiac hypertrophy, and exposure to circulating GDF-15 limited cardiac myocyte hypertrophy. On the other hand, mice deficient in GDF-15 exhibited both increased hypertrophy and loss of cardiac function in response to pressure overload compared with wild-type mice (Xu et al., 2006). Moreover, GDF-15 serum and mRNA levels were found to be elevated in patients who developed quadriceps atrophy following cardiac surgery (Bloch et al., 2013). Similarly, elevated GDF-15 expression was noted in intensive care unit-acquired weakness patients in whom increased GDF-15 was associated with reduced expression of several muscle microRNAs shown to be involved in skeletal muscle growth (Bloch et al., 2015). These observations are consistent with the GDF-15 anti-hypertrophy/pro-apoptosis role described in some cancers (Corre et al., 2013).

Thus, apparently, GDF-15 has both an environment/situation-mediated and a direct effect on skeletal muscles, resulting in reduced muscle mass and therefore it is potentially involved in sarcopenia. However, the lack of clear knowledge about the GDF-15 receptor, and therefore, the signaling pathways that mediate GDF-15 action (Corre et al., 2013) make the development of therapeutic interventions with GDF15 or anti-GDF15 agents questionable. Nevertheless, the growing body of evidence suggesting the definite involvement of GDF-15 in muscle pathology makes it a promising sarcopenia biomarker (Fig. 3).

3.3. Positive regulators of muscle growth

3.3.1. FST

FST, a single chain glycoprotein, is known as a strong inhibitor of myostatin-mediated muscle wasting: FST-overexpressing transgenic mice exhibit a significant increase in muscle mass (Lee and McPherron, 2001). This occurred much more in myostatin knockout mice and myostatin-null mice carrying a FST transgene, which displayed about four times the muscle mass of the wild-type mice (Lee et al., 2001; Lee, 2007). These observations imply the existence of other muscle growth regulators besides myostatin that FST can modulate. There are three reported FST isoforms: FST288, FST315, and FST300 (or FST303); of these, FST315 protein possesses endocrine-like activity and is found mainly in circulation (Kumar, 2005). Importantly, FST proteins were found to bind to myostatin in the serum, thus, making myostatin often undetectable (Hill et al., 2002).

Although the mechanism FST-induced enhancement of muscle healing is not clear, it appears to be related to an increase in the myogenic potential of muscle progenitor cells, most likely due to FST-mediated inhibition of myostatin, activin A, and TGFβ (Gilson et al., 2009; Lee et al., 2005; Zhu et al., 2011). In addition, muscle hypertrophy in FST-overexpressed mice was accompanied by enhanced AKT phosphorylation, mTOR signaling, and protein synthesis, which were inhibited by rapamycin (Winbanks et al., 2012). The same study reported that inhibition of Smad3 activity by FST is critical for activation of AKT/mTOR signaling since constitutively active Smad3 suppressed FST-induced muscle growth and mTOR activation. These observations suggest that Smad3 and AKT directly interact in this system independently of myostatin. Moreover, a molecular crosstalk between the FST, Wnt/β-catenin, and TGFβ signaling pathways in muscle growth has been recently uncovered (Han et al., 2014) (Fig. 3). These findings could be of a crucial importance in light of the deep involvement of the Wnt/β-catenin signaling system in regulating muscle fiber growth and maintenance (Cisternas et al., 2014).

The well-established capability of FST to ameliorate muscle growth reduction clearly implicates it as a potentially powerful drug for treatment of muscle injury and wasting. Indeed, an engineered FST315 variant has been created by fusing FST315 lacking heparin binding activity to the N-terminus of a murine IgG1 Fc (FST315–ΔHBS-Fc): its weekly administration in normal mice resulted in increased body weight and SMM (Yaden et al., 2014). In addition, in several models of muscle injury, FST315–ΔHBS-Fc induced an accelerated restoration of myofibers and muscle function, thus, demonstrating that it is a potent inducer of muscle remodeling and regeneration (Yaden et al., 2014). Moreover, transgenic mice expressing a FST-derived myostatin inhibitor, designated FS I-I, exhibited increased skeletal muscle hypertrophy in FST-overexpressed mice was accompanied by increased body weight and SMM (Yaden et al., 2014). In addition, in several models of muscle injury, FST315–ΔHBS-Fc induced an accelerated restoration of myofibers and muscle function, thus, demonstrating that it is a potent inducer of muscle remodeling and regeneration (Yaden et al., 2014). In addition, in several models of muscle injury, FST315–ΔHBS-Fc induced an accelerated restoration of myofibers and muscle function, thus, demonstrating that it is a potent inducer of muscle remodeling and regeneration (Yaden et al., 2014). Moreover, transgenic mice expressing a FST-derived myostatin inhibitor, designated FS I-I, exhibited increased skeletal muscle hypertrophy, and strength. Notably, in mice resulting from crossing FS I-I transgenic mice with mdx mice, a model for human Duchenne muscular dystrophy, the skeletal muscles were enlarged and there was reduced cell infiltration along with recovering of muscle strength (Nakatani et al., 2008). These findings justify further examination of FST or FST mimetics in clinical use for treating muscle injuries/atrophy, sarcopenia prophylaxis, and for applying FST as a potential sarcopenia biomarker.

3.3.2. BMPs

BMPs are additional well-known members of the TGFβ family of cytokines. About 20 different BMPs with various, even opposing functions, including the regulation of cell differentiation, proliferation, survival, migration, and homeostasis are known and have been extensively studied, with an emphasis on their differentiation potential with regard to bone precursor cells (Ruschke et al., 2012). BMPs are secreted growth factors and recent studies revealed their pleiotropic effects in tissues other than bone including skeletal muscle where they have been found to be critical enhancers of muscle mass, competing with the TGFβ/myostatin/activin pathway. In particular, BMPs positively regulate the transcription of target genes utilizing Smad proteins 1, 5, and 8, which are also capable of forming a complex with Smad4 (Massagué et al., 2005). In addition, they negatively regulate the activity of Smad3 proteins 6 and 7, which are capable of preventing receptor-mediated activation of Smad1/5/8 (Hayashi et al., 1997). Thus, the complexity of the TGFβ signaling network includes cross-regulation between the myostatin/activin–Smad2/3 and BMP–Smad1/5/8 axes,
supposed to have the potential to operate in parallel and reciprocally (Sartori et al., 2014) (Fig. 3). This suggests that stimulation of the BMP–Smad1/5/8 signaling axis may offer therapeutic benefits in preventing or ameliorating pathology-associated muscle wasting. Indeed, it was observed that activation of the BMP–Smad1/5/8 axis is accompanied by enhanced mouse skeletal muscle growth and that it prevents muscle wasting in vivo (Winbanks et al., 2013). Although these observations demonstrate the deep involvement of BMPs in muscle physiology, to date, there are no data concerning the participation of BMPs in sarcopenia.

3.3.3. Irisin

As mentioned previously, myokines, adipokines, and adipomyokines regulate muscle growth in an auto-, para- and endocrine manner. Recently, Boström et al. (2012) identified a new muscle tissue-secreted peptide, irisin, described as the extracellular cleaved product of fibronectin type III domain containing protein 5 (FNDC5). The C-terminal tail of the protein is located in the cytoplasm, whereas the extracellular portion, a 112 amino acid N-terminal part is supposed to be cleaved and released to the circulation. In the same study, mRNA expression and plasma levels of irisin were found to be elevated in mice in response to exercise. Data collected soon after its discovery confirmed levels of irisin were found to be elevated in mice in response to exercise. It is also secreted by adipocytes (Roca-Rivada et al., 2013), and therefore, it was categorized as an adipo-myokine (Raschke and Eckel, 2013). Furthermore, irisin was found to increase the browning of murine white/beige adipose tissue and enhanced the thermogenesis of white adipose tissue by increasing energy expenditure, thereby contributing to reduced body weight, and improved glucose homeostasis and insulin resistance (Boström et al., 2012; Moreno-Navarrete et al., 2013; Yan et al., 2014). Moreover, decreased circulating irisin concentrations and reduced expression of its structural gene, FNDC5, in muscle and adipose tissue were observed in obese and type-2 diabetic subjects (Liu et al., 2013; Moreno-Navarrete et al., 2013; Zhang et al., 2014). Along with the 100% homology between murine and humans, the aforementioned observations imply that irisin is an attractive treatment option for metabolic diseases such as obesity and type-2 diabetes. Indeed, the ability of anti-diabetic drug metformin to elevate irisin blood levels in obese/diabetic db/db mice in correlation with up-regulated intracellular FNDC5 mRNA/protein expression and the release of irisin in murine C2C12 myotubes (Li et al., 2014) lends support to this notion.

Moreover, accordingly, a positive correlation between circulating irisin and FST (but not with myostatin or activin A) levels was observed in healthy men and obese individuals. Interestingly, in these subjects, irisin mRNA expression was positively correlated with FST mRNA expression in muscle biopsy samples (Vamvini et al., 2013). These data are the first to demonstrate the possible reciprocal regulation of irisin and FST in human skeletal muscle by yet to be deciphered mechanisms (Fig. 3). Intriguingly, a significant negative correlation between decreased irisin and elevated serum levels of sclerostin was documented recently in adults with prediabetes (Klangiareonchai et al., 2014). Sclerostin is produced by osteocytes and inhibits the activity of the Wnt signaling pathway, leading to a reduction in osteoblast differentiation and bone formation, as well as the stimulation of adipogenesis (Sapir-Koren and Livshits, 2014). Thus, the observed correlation between irisin and sclerostin suggests an unexpected irisin-mediated link among muscle, fat, and bone metabolism.

However, there are some concerns regarding the functional role of irisin in humans. For example, the browning of white adipose tissue and the thermogenic effects of irisin in humans as well as the validity of its serum determination remain controversial. In addition, the initially reported exercise-dependent increase of irisin in humans could not be confirmed in several new studies (Crujeiras et al., 2014; Erickson, 2013; Hofmann et al., 2014; Irving et al., 2014; Raschke and Eckel, 2013). Additional evidence against the potential beneficial effect of irisin in humans is the observation that primary human pre- adipocytes displayed browning in response to bone morphogenetic protein 7 (BMP7) but not to irisin (Raschke et al., 2013). Moreover, the human FNDC5 gene was found to possess a mutation in the conserved start codon ATG to ATA, thus, suggesting that it is a transcribed pseudogene unable to be translated into the full-length FNDC5 protein and potentially to be processed to irisin (Raschke et al., 2013). However, the fact that FNDC5 is translated using an alternative start codon, similarly to a large number of other genes (Ivanov et al., 2011), does not necessarily lead to impaired functionality. Indeed, recent data indicated correlations between serum irisin levels and various human disorders (Boström et al., 2014; Liu, 2015). Moreover, the obvious involvement of irisin in muscle physiology suggests its application as a potential sarcopenia biomarker.

3.3.4. BDNF

BDNF is a member of the neurotrophin family where structurally related growth factors play a major role in neuronal growth, differentiation, and plasticity. BDNF was also detected in and released by primary human myotubes, thus confirming its myokine nature (Raschke et al., 2013). In skeletal muscle, BDNF is involved in the development and differentiation of myoblasts and muscle fibers as well as in the regulation of motoneuron survival, the presynaptic release of neurotransmitters, and the maintenance of the postsynaptic region in skeletal myofibers (Raschke and Eckel, 2013; Sakuma et al., 2015). BDNF exerts its effects via the tropomysosin-related kinase-B receptor (TrkB) and the p75 neurotrophin receptor (p75NTR) (Fig. 3). The synthesis of BDNF occurs primarily in the central nervous system (CNS), initially as a precursor molecule consisting of 250 amino acid residues, a length twice the size of mature BDNF. BDNF regulates synaptic transmission in the CNS as well as neuromuscular transmission by interacting with pre-synaptic adenosine A2A receptors and muscarinic receptors (Bayduyk and Xu, 2014). It has also been demonstrated that p75NTR (but not TrkB) is a marker for human skeletal muscle precursor cells at a high differentiation potential, and that it positively regulates myogenesis and myofiber maturation (Colombo et al., 2011). Accordingly, the blockade of p75NTR signaling in vivo in an injury mouse model hampered muscle regeneration (Deponti et al., 2009).

An interesting aspect of the BDNF link to muscle pathology was revealed in a study of diaphragm muscle, which turned out to be highly susceptible to sarcopenia in mice (Greising et al., 2014). One of the proposed mechanisms of such susceptibility might be the altered trophic interactions between motor neurons and muscle fibers maintained by BDNF. Indeed, by applying a specific inhibitor of TrkB, it was shown that inhibition of BDNF/TrkB signaling impaired neuromuscular transmission only in young adults but not in old mice, thus suggesting that the loss of endogenous BDNF precedes the reduced activity of the high-affinity TrkB activity in aging mouse diaphragm muscle (Greising et al., 2015).

Some recent intriguing observations suggest an unexpected linking of BDNF to immunity, inflammation, and muscle physiology/pathology. Previous data collected from both animal and human studies indicated that various immune cells could produce BDNF (Papathanassoglou et al., 2014). In addition, acute exercise was found to be accompanied by fast up and down BDNF and pro-BDNF protein levels as well as p75NTR receptor expression in human immune cells proportionally to the exercise intensity and duration (Brunelli et al., 2012). In an animal model,
running exercise protected against lipopolysaccharide (LPS)-induced down-regulation of BDNF signaling in the striatum nucleus, and blocking BDNF activity with a TrkBR antagonist eliminated the protective effects of exercise (Wu et al., 2011). These findings suggest that the anti-inflammatory effects of exercise are mediated by activating the BDNF/TrkBR signaling pathway. More recently, the preferential accumulation of BDNF-releasing activated T lymphocytes and macrophages near p75NTR-positive myofibers within regeneration areas has been demonstrated (Colombo et al., 2013). These observations support the hypothesis that BDNF may serve as a mediator for regeneration via a crosstalk between immune and muscle cells.

Altogether, in addition to its well-known role in neurobiology, BDNF was found to be produced in skeletal muscle cells whose levels are increased by contraction, most probably acting in an autocrine and/or paracrine manner within skeletal muscle and playing an important role in muscle repair, regeneration, and differentiation. It acts also in an endocrine manner by participating in fat oxidation and other metabolic events, as well as in immune regulation and inflammation (Pedersen, 2013), all together, suggesting its involvement in the pathogenesis of sarcopenia. However, since muscle-derived BDNF appears not to be released into circulation (Matthews et al., 2009), its usage as a sarcopenia biomarker remains questionable.

In summary, in normalcy, the dynamic balance maintains skeletal muscle growth and function. The shift in the balance to the negative regulators could lead to muscle pathology, thus, being one of the major mechanisms responsible for developing sarcopenia (Fig. 3).

3.4. Aging-associated impairment of NMJs: CAF

In addition to the proposed disbalance between negative and positive muscle growth regulators, other factors are supposedly involved in sarcopenia pathogenesis. Among them is the aging-associated decline in neurophysiological functions mainly due to impairment of NMJs (Gonzalez-Freire et al., 2014; Rudolf et al., 2014). The main player in NMJ function is acetylcholine (ACh), capable of triggering acetylcholine receptors (AChR) located in the post-synaptic membrane, which produce an action potential, consequently, activating voltage-gated dihydropyridine receptors (DHPR) located in the sarcolemma and by induction, ryanodine receptors (RyR). Calcium released from the sarcoplasmic reticulum through the RyRs binds to troponin C (see below) and allows cross-bridge cycling and force production (Punga and Ruegg, 2012). Although the reasons for age-related NMJ dysfunction in muscle are not clear, many factors have been suggested to be involved in the functional impairment of NMJs, e.g., mitochondrial dysfunction, oxidative stress, inflam-aging, and others (Gonzalez-Freire et al., 2014; Rudolf et al., 2014). These events are thought to be associated with neuromuscular fatigue – progressively impaired transduction of action potentials through the NMJ during exercise. This mechanism has been identified as a factor that limits exercise intensity and duration, and as a contributor to reduced response to exercise training during aging (Nishimune et al., 2014).

It has been also suggested that the onset of a pathogenic pathway ending in sarcopenia might be the destabilization of the NMJs through enhanced proteolytic cleavage of extracellular proteoglycan agrin (Fig. 4). Synthesized in motoneurons, agrin interacts with low-density lipoprotein receptor-related protein 4 (LRP4) to activate the receptor tyrosine muscle-specific kinase (MuSK), which is transported along the axons and finally released into synaptic basal lamina, where it induces postsynaptic differentiation including AChR clustering and stabilization. Agrin is inactivated by cleavage by neutrophysin, a synaptic protease, thereby releasing a soluble 22 kDa CAF to circulation, making it detectable in human serum (Bolliger et al., 2010; Drey et al., 2013; Hettwer et al., 2013). Remarkably, young transgenic mice overexpressing neutrophysin in spinal motoneurons are characterized by early declines in muscle mass and strength, with histopathological traits mimicking those of sarcopenia such as type II fiber loss, increased heterogeneity of fiber diameter, a higher frequency of centralized nuclei, fiber-type grouping, and type I to type II fiber transition (Büttikofer et al., 2011). These findings indicate that an excessive cleavage of agrin results in elevated levels of CAF as well as NMJ fragmentation and fiber denervation, which may contribute to the pathogenesis of sarcopenia (Fig. 4). Recent human observations support this notion. Indeed, serum CAF levels were shown to be significantly higher in sarcopenic versus non-sarcopenic subjects (Hettwer et al., 2013; Marzetti et al., 2014). In addition, a significant inverse correlation between serum levels of CAF and neuromuscular fatigue was determined by measuring the vastus lateralis muscle’s physical working capacity threshold (Stout et al., 2015). In addition, CAF serum concentrations were found to be a potential marker for the loss of appendicular lean mass in older adults (Drey et al., 2013).

Taken together, these findings suggest that assessment of CAF from blood samples as a biomarker may provide clinically informative measures of NMJ-related skeletal muscle status in sarcopenia. However, despite the extensive evidence concerning muscle denervation in older persons, it remains unclear whether denervation precedes sarcopenia or vice versa (Gonzalez-Freire et al., 2014; Rudolf et al., 2014). Therefore, better understanding the mechanisms underlying NMJ dysfunction is important.

3.5. The muscle contractility regulatory proteins: sTnT

Troponins comprise a family of key regulatory proteins associated with the contractility process of cardiac and skeletal muscle tissues (Chase et al., 2013). In striated muscles, contraction is achieved by activating very complex machinery composed of several proteins and their isoforms, and is regulated by calcium (Ca2+) on the troponin complex composed of troponin C – the calcium-binding subunit, troponin I – the inhibitory subunit, and the tropomyosin-binding subunit troponin T (TnT) including its isoform – skeletal muscle-specific troponin T (sTnT). This complex stands at the center of the contractile machinery and regulates the on/off binding or unbinding of calcium needed for the repetitive cycles of contraction and relaxation (Chase et al., 2013).

Importantly, troponins normally are not found in the blood, except for trace amounts due to normal muscle turnover or muscle damage (Chase et al., 2013). Skeletal muscles are surrounded and protected by several layers of connective tissue, which aid in maintaining intact muscle integrity. If this barrier is insulted, internal components of muscle, notably sTnT, can leak out to the blood. Thus, the presence of sTnT in blood should be interpreted as pathological. Since sarcopenia, cachexia, and some neuromuscular diseases, such as amyotrophic lateral sclerosis, myasthenia gravis, and spinal muscular atrophy display motoneuron damage or death (Dupuis and Echaniz-Laguna, 2010), interventions to enhance the activation and function of the sarcomere may provide benefits regarding skeletal muscle performance and physical function. As such, it is feasible to suggest that physical activity would lead to a reduction in troponin leakage from sarcomere. Indeed, a significant improvement in physical performance, including an increase in grip strength, has been observed after a 10-week strength-training regimen in community-dwelling old individuals. Strikingly, this beneficial effect of training was associated with a ~2-fold reduction in serum levels of sTnT (Abreu et al., 2014), suggesting the use of sTnT as a novel biomarker of muscle health and pathology (sarcopenia).
Fig. 4. Schematic representation of the possible involvement of CAF in NMJ dysfunction. A. Normal, agrin, released from the motoneuron into the synapse, interacts with lipoprotein receptor-related protein 4 (LRP4) that binds, clusters, and activates muscle-specific kinase (MuSK). This event activates acetylcholine receptors (AChRs) in synaptic basal lamina of muscle fiber followed by their clustering and stabilization, thus, providing an appropriate signal inside the muscle fiber resulting in maintenance of muscle fiber diameter stabilization, random distribution of central nuclei, appropriate muscle growth and function (Gonzalez-Freire et al., 2014; Rudolf et al., 2014). B. In pathology/aging, neutriprysin cleaves agrin, thereby releasing soluble C-terminal agrin faragment (CAF) followed by its leakage from the synapse to the circulation. Cleaved agrin is unable to activate ACHR. As a result, ACHR became non-stable, leading to increased heterogeneity of fiber diameter, a higher frequency of centralized nuclei, muscle fiber-type grouping, and type I to type II fiber transition (Bütikofer et al., 2011). Consequently, NMJ became dysfunctional, resulting in reduced muscle growth and function (sarcopenia/cachexia).

In line with the beneficial influence of exercise training on muscle flexibility, balance, and strength is the capability of a recently engineered novel drug, referred to as tirasemtiv or CK-2017357, to increase submaximal force and attenuate its decline in a rat model (Russell et al., 2012). Mechanistically, the drug was shown to directly target the troponin–tropomyosin regulatory complex in fast-twitch skeletal muscle fibers and render the sarcomere more sensitize to calcium. Moreover, in the same study, grip strength was found to be significantly increased after a single dose of tirasemtiv in the compromised animals but not in control rats, suggesting that this intervention is most effective for weakness resulting from impaired motoneuron or neuromuscular function. Indeed, tirasemtiv efficiently amplified skeletal muscle response to nerve activation in humans (Hansen et al., 2014), thus, suggesting it as a promising drug for treatment of sarcopenia.

Table 1: Potential sarcopenia biomarkers.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Suggested role in sarcopenia pathogenesis</th>
<th>Predicted changes according to proposed mechanism</th>
<th>Observed changes in sarcopenia individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myostatin – TGFβ family member, adipomyokine</td>
<td>Negative regulation of muscle growth</td>
<td>Increase</td>
<td>Increase (Yarasheski et al., 2002)</td>
</tr>
<tr>
<td>Activins A and B – TGFβ family members, adipomyokines</td>
<td>––</td>
<td>Increase</td>
<td>No changes (Ratkevicius et al., 2011)</td>
</tr>
<tr>
<td>GDF-15 – TGFβ family member, myokine</td>
<td>––</td>
<td>Increase</td>
<td>No data</td>
</tr>
<tr>
<td>TGFβ – TGFβ family member, adipomyokine</td>
<td>––</td>
<td>Increase</td>
<td>No data</td>
</tr>
<tr>
<td>BMPs – TGFβ family members</td>
<td>Positive regulation of muscle growth</td>
<td>Decrease</td>
<td>No data</td>
</tr>
<tr>
<td>FST – adipomyokine</td>
<td>––</td>
<td>Decrease</td>
<td>No data</td>
</tr>
<tr>
<td>BDNF – TGFβ family member, myokine</td>
<td>––</td>
<td>Decrease</td>
<td>No data</td>
</tr>
<tr>
<td>Irisin – TGFβ family member, adipomyokine</td>
<td>––</td>
<td>Decrease</td>
<td>No changes (Choi et al., 2014)</td>
</tr>
<tr>
<td>CAF – agrin fragment cleaved by neutripryspin</td>
<td>Existence of CAF instead of agrin in NMs leads to muscle dysfunction</td>
<td>Increase</td>
<td>Increase (Hettwer et al., 2013)</td>
</tr>
<tr>
<td>sTnT – component of contractile machinery</td>
<td>Existence of sTnT in sarcomere leads to contractile insufficiency</td>
<td>Increase</td>
<td>Increase (Marzetti et al., 2014)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Increase in men (Drey et al., 2013)</td>
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<td></td>
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<td>No data</td>
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</tbody>
</table>
4. Concluding remarks

Sarcopenia is a major public health problem that is anticipated to grow as our population ages. Unfortunately, we are still at an early stage of understanding the molecular mechanisms leading to sarcopenia. Clearly, deciphering these mechanisms is essential for achieving scientific progress in this area and for exploiting molecular research for the early identification of sarcopenia and for creating the basis for therapeutic interventions for this clinical entity. Based on the latest achievements in this field, as reviewed above, we propose that the prevalence of negative regulators of muscle growth, such as TGFβ, myostatin, activins A and B, and GDF-15 over positive regulators, in particular, BMPs, BDNF, irisin, and FST constitute a main mechanism of sarcopenia pathogenesis. Two additional factors, namely, age-dependent NMJ dysfunction, accompanied by an augmented production of CAF, and contractile insufficiency associated with elevated expression of sTNF, apparently augment the development of sarcopenia. Although there are only scarce data presently available regarding the correlation of circulating levels of these molecules with sarcopenia manifestation and progression (Table 1), their profound involvement in muscle physiology/pathology is probably clearly established and this suggests that creating drugs capable of selectively affecting these molecules/mechanisms would prevent and/or ameliorate sarcopenia.

Obviously, the aforementioned biomarkers represent only a fraction of the wide milieu of molecules potentially involved in sarcopenia pathogenesis. For example, a bioinformatics study indicated that the secretome of human muscle cells includes more than 300 proteins (Bortoluzzi et al., 2006). The transcriptome and proteome studies of human muscle have demonstrated that the expression of many genes and proteins up-regulates in response to physiological/pathological conditions (Haugen et al., 2010; Quinn et al., 2002), apelin (Besse-Patin et al., 2011), caveolin-3 (Hadj Sassi et al., 2012), follistatin-like protein 1 (Boström et al., 2004), caveolin-3 (Hadj Sassi et al., 2012), follistatin-like protein 1 (Boström et al., 2004), adiponectin (Liu and Sweeney, 2014), crosstalk between mTOR and AMPK is required for maintaining the contractile state of muscle fibers (Sakuma et al., 2013). Indeed, from a systems biology viewpoint, the processes underlying sarcopenia are too complex to be captured by a single parameter (Boström et al., 2004). Therefore, it is necessary to exploit recent advances in systems biology, specifically the use of integrative omics approaches (Haugen et al., 2010; Quinn et al., 2002)

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Follistatin induces muscle hypertrophy through satellite cell proliferation and inhibition of both myostatin and activin. J. Appl. Physiol. 105, 584–593.

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