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Mechanisms of sarcopenia in liver cirrhosis and the role of myokines

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Abstract

Sarcopenia is a syndrome characterized by a decline in skeletal muscle quantity and/or quality, strength and performance, leading to unfortunate events, such as injurious falls or even death. It is not identical to frailty and malnutrition, even though there is a significant overlap among these syndromes. In patients with liver cirrhosis (LC), sarcopenia is classified as secondary and has been associated with increased morbidity and mortality during the pre- and post-transplantation period. It can be a result of malnutrition, hyperammonemia, low physical activity, endocrine abnormalities, accelerated starvation, metabolic disturbances, altered gut function leading to chronic inflammation, and alcohol abuse. Myokines are peptides mainly synthesized by contracting muscle and adipose tissue cells and may play a key role in the pathophysiology of sarcopenia. More than a hundred myokines have been recognized, but only a few have been investigated. They can be classified as negative regulators, such as myostatin, tumor growth factor-β, activins, growth differentiation factor-11, and positive regulators of muscle growth including follistatin, bone morphogenic proteins, and irisin. So far, only myostatin, follistatin, irisin and decorin have been studied in LC-associated sarcopenia. In this review, we focused on the mechanisms of cirrhosis-related sarcopenia and the role of myokines that have already been studied in the literature, either as markers helping in the diagnostic evaluation of sarcopenia, or as prognostic factors of survival. Standard therapeutic options to prevent or treat sarcopenia in LC are also being reported, as well as the possible therapeutic implication of myokines.

Keywords Sarcopenia, liver cirrhosis, myokines

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Introduction

The term sarcopenia is derived from Greek "sarx" signifying flesh and "penia" meaning poverty or loss. Irwin Rosenberg coined the term in 1989 [1] to describe an age-related decline in lean body mass (LBM). However, it is currently used to refer to a loss of muscle mass, strength and function of any cause (age, disease, low calorie intake, lack of exercise) not limited entirely to older people [2].

According to the European Society for Clinical Nutrition and Metabolism, sarcopenia is a state of muscle mass, strength and function defacement that carries significant risks for deleterious clinical outcomes [3]. Similarly, the updated criteria of the European Working Group on Sarcopenia in Older People (EWGSOP2), defines sarcopenia as a syndrome of progressive and generalized loss of muscle quantity and/or quality and strength, with high risk of injurious and non-injurious falls, fractures, disability and death [4]. In the latter definition, reduced muscle strength is used to identify sarcopenia as probable [4]. Diminished muscle quantity and/or quality

are used in the diagnostic approach to confirm sarcopenia. According to the EWGSOP definition, substandard physical performance is suggestive of severe sarcopenia [4].

The criteria and methods used to diagnose sarcopenia are heterogeneous and are summarized in Table 1. To date, evaluation of the cross-sectional surface area of abdominal skeletal muscles and myosteatosis by computed tomography (CT) is considered one of the most valid noninvasive methods to determine muscle quantity and quality [4,5]. However, patient's radiation exposure, and the demand for trained personnel and expensive software to interpret the results, render CT a difficult tool in routine clinical practice. Alternative methods that assess the quantity and quality of skeletal muscle, such as dual-energy X-ray absorptiometry (DEXA) [4,5], tetrapolar bioelectrical impedance analysis (BIA), or magnetic resonance imaging [4,5], are usually unavailable in daily practice. Moreover, they are affected by water retention usually present in decompensated cirrhosis, which may limit the validity of measurements. Anthropometric methods such as mid-arm muscle circumference (MAMC) and handgrip strength measurements are simple, rapid to perform at bedside, and not affected by the hyperhydration of cirrhosis. Unfortunately, there is not a gold standard reference method for sarcopenia assessment in cirrhotics. Moreover, because of the water retention, the cutoff values of the aforementioned methods in cirrhotics need to be further validated.

Table 1 Sarcopenia diagnostic tools (EWGSOP2 Table modified [4,5])

Case finding	Strength, assistance with walking, rising from a chair, climbing stairs, and falls (SARC-F questionnaire)
Skeletal muscle strength	Hand grip strength Knee extension Chair stand test Maximum expiratory flow rate
Skeletal muscle quantity and quality*	Cross-sectional area at the 3rd lumbar vertebra level (skeletal muscle index) or Psoas muscle mass (psoas muscle index) 1. Computed tomography or 2. Magnetic resonance imaging 3. Whole body skeletal muscle mass (SMM) by bioelectrical impedance analysis 4. Appendicular SMM by dual-energy X-ray absorptiometry 5. Ultrasound 6. Anthropometric measurements** Mid arm circumference Triceps skin fold Mid arm muscle circumference Mid arm muscle area
Muscle performance	Gait speed Short physical performance battery test Timed up and go test 400-m walk or long-distance corridor walk

*Muscle quality assessment (myosteatosis) can be assessed by computed tomography or magnetic resonance imaging

**Anthropometric measurements are not recommended by EWGSOP2 but are recommended by EASL practice guidelines

Serum peptides (myokines), which are easily obtained and measured, may represent reliable means for sarcopenia screening. A panel of myokines, or a combination with other routine measurements, could form a relatively inexpensive diagnostic tool to detect and stage sarcopenia in liver cirrhosis (LC). Moreover, a deeper knowledge of the pathophysiological mechanisms involved in the mode of action of myokines may help render them future therapeutic targets for sarcopenia treatment.

Sarcopenia in LC

Epidemiology

According to the literature, the prevalence of sarcopenia in patients with LC ranges from 40-68% [6-8]. In 2017, Kim *et al* coordinated a meta-analysis that included 20 studies estimating the prevalence of sarcopenia in patients with LC, and concluded that it came to approximately 48.1% (61.6% in men and 36% in women) [6]. A Japanese retrospective study, conducted in 2015, included 130 patients with LC and showed a 68% rate of sarcopenia [8]. Muscle mass measurement by CT was used to identify patients with sarcopenia. Sarcopenia was more frequent in males with low body mass index (BMI) and alcoholic liver disease, and it was found to be a poor prognostic factor that was significantly associated with mortality [8]. The wide range of prevalence rates reported in the literature may be due to misdiagnosis of sarcopenia. This is a result of the misuse of the diagnostic algorithm for sarcopenia (most of the studies utilize only the presence of low muscle mass for sarcopenia diagnosis), the use of different quantitative and qualitative methods of muscle mass assessment and the composition of the cirrhotic population, as the prevalence of sarcopenia increases with progression of LC [4-7].

Impact of sarcopenia on LC

Sarcopenia is not only an independent factor affecting long-term mortality [9], but also a strong prognostic factor for numerous complications during the pre- and post-liver transplantation period [10]. Sarcopenia's high prognostic value was also highlighted by studies that incorporated skeletal muscle index (SMI) with the model for end-stage liver disease (MELD) score, creating the MELD-sarcopenia model [11]. This model appears more accurate in predicting 1-year survival than MELD score alone, especially in patients with less severe liver disease (MELD <15). A 2016 meta-analysis including several studies of patients awaiting a liver transplant showed that sarcopenic patients had a greater risk of mortality, even after adjustment for MELD score [12]. Hence, sarcopenic patients with low MELD scores should be prioritized for transplantation [13].

Sarcopenia has been associated with a significant increase in post transplantation complications, in particular risk of infection, need for blood transfusion, longer intensive care unit and hospital stay, and prolonged rehabilitation periods [14-16]. It has been acknowledged that sarcopenic patients undergoing

liver transplantation have a lower probability of survival within the first year after transplantation, compared to non-sarcopenic patients [17]. Furthermore, in patients who have already presented with sarcopenia before transplantation, the condition is not reversed after the operation [18,19]. *De novo* perioperative sarcopenia has also been described in subjects who did not present with decreased muscle mass before transplantation, but developed it after the procedure [20].

Sarcopenic patients are vulnerable to infections, and sepsis-related death is the leading fatal complication in this population [21-22]. The pathophysiological mechanisms have not been fully elucidated; however, impaired immunity, long hospitalizations and consequent complications that accompany sarcopenia certainly play an essential role in the development of sepsis. More specifically, patients below the lowest quartile of mid-arm muscle circumference showed a 32% risk of infection, compared to 8% for those above this cutoff [23]. A different cross-sectional study included patients in the post-liver transplantation period, and concluded that the risk of infection was 4 times higher in patients within the lowest quartile of muscle mass compared to those within the highest quartile [24].

The social impact of sarcopenia on patients with LC should not be underestimated. Loss of muscle mass, strength and performance impede their daily routine activities. Fatigue and muscle cramps do not allow people with sarcopenia to work, concentrate, exercise or socialize, and as a result their quality of life deteriorates [25].

Mechanisms that cause sarcopenia in LC

Multiple pathophysiological mechanisms have been implicated in the pathogenesis of sarcopenia. A constant imbalance between muscle synthesis and breakdown in LC is combined with an impaired proliferation and differentiation of myocyte precursor satellite cells [26]. Satellite cells are myogenically derived progenitor cells that help recover and regenerate the adult skeletal muscle cells [27]. The multiple pathophysiological mechanisms implicated in sarcopenia in patients with LC are discussed below.

Malnutrition

Cirrhotics may experience decreased appetite as a result of upregulation of leptin and tumor necrosis factor-α (TNF-α), and early satiety due to increased intra-abdominal pressure from ascites, and impaired gastric and intestinal motility [28]. Heavy alcohol consumption results in poor eating, salt restriction worsens patients' taste for food and long periods of fasting for clinical investigations or complications of LC are the rule. Moreover, patients with alcoholic cirrhosis have a reduced appetite because of ethanol's direct toxic effect on the nervous and gastrointestinal system. All the above contribute to the restriction of calorie intake [29]. In addition, patients with chronic inflammation have reduced synthesis of bile salts and impaired bile flow, leading to intestinal bacterial overgrowth (see below) (Fig. 1). This phenomenon culminates in impaired intestinal motility, nutrient malabsorption, e.g., of fat-soluble vitamins (A, D, E, K), and protein deficiency [30]. Clinicians usually mention all the other complications of decompensated cirrhosis (ascites, gastroesophageal bleeding, hepatic encephalopathy, jaundice), but they often fail to report malnutrition, which may feature in up to 80% of cases in advanced disease [31].

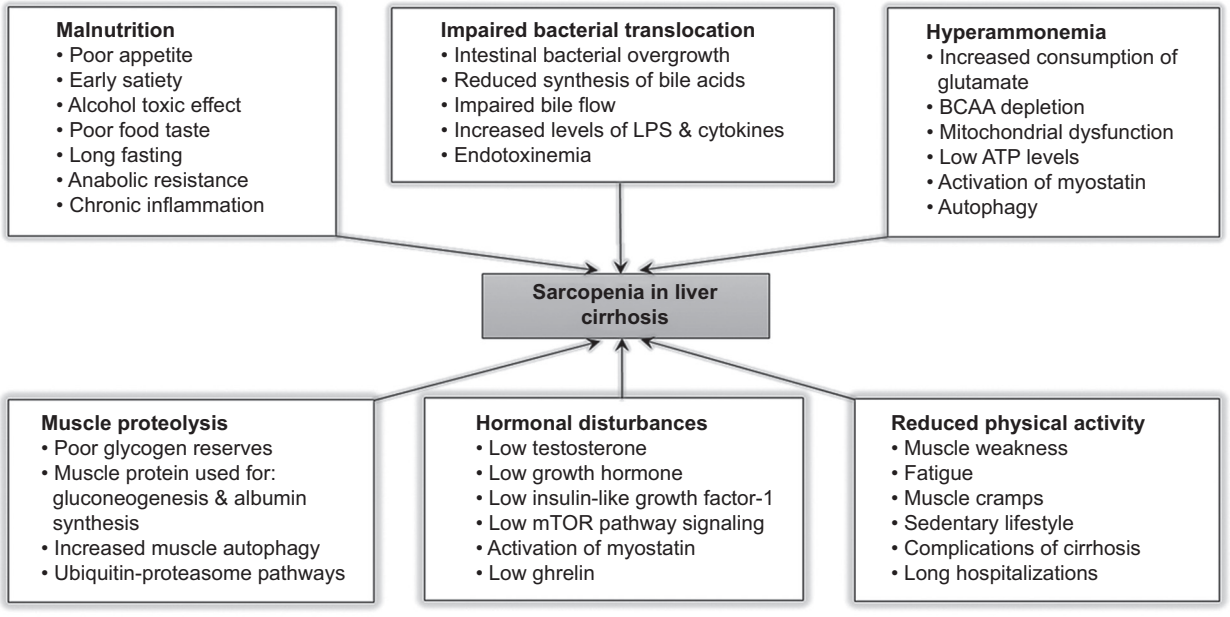

Figure 1 Mechanisms of sarcopenia in liver cirrhosis
LPS, lipopolysaccharides; BCAA, branched-chain amino acids

Metabolic dysfunction

LC is accompanied by a constant catabolic process. This is a state of accelerated starvation, during which the essential amino acids (derived from nutritional sources or proteolysis) required for muscle protein synthesis are diverted to the synthesis of more important proteins, such as albumin, or to gluconeogenesis [32]. People with LC have poor glycogen reserves and even short periods of fasting force the liver to turn to gluconeogenesis for glucose production, using muscle proteins as an amino acid source with subsequent muscle loss [33]. Long and frequent fasting periods, combined with the anabolic resistance usually seen in patients with LC, may evolve to progressive muscle loss (Fig. 1) [32]. The overnight fast in LC is equivalent to a 72-h fast in healthy subjects. Therefore, these patients are advised to consume a late-night snack [33] to compensate for the overnight fasting period.

It has been demonstrated that circulating branched-chain amino acids (BCAAs) are depleted during gluconeogenesis. Moreover, BCAAs are the only amino acids used by skeletal muscles, given the use by muscle of branched-chain keto-dehydrogenase. The depletion of BCAAs further enhances autophagy in skeletal muscles [34]. Autophagy is a normal cellular process to dispose of damaged organelles and abnormal proteins, but in LC uncontrolled autophagy contributes to muscle wasting (Fig. 1) [35].

Another mechanism leading to muscle proteolysis and depletion is the ubiquitin proteasome pathway. However, the role of this pathway in sarcopenia of cirrhosis is obscure. Some investigators found that ubiquitin-mediated proteolysis is decreased or unchanged and others reported increased expression of genes in the proteasome pathway in LC [36]. Furthermore, in the case of alcohol abuse, acetaldehyde, a cytotoxic ethanol metabolite, impairs urea cycle enzymes, such as ornithine transcarbamylase, and induces hyperammonemia even without severe liver disease or portosystemic shunting [32].

Hyperammonemia effect

Research data have indicated that the incidence of muscle depletion was 30%, 49% and 56% in cirrhotic patients with no, minimal or greater hepatic encephalopathy, respectively [37]. Skeletal muscle ammonia levels are significantly increased in cirrhosis, leading to activation of detrimental signaling pathways that contribute to sarcopenia (Fig. 1). More specifically, liver–muscle axis deterioration has been illustrated *in vivo* and myostatin has been suggested as the mediator of this axis [38]. Hyperammonemia increases the expression of myostatin, a known potent inhibitor of skeletal muscle growth, via upregulation of the transcription factor nuclear factor-κB (NF-κB) (Fig. 2) [38]. Ammonia is a cytotoxic molecule and is mainly metabolized in the liver, through the urea cycle, to urea that is then excreted by the kidneys. In LC ammonia levels are elevated, mainly because of hepatocellular dysfunction and portosystemic shunts. Skeletal muscle is an alternate site of ammonia metabolism. Vigorous

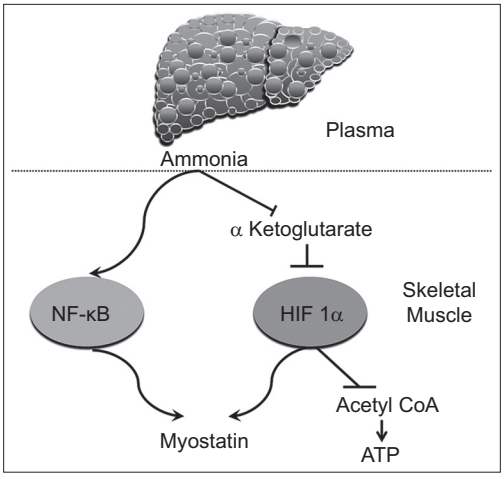

Figure 2 Ammonia upregulates myostatin by a nuclear factor κB (NF-κB)-mediated mechanism and decreases α-ketoglutarate by cataplerosis. Low α-ketoglutarate stabilizes hypoxia-inducible factor 1α (HIF1α), which can subsequently activate myostatin and lower ATP by inhibiting acetyl CoA (modified from Dasarathy *et al* [36])

striated muscles lower serum ammonia levels through the glutamine synthetase reaction, which utilizes ammonia and glutamate to produce glutamine. Hence, ammonia is taken up by the skeletal muscle, which functions as a metabolic "reservoir" converting ammonia to glutamine [39]. However, glutamine is a substrate of ammonia generation by tissues that utilize glutamine, consequently accelerating ammonia production. In addition to the myostatin inhibition in muscle synthesis, high ammonia in the mitochondria is converted to glutamate through "cataplerosis" of α-ketoglutarate and subsequently to glutamine in the skeletal muscles (Fig. 2). The depletion of the α-ketoglutarate substrate stabilizes hypoxia-inducible factor 1α, which activates myostatin, resulting in inhibition of mammalian target of rapamycin complex 1 (mTORC1), and lowers ATP synthesis, leading to further muscle loss [40] (Fig. 2). In other words, hyperammonemia promotes increased consumption of glutamate for glutamine synthesis and activates BCAA catabolism through the BCAA aminotransferase reaction [41]. High rates of BCAA catabolism and depletion have been demonstrated in LC. BCAAs consist of an essential energy source by promoting protein synthesis, thus supporting ammonia detoxification. Decreased BCAA levels result in muscle mass loss and increased ammonia levels. Moreover, increased expression of autophagy markers has been recorded as a response to hyperammonemia *in vivo* and *in vitro*, consistent with impaired muscle proteolysis. Following ammonia withdrawal, the autophagy markers decrease, a finding that may explain the restoration of muscle proteostasis and reversion of sarcopenia [42].

In conclusion, hyperammonemia leads to muscle wasting through overexpression of muscle inhibitors, mitochondrial dysfunction and increased autophagy [43]. Nevertheless, muscle loss further favors ammonia accumulation, which subsequently leads to more muscle wasting. Ammonia is evidently the mediator of a vicious cycle between sarcopenia and hepatic encephalopathy.

Pathological bacterial translocation

Abnormal bacterial translocation is a feature of advanced liver disease. It is attributed to disturbances in gut microbiota and loss of epithelial cell tight junctions [44]. Because of the damaged intestinal barrier and permeability, bacterial products or viable bacteria translocate from the gut lumen into mesenteric lymph nodes and disseminate to the bloodstream, resulting in increased levels of lipopolysaccharides and cytokines such as TNF-α, interleukin (IL)-1, and IL-6) (Fig. 1) [44]. IL-6 induces activation of the JAK kinase, which in turn activates transcription factor STAT3, causing muscle atrophy [45]. IL6 and TNF-α promote NF-κB expression, leading also to muscle wasting [45]. Increased endotoxemia is further enhanced by immune dysfunction, hepatocellular damage and the portosystemic shunting usually present in cirrhosis [46]. Translational studies have also revealed that lipopolysaccharides decreased the myogenic differentiation of myoblasts and increased NF-κB DNA-binding activity and myostatin expression [47]. Anti-TNF-α treatment reversed the impairment of myogenesis [47].

Hormonal disturbances

Insulin and insulin-like growth factor-1 (IGF-1) are potent anabolic factors and promote muscle growth. Insulin is produced by the pancreas, but IGF-1 is synthesized in the liver and its synthesis is regulated by growth hormone (GH) (Fig 1). Testosterone is known to induce protein synthesis; therefore, patients on hormone replacement therapy have increased muscle mass and strength. Both GH and testosterone inhibit myostatin expression and promote IGF-1 and m-TOR activation, thus promoting myocyte synthesis [36]. Cirrhosis is characterized by low serum testosterone, GH and IGF-1 levels. Deficiency of these hormones in cirrhosis results in increased myostatin expression and impaired muscle growth [36,39]. Insulin and IGF-1 activate both the mitogen-activated protein kinase/extracellular signal-regulated kinase (RAS-MAPK-ERK) and the PI3K-AKT-mTOR pathways [45].

Ghrelin, a hormone mainly secreted by entero-endocrine cells in the stomach, is considered an appetite stimulator. Its levels increase before the start of a meal and decrease rapidly afterwards [48]. Ghrelin regulates energy balance, and promotes fat deposition and food intake (Fig. 1). Patients with LC showed lower postprandial ghrelin concentrations after 4 h compared to healthy controls, and the increase in ghrelin concentrations from the minimal post-meal value to 4 h later was negatively correlated with weight loss in the patients with LC [48,49].

Reduced physical activity

Most patients with LC, especially those in the end stage of the disease or on the waiting list for liver transplantation, live a sedentary life. Even if a light exercise program is recommended, they are not compliant because they feel fatigue (Fig. 1). Hence, muscle weakness and the feeling of fatigue limit daily physical activity. Reduced physical activity further worsens muscle loss and increases the episodes of cramps, which are very common in this population. However, those who can adhere to an exercise program show an amelioration of muscle protein synthesis through the stimulation of the mTOR signaling pathway and IGF-1 enhancement [50].

Myokines

Skeletal muscle is not only a motor mechanism, but may also be considered as an endocrine system that secretes a plethora of myokines [51]. Myokines are peptides produced and released by myocytes. They are muscle contraction-regulated molecules, implying that their serum concentrations increase after exercise. In addition, their expression (mRNA levels) increases during skeletal muscle contraction (muscle biopsies) [52]. More than 100 myokines have been recognized [51]. Myokines control muscle metabolism through autocrine mechanisms. The liver, adipose tissue and brain receive paracrine signals through myokine receptors. In addition, myokines play a substantial role in immune regulation, since they may exert anti-inflammatory or immunoprotective effects [53]. They can be classified as positive regulators, including follistatin (FST), bone morphogenic proteins (BMPs) and irisin, and negative regulators of muscle growth differentiation and repair, such as myostatin, transforming growth factor-β (TGF-β), activins and growth differentiation factor (GDF)-11 [32,45,52]. The negative balance between differentiation, proliferation and repair of myocytes on the one hand, and damage of muscle synthesis on the other, may lead to sarcopenia. In the following sections, only myokines that have been studied so far in LC will be discussed. Our comprehension of the pathophysiological and cellular mechanisms implicated in sarcopenia is crucial, since novel treatment strategies related to myokines may halt the progression of muscle wasting.

Myostatin – GDF-8

Mode of action

The myokine that was first recognized was myostatin, a member of the TGF-β family [54]. The myostatin gene is expressed in abundance in myocytes, and it is considered as a negative regulator of muscle protein synthesis [54]. Myostatin may also be labeled an adipo-myokine, as it may also be secreted by human adipocytes [32,52]. The targeted inactivation of myostatin enhanced hypermuscularity in mice [55].

Furin protein convertase cleaves myostatin into two fragments: a biologically active C-terminal (myostatin ligand) and an N-terminal particle (myostatin prodomain) [55]. When these particles are released into the circulation, they are bound to other suppressive proteins (FST) that render them inactive [56]. Metalloproteinases cleave the fragment–protein complex and

thus release the active form of myostatin [57]. The myostatin/activin/GDF11/TGF-β family ligands bind activin RIIB/RIIA/TGF-βRII receptors and activate activin receptor-like kinase (ALK) 4/5/7, which subsequently phosphorylates Smad2/3 molecules and forms a complex with Smad-4 [44] (Fig. 3). This complex in its turn inhibits the IGF-1/PI3K/Akt-mediated mTOR signaling pathway [58], leading to protein breakdown (Fig. 3). In addition, myostatin regulates other signaling pathways and transcription factors such as beta-catenin, stimulation of forkhead box O (FOXO) and 5' adenosine monophosphate-activated protein kinase [59]. Thus, myostatin was thought to be a potent muscle synthesis inhibitor, as indicated by myostatin gene knock-out mice that rapidly increased muscle mass [60]. Moreover, inhibition of myostatin by antibody administration was found to reverse muscle reduction. An increase in muscle strength was demonstrated in experimental animals following the administration of myostatin antibodies [61].

Other molecules related to myostatin action

Activins A and B also belong to the TGF-β family ligands and, through their interaction with ActRII receptors on myocytes, lead to muscle wasting. Studies in mice demonstrated that activins A and B were 100 times more robust in inducing muscle loss as compared with myostatin [62]. Activin-A signaling pathway decreased AKT-mediated mTOR muscle mass and function [45]. Genetic results illustrate that both ActRIIB- and ActRIIA-deficient mice exhibit a hypertrophic phenotype [63]. Maximal anabolic response and inhibition of myostatin/activins A and B is achieved only by simultaneous blockade of both ActRIIA and ActRIIB receptors [63]. These findings have important clinical implications for the production of an antibody that could block both receptors for clinical purposes.

BMPs, which are also TGF-β-related molecules, have an opposite function. They bind BMP type II receptors and induce ALK2/3/6 to phosphorylate and activate Smad 1/5/8. These ligands form a complex with Smad4 and upregulate mTOR, thus upregulating protein synthesis [45] (Fig. 3).

Controversies in myostatin action

Myostatin's role is more complicated than was originally suggested. Paradoxically, Laksman *et al* found that myostatin

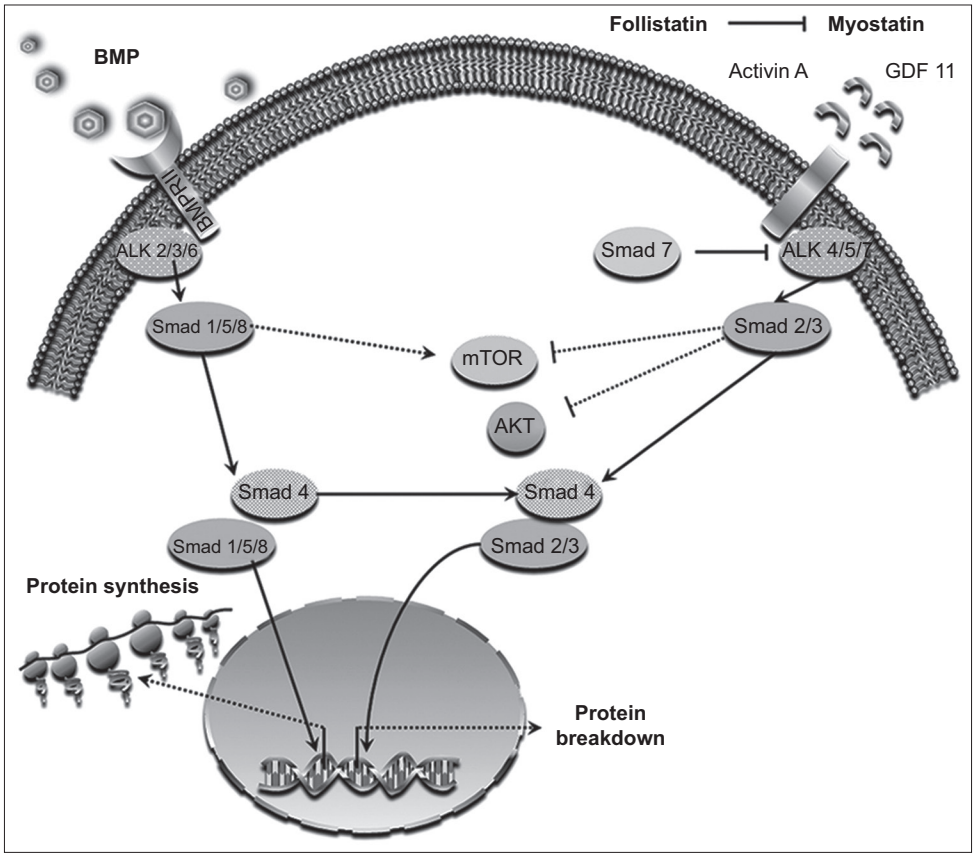

Figure 3 The transforming growth factor-β (TGF-β) superfamily activates 2 different groups of transcription factors with antagonistic function. On the one hand, myostatin (GDF8), activins A/B, TGF-β, and growth differentiation factor-11 (GDF11) bind to type II receptors ActRIIB/IIA, which subsequently activate ALK4/5/7 to form Smad2/3/Smad4 complex leading to protein breakdown. On the other hand, morphogenetic proteins (BMPs) bind BMPRII and ALK2/3/6 receptors and activate Smad 1/5/8 to form a complex with Smad4, leading to protein synthesis. The TGF-β/myostatin/activins ligands are restrained by myokines such as follistatin. Finally, both Smad2/3 and Smad1/5/8 transcription factors regulate mTOR activity by inhibiting or enhancing it, respectively. Dotted lines describe non-well-defined pathways (Sartori *et al* [45] modified)

levels were higher in young as opposed to older men, and were higher in men receiving testosterone [64]. Peng *et al* analyzed serum myostatin in healthy community-living older adults and illustrated that low serum myostatin levels were associated with low skeletal muscle mass in men, but not in women [65]. Adding to that, Arietta *et al* conducted an interventional study that included ambulatory elderly individuals and controls and demonstrated that serum myostatin was elevated in patients with better physical fitness before and after 6 months of physical exercise intervention [66].

Role of myostatin in sarcopenia of LC or portal hypertension

In animal models undergoing portacaval shunts (PCS) (mimicking portosystemic collaterals in humans because of portal hypertension), decreases in body weight, skeletal muscle weight and grip strength were recorded [67]. Skeletal muscle expression of myostatin mRNA and its receptor activin IIB were higher in animals with PCS than in controls. In addition, markers of skeletal muscle protein and markers of satellite cell proliferation and differentiation were diminished, while components of the ubiquitin proteasome pathway that indicated proteolysis were increased [67]. This elegant study proved *in vivo* almost all assumptions about the pathophysiology of sarcopenia in cirrhosis.

Human and murine muscle biopsies and muscle cell cultures showed that myostatin is the main regulator of protein synthesis and muscle reduction, and that hyperammonemia upregulates the transcriptional expression of myostatin [67]. Apart from high ammonia levels, myostatin upregulation in LC is driven by a reduction in GH and testosterone, and the increased production of TNF-α [39].

In human studies, Nishikawa *et al* enrolled 198 patients with LC graded Child-Pugh A, B or C and followed them for 7 years [68] (Table 2). The investigators showed that myostatin levels increased with the severity of LC, and that higher levels were associated with an adverse clinical outcome. They also demonstrated a positive correlation of myostatin with serum ammonia values and a negative correlation with psoas muscle mass cross-sectional area, albumin, prothrombin time and BCAA/tyrosine ratio [68]. The study of Nishikawa *et al* drew important conclusions in humans with LC about the role of myostatin and its association with ammonia. It also illustrated that the severity of liver disease was accompanied by a deteriorating nutritional status.

In another small study of 36 patients waiting for liver transplant evaluation, myostatin levels were elevated compared to the control group [69] (Table 2). Serum myostatin was also used for the prediction of hepatocellular carcinoma (HCC) in 1077 patients with alcoholic cirrhosis who were followed up for a median of 2.5 years [70]. Serum myostatin, age, sex and platelet count were independent predictors of HCC development, and high myostatin levels were associated with a higher cumulative probability of HCC at 5 years [70].

In a very recent study, 262 patients with LC (Child-Pugh A, B and C) were included [71] (Table 2). Myostatin, FST and irisin concentrations were measured at baseline. According to the results, low BMI, high myostatin and low irisin levels were independent predictors of sarcopenia. Myostatin serum levels were better associated with the severity of liver disease compared to irisin levels. Myostatin levels were predictive of 4-year mortality, regardless of the presence of sarcopenia, mainly in the advanced stages of LC [71].

Previous research by our group evaluated myostatin as a biomarker of sarcopenia in 115 patients with LC (71.3% decompensated) and found that myostatin levels are associated with the presence of sarcopenia or low skeletal mass index after adjusting for multiple confounding factors [72] (Table 2). To diagnose sarcopenia using myostatin without muscle mass measurement, we found that myostatin concentrations in combination with serum creatine phosphokinase or albumin showed good performance in excluding sarcopenia in patients with cirrhosis. The best diagnostic performance of the biomarker was seen in advanced stages of LC with MELD score ≥15 [72].

Choi *et al* investigated sarcopenic patients with HCC and found a positive correlation between serum myostatin levels and psoas muscle index, while there was no difference in the overall survival rate between the high and the low myostatin level group [73] (Table 2). The discrepancy between this finding and the results of Nishikawa *et al* [68] or Boga *et al* [71] might be attributable to differences between the study populations, as Choi *et al* included only patients with HCC. It is possible that cancer development may have changed the regulatory pathway of myostatin [74].

FST

Mode of action

FST is a glycoprotein that is bound to myostatin's precursor peptide, making myostatin often undetectable [75]. There are 3 isoforms: FST 288, FST315 and FST300. FST antagonizes the inhibitory effect of myostatin, activins and GDF-11 [45,75,76]. (Fig. 3). FST interacts with 3 different molecular pathways; Smad3, AKT and the recently recognized molecular crosstalk between Wnt/-catenin pathway [45]. FST suppresses Smad3 but also reinforces AKT phosphorylation/mTOR signaling, resulting in muscle mass increase [77]. In patients who exercised for a certain period and managed to increase their muscle strength and performance, a marked increase in serum FST was noted [78]. When an engineered FST molecule (FST315-HBS-Fc) was administered in normal mice, a substantial increase in body weight and skeletal muscle mass was observed [79].

FST is capable of reversing myostatin-induced sarcopenia. In the previously mentioned *in vivo* study in animal models undergoing PCS, administration of FST to the animals resulted in decreased expression of myostatin, and therefore an increase in muscle protein synthesis, and markers of satellite cell function contributed to the reversal of sarcopenia [67].

Table 2 Studies of the role of myokines in patients with chronic liver disease

Myokine	No of patients	Disease status	Follow up	Results	Study [ref.]
Myostatin (MS)	198	Liver cirrhosis Child-Pugh A, B, C	7 years	High MS levels were associated with low survival Positive correlation with ammonia levels Inverse correlation with skeletal muscle mass, albumin, prothrombin time and BCAA/tyrosine ratio	Nishikawa *et al* [68]
	36	End stage liver disease	No	Elevated MS levels in patients compared to controls	Garcia *et al* [69]
	1077	Alcoholic cirrhosis	5 years	Independent predictor of HCC development High risk of 5-year HCC in those with high MS levels	Kim *et al* [70]
	262	Liver cirrhosis Child-Pugh A, B, C	4 years	MS was a good diagnostic marker of sarcopenia Positive association with severity of liver disease Good predictor of 4-year mortality in Child-Pugh B & C	Boga *et al* [71]
	115	Liver cirrhosis Child-Pugh A, B, C	No	Positive correlation of MS levels and SMI Good diagnostic marker for the diagnosis of sarcopenia (in combination with other parameters)	Alexopoulos *et al* [72]
	238	HCC	3 years	Positive correlation of MS levels and SMI No difference in survival between high and low MS group	Choi *et al* [73]
Follistatin (FST)	238	HCC	3 years	Negative correlation of FST levels and SMI Independent factor of poor survival	Choi *et al* [73]
	12	TIPS	No	Positive correlation with SMI and fat free muscle area	Praktiknjo *et al* [80]
	8	Liver cirrhosis	No	Infusion of glucagon and somatostatin increased FST levels	Rinnov *et al* [81]
	262	Liver cirrhosis Child-Pugh A-C	4 years	No association with sarcopenia or severity of liver cirrhosis	Boga *et al* [71]
Irisin	41	Nonalcoholic fatty liver disease	No	Positive correlation with markers of fibrosis Irisin levels higher in advanced fibrosis	Armandi *et al* [89]
	187	Liver cirrhosis	No	Positive correlation between irisin levels and SMI irisin was one of the biomarkers independently associated with sarcopenia	Zhao *et al* [90]
	262	Liver cirrhosis Child-Pugh A, B, C	4 years	Irisin levels lower in sarcopenic vs nonsarcopenic Irisin levels predicted presence of sarcopenia A good predictor of 4-year mortality in Child-Pugh A	Boga *et al* [71]
	88	Decompensated liver cirrhosis	No	No correlation with sarcopenia No correlation with severity of liver disease	Kukla *et al* [91]
Decorin	39	Liver cirrhosis	No	High levels in patients with normal compared to those with low SMI	Bekki *et al* [84]

SMI, skeletal mass index; BCAA, branched-chain amino acid; HCC, hepatocellular carcinoma; TIPS, transjugular intrahepatic portosystemic shunt

Role of FST in sarcopenia of LC or portal hypertension

In humans, FST was assessed in end-stage LC patients who underwent transjugular intrahepatic portosystemic shunt (TIPS). Sarcopenia was assessed by measurement of the fat-free muscle area (FFMA) before the TIPS placement. FST was calculated in only 12 patients and displayed a good correlation with both transversal psoas muscle thickness and FFMA [80] (Table 2). Infusion of glucagon and somatostatin mimicking the exercise effect in 8 LC patients resulted in an increase in FST levels, however, these rapidly decreased in cirrhotic individuals compared to healthy controls [81] (Table 2).

There are also conflicting results regarding the role of FST in sarcopenia in patients with LC. In the above-mentioned study by Boga *et al*, which studied the levels of different myokines in LC, FST levels at rest were similar in sarcopenic and non-sarcopenic patients with LC, and differed neither between patients and controls, nor between the early and late stages of LC [71] (Table 2).

Decorin

A small leucine-rich proteoglycan known as decorin is an important component of extracellular matrix. It stabilizes collagen and blocks TGF-β signaling, while exerting tumor suppression properties by inhibiting tyrosine kinase [82]. It has been demonstrated that decorin inhibits myostatin and promotes muscle hypertrophy [83]. However, its role as a sarcopenia biomarker has not yet been established.

In a study of 39 patients with LC [84], serum decorin levels were significantly associated with skeletal muscle mass and were found to be higher in those with normal compared to those with low skeletal muscle index, as assessed by CT (Table 2). Decorin and BMI were demonstrated to be independent negative risk factors for low SMI in multivariate analysis [84]. No other studies exploring the role of decorin in patients with LC have been published so far.

Irisin

Mode of action

Muscle cells generate irisin in response to exercise and its structure is very similar to that of FST. Not only muscle tissue, but also adipocytes produce irisin. Irisin is therefore included in the list of adipomyokines [85].

Irisin counteracts myostatin's actions through activation of PI3K/AKT/mTOR and RAS/MAPK/ERK pathways [32,45]. Moreover, it suppresses adipogenesis and cholesterol synthesis, while increases insulin sensitivity and lowers atherosclerosis burden [86]. Irisin levels were found to be positively associated with FST at both the mRNA and circulating protein level [87].

Role of irisin in liver fibrosis

It has been shown *in vitro* that irisin has antifibrotic effects in the liver [88]. In particular, in a study where recombinant irisin was used to treat human immortalized hepatic stellate cells (HSC)—which play a crucial role in the progression of hepatic fibrosis—suppression of the expression of fibrosis markers was observed, leading to inhibition of the HSC functions [88]. Contrary to the previous study, irisin levels were positively correlated with markers of fibrosis, such as N terminal type III collagen propeptide and type VI collagen cleavage product, in a study of non-obese, non-diabetic humans with nonalcoholic fatty liver disease (NAFLD) [89]. In the same study, irisin levels were higher in more advanced stages of fibrosis—a finding suggesting that irisin is upregulated in high fibrosis states [89].

Role of irisin in sarcopenia of LC

In other human studies, researchers tried to examine the associations between serum irisin concentrations and sarcopenia in patients with LC. Zhao *et al* conducted an observational cross-sectional study that included 187 patients with cirrhosis, and found lower irisin levels in sarcopenic compared to non-sarcopenic patients with cirrhosis [90] (Table 2). In addition, a positive correlation between irisin levels and SMI assessed with CT was identified. Moreover, irisin was one of the biomarkers that were independently associated with sarcopenia [90]. Boga *et al* also demonstrated that irisin levels, along with myostatin and BMI, predicted sarcopenia after adjustment for multiple factors in 262 patients with LC, with or without sarcopenia [71] (Table 2). Irisin levels were lower in sarcopenic than in non-sarcopenic patients with LC, and lower in patients with LC compared to healthy controls [71]. Contrary to the previous studies, Kukla *et al* evaluated serum irisin concentrations in patients with LC and found no significant difference in the levels of irisin in patients with or without ascites, or between early or late stages of LC. Furthermore, they found no association between irisin levels and muscle mass, as assessed through anthropometry or CT [91].

Therapeutic interventions for preventing and treating sarcopenia

Given the multifactorial nature of sarcopenia in LC, nutritional, pharmacological, and physical interventions may be required to prevent or reverse it. Targeting the underlying liver disease is the first therapeutic option. The administration of antivirals for chronic viral hepatitis-related LC and the abstinence from alcohol in alcoholic cirrhosis are recommended, as progression of liver disease may worsen malnutrition and further deteriorate sarcopenia.

A high calorie and protein diet (at least 35 kcal/kg/day and 1.2-1.5 g/kg/day, respectively), according to latest guidelines from the European Association for the Study of the Liver [5], and frequent, small meals are encouraged. However, adequate calorie and protein intake is difficult to achieve in malnourished

sarcopenic patients with advanced liver disease; therefore, enteral or parenteral feeding has been discussed, with no documented improvement in survival [5]. Improved nitrogen balance and decreased lipid oxidation is achieved by taking breakfast and a late evening snack [5,33]. Vegetable is preferred to animal protein. Furthermore, cirrhotic patients with a history of hepatic encephalopathy benefit from ammonia-lowering measures, such as lactulose and rifaximin. Prolonged supplementation of BCAAs and leucine-enriched supplements (0.20-0.25 g/kg/day) [5] contribute to both energy intake and protein synthesis, as well as to ammonia lowering. However, no published study has shown prevention or reversal of sarcopenia using BCAAs. Nutritional supplements, along with behavioral interventions, are a prerequisite for appropriate therapy. Regarding physical activity, moderate intensity resistance and endurance exercise, whenever possible, can be beneficial in cirrhosis [5]. Serial assessments of sarcopenia by the most appropriate and available tool are necessary. Whenever CT is available, for example from HCC screening, its use is recommended serially for the re-evaluation of sarcopenia. Anthropometric measurements, DEXA and BIA are alternative means, if available [5]. Specifically, handgrip strength testing is a simple, effective and inexpensive method that can be used once or serially to detect sarcopenia [5].

Future perspectives on drugs for treating sarcopenia

Given that blocking myostatin or interacting proteins induced muscle mass gain in animal models, investigators have tested myostatin inhibitors in people with genetic muscular disorders, or in elderly persons after a major debilitating event. In particular, administration of the myostatin inhibitor domagrozumab to 120 ambulatory boys with Duchenne muscular dystrophy (DMS) significantly increased lean body mass versus placebo in the appendicular skeleton at week 49 [92]. Moreover, in another study evaluating functional skills in persons with DMS with and without domagrozumab administration, it was shown that domagrozumab-treated patients showed better functionality compared to placebo-treated patients [93].

The administration of myostatin antibody (Ly2495655) landogrozumab, which binds to and neutralizes myostatin, in a single intravenous dose of 3 mg/kg or 30 mg/kg in a study of older adults [94] resulted in increases in thigh muscle volume and in lean body mass compared to placebo. The beneficial results were maintained for a longer period after administration of the high dose [94]. In addition, total fat body index decreased from baseline. Likewise, an amelioration in gait speed, stair climbing, and chair rise tests were demonstrated in another study when the same drug was administered to patients older than 75 years who had had a recent fall, and showed low muscle strength and power [95].

The administration of ACE-083, a locally acting, FST-based fusion protein, to adults with Charcot-Marie-Tooth disease resulted in an increase in total muscle and contractile muscle volume, although no functional improvement was observed [96]. Similar results were reported when the same drug was administered in facioscapulohumeral muscular dystrophy [97].

The administration of the monoclonal antibody bimagrumab, which inhibits ActIIA/ActIIB receptors and myostatin, demonstrated a beneficial effect on 6-min walking performance in elderly adults, according to a meta-analysis [98]. In another study, therapeutic intervention with bimagrumab 70, 210, 700 mg or placebo improved recovery in 250 elderly persons who had undergone internal fixation or hemiarthroplasty for a recent femoral fracture [99]. A significant increase in lean body mass from baseline compared to placebo administration was found in the arm receiving a high dose of bimagrumab, but not in the low-dose arm [99].

Unfortunately, none of the aforementioned studies have included patients with LC. Therefore, future research should also explore whether such agents could be a safe and effective method of preventing and or treating sarcopenia in LC.

Concluding remarks

Sarcopenia usually accompanies advanced liver disease, it is rarely reversible and it is associated with adverse outcomes and mortality during the pre- and post-liver transplantation period. The underlying pathogenetic mechanisms are complex, and include undernutrition, anabolic resistance in LC, metabolic disturbances leading to a starvation-like state, hyperammonemia, impaired bacterial translocation and its consequences, physical inactivity, hormonal changes and chronic inflammation. Myokines are mainly produced by muscle cells and are regulators of muscle synthesis and degradation. Myostatin is the most thoroughly studied myokine in LC and is mainly associated with sarcopenia. Other myokines, such as FST or irisin, are associated with muscle homeostasis (proteostasis) pathways, but also with fibrogenesis and glucose homeostasis. A panel of myokines closely associated with sarcopenia and not affected by other parameters could be a relatively inexpensive diagnostic tool for the early and accurate detection of accelerating muscle wasting in LC. Pharmaceutical agents based on the knowledge of the action of myokines are under investigation in patients with several genetic muscle disorders and the elderly. These agents may also allow researchers and clinicians to provide targeted preventive and therapeutic strategies for sarcopenia in patients with LC.

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