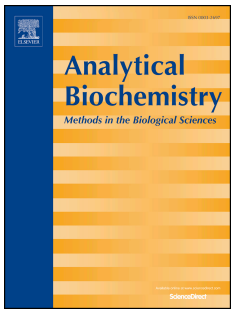
Journal Pre-proof



Sarcopenia in liver cirrhosis: Prevalence, pathophysiology and therapeutic strategies

Rebecca Fox, Kate Stenning, Adrian Slee, Jane Macnaughtan, Nathan Davies

PII: S0003-2697(22)00037-9

DOI: https://doi.org/10.1016/j.ab.2022.114581

Reference: YABIO 114581

To appear in: *Analytical Biochemistry*

Received Date: 19 July 2021

Accepted Date: 31 January 2022

Please cite this article as: R. Fox, K. Stenning, A. Slee, J. Macnaughtan, N. Davies, Sarcopenia in liver cirrhosis: Prevalence, pathophysiology and therapeutic strategies, *Analytical Biochemistry* (2022), doi: https://doi.org/10.1016/j.ab.2022.114581.

Sarcopenia in Liver Cirrhosis: Prevalence, Pathophysiology and Therapeutic Strategies

Rebecca Fox [1,3],

Kate Stenning [1,] ,

Adrian Slee [1],

Jane Macnaughtan[2]

*Nathan Davies [2]

[1] UCL Division of Medicine, University College London, London, UK.

[2]UCL Institute for Liver and Digestive Health, University College London, UK.

[3] School of Medicine, Trinity College Dublin, University of Dublin, Dublin, Ireland.

foxre@tcd.ie

kate.stenning.18@alumni.ac.uk

a.slee@ucl.ac.uk

j.macnaughtan@ucl.ac.uk

Nathan.davies@ucl.ac.uk

*Author for correspondence

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions: Conceptualization, R.F, J.M, A.S; methodology, R.F, J.M, A.S data curation, R.F.; writing—original draft preparation, R.F, K.S.; writing—review and editing, J.M, A.S, N.D.

All authors have read and agreed to the final version of the manuscript.

Abstract:

Sarcopenia, characterized by a loss of muscle strength, quantity/quality, and physical performance is associated with increased mortality and poor clinical outcomes in concomitant presentation with liver cirrhosis (LC). A number of mechanisms are involved in sarcopenia development in LC, many of which are secondary to liver dysfunction and/or iatrogenic involvement in treating LC. Sarcopenia severity in this population appears to be affected by patient gender, as well as the primary aetiology of LC (alcohol, non-alcoholic fatty liver disease etc.) with patient demographics shifting in recent years. Clinical detection of sarcopenia in this population may involve a combination of assessment tools, in addition to measuring muscle mass and strength separately. Muscle mass may be assessed using radiography, bioelectric impedance, ultrasound, or anthropometrics. Hand-grip strength, on the other hand, may be a useful tool for evaluating muscle strength. The role of malnutrition in sarcopenia is also a relevant factor, and screening tools such as MELD and SARC-F may be clinically useful tools for more complete diagnosis of sarcopenia in these patients. Myostatin and titin-N may represent potential diagnostic biomarkers. Lastly, physical activity and nutrition remain key elements of treatment. Further research is being conducted regarding the role of resistance vs aerobic exercise as well as the function of complementary nutrition. Continued study into the role of nutrition, physical activity and other complementary therapies will be important future endeavours in the treatment of sarcopenia in LC.

Keywords: liver cirrhosis; sarcopenia; nutrition; anthropometry

1. Introduction

Liver cirrhosis (LC) is one of the most common causes of morbidity and premature mortality in adults worldwide (*1, 2*). Increasing prevalence is observed, in particular, due to a rise alcohol consumption and obesity. Conventional predictors of mortality have focused on markers of liver synthetic function such as the model for end-stage liver disease (MELD) score. More recent data demonstrates that sarcopenia, defined as a decrease in muscle strength, quantity/quality, and physical performance (*3*) has been found to be an independent prognostic determinant in LC, highlighting it's key role in pathogenesis. Sarcopenia has also been found to be associated with increased risk of post-liver transplant infection and prolonged mechanical ventilation. Sarcopenia is a common finding in cirrhosis affecting 65-100% of patients with advanced liver disease. Of these patients, approximately 20% of compensated, 80% of decompensated, and up to 100% of liver transplant candidates are sarcopenic (*4, 5*). Furthermore, sarcopenia occurs irrespective of LC aetiology (*6*). However, there is likely an effect of gender due to differences in adipose and muscle tissue between males and females (*7*).

Prevalence of sarcopenia increases with progressive disease although diagnostic testing is of variable nature and sensitivity. Multiple mechanisms for sarcopenia in LC include: malnutrition, altered skeletal muscle proteostasis, gut microbiome dysbiosis, and systemic inflammation (*8-10*) (Figure 1). However, the interplay between these factors remains unclear.

This review will assess key pathophysiologic elements underlying sarcopenia in LC, and examine current diagnostic procedures in order to further establish specific criteria for sarcopenia in LC, focusing on potential therapeutic options.

2. Methodology

A PubMed search was conducted from inception to April 2021 including the following search terms: 'sarcopenia', 'malnutrition', 'liver', 'cirrhosis', and 'body composition'. A manual selection of articles further detailing aetiology, diagnosis and epidemiology of sarcopenia in cirrhosis was performed.

3. Results

3.1.0 Sarcopenia Pathophysiology in Liver Cirrhosis

Multiple factors contribute to the development of sarcopenia in LC. These include inadequate oral intake, early post-prandial satiety in the context of abdominal ascites, disordered GI transit and a heightened catabolic state particularly in reference to nitrogen metabolism. Furthermore, ingestion of alcohol with a direct toxic effect on muscle is an important contributor.

3.1.1. *Altered Proteostasis*

Skeletal muscle is maintained through a balance of anabolic and catabolic signals, though these signals become disrupted in sarcopenia (*11*). Predominant hepato-muscular disruptors include: hyperammonaemia, lipopolysaccharide-induced systemic inflammation, myostatin, reduced anabolic hormones, reduced amino acid availability and mitochondrial dysfunction (Figure 1)

3.1.2 *Gluconeogenesis*

Reduced hepatic glycogen storage is a key feature of LC. This lack of available glycolytic reserve precipitates an early onset of fasting-type metabolism; resulting in earlier gluconeogenesis (*12*). Amino acid and lipid oxidation are therefore increased, demonstrated by decreased respiratory quotients in these patients (*13*). However, nocturnal nutritional supplementation with carbohydrate offsets this early metabolic shift, mitigating muscle and adipose losses in cirrhosis patients (*14, 15*). Previously, high protein intake in LC was believed to promote HE (*16, 17*). However, this theory has been replaced by more recent evidence suggesting the importance of amino acid provision to alleviate muscle catabolism (*15, 18*). The European Society for Enteral and Parenteral Nutrition (ESPEN) guidelines follow this, and suggest a greater energy intake (35-40kcal/kg/d) and amino acid provision (1.2–1.5 g kg^{-1} d^{-1}), while carbohydrate should make up 50% - 60% of total caloric intake and the remainder comprised of fat (*15*).

3.1.3 *Hormone Status*

Several factors regulate muscle protein turnover (synthesis and breakdown) including testosterone, and Growth Hormone (GH), which stimulates hepatic insulin-like growth factor-1 (IGF-1) production. However, diminished production and action of these hormones are demonstrated in LC due to reduced hepatic function and altered hypothalamic-pituitary axis function (*19-21*).

IGF-1 is mainly produced in the liver, along with respective binding proteins and is secreted in response to pituitary GH signals. IGF-1 reductions have been correlated with cirrhosis severity according to Child-Pugh, and MELD scores (*22, 23*). Specifically, IGF-1 upregulates the protein-synthetic mTOR pathway while simultaneously inhibiting myostatin (*24*). Improved muscle mass has been demonstrated following exogenous IGF-1 administration in rats (*25*). Furthermore, one human clinical trial administering synthetic IGF-1 found improved mitochondrial function and increased serum albumin in LC patients. However, these effects were more pronounced in patients who had better baseline nutrition, higher hormone bioavailability, and those with alcohol related LC (*26*)

Low testosterone levels have been demonstrated to be an independent predictor of mortality in LC (*27, 28*). Male LC patients have been shown to have particularly low testosterone levels. This is due to a combination of alterations in the hypothalamic-pituitary gonadal axis, steroid hormone binding globulin (SHBG) production and increased peripheral androgen aromatization (*29-31*). Low testosterone levels lead to reduced activation of the mTOR Protein kinase B (PCK/AKT) pathway, thus altering muscle proteostasis (*35*). There is increased SHBG production in LC, thus potentially limiting testosterone bioactivity by keeping it in its bound, rather than free form (*32*).It has also been shown that testosterone supplementation therapy appears to increase muscle mass in male LC patients (*34*). Further, low testosterone levels correlate with increased aromatase concentrations, potentially mediating sarcopenia progression through reduced anabolic activity in LC patients (*27, 36*).

3.1.4 Hyperammonaemia& Hepatic Encephalopathy (HE)

Ammonia is a cytotoxic by-product of amino acid breakdown and bacterial metabolism; requiring intrahepatic conversion to urea for excretion (*37*). Reduced hepatic urea synthesis due to LC may create excess circulating ammonia (*38*). Furthermore, it has been shown that hyperammonaemia is caused by portal-systemic shunting of portal-drained viscera (PDV) (namely, the colon) and renal-ammoniagenesis (*39*). PDV ammonia production is largely a function of glutamine metabolism. Further, the cirrhotic liver cannot maintain adequate ammonia detoxification in this setting. Skeletal muscle has been shown to remove greater amounts of ammonia from circulation to compensate this. However, as a result, muscle releases glutamine as a nitrogen carrier following ammonia metabolism, which can contribute to ammonia-genesis in both renal and PDV- thus mitigating the effort (*39*). Figure 2). In addition, there is increasing evidence to suggesting a link between sarcopenia and HE (*40-43*). This is likely related to a combination of alterations in ammonia handling capacity of muscle associated

with reduced functional mass (*37*, *44*, *45*). In particular, muscle removes ammonia via the enzyme glutaminase; converting ammonia to glutamine. Thus, if there is reduced muscle mass, there is reduced functional enzyme capacity to handle ammonia efficiently.

Recent interest has been placed on the probable deleterious effects of ammonia on mitochondrial function and skeletal muscle proteostasis. First, extra-hepatic glutamine production from ammonia requires a reaction with a-ketoglutarate, using branched chain amino acids (BCAA) as substrate, particularly leucine. However, this cataplerosis results in reduced ATP synthesis as some a -ketoglutarate is used in this reaction rather than for its primary function in the citric acid cycle (CAC) (*46*, *47*). Furthermore, energy depletion through inner mitochondrial electron transport chain leakage has also been described in hyperammonaemia (*47*). Mentioned by Dasarathy & Hatzglou, this provides a mechanistic basis for the anabolic resistant, bioenergetically dysfunctional phenotype seen in cirrhosis (*48*). Therefore, utilizing this relationship between hyperammonaemia and cataplerosis, replacing CAC intermediates may aid in reversing mitochondrial damage and therefore maintaining skeletal muscle mass (*47*, *48*).

Reduced serum ratio of BCAA to aromatic amino acids is a consequence of peripheral ammonia detoxification (*46*). Serum BCAA levels are therefore decreased due to a probable combination of increased leucine requirements for glutamate formation in addition to increased branched chain alpha-ketoacid dehydrogenase expression– activated by inflammatory cytokines and cortisol (*49*, *50*). As BCAAs are rate-limiting components of muscle protein synthesis, inadequate concentrations of BCAA's may be a further pathogenic contributor in sarcopenia. Results of two studies have shown positive effects on muscle mass with BCAA supplementation in combination with exercise (*51*, *52*), Furthermore, leucine supplementation may be particularly useful, showing functional benefit (*53*).

Ethanol, a common pathogenic factor in cirrhosis, plays a role in sarcopenia. Ethanol exposure has shown proportional reductions in hepatic ureagenesis, while also increasing intramuscular ammonia concentrations in mice (*54*). Further, elevated ethanol and ammonia synergistically reduce muscle mass by impairing mTORC1 signalling and increasing autophagy in skeletal muscle (*54*). Furthermore, when tissues were treated with ammonia in ethanol-naïve tissues, perturbations in muscle protein synthesis were reduced. This suggests variation in cirrhosis-sarcopenia pathology based on aetiology.

3.1.5 Myostatin

Myostatin is a growth and differentiation factor involved in negatively regulating satellite cell differentiation through mTORC1 activation (*55*). Cirrhotic patients have elevated serum and intracellular myostatin concentrations compared to those with normal hepatic function (*38*, *53*, *56*). This elevation likely contributes to sarcopenia progression in cirrhosis, as myostatin inhibition is linked to improved muscle strength and function (*57*). A murine model has demonstrated that excess ammonia was also associated with increased myostatin production through elevated nuclear translocation of the p65NFkB transcription factor (*9*). Testosterone and IGF-1 are involved in myostatin suppression in healthy individuals, therefore their deficiency in cirrhosis may be a factor in elevating serum myostatin in these patients (*58*). Thus, myostatin inhibition may be a promising future treatment in this condition.

3.1.6 Systemic Inflammation and the Liver-Gut Axis

Systemic inflammation, resulting in inflammatory cytokine presence may further promote sarcopenia. Interest has been placed on the role of the liver-gut axis, emphasizing the gut microbiome and intestinal barrier dysfunction. Increased gut permeability resulting in bacterial translocation represents one of the mechanisms by which inflammatory cytokines including tumour necrosis factor alpha (TNF-a), interleukin 6 (IL-6), and interleukin 1 beta (IL-1B) become increased in cirrhosis (*59*). These pro-inflammatory cytokines are associated with increased muscle wasting through mechanisms including

IGF-1 and insulin suppression, thereby increasing FoxO activation and decreasing Akt function. In addition, pro-inflammatory cytokines increase calpain, E3 ligase and Nf-kB expression- thus altering protein metabolism (*60, 61*).

Further, bile acid metabolism is altered in cirrhosis arising from such inflammation. This may incur disproportionate growth of inflammatory intestinal bacteria, as well as reduce nutrient absorption, particularly fat-soluble vitamins such as vitamin D (*62, 63*). Furthermore, in advanced liver cirrhosis, a number of important hepatic immune mechanisms may be altered, such as the phagocytic reticuloendothelial system of Kupffer cells- thus potentially contributing further to endotoxemia (*64, 65*). These mechanisms appear to encourage a cascade of decompensation in LC, further exacerbating sarcopenia progression through autophagy pathway activation (*66, 67*). However, small intestinal bacterial overgrowth (SIBO) may mediate endotoxemia and/or bacterial peritonitis in LC (*59*). SIBO may result from reduced bile acid synthesis, altered mucosal immunity, and impaired gastrointestinal motility caused by vagal dysfunction (*68*).

3.1.7 Transjugular Intrahepatic Portosystemic Shunt (TIPS)

Portal hypertension presents in approximately 60% of LC patients (*42*). Body composition improvements in LC have been observed after TIPS procedures aimed at alleviating portal hypertension. This procedure is recommended by the European Association for Study of the Liver (EASL) for patients who have refractory ascites (*69*). In one study, 21 patients showed increased total body cell mass evaluated by total body potassium and weight gain after TIPS procedures (*70*). Similar lean body mass increases were shown in two other studies evaluating TIPS - demonstrating potential efficacy in mitigating sarcopenia progression (*71, 72*). Possible sarcopenia reversal mechanisms may be related to reduced intestinal permeability by reducing portal hypertension, therefore limiting inflammatory cytokine release (*64, 70*). These inflammatory cytokines, namely IL-1a, IL-6, and TNF-a may promote anti-insulin effects and induce catabolism on skeletal muscle (*73*). Conversely, greater likelihood of HE was shown in individuals who received TIPS with(*74*) pre-existing sarcopenia as diagnosed using L3 CT (*42*). This may reveal the negative contribution of sarcopenia to the patient's overall metabolic muscle reserve, representing the importance of pre-procedure body composition assessment.

3.1.8 Serum 25-OH-Vitamin D

The liver plays a critical role in endogenous vitamin D metabolism, acting as the site of hydroxylation to form 25-OH-Vitamin D, which is used as a biomarker for an individual's vitamin D status (*75, 76*). Vitamin D deficiency is more common in individuals with chronic liver disease compared to healthy individuals(*74*), and is associated with increased mortality(*77, 78*). Recent studies have shown potential links between low serum vitamin D and sarcopenia in LC. It has been demonstrated that as vitamin D status declines, rates of frailty and sarcopenia increase proportionally; with severe vitamin D deficiency independently associated with functional frailty and sarcopenia in chronic liver disease (*79*). The mechanism behind vitamin D and sarcopenia is not yet clear. However, it is well known that vitamin D receptors are present throughout skeletal muscle. In murine models, vitamin D receptor knockout mice have shown reduced muscle mass and strength, progressing with further vitamin D deficiency(*80*). In addition, sarcopenia is associated with reduced size and number of type 2 muscle fibres-associated with higher force of contraction and bulk compared to type 1 muscle fibres (*81*). Muscle biopsy assessments in vitamin D deficient patients have shown predominantly type 2 muscle fibre atrophy(*82*). These results altogether give emerging evidence of the potential effect of low vitamin D status on sarcopenia development.

3.2 Aetiologic Differences

Alcohol remains the most common cause of LC in Europe (*83*). However, NASH, viral hepatitis, and biliary duct disease are all epidemiologically important contributors to cirrhosis and concomitant sarcopenia. It has been demonstrated that rates of NASH-related cirrhosis have doubled in the past two decades, with non-alcoholic fatty liver disease (NAFLD) being one of the most common causes of chronic liver disease in the United States (*84*). Higher mortality rates occur in cirrhosis patients with sarcopenic obesity and myosteatosis than in non-obese phenotypes (*85*).

Myosteatosis is the pathological accumulation of adipose in skeletal muscle (*86*). The prevalence of this condition ranges from 30% to upwards of 50% of LC patients, with older age and worse liver function being predictive features (*44, 85, 87*). This condition has become an increasingly important prognostic indicator in patients with LC. While not synonymous with sarcopenia, myosteatosis may act synergistically in muscle functional deficits (*88*) Myosteatosis and sarcopenia have been shown to be independent risk factors for developing complications such as HE (*44*), as well as being prognostic factors for overall survival(*85*) (*89*).

Further, NASH is considered a hepatic manifestation of metabolic syndrome (*90, 91*), and concomitant excess visceral fat is associated with proinflammatory cytokine secretion, and reduced IGF-1 release (*92*). These factors may therefore be a combined force in promoting skeletal muscle catabolism. Thus, perhaps stratifying LC patients based on primary aetiology may be useful in assessing sarcopenia. In addition, there appears to be a gender effect in sarcopenia. One study indicated that poorer outcomes were found for men with sarcopenia, while worse female outcomes were related to greater losses of low subcutaneous adipose tissue rather than skeletal muscle mass (*7*). It should be noted that in this particular study, there were proportionally more men than women enrolled (67% male). This result may highlight key physiologic differences between men and women in this disease context. Therefore, when possible, future studies should stratify sample groups based on this parameter.

3.3 Sarcopenia Diagnosis

3.3.1 Muscle Mass

Body composition assessment is crucial in accurately diagnosing the sarcopenic patient. Multiple methodologies exist for this assessment, though the validity of each depends on the technology itself, as well as the complex set of comorbidities in the cirrhotic patient.

Computed tomography (CT) and Magnetic resonance imaging (MRI) are currently the gold-standard for evaluating body composition in LC patients (*3, 93*). Sarcopenic status can be derived from adipose and lean tissue quantities measured from axial L3 spinal level images, translating into a skeletal muscle index (*93, 94*). In addition, myosteatosis may also be characterized using CT and MRI (*95*). Alternately, psoas-major muscle cross-sectional area has been used to effectively determine sarcopenia in LC. This is probably due to its role as a postural muscle not subject to disuse atrophy in the ambulatory patient. Furthermore, retroperitoneal muscle is not subject to distortion in the presence of ascites (*96, 97*). While CT and MRI have high internal and external validity, their cost as well as CT ionizing radiation risk limits their use in body composition assessment to secondary, opportunistic tests in the shadow of a primary investigation (*6*).

Another gold standard body composition technique is dual - energy x - ray absorptiometry (DXA). However, DXA is affected by changes in patient fluid status and so may not be the best test for sarcopenia in decompensated LC with ascites or other fluid disturbances (*6*).

Bioelectric impedance analysis (BIA) is convenient and therefore commonly used for assessing body composition both clinically and in research settings. It utilizes differing electrical resistance between adipose (high impedance) and lean tissue and fluid (lower impedance) compartments. Therefore, excess-fluid related conditions such as oedema and ascites, common in decompensated LC, reduce the test's sensitivity. Nevertheless, the inclusion of the phase angle (PA) principle when calculating BIA

may mitigate this issue (*98*). The PA is a ratio of BIA reactance (Xc) to resistance (R), from which relative nutritional status, muscle mass and cellular health can be determined (*99-102*). Furthermore, the BIA PA has been shown to be predictive of mortality in cirrhosis patients (*103*). Thus, as BIA can be performed at the bedside, it may be a useful diagnostic tool for sarcopenia when incorporating the PA parameter. However, validation in this population is necessary.

Ultrasound has been recently implemented as clinical diagnostic tool for sarcopenia (*3*). It has been validated against gold-standard techniques such as CT and has proven valid for body composition assessment in patients with cardiac and respiratory illnesses (*104, 105*). The technique has also proven useful in one study evaluating thigh muscle cross-sectional area in LC (*6*), though its validation requires further study.

Anthropometric assessments including skinfold measurements and BMI may allow accurate sarcopenia diagnosis in those with compensated cirrhosis, but may be less useful in those with more advanced disease stages (*106*). In out-patient settings for those awaiting liver transplant, mid-upper arm circumference (MUAC) was associated with sarcopenia, presenting in 5.36% of patients (*106*). Within this cohort (n=261), HE and ascites were present in 11.88% and 8.81% of patients, respectively, while mean MELD score was 9.82. However, this study demonstrated much lower proportions of sarcopenia compared to other studies with similar demographics (*106*). On the other hand, a combined approach using anthropometry (BMI, kg/m^2) and ultrasound-measured thigh muscle thickness was highly correlated with CT results (*6*). Therefore, accurate assessment of sarcopenia may best be achieved through a combination of anthropometric and imaging techniques.

3.3.2 Muscle Strength

Hand-grip strength is a convenient measure of muscle strength in sarcopenia (*3*). This measure is also a useful overall strength indicator in LC (*107*). Similarly, hand-grip strength may detect loss of strength in the context of concomitant myosteatosis where fat infiltration into muscle may confound MUAC measures by increasing apparent muscle volume (*108*). However, the technique requires active patient participation which may be difficult in HE (*109*)

3.3.4 Malnutrition and Functional Assessment Tools

Assessment tools are widely used to evaluate nutritional status and may require validation for sarcopenia in LC patients specifically. For example, the subjective global assessment (SGA) is regularly used to assess cirrhotic patient's nutritional status. However, it has been demonstrated that SGA is unable to strongly predict sarcopenia presence (*110*). Conversely, the Royal Free Hospital Global Assessment (RFH-GA) is recognized in the UK as the gold-standard nutrition assessment tool for liver disease patients (*111*). This tool has been validated for accurate detection of malnutrition in this patient subset, and includes triceps skinfold thickness, MUAC, BMI, and dietary intake information (*111*). Furthermore, it may predict post liver transplant infection and length of ICU stay more effectively than CT-measured psoas muscle area alone (*96*).

Conversely, the SARC-F questionnaire may be a practical tool for sarcopenia case -finding (*3*). SARC-F is based on patients' self-perceptions of functional performance ability for various tasks such as climbing stairs and walking (*3*). On the other hand, the Ishii screening test is also designated as a validated sarcopenia screening tool, incorporating patient age, grip strength and calf-circumference (*112*). However, in decompensated LC, lower limb oedema may impede accurate calf circumference measurement.

3.3.5 Biomarker Testing

Currently, there is no decisive biomarker for sarcopenia diagnosis. However, as sarcopenia is a multifactorial disease, particularly in the setting of LC, it may, in fact, be that a combination of biomarkers may be used in conjunction with current imaging and anthropometric practices to both

diagnose sarcopenia and measure treatment efficacy. For example, serum myostatin may be a useful indicator of muscle deterioration in LC. Results by Nishikawa et al. indicate serial myostatin measurements may be useful to measure patient responses to therapeutic interventions(*9*).

Titin is an abundant protein found in striated muscle. This protein is degraded by various proteolytic enzymes such as calpain following muscle damage. N-terminal fragments of titin (titin-N)_are detectable in urine, and have been shown to be a biomarker reflecting muscle catabolism in Non-Alcoholic Fatty Liver Disease (*113*). Thus, titin-N may be a potentially useful, non-invasive biomarker in sarcopenia in LC where muscle proteolytic mechanisms are similarly at play.

3.4 Treatment Approaches

A number of potential therapeutic strategies have been evaluated for sarcopenia in LC, based on mechanisms mentioned above.

3.4.1 Exercise & Physical Activity

Generally speaking, aerobic exercise improves functional muscle capacity, while resistance exercise is beneficial for improving skeletal muscle mass(*114*). A number of studies have been performed assessing muscle mass and strength in response to exercise in LC patients (*115-120*), however, only half of these studies have found significant muscle improvements (*115-117*). Further, only one study has shown functional improvements following a 12 week body-weight resistance exercise programme (*121*). Currently, there is inconclusive evidence regarding optimal post-exercise nutritional supplementation to maintain potential skeletal muscle improvements. Thus, while long-term data in LC patients is still required, it has been shown that a moderate-intensity exercise regimen of both aerobic and resistance activities may be the most beneficial for sarcopenic LC patients.

3.4.2 Nutritional Intake

In terms of optimal nutritional intake for sarcopenic LC patients, maintaining adequate energy and protein intakes are key. According to EASL guidelines, patients should consume at least 35-40kcal/kg body weight/d, while protein intake should fall between 1.2-1.5g/kg bodyweight/d(*15, 122*). These recommendations are to ensure adequate maintenance and prevent loss of muscle mass. However, it may not be feasible for some patients to achieve this level of nutrition due to LC side effects such as nausea, reduced GI motility etc.

Due to the evidence suggesting potential benefit of BCAA supplementation along with adequate protein and energy intake, The European Association for the Study of the Liver (EASL) clinical practice guidelines have more recently included recommendations for long-term oral BCAA supplementation (0.25g/kg/d) for advanced cirrhosis patients for improvement of event-free survival or quality of life (*122*).

3.4.3 Other Therapies

Selective androgen receptor modulators (SARMs) use has increased, with drugs such as Enobosarm, producing testosterone-like anabolic effects in patients with cachexia (*123*). Further, both transdermal and intramuscular testosterone infusions have been shown to safely improve muscle mass and strength in two clinical trials in men with compensated cirrhosis (*34, 124*), potentially indicating direct testosterone use in sarcopenia reversal. However, further research is required to establish its role as a sarcopenia treatment in LC, particularly as exogenous administration of testosterone is associated with side effects such increased risk of hepatocellular carcinoma (*125*)

Growth hormone (GH) may also be a promising sarcopenia therapy, showing reduced GH resistance and increased lean body mass. Improved GH resistance may be beneficial particularly with downstream effects of improving IGF-1 status (*126*). However, there is minimal long-term evidence available describing the long-term impacts of GH administration in LC.

By reducing portal hypertension and increasing intestinal motility (*64*), beta-blockers have been demonstrated to reduce infection risk in LC likely by reducing SIBO and risk of bacterial peritonitis by reducing portal hypertension (*127, 128*). Thus, this may be a clinically relevant therapy for limiting the effects of decompensation, and therefore sarcopenia. Beta-blockers have also been tested in heart failure patients for cachexia, showing partial symptom reversal (*129*). This may demonstrate the role of sympathetic activity on muscle loss, and may therefore be similarly useful for sarcopenia treatment.

4. Conclusions

Sarcopenia in LC arises due to multiple factors related both directly and indirectly to hepatic dysfunction. Malnutrition, altered proteostasis, systemic inflammation and iatrogenic factors are likely the most important elements in this disease. With such complex interrelationships, discrete biomarker identification may become increasingly important. Validation of body composition assessment techniques, and better-defined diagnostic thresholds in this patient population are warranted. Lastly, further study into the role of physical activity and complementary nutrition may be useful for limiting sarcopenia progression in this patient population. These refinements will improve sarcopenia diagnosis in LC and improve patient outcomes.

Figure 1. Mechanistic Overview of Sarcopenia in Liver Cirrhosis. Adapted from Dasarathy et. al 2012. Poor nutritional intake limits available substrate required for muscle protein synthesis. Bacterial endotoxin, portal hypertension (HTN), and impaired metabolite clearance all contribute to systemic inflammation. Circulating inflammatory cytokines (IL1, IL-6, TNF-a) potentially play a role in muscle catabolism by ubiquitin proteasome (UPP) and autophagy pathway upregulation. Hyperammonaemia, and reduced testosterone may upregulate myostatin production, preventing proper skeletal muscle growth. Metabolic changes including reduced hepatic glycogen storage leads to upregulated gluconeogenesis, and therefore increased skeletal muscle amino acid catabolism. Reduced growth hormone (GH) secretion leads to reduced insulin-like growth factor-1 (IGF-1), leading to reduced muscle protein synthesis. Lastly, hyperammonaemia is converted in skeletal muscle to glutamine using branched-chain amino acids (BCAA), therefore reducing anabolic substrate for muscle. Illustration of gastrointestinal tracts by Unknown Author is licensed under CC BY-SA.

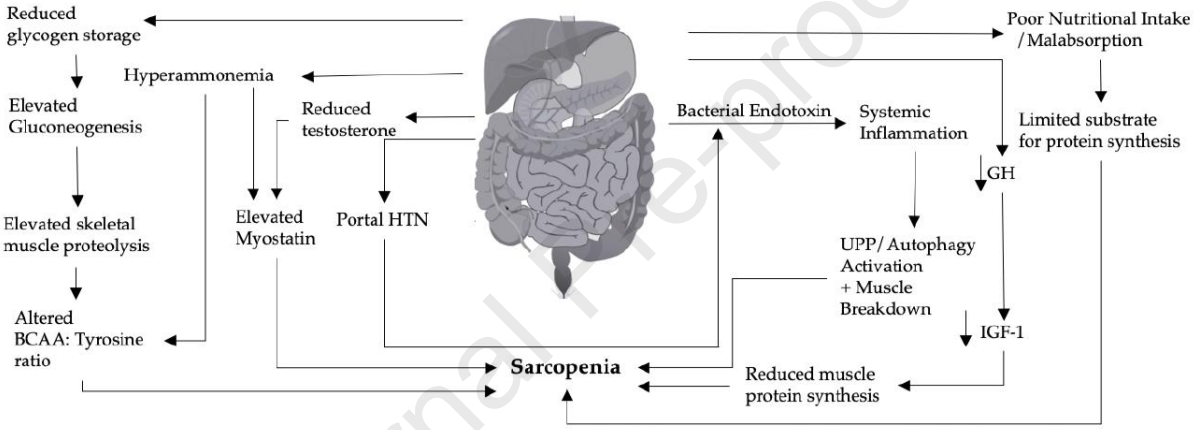
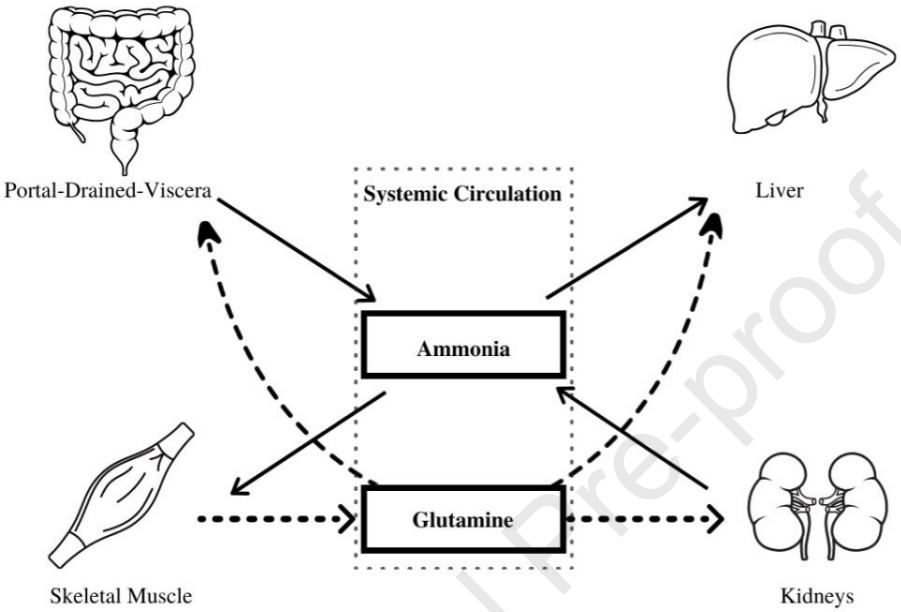


Figure 2. Relationship of increased ammonia production from renal and portal-drained-viscera with increased glutamine production from skeletal muscle.



References

1. A. A. Mokdad *et al.*, Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis. *BMC medicine* **12**, 145 (2014).

2. R. Williams *et al.*, Addressing liver disease in the UK: a blueprint for attaining excellence in health care and reducing premature mortality from lifestyle issues of excess consumption of alcohol, obesity, and viral hepatitis. *The Lancet* **384**, 1953-1997 (2014).

3. A. J. Cruz-Jentoft *et al.*, Sarcopenia: revised European consensus on definition and diagnosis. *Age and ageing* **48**, 16-31 (2019).

4. P. Tandon *et al.*, Severe muscle depletion in patients on the liver transplant wait list: its prevalence and independent prognostic value. *Liver Transplantation* **18**, 1209-1216 (2012).

5. A. J. Montano-Loza *et al.*, Severe muscle depletion predicts postoperative length of stay but is not associated with survival after liver transplantation. *Liver Transplantation* **20**, 640-648 (2014).

6. P. Tandon *et al.*, A Model to Identify Sarcopenia in Patients With Cirrhosis. *Clinical Gastroenterology and Hepatology* **14**, 1473-1480.e1473 (2016).

7. M. Ebadi *et al.*, Low subcutaneous adiposity associates with higher mortality in female patients with cirrhosis. *Journal of hepatology* **69**, 608-616 (2018).

8. H.-S. Lim, H.-C. Kim, Y.-H. Park, S.-K. Kim, Evaluation of malnutrition risk after liver transplantation using the nutritional screening tools. *Clinical nutrition research* **4**, 242-249 (2015).

9. H. Nishikawa, Y. Osaki, Liver Cirrhosis: Evaluation, Nutritional Status, and Prognosis. *Mediators Inflamm* **2015**, 872152 (2015).

10. A. Cárdenas *et al.*, Renal failure after upper gastrointestinal bleeding in cirrhosis: incidence, clinical course, predictive factors, and short-term prognosis. *Hepatology* **34**, 671-676 (2001).

11. P. Periyalwar, S. Dasarathy, Malnutrition in cirrhosis: contribution and consequences of sarcopenia on metabolic and clinical responses. *Clinics in liver disease* **16**, 95-131 (2012).

12. O. Owen *et al.*, Nature and quantity of fuels consumed in patients with alcoholic cirrhosis. *The Journal of clinical investigation* **72**, 1821-1832 (1983).

13. C. Glass *et al.*, Sarcopenia and a physiologically low respiratory quotient in cirrhotic patients: a prospective controlled study. *American Journal of Physiology-Heart and Circulatory Physiology*, (2013).

14. L. D. Plank *et al.*, Nocturnal nutritional supplementation improves total body protein status of patients with liver cirrhosis: a randomized 12-month trial. *Hepatology* **48**, 557-566 (2008).

15. ESPEN, ESPEN Guidelines on Parenteral Nutrition: Hepatology. *Clinical Nutrition* **28**, 436 - 444 (2009).

16. S. M. Riordan, R. Williams, Treatment of hepatic encephalopathy. *New England Journal of Medicine* **337**, 473-479 (1997).

17. K. D. Mullen, F. L. Weber, in *Seminars in liver disease*. (© 1991 by Thieme Medical Publishers, Inc., 1991), vol. 11, pp. 292-304.

18. J. Córdoba *et al.*, Normal protein diet for episodic hepatic encephalopathy: results of a randomized study. *Journal of hepatology* **41**, 38-43 (2004).

19. N. Assy *et al.*, Growth hormone-stimulated IGF-1 generation in cirrhosis reflects hepatocellular dysfunction. *Journal of hepatology* **49**, 34-42 (2008).

20. A. Adamek, A. Kasprzak, Insulin-Like Growth Factor (IGF) System in Liver Diseases. *Int J Mol Sci* **19**, (2018).

21. D. J. Handelsman, S. Strasser, J. A. McDonald, A. J. Conway, G. W. McCaughan, Hypothalamic-pituitary-testicular function in end-stage non-alcoholic liver disease before and after liver transplantation. *Clinical endocrinology* **43**, 331-337 (1995).

22. A. Khoshnood *et al.*, A survey of correlation between insulin-like growth factor-I (igf-I) levels and severity of liver cirrhosis. *Hepatitis monthly* **13**, (2013).

23. B. Castro *et al.*, Insulin-like growth factor I correlates with MELD and returns to normal level after liver transplantation. *Ann Transplant* **18**, 57-62 (2013).

24. R. A. Frost, C. H. Lang, Multifaceted role of insulin-like growth factors and mammalian target of rapamycin in skeletal muscle. *Endocrinology and Metabolism Clinics* **41**, 297-322 (2012).

25. I. Castilla-Cortazar *et al.*, Impaired intestinal sugar transport in cirrhotic rats: correction by low doses of insulin-like growth factor I. *Gastroenterology* **113**, 1180-1187 (1997).

26. R. Pérez *et al.*, Mitochondrial protection by low doses of insulin-like growth factor-Iin experimental cirrhosis. *World journal of gastroenterology: WJG* **14**, 2731 (2008).

27. M. Sinclair *et al.*, Low testosterone as a better predictor of mortality than sarcopenia in men with advanced liver disease. *Journal of gastroenterology and hepatology* **31**, 661-667 (2016).

28. M. Sinclair *et al.*, Low serum testosterone is associated with adverse outcome in men with cirrhosis independent of the model for end-stage liver disease score. *Liver Transplantation* **22**, 1482-1490 (2016).

29. C. Gluud, Serum testosterone concentrations in men with alcoholic cirrhosis: background for variation. *Metabolism* **36**, 373-378 (1987).

30. D. Van Thiel, S. Kumar, J. Gavaler, R. Tarter, Effect of liver transplantation on the hypothalamic-pituitary-gonadal axis of chronic alcoholic men with advanced liver disease. *Alcoholism: Clinical and Experimental Research* **14**, 478-481 (1990).

31. G. G. GORDON, J. OLIVO, F. RAFII, A. L. SOUTHREN, Conversion of Androgens to Estrogens in Cirrhosis of the Liver1. *The Journal of Clinical Endocrinology & Metabolism* **40**, 1018-1026 (1975).

32. Y. Maruyama *et al.*, Mechanism of feminization in male patients with non-alcoholic liver cirrhosis: role of sex hormone-binding globulin. *Gastroenterol Jpn* **26**, 435-439 (1991).

33. C. Moctezuma-Velázquez *et al.*, Association between low testosterone levels and sarcopenia in cirrhosis: a cross-sectional study. *Annals of hepatology* **17**, 615-623 (2018).

34. M. Sinclair, M. Grossmann, R. Hoermann, P. W. Angus, P. J. Gow, Testosterone therapy increases muscle mass in men with cirrhosis and low testosterone: a randomised controlled trial. *Journal of hepatology* **65**, 906-913 (2016).

35. S.-H. I. R. S. Lee, Testosterone-induced muscle hypertrophy is associated with an increase in satellite cell number in healthy, young men. *American Journal of Physiology. Endocrinology and Metabolism* **285**, E197-E205 (2003).

36. W. Liu *et al.*, Myostatin is a skeletal muscle target of growth hormone anabolic action. *The Journal of Clinical Endocrinology & Metabolism* **88**, 5490-5496 (2003).

37. S. W. O. Damink, R. Jalan, C. H. Dejong, Interorgan ammonia trafficking in liver disease. *Metabolic brain disease* **24**, 169-181 (2009).

38. J. Qiu *et al.*, Hyperammonemia in cirrhosis induces transcriptional regulation of myostatin by an NF-κB–mediated mechanism. *Proceedings of the National Academy of Sciences* **110**, 18162 (2013).

39. S. W. O. Damink *et al.*, Interorgan ammonia and amino acid metabolism in metabolically stable patients with cirrhosis and a TIPSS. *Hepatology* **36**, 1163-1171 (2002).

40. T. Hanai *et al.*, Sarcopenia predicts minimal hepatic encephalopathy in patients with liver cirrhosis. *Hepatology Research* **47**, 1359-1367 (2017).

41. M. Merli *et al.*, Muscle depletion increases the risk of overt and minimal hepatic encephalopathy: results of a prospective study. *Metabolic Brain Disease* **28**, 281-284 (2013).

42. S. Nardelli *et al.*, Sarcopenia Is Risk Factor for Development of Hepatic Encephalopathy After Transjugular Intrahepatic Portosystemic Shunt Placement. *Clin Gastroenterol Hepatol* **15**, 934-936 (2017).

43. L. Augusti *et al.*, Lower values of handgrip strength and adductor pollicis muscle thickness are associated with hepatic encephalopathy manifestations in cirrhotic patients. *Metabolic Brain Disease* **31**, 909-915 (2016).

44. S. Nardelli *et al.*, Muscle alterations are associated with minimal and overt hepatic encephalopathy in patients with liver cirrhosis. *Hepatology* **70**, 1704-1713 (2019).

45. O. P. Ganda, N. B. Ruderman, Muscle nitrogen metabolism in chronic hepatic insufficiency. *Metabolism* **25**, 427-435 (1976).

46. M. Holecek, M. Vodenicarovova, Muscle wasting and branched-chain amino acid, alpha-ketoglutarate, and ATP depletion in a rat model of liver cirrhosis. *Int J Exp Pathol* **99**, 274-281 (2018).

47. G. Davuluri *et al.*, Hyperammonaemia-induced skeletal muscle mitochondrial dysfunction results in cataplerosis and oxidative stress. *The Journal of physiology* **594**, 7341-7360 (2016).

48. S. Dasarathy, M. Hatzoglou, Hyperammonemia and proteostasis in cirrhosis. *Current opinion in clinical nutrition and metabolic care* **21**, 30-36 (2018).

49. M. D. Nawabi, K. P. Block, M. C. Chakrabarti, M. Buse, Administration of endotoxin, tumor necrosis factor, or interleukin 1 to rats activates skeletal muscle branched-chain alpha-keto acid dehydrogenase. *The Journal of clinical investigation* **85**, 256-263 (1990).

50. M. Holeček, Leucine metabolism in fasted and tumor necrosis factor-treated rats. *Clinical nutrition* **15**, 91-93 (1996).

51. A. Hiraoka *et al.*, Efficacy of branched-chain amino acid supplementation and walking exercise for preventing sarcopenia in patients with liver cirrhosis. *Eur J Gastroenterol Hepatol* **29**, 1416-1423 (2017).

52. E. Roman *et al.*, Randomized pilot study: effects of an exercise programme and leucine supplementation in patients with cirrhosis. *Dig Dis Sci* **59**, 1966-1975 (2014).

53. C. Tsien *et al.*, Metabolic and molecular responses to leucine-enriched branched chain amino acid supplementation in the skeletal muscle of alcoholic cirrhosis. *Hepatology* **61**, 2018-2029 (2015).

54. S. Kant *et al.*, Ethanol sensitizes skeletal muscle to ammonia-induced molecular perturbations. *J Biol Chem*, (2019).

55. B. Elliott, D. Renshaw, S. Getting, R. Mackenzie, The central role of myostatin in skeletal muscle and whole body homeostasis. *Acta Physiologica* **205**, 324-340 (2012).

56. P. S. García, A. Cabbabe, R. Kambadur, G. Nicholas, M. Csete, Elevated myostatin levels in patients with liver disease: a potential contributor to skeletal muscle wasting. *Anesthesia & Analgesia* **111**, 707-709 (2010).

57. V. Siriett *et al.*, Antagonism of myostatin enhances muscle regeneration during sarcopenia. *Molecular Therapy* **15**, 1463-1470 (2007).

58. R. J. Urban *et al.*, Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis. *American Journal of Physiology-Endocrinology And Metabolism* **269**, E820-E826 (1995).

59. T. M. Bauer *et al.*, Small intestinal bacterial overgrowth in human cirrhosis is associated with systemic endotoxemia. *The American journal of gastroenterology* **97**, 2364 (2002).

60. B. Sharma, R. Dabur, Role of Pro-inflammatory Cytokines in Regulation of Skeletal Muscle Metabolism: A Systematic Review. *Curr Med Chem* **27**, 2161-2188 (2020).

61. P. Londhe, D. C. Guttridge, Inflammation induced loss of skeletal muscle. *Bone* **80**, 131-142 (2015).

62. J. M. Ridlon, J. M. Alves, P. B. Hylemon, J. S. Bajaj, Cirrhosis, bile acids and gut microbiota. *Gut Microbes* **4**, 382-387 (2013).

63. M. Garcia-Alvarez *et al.*, Relationship of vitamin D status with advanced liver fibrosis and response to hepatitis C virus therapy: a meta-analysis. *Hepatology* **60**, 1541-1550 (2014).

64. T. Reiberger *et al.*, Non-selective betablocker therapy decreases intestinal permeability and serum levels of LBP and IL-6 in patients with cirrhosis. *Journal of Hepatology* **58**, 911-921 (2013).

65. A. Rimola *et al.*, Reticuloendothelial system phagocytic activity in cirrhosis and its relation to bacterial infections and prognosis. *Hepatology* **4**, 53-58 (1984).

66. C. H. Lang, R. A. Frost, A. C. Nairn, D. A. MacLean, T. C. Vary, TNF-α impairs heart and skeletal muscle protein synthesis by altering translation initiation. *American Journal of Physiology-Endocrinology and Metabolism* **282**, E336-E347 (2002).

67. C. W. Keller *et al.*, TNF-α induces macroautophagy and regulates MHC class II expression in human skeletal muscle cells. *Journal of Biological Chemistry* **286**, 3970-3980 (2011).

68. M. T. Hendrickse, P. J. Thuluvath, D. R. Triger, Natural history of autonomic neuropathy in chronic liver disease. *The Lancet* **339**, 1462-1464 (1992).

69. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. *Journal of Hepatology* **69**, 406-460 (2018).

70. M. Plauth *et al.*, Weight gain after transjugular intrahepatic portosystemic shunt is associated with improvement in body composition in malnourished patients with cirrhosis and hypermetabolism. *Journal of Hepatology* **40**, 228-233 (2004).

71. J. Montomoli *et al.*, Body composition changes after transjugular intrahepatic portosystemic shunt in patients with cirrhosis. *World J Gastroenterol* **16**, 348-353 (2010).

72. C. Tsien, S. N. Shah, A. J. McCullough, S. Dasarathy, Reversal of sarcopenia predicts survival after a transjugular intrahepatic portosystemic stent. *Eur J Gastroenterol Hepatol* **25**, 85-93 (2013).

73. M.-A. Aller, J.-L. Arias, A. Cruz, J. Arias, Inflammation: a way to understanding the evolution of portal hypertension. *Theoretical Biology and Medical Modelling* **4**, 44 (2007).

74. C. Konstantakis, P. Tselekouni, M. Kalafateli, C. Triantos, Vitamin D deficiency in patients with liver cirrhosis. *Annals of Gastroenterology: Quarterly Publication of the Hellenic Society of Gastroenterology* **29**, 297 (2016).

75. D. D. Bikle, Vitamin D metabolism, mechanism of action, and clinical applications. *Chemistry & biology* **21**, 319-329 (2014).

76. J. G. Haddad, T. Stamp, Circulating 25-hydroxyvitamin D in man. *The American journal of medicine* **57**, 57-62 (1974).

77. A. R. Buonomo *et al.*, Severe vitamin D deficiency increases mortality among patients with liver cirrhosis regardless of the presence of HCC. *in vivo* **33**, 177-182 (2019).

78. R. Paternostro *et al.*, Low 25-OH-vitamin D levels reflect hepatic dysfunction and are associated with mortality in patients with liver cirrhosis. *Wiener Klinische Wochenschrift* **129**, 8-15 (2017).

79. C. Saeki *et al.*, Low Serum 25-Hydroxyvitamin D Levels Are Related to Frailty and Sarcopenia in Patients with Chronic Liver Disease. *Nutrients* **12**, 3810 (2020).

80. C. M. Girgis *et al.*, Vitamin D receptor ablation and vitamin D deficiency result in reduced grip strength, altered muscle fibers, and increased myostatin in mice. *Calcified tissue international* **97**, 602-610 (2015).

81. J. Talbot, L. Maves, Skeletal muscle fiber type: using insights from muscle developmental biology to dissect targets for susceptibility and resistance to muscle disease. *Wiley Interdisciplinary Reviews: Developmental Biology* **5**, 518-534 (2016).

82. L. Ceglia, S. S. Harris, Vitamin D and its role in skeletal muscle. *Calcified tissue international* **92**, 151-162 (2013).

83. G. Vernon, A. Baranova, Z. Younossi, Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Alimentary pharmacology & therapeutics* **34**, 274-285 (2011).

84. M. N. Kabbany *et al.*, Prevalence of nonalcoholic steatohepatitis-associated cirrhosis in the United States: an analysis of national health and nutrition examination survey data. *The American journal of gastroenterology* **112**, 581 (2017).

85. A. J. Montano-Loza *et al.*, Sarcopenic obesity and myosteatosis are associated with higher mortality in patients with cirrhosis. *Journal of cachexia, sarcopenia and muscle* **7**, 126-135 (2016).

86. I. Miljkovic, J. M. Zmuda, Epidemiology of myosteatosis. *Current opinion in clinical nutrition and metabolic care* **13**, 260 (2010).

87. D. Gallagher *et al.*, Adipose tissue in muscle: a novel depot similar in size to visceral adipose tissue. *The American journal of clinical nutrition* **81**, 903-910 (2005).

88. R. Correa-de-Araujo *et al.*, Myosteatosis in the context of skeletal muscle function deficit: an interdisciplinary workshop at the national institute on aging. *Frontiers in Physiology* **11**, 963 (2020).

89. A. J. Montano-Loza *et al.*, Sarcopenic obesity and myosteatosis are associated with higher mortality in patients with cirrhosis. *Journal of cachexia, sarcopenia and muscle* **7**, 126-135 (2016).

90. A. Wree, A. Kahraman, G. Gerken, A. Canbay, Obesity affects the liver–the link between adipocytes and hepatocytes. *Digestion* **83**, 124-133 (2011).

91. D. Cabrera *et al.*, Diet-induced nonalcoholic fatty liver disease is associated with sarcopenia and decreased serum insulin-like growth factor-1. *Digestive diseases and sciences* **61**, 3190-3198 (2016).

92. I. Beyer, T. Mets, I. Bautmans, Chronic low-grade inflammation and age-related sarcopenia. *Current Opinion in Clinical Nutrition & Metabolic Care* **15**, 12-22 (2012).

93. M. Giusto *et al.*, Sarcopenia in liver cirrhosis: the role of computed tomography scan for the assessment of muscle mass compared with dual-energy X-ray absorptiometry and anthropometry. *European journal of gastroenterology & hepatology* **27**, 328-334 (2015).

94. J. Benjamin *et al.*, Characterization of body composition and definition of sarcopenia in patients with alcoholic cirrhosis: A computed tomography based study. *Liver International* **37**, 1668-1674 (2017).

95. A. Faron *et al.*, Body composition analysis using CT and MRI: Intra-individual intermodal comparison of muscle mass and myosteatosis. *Scientific Reports* **10**, 1-10 (2020).

96. M. Kalafateli *et al.*, Malnutrition and sarcopenia predict post-liver transplantation outcomes independently of the Model for End-stage Liver Disease score. *J Cachexia Sarcopenia Muscle* **8**, 113-121 (2017).

97. M. Pahor, T. Manini, M. Cesari, Sarcopenia: clinical evaluation, biological markers and other evaluation tools. *JNHA-The Journal of Nutrition, Health and Aging* **13**, 724-728 (2009).

98. A. Molfino, S. Johnson, V. Medici, The Challenges of Nutritional Assessment in Cirrhosis. *Current nutrition reports* **6**, 274-280 (2017).

99. S. A. Fernandes, A. A. de Mattos, C. V. Tovo, C. A. Marroni, Nutritional evaluation in cirrhosis: Emphasis on the phase angle. *World J Hepatol* **8**, 1205-1211 (2016).

100. R. N. Baumgartner, W. C. Chumlea, A. F. Roche, Bioelectric impedance phase angle and body composition. *Am J Clin Nutr* **48**, 16-23 (1988).

101. T. Cederholm *et al.*, ESPEN guidelines on definitions and terminology of clinical nutrition. *Clinical Nutrition* **36**, 49-64 (2017).

102. M. C. G. Barbosa-Silva, A. J. Barros, J. Wang, S. B. Heymsfield, R. N. Pierson Jr, Bioelectrical impedance analysis: population reference values for phase angle by age and sex–. *The American journal of clinical nutrition* **82**, 49-52 (2005).

103. G. Belarmino *et al.*, Phase angle obtained by bioelectrical impedance analysis independently predicts mortality in patients with cirrhosis. *World journal of hepatology* **9**, 401-408 (2017).

104. W. Nijholt, A. Scafoglieri, H. Jager-Wittenaar, J. S. Hobbelen, C. P. van der Schans, The reliability and validity of ultrasound to quantify muscles in older adults: a systematic review. *Journal of cachexia, sarcopenia and muscle* **8**, 702-712 (2017).

105. C. Ismail *et al.*, Diagnostic ultrasound estimates of muscle mass and muscle quality discriminate between women with and without sarcopenia. *Frontiers in physiology* **6**, 302 (2015).

106. L. A. A. Santos *et al.*, Anthropometric measures associated with sarcopenia in outpatients with liver cirrhosis. *Nutrition & Dietetics* **0**, (2019).

107. T. R. da Silveira, Comparison between handgrip strength, subjective global assessment, and prognostic nutritional index in assessing malnutrition and predicting clinical outcome in cirrhotic outpatients. *Nutrition* **21**, 113-117 (2005).

108. M. Visser *et al.*, Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident mobility limitations in well-functioning older persons. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* **60**, 324-333 (2005).

109. A. Duarte-Rojo *et al.*, Exercise and physical activity for patients with end-stage liver disease: Improving functional status and sarcopenia while on the transplant waiting list. *Liver Transplantation* **24**, 122-139 (2018).

110. C. Moctezuma-Velazquez *et al.*, Limited performance of subjective global assessment compared to computed tomography-determined sarcopenia in predicting adverse clinical outcomes in patients with cirrhosis. *Clinical Nutrition*, (2018).

111. M. Y. Morgan, A. M. Madden, C. T. Soulsby, R. W. Morris, Derivation and validation of a new global method for assessing nutritional status in patients with cirrhosis. *Hepatology* **44**, 823-835 (2006).

112. H. Nishikawa *et al.*, Serum Zinc Concentration and Sarcopenia: A Close Linkage in Chronic Liver Diseases. *J Clin Med* **8**, (2019).

113. N. Oshida *et al.*, Urinary Levels of Titin-N Fragment, a Skeletal Muscle Damage Marker, are Increased in Subjects with Nonalcoholic Fatty Liver Disease. *Scientific Reports* **9**, 19498 (2019).

114. T. R. Lundberg, R. Fernandez-Gonzalo, T. Gustafsson, P. A. Tesch, Aerobic exercise alters skeletal muscle molecular responses to resistance exercise. *Medicine & Science in Sports & Exercise* **44**, 1680-1688 (2012).

115. M. Debette-Gratien *et al.*, Personalized adapted physical activity before liver transplantation: acceptability and results. *Transplantation* **99**, 145-150 (2015).

116. L. Zenith *et al.*, Eight weeks of exercise training increases aerobic capacity and muscle mass and reduces fatigue in patients with cirrhosis. *Clinical Gastroenterology and Hepatology* **12**, 1920-1926. e1922 (2014).

117. E. Román *et al.*, Effects of an exercise programme on functional capacity, body composition and risk of falls in patients with cirrhosis: a randomized clinical trial. *PLoS One* **11**, e0151652 (2016).

118. E. Román *et al.*, Randomized pilot study: effects of an exercise programme and leucine supplementation in patients with cirrhosis. *Digestive diseases and sciences* **59**, 1966-1975 (2014).

119. C. Kruger *et al.*, Home exercise training improves exercise capacity in cirrhosis patients: role of exercise adherence. *Scientific reports* **8**, 1-10 (2018).

120. A. Berzigotti *et al.*, Effects of an intensive lifestyle intervention program on portal hypertension in patients with cirrhosis and obesity: the SportDiet study. *Hepatology* **65**, 1293-1305 (2017).

121. F. R. Williams *et al.*, Home-Based exercise in patients awaiting liver transplantation: a feasibility study. *Liver Transplantation* **25**, 995-1006 (2019).

122. E. A. f. t. S. o. t. Liver, EASL Clinical Practice Guidelines on nutrition in chronic liver disease. *Journal of hepatology* **70**, 172-193 (2019).

123. R. Srinath, A. Dobs, Enobosarm (GTx-024, S-22): a potential treatment for cachexia. *Future Oncology* **10**, 187-194 (2014).

124. A. Yurci *et al.*, Effects of testosterone gel treatment in hypogonadal men with liver cirrhosis. *Clinics and research in hepatology and gastroenterology* **35**, 845-854 (2011).

125. N. Nagasue *et al.*, Active uptake of testosterone by androgen receptors of hepatocellular carcinoma in humans. *Cancer* **57**, 2162-2167 (1986).

126. J. D. Wallace *et al.*, GH Treatment in Adults with Chronic Liver Disease: A Randomized, Double-Blind, Placebo-Controlled, Cross-Over Study. *The Journal of Clinical Endocrinology & Metabolism* **87**, 2751-2759 (2002).

127. M. Senzolo *et al.*, β-Blockers protect against spontaneous bacterial peritonitis in cirrhotic patients: a meta-analysis. *Liver International* **29**, 1189-1193 (2009).

128. M. Merli *et al.*, The chronic use of beta-blockers and proton pump inhibitors may affect the rate of bacterial infections in cirrhosis. *Liver International* **35**, 362-369 (2015).

129. A. L. Clark *et al.*, Effect of beta-adrenergic blockade with carvedilol on cachexia in severe chronic heart failure: results from the COPERNICUS trial. *Journal of cachexia, sarcopenia and muscle* **8**, 549-556 (2017).