The Benefits of Neuromuscular Electrical Stimulation in the Muscular and Functional Capacity of Patients With Liver Cirrhosis: Protocol for a Randomized Clinical Trial

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ABSTRACT: Cirrhosis causes systemic and metabolic changes that culminate in various complications, such as compromised pulmonary function, ascites, hepatic encephalopathy, weight loss, and muscle weakness with significant physical function limitations. Our aim is to evaluate the effects of training with neuromuscular electrical stimulation (NMES) on the muscular and functional capacity of patients with cirrhosis classified as Child-Pugh B and C. A total of 72 patients diagnosed with cirrhosis will be recruited and randomized to perform an NMES protocol for 50 minutes, 3 times a week, for 4 weeks. The evaluations will be performed at the beginning and after 12 sessions, and patients will be submitted to a pulmonary function test, an ultrasound evaluation of the rectus femoris, an evaluation of peripheral muscle strength, a submaximal exercise capacity test associated with an evaluation of peripheral tissue oxygenation, a quality of life evaluation, and orientation about monitoring daily physical activities. The evaluators and patients will be blinded to the allocation of the groups. Training Group will be treated with the following parameters: frequency of 50 Hz, pulse width of 400 μs, rise and fall times of 2 s, and on:off 1:1; Sham Group: 5 Hz, 100 μs, on:off 1:3. The data will be analyzed using the principles of the intention to treat. This study provides health professionals with information on the benefits of this intervention. In this way, we believe that the results of this study could stimulate the use of NMES as a way of rehabilitating patients with more severe cirrhosis, with the objective of improving these patients' functional independence.

KEYWORDS: Electric stimulation, liver cirrhosis, muscle strength, exercise capacity, rehabilitation

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Introduction

The pathological process of cirrhosis causes systemic and metabolic changes that culminate in various complications, such as compromised pulmonary function, ascites, hepatic encephalopathy, jaundice, weight loss, muscle weakness, and cardiac changes with significant physical function limitations and impacts on the quality of life of individuals.[1,2]

Mechanisms to explain compromised exercise capacity in these patients include skeletal and cardiac muscle dysfunction, resulting in diminished oxygen extraction by active muscles.[3] Weight loss and muscle weakness are the most common symptoms of cirrhosis, contributing to a worse functional state. Studies have diagnosed loss of weight and peripheral muscle strength of 40% in patients with cirrhosis, showing an inverse correlation between strength and the hepatic disease severity, and affirmed that sarcopenia is a strong independent predictive factor of mortality.[4,5]

Peripheral muscle strength loss influences the capacity to exercise and levels of physical activity. Various studies report that patients with cirrhosis walk shorter distances than healthy individuals and that patients with cirrhosis have daily step counts similar to those of individuals with chronic diseases, such as chronic obstructive pulmonary disease, end-stage chronic kidney disease, and congestive heart failure; in addition to finding sedentary or inactive lifestyles in 75% of the sample, when evaluated with equipment such as accelerometers.[4,6,7] Low scores in surveys on daily physical activities and general health status corroborate this data, demonstrating that patients with cirrhosis need considerable assistance with their daily activities.[6]

Studies that evaluate exercise protocols in this population include individuals in the initial stages, with lower severity and no complications such as ascites, esophageal varices, and encephalopathy.[8–12] Jones et al[4] suggested in a review that aerobic training causes improvements in muscular strength and resistance, and exercise capacity, although the analyzed studies were heterogeneous in their protocols, interventions, durations, and intensities, in addition to having small sample sizes. Also, possible adverse events and safety in these types of interventions were not evaluated or reported in these studies.

Macías-Rodríguez et al[12] concluded that a supervised physical exercise program associated with nutritional therapy improves the nutritional state and significantly reduces the hepatic venous pressure gradient in stable patients with cirrhosis when

compared to patients with cirrhosis treated with only nutritional therapy. This demonstrated that exercise could be an attractive approach to portal hypertension. However, the authors point out that these results cannot be applied to patients with more advanced stages of the disease.

Recently, Román et al[11] compared an aerobic exercise program of moderate intensity with a relaxation program. They found an increase in the anaerobic threshold in the cardiopulmonary exercise test, a reduction in the estimate of falls measured by the Timed Up&Go test, a decrease in fat, and an increase in muscle mass in the exercise group. However, the study had limitations such as a small sample size (n = 14 for the exercise group, and n = 9 for the relaxation group) and a low severity of the individuals: Model for End-Stage Liver Disease (MELD) (8.2 ± 0.4, 9.1 ± 0.4) and Child-Pugh (A).

Currently, neuromuscular electrical stimulation (NMES) has been suggested as an option for patients with chronic diseases, such as heart failure,[13] chronic obstructive pulmonary disease,[14] and recently renal failure.[15] Interventions of this type in these populations have shown improvements in peripheral muscle strength,[15] respiratory muscle strength,[14] and functional capacity.[14,15] However, there are no reports in the literature of studies investigating the effects of NMES in patients with more severe cirrhosis, classified as Child-Pugh B and C.

Considering that patients with cirrhosis show systemic changes, including reduced peripheral muscle strength and exercise capacity, which affect normal daily activities, our aim is to evaluate the effects of training with NMES on the muscular and functional capacity of patients with cirrhosis classified as Child-Pugh B and C. Our hypothesis is that an NMES protocol has an effect on peripheral muscle strength, exercise capacity, peripheral tissue oxygenation, and daily life activities of patients with cirrhosis classified as Child-Pugh B and C.

Methods

The study will follow the recommendations of CONSORT[16] for clinical trials. This is a controlled, double blind, randomized clinical trial, with parallel groups. The selected patients will be split equally into training group (TG) and control group (CG). The protocol was registered on the Brazilian clinical trial platform (ReBEC) with study ID RBR-3SST2S and was approved by the ethics committee with CAAE 52887815.6.0000.0118. The study will be performed at the Hepatology Clinic at University Hospital at the Federal University of Santa Catarina (HU-UFSC) and at the Biomechanical Laboratory at the State University of Santa Catarina (UDESC) in Florianopolis, SC.

Trial Participants

The study population is composed of patients with cirrhosis classified as Child-Pugh B and C. The inclusion criteria will be: (1) patients diagnosed with histologically confirmed cirrhosis and/or by the combined clinical, image, and laboratory findings of Child-Pugh B and C patients with portal hypertension symptoms; (2) absence of acute cirrhosis symptoms (characterized by rapid onset of hepatic encephalopathy, massive ascites, gastrointestinal hemorrhage, bacterial infection, or recent hospital stay); (3) patients who do not have uncontrolled hypertension, recent ischemic heart disease, unstable angina, severe heart arrhythmia, and hepatopulmonary syndrome; (4) patients who did not use antibiotics for 7 days prior to the study; (5) patients without respiratory, orthopedic, or neurological diseases that could limit the performance of the protocol; and (6) patients who do not practice any type of physical training and have not done so for at least 6 months.

Patients will be excluded if they (1) are unable to perform any of the evaluations of the study (inability to cooperate or comprehend), (2) have significant edema in the lower limbs on evaluation day or during the treatment protocol, (3) develop a bacterial infection during the study, and (4) have clinical state decompensation during the study.

Sample Size

The sample size calculation was performed a priori using the program GPower 3.1, based on the results obtained by Brüggemann et al.[15] In the group that performed NMES, the values of right peripheral muscle strength (RPMS) and left peripheral muscle strength (LPMS) used were, for pre-training, an initial RPMS of 155.35 ± 65.32 N m and an initial LPMS of 156.60 ± 66.51 N m; and post-training, a final RPMS of 161.60 ± 68.73 N m; and a final LPMS of 164.10 ± 69.76 N m. The respective correlation values were 0.98 for initial and final RPMS and 0.98 for initial and final LPMS. In addition, the values for exercise capacity used were 435.55 ± 95.81 m and 457.25 ± 90.64 m for pre- and post-training, respectively, with a correlation of 0.90. Considering an error of 5%, and statistical power of 80%, sample sizes of 32 patients for RPMS, 23 patients for LPMS, and 25 patients for exercise capacity were determined. Using the largest value of 32 patients, and increasing that by 10% to account for possible sample losses, 36 patients will be necessary for each group.

Recruitment and Randomization

All potentially eligible patients will be recruited from the hepatology clinic in the HU-UFSC, after being informed about the procedures and objectives of the study and signing an informed consent document (ICD). Randomization will be stratified by sex, using blocks of four, with the Lucky Wheel application, and performed by a non-evaluator researcher (Figure 1).

Protocol

NMES of vastus lateralis and medialis will be performed bilaterally 3 times a week (12 sessions) with a duration of 50 minutes. It will be applied in a sitting position, using a dual-channel, portable stimulator (CarciFesmed IV, São Paulo, Brazil) with the following parameters: frequency of 50 Hz, pulse width of

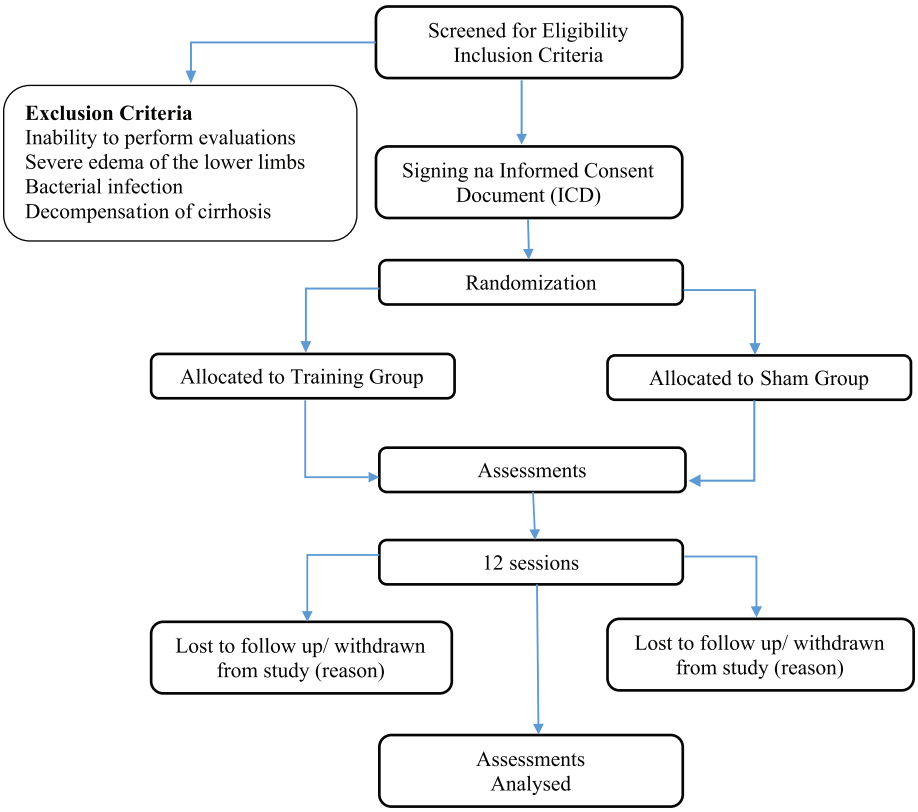

Figure 1. Flow of patients through the study.

400 μs, rise and fall times of 2 seconds, and on:off stimulation time starting with 1:2 relation during the first week (stimulation time of 10 seconds and rest time of 20 seconds, with the objective of adapting and minimizing the effects of muscular fatigue), and changing the relation to 1:1 during the second week (stimulation time of 10 seconds and rest time of 10 seconds).

Each session will involve a warm-up period performed with 20% of the intensity used in the last session, with a gradual increase of 20% per minute until the fifth minute, followed by a training period of 50 minutes and ending with a recovery period of 5 minutes with a gradual 20% decrease in intensity per minute. The intensity during the training period will be the maximum tolerated by patients, in order to increase it incrementally at each session, and will be recorded for later calculations of the average intensity used along the duration of the training.

The self-adhesive surface electrodes will be positioned on the vastus lateralis and medialis muscles (along the direction of the muscle fibers, positioned 3 cm above the upper edge of the patella and 5 cm below the inguinal fold). Blood pressure, heart rate, and the Borg Rating of Perceived Exertion Scale[17] will be measured at the beginning and end of each session. Training will be performed by 2 trained physical therapists.

Control Group

In the CG, the electrodes will be placed in the same position as the TG, but muscular contractions will not be permitted during training and there will be no increase in current intensity. The parameters used will include a frequency of 5 Hz and on:off stimulation time with a relation of 1:3 (stimulation time of 10 seconds and rest time of 30 seconds) with a pulse duration of 100 μs. The GS will also have the same warm-up and cool-down periods as the TG, although with no increase in intensity.

Both protocols will be monitored for 1 hour.

Outcomes

The evaluators and the patients involved in this study will not know the groups to which the patients will be allocated. The evaluations will be performed at the beginning and after 12 sessions, always by the same evaluator, split into 2 parts. On the first day after signing the ICD, patients will be submitted to a pulmonary function test, an ultrasound evaluation of the rectus femoris, an evaluation of peripheral muscle strength, a submaximal exercise capacity test associated with an evaluation of peripheral tissue oxygenation, a quality of life evaluation, and orientation about monitoring daily physical activities. On the second day, the patient's venous blood will be collected.

Primary Outcomes

Peripheral muscle strength

In order to measure maximum torque, an isokinetic dynamometer (Biodex System 4 Pro, New York, USA) will be used in isometric mode. The patient will be seated on a chair with

torso, hips and the evaluated lower limb secured by straps to avoid compensation, with the limb flexed to 85° relative to the hip, and lateral femoral condyle aligned with the rotation axis.

The maximum voluntary isometric contraction will start from 60° of knee flexion,[18] considering 0° total extension, and be held for 5 seconds with 60 seconds rest between contractions. The patient will perform 5 contractions, and from the 2 that have less than 10% variation, the larger will be used.

Exercise capacity and peripheral muscle tissue oxygenation

The patients will perform two 6-minute walk tests (6MWT), meeting the standard criteria set by the European Respiratory Society/American Thoracic Society.[19] The largest measured distance will be used for analysis, with reference values used described by Britto et al.[20] Before and after each test, blood pressure, heart rate, breathing rate, oxygen pulse saturation, and the Borg Rating of Perceived Exertion Scale (modified Borg scale) data will be collected.

During 6MWT, patients' peripheral muscle tissue oxygenation will be assessed by the near infrared spectroscopy (NIRS) method (Oxymon, Artinis®, The Netherlands). In a noninvasive, continuous, and direct way, it provides information about peripheral tissue oxygenation and adaptations caused by exercise.[21] The equipment sensor will be positioned over the lateral vastus muscle of the dominant lower limb, two-thirds the distance from the anterior superior iliac spine to the lateral border of the patella according to the SENIAM (http://www.seniam.org/) guidelines. The variables measured in real time will be oxyhemoglobin (O_2Hb), deoxygenated hemoglobin (HHb), total hemoglobin (THb), and tissue oxygenation saturation index (TSI-%).[21]

Secondary Outcomes

Rectus femoris muscle thickness

The rectus femoris muscle thickness will be measured using an ultrasonography of the thigh with BX-2000 (Body Metrix, Livermore, USA) equipment, in mode A, using the 2.5 MHz transducer. The position of the measurement will be chosen following the International Society for the Advancement of Kinanthropometry (ISAK) guidelines.[22] The patient will be seated, with hip and knees at 90°. Initially, the midpoint (MP) between the inguinal fold and the upper patella edge will be found and then the midpoint between the MP and the upper patella edge will be measured (MP1). The scan area will correspond to the distance between the points MP and MP1.

With the lower limb extended and relaxed, the transducer will be positioned perpendicular to the long axis of the thigh, at an angle of 90 degrees, in scan mode, and then slid in the proximal to distal direction between points MP and MP1 for 6 seconds, according to the manufacturer's instructions. The measurement will be obtained by means of a planimetric technique, after establishing the interior echogenic line of the rectus femoris over the still image. The area will be calculated using the average of 3 consecutive, reproducible measurements with a maximum difference of 10% between measurements.[23]

Blood markers

The variations of interleukin (IL) cytokine levels of IL-6, IL-10, and IL-1RA in serum will be measured before and after 12 training sessions. Samples of 4 mL of blood will be collected and stored in a vacutainer previously treated with lithium heparin and then centrifuged for 7 minutes (400G, 6600 r/min at 4°C) to separate the plasma. Then, aliquots of 350 µL will be stored in a freezer at −80°C, in order to measure the markers to be studied using enzyme-linked immunosorbent assay (ELISA) method kits (DuoSet® ELISA, R & D Systems, Minneapolis, MN, USA).

Pulmonary function test

Pulmonary function will be measured using a calibrated portable digital spirometer, NDD EasyOne (Zurich, Switzerland), according to the recommended methods and criteria from ATS/ERS.[24] The criteria for normal pulmonary test results will consist of forced vital capacity (FVC) and forced expiratory volume in the first second (FEV_1) ⩾80% of predicted value and FEV_1/FVC ⩾0.7.[25]

Physical activity

Physical activity will be evaluated using a 3-axis accelerometer (Actigraph GTX3 Pensacola, FL, USA). Patients will be monitored for 7 consecutive days, for 12 hours each day, starting soon after waking up. They will be instructed regarding the correct positioning of the device and will receive a manual with clear instructions and figures. In addition, they will be instructed to perform their daily activities normally without any changes while using the device.[26]

Satisfaction scale

In order to evaluate satisfaction and diagnose if the patient noticed an improvement in the conditions evaluated, during the re-evaluation patients will respond to a Likert scale. This was created by the researchers and is composed of 7 questions, with 5 set, linear, ordinal answers for each, allowing the patient to express how much they agree or disagree with the specific statement in the question.[27]

Data analysis

The data will be analyzed using the principles of the intention to treat. To compare the behavior of the result variables between groups, the independent student's *t* test and the Mann-Whitney

U test will be used. To compare the pre- and post-training averages, the repeated-measures analysis of variance (ANOVA) test and Tukey's post hoc test will be used. To show relations between peripheral muscle strength and exercise capacity, and between peripheral muscle strength and muscle thickness, the Pearson and Spearman correlation coefficients will be used. The Shapiro-Wilk test will be used to test normality of the data. A significance level of 5% ($P < .05$) will be used.

Discussion

It is estimated that cirrhosis results in approximately 30 000 deaths each year in the United States, out of 5.5 million cases.[28] In Brazil, the current data is limited, and in 2008, there was an estimated 0.35% of the population affected, or 350 cases per 100 000 inhabitants, with 37% of these resulting from hepatitis C, 11% from hepatitis B, and 52% from alcohol or other causes.[29]

Patients with cirrhosis have peripheral muscle strength loss in both upper and lower limbs when compared to healthy individuals. Strength and muscle loss is present in 40% of patients, with sarcopenia being a strong, independent predictive factor of death.[30]

Computerized tomography is the gold standard to diagnose sarcopenia, even though it is expensive and exposes the patient to radiation.[31] Tandon et al[31] demonstrated that ultrasonographies of sarcopenia are strongly associated with tomographic sections, and the authors state that an ultrasonography of the thigh is a precise, reproducible, and reliable bedside measure, without exposing the patient to radiation. The ultrasonography of the thigh is becoming a useful accessory for physiotherapy practice, as it is a safe, precise, and reliable kinesiological method.[32]

Considering that cirrhosis is a progressive disease, Pereira et al[33] state that the advancement of hepatic diseases tends to further reduce the functional capacity of those afflicted, because patients with a Child-Pugh score of C had worse performance on the 6MWT compared with patients with Child-Pugh scores of A and B. Recently, Pereira et al[34] demonstrated in a survivability analysis that individuals with cirrhosis who walk a distance smaller than 410 m had a survivability rate of 55%.

Wu et al[35] found low levels of self-reported daily physical activity by means of surveys to be potential mortality risk factors in patients with cirrhosis. This analysis is a subjective measure, as demonstrated by Dunn et al,[7] who used self-reporting scales (Rosow-Breslau scale and Karnofsky performance scale) and an accelerometer (Bodymedia SenseWear Minifly ArmBand) to show that while patients had near normal daily physical activity levels on the scales, the accelerometers showed levels of inactivity and sedentarism similar to other individuals with chronic diseases. This explains our concern regarding the use of a triaxial accelerometer to evaluate daily physical activity in these patients.

Regarding studies that report peripheral tissue oxygenation variables, one study was found that evaluated 8 patients during an incremental cycle ergometer test. The results indicate that patients with cirrhosis have a limited exercise capacity due to a reduced ability to extract O_2 and not due to hemodynamic failure. According to the authors, the limitations of the study include small sample size and high variability of the etiology of cirrhosis.[3] In this way, our study contributes to the knowledge of baseline peripheral tissue oxygenation, as well as making it possible to verify if NMES is able to change this factor.

Studies that analyze and recommend exercise protocols are still few. In addition, few studies involve more severe patients.[36] Zenith et al[9] after 8 weeks of supervised aerobic training on a cycle ergometer, 3 times a week, found an improvement of Vo_{2max} of 5.3 mL/kg/min in the exercise group compared with the CG (ordinary care), in addition to muscle tissue increase. The study sample only involved patients with Child-Pugh scores of A and B, which are less severe in relation to cirrhosis conditions.

Román et al[10] with an exercise protocol of 1 hour on a treadmill and bicycle ergometer (60%-70% of maximum functional capacity) 3 times per week for 12 weeks, combined with leucine supplements, showed an increase in exercise capacity in the exercise group, as measured by 6MWT and the Step Test, and decreased thigh circumference, as well as improved quality of life on the survey SF-36. The researchers also stated that no adverse effects were observed due to the exercise protocols, although 70% of the patients in the sample were Child-Pugh A.

Currently, the literature does not contain studies investigating the safety of aerobic exercises in patients with cirrhosis, especially those that are Child-Pugh B and C and have worse clinical conditions, and these patients are candidates for receiving alternative protocols such as NMES, which is the proposal of our project.

Recently, the use of NMES has seen an increase as an exercise for patients with chronic disease, who have muscular disorders secondary to systemic changes, such as in cardiac failure,[13] in chronic obstructive pulmonary disease,[14] and in patients with chronic kidney failure.[15] Studies have demonstrated that these populations benefit from NMES, showing improvements in peripheral muscle strength,[15] respiratory muscle strength,[14] functional capacity,[14,15] and quality of life.[13,14] No studies were found that consider the use of NMES in patients with cirrhosis.

The protocol of this study was created with the objective of investigating the effects of an alternative to aerobic exercise as a treatment for patients with cirrhosis, using accurate methods that have been inadequately explored to evaluate this population. Therefore, it was sought to determine the efficacy of NMES in Child-Pugh B and C patients with cirrhosis in regard to peripheral muscle strength, exercise capacity, physical activities, and peripheral tissue oxygenation. This study

provides health professionals with information on the benefits of this intervention, especially on more severe patients who are currently not included in intervention studies. In this way, we believe that the results of this study could stimulate the use of NMES as a way of rehabilitating patients with more severe cirrhosis, with the objective of improving these patients' functional independence.

Author Contributions

CLdM and TMAdC took the lead in writing the manuscript. TDP, CCP, MNL contributed to the final manuscript. EP supervised this work.

REFERENCES

1. Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. *Lancet*. 2014;383:1749–1761. doi:10.1016/S0140-6736(14)60121-5.
2. Galant LH, Junior F, Luiz A, Dias AS, Marroni CA. Functional status, respiratory muscle strength, and quality of life in patients with cirrhosis. *Braz J Phys Ther*. 2012;16:30–34. doi:10.1590/S1413-35552012000100006.
3. Nielsen HB, Secher NH, Clemmesen O, Ott P. Maintained cerebral and skeletal muscle oxygenation during maximal exercise in patients with liver cirrhosis. *J Hepatol*. 2005;43:266–271. doi:10.1016/j.jhep.2005.02.039.
4. Jones JC, Coombes JS, Macdonald GA. Exercise capacity and muscle strength in patients with cirrhosis. *Liver Transpl*. 2012;18:146–151. doi:10.1002/lt.22472.
5. Hanai T, Shiraki M, Ohnishi S, et al. Rapid skeletal muscle wasting predicts worse survival in patients with liver cirrhosis. *Hepatol Res*. 2016;46:743–751. doi:10.1111/hepr.12616.
6. Leitão AV, Castro CL, Basile TM, Souza TH, Braulio VB. Avaliação da capacidade física e do estado nutricional em candidatos ao transplante hepático. *Rev Assoc Med Bras*. 2003;49:424–428. doi:10.1590/S0104-42302003000400035.
7. Dunn MA, Josbeno DA, Schmotzer AR, et al. The gap between clinically assessed physical performance and objective physical activity in liver transplant candidates. *Liver Transpl*. 2016;22:1324–1332. doi:10.1002/lt.24506.
8. Soriano G, Román E, Torrades MT, et al. Study of the benefit of an exercise programme and branched amino acid supplements in patients with liver cirrhosis. *Trauma (Spain)*. 2013;24:6–11.
9. Zenith L, Meena N, Ramadi A, et al. Eight weeks of exercise training increases aerobic capacity and muscle mass and reduces fatigue in patients with cirrhosis. *Clin Gastroenterol Hepatol*. 2014;12:1920–1926. doi:10.1016/j.cgh.2014.04.016.
10. Román E, Torrades MT, Nadal MJ, et al. Randomized pilot study: effects of an exercise programme and leucine supplementation in patients with cirrhosis. *Dig Dis Sci*. 2014;59:1966–1975. doi:10.1007/s10620-014-3086-6.
11. Román E, García-Galcerán C, Torrades T, 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*. 2016;11:e0151652. doi:10.1371/journal.pone.0151652.
12. Macías-Rodríguez RU, Ilarraza-Lomelí H, Ruiz-Margáin A, et al. Changes in hepatic venous pressure gradient induced by physical exercise in cirrhosis: results of a pilot randomized open clinical trial. *Clin Transl Gastroenterol*. 2016;7:e180. doi:10.1038/ctg.2016.38.
13. Van Buuren F, Mellwig KP, Fründ A, et al. Electrical myostimulation: improvement of quality of life, oxygen uptake and left ventricular function in chronic heart failure. *Rehabilitation (Stuttg)*. 2014;53:321–326. doi:10.1055/s-0033-1358734.
14. Vieira PJ, Chiappa AMG, Cipriano G, Umpierre D, Arena R, Chiappa GR. Neuromuscular electrical stimulation improves clinical and physiological function in COPD patients. *Respir Med*. 2014;108:609–620. doi:10.1016/j.rmed.2013.12.013.
15. Brüggemann AK, Mello CL, Dal Pont T, et al. Effects of neuromuscular electrical stimulation during hemodialysis on peripheral muscle strength and exercise capacity: a randomized clinical trial. *Arch Phys Med Rehabil*. 2017;98:822–831.
16. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMC Med*. 2010;8:18. doi:10.1186/1741-7015-8-18.
17. Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc*. 1982;14:377–381.
18. Murphy AJ, Wilson GJ, Pryor JF, Newton R. Isometric assessment of muscular function: the effect of joint angle. *J Appl Biomech*. 1995;11:205–215.
19. Holland AE, Spruit MA, Troosters T, et al. An official European Respiratory Society/American Thoracic Society technical standard: field walking tests in chronic respiratory disease. *Eur Respir J*. 2014;44:1428–1446.
20. Britto RR, Probst VS, Andrade AF, et al. Reference equations for the six-minute walk distance based on a Brazilian multicenter study. *Braz J Phys Ther*. 2013;17:556–563. doi:10.1590/S1413-35552012005000122.
21. Van Beekvelt MC, Colier WN, Wevers RA, Van Engelen BG. Performance of near-infrared spectroscopy in measuring local O2 consumption and blood flow in skeletal muscle. *J Appl Physiol*. 2001;90:511–519. doi:10.1152/jappl.2001.90.2.511.
22. Marfell-Jones M, Stewart A, De Ridder H. *ISAK Accreditation Handbook*. Upper Hutt, New Zealand: International Society for the Advancement of Kinanthropometry; 2012.
23. Seymour JM, Ward K, Sidhu PS, et al. Ultrasound measurement of rectus femoris cross-sectional area and the relationship with quadriceps strength in COPD. *Thorax*. 2009;64:418–423. doi:10.1136/thx.2008.103986.
24. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26:319–338. doi:10.1183/09031936.05.00034805.
25. Pereira CAC, Sato T, Rodrigues SC. Novos valores de referência para espirometria forçada em brasileiros adultos de raça branca. *J Bras Pneumol*. 2007;33:397–406. doi:10.1590/S1806-37132007000400008.
26. Pruitt LA, Glynn NW, King AC, et al. Use of accelerometry to measure physical activity in older adults at risk for mobility disability. *J Aging Phys Act*. 2008;16:416–434. doi:10.1123/japa.16.4.416.
27. Bowling A. *Research Methods in Health*. Buckingham, UK: Open University Press; 1997.
28. Vong S, Bell PB. Chronic liver disease mortality in the United States, 1990–1998. *Hepatology*. 2004;39:476–483. doi:10.1002/hep.20049.
29. Carvalho JRD, Portugal FB, Flor LS, Campos MR, Schramm JMDA. Método para estimação de prevalência de hepatites B e C crônicas e cirrose hepática-Brasil, 2008. *Epidemiol Serv Saúde*. 2014;23:691–700. doi:10.5123/S1679-49742014000400011.
30. Wiesinger GF, Quittan M, Zimmermann K, et al. Physical performance and health-related quality of life in men on a liver transplantation waiting list. *J Rehabil Med*. 2001;33:260–265. doi:10.1080/165019701753236446.
31. Tandon P, Low G, Mourtzakis M, et al. A model to identify sarcopenia in patients with cirrhosis. *Clin Gastroenterol Hepatol*. 2016;14:1473–1480. doi:10.1016/j.cgh.2016.04.040.
32. Parry SM, El-Ansary D, Berney SC, et al. The role of neuromuscular ultrasound imaging in the critically ill population and relationship to muscle strength and physical function. *Am J Respir Crit Care Med*. 2014;189:A4505.
33. Pereira JLF, Figueredo TCM, Junior LAF, et al. Capacidade funcional e força muscular respiratória de candidatos ao transplante hepático. *Rev Bras Med Esporte*. 2011;17:315–318. doi:10.1590/S1517-86922011000500004.
34. Pereira JLF, Galant LH, Rossi D, et al. Functional capacity, respiratory muscle strength, and oxygen consumption predict mortality in patients with cirrhosis. *Can J Gastroenterol Hepatol*. 2016;2016:6940374. doi:10.1155/2016/6940374.
35. Wu LJ, Wu MS, Lien GS, Chen FC, Tsai JC. Fatigue and physical activity levels in patients with liver cirrhosis. *J Clin Nurs*. 2012;21:129–138. doi:10.1111/j.1365-2702.2011.03900.x.
36. Toshikuni N, Arisawa T, Tsutsumi M. Nutrition and exercise in the management of liver cirrhosis. *World J Gastroenterol*. 2014;20:7286–7297. doi:10.3748/wjg.v20.i23.7286.