Efficacy of neuromuscular electrical stimulation with combined low and high frequencies on body composition, peripheral muscle function and exercise tolerance in patients with chronic kidney disease undergoing haemodialysis: a protocol for a randomised, double-blind clinical trial

ABSTRACT

Introduction  Neuromuscular electrical stimulation (NMES) as an adjunctive strategy to increase isolated muscular strength or endurance has been widely investigated in patients with chronic kidney disease (CKD) undergoing haemodialysis (HD). However, the efficacy of combined low and high frequencies, to improve both muscular strength and endurance, is unknown. This trial aims to evaluate the efficacy of this combined NMES strategy in this population.

Methods and analysis  This is a randomised controlled trial with blinded assessments and analysis. A total of 56 patients with CKD undergoing HD will be recruited and randomised to an NMES protocol. The evaluations will be performed on three different days at baseline and after 24 sessions of follow-up. Assessments will include the background, insulin-like growth factor, lactate measurement, malnutrition and inflammation score evaluation, an electrical bioimpedance examination, global muscular evaluation by means of the Medical Research Council scale, handgrip strength evaluation, muscular isokinetic evaluation of lower limbs, 6 min step test performance and quality of life (QoL) questionnaire with emphasis on physical function. The patients will be allocated in one of the following four groups: 1) combined low and high frequencies; 2) low frequency; 3) high frequency; and 4) sham stimulation with minimal intensity to generate only sensory perception (with no visible contraction). In all groups, the intensity throughout the session will be the highest tolerated by patient (except for control group). The primary endpoint is the change of peripheral muscle function (muscular strength and endurance). The secondary endpoints will be the changes of body composition; muscle trophism; exercise tolerance; QoL; and nutritional, inflammatory, and metabolic markers. The findings of this study are expected to provide valuable knowledge on how to optimise the NMES intervention, with improvements in both muscle strength and endurance.

Ethics and dissemination  This protocol has been approved by the Ethics Committee on Research with Humans of Hospital Sírio-Libanês (approval no. 24337707). Written informed consent will be obtained from each participant. The results of the study will be published in peer-reviewed journals.

Trial registration number  NCT03779126

INTRODUCTION

Different body systems are affected with the progress of chronic kidney disease (CKD) and with the start of the haemodialysis (HD), especially the cardiovascular and musculoskeletal systems. Different causes are attributed for these alterations, such as: metabolic acidosis, low protein intake, systemic inflammation and increase of sedentary behaviour. The peripheral muscles evolve with the reduction in their oxidative capacity, quality and...
Neuromuscular electrical stimulation (NMES) has been used to early mobilisation as functional electrical stimulation, and proved to be a useful strategy for rehabilitation with chronic diseases. A previous randomised controlled trial (RCT) was conducted comparing the effects of exercise training using cycle ergometer with NMES in patients undergoing HD. The results evidenced improvement on peripheral muscle strength and exercise tolerance in both interventions with no difference between them but with statistic difference when compared with the control group. The key point observed in this study was that the NMES can bring similar outcomes when compared with exercise training with cycle ergometer.

Different studies have used NMES with high frequency to treat patients undergoing HD, and the results observed were a reduction in genomic damage and an increase in muscle strength, angle of pennation and exercise tolerance. However, the parameters used for NMES have not been standardised. In a recent RCT, the effect of NMES with high frequency has been investigated in acute kidney disease, showing improvement on peripheral muscle strength in the intervention group. An improvement on quality of life (QoL) has also been reported in patients undergoing HD, using NMES with high frequency. In a recent study, the effect of NMES with high frequency was compared with low frequency in patients undergoing HD. It could be observed that there was an increase on peripheral muscle strength, exercise tolerance and reduction of level of interleukin-10 on the high-frequency group. On the other hand, in the low-frequency group, it was observed that there was an increase in the exercise tolerance and levels of insulin-like growth factor (IGF-1). The authors speculated that the improvement in exercise tolerance observed in both groups could be explained by different mechanisms of changes on muscular function: in the high-frequency group, the exercise tolerance could be improved by an increase on muscular strength, while in the low-frequency group, this improvement could result from an increase on muscular endurance. However, in this study, the muscular endurance measures were not performed, compromising the confirmation of this hypothesis.

Based on this, it could be expected that the rehabilitation with NMES using combined low and high frequencies could be effective to improve both muscular strength and endurance, contributing to a better treatment approach of individuals undergoing HD. To the best of our knowledge, the effects of NMES combined with low and high frequencies in individuals undergoing HD, or in any population of healthy individuals, and with other chronic diseases have not yet been investigated. Therefore, the aim of this study is to assess the efficacy of combined NMES with low and high frequencies on body composition, peripheral muscle function and exercise tolerance in patients undergoing HD.

METHODS

The protocol was structured according to the Spirit 2013 checklist (online supplemental material 1).

Study design

This study was approved by the ethics committee of a private hospital in São Paulo, Brazil (number 24337707), and the protocol design was registered in the Clinical Trials database. This is a randomised clinical trial (RCT) designed with blinded assessments and analyses including four groups and which will be carried out over an 8-week period of NMES, comparing the effects of different strategies: (1) combined low and high frequencies, (2) low frequency, (3) high frequency and (4) sham stimulation group. The research structure is shown in figure 1.

The patients will be recruited by convenience from the HD centre of a private hospital in São Paulo, Brazil, according to inclusion and exclusion criteria. At the beginning of recruitment, detailed information about the study, including the research objectives, study procedure and potential benefits and risks, will be provided to all eligible patients. If the patients agree to participate, they will be asked to sign a written informed consent form (online supplemental material 2).

The inclusion criteria will be patients (1) with CKD undergoing kidney replacement therapy through HD, (2) older than 18 years, (3) without pacemaker or other electrical device, (4) without cognitive or motor deficit that makes it impossible to perform the volitional tests within the criteria of technical acceptability, and (5) who did not practice regular physical activity (Garber C, 2011). The exclusion criteria for follow-up are (1) inability to perform any of the evaluations of the study, (2) absence from more than two consecutive sessions or more than four sessions in total (16.7%), and (3) need for hospitalisation for any reason.

Sample size

The sample size calculation was performed using a statistics programme (SigmaStat V.3.5; San Jose, California, USA), based on the results obtained by Dobsak et al, who found, in the group that performed NMES, a baseline value of peripheral muscle strength of 185.4±53.0 kgf and a post-treatment value of 222.4±36.6 kgf. Considering an error of 5% and statistical power of 80%, a sample size of 14 patients in each group will be necessary, totalling the inclusion of 56 patients.
**Randomisation and allocation concealment**

The randomisation will be performed using opaque envelopes, and the patients will be stratified by gender, age (for decades) and use of protein nutritional supplementation. After the assessments, an envelope with printed random numbers will be drawn by an independent staff member to determine the group assigned to that participant. Random block sizes of 4 will be used, ensuring a 1:1 ratio between the experimental and sham groups.

**Blinding**

Researchers involved in the assessments will not have access to information about randomisation; and similarly, researchers responsible for treatment will not have access to performance data of evaluations during the study. The patients will also be abstained from information about their performance during the study as well as their allocation group. The investigator must report all code breaks (with reason) as they occur on the corresponding case report form page.

**Intervention**

The protocol consists of NMES of vastus lateralis and vastus medialis bilaterally for 1 hour, three times a week, for 8 weeks, totalling 24 sessions. It will be applied in a sitting position or lying supine according to the individual’s needs for HD session, maintaining a knee flexion angle between 60° and 80°, using a dual-channel portable stimulator (Neurodyn II; Ibramed, São Paulo, Brazil). NMES should be initiated in the second third of the HD session, to avoid the initial and final periods, where haemodynamic conditions may be unfavourable. The NMES will be done through eight self-adhesive surface electrodes (50×90 mm), which will be positioned along

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**Figure 1** Flow diagram of the progress through the phases of a parallel randomised trial of two groups (ie, enrolment, intervention allocation, follow-up and data analysis). Intervention 1, combined frequency; intervention 2, high frequency; and intervention 3, low frequency.
the direction of the muscle fibres, on the vastus lateralis, one positioned 3 cm above the upper edge of the patella and 5 cm below the inguinal fold towards the anterosuperior iliac crest, and vastus medialis, one positioned 3 cm above the upper edge of the patella and another 5 cm below in the oblique direction. The patients will be allocated in one of the following four groups: (1) combined low-frequency and high-frequency group—30-min of NMES with a low frequency of 20 Hz, followed by 30-min with a high frequency of 70 Hz; (2) low-frequency group—60-min with a low frequency of 20 Hz; (3) high-frequency group—60-min with a high frequency of 70 Hz; and (4) sham stimulation group—60-min with a frequency of 5 Hz and minimal intensity to generate only sensory perception (with no visible contraction). In all groups, the intensity throughout the session will be the highest tolerated by patient (except for sham stimulation group); the pulse width will be 400 μs; time relation on/off—10–20 s (1:2); and rise and fall times of 1 s. If accommodation occurs, the intensity will be increased according to the patient tolerance. However, if the patient does not respond to the increase of intensity and reports discomfort, the session will be interrupted.

A warm-up period will be performed in each session with an initial intensity titrated in 20% of the intensity used in the previous session, with a gradual increase of 20%/min until the fifth minute. A cool-down period of 5 min with a 20% gradual intensity decrease will be performed, except for the transition between low and high frequencies in the combined group (figure 2). In the sham stimulation group, the position of patient and the electrodes will be the same as in the other groups, but there will be no increase in intensity during the session. Besides, there should be no muscle contraction during NMES. The parameters will be frequency of 5 Hz, pulse width of 400 μs and the minimum intensity perceived by the patient. A trained physical therapist will accompany the patient throughout all NMES session and will collect the following data: perception of dyspnoea (Borg D) and lower limb fatigue (Borg F) by the modified Borg Scale; blood pressure (BP) which will be measured before, at 30 min, and after NMES; symptoms of pain, which will be measured at the end of the therapy using an visual analogue scale; heart rate (HR), which will be monitored every 10 min using a pulse oximeter (Infinity Gamma XL; Drager, Lübeck, Germany).

Outcome measures
Evaluations will be performed at baseline and after 24 sessions of NMES, on three alternate days before the HD sessions. On the first day, patient’s anamnesis will be performed; venous blood sample will be collected; inflammation and nutrition status assessment will be carried out; peripheral muscle function and QoL will be evaluated. On the second day, a body composition assessment and exercise tolerance evaluation will be performed. On the third day, assessment of peripheral muscle function will be conducted using isokinetic dynamometry of knee extensors bilaterally. The same researcher will assess the evaluations at the beginning and at the end of the protocol.

Biochemical markers
The venous blood will be collected by the nursing team of the HD centre through venous and/or arterial access to perform HD, so that a new puncture will not be necessary, and serum values of urea, creatinine, lactate, ferritin, albumin and IGF-1 will be analysed in the clinical analysis laboratory of a private hospital in São Paulo, Brazil. The values will be expressed in nanogram per millilitre.

Anthropometry
To perform anthropometry, a previously calibrated digital scale (Personal; Filizola, São Paulo, Brazil) will be used. The patients will be instructed to wear light clothes, remove their shoes when climbing on the scale and remain erect, with the head directed straight ahead until the scale stabilises the body mass. To measure height, a stadiometer (Personal, Filizola) will be used, and the subject must also be without shoes, with heels together and as erect as possible. Once anthropometric values (body mass and height) are obtained, the body mass index will be calculated using the following equation: body mass/height². The values will be expressed in kilogram per square metre.

Nutritional and inflammation status
Malnutrition and inflammation score (MIS) will be used to assess the inflammatory and nutritional status. The score assesses the reduction of body weight after HD session, self-reported functional capacity, subject assessment of fatigue and muscle mass reduction, caloric intake and gastrointestinal symptoms. Serum values of ferritin and albumin that make up MIS will be collected on the same day as the score is applied. The values will be expressed in points.

Body composition and cellular integrity
To assess body composition, the electrical bioimpedance (EB) will be performed (body composition monitor; Fresenius Medical Care, Renal Pharma, Wanchi, Hong Kong). To perform the exam, patients will be instructed...
to fast for 4 hours; abstinence from alcohol, physical activity and sauna for 8 hours; and emptying the bladder before performing the test. The patient must remain lying in the supine position. All metallic objects must be removed from patient’s proximity. A pair of self-adhesive and single-use electrodes will be adhered on the dorsal region of the hands and another pair on the dorsal region of the feet. In these places, the skin must be intact and be cleaned with 70% alcohol before the evaluation. The data obtained with EB will be saved on a magnetic card and later analysed by the software Frese-nius Medical Care (Renal Pharma). They will be analysed as variables: lean tissue index, fat tissue index, overhydration and phase angle (PA). The PA value will be obtained at the frequency of 50 kHz (figure 3). The values will be expressed in kilogram per square metre, percentage and degrees, respectively.

Peripheral muscle function

Medical Research Council (MRC)

Global peripheral muscle strength will be assessed using MRC. The patients will be asked to make six specific voluntary bilateral movements of the upper and lower limbs, and strength will be assessed and graded, with range from 0 (absence of muscle contraction) to 5 (muscle contraction capable of overcoming strong resistance). The values will be expressed in points.

Handgrip strength (HGS)

The HGS will be assessed using a manual hydraulic dynamometer (SH 5001; Saehan Corporation, Masan, Yangleok-Dong, South Korea), respecting the recommendations by the American Society of Hand Therapists. Patients will be instructed to remain seated on a chair, with the shoulders positioned in a neutral position, one hand resting on the thigh and the elbow of the limb to be measured kept flexed at 90°, with the forearm in neutral rotation. For all subjects, the dynamometer handle will be individually adjusted according to the hand size so that the shaft closest to the dynamometer body is positioned under the second phalanges of the index. Three evaluations on the dominant hand or on the limb without fistula will be measured, with 1 min of rest between each one. The best mark among three acceptable evaluations will be considered as the measure of HGS (figure 4A). The values will be expressed in kilogram-force.

Isokinetic dynamometry

To measure the maximum torque and fatigue index, an isokinetic dynamometer (Biodex System 3, dynamometer; Biodex Medical System, New York, USA) will be used. The patients will be seated on a chair with torso and hips stabilised by straps in order to avoid compensations in/during the execution of the movements. The assessment consists of three batteries of knee extension and flexion of both limbs: the first series with five repetitions at an angular speed of 60°, the second series with 10 repetitions at an angular speed of 180° and the third series with 30 repetitions at an angular speed of 240°. The series will have 30 s of rest between them. The patients will receive standardised verbal encouragement at the beginning, in the middle and at the end of each series. The incentive should be standardised for all individuals. The technical acceptability criterion will be a coefficient of variation of less than 30 at an angular speed of 60°. The muscle strength value will be obtained by means of the maximum torque value obtained during knee extension of the dominant limb at an angular speed of 60° in the series with five repetitions. The fatigue index will be calculated considering the data obtained in the third
series of 30 repetitions. The average peak torque of the first 10 repetitions and of the last 10 repetitions will be calculated. With these data, the decline of peak torque value will be obtained; the results should be expressed in percentage. Isokinetic dynamometry evaluation is shown in figure 4B. The values will be expressed in nanometre.

Exercise tolerance
The exercise tolerance evaluation will be performed using the 6 min step test, respecting the recommendations of the European Respiratory Society and the American Thoracic Society. This instrument is valid, reproducible and safe in healthy individuals and subjects with chronic diseases. For this test, a 15 cm-high step will be used against the wall, in order to ensure that the step does not move during the test. The patient will be instructed to place both feet on the step, then both feet on the floor, one foot at a time, and to perform as many repetitions as possible. Support will not be allowed continuously, only in case of imbalance. The patient will receive standardised verbal stimulus every minute. The test could be paused for the subjects if they feel it is necessary; however, the chronometer should be kept running. The Borg D, Borg F, BP, HR, respiratory rate (RR) and oxygen pulse saturation will be measured at the beginning, at the end and after 2 min of recovery.

For the predicted step values, the equation proposed by Arcuri et al will be used, with the adjustment for age and gender of the individuals. The test is shown in figure 5. The values will be expressed in number of steps and percentage of predicted.

QoL assessment
QoL will be assessed using the Kidney Disease Quality of Life Short Form. This questionnaire is a disease-specific measure that assesses patient’s perception of the impact of CKD on physical, socioeconomic and psychological aspects. The translation into Portuguese was performed as well as the cross-cultural validation of the instrument. The self-reported physical function has specific items regarding the perception of exercise tolerance, peripheral muscle strength and functional limitations. This domain will be used to statistical purposes, to avoid confusion factor, due to the risk of influence of other aspects covered by the questionnaire. The questionnaire will be applied by the same evaluator. The values will be expressed in points.

Statistical analysis
All statistical analyses will be performed using SigmaStat V.3.5 (SYSTAT Software, San Jose, California, USA). Data distribution will be analysed by Visual Inspection of Quantile–Quantile Plots and Density Plots. According to normality data distribution, data will be presented as mean and SD, or median and IQR. Student’s t-test will be used if the primary and secondary outcome measures conform to normal distribution or Wilcoxon signed-rank test to non-normal distribution. The intergroup rank-sum test will be used to compare the difference between two groups for primary and secondary outcome measures. All reported p values will be two-sided, and CIs will be at the 95% level. Linear mixed modelling will be used for each continuous dependent variable, with patients having correlated or uncorrelated random intercept and slope, with the following variables with fixed effect: variables of stratification, group of randomisation and the interaction of the group of randomisation by time. Log transformation will be used when the model does not converge. Multiple imputations will replace the missing values as a sensitivity analysis, and repeat linear mixed analyses will be performed on several imputed data sets. Rubin’s rule will be applied to consider the data sets’ variance. A p value of <0.05 will be considered statically significant.

ETHICS AND DISSEMINATION
This study will adhere to the principles of the Declaration of Helsinki. This study will be conducted at the HD centre of a private hospital in São Paulo, Brazil. The study will be approved by the ethics committee of the Hospital Sírio-Libanês, São Paulo, Brazil.

MODIFICATION OF THE PROTOCOL
Any modifications to the protocol, including changes of study objectives, study design, patient population, sample sizes, study procedures or significant administrative aspects, will require a formal application to the hospital as the clinical trial registry.

CONFIDENTIALITY
All study participants will be given an identification number throughout the trial to assure confidentiality. All
participants’ information will be stored in locked cabinets with limited access.

DISSEMINATION
The results of this study will be published in open-access and peer-reviewed journals and presented at relevant conferences.

PATIENT AND PUBLIC INVOLVEMENT
No patient was involved in the study.

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Contributors
IGM conceived the protocol and prepared the figures; WPY wrote the statistical analysis plan; CPB, DSF, IGM, LMF and WPY wrote the protocol; CL and CMMdB contributed to the study design. All authors read and approved the final manuscript.

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Competing interests
None declared.

Patient and public involvement
Patients and/or the public were not involved in the design, conduct, reporting or dissemination plans of this research.

Patient consent for publication
Not applicable.

Ethics approval
This study involves human participants and was approved by the ethics committee on research with humans of Hospital Sírio-Libanés (approval number 24337707). The participants gave informed consent to participate in the study before taking part.

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Supplemental material
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REFERENCES
1 Di Lullo L, House A, Gorini A, et al. Chronic kidney disease and cardiovascular complications. Heart Fail Rev 2015;20:259–72.
2 Schardong J, Marcolino MAZ, Plentz RDM. Muscle atrophy in chronic kidney disease. Adv Exp Med Biol 2018;1088:393–412.
3 Kooman JP, Dekker MJ, Usyvat LA, et al. Inflammation and premature aging in advanced chronic kidney disease. Am J Physiol Renal Physiol 2017;313:F938–50.
4 Musso CG, Jauregui JR, Macias Núñez JF. Frailty phenotype and chronic kidney disease: a review of the literature. Int Urol Nephrol 2015;47:1801–7.
5 Roshanravan B, Gamboa J, Wilund K. Exercise and CKD: skeletal muscle dysfunction and practical application of exercise to prevent and treat physical impairments in CKD. Am J Kidney Dis 2017;69:837–52.
6 Rosa CSdaC, Nishimoto DY, Souza GDE, et al. Effect of continuous progressive resistance training during hemodialysis on body composition, physical function and quality of life in end-stage renal disease patients: a randomized controlled trial. Clin Rehabil 2018;32:899–908.
7 Fernandes AdeO, Sena YADS, Xavier VB, et al. Functional and respiratory capacity of patients with chronic kidney disease undergoing cycle ergometer training during hemodialysis sessions: a randomized clinical trial. Int J Nephrol 2019;2019:7857824–7.
8 Ferrari F, Helal L, Dipp T, et al. Intradialytic training in patients with end-stage renal disease: a systematic review and meta-analysis of randomized clinical trials assessing the effects of five different training interventions. J Nephrol 2020;33:251–266.
9 Eidekam I, Haaber AB, Feldt-Rasmussen B, et al. Exercise training and the progression of chronic renal failure. Nephron 1997;75:36–40.
10 Dobask P, Homolka P, Svojanovsky J, et al. Intra-dialytic electrostimulation of leg extensors may improve exercise tolerance and quality of life in hemodialyzed patients. Artif Organs 2012;36:71–81.
11 Koh KP, Fassett RG, Sharman JE, et al. Effect of intradialytic versus home-based aerobic exercise training on physical function and vascular parameters in hemodialysis patients: a randomized pilot study. Am J Kidney Dis 2010;55:89–99.
12 Parry SM, Berney S, Koopman R, et al. Early rehabilitation in critical care (eRiCC): functional electrical stimulation with cycling protocol for a randomised controlled trial. BMJ Open 2012;2:e001891.
13 Schardong J, Stein C, De la Maa Plentz R. Neuromuscular electrical stimulation in chronic kidney failure: a systematic review and meta-analysis. Arch Phys Med Rehabil 2020;101:700–11.
14 Roxy RS, Xavier VB, Miorin LA, et al. Impact of neuromuscular electrical stimulation on functional capacity of patients with chronic kidney disease on hemodialysis. J Bras Nefrol 2016;38:344–50.
15 Schardong J, Dipp T, Bozetto CB, et al. Effects of intradialytic neuromuscular electrical stimulation on strength and muscle architecture in patients with chronic kidney failure: randomized clinical trial. Artif Organs 2017;41:1049–58.
16 Di Iorio B, Torraca S, Gustafsson P, et al. High-Frequency external muscle stimulation in acute kidney injury (AKI): potential shortening of its clinical course. Clin Nephrol 2013;79 Suppl 1:S37–45.
17 Klassen A, Racasan S, Gherman-Capriola M. High-tone external muscle stimulation in end-stage renal disease: effects on quality of life in patients with peripheral neuropathy. Clin Nephrol 2013;79 Suppl 1:S52–33.
18 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;98:825–31.
19 Garber CE, Blissmer B, Deschene MR, et al. American College of Sports Medicine. American College of sports medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, muscular, bone, and metabolic fitness in apparently healthy adults: guidance for prescribing exercise. Med Sci Sports Exerc 2011;43:1334–59.
20 Borg GA. Psychophysical bases of perceived exertion. Med Sci Sports Exerc 1982;14:377–81.
21 Kosoku A, Uchida J, Nishide S, et al. Association of sarcopenia with depression and body mass index in kidney transplant recipients. Sci Rep 2020;10:266.
22 Fetter RL, Bigogno FG, Oliveira FGP. Adaptation transcultural para portugues de instrumentos de evaluación do estado nutricional de pacientes en diálisis. Jornal Brasileiro de Nefrologia 2014;36.
23 Eickember M, Oliveira CC, Carneiro RAK. Bioimpedância elétrica E sua aplicação em avaliação nutricional. Revista de nutrição 2011;24:883–93.
24 Hough CL, Lieu BK, Caldwell ES. Manual muscle strength testing of critically ill patients: feasibility and interobserver agreement. Crit Care 2011;15:F43.
25 Fess EE. Grip strength. In: Casanova JS, ed. Clinical assessment recommendations. 2nd edition. Chicago: American Society of Hand Therapists, 1992.
26 Lopes AD, Grams ST, da Silva EF, et al. Reference equations for handgrip strength: normative values in young adult and middle-aged subjects. Clin Nutr 2018;37:914–8.
27 Franssen FME, Broekhuizen R, Janssen PP, et al. Limb muscle dysfunction in COPD: effects of muscle wasting and exercise training. *Med Sci Sports Exerc* 2005;37:2–9.
28 Murphy AJ, Wilson GJ, Pryor JF, et al. Isometric assessment of muscular function: the effect of joint angle. *J Appl Biomech* 1995;11:205–15.
29 Smidt GL, Rogers MW. Factors contributing to the regulation and clinical assessment of muscular strength. *Phys Ther* 1982;62:1283–90.
30 Pincivero DM, Lephart SM, Karunakara RA. Reliability and precision of isokinetic strength and muscular endurance for the quadriceps and hamstrings. *Int J Sports Med* 1997;18:113–7.
31 Neder JA, Nery LE, Shinzato GT, et al. Reference values for concentric knee isokinetic strength and power in nonathletic men and women from 20 to 80 years old. *J Orthop Sports Phys Ther* 1999;29:116–26.
32 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;44:1428–46.
33 Pessoa BV, Arcuri JF, Labadessa IG, et al. Validity of the six-minute step test of free cadence in patients with chronic obstructive pulmonary disease. *Braz J Phys Ther* 2014;18:228–36.
34 Carvalho LP, Di Thomazzo-Luporini L, Aubertin-Leheudre M, et al. Prediction of cardiorespiratory fitness by the six-minute step test and its association with muscle strength and power in sedentary obese and lean young women: a cross-sectional study. *PLoS One* 2015;10:e0145960.
35 Arcuri JF, Borghi-Silva A, Labadessa IG, et al. Validity and reliability of the 6-minute step test in healthy individuals: a cross-sectional study. *Clin J Sport Med* 2016;26:69–75.
36 Wegrzynowska-Teodorczyk K, Mozdzanowska D, Josiak K, et al. Could the two-minute step test be an alternative to the six-minute walk test for patients with systolic heart failure? *Eur J Prev Cardiol* 2016;23:1307–13.
37 José A, Dal Corso S. Step tests are safe for assessing functional capacity in patients hospitalized with acute lung diseases. *J Cardiopulm Rehabil Prev* 2016;36:56–61.
38 Duarte PS, Miyazaki MCOS, Ciconelli RM, et al. Tradução e adaptação cultural do instrumento de avaliação de qualidade de vida para pacientes renais crônicos (KDQOL-SF TM). *Revista da Associação Médica Brasileira* 2003;49:375–81.
39 Sesso CR, Lopes AA, Thomé FS. Inquérito brasileiro de diálise crônica 2015;38:54–61.