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

Igor Gutierrez Moraes, Camila Porto Brito, Davi de Souza Francisco, Larissa Martínez Faria, Claudio Luders, Christina May Moran de Brito, Wellington Pereira Yamaguti

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-Libanés (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...
function. The interventions using physical exercise are recommended, aiming to improve muscle function and exercise tolerance in this population. Initially, exercise training using cycle ergometer was implemented to improve exercise tolerance in this population, however, in periods outside of HD. Later, cycle ergometer started to be used during HD, although the use of standard types of exercise training may be limited by actual health conditions and haemodynamic instability.

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 individuals’ 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
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 20; 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 stabilizes 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/height^2. 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 fat 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 Fresenius 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 of 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.
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.

Author affiliations
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2Haemodialysis Centre, Hospital Sírio-Libanes, São Paulo, Brazil

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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-Libanes (approval number 24337707). The participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

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REFERENCES
# SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

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<th>Item No</th>
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<td>Title</td>
<td>1</td>
<td>Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym</td>
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<td>Trial registration</td>
<td>2a</td>
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<td></td>
<td>5c</td>
<td>Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities</td>
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<tr>
<td></td>
<td>5d</td>
<td>Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)</td>
<td>Not applicable</td>
</tr>
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### Introduction

**Background and rationale**  
6a Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention  
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6b Explanation for choice of comparators  
Page: 6 / Line 8

**Objectives**  
7 Specific objectives or hypotheses  
Page: 6 / Line 21

**Trial design**  
8 Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)  
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### Methods: Participants, interventions, and outcomes

**Study setting**  
9 Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained  
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**Eligibility criteria**  
10 Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)  
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**Interventions**  
11a Interventions for each group with sufficient detail to allow replication, including how and when they will be administered  
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11b Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)  
Page: 7 / Line 29

11c Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)  
Not applicable

11d Relevant concomitant care and interventions that are permitted or prohibited during the trial  
Not applicable

**Outcomes**  
12 Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended  
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**Participant timeline**  
13 Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)  
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<th><strong>Sample size</strong></th>
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<th>Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations</th>
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<td><strong>Allocation:</strong></td>
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<tr>
<td><strong>Sequence generation</strong></td>
<td>16a</td>
<td>Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions</td>
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<td><strong>Allocation concealment mechanism</strong></td>
<td>16b</td>
<td>Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned</td>
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<td><strong>Implementation</strong></td>
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<td>Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions</td>
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<td>17a</td>
<td>Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how</td>
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<tr>
<td></td>
<td>17b</td>
<td>If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant’s allocated intervention during the trial</td>
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<tr>
<td><strong>Methods: Data collection, management, and analysis</strong></td>
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<tr>
<td><strong>Data collection methods</strong></td>
<td>18a</td>
<td>Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol</td>
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<td></td>
<td>18b</td>
<td>Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols</td>
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### Data management

| Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol | Page: 10 / Line 6 |

### Statistical methods

| Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | Page: 14 / Line 6 |
| Methods for any additional analyses (eg, subgroup and adjusted analyses) | Page: 14 / Line 18 |
| Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | Page: 14 / Line 23 |

### Methods: Monitoring

| Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | Page: 15 / Line 31 |
| Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | Page: 1 / Line 22 |

### Harms

| Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct | Not applicable |

### Auditing

| Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | Not applicable |

### Ethics and dissemination

<p>| Plans for seeking research ethics committee/institutional review board (REC/IRB) approval | Page: 2 / Line 32 |
| Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) | Page: 15 / Line 1 |</p>
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<tr>
<td>Consent or assent</td>
<td>26a</td>
<td>Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)</td>
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<td></td>
<td>26b</td>
<td>Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable</td>
<td>Not applicable</td>
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<td>Confidentiality</td>
<td>27</td>
<td>How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial</td>
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<td>Ancillary and post-trial care</td>
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<td>Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation</td>
<td>Not applicable</td>
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<td>Dissemination policy</td>
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<td>Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions</td>
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<td></td>
<td>31b</td>
<td>Authorship eligibility guidelines and any intended use of professional writers</td>
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<td>31c</td>
<td>Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code</td>
<td>Not applicable</td>
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<td>Appendices</td>
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<td>33</td>
<td>Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable</td>
<td>Not Applicable</td>
</tr>
</tbody>
</table>

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.
Informed consent form

Study title: 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 randomised, double blind clinical trial

Phone: 55 11 97994-1836 or 55 11 3994-5331

You are invited to participate in a research project. Please read this document carefully before signing. If there are any words or phrases that you cannot understand, talk to the researcher responsible for the study or a member of the research team to clarify them.

This free and informed consent form aims to explain everything about the study and ask for your permission to participate.

Note: If the patient cannot read and/or understand this informed consent form, it may be signed and dated by a family member or legal guardian of the patient.

Study objectives
This study aims to evaluate the benefits of electrical stimulation of the thigh muscle during haemodialysis sessions in improving muscle performance, physical capacity and quality of life of patients undergoing haemodialysis.

Study duration
The forecast for your study participation will be two months during the haemodialysis period.

Study description
Approximately 56 individuals will participate in the study.

This study will be carried out at the haemodialysis centre and rehabilitation centre of Hospital Sírio-Libanês.

Requirements to participate: patients with chronic kidney disease dependent on classical haemodialysis; age ≥ 18 years; without pacemaker; no cognitive or motor impairment.

You cannot participate in this study if you cannot satisfactorily perform the assessments within the technical acceptability criteria or if you have more than two consecutive absences during the protocol or four absences in total.
Study procedure

After understanding and agreeing to participate, some procedures will be carried out as described below:

1) Application of a questionnaire to identify personal background, habits, and quality of life.

2) Assessment of weight and height.

3) Body composition assessment: To determine total water volume, muscle, fat, body mass and urea volume of distribution.

4) Handgrip Strength Test: This test will be performed to measure the strength of the arm muscles. To perform it, you will be seated in a chair and will press a manual dynamometer (a device with two parallel adjustable rods) with the maximum force possible according to the evaluator’s verbal orders. The arm without a fistula will be tested, performing three measurements, with a one-minute interval between them.

5) Medical Research Council (MRC): six specific movements of both arms and legs are voluntarily performed, and the strength is evaluated and graded which can vary from 0 (absence of movement) to 5 points (normal muscle strength) for each movement, totaling a maximum value of 60 points.

6) Muscle function: patients will be followed up at the Rehabilitation Centre at Hospital Sírio-Libanês to assess maximum strength and resistance during the knee-extending movement.

7) Six-minute step test: will be applied using a 15 centimeters high step leaning against the corner of the wall or in order to ensure that the step does not move during the test. You will have to go up and down the step for over six minutes, as many times as possible. Breaks will be allowed throughout the test according to your tolerance.

8) Laboratory tests: will be evaluated (urea, creatinine, ferritin, albumin, lactate, and IGF-1). All exams will be collected through the arteriovenous fistula without requiring a new puncture.

9) Finally, participants will be divided into four groups: low-frequency electrical stimulation, high-frequency electrical stimulation, low-and-high-frequency electrical stimulation, and minimal-frequency electrical stimulation. All groups receive the application of electric current in the thigh muscles for 60 minutes, three times a week for two months. Electrical stimulation will start from the second hour of haemodialysis. The results of all exams will be provided to you immediately upon completion of each assessment.

Potential risks, side effects, and discomfort

You will not feel any discomfort to answer the quality-of-life questionnaire as the questions are very simple. It is possible that you feel light tiredness and/or dizziness, pain in your legs during the six-minute step test. However, these symptoms might stop immediately after the end of each evaluation, as the tests will be performed with sufficient rest intervals for you to be able to recover and avoid the emergence of these undesirable symptoms. During electrical stimulation, you may feel tired in your legs. These exams are constantly performed on patients with kidney diseases and do not suffer significant risks or adverse situations.
Benefits for the participant

There is no direct benefit to the participant in this study. This is an evaluation study testing the hypothesis that electrical stimulation of the thigh muscles during haemodialysis sessions improves muscle performance, physical capacity and quality of life in patients undergoing haemodialysis.

Only at the end of the study can we conclude the presence of some benefit. However, the results obtained from this study may help to assess and treat patients on haemodialysis more accurately.

Compensation

There are no personal expenses for the participant in any phase of the study, including exams and training with electrical stimulation. There is also no financial compensation related to your participation. If there is any additional expense, it will be absorbed by the research budget.

Voluntary participation/withdraw from the study

Your participation in this study is entirely voluntary. You only participate if you want to.

Non-participation in the study will not imply any loss. After signing the consent, you can withdraw it at any time and stop participating in the study if you wish, without any prejudice.

New information

Any new information that may affect your safety or influence your decision to continue participating in the study will be provided to you in writing. If you decide to continue in this study, you will have to sign a new (revised) Informed Consent Form to document your knowledge of further information.

In case of research-related-damages

There is no compensation for damages for your participation in the research.

Use of medical records and confidentiality

All information collected and tested results will be analysed in a strictly scientific manner, maintaining the confidentiality (secret) of the participant at all times; that is, at no time will the data that identifies him be disclosed unless required by law.

Regulatory agencies and the Research Ethics Committee may inspect medical records bearing your identification and this signed consent form.

The results of this research may be presented at meetings or publications. However, your identity will not be revealed in these presentations.

Who should I contact if I have questions

At any stage of the study, you will have access to the professionals responsible for the research to clarify any doubts. Those responsible for the study at this institution are the physiotherapists Camila Porto Brito, Igor Gutierrez Moraes and Welington Yamaguti, who can be found at the Hospital Sírio-Libanês Rehabilitation Centre or on their respective telephones: 55 (11) 3994-4395 or 55 (11) 97994-1836.

In case of doubts or concerns about your rights as a participant in this study you can contact the Research Ethics Committee of this hospital through the telephone number 55 (11) 3994-8318 or e-mail: cepesq@hsl.org.br.
Consent form

I agree to participate in the study entitled: “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 randomised, double blind clinical trial”.

I have read and understood the consent document and the purpose of the study, as well as its possible benefits and risks. I had the opportunity to ask about the research, and all my doubts were clarified.

I understand that I am free to decide not to participate in this research.

I authorize using my evaluation data obtained by the researcher, regulatory authorities and the institution's Research Ethics Committee.

I will receive a signed and dated copy of this document.

I understand that I am not abdicating any of my legal rights by signing this document.

Volunteer name print: __________________________ Date: ____________

Volunteer signature: __________________________ Date: ____________

Signature of person obtaining consent: __________________________ Date: ____________

Initials of the participant or responsible: __________________________

Researcher rubric: __________________________