SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

Section/item	Item No	Description
Administrative information		
Title	1	Supervised exercise protocol for lower limbs in subjects with chronic venous disease: an evaluator-blinded randomized clinical Trial (page 1)
Trial registration	2a	RBR-57xtk7, Brazilian clinical trial database (September 19th, 2016) (page 18)
	2b	All items from the World Health Organization Trial Registration Data Set - the study follows all trial registration data set from WHO
Protocol version	3	28/10/2018– version 1 (page 17)
Funding	4	This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. Guilherme Augusto de Freitas Fregonezi is a fellow of the *Conselho nacional de Desenvolvimento Científico e Tecnológico* (*CNPq*) – process number 307353/2015-0. Vanessa Regiane Resqueti Fregonezi is a fellow of the *Conselho nacional de Desenvolvimento Científico e Tecnológico* (*CNPq*) – process number 310091/2015-2. (page 19)

Roles and responsibilities

5a **Names, affiliations, and roles of protocol contributors**

Authors: Volpe, Esther Fernandes Tinoco[1,2] ; Resqueti, Vanessa R[1,2] ; Ana Aline Marcelino da Silva [1,2]; Peroni Gualdi, Lucien [1,3]; Fregonezi, Guilherme A.F[1,2]

Afiliação Institucional: 1. PneumoCardioVascular Lab/HUOL Hospital Universitário Onofre Lopes, Empresa Brasileira de Serviços Hospitalares (EBSERRH) Departamento de Fisioterapia Universidade Federal do Rio Grande do Norte, Natal, Rio Grande do Norte, Brasil; 2. Laboratório de Inovação Tecnológica em Reabilitação, Departamento de Fisioterapia, Universidade Federal do Rio Grande do Norte, Natal, Rio Grande do Norte, Brasil ; 3. Faculdade de Ciências da Saúde do Trairi, Universidade Federal do Rio Grande do Norte (UFRN), Santa Cruz, Rio Grande do Norte, Brasil.

Esther Fernandes Tinoco Volpe was responsible for reviewing the literature, the development of the intervention protocol and for writing the full manuscript.

Vanessa R Resqueti was responsible for the development of the intervention protocol and reviewing the full manuscript.

Ana Aline Marcelino da Silva: will perform the blind evaluation.

Lucien Peroni Gualdi was responsible for writing and reviewing the full manuscript.

Guilherme A. F. Fregonezi was responsible for the final review and approval of the manuscript. (page 19)

5b Name and contact information for the trial sponsor - there is no trial sponsor.

5c **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.**

The study has no specific sponsor or funding from any public or private agency. Guilherme Fregonezi and Vanessa Resqueti are fellows of the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (*CNPq*) however, the national public agency has no authority over the protocol activities.

5d

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):

The protocol will be performed in a single center coordinated by professor GuilhermeA F Fregonezi. All previously described research will be responsible for the study performance in specific tasks.

A previously trained therapist (Ana Aline Marcelino da Silva) will perform the initial and final evaluation; Esther Volpe will perform the protocol and data entry in the database. Data management will be performed by Lucien Gualdi, Esther Volpe and Vanessa Resqueti.

No other individual or group will be allowed to see data without the study coordinator's permission.

Introduction

Background and rationale

6a

Triceps surae muscle changes and range of motion decrease which results from pathophysiological changes seen in subjects with CVI leading to important impairment of functional activities related to lower limbs in these individuals. Several studies have shown the benefits of exercise therapy focusing on triceps surae muscle strengthening for improving the function of the calf muscle pumping. Randomized studies using exercise programmes for lower limbs in subjects with CVI are still rare in the literature, leading to a weak indication of this modality to treat this population. The aim of this study is to investigate the effects of a supervised exercise programme to improve functional capacity and quality of life in individuals with CVI. (page 3)

6b

Explanation for choice of comparison subjects:

This study will perform a comparison between subjects submitted to a supervised exercise protocol and individuals that will receive educational information regarding the disease and usual treatment without any exercise intervention. The outcomes: plantar flexors strength and performance, ankle range of motion and exercise capacity were chosen because they were related to the main CVI-related impairments. Quality of life was chosen because it represents a more comprehensive concept of health.

Objectives	7	**Specific objectives and hypothesis:** Objective: Primary objective: To assess the efficacy of two months a supervised exercise programme to improve plantar flexors strength and performance and health-related quality of life compared to a usual treatment in individuals with CVI. Secundary objectives: To access the efficacy of two months a supervised exercise programme to improve ankle range of motion and exercise capacity compared to a usual treatment in individuals with CVI. Hypothesis: The authors hypothesized that a supervised lower limbs muscle-training program with triceps surae muscle strengthening in subjects with CVI will improve the calf strength and resistance and health-related quality of life in these individuals. (page 5)
Trial design	8	**Description of trial design including type of trial (e.g. parallel group, crossover, factorial, single group), allocation ratio, and framework (e.g. superiority, equivalence, non-inferiority, exploratory):** This is an evaluator-blinded randomized clinical trial. Individuals will be randomly allocated in two groups: 1) treatment group (TG) which will be submitted to a supervised exercise programme for the lower limbs; 2) control group (CG) in which usual treatment will be maintained without the performance of any supervised exercise modality. Participants of both groups will participate in a health education speech. (pages 5 and 6)

Methods: Participants, interventions, and outcomes

Study setting	9	**Description of study settings (e.g. community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained:** Evaluations will be performed at the Pneumocardiovascular laboratory, and the intervention protocol will be performed at the Physical Therapy Office, both located in a University Hospital in the city of Natal/RN, Brazil. (page 5)

Eligibility criteria	10	**Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (e.g. surgeons, psychotherapists):** Individuals of both genders aged between 35 and 69 years old, with chronic venous insufficiency diagnosis through venous vascular echo-Doppler examination, CEAP criteria between 2 to 6, without peripheral arterial disease (PAD) (ankle-brachial index ≤ 0.9) will be included in the study. Subjects who do not agree to participate in the study, present ulcers with diameter greater than 4 cm or with clinical signs and/or confirmed diagnosis of infection will be excluded. Individuals unable to attend the physiotherapy service twice a week and/or presenting clinical manifestations that are incompatible with moderate to intense exercise (according to the guidelines of the American College of Sports Medicine - ACSM) (ASCM, 2005), such as acute or uncontrolled congestive heart failure, uncontrolled or unstable angina, uncontrolled cardiac dysrhythmia causing hemodynamic symptoms, severe symptomatic aortic stenosis, recent deep venous thrombosis, recent pulmonary embolism, acute pericarditis or myocarditis, dissecting aneurysms (known or suspected), unstable or uncontrolled blood pressure (Systolic pressure > 160 mmHg, diastolic pressure > 100 mmHg), acute systemic infection, or uncontrolled diabetes, as well as subjects with limiting musculoskeletal diseases or difficulty to understand the activities will also be excluded. (pages 6 and 7)

Interventions	11a	All subjects will be invited to participate in an educational speech about the disease, risk factors, lifestyle changes, and lower limb care (hygiene, exercises, dressings), as well as the benefits of using compressive techniques. The speech will be performed by a physical therapist. Compression stocking for those subjects that are not using compressive techniques will be prescribed based on clinical severity with 20 to 30 mmHg for patients with CEAP C2 to C3, 30 to 40 mmHg for those with CEAP C4 to C6 and 40 to 50 mmHg for those with recurrent ulcers (Eberhard, Raffetto , 2014). The exercise protocol will consist of aerobic training, strengthening, step-up/down exercises and flexibility. Aerobic exercises will be performed using an ergometric bicycle and/or a cycle ergometer. Muscle strengthening will be performed through resistive load to the triceps surae muscles. Flexibility of the triceps surae and tibialis anterior will be performed by active stretching 24 hours after the training. The protocol will last around 40 minutes and will be performed twice a week, totaling 16 exercise sessions. Heart rate and blood pressure will be checked at the beginning and end of the training, as well as at the end of each series. The individual will be asked about perceived fatigue measured by the modified BORG scale (BORG, 1982). Subjects will perform 5 minutes of cycling without load at the beginning of the protocol for warm-up. Aerobic training will be subsequently performed using the bicycle for 15 minutes. The load will be set to reach moderate intensity (between 4 and 6 on the modified BORG scale 0-10). For strengthening of the triceps surae muscles, calf raise exercises will be performed in its full range. Submaximal load will be calculated individually based on momentary muscle failure (characterized as the inability to perform concentric contraction without significant posture change and repetition duration changes against a certain resistance), and exercise prescription will follow the standardization of 3 sets of 10 repetitions. The exercises will be performed without any load during initial sessions. Successive load progression will be made maintaining the same volume according to the patient's performance. The load will be applied using an adjustable weight vest, according to a calculation for each subject. The step up exercise will be performed on a rubber step at a height of 20 cm. Subjects should go up and down the step with one foot at a time using free cadence. They will be instructed to perform the movement as fast as possible for 12 repetitions. Progression will be according to individual tolerance. During the program execution, the load may be decreased, the rest time increased or the session interrupted if the subject reports very intense perceived fatigue (7 or above) through the BORG scale, complains of limiting pain or presents any symptom incompatible with physical activity. The exercise protocol is described in the supplementary file. Participants will only perform exercises supervised by the physiotherapist responsible for the study protocol. Subjects will receive a written and illustrated guide for performing active stretching of triceps surae and tibialis anterior muscles at home 24 hours after supervised training (Hallegraeff et al., 2012). (pages 8-10)

11b **Criteria for discontinuing or modifying allocated interventions for a given trial participant (e.g. drug dose change in response to injury, participant request, or improving/worsening disease):**

Individuals showing exercise limitation due to pain, those who changed usual medication, submitted to any alternative treatment or those who miss three consecutive intervention sessions will be excluded from the study. Collected data will be included in the database for further analysis even after exclusion.

11c **Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (e.g. drug tablet return, laboratory tests**):

Subjects will receive a follow-up guide containing questions regarding compressive therapy, stretching and lower limb positioning during rest. (page 10)

11d **Relevant concomitant care and interventions that are permitted or prohibited during the trial:**

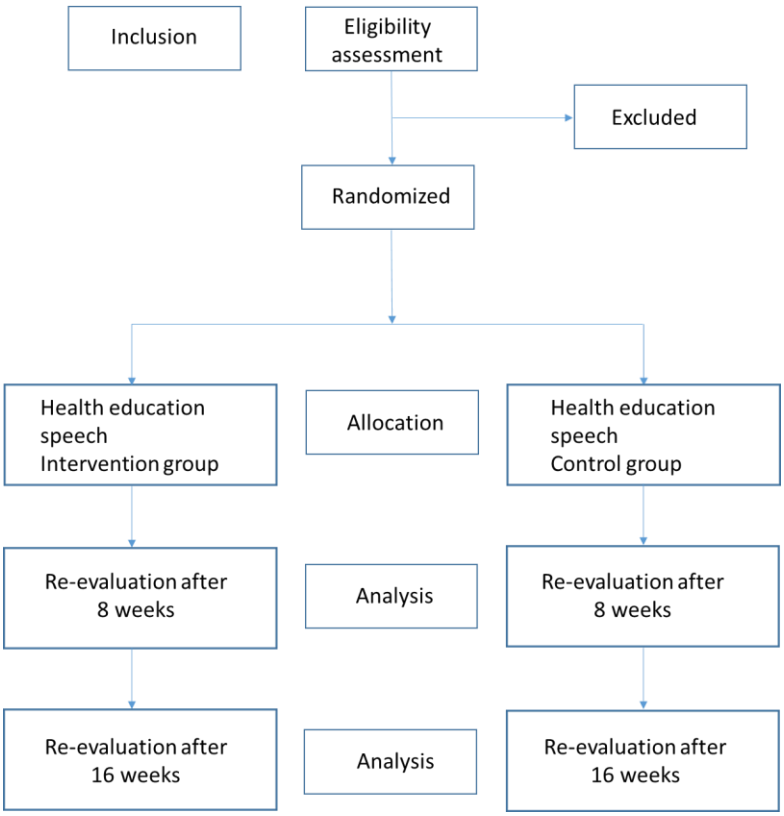
Controls will be instructed to be instructed to maintain their usual activities and treatments. During this period, individuals (independent of the allocation group) will not be able to perform any elective surgery or other treatment modality for venous disease other than the one usually used. (page 11)

Outcomes

12 **Primary, secondary, and other outcomes, including the specific measurement variable (e.g. systolic blood pressure), analysis metric (e.g. change from baseline, final value, time to event), method of aggregation (e.g. median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended:**

The study will compare the evaluation and revaluation results between subjects submitted to the protocol and a control group by the following outcomes: range of motion (mean increase of joint angulation), physical performance by the heel rise test (mean increase of elevations and time of test performance) physical capacity by the step test (ST6) (mean increase of ups and downs on the step) and quality of life by the VEINES-QOL (values increase for the domains 1, 4, 5 and 8; and values decrease for the domains 3, 6 and 7). Positive changes of these parameters will be able to guide the therapist on the appropriate treatment to patients with CVI. (pages 11-14)

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):** All protocol dates are described along the main manuscript. (Figure 1 of the main manuscript).



Sample size	14	**Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations**: The sample size is based on a prior study conducted by the authors using the difference between two independent means and the standard deviation of the heel rise test. Calculated effect size was 1.67. An α error of 0.05 and 95% power were considered. After the analysis the final sample size resulted in 18 subjects. The G*Power version 3.1 statistics program was used. (page 7)
Recruitment	15	**Strategies for achieving adequate participant enrolment to reach target sample size:** Subjects will be recruited at the medical clinic of the University Department of Clinical Medicine in the city of Natal/RN- Brazil. This outpatient facility has five physicians specialized in vascular surgery to whom inclusion and exclusion criteria were presented by personal contact. Physicians were asked to refer all subjects who met the inclusion criteria of the study. (pages 7 and 8)

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	**Method of generating the allocation sequence (e.g. computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (e.g. blocking) should be provided in a separate document that is unavailable to those who enroll participants or assign interventions:** The randomization.com program will be used by the researcher responsible for the study to randomize the participants. The program will randomly allocate individuals into two groups (control or intervention). Stratification procedures to ensure the balance between the groups in two strata (CEAP 2 and 3) and (CEAP 4 to 6) will be used. The subjects will be able to access the randomization result after the end of the evaluation. The responsible researcher will contact the individuals by telephone to initiate the treatment. (page 8)
Allocation concealment mechanism	16b	**Mechanism of implementing the allocation sequence (e.g. central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned:** The researcher responsible for the study will contact subjects to initiate the treatment by phone.

Implementation	16c	**Who will generate the allocation sequence, who will enroll participants, and who will assign participants to interventions:** The researcher responsible for the study will generate the allocation sequence, subjects' enrollment in the physical therapy service and the exercise protocol application.
Blinding (masking)	17a	**Who will be blinded after assignment to interventions (e.g. trial participants, care providers, outcome assessors, data analysts), and how:** The researcher who will perform the initial and final evaluations will be blinded to the subjects' allocation groups. Participants will be instructed to not make any comments regarding the allocation group. The evaluator will not have access to the treatment site where the protocol will be applied to reduce the possibility of interfering with its blinding. (page 8)
	17b	**If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial:** Non-blinding will not be allowed and the evaluator will have no access to the allocation group until the end of the study.

Methods: Data collection, management, and analysis

Data collection methods	18a	**Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (e.g. duplicate measurements, training of evaluators) and a description of study instruments (e.g. 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:** Data collection at baseline and revaluation will be performed by a previously trained physical therapist that will use a protocol for the outcomes regarding the questionnaires (VEINES-QOL, sociodemographic characteristics and CEAP clinical classification), equipment and software (physical tests and ankle-brachial index). For the ABI the highest of the three measures for each limb will be used. For the performance of physical tests, the evaluator will give the participants a brief explanation allowing the individuals to simulate the movement before the test. Data regarding SEMG and Physioflow will be collected according to the recommendations of the superficial electromyography for non-invasive muscle evaluation (SENIAM), the European Union project for Biomedical Health, the research program and the manufacturers' recommendations (PhysioFlow Q-Link). All data will be available in the patients' evaluation form and in the computer folder. Data access will only be permitted to researchers previously allowed by the study coordinator. The exercise protocol application will be performed by a physical therapist specialized in exercise physiology. All data registered during the treatment protocol will be attached to the patients' file.

18b **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**:

A follow-up report will be available to all subjects of the intervention group. The document will include evaluation and re-evaluation information for the next medical appointment. The evaluator will refer to the individual's physician for those excluded from the study due to ABI index above or below pre-established values.

Data management

19 **Plans for data entry, coding, security, and storage, including any related processes to promote data quality (e.g. double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol**:

Data will be stored in one of the laboratory computers and double entry will be performed by two study researchers. Data access will be limited to the study researchers and any other access must be authorized by the coordinator.

Statistical methods

20a **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:**

The Shapiro-Wilk test will be used to verify the sample normality. Two-way ANOVA with Benferroni post hoc test will be used for intra and intergoup analysis. GraphPad Prism version 6.0 (GraphPad Software Inc., San Diego California USA) will be used for the analysis. The significance level was set at 95% ($p<0.05$).

20b **Methods for any additional analyses (e.g. subgroup and adjusted analyses):**

The study has no additional analyses planned.

20c **Definition of analysis population relating to protocol non-adherence (e.g. as randomised analysis), and any statistical methods to handle missing data (e.g. multiple imputation):**

Even those subjects excluded from the study due to three consecutive absences will be included in the analysis according to the original group allocation according to the recommendations of CONSORT.

Methods: Monitoring

Data monitoring	21a	**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:** Data will be monitored by the study coordinator and posteriorly accessed by all the researchers involved in the study by previous authorization. There will not be an independent database as the study has no sponsor.
	21b	**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:** An intermediate analysis may be performed for those individuals who miss the protocol for three consecutive days or those who refuse to participate in the study for any reason. Subjects reporting any discomfort regarding exercise performance will be assessed by a physician, and the decision to continue in the protocol will be made by the physician and the study coordinator. Intermediate analysis will be not possible for those individuals who continue in the study protocol. All interim data will be kept by the study coordinator. If the study needs to be closed for any reason, the decision on how to analyze and publish data will be made by the study coordinator.
Harms	22	**Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct:** Any adverse effect that occurs during the protocol performance will be reported in the follow-up guide and the information will be referred to the patient's physician.
Auditing	23	**Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor:** The responsible researcher will supervise the protocol application. Participants will be instructed to perform flexibility exercises the day after the protocol performance. Flexibility exercise instruction and monitoring of eventual doubts will be conducted by the responsible researcher.
Ethics and dissemination		
Research ethics approval	24	**Plans for seeking research ethics committee/institutional review board (REC/IRB) approval:** The study was approved by the ethics and research committee of the responsible institution (number 1.541.241) and registered on the Brazilian Clinical Trials Database (REBEC) (RBR57xtk7). (page 18)

Protocol amendments	25	**Plans for communicating important protocol modifications (e.g. changes to eligibility criteria, outcomes, analyses) to relevant parties (e.g. investigators, REC/IRBs, trial participants, trial registries, journals, regulators):** If any important protocol change is necessary, the researchers will communicate it to the institution ethics committee, as well as the Brazilian clinical trials database for approval.
Consent or assent	26a	**Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how? (see Item 32):** All participants will sign the informed consent form that will be explained by the evaluator before the evaluation.
	26b	**Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable:** Not applicable.
Confidentiality	27	**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:** All collected data will be kept confidential and only the study researchers will have access to it.
Declaration of interests	28	**Financial and other competing interests for principal investigators for the overall trial and each study site:** The study investigators have no financial or other conflicting interests. Professors Guilherme Fregonezi and Vanessa Resqueti are *CNPq* fellows. However, this public agency has no authority to interfere in the protocol or its results.
Access to data	29	**Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators:** All previously described researchers will have full access to the final database. The use of data will be discussed in monthly meetings.
Ancillary and post-trial care	30	**Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation:** Medical assistance will be provided to any participant who presents any injury caused by the study participation in accordance with the resolution 466/12 of the National Health Council.

Dissemination policy	31a	**Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (e.g. via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions:** The results will be disseminated by scientific events presentations and publication in peer reviewed journals.
	31b	**Authorship eligibility guidelines and any intended use of professional writers:** The TRIALS
	31c	**Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code:** Not applicable.

Appendices

Informed consent materials	32	**Model consent form and other related documentation given to participants and authorised surrogates:** The consent form model followed the Brazilian model for informed consent and was approved by the responsible ethics committee.
Biological specimens	33	**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**: Not applicable.