Effects of Different Ischemic Preconditioning Occlusion Pressures on Muscle Damage Induced by Eccentric Exercise: A Study Protocol for a Randomized Controlled Placebo Clinical Trial

Eduardo Pizzo Junior (✉ eduardopizzojr@hotmail.com)
Universidade Estadual Paulista Julio de Mesquita Filho Faculdade de Ciencias e Tecnologia Campus de Presidente Prudente
https://orcid.org/0000-0001-6028-6432

Allysiê Priscilla de Souza Cavina
Universidade Estadual Paulista Julio de Mesquita Filho Faculdade de Ciencias e Tecnologia Campus de Presidente Prudente

Leonardo Kesrouani Lemos
Universidade Estadual Paulista Julio de Mesquita Filho Faculdade de Ciencias e Tecnologia Campus de Presidente Prudente

Taíse Mendes Biral
Universidade Estadual Paulista Julio de Mesquita Filho Faculdade de Ciencias e Tecnologia Campus de Presidente Prudente

Carlos Marcelo Pastre
Universidade Estadual Paulista Julio de Mesquita Filho Faculdade de Ciencias e Tecnologia Campus de Presidente Prudente

Franciele Marques Vanderlei
Universidade Estadual Paulista Julio de Mesquita Filho Faculdade de Ciencias e Tecnologia Campus de Presidente Prudente

Study protocol

Keywords: Ischemic Preconditioning, Exercise, Musculoskeletal Pain, Creatine Kinase, Muscle Fatigue, Stress, Physiological.

DOI: https://doi.org/10.21203/rs.3.rs-65492/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License
Abstract

Introduction: due to its mechanical advantage and less metabolic demand, eccentric exercise (EE) has been widely used in rehabilitation and for improving physical fitness. However, EE can induce muscle damage, leading to structural alterations and reduced muscle function, making it necessary to seek alternatives to reduce this damage caused by stress. Thus, ischemic preconditioning (IPC) could represent an aid to reduce the damage caused by EE, because it can mitigate the ischemia-reperfusion injury, and can be used to accelerate the post-exercise recovery process.

Objectives: to compare the effects of IPC, using different occlusion pressures, on the acute and delayed responses of perceptual outcomes and on the markers of muscle damage, in addition to verifying whether the technique causes deleterious effects on performance in recovery after eccentric exercise.

Methods: a randomized controlled placebo clinical trial will be carried out with 80 healthy men aged 18 to 35 years who will be randomly divided into four groups: IPC using total occlusion pressure (TOP), IPC with 40% more than TOP, placebo (10 mmHg), and control. The IPC protocol will consist of four cycles of ischemia and reperfusion of five minutes each. All groups will perform an EE protocol, and assessments will be carried out before, immediately after, and 24, 48, 72, and 96 hours after the end of the EE to evaluate creatine kinase (CK), blood lactate, perception of recovery using the Likert scale, being sequentially evaluated, pain by the visual analog scale (VAS), pain threshold using a pressure algometer, muscle thickness by ultrasound, muscle tone, stiffness and elasticity by myotonometry, vectors of cell integrity through electrical bioimpedance (BIA), and maximal voluntary isometric contraction (MVIC) using the isokinetic dynamometer. The trial was registered at ClinicalTrials.gov (NCT04420819).

Discussion: the present study aims to present an alternative technique to reduce muscle damage caused by EE, which is easy to apply and low cost. If the benefits are proven, IPC could be used in any clinical practice that aims to minimize the damage caused by exercise, presenting an advance in the prescription of EE and directly impacting on the results of post-exercise recovery.

Trial registration number: ClinicalTrials.gov, (30765020.3.0000.5402). Registered on 19 May 2020.

Introduction

Resistance training is the most commonly used training method for maintaining and improving strength and muscle mass (1), being characterized by concentric or eccentric muscle contractions (2, 3). Evidence indicates that eccentric training programs have been widely used in musculoskeletal rehabilitation or for improving physical fitness (4, 5). The lower metabolic demand and lower activation of motor units make eccentric exercise (EE) an alternative to obtain better hypertrophic adaptations when compared to concentric exercise, promoting greater mechanical efficiency and generating a greater amount of strength (2, 6).

Thus, this exercise presents an interesting alternative for clinical use and should be encouraged in the rehabilitation process, to improve performance and prevent injuries. However, it must be considered that EE produces marked acute responses, structural damage, and reduced muscle function (7). Due to the high force exerted on these deformable units, deformation occurs, which causes muscle stiffness, and may influence the risk of injuries (8).
Exercise-induced muscle damage (EIMD) is characterized by morphological alterations, decreased performance with reduced range of motion and strength, edema, delayed-onset muscle pain, and muscle proteins in the blood, especially creatine kinase (CK).

Therefore, considering that muscle damage can be harmful, several strategies are applied to minimize it, including massage, photobiomodulation, cryotherapy and compressive clothing, which are widely used in the post-exercise recovery period \(^9^-^1^2\). Some pre-exercise strategies are also used as a way to minimize these damages, especially ischemic preconditioning (IPC) \(^1^3\), which is characterized by the application of short periods of circulatory occlusion (ischemia) and reperfusion of a limb in the minutes or hours preceding the exercise, through insufflations and deflations of a pressure cuff \(^1^4^-^1^5\).

IPC was first presented by Takarada et al. and consists of three to four series of ischemia and reperfusion, with ischemia being maintained for five minutes and reperfusion for three minutes \(^1^6\). Patterson et al. demonstrated that this strategy can be administered from one to two times a day, applying three to five cycles of ischemia and reperfusion, with five minutes of ischemia and reperfusion varying from three to five minutes \(^1^7\).

This strategy has been considered attractive, because in addition to being low-cost and non-invasive, it has easy clinical applicability and provides an ergogenic aid to improve physical performance \(^1^5\). Recent studies have emphasized the effectiveness of IPC in assisting post-exercise recovery \(^1^8\), which can reduce the damage caused by ischemia-reperfusion injury after EIMD. These responses occur due to an increase in local blood flow that activates ATP-sensitive potassium channels, increasing levels of adenosine and/or reducing the inflammatory response \(^1^3^-^1^9,^2^0\).

Thus, its application before exercise can reduce the magnitude of muscle damage and subsequent pre-inflammatory responses, directly stimulating the body's physiological defense system and improving tissue integrity \(^1^3,^2^1\). Therefore, some form of post-exercise recovery measurement is required, which can be acquired through a Likert recovery perception scale that ranges from 0 to 10 \(^2^2\).

However, studies have been trying to determine the best prescription parameters for this strategy. Cocking et al. \(^2^3\) and Lindsay et al. \(^2^4\) analyzed the IPC response dose. The first study \(^2^3\) observed whether different IPC protocols improve muscle endurance, with a four-cycle and an eight-cycle protocol. The results showed that the four-cycle protocol demonstrated an improvement in resistance performance compared to the eight-cycle protocol. The second study \(^2^4\), on the other hand, verified whether IPC applied different numbers of times per day improves performance, however, daily frequency was not a determining factor for improving performance \(^2^3,^2^4\). Another factor that can determine the results of IPC is the pressure of occlusion generated in the period of ischemia, however, no studies were found that analyzed these effects in the recovery period after an EIMD.

Based on the above, it is hypothesized that IPC will provide a protective effect against eccentric EIMD, reducing muscle pain and improving clinical indicators, without, however, impairing muscle function. In addition, these responses are expected to be more evident in the higher occlusion pressure since there is a greater increase in blood flow and high levels of post-ischemic adenosine. In addition, factors related to the IPC response dose may influence responses in post-exercise recovery. Therefore, the aim of the present study is to compare the effects of IPC, using different occlusion pressures, on acute and delayed responses to perceptual outcomes and on muscle...
damage markers, in addition to verifying whether the technique causes deleterious effects on performance in post-exercise recovery.

**Methods**

**Study design**

This is a simple blind placebo controlled clinical trial conducted at the Center for Studies and Care in Physiotherapy and Rehabilitation at Universidade Estadual Paulista (FCT/UNESP), Presidente Prudente, SP, Brazil. The trial was registered at ClinicalTrials.gov (NCT04420819) and approved by the Research Ethics Committee of FCT / UNESP, Presidente Prudente, SP, Brazil (CAAE: 30765020.3.0000.5402).

The study protocol follows the SPIRIT 2013 checklist (Standard Protocol Items: Recommendations for International Trials)\(^{(25)}\) (Supplementary File 1) and the TiDieR (Template for Intervention Description and Replication)\(^{(26)}\), so that the information and quality of reports of interventions are well described\(^{(27)}\).

**Participants and population analysis**

For this study, the sample will consist of 80 healthy male individuals classified as active by the International Physical Activity Questionnaire (IPAQ)\(^{(28)}\), aged between 18 and 35 years, who will be recruited from a database of the Sports Physiotherapy Laboratory of FCT/UNESP and the community, through dissemination folders and posters on the institution's premises, social networks, and advertisements in the local media. These procedures are recommended by Treweek et al.\(^{(29)}\) as strategies to improve participant recruitment.

Individuals who exhibit one or more of the following characteristics will not be included: (1) the presence of any health condition that contraindicates or prevents EE; (2) diabetes and diagnosed arterial hypertension; (3) inflammatory, psychiatric, cardiovascular, and/or respiratory rheumatological disease; (4) being an alcoholic, using drugs, and/or being a smoker; (5) history of knee surgery (for example, meniscal repair and ligament reconstruction) or recent musculoskeletal injury to the lower limbs that may impair performance during tests or interventions (for example, muscle injury, tendinopathy, patellofemoral pain in the lower limbs, and/or back pain in the previous six months); (6) involvement in any type of training program during the study period; (7) engaging in a lower limb strength training program during the three months prior to participating in the study; (8) use of ergogenic supplements to improve physical performance and/or muscle mass and/or vasoactive drugs; (9) having one or more risk factors predisposing to thromboembolism\(^{(30)}\).

Participants will be excluded from the study if they: present any health problem that does not allow continuity, use medication, electrotherapy or other therapeutic means that may interfere with any result, perform unusual or strenuous physical activities during the evaluation period, or wish to leave the study.

Participants will be instructed in advance not to perform any physical activity or to use any therapeutic form of pain relief or performance improvement during the collection. They will also be instructed not to consume alcohol during the procedure. If there are episodes of musculoskeletal injuries, individualized physiotherapeutic treatment will be offered.

The flowchart of the study design and the composition of the groups is illustrated in figure 1.
Randomization

The randomization sequence will be developed by a researcher who will not be involved in recruiting, evaluating, or training participants using software (Microsoft Office Excel 2007) and will be placed in sequential numbering in opaque and sealed envelopes. There will be blinding of the evaluators and statistical analysis procedures. The experimental protocol will be supervised by trained physiotherapists who are not involved in the randomization process or assessments. Due to the nature of the interventions, the participants who perform the exercise will not be blind to the allocation of the groups.

Participants who meet the eligibility criteria will be randomly allocated in a 1:1:1:1 ratio to one of the four groups, namely:

1. **IPC-TOP**: this group will carry out baseline assessments, perform IPC using exactly the TOP, then perform post-IPC assessments and start EE. Post-exercise assessments will take place immediately after the end of EE and will be repeated at 24h, 48h, 72h, and 96h.

2. **IPC-40%**: this group will perform baseline assessments, perform IPC using 40% more occlusion than TOP, then perform post-IPC assessments and start EE. Post-exercise assessments will take place immediately after completion of the EE and will be repeated at 24h, 48h, 72h, and 96h.

3. **IPC-10mmHg**: this group will carry out baseline assessments, perform the occlusion-perfusion intervention with 10mmHg restriction characterizing the placebo, and then perform post-IPC assessments and start EE. Post-exercise assessments will take place immediately after completion of the EE and will be repeated at 24h, 48h, 72h, and 96h.

4. **CONTR**: this group will carry out the baseline assessments, and immediately afterwards start the EE. Post-exercise assessments will take place immediately after the end of the EE and will be repeated at 24h, 48h, 72h, and 96h.

Study design

Data collection will be carried out at the Center for Studies and Assistance in Physiotherapy and Rehabilitation (CEAFIR) of FCT/UNESP, respecting the time from 17h to 22h. All procedures will be performed under standard conditions (temperature: 21-23°C; relative humidity: 40-60%). Each participant will attend the clinic for five consecutive days. Initially, participants will be assessed for anthropometric characteristics, using a scale (Tanita BC 554, Iron Man/Inner, Arlington Heights Illinois, USA) and a stadiometer (Sany - American Medical do Brasil, São Paulo, Brazil) from which the body mass index (BMI) will be calculated.

After these initial procedures, the TOP evaluation will be carried out. After a 10-minute rest, baseline outcome assessments will be performed. Initially, CK and blood lactate will be collected, followed by application of the scales of perception of recovery and muscle pain and the pain threshold. In the supine position, the participant will rest for 10 minutes before the ultrasound, myotonometry, and bioelectrical impedance (BIA) evaluations are performed. Subsequently, MVIC will be evaluated. Next, the participants will perform the previously randomized 40-minute IPC protocol. Evaluations will be carried out immediately after the IPC protocol of all analyzed outcomes except for ultrasound. The same order of execution of the evaluations will be maintained. Thereafter, EE will be initiated and immediately after the end of the EE, all outcomes will be collected again. Subsequent visits will be carried out 24, 48, 72, and 96 hours after the EE, in which the same outcomes mentioned above will be collected, maintaining the same order of execution of the evaluations. The study design is outlined in figure 2.
TOP determination

After assessing the anthropometric parameters, the participants will be asked to lie down for ten minutes (31). All participants will be instructed to avoid strenuous exercise and alcohol intake within 48 hours of the TOP assessment.

For the determination of TOP, a transducer with Doppler equipment (DV-2001; Medpej, Ribeirão Preto, São Paulo, Brazil) will be used, which will be positioned over the posterior tibial artery to capture the auscultatory pulse located at the average distance between the medial malleolus tibia and Achilles tendon. A blood pressure cuff will be attached to the participant’s thigh close to the region of the inguinal crease of the dominant member (30) and will then be inflated to the point where the auscultatory pulse of the tibial artery is interrupted. TOP will be defined as the pressure at the moment when the arterial pulse is abolished, indicated by the absence of an auscultatory signal.

The vascular occlusion will be performed using an adapted blood pressure cuff (nylon, velcro, 175 mm wide and 920 mm long, JPJ - Hospital Materials Industry, São Paulo, Brazil). We opted for a wider cuff, as it has been proven that the width of the cuff has great influence on the pressure required to achieve total blood flow restriction (32).

IPC Protocol

The IPC protocol will be applied to the inguinal region of the dominant limb with the participants relaxed and comfortably positioned in the supine position. The same cuff used to determine TOP will be used and the protocol will consist of four cycles of total ischemia (TOP determined individually) of five minutes, followed immediately by four cycles of five minutes of vascular reperfusion (0 mmHg), totaling 40 minutes, as shown in figure 3.

To perform the IPC protocol, one of the study groups will use 40% more than the TOP, according to the study by Lopes et al. (33), the pressure at which the blood flow stops passing to the tibial artery varies from 140 to 160 mmHg and most studies performing IPC use pressures between 200 to 220 mmHg, thus, the values are corresponding (33). The other study group will use the exact TOP, since at this value there is an absence of blood flow.

Placebo preconditioning

Placebo preconditioning will be performed on the dominant thigh with the same cuff, similar to the IPC protocol described above (Figure 3), but with four cycles of five minutes of placebo occlusion (10mmHg), alternating with four cycles of five minutes of reperfusion (0 mmHg) (34, 35).

It should be mentioned that in the three study groups, participants will be previously informed that the applied occlusion pressure will be sufficient to improve performance and avoid muscle damage. In addition, all procedures will be performed individually, to prevent participants from talking to each other about the compression generated by the cuff.

EE Protocol

The EE will be performed on an isokinetic dynamometer (Biodex System 4 Pro, New York, New York, USA) for the knee extensor muscles of the dominant limb. Initially, five submaximal knee extension contractions will be performed for familiarization. Before each repetition, the dominant leg will be positioned at 30º of knee flexion.
The participant will be instructed to perform a knee extension, while the dynamometer, with its resistance, returns the leg to 90° flexion, at a speed of 60°/s (1.04 rad/s) executing a range of movement of 60° (30-90° of knee flexion).

The protocol is based on the study by Machado et al. (22), starting five minutes after familiarization and consisting of 5 sets of 15 maximum eccentric contractions of knee extension, with 30 seconds of rest between sets, totaling 75 repetitions. The speed and range of movement will be similar to the familiarization and verbal encouragement will be given throughout the protocol. According to the authors, this protocol is capable of promoting muscle damage (22).

**Primary and secondary outcomes and assessment points**

Primary outcomes will be related to muscle damage and muscle function through the quantification of creatine kinase, blood lactate, MVIC, and muscle thickness. In addition, there will be four measures of secondary outcomes: assessment of pain and pain threshold, assessment of recovery perception, myotonometry (muscle tone, stiffness, and elasticity), and electrical bioimpedance (resistance, reactance, and phase angle). All outcomes will be collected at the initial assessment with the exception of the perception of recovery, and immediately and 24, 48, 72, and 96 hours after the end of the EE. Ultrasonography will not be performed immediately after the exercise. The description of the specific moments of collection of outcomes can be seen in Table 1.
Table 1. Time points for outcomes

| Outcomes                              | Initial assessment | Immediately after exercise | Recovery        |
|---------------------------------------|--------------------|----------------------------|-----------------|
|                                       | 24 hours           | 48 hours                   | 72 hours        | 96 hours        |
| **Primary**                           |                    |                            |                 |
| **Muscle damage**                     |                    |                            |                 |
| CK                                    | √                  | √                          | √               | √               |
| **Blood lactate**                     |                    |                            |                 |
| [Lact+]                               | √                  | √                          | √               | √               |
| **Muscle strength**                   |                    |                            |                 |
| MVIC                                  | √                  | √                          | √               | √               |
| **Muscle thickness**                  | Ultrasound         |                            |                 |
| √                                     | √                  | √                          | √               | √               |
| **Secondary**                         |                    |                            |                 |
| **Pain**                              |                    |                            |                 |
| VAS                                   | √                  | √                          | √               | √               |
| **Pain threshold**                    |                    |                            |                 |
| Algometer                             | √                  | √                          | √               | √               |
| **Perception of recovery**            | Likert scale       |                            |                 |
| √                                     | √                  | √                          | √               | √               |
| **Muscle tone, stiffness and elasticity** | Myotometry         |                            |                 |
| √                                     | √                  | √                          | √               | √               |
| **Resistance, reactance and phase angle** | Electrical bioimpedance |                  |                 |
| √                                     | √                  | √                          | √               | √               |

Details of procedures

Creatine Kinase (CK)

Plasma CK concentration will be obtained by means of 32 μL of capillary blood collected from the digital pulp. This puncture will take place by means of a lancet with automatic trigger, after cleaning the location with 95% ethyl alcohol and drying with cotton. The blood sample will be drained into a heparinized capillary tube and then pipetted into a reactive CK strip for analysis on the Reflotron Plus System (Roche Diagnostics, Mannheim, Germany) using the reflection photometry method at 37 °C (test temperature). The test strips will be kept at a storage temperature of 2 to 8 °C, according to the manufacturer's instructions. The Reflotron System method allows fast and reliable measurement of CK levels (36).

Blood lactate concentration

To assess the participant's blood lactate concentration, 25 ml of blood will be collected from an ear lobe capillary. Heparinized capillaries and polyethylene Eppendorf tubes (1.5 ml) containing 50 μL of sodium fluoride (NaF - 1%) will be used. The analyses will be performed on a lactimeter (YSI, Yellow Springs - 1,500) (37) and the lactate values will be expressed in mmol/L. The lactate will be collected at the following moments: pre IPC protocol, pre...
EE, immediately after the end of EE, and in the 1st, 3rd, 5th, 7th, 9th, 11th, 13th, and 15th minutes, and 24 h, 48 h, 72 h, and 96 h after the intervention.

**Perception of recovery**

The perception of recovery of the lower limb submitted to the EE protocol will be assessed using a 10-point Likert Scale, where 1 indicates “not recovered” and 10 “fully recovered” (22). The scale will be presented to the participants and, so that they are not influenced by the researcher, they will answer the question: "From 1 to 10 points, how do you rate the perception of recovery felt in your lower limb at this moment?" (22,382). This scale will be applied immediately after the end of the EE, and 24, 48, 72, and 96 hours after the intervention.

**Muscle pain and pain threshold**

Muscle pain will be measured using the visual analog scale (VAS). Participants will be asked to rate their exercise-induced leg pain, on the scale, which ranges from 0 “no pain” to 10 “extreme pain” (39).

To assess the pain threshold, a pressure algometer will be used, which is a reliable and validated instrument (FPX 50/220; Wagner instruments, Greenwich, Connecticut, USA) (40). The pressure algometer will be applied 4 cm above the base of the patella, 15 cm below the antero-superior iliac spine (ASIS), midpoint between the patella and the antero-inferior iliac spine (AIIS), and 2 cm medial and 2 cm lateral in relation to the midpoint. The pain threshold will be defined in kgf and will not exceed 2.55 kgf, as suggested by Jönhagen et al. (41).

**Muscle thickness**

The evaluation of the muscular structure will be carried out using ultrasound images of the participant's dominant lower limb, which will be captured using Siemens Sonoline Sienna equipment (Issaquah, WA, USA), together with a linear matrix transducer (48 mm, 7, 5 MHz) to determine the thickness of the rectus femoris (RF) and vastus lateralis (VL) muscles. Anatomical reference points and skin marks will be drawn on transparent sheets to ensure similar positioning of the ultrasound transducer in the same location in the evaluations.

The images will be taken between the midpoint of the greater trochanter and the lateral condyle of the femur (42). The ultrasound transducer will be covered with water-soluble transmission gel and positioned perpendicular to the skin over the RF and VL and oriented parallel to the muscle fascicles (42). The alignment of the transducer will be considered adequate when several fascicles can be drawn without interruption through the image (42).

**Myotonometry**

MyotonPRO (MyotonAS, Tallinn, Estonia) will be used to measure tonus, stiffness, and elasticity of the quadriceps femoris muscle (43). The device will be positioned at 2/3 between the ASIS and the upper pole of the patella (44), at the midpoint between them and 2 cm to the medial and 2 cm to the side, 15 cm from the AIIS and 4 cm from the base of the patella.

The device will be positioned perpendicular to the evaluated region, with light pressure. This preload is controlled and corresponds to 0.18 N of initial compression of the subcutaneous tissue, after which an additional impulse of 0.40 N will be released, with a duration of 15 ms. This impulse will induce oscillation in the tissue, which will be damped or deteriorated (45).
BIA

BIA will be assessed using tetrapolar electrodes (BIA Analyzer, Nutritional Solutions Corporation, Harrisville, MI, USA, f = 50 kHz, and 800 µA) \(^{(46, 47)}\). Global and localized evaluations will be carried out. The global assessment will take place with the participant in the supine position; the electrodes will be positioned on the dominant hand (base of the 3rd metacarpal phalanx and between the styloid process of the radius and the head of the ulna) and on the dominant foot (at the base of the 3rd metacarpal phalanx and in the anterior region of the ankle, between the malleoli). The localized evaluation will be carried out to evaluate the quadriceps femoris muscles; the electrodes will be positioned five centimeters below the EIAS and above the base of the patella. The BIA components analyzed will be resistance R, reactance (Xc), and phase angle (phA) \(^{(47, 48)}\). The analysis of the tolerance ellipse will also be performed. The Bioscan program: BL-960141 (Biologica, Barcelona, Spain) will be used for the analyses \(^{(46, 47)}\).

MVIC

For MVIC evaluation, the participant will be positioned with the dominant lower limb on the Biodex System Pro isokinetic dynamometer (Biodex Medical System, Shirley – NY, USA). According to the protocol suggested by Baroni et al. \(^{(49)}\), prior to the evaluation, the volunteer will be submitted to a warm-up, which will consist of ten repetitions of concentric contractions of knee flexion-extension at 180º/s throughout the range of motion.

Muscle function will be assessed by means of the highest torque value obtained between three repetitions of five seconds of maximum voluntary contraction at 60º of knee flexion (with 0º corresponding to the maximum extension). A two-minute interval between repetitions will be administered in order to minimize the possible effects of fatigue. The participant will be instructed to give their maximum strength and will be verbally encouraged by the researcher in each maximum voluntary contraction.

Sample calculation

The sample calculation was performed based on the study by Bailey et al.\(^{(50)}\), using the CK variable, since this variable expresses the amount of muscle protein in the blood representative of indirect muscle damage. Considering the standard deviation in the CK concentration of 200 µL, using a two-tailed hypothesis test with 80% test power and 5% significance level, a sample size of 20 participants per group was stipulated, totaling 80 participants.

Statistical analysis

The normality of the data will be verified by the Kolmogorov Smirnov test. If normality is detected, the sample characterization variables will be presented as mean and standard deviation, if not, as median and interquartile range. To compare the characterization of the sample, one-way ANOVA with Tukey's post-test or Kruskal-Wallis with Dunn's post-test will be used depending on the normality of the data.

The comparisons of the outcomes between the four groups studied and the moments will be performed using the technique of analysis of variance for repeated measures model in the two-factor scheme, which will provide information on the effects of time, group, and interaction. The repeated measurement data will be checked for sphericity violation using the Mauchly's test and the Greenhouse-Geisser correction will be used when the sphericity is violated. For moment analysis, Bonferroni's post-test for parametric distribution or Dunn's post-test for
non-parametric distribution will be used and the analysis between the groups will be performed using One-Way ANOVA or the Kruskal Wallis test.

In addition, the effects of the groups studied will be verified for all the outcomes assessed by calculating the effect size (ES) using Cohen’s d, considered as “null” (<0.2), “small” (≥0.2), “moderate” (≥0.6), “large” (≥1.2), or “very large” (≥2.0) (51).

An intention-to-treat analysis will be performed using the patient’s most recent assessment in case of withdrawals or absence of data. The level of significance will be p <0.05 for all tests. The statistical program SPSS (version 24.0) (SPSS Inc., Chicago, IL, USA) will be used for the analyses.

Discussion

Potential impact and significance of the study

This IPC protocol follows the application time pattern found in the literature, however, with different occlusion pressures. Thus, the study findings could bring a new strategy to reduce muscle damage caused by exercise, based on the dose response. Thus, if the hypothesis of the present study is proven, physically active people could benefit from the technique, since it is easily accessible and applicable, in addition to being low cost.

It is also worth noting that the study aims to analyze the participants’ perceptions of the different IPC protocols, especially pain and recovery. Thus, these results may add elements that favor the use of IPC protocols and guarantee the adherence of this pre-exercise strategy.

Strengths And Weaknesses Of The Study

A strong point of the study is the comparison between two different IPC protocols with a placebo group and a control group, thus making it possible to observe the true results found from each intervention. The fact that the technique uses only a pressure cuff makes it easy to apply and access, so that it can be implemented in prevention and rehabilitation centers. Another positive point of the study is the monitoring of the effects of the technique for up to 96 hours after the intervention, in addition to the high methodological quality of the study characterized by prospective registration, randomization, blinding, and intention to treat approach. However, a limitation of the study is the fact that the therapist and participants are not blind. Finally some participants may be injured due to the strenuous protocol of eccentric exercise performed.

Contribution And Clinical Applicability

As already described, the technique is easy to apply, low cost, and non-invasive. It can be applied in different environments of prevention, rehabilitation, and functional recovery. The data obtained in the present study could be used for better application of the technique in physiotherapy, especially in sports clinical practice, considering the periods of training and, especially, competition, providing protection from EIMD.

It is also worth noting that this study includes the items on the checklist for protocol studies in order to minimize bias, and was prospectively recorded. The outcomes will be disseminated through publications in scientific journals and presentations at congresses in the area.
Abbreviations

BIA
Electrical bioimpedance
CEAFIR
Center for Studies and Assistance in Physiotherapy and Rehabilitation
CK
Creatine kinase
MVIC
maximal voluntary isometric contraction
EIMD
Exercise-induced muscle damage
LOMP
Late-onset muscle pain
EE
Eccentric Exercise
ASIS
Antero-superior iliac spine
AIIS
Antero-inferior iliac spine
VAS
Visual analog scale
I-R
Ischemia-reperfusion
IPC
Ischemic preconditioning
TOP
Total occlusion pressure
RF
Femoral rectum
TR
Resistance training
VL
Vastus lateralis

Declarations

Trial status

Number Protocol: NCT04420819

Patient recruitment is currently underway.

Study start date: August 2020
Primary completion date: December 2020

Study Completion date: July 2021

Acknowledgements

Not applicable.

Authors’ contributions

EPJ and FMV are responsible for the study design. EPJ, APSC, LKL, TMB, CMP and FMV commented on the various versions of this study protocol. EPJ, APSC, LKL and TMB will be involved in recruiting and collecting data. All authors approved the final manuscript.

Ethics approval and consent to participate

Ethical approval has been granted by the Human Ethics Committee of the São Paulo State University (CAAE: 30765020.3.0000.5402). A statement confirming informed consent will be obtained from all study participants.

Consent for publication

Not applicable.

Availability of data and materials

The spreadsheets containing the raw numeric data will be stored on two external hard drives and two "clouds". After analyzing the data, scientific articles related to the study will be made, and after publication, the data will remain preserved and will also be shared and made available in its entirety for a period of 5 years.

Competing interests

The authors declare that they have no conflict of interest.

Funding

Not applicable.

References

1. Walker S, Blazevich AJ, Haff GG, Tufano JJ, Newton RU, Häkkinen K. Greater strength gains after training with accentuated eccentric than traditional isoinertial loads in already strength trained men. Front Physiol. 2016;7(149):1–12.

2. American College of Sports Medicine. American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. Med Sci Sports Exerc. 2009;41(3):687–708.

3. Franchi MV, Atherton PJ, Reeves ND, Flück M, Williams J, Mitchell WK, Selby A, Beltran Valls RM, Narici MV. Architectural, functional and molecular responses to concentric and eccentric loading in human skeletal muscle. Acta Physiol (Oxf). 2014;210(3):642–54.
4. Suchomel TJ, Nimphius S, Bellon CR, Stone MH. The importance of muscular strength: training considerations. Sports Med. 2018;48(4):765–85.

5. Douglas J, Pearson S, Ross A, McGuigan M. Chronic adaptations to eccentric training: a systematic review. Sports Med. 2017;47(5):917–41.

6. Komi PV, Kaneko M, Aura O. EMG activity of the leg extensor muscles with special reference to mechanical efficiency in concentric and eccentric exercise. Int J Sports Med. 1987;8(Suppl 1):22–9.

7. Baroni B, Pinto R, Herzog W, Vaz M. Eccentric resistance training of the knee extensor muscle: training programs and neuromuscular adaptations. Isokinet Exerc Sci. 2015;23(3):183–98.

8. Rodriguez ECP, Watsford ML, Bower RG, Murphy AJ. The relationship between lower body stiffness and injury incidence in female netballers. Sports Biomech. 2017;16(3):361–73.

9. Bishop PA, Jones E, Woods AK. Recovery from training: a brief review. J Strength Cond Res. 2008;22(3):1015–24.

10. Machado AF, Micheletti JK, Lopes JSS, Vanderlei FM, Leal-Junior ECP, Netto Junior J, Pastre CM. Phototherapy on management of creatine kinase activity in general versus localized exercise: a systematic review and meta-analysis. Clin J Sport Med. 2018 [Epub ahead of print].

11. Machado AF, Ferreira PH, Micheletti JK, Almeida AC, Lemes IR, Vanderlei FM, Netto Junior J, Pastre CM. Can water temperature and immersion time influence the effect of cold water immersion on muscle soreness? A systematic review and meta-analysis. Sports Med. 2016;46(4):503–14.

12. Howatson G, Van Someren KA. The prevention and treatment of exercise-induced muscle damage. Sports Med. 2008;38(6):483–503.

13. Franz A, Behringer M, Nosaka K, Buhren BA, Schrumpf H, Mayer C, et al. Mechanisms underpinning protection against eccentric exercise-induced muscle damage by ischemic preconditioning. Med Hypotheses. 2017;98:21–7.

14. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation. 1986;74(5):1124–36.

15. Slyszt JT, Burr JF. Impact of 8 weeks of repeated ischemic preconditioning on running performance. Eur J Appl Physiol. 2019;119(6):1431–7.

16. Takarada Y, Takazawa H, Ishii N. Applications of vascular occlusion diminish disuse atrophy of knee extensor muscles. Med Sci Sports Exerc. 2000;32(12):2035–9.

17. Patterson SD, Hughes L, Warmington S, Burr J, Scott BR, Owens J, et al. Blood flow restriction exercise: considerations of methodology, application, and safety. Front Physiol. 2019;10:533.

18. Beaven CM, Cook CJ, Kilduff L, Drawer S, Gill N. Intermittent lower-limb occlusion enhances recovery after strenuous exercise. Appl Physiol Nutr Metab. 2012;37(6):1132–9.

19. Page W, Swan R, Patterson SD. The effect of intermittent lower limb occlusion on recovery following exercise-induced muscle damage: a randomized controlled trial. J Sci Med Sport. 2017;20(8):729–33.

20. Wang WZ, Baynosa RC, Zamboni WA. Therapeutic interventions against reperfusion injury in skeletal muscle. J Surg Res. 2011;171(1):175–82.

21. Franz A, Behringer M, Harmsen JF, Mayer C, Krauspe R, Zilkens C, et al. Ischemic preconditioning blunts muscle damage responses induced by eccentric exercise. Med Sci Sports Exerc. 2018;50(1):109–15.
22. Machado AF, Almeida AC, Micheletti JK, Vanderlei FM, Tribst MF, Netto Junior J, Pastre CM. Dosages of cold-water immersion post exercise on functional and clinical responses: a randomized controlled trial. Scand J Med Sci Sports. 2017;27(11):1356–63.
23. Cocking S, Wilson MG, Nichols D, Cable NT, Green DJ, Thijssen DHJ, et al. Is there an optimal ischaemic preconditioning dose to improve cycling performance? Int J Sports Physiol Perform. 2018;13(3):274–82.
24. Lindsay A, Petersen C, Ferguson H, Blackwell G, Rickerby S. Lack of a dose response from 7 days of ischemic preconditioning in moderately trained cyclists. Sports Med Int Open. 2018;2(4):E91-7.
25. Chan AW, Tetzlaff JM, Altman DG, Laupacis A, Gotzsche PC, Krle A-Jeric K, et al. SPIRIT 2013 Statement: defining standard protocol items for clinical trials. Rev Panam Salud Publica. 2015;38(6):506–14.
26. Yamato T, Maher C, Saragiotto B, Moseley A, Hoffmann T, Elkins M, et al. The TIDieR checklist will benefit the physical therapy profession. Braz J Phys Ther. 2016;20(3):191–3.
27. Yamato TP, Maher CG, Saragiotto BT, Moseley AM, Hoffmann TC, Elkins MR, et al. The TIDieR checklist will benefit the physiotherapy profession. Physiother Can. 2016;68(4):311–2.
28. Matsudo S, Araújo T, Marsudo V, Andrade D, Andrade E, Oliveira, IC, et al. Questionário internacional de atividade física (IPAQ): estudo de validade e reproducibilidade no Brasil. Ver Bras Ativ Fis Saúde. 2001;6(2):5–18.
29. Treweek S, Lockhart P, Pitkethly M, Cook JA, Kjeldstrom M, Johansen M, et al. Methods to improve recruitment to randomised controlled trials: Cochrane systematic review and meta-analysis. BMJ Open. 2013;3:e002360.
30. Dankel SJ, Buckner SL, Counts BR, Jesse MB, Mouser JG, Mattocks KT, et al. The acute muscular response to two distinct blood flow restriction protocols. Physiol Int. 2017;104(1):64–76.
31. Hughes L, Jeffries O, Waldron M, Rosenblatt B, Gissane C, Paton B. Influence and reliability of lower-limb arterial occlusion pressure at different body positions. PeerJ. 2018;6:e4697.
32. Crenshaw AG, Hargens AR, Gershuni DH, Rydevik B. Wide tourniquet cuffs more effective at lower inflation pressures. Acta Orthop Scand. 1988;59(4):447–51.
33. Lopes TR, Sabino-Carvalho JL, Ferreira THN, Succi JE, Silva AC, Silva BM. Effect of ischemic preconditioning on the recovery of cardiac autonomic control from repeated sprint exercise. Front Physiol. 2018;9:1465.
34. Barbosa TC, Machado AC, Braz ID, Fernandez IA, Vianna LC, Nobrega AC, et al. Remote ischemic preconditioning delays fatigue development during handgrip exercise. Scand J Med Sci Sports. 2015;25(3):356–64.
35. Seeger JPH, Timmers S, Ploegmakers DJM, Cable NT, Hopman MTE, Thijssen DHJ. Is delayed ischemic preconditioning as effective as running performance during a 5 km time trial as acute IPC? J Sci Med Sport. 2016;20(2):208–12.
36. Horder M, Hafkenscheid JCM, Carstensen CA. Creatine kinase determination: a European of creatine kinase determination in serum, plasma and whole blood with the Reflotron System. Eur J Clin Chem Clin Biochem. 1991;29(10):691–6.
37. Bastos FN, Vanderlei LC, Nakamura FY, Bertollo M, Godoy MF, Hoshi RA, et al. Effects of cold water immersion and active recovery on postexercise heart rate variability. Int J Sports Med. 2012;33(11):873–9.
38. Brancaccio P, Maffulli N, Buonauro R, Limongelli FM. Serum enzyme monitoring in sports medicine. Clin Sports Med. 2008;27(1):1-s18.
39. Ferreira-Valente MA, Pais-Ribeiro JL, Jensen MP. Validity of four pain intensity rating scales. Pain. 2011;152(10):2399–404.
40. Kinser AM, Sands WA, Stone MH. Reliability and validity of a pressure algometer. J Strength Cond Res. 2009;23(1):312–4.

41. Jönhagen S, Ackermann P, Saartok T. Forward lunge: a training study of eccentric exercises of the lower limbs. J Strength Cond Res. 2009;23(3):972–8.

42. Baroni BM, Geremia JM, Rodrigues R, De Azevedo Franke R, Karamanidis K, Vaz MA. Muscle architecture adaptations to knee extensor eccentric training: rectus femoris vs. vastus lateralis. Muscle Nerve. 2013;48(4):498–506.

43. Ho CS, Lee MC, Chang CY, Chen WC, Huang WC. Beneficial effects of a negative ion patch on eccentric exercise-induced muscle damage, inflammation, and exercise performance in badminton athletes. Chin J Physiol. 2020;63(1):35–42.

44. Aird L, Samuel D, Stokes M. Quadriceps muscle tone, elasticity and stiffness in older males: reliability and symmetry using the MyotonPRO. Arch Gerontol Geriatr. 2012;55(2):31–9.

45. Feng YN, Li YP, Liu CL, Zhang ZJ. Assessing the elastic properties of skeletal muscle and tendon using shearwave ultrasound elastography and MyotonPRO. Sci Rep. 2018;8(1):17064.

46. Nescolarde L, Piccoli A, Román A, Núñez A, Morales R, Tamayo J, et al. Bioelectrical impedance vector analysis in haemodialysis patients: Relation between oedema and mortality. Physiol Meas. 2004;25(5):1271–80.

47. Piccoli A, Rossi B, Pillon L, Buccianti G. A new method for monitoring body fluid variation by BIA: The RXc graph. Kidney Int. 1994;46(2):534–9.

48. Norman K, Stobäus N, Pirlich M, Bosy-Westphal A. Bioelectrical phase angle and impedance vector analysis—clinical relevance and applicability of impedance parameters. Clin Nutr. 2012;31(6):854–61.

49. Baroni BM, Leal Junior EC, De Marchi T, Lopes AL, Salvador M, Vaz MA. Low level laser therapy before eccentric exercise reduces muscle damage markers in humans. Eur J Appl Physiol. 2010;110(4):789–96.

50. Bailey DM, Erith SJ, Griffin PJ, Dowson A, Brewer DS, Gant N, Williams C. Influence of cold-water immersion on indices of muscle damage following prolonged intermittent shuttle running. J Sports Sci. 2007;25(11):1163–70.

51. Cohen J. Statistical Power Analysis for the Behavioral Sciences. 2 ed. New York: Routledge; 1988.