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STUDY PROTOCOL

Effects of ischemic preconditioning on neuromuscular and biochemichal variables in paralympics athlets during a detraining period: Study protocol

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Abstract

Background: Ischemic pre-conditioning (IPC) is one of the forms of imposing this ischemia, alternating complete vascular occlusion and reperfusion before exercise, to pre-condition varied physiological functions. In the sporting context, there are moments when an athlete interrupts his physical activities, resulting in a reduction in strength and muscle mass. Accordingly, a hypothesis arises that the IPC will be able to reduce training effects, speeding up the process of the athlete's return to his sporting activities. **Objective:** To evaluate the effects of IPC on various neuromuscular forces (isometric force, explosive force and electromyography) and markers of oxidative stress and muscle damage (Creatine Kinase, Malondialdehyde and antioxidant capacity), in paralympic athletes, subject to periods of discontinuity from training loads. **Methods:** The sample will be composed of 38 young adult athletes (20 to 40 years old), of both sexes, who will compete in competitions at a national level in their own way. These will be randomly divided into 2 groups: experimental (IPCG: n = 19), and control (CG: n = 19). The GIPC will be subject to 4 weeks of IPC twice a day (40 weeks). Both groups will be validated before, and after 2 and 4 weeks of surgery, as regards: body composition, lower limb strength, explosive strength and electromyography (EMG), as well as biochemical markers Creatine Kinase (CK), Malondialdehyde (MDA) and antioxidant capacity (CAT). These data will be analyzed in the Statistical Package for the Social Science (SPSS - 25.0). Initially, we will test the normality (Shapiro-Wilk test), homogeneity (Levene test) and sphericity (Mauchly test) of the data, followed by ANOVA (repeat tests) to analyze the effects of the protocols, before and after 2 and 4 weeks of intervention followed Bonferroni's post hoc tests, adopting a significance level of P 0.05, in all analyses. **Expected results:** We hope that the GIPC intervention will alleviate or inhibit the reduction of strength and muscular hypotrophy in our athletes during the period of discontinuation of training loads.

Keywords: Ischemia; reperfusion; muscle strength; biomarkers.

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BACKGROUND

Ischemic Preconditioning (IPC) is a method that consists of periods or cycles (generally 3 or 4 x 5 min) of ischemia, followed by periods of reperfusion of equal duration[1]. This exposure to brief circulatory occlusion and reperfusion periods protects local or systemic organs against subsequent ischemia-reperfusion injuries[2]. Since the discovery of this phenomenon in 1986, research has focused mainly on the clinical utility of IPC in protecting against organ damage and cellular injury, such as during myocardial infarc-

tion or perioperative periods[3]. In recent years, IPC has also been investigated in the sports area, where it has already been shown to be capable of attenuating the depletion of adenosine triphosphate (ATP), glycogen, and lactate production during prolonged ischemia. Furthermore, IPC can improve blood flow to skeletal muscles by inducing vasodilation, increasing functional sympatholysis, and preserving endothelium and microvascular function during stress. Based on these findings, IPC has attracted researchers' interest, such as a new intervention capable of improving performance during exercise[4].

Other studies have also investigated the effect of IPC on resistance exercise, high-intensity interval training, and isometric exercises, which were able to promote acute improvements in muscular strength and aerobic conditioning in men and women[5]. This, according to Tapuria et al.[6], is probably because acute molecular and vascular adaptations can promote local vasodilation, increase blood flow, and, finally, improve oxygen (O2) delivery, optimizing performance in various types of exercise. In the physiotherapeutic context, IPC has shown itself to be of particular interest because it can prevent or minimize muscle atrophy due to disuse in patients affected by injuries. Thus, Zargi et al.[7] found that IPC followed by a low-load knee extension exercise was able to mitigate the loss of volume, strength and function of the quadriceps femoris muscle, after ACL reconstruction.

Despite these evident benefits of IPC, the problem of the present study is the fact that, in the sporting context, there are times when the athlete interrupts his physical activities, such as in cases of injuries, or in transitional periods of training, with consequent reduction in strength and muscle mass. Detraining is the partial or complete loss of adaptations induced by physical training due to a reduction or cessation in exercise frequency, intensity, or duration. Just two weeks of reduced physical activity can induce catabolic events in skeletal muscle, decreasing muscle mass[8]. According to Hood[9], the relatively short half-life of mitochondrial proteins (approximately one week) can cause decreases in mitochondrial function and capacity after a short period of detraining. Likewise, a decrease in muscle oxidative capacity[10,11] and reduced mitochondrial enzymatic activities[10,12] were found after a few days/weeks of training interruption.

Therefore, studying IPC is justified both from a theoretical-scientific point of view and from the point of view of physiotherapeutic practice since the regression of physical capabilities as a result of the interruption of a training program is obvious. Furthermore, to date, no studies have been found in the literature testing the hypothesis that any IPC protocol inhibits atrophy. According this context, the objective of this study will be to evaluate the effects of IPC on neuromuscular variables (isometric strength, explosive strength, and EMG) and biochemical variables (markers of oxidative stress and muscle damage) in athletes undergoing periods of discontinuity in training loads.

METHODS

Characterization of the study

In controlled and randomized clinical studies, according to Berwanger et al.[13], the assignment of participants to treatment or exposure is under the investigator's control, being randomly assigned to any of the groups. Therefore, the groups will remain bal-

anced for known and unknown characteristics. Hence, the only difference between the groups is the intervention (experimental and control), ruling out the possibility of systematic errors.

Population and sample

The sample will be made up of 38 young adult athletes (20 to 40 years old) of both sexes, practicing Blind Football, Goalball, and Athletics, who compete in national and international level competitions in their modalities, divided into randomly two groups: 1 - IPC group (IPCG: n = 19) and 2 - control group (CG: n = 19).

Sample calculation

The sample size was calculated using the G*Power software, version 3.1.9 (Universitat Kiel, Germany), based on the study by Tanaka et al.[14], which investigated the impact of IPC on sustained isometric knee extension time until exhaustion. A two-sided significance level of 0.05, a correlation between groups of 0.5, and a power of 0.95 indicated that 38 participants would be needed.

Inclusion and exclusion criteria

Criteria Inclusion: the members of the groups must be athletes from sports requiring lower limb exercises and participating in Paralympic sports as long as their deficiencies do not involve lower limb impairment. Athletes must 1) be in a transitional period of at least 4 weeks between the end of a season and the beginning of the following season of training and competitions and 2) not have suffered an injury or interrupted training for any other reason in the 4 months before the study; 3) Do not use supplements during the study period.

Criteria Exclusion: athletes who 1) perform physical exercises that stimulate increased strength and hypertrophy in the transitional period will be excluded; 2) miss at least 3 consecutive or discontinued training sessions; 3) Start other physiotherapeutic procedures during the study period; 4); who experience severe pain when carrying out the procedure; 5) withdraw from the study.

Ethical aspects

The project was submitted to the Research Ethics Committee of the Health Sciences Center of the Federal University of Paraíba (CEP/CCS/UFPB), protocol no. 6.335.329 and CAAE: 71722623.7.0000.5188. Athletes will only be able to participate in the research by signing the Free and Informed Consent Form, in accordance with standards for research involving human beings—Resolution Nº. 466/2012 of the National Health Council.

Ischemic preconditioning protocol

The intervention protocol was based on the modified study by Lindner et al.[15], in which the GIPC will perform three series of 5 minutes of IPC, with 5 minutes of reperfusion, between series, in quadriceps muscles (rectus femoris, vastus medialis and vastus lateralis) and hamstrings (biceps femoris and semitendinosus), totaling 30 minutes, twice a day (morning and afternoon), from Monday to Friday, for four weeks (40 sessions). The pressure will promote total blood flow occlusion in the quadriceps muscles

(rectus femoris, vastus medialis, and vastus lateralis) and hamstrings (biceps femoris and semitendinosus). A blood flow restricted cuff (Cardiomed, Scientific Leg Cuff, Canadá), (dimensions 12.5 X 84 cm) will adequately obstruct blood flow in the distal region of the lower limbs. During treatment in the GIPC and CG, occlusion will be performed, with blood pressure cuffs applied to both lower limbs in the sublingual region of the upper thigh. The pressure will be established, individually, at 80% of the Vascular Occlusion Pressure (VOP) during the occlusion phase and 0% during the reperfusion phase. On the other hand, the pressure in the GC will be 50 mmHg during the occlusion phase and 0 mmHg during the reperfusion phase. GIPC and CG participants will be instructed to write down and report weekly on all physical activities (walking, domestic, and work activities) carried out throughout the day that require some physical effort.

Body composition

The analysis of body mass (BM), fat mass (FM), and skeletal muscle mass (MM) for the entire body and segmented by limbs, will be carried out using Dual Energy X-Ray Absorptiometry / DXA (Lunar Advance DF+ 13.4038 Radiation; GE Lunar Corporation —USA) from PAPGEF/UFPB, and an experienced researcher will analyze the data. The results of the DXA evaluation will be obtained through a full-body scan. Athletes will be instructed to go barefoot and wear shorts and a t-shirt, positioned supine on the equipment table, keeping the arms along the body, without using metallic material[16].

Dynamometry of knee extensors and flexors

Participants will initially warm up for 5 minutes on an exercise bike at a speed of 20 km/h, then test the isometric strength of the knee flexors and extensors. To do this, the subjects will position themselves seated, with their hips flexed at 110º, keeping the knee evaluated at an angle of 60° for the extensors and 30° for the flexors, using a Bonett chair (adapted), with a portable digital dynamometer (model DD-300, Instrutherm Ltd., BR) for measuring Maximum Voluntary Isometric Contraction – MVIC[17,18]. During the test, to stabilize the subjects, belts will be used on the trunk (diagonal), the pelvis, and the thigh (transverse), in addition to a leather strap on the ankle connected to a load cell, using a steel cable, forming a 90º angle with the leg. Strength measurements for knee flexion and extension will consist of a series of 3 MVIC, each maintained for 5 seconds, with a 30-second rest interval[19]. In each of them, the subjects received a vocal stimulus ("strength, force, force") by the same evaluator to find the average of the force peaks used in the statistical analysis[18].

Explosive strength (vertical jump)

The vertical jump test will be carried out to evaluate the explosive strength of the lower limbs, following the squat jump technique, with countermovement, in which the performer remains motionless in the squat position for a few seconds and waits for the command to jump, vertically, trying to reach the maximum height from the ground. Athletes will flex their knees to 90° followed by extension and keep their lower limbs extended during the jump, hands positioned on the waist so as not to use them when pushing; the landing will be performed with both feet simultaneously, remaining with the ankles in dorsiflexion, according to the protocol described by Coratella et al.[20]. Three

jumps will be performed, with 30-second intervals between them, using a contact mat (44 x 44 cm) and analyzed using the MultiSprint Full software version 3.5.7 (Hidrofit Ltda, Brazil). To familiarize themselves with the vertical jump test, with countermovement, athletes will perform five vertical jumps before the series is considered valid.

Electromyography

To record the electrical signal from the quadriceps muscles (rectus femoris, vastus medialis, and vastus lateralis) and Hamstrings (biceps femoris and semitendinosus), a 12-channel electromyograph (Ultium – EMG System - Noraxon – USA), Bluetooth, with the following technical characteristics: hardware with 24-bit analog-to-digital (A/D) conversion board, 1000 times gain amplifier, 20 to 500 Hz bandpass filter (2nd order Butterworth), mode rejection rate common (RRMC) >100 Db, signal noise ratio (< 1 µV RMS), input impedance 109 M , sensors with accelerometers, surface electrodes, bipolar, active, simple differential, pre-amplification 20 times and software (my research TM3) for collecting and analyzing signals with a sampling frequency of 4000 Hz.

The EMG capture protocol for the quadriceps and hamstrings will be carried out according to Surface Electromyography for the Non-Invasive Assessment of Muscles[21], with the electrodes positioned with bars perpendicular to the muscle fibers. After locating and marking the electrode fixation points with the individuals in the test position, trichotomy, abrasion, and skin cleaning will be carried out with 70% alcohol, and all preparation procedures and acquisition of EMG signals will be carried out by the same evaluator. Each individual will perform three attempts of 5 seconds during a maximal voluntary isometric contraction (MVIC), being stimulated with the command "force, force, force" during the contraction, and the central 3 seconds of each contraction will be used to process the data. , using the average Root Mean Square (RMS) values normalized by the peak of the EMG signal[21].

Biochemical markers of muscle damage and oxidative stress

Assessment of serum Creatine Kinase (CK) activity

A nursing technician will collect venous blood to determine serum CK activity. A volume of 5 ml of blood was removed from the antecubital vein and placed in test tubes without any anticoagulant, which was placed in a container with ice and taken to the laboratory. Approximately 20 minutes after each collection, the samples will be centrifuged at 3000 rpm for 15 minutes, and the supernatant will be placed in Eppendorfs tubes and refrigerated at -20° C until analysis. The plasma concentration of CK will be quantified by acquiring the commercial kit CK NAC Liquiform (Labtest, Minas Gerais, Brazil) using the UV-IFCC method[22]. The absorbance will be checked on the automatic analyzer Labmax 240 Premium® at a wavelength of 340nm from Hirose Electronic System Co., Ltd. (Tochigi, Japan).

Analysis of total antioxidant capacity

After collection, plasma samples will be placed in microtubes for subsequent analysis based on the method described by Brand-Williams, Cuvelier, and Berset[23], in which an aliquot of 1.25 mg of 2,2 diphenyl-1-picrylhydrasyl (DPPH) is diluted in 100 ml of ethanol (absolute ethyl alcohol 99.5%), kept refrigerated and protected from light. 3.9 ml

of DPPH solution will be added to 100 µL of plasma in tubes, which will then be vortexed and left to rest for 30 minutes. Subsequently, they will be centrifuged at 10,000 rpm at 20ºC for 15 minutes. The supernatant will be used to read on a spectrophotometer (Biospectro SP-22, Curitiba, Brazil) at a wavelength of 515 nm, and the results will be expressed as a percentage of antioxidant activity (AAO):

$$AAO = 100 – [DPPH•R]t / [DPPH•R]B\ 100)$$

Where [DPPH•R]t and [DPPH•R]B correspond to the concentrations of DPPH• remaining after 30 minutes, evaluated in the sample (t) and the blank (B) prepared with distilled water[23].

Malondialdehyde

The oxidizing activity will be quantified through the reaction of thiobarbituric acid (TBARS) with the decomposition products of hydroperoxides, according to the method described by Ohkawa, Ohishi, and Yagi[24]. For this, 250 µL of plasma will be incubated in a water bath at 37°C for 60 minutes, and then the sample will be precipitated with 35% AA perchloric acid and centrifuged at 14000 rpm for 20 minutes at 4°C. The supernatant will be transferred to new microtubes, to which 400µl of 0.6% thiobarbituric acid will be added and incubated at 100°C for 60 minutes. After cooling, the material will be read using an ultraviolet spectrophotometer (Biospectro SP-22, Curitiba, Brazil) at a wavelength of 532nm at room temperature.

Ankle-Brachial Index (ABI)

The ABI, a diagnostic test for screening and detecting peripheral arterial disease (PAD), is also a risk enhancer in the American Heart Association/American College of Cardiology guidelines on the primary prevention of atherosclerotic cardiovascular disease. It is associated with cardiovascular morbidity and mortality when the ratio is less than 0.9 and greater than 1.3, according to the measurement of Systolic Blood Pressure (SBP) of the upper limbs (brachial artery) and lower limbs at ankle height – arteries: posterior tibial or pedal artery[25]. To measure systolic blood pressure (SBP), a portable high-frequency vascular Doppler will be used: 5 to 10 MHz (MedPeg® DV – 2001, Ribeirão Preto, SP, Brazil). Participants will position themselves on a stretcher, in the supine position, and will remain at rest for 10 minutes, being instructed not to cross their arms or legs and not to speak during the procedure. Subsequently, the SBP measurements of each blood vessel will be obtained rotationally, with 2-minute intervals between them[26], and the ABI will be calculated bilaterally, using the following formula: right ABI = SBP of the right ankle/SBP of the right arm; Left ABI = right ankle SBP/left arm SBP.

Statistical treatment

The data will be analyzed using the Statistical Package for the Social Sciences software (25.0 IBM, Chicago, IL). After performing the Shapiro Wilk, Levene, and Mauchly tests to evaluate the normality, homogeneity, and sphericity of the data, respectively, an ANOVA for repeated measures will be carried out to verify the effects of the intervention protocols (Pre and post-2 and four weeks of intervention) in the variables lean lower limb mass, CK, MDA, CAT, explosive strength (vertical jump), isometric strength

and EMG of the quadriceps muscles (rectus femoris, vastus medialis and vastus lateral) and Hamstrings (biceps femoris and semitendinosus), followed by the Bonferroni post hoc test, adopting a significance level of $P \leq 0.05$, in all analyses. The Kruskal-Wallis test will be used if the data does not meet the normality assumptions.

RESULTS

It is expected that the intervention in the IPCG will attenuate or may inhibit the reduction of strength and muscular hypotrophy in athletes in a period of discontinuation of training loads, potentially leading to significant improvements in their performance and health.

Conflict of interest: The authors declare no conflict of interest in this study.

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