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Effects of Ischemic Preconditioning on Functional and Neuromuscular Performance of Lower Limbs of Male Amateur Soccer Players: Protocol for a Randomized Trial

I. Martins de França[1], M. S. Cerqueira[1,2], S. Bezerra da Nobrega Neto[1], C. Augusto de Menezes Araújo[1], Y. M. Barros de Sousa Oliveira[1], W. H. de Brito Vieira[1]

[1] *Neuromuscular Performance Analysis Laboratory, Department of Physical Therapy, Federal University of Rio Grande do Norte (UFRN), Natal (RN), Brazil*

[2] *Research Group in Neuromuscular Physiology, Department of Biological Sciences, Universidade Estadual do Sudoeste da Bahia (UESB), Jequié (BA), Brazil*

CORRESPONDING AUTHOR:
Wouber Hérickson de Brito Vieira
Neuromuscular Performance Analysis Laboratory
Department of Physical Therapy
Federal University of Rio Grande do Norte (UFRN)
Av. Senador Salgado Filho 3000
Lagoa Nova, Natal
Rio Grande do Norte 59072-970, Brazil
E-mail: hericksonfisio@yahoo.com.br

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SUMMARY

Background. Although ischemic preconditioning (IPC) has been used to improve performance in different sports, no consensus about the effectiveness of IPC on performance of amateur soccer players is established.

Objective. To investigate the effects of IPC on functional and neuromuscular performance of lower limbs of male amateur soccer players.

Methods. Male amateur soccer players aged 18 to 35 will be randomly allocated to IPC or IPC sham group. IPC group will receive four cycles of five minutes of ischemia (total blood flow restriction pressure [BFRP] of the posterior tibial artery) alternated with five minutes of reperfusion (*i.e.*, BFRP of 0 mmHg). IPC sham group will receive the same protocol but a minimum BFRP (10 mmHg). We will assess functional performance (shuttle run and side hop test), neuromuscular function of knee flexors and extensors (isometric peak torque and isokinetic performance at 60°/s and 240°/s), rate of force development, thigh circumference, and Feeling Scale.

Results. The results will indicate whether IPC improves functional and neuromuscular performance of amateur soccer players.

Conclusions. This study is a clinical trial protocol based on SPIRIT recommendations and aims to evaluate the neuromuscular and functional performance of amateur soccer players.

Study registration. The study was registered in the Brazilian Registry of Clinical Trials (Register Number: RBR-3djfww).

KEY WORDS

Agility; blood flow restriction; ischemic preconditioning; soccer; torque.

INTRODUCTION

Soccer is characterized by intermittent activities, high physical demands (strength, endurance, speed, and agility) (1). Therefore, this activity constantly challenges the recovery and energy systems because of the metabolic and neuromuscular involvement, which may impair strength, power, and endurance of the recruited muscles (1).

In this way, soccer players are constantly aiming to improve their neuromuscular performance, which involves physical capabilities (torque, power, total work), neuromuscular fatigue, injury risks and adaptations to training cycles (2). There are several ways to assess neuromuscular performance in soccer players, including isokinetic assessment and more recently, the rate of force development (TDF) (3, 4). These methods are able to assess the muscle strength produced, in addition to verifying changes in performance and risk of injuries, which becomes a useful tool for soccer practice (5, 6).

Ischemic preconditioning (IPC) is a method to improve performance acutely and prevent muscle fatigue (7-9). IPC consists of short cycles of vascular occlusion (2 to 5 minutes) followed by reperfusion, induced by the insufflation and deflation of a pressure cuff (7-9). Recently, IPC has been used to protect cardiac and skeletal muscles against ischemia-reperfusion (I/R) injury (LIU ET ALT), defined as metabolic and contractile damage that occurs when blood supply returns to the tissue after a period of prolonged ischemia (10-12). IPC could improve metabolic efficiency by increasing mitochondrial electron flow, blood flow, and oxygen release (augmented post-ischemia adenosine) and attenuating ATP depletion and lactate production (7, 13, 14).

Moreover, the effects of IPC on mitochondria and blood vessels may also improve the resynthesis of ATP in the active muscle and increase blood flow, O_2 release, and metabolite removal (7, 13, 14). Bailey *et al.* showed that IPC applied before a maximal running test attenuated blood lactate accumulation and positively affected running performance in healthy men (15). IPC may also improve performance and strength through ATP production (glycolytic and phosphogenic pathways) and hyperemia resulting from reperfusion, respectively (7, 16). Paradis-Deschenes *et al.* applied IPC before five sets of five maximum voluntary extensions in strength-trained athletes and observed improved strength during reperfusion (17).

Evidence on the effects of IPC is conflicting. Some studies suggest that IPC improves muscle function, performance, and maximum oxygen consumption (8, 15, 17), whereas others suggest that IPC is not superior to sham (14). This conflict may occur due to limitations, such as intervals of less than 45 min between ischemia and exercise (*i.e.*, minimum time needed to obtain post-ischemic effects), lack of protocol standardization, individualization of blood flow restriction pressure, and use of no-intervention instead of a sham group (9).

Few studies had assessed functional and neuromuscular performance of amateur soccer players after IPC, even though soccer is a popular sport that requires improved performance (18). The systematic review of Caru *et al.* showed that IPC might be more beneficial for healthy individuals who wish to improve aerobic fitness than athletes (19). Therefore, there are still many gaps in the literature on the use of IPC in athletes and in modalities with a predominance of anaerobic metabolism (19). In this sense, investigating the effects of IPC on different sports is needed, preferably using the most recent IPC recommendations to minimize bias. Thus, our study aims to investigate the effects of IPC on functional and neuromuscular performance of lower limbs of amateur soccer players.

METHODS

Study design

This prospective two-arm randomized placebo-controlled trial will be conducted using concealed allocation, blind researchers and volunteers, and intention-to-treat analysis at the Department of Physical Therapy of the Federal University of Rio Grande do Norte. The protocol was developed according to the Standard Protocol Items: Recommendations for International Trials (2013).

Ethical issues

The protocol was approved by the research ethics committee of the Federal University of Rio Grande do Norte (number: 32025220.8.0000.5537 – Date of approval: September 01, 2020). The protocol follows the recommendations of the SPIRIT (Standard Protocol Items: Recommendations for International Trials) 2013 checklist guidelines.

Eligibility criteria

Male amateur soccer players aged between 18 and 35 years and regularly practicing resistance training and soccer will participate in the study. We will include soccer players with body mass index between 18.5 and 30 kg/m^2, no lower limb surgeries in the past six months, and without diabetes mellitus, uncontrolled blood pressure, musculoskeletal injuries in the lower limbs in the last three months, or inflammatory, rheumatic, cardiovascular, or pulmonary disease precluding from performing the protocol. Those presenting a health problem preventing from continuing in the study, using any drugs, electrotherapy, or other therapeutic resources that might interfere with any outcomes; or performing any unusual or strenuous physical activities during the study will be excluded.

The following non-adherence and retention criteria will be used: leave the study, miss data collection visits, or experience considerable muscle soreness or discomfort that precludes from performing the procedures. Volunteers will also be instructed to avoid strenuous exercise, therapeutic methods to relieve muscle soreness, activities different from their routine, or alcohol during the procedures.

Experimental procedures

Each volunteer will visit the laboratory four times. On the first visit, the volunteer will respond to an assessment form containing anthropometric, blood pressure, and personal data (frequency and duration of soccer practice, history of injuries and illnesses, field position, and thigh circumference of the non-dominant limb). Total restriction pressure (TRP) will be measured in the same visit after 10 min o rest in supine position.

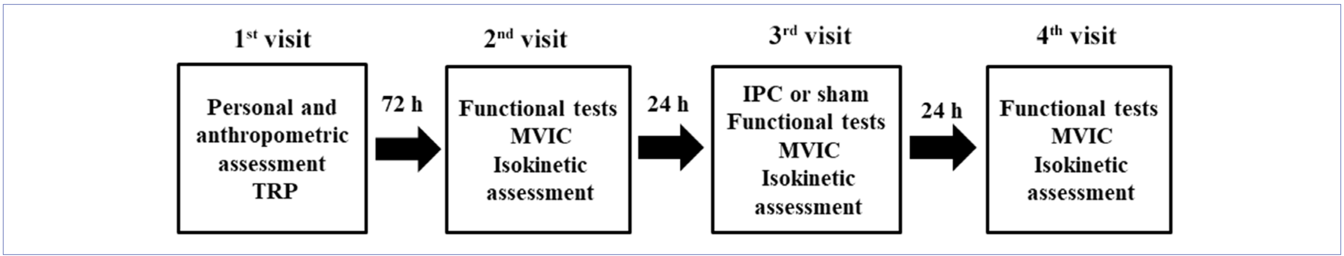

Figure 1. Study design.

IPC: ischemic preconditioning; TRP: total restriction pressure; MVIC: Maximum voluntary isometric contraction.

On the second visit, which will take place at least 72 h after the first visit, the volunteer will perform a warm-up running for five minutes, functional tests (shuttle run and side hop tests), maximum voluntary isometric contraction (MVIC), and isokinetic performance at two speeds (60°/s and 240°/s). IPC or IPC sham will be applied in the third visit, which will take place at least 24 h after the second visit. After the protocols, an interval of 45 min will be applied, followed by functional tests, MVIC, and isokinetic assessments. The fourth visit (functional tests, MVIC, and isokinetic performance) will be conducted 24 hours after the third visit. The study design is shown in **figure 1**. Assessments will be conducted during the same period of the day to minimize the influence of the circadian cycle.

Randomization and allocation

Volunteers will be randomly assigned to IPC or IPC sham groups (**figure 2**) using a random sequence generated on www.randomization.com by a researcher not involved in the study. Allocation will be concealed in sequentially numbered and opaque envelopes prepared before the study.

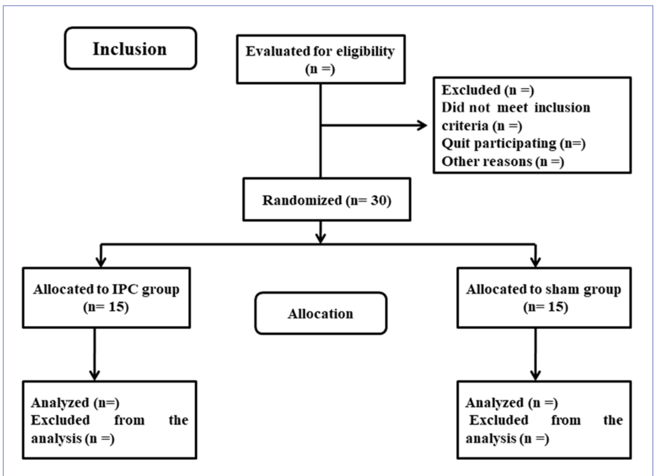

Figure 2. Study flowchart.

IPC: ischemic preconditioning

The researchers responsible for assessing outcome measures will not have access to allocation or be present during the application of the protocol.

The researchers responsible for applying IPC or IPC sham will not participate in assessing outcome measures and will open the opaque envelopes only immediately before intervention. interventions will also be performed in a separate room to avoid contact with other volunteers or researchers. Only one researcher will be responsible for the anthropometric and tests assessment. Two trained researchers will apply the IPC or IPC sham. The researcher responsible for statistical analysis will also be blinded to interventions and receive the database with labels (A and B).

Sample calculation

Based on a sample size calculated in a recent clinical trial by Huang *et al.* using isokinetic muscle strength as outcome, 30 volunteers (15 for each group) will be needed for a power of 80%, alpha of 0.05, and 20% of losses (20).

Demographic and anthropometric data

We will record total body mass, height, thigh circumference, body mass index, total restriction pressure, age, educational level, comorbidities, dominant limb, field position, life habits, and volunteers' medications.

Individualized determination of total restriction pressure

TRP of the posterior tibial artery of the non-dominant limb will be determined after 10 min of rest in supine position using a doppler ultrasound (DV 6010B Medmega, Franca - SP). The transducer will be positioned on the ankle (medium distance between the medial malleolus and Achilles tendon) with the volunteer in dorsal decubitus (21). The pressure cuff (Aneroid sphygmomanometer premium; cuff width 20 cm) will be positioned around the subinguinal region of the thigh and inflated according to a previous protocol (21). Auditory and visual signals will indicate the presence of arterial pulse (13).

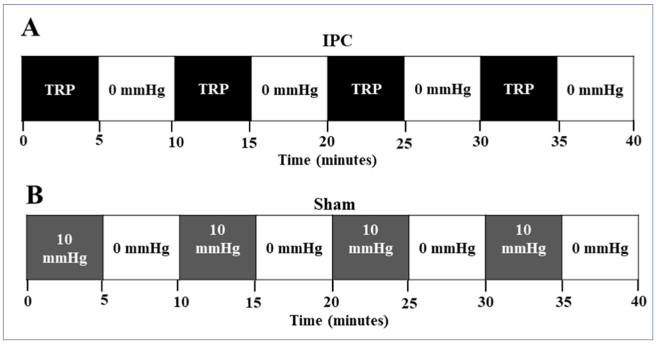

Figure 3. Protocols for IPC and IPC sham groups. **(A)** IPC Protocol, **(B)** IPC sham protocol.

IPC: Ischemic Preconditioning; TRP: total restriction pressure.

Primary outcome

Functional performance

The side hop and shuttle run test will assess functional performance of the non-dominant limb. The side hop test will assess strength and confidence in the lower limbs (22). In two attempts, the volunteers will have to hop transversely 30 cm for 30s as many times as possible as quickly as possible using the non-dominant limb; the attempt with the shortest time will be recorded (22). Attempts will not be accepted if the volunteer lands within the 30 cm, and the test will be repeated until two valid attempts are achieved (22). Familiarization will be performed on the second visit.

The shuttle run test will assess the agility of volunteers (23). The test will be performed on a flat surface and with feet shod. The volunteer must cover a 10-m corridor marked by two cones, make a 180° turn without touching the cone, and return to the starting point in the shortest possible time (23). Two attempts will be allowed with a two-minute rest in between, and the best result will be included for analysis (23).

Neuromuscular performance

Neuromuscular performance will be assessed using MVIC and isokinetic performance. In the MVIC, the volunteer will position the non-dominant lower limb on the isokinetic dynamometer (Biodex Multi-Joint System 4, Biodex Biomedical System Inc®, New York, USA) according to recommendations of the manufacturer for knee extension. Three MVIC will be performed with the knee flexed at 60º (0º being full knee extension) (24, 25). We will previously instruct the volunteer to isometrically contract the knee extensor muscles as quickly and strongly as possible after the command "Go", maintain the contraction for three seconds, and relax after the command "Stop" (24, 25). One minute of rest between each contraction will be allowed to minimize possible fatigue effects (24, 25).

Strength, power, and localized muscle fatigue will also be assessed using the isokinetic dynamometer. The volunteer will be positioned on the dynamometer chair following recommendations of the manufacturer. We will conduct a familiarization session for concentric flexion and extension at the same speeds used in the tests. One test at angular speed of 60°/s (one cycle of five repetitions) will be executed to analyze peak torque (PT), total work, and power, whereas another at angular speed of 240°/s (one cycle of 30 repetitions) will be conducted to analyze the fatigue index of knee flexors and extensors of the non-dominant limb (26, 27). During maximum contractions, the volunteer will receive visual (computer monitor) and verbal incentives from researchers to perform maximal contractions with the greatest speed possible (26, 27).

Secondary outcomes

Rate of force development

RFD will be extracted from the MVIC with highest PT. RFD derives from the force curve analysis recorded during an explosive voluntary contraction and provides important physiological information about the role of neural (0 to 50 ms) and muscle factors (between 100 and 200 ms) on force production and neuromuscular fatigue (28).

To determine RFD from MVIC curves, the beginning of the contraction will be identified as the time point at which the force curve exceeds 2.5% of the difference between baseline force and the PT (28). The average slope of the force-time curve (Δforce/Δtime) over the time intervals of 0 to 30 ms, 0 to 50 ms, 0 to 100 ms, and 0 to 200 ms (MATLAB 7.6.0 software, R2008a, MathWorks, Natick, MA) relative to the onset of contraction will represent RFD (25, 27, 28).

Thigh circumference

Thigh circumference will be assessed on the non-dominant limb (mean distance between the greater trochanter and lateral epicondyle of the femur) using a standard measuring tape (29).

Perceived exertion

Perceived exertion will be assessed using the modified 10-point Borg scale immediately after assessing neuromuscular performance on the second, third, and fourth visits. The scale consists of a score ranging from 0 to 10, which 0 represents no effort and 10 represents maximum effort that prevents the volunteer from continuing the exercise (30).

Affective response

The Feeling Scale will assess whether IPC in the thigh is pleasant or unpleasant (28). This scale consists of 11 points, ranging from -5 (very bad) to 5 (very good), and will be applied immediately after IPC or IPC sham (27).

IPC group

The IPC protocol will be performed on the non-dominant limb with the volunteer comfortably positioned supine. A pneumatic tourniquet 96 cm long and 13 cm wide (Premium aneroid sphygmomanometer; 20 cm pressure cuff) will be applied around the subinguinal region of the upper thigh. The protocol will last approximately 40 min: four cycles of five minutes of total restriction alternated with four cycles of five minutes of reperfusion (0 mmHg) (16). The ischemia and reperfusion cycles can be seen in **figure 3**.

IPC sham group

The IPC sham protocol will also be performed for 40 min and with the same equipment and position used in the IPC group; however, a maximum pressure of 10 mmHg will be applied, and total restriction pressure will not be reached (31, 32).

Statistical analysis

Data normality will be verified using Shapiro-Wilk and Levene tests. Data will be presented as mean, standard deviation, and 95% confidence intervals. For normal data distribution, two-way repeated measures ANOVA will verify differences between groups and time, followed by Bonferroni *post hoc*. The mean difference between groups and 95% confidence intervals will be reported for all outcomes; Cohen's f will assess effect-sizes.

For non-normal distribution, data will be presented as median and interquartile range, and the Friedman test will be used. Effect-sizes will be calculated using Cohen's r. All volunteers will be included following an intention-to-treat analysis. Statistical significance will be set at $p < 0.05$, and analyzes will be performed using SPSS® software (version 22.0, IBM, New York).

DISCUSSION

This protocol will examine the effects of IPC on functional and neuromuscular performance of the lower limbs of male amateur soccer players. The interest in studying strategies to improve sports performance has grown substantially, and an expressive quantity systematic reviews on the effects of IPC have been published in the last decade (9, 19).

Sports professionals try to ensure as much as possible that post-exercise recovery strategies are appropriate to maintain or improve performance during competition. The use of tools to acutely improve performance and prevent muscle fatigue are particularly relevant in sports, especially for elite athletes, which small differences in performance separate success from failure (33, 34).

In this way, the evaluation of the soccer players performance becomes an important point, both to verify the physical state and to prevent injuries (35). The isokinetic evaluation is widely used, being considered one of the indispensable tools for the identification of alterations and muscle asymmetries in players (35). In addition, TDF has also been used to assess strength and identify muscle asymmetries (4). In addition, it is able to assess the muscle strength produced in a short period of time and more subtle changes in strength and muscle activation, which makes it quite relevant, since it analyzes in more detail the specific actions of soccer, which require the rapid production of strength in their executions (5, 6).

Due to soccer energy needs caused by great physical efforts, there is a wear of metabolic and neuromuscular pathways, especially after game seasons, which can cause performance reduction of these players (1). Thus, if applied before games or training, IPC could reduce the metabolic damage and improve the performance of these players. Despite some positive results, IPC effects on exercise performance are very conflicting (8, 14, 15, 17) and few studies have investigated whether IPC influences functional and neuromuscular performance of soccer players (18). Although literature indicates the need for performing exercises or tests 45 min after IPC, few studies used this approach, and many failed to standardize and individualize total restriction pressure (9, 19).

CONCLUSIONS

This study is a randomized clinical trial, with blinding of evaluators and volunteers and with allocation concealment. The sample size was calculated to provide adequate statistical power. The PRT used will be individualized, allowing the IPC effect for each volunteer, respecting their anatomical and physiological differences. Several outcomes will be included to measure the torque (isokinetic assessment, RFD), functional performance (side hope test, shuttle run test) and anthropometric measurements, making the assessment broader and more focused on soccer. It is described in detail, based on SPIRT recommendations for protocols, so that the study results can be reproduced, interpreted, and compared any time.

As a limitation of this study, the percentage of occluded blood flow will not be assessed; therefore, the exact amount of blood flow restriction will not be calculated. Also, fatigue will be assessed using the isokinetic dynamometer, instead of electromyography.

FUNDINGS

None.

DATA AVAILABILITY

N/A.

CONTRIBUTIONS

IMF, MSC: conceptualization. IMF, MSC, YMB, WHB: design and coordination. IMF: writing. SBNN, CAMA, WHBV: revision. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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