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Local, Distal, Proximal, and Contralateral Effects of Low-Load Blood Flow Restriction Training on Upper Extremity Neuromuscular Performance of Healthy Women: a Randomized Placebo-Controlled Trial Protocol

M. T. M. Jales[1], W. S. Lima Júnior[2], Y. T. Pinheiro[2], G. M. Barbosa[2], M. C. de Souza[2], C. A. A. Lins[2]

[1] *Faculty of Health Sciences of Trairi, Federal University of Rio Grande do Norte, (UFRN/FACISA), Santa Cruz, Rio Grande do Norte, Brazil*

[2] *Rehabilitation Sciences, Faculty of Health Sciences of Trairi, Federal University of Rio Grande do Norte, Santa Cruz, Rio Grande do Norte, Brazil*

CORRESPONDING AUTHOR:
Caio Alano de Almeida Lins
Postgraduate Program in Rehabilitation Sciences
Faculty of Health Sciences of Trairi
Federal University of Rio Grande do Norte
Rua Vila Trairi S/N
Rio Grande do Norte, Brazil
E-mail: caiouzl@hotmail.com

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SUMMARY

Introduction. Low-load blood flow restriction (BFR) training may induce positive neuromuscular adaptations, but proximal BFR effects are unclear. This study aims to investigate chronic effects of low-load resistance training (LLRT) with BFR on upper extremity neuromuscular performance of healthy women.

Methods. This protocol for clinical trial will include 78 volunteers randomized into three groups of 26 participants: LLRT (LLRT without BFR); LLRT + placebo blood flow restriction (20% BFR); and LLRT + 60% BFR. All groups will perform four sets of 15 repetitions at 20% of one-repetition maximum for each of the following muscles: serratus anterior, lateral shoulder rotators, and lower trapezius. Participants will be assessed before protocol, after completing eight weeks of protocol, and after a four-week follow-up. Primary outcome will be muscle strength, and secondary outcomes will be muscle excitation, perimetry, pain, subjective perceived exertion, affective valence with exercise, and power of upper extremity muscles.

Discussion. Exercises are often used to prevent and treat upper limb disorders. However, only two studies analyzed the effects of these exercises associated with BFR. Therefore, this protocol aims to fill the gaps in these studies and propose more reliable results on the subject.

Trial registration. EnsaiosClinicos.gov.br (Identifier: RBR-3pd52f).

KEY WORDS

Electromyography; muscle strength; resistance training; shoulder joint; vascular occlusion.

INTRODUCTION

Resistance training is recommended to increase muscle strength and muscle cross-sectional area (*i.e.*, muscle hypertrophy) and can benefit morphological and neuromuscular (*e.g.*, power and endurance) components (1). The American College of Sports Medicine recommends eight to 12 repetitions of 60-70% of one-repetition maximum (1RM) for increasing muscle strength in beginner or intermediate healthy individuals (1). In the 2000s, a new resistance training modality using blood flow restriction (BFR) was suggested as alternative to conventional resistance training (*e.g.*, 60-70% of 1RM) (2). BFR combines a pressure cuff with low-load (20-40% of 1RM) resistance exercises and high number of repetitions (*i.e.*, > 12 repetitions) (2, 3).

Some mechanisms acting simultaneously are proposed to explain the effects of exercise with BFR, among them are the metabolic stress, that increases under ischemia/hypoxia conditions and activates other mechanisms such as systemic hormonal release of growth hormone and insulin-like growth factor type 1. Exercise with BFR can also increase the production of reactive oxygen species such as nitric oxide, which can stimulate satellite cells and protein synthesis (4, 5). High-load training and low-load BFR training may induce similar hypertrophy levels in healthy people (6). However, literature regarding muscle strength gains in this population is conflicting, with previous studies showing superiority (6, 7) or similarity (8) of high-load resistance training *versus* low-load BFR training. Furthermore, it is suggested that the occurrence of fatigue during low-load resistance training is similar between the conditions of restricted blood flow and free blood flow (9).

Low-load BFR training increases strength and muscle mass of healthy adults compared with low-load training alone (10). However, while most studies reported neuromuscular adaptations on muscles distal to BFR, other investigations observed effects contralateral, distal, and proximal to BFR (11-18). Two studies reported effects of BFR training on gains in strength and muscle mass in muscles moving glenohumeral and scapulothoracic joints (19, 20). Despite this, no study used placebo, blinded participants, or included training of essential muscles for shoulder rehabilitation, such as serratus anterior and lower trapezius (21).

Thus, this study aims to investigate local, proximal, and distal chronic effects of low-load BFR training on neuromuscular performance of upper extremity muscles in healthy women. We hypothesize that BFR resistance training will increase strength of muscles proximal, contralateral, and distal to BFR and improve upper limb power and muscle excitation compared with low load resistance training alone and placebo BFR training.

METHODS

Design

This study proposes a randomized and blinded clinical trial protocol that will be conducted at the Faculty of Health Sciences of Trairi. The study was prospectively registered in the Brazilian Clinical Trials Registration Platform (Identifier: RBR-3pd52f) and was approved by the local Research Ethics Committee (No: 4.216.594 – on August 17, 2020). All procedures will be performed according to the Declaration of Helsinki.

Participants will be randomly assigned to three groups (26 per group): low-load resistance training (LLRT), low-load resistance training with 20% of BFR (LLRT + placebo application [pBFR]), and low-load resistance training with 60% of BFR (LLRT + BFR). The study will follow recommendations of the Template for Intervention Description and Replication (TiDieR) checklist (22) and the Standard Protocol Items: Recommendations for International Trials (SPIRIT) (23). The study will be reported according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines (24).

Participants

Participants will be recruited using social media, announcements in local media, university community newsletters, banners, or flyers posted in strategic locations around town. Eligibility screening of those interested in participating will be performed by phone. Eligible participants will receive oral and written instructions about study aims and procedures and sign a consent form.

Personal information (name, address, and telephone number), anthropometric (age, height, and body mass), sociodemographic (profession, race, and educational level), and clinical data will be assessed. Personal data will be numerically coded, and information will be stored in a database, accessed only by the researcher responsible for randomization and blinding. Steps of the study are shown in **figure 1**.

Inclusion criteria

- Women aged between 18 and 35;
- irregularly active or sedentary (International Physical Activity Questionnaire) (25);
- body mass index between 18.5 and 30 kg/m^2;
- no previous experience with BFR training;
- no diabetes mellitus or high blood pressure;
- non-smoker (26);
- no regular use of vasoactive medications or nutritional supplements (27);
- no upper limb injuries in the last six months (26);
- no rheumatological, severe cardiovascular, or severe pulmonary inflammatory conditions that would prevent performing assessments and exercise protocol (27);

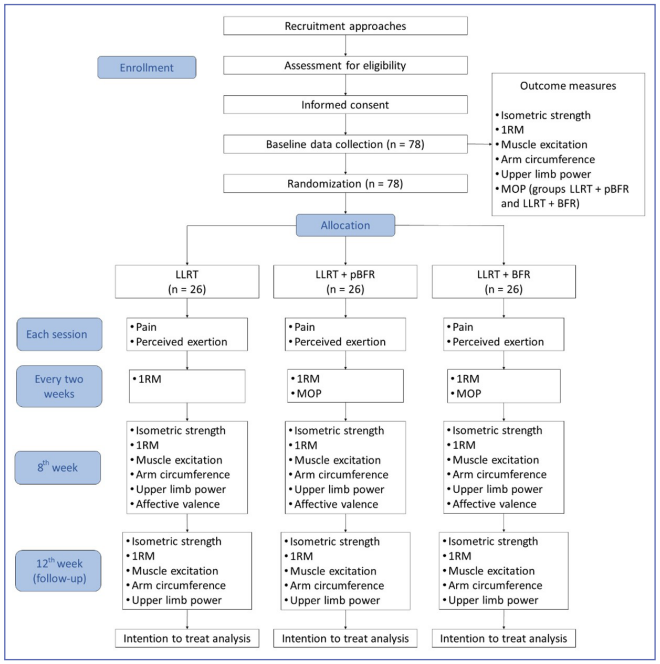

Figure 1. Study flow diagram.

- no psychiatric illness or malignant tumor and no previous zika or chikungunya infection in the last year (27);
- no regular upper limb physical training in the last six months (28).

Exclusion criteria

- Refuse to remain in the study (27);
- presence of incapacitating health conditions precluding participants from continuing the protocol (27);
- use of analgesic or anti-inflammatory medicines or both that may interfere with any outcome (27);
- unusual or strenuous physical activities during the study (27);
- missing two consecutive training days (26);
- leave the study before completing 70% of the training program (29).

Research team

This study will involve four researchers: one responsible for randomizing participants, one to conduct assessments, one to perform interventions, and one to perform statistical analyses.

Randomization and blinding

Included participants will be randomly assigned to one of three groups: LLRT, LLRT + pBFR, and LLRT + BFR. Randomization will be via www.randomization.com. Concealed allocation using individual, opaque, sealed envelopes will be conducted to avoid selection bias. Randomization will be performed by a researcher not involved in other procedures. Allocation of participants will be revealed before the first intervention, ensuring confidentiality. In addition, data collected during evaluations will not be revealed to researchers responsible for interventions. After study completion, the statistician will receive a worksheet with blinded data.

Interventions

LLRT protocol

Three exercises will be performed individually during each training session: shoulder external rotation (30), supine serratus punch (31), and prone horizontal abduction with shoulder externally rotated (32). For the first exercise, participants will be positioned in lateral decubitus, with shoulder adducted and internally rotated, 90° elbow flexion, and a towel positioned between elbow and trunk (**figure 2 A**). Participants will be encouraged to perform lateral rotation against resistance, avoiding compensations. In the second exercise, participants will be positioned supine, with 90° shoulder flexion, and elbow fully extended (**figure 2 B**). The exercise will initiate with shoulder retraction, and the participant will be asked to perform shoulder protraction against resistance with elbow fully extended. For the third exercise, participants will be positioned prone with elbow fully extended and with approximately 140° of shoulder abduction (**figure 2 C**). Participants will be instructed to lift the arm against resistance. Elbow will also remain extended throughout the exercise. Resistance for all exercises will be applied using a dumbbell. These exercises were chosen because they primarily recruit lateral rotators (30), serratus anterior (31), and lower trapezius (32), which are frequently included in rehabilitation protocols for individuals with shoulder pain (30, 33-35). Exercises will be performed in a predetermined random sequence for each participant; this sequence will be maintained throughout protocol. Cadence will be 1.5 seconds in each movement phase (concentric and eccentric) and controlled by a metronome, totaling three seconds per repetition (36).

1RM for all exercises will be predicted using Brzycki's formula (44): $predicted\ 1RM = 100 \times Load / [102.78 - (2.78 \times Repetitions)]$, in which load of 20% of predicted 1RM will be used for training (36). Predicted 1RM will be calculated every two weeks to follow physiological adaptations to exercise.

All groups will perform four sets of 15 repetitions for each exercise on each side, with a 30-second interval between sets and 1 minute between exercises, changing only the percentage of occlusion in LLRT + pBFR and LLRT + BFR groups.

Figure 2. Exercises will be performed individually during each training session.

Training sessions will be held twice a week, 48 hours apart (37), for eight weeks, totaling 16 sessions. To correct possible compensations and keep participants safe during exercises, a trained therapist will monitor the training program.

BFR protocol

Initially, maximum occlusive pressure (MOP) will be calculated. Blood pressure will be assessed after ten minutes of rest in supine position and with arms relaxed. An adapted sphygmomanometer (P.A. MED, São Paulo, Brazil), 52 cm length and 7 cm wide will be used. MOP will be determined with participants in supine position, upper limbs extended at body side and supported on the stretcher, with hand palms, face, and eyes facing forward, and lower limbs parallel with fingers facing forward (38). From this position, a portable vascular doppler (Dv 2001 – MedPej, São Paulo, Brazil) will be placed near wrist and over the radial artery, and the cuff will be slowly inflated to the point where blood pulse is abolished. The value displayed on manometer at this point will represent MOP (39). For LLRT + pBFR, the value corresponding to 20% of MOP will be considered for the exercise protocol (40). Regarding LLRT + BFR, 60% of MOP will be used (41). BFR will be performed in only one limb, which will be randomly chosen. The cuff will be deflated between exercises (26), and MOP will be reevaluated every two weeks to adjust pressure if necessary (42). Strengthening with BFR is considered safe (43), and participants will be encouraged to complete all sessions. Any adverse effects (*e.g.*, signs of deep vein thrombosis or rhabdomyolysis, persistent numbness, signs of venous injury, or any other harmful effect) (43) will be recorded. The researcher responsible for the intervention can also interrupt the intervention, if necessary.

Assessments

Muscle strength, muscle excitation, power, and arm circumference will be assessed at three time points: before training (T_0), after eight weeks of training (T_8), and after a four-week follow-up (T_{12}). Participants will be questioned at each session regarding subjective pain and perceived exertion. Participants will be contacted by phone and reminded to attend assessment days (**table I**).

Table I. Primary and secondary outcomes and assessments during the study.

	Enrolment	Baseline (T_0)	Intervention	Post-intervention (T_8)	Follow-up (T_{12})
Study Phase	**Three weeks before training**	**Day 0**	**Week 1 to 8 (2x/week)**	**Week 8 (2-3 days after the last intervention)**	**Week 12**
Enrolment					
Eligibility screening	X				
Informed consent		X			
Allocation		X			
Interventions					
LLRT			X		

	Enrolment	Baseline (T_0)	Intervention	Post-intervention (T_8)	Follow-up (T_{12})
Study Phase	**Three weeks before training**	**Day 0**	**Week 1 to 8 (2x/week)**	**Week 8 (2-3 days after the last intervention)**	**Week 12**
LLRT + pBFR			X		
LLRT + BFR			X		
Assessments					
Primary outcome					
Isometric strength		X		X	X
Secondary outcomes					
Muscle excitation		X		X	X
Upper limb power		X		X	X
Arm circumference		X		X	X
Pain			X		
Perceived exertion			X		
Affective valence				X	

LLRT: low-load resistance training; pBFR: placebo blood flow restriction; BFR: blood flow restriction.

Primary outcome

Isometric strength

A digital hand-held dynamometer (Lafayette Instrument Company, Lafayette, IN, US) will be used to evaluate isometric muscle strength of elbow flexors, lateral rotators, shoulder abductors (scapular plane), serratus anterior, and lower trapezius. Handgrip strength will be quantified using a hydraulic hand dynamometer (JAMAR, Hydraulic Hand Dynamometer® - Model PC-5030J1, Fred Sammons, Inc., Burr Ridge, IL, US). Procedures will be performed bilaterally and separately for each limb, and participants will perform up to two submaximal contractions for familiarization. Three five-second repetitions of maximal isometric contraction and 30-second rest between repetitions will be performed (44). For handgrip strength, participants will perform three grips of six seconds each, interspersed by one-minute rest (45). Quick and emphatic verbal commands (*i.e.*, "go! go! go!") will be used to stimulate maximum force production during the test (46). A universal goniometer (Fibra cirúrgica, Santa Catarina, Brazil) will be used to verify joint positioning. Coefficients of variation between repetitions will be calculated and, if necessary, additional measurements will be taken to guarantee a variability lower than 10% (47). Mean values (in kilogram-force) obtained in each limb will be included in data analysis and normalized by body mass. Positioning, fixation of apparatus, and execution of each test are shown in **table II**.

Table II. Evaluation of upper limb isometric muscle strength.

Test	Positioning	Dynamometer setting	Execution
Elbow flexors (44, 45)	Sitting, arm beside trunk, supine forearm, and elbow at 90°.	Dynamometer will be placed in the anterior forearm region, between radial styloid process and ulna, fixed around chair by an inelastic band.	Participants will perform maximum force by flexing elbow against resistance imposed by belt.
Sloulder lateral rotators (46)	Sitting, arm in adduction and neutral rotation, elbow flexed at 90° degrees with towel roll between elbow and torso, and forearm in neutral position.	Dynamometer will be attached to a bracket fixed to the wall and positioned on the posterior region of participant's forearm, three centimeters proximal to radial styloid process.	Participants will perform shoulder lateral rotation, applying maximum force against resistance imposed by the wall.

Test	Positioning	Dynamometer setting	Execution
Shoulder abductors (scapular plane) (47)	Shoulder elevated 90° in scapular plane, thumb pointing up, and elbow extended.	Dynamometer will be attached to an inelastic strip attached to the ground, positioned one centimeter proximal to radiocarpal joint.	Participants will perform maximum force toward ceiling, trying to raise the arm against resistance imposed by the band.
Anterior serratus (48, 49)	Supine with shoulder and elbow flexed at 90°. To find the initial position, participants will perform maximum protraction and retraction of scapula. Midpoint between these two movements will be considered for initial position of the test.	Hand-held dynamometer will be positioned on olecranon, perpendicular to the stretcher. An inelastic belt will be positioned around the stretcher to fixate the device.	Participants will perform maximum force toward ceiling against resistance imposed by the belt.
Lower trapezius (50)	Participants in prone, 145° shoulder abduction, thumb toward ceiling, and contralateral hand under forehead.	Hand-held dynamometer will be fixed by a belt attached to the ground, positioned laterally to distal portion of radius, just above radial styloid process.	Participants will be instructed to raise arm toward ceiling against resistance imposed by belt.
Handgrip (41, 51-53)	Sitting in a chair without armrests, feet flat on floor, shoulder adducted, elbow flexed at 90°, forearm in neutral position, and wrist between 0° and 30° of extension.	Participants will hold a hand hydraulic dynamometer with hands.	Participants will be instructed to perform maximum grip by bringing two rods together with maximum force.

Source: original from authors.

Secondary outcomes

Muscle excitation

Muscle excitation will be assessed simultaneously with muscle strength assessment. Acquisition and processing of electromyographic signals will be performed using a four-channel signal conditioning module (MCS 1000) (EMG System do Brasil®, Brazil) with analog-to-digital converter - A/D (CAD, 12/36-60K) of 12-bit resolution.

The equipment has a common-mode rejection ratio of > 80 Db, sampling frequency of 2000 Hz, and signals will be filtered between 20 and 500 Hz. Signals will be amplified 1000 times (50 times in converter and 20 times in electrodes). The electromyograph will be connected to a computer, which will receive signals that will be analyzed using EMGLab software (EMG System do Brasil®, Brazil).

Muscle excitation of serratus anterior, lower trapezius, and anterior deltoid muscles will be acquired after skin preparation (*i.e.*, trichotomy and cleaning with 70% alcohol). Simple differential surface electrodes, composed of Ag/AgCl associated with conductive gel (bipolar configuration, 4 cm × 2.2 cm of adhesive area, and 1 cm of conductive area) and separated by an interelectrode distance of 2 cm (Noraxon®, US), will be used. A monopolar reference electrode will also be used (Ag/AgCl with conductive gel, diameter of 3.8 cm of adhesive area, and 1 cm conductive area) (Noraxon®, US).

According to SENIAM recommendations (48), signal of serratus anterior muscle will be acquired with electrode positioned longitudinally anterior to latissimus dorsi and posterior to pectoralis major between sixth and eighth ribs. For lower trapezius, the electrode will be positioned 2/3 from scapular spine to eighth thoracic vertebra. For anterior deltoid, electrode will be positioned two centimeters distal and anterior to acromion. Reference electrode will be placed on the prominence of seventh cervical vertebra.

Root mean square (RMS) during muscle strength assessment will be normalized using peak RMS obtained during isometric contraction (49). Electromyographic signals corresponding to greatest isometric torque contraction using dynamometer will be included in data analysis.

Upper limb power

Unilateral seated shot-put test will assess upper limb power (50). Participants will sit on floor with torso, scapula, and

head against the wall, knees flexed at approximately 90°, and feet flat on the floor. Participants will hold a medicine ball (~ 3 kg) at shoulder height and will be instructed to push the ball forward as far as possible, keeping head and scapula on the opposite side in contact with wall and contralateral arm close to the body. The ball will be covered with chalk to facilitate measurements. After familiarization with two submaximal repetitions, participants will perform three maximum repetitions on each side with 30-second intervals between repetitions. Distance between wall and ball after first touch on the ground will be measured in centimeters and mean of three measurements will be used for analysis.

Arm circumference
Circumference of both arms will be measured using perimetry, adapted from Chapman *et al.* (51). Participants will be standing with arms at body side and ventral face of hands facing thighs. A non-elastic flexible measuring tape and semi-permanent marker will be used to measure arm circumferences (in centimeters) at three, six, nine, 12, and 15 centimeters above elbow crease line. Mean of all measurements will be considered for analysis.

Pain
Muscle pain will be monitored before and after each training exercise of all protocols and throughout the intervention program using the numerical pain rating scale. Participants will rate pain intensity with exercises by selecting a number from zero to ten, in which zero represents "no pain" and ten represents "worst possible pain" (52). At the end of intervention period, a pain diary will be created for each exercise and represented as a graph.

Perceived exertion
OMNI-RES Scale (53) will assess subjective perceived exertion at the end of each training session. This scale ranges from zero to ten, in which zero represents an "extremely easy" degree of exertion and ten an "extremely hard" degree of exertion.

Affective Valence
Affect will be determined after the last week of training using the Feeling Scale (54). This is an 11-point scale, with items ranging from + 5 (very good) to - 5 (very bad). Instructions for participants will be as follows: use numbers on this scale to indicate how you felt while performing this activity; if you felt exercise was very good (pleasant or comfortable), then the corresponding number will be "+ 5"; if you felt exercise was very bad (unpleasant or uncomfortable), then the corresponding number will be "- 5". If you feel neutral (between pleasure and displeasure /comfort and discomfort), then the corresponding number will be "0". **Table III** shows time points of assessments.

Researcher training

Researchers will be trained before initiating the study to standardize the application of exercise protocols, BFR, and assessment tools. Test-retest and intra-rater reliability for muscle strength, power, and upper limb circumference will be performed before initiating the study.

Sample size calculation

Sample size was calculated based on a previous study (55). Total sample size was estimated as 71 participants, considering mean difference of relative isometric strength (primary outcome) between groups of 0.15 Nm/cm^2, standard

Table III. Evaluation of primary and secondary outcomes.

Outcomes	Baseline (T_0)	After each intervention	After 8 weeks of intervention (T_8)	Four weeks after intervention (T_{12})
Primary				
Isometric strength	√		√	√
Secondary				
Muscle excitation	√		√	√
Upper limb power	√		√	√
Arm circumference	√		√	√
Pain		√		
Perceived exertion		√		
Affective valence			√	

deviation of 0.33 Nm/cm², 80% statistical power, and 5% significance level. Including a 10% dropout rate, 78 participants will be needed (26 per group).

Statistical analysis

Kolmogorov-Smirnov and Levene tests will be used to verify data normality and homogeneity of variance, respectively. Quantitative results will be represented as mean ± SD and qualitative results as absolute values, percentages, and 95% confidence intervals (95% CI).

Intergroup comparisons will be evaluated using linear mixed model (mixed ANOVA) or Kruskal-Wallis test, depending on data normality. When a significant F-value is found, the Bonferroni *post-hoc* test will be applied to identify the differences. Significance level at 5% ($p < 0.05$) and 95% CI will be adopted for all statistical analyses. All participants will be evaluated using intention-to-treat analysis. For missing data, results will be imputed by repeating the value of the last assessment.

DISCUSSION

Exercises for glenohumeral and scapulothoracic regions are frequently used to increase strength (35), improve function (33), and prevent (56, 57) and rehabilitate shoulder dysfunction (58-61) in different populations. Regarding upper limbs, only two studies investigated BFR effects for muscles of shoulder complex (55, 20). Methodological limitations, such as lack of placebo group and conflicting results, hinder recommending BFR for improving upper limb function.

The present protocol proposes a similar training volume between groups and exercises commonly used to prevent and treat shoulder dysfunctions (*e.g.*, rotator cuff tears (58), glenohumeral instability (59), or subacromial pain (61)). Another relevant aspect of our study is the inclusion of control and placebo groups. In particular, placebo group will receive a minimal percentage of BFR that is unable to change results significantly (40, 41). Finally, upper limb power will also be investigated and a four-week follow-up period will indicate whether training results are maintained.

As limitations, we highlight the impossibility of controlling hormonal changes of participants and measuring trophism changes of target muscles because of lack of adequate equipment, such as MRI or ultrasound.

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DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

All authors contributed significantly to the construction of the study. MTMJ, WSLJ, YTP: writing, approval of the final manuscript. GMB, MCS: assistance in protocol development. CAAL: contextualization and final review of the study.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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