Acute and Chronic Effects of Exercise in Health

Resistance training with blood flow restriction and cognition in elderly women (project "forte-mente-ativa"): study protocol

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Abstract - Aim: In the process of aging, there is a decrease on muscle strength and cognitive function. Resistance training combined with blood flow restriction (BFRRT) has been shown capable of maintaining or improve aspects of physical health. However, the effects of BFRRT the cognitive function of the elderly are not clear. This study aimed to describe the design of a randomized controlled clinical trial, that will investigate the effects of BFRRT on cognitive function, physical performance and physiological and morphological aspects in elderly women. **Methods:** Forty participants will be randomized into one of the following groups: low load resistance training, blood flow restriction resistance training, moderate load resistance training or Control. All intervention groups will complete 16 weeks of resistance training, three times week (45 minutes each), with training consisting of four exercises for the upper and lower body, including three sets of ten repetitions each. No exercise will be performed by the Control group. Cognitive function will be the primary outcome of the study. Secondary outcomes will include body composition, muscle strength, functional capacity, double-task, level of physical activity, static and dynamic balance, brain activity, BDNF neurotrophic factor, anxiety, depression and sleep state). **Conclusion:** This project will contribute to the existing knowledge and will have a social impact regarding the use of physical exercise as a non-pharmacological tool for the mental and physical health older individuals. Trial Registration: Brazilian Registry of Clinical Trials number RBR-7BC8ZP.

Keywords: strength training, aging, vascular ischemia, cognitive function, study protocol.

Introduction

Aging is a dynamic and progressive process characterized by functional, physical, morphological and cognitive changes that can negatively impact health status. Although these events occur naturally, the heterogeneity in the pattern of these changes may expose certain individuals to a greater risk of harmful consequences to health, such as frailty and cognitive decline[1]. Thus, strategies that are able to promote beneficial changes in these aspects are necessary to mitigate age-related deficits.

Physical exercise is one of the most promising non-pharmacological strategies to delay or prevent some of the age-related deleterious effects on physical and cognitive health-related outcomes in the elderly population. Moderate-to-high load resistance training (MHIRT) has been reported to increase muscle strength, functional capacity[2], and reduce the risk of cognitive declines associated with aging[3]. However, clinically limited individuals are often not able to exercise at such high intensities in order for the desired physiological changes to occur.

An alternative to MHIRT is the manipulation of some of the training variables, such as reducing training load and combining the resistance exercise with the blood flow restriction, as it causes less overload for the musculoskeletal system, reducing the risk of injuries and promoting similar responses to the MHIRT[4]. A recent meta-analysis[5] reported that resistance training with blood flow restriction (BFRRT) is effective to promote muscle mass and strength improvements in older adults. BFRRT has also been shown to improves perfusion and oxygen delivery for skeletal muscles in elderly individuals[6]. It has been hypothesized that such phenomena are likely to occur in the brain, leading to improvements on cognitive function (CF).

Some studies have analyzed the effects of moderate load resistance training (MLRT) in cognitive function and physical performance of elderly subjects; however, it is not clear how, or if, BFRRT alters the cognitive function in older individuals. A recent study investigated the acute effects of BFRRT on cognitive function of elderly, using the Stroop test, and have reported that a single BFRRT session (composed of 4 sets, being 1 of 30 repetitions and 3 of 15 repetitions, with 30% of 1RM and 50% total blood flow restriction) was able to reduce the execution time of the test, indicating an improvement in the cognitive function[7].

A manuscript from Torpel et al.[8] proposed molecular and functional mechanisms that may be associated with a better performance on cognitive function after BFRRT, such as an increased release of neurotrophic hormones (e.g., Insulin-like Growth Factor-1 [IGF-1], Growth Hormone [GH], Vascular Endothelial Growth Factor [VEGF], Brain-Derived Neurotrophic Factor [BDNF]), blood lactate, and greater brain activation than traditional resistance training. Despite current evidence suggesting beneficial changes[9], to our knowledge no study has tested the long-term effects of BFRRT on CF.

This study will describe the design of a randomized controlled clinical trial (Project "Forte-Mente-Ativa") that will investigate the effects of 16 weeks of BFRRT on cognitive function, physical performance and physiological and morphological aspects in elderly women. Our hypothesis is that BFRRT will promote similar improvements compared to moderate load resistance training (MLRT), but greater than the low load resistance training (LLRT) and Control groups for all outcome measures.

Methods

Study design

The "Forte-Mente-Ativa" Project (Strong-Mind-Active, in English) will be a randomized controlled single-blinded clinical trial, approved by the Human Ethics Research Committee of the Federal University of Paraiba (protocol nº: 11399019.7.0000.5188), registered on the Brazilian Registry of Clinical Trials (REBEC ID: RBR-7BC8ZP), that and will follow the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT)[10] guidelines.

Participants and eligibility criteria

Elderly women aged between 60 - 80 years will be invited to participate on the project. Upon recruitment, the lead researcher will explain the study aims, provide further information if requested by participants, and the participant will sign a consent form. Participants will be eligible to enter the study if they: 1) are female; 2) are between 60 to 80 years old; 3) do not have musculoskeletal issues in the lower or upper limbs or any contraindications to perform resistance exercise; 4) are physically active (cut off will be considered as moderate to vigorous physical activity as 1041 counts/min, measured by an accelerometer[11]); 5) do not have a history of any neurological, psychological or cardiovascular diseases (except controlled hypertension); 6) do not a have history of any craniocerebral trauma with more than 30 minutes of conscience loss; 7) are non-smokers and non-alcohol users; 8) have no motor, auditive or visual (non-corrected) issues; 9) have completed at least elementary school; 10) present an ankle-brachial index outside the range of 0.9 and 1.4, indicating predisposition of peripheral artery disease[12]; 11) score 24 or higher in the Mini-Mental State Examination (MMSE). Participants who fail to complete at least 75% of the training sessions or miss any of the tests will be excluded from the study.

Sample size calculation

The present study is the first to investigate the effects of resistance training with blood flow restriction on the cognitive function in health elderly women, which limits a formal analysis to calculate sample size. Therefore, based on the results of different meta-analysis[13,14], we used the following criteria to determine sample for the current study: effect size = 0.29, alpha level = 5%; power (two-tailed) = 80%. According to these estimates and assuming a dropout rate of ≈ 20%, we will aim to recruit 46 women (12 per group) to have a final sample of, at least, 38 women.

Recruitment and randomization

Participants will be recruited from the city of João Pessoa-PB during visits to community exercise groups in the city squares, advertisements placed around the University, social media and by contacting volunteers from previous research projects. Randomization will be performed after baseline assessment (pre-training) in a 1:1:1:1 fashion through a sequence of randomly permuted blocks, stratified by strength levels and age, which will be determined by an independent researcher using the website http://www.randomizer.org. Due to the nature of the interventions under investigation, double blinding of treatments will not be possible. The lead researcher will be blinded regarding participants allocation into groups, primary outcome (cognitive function) and at least one secondary outcome (one maximal repetition test [1-RM]) during all phases of data collection and analysis.

Intervention

The intervention groups (moderate load resistance training [MLRT], blood flow restriction resistance training [BFRRT], low load resistance training [LLRT]) will perform a 16-week resistance training program (3 times per week) on alternate days. The training program will consist of four exercises for the upper and lower limbs (Squat,

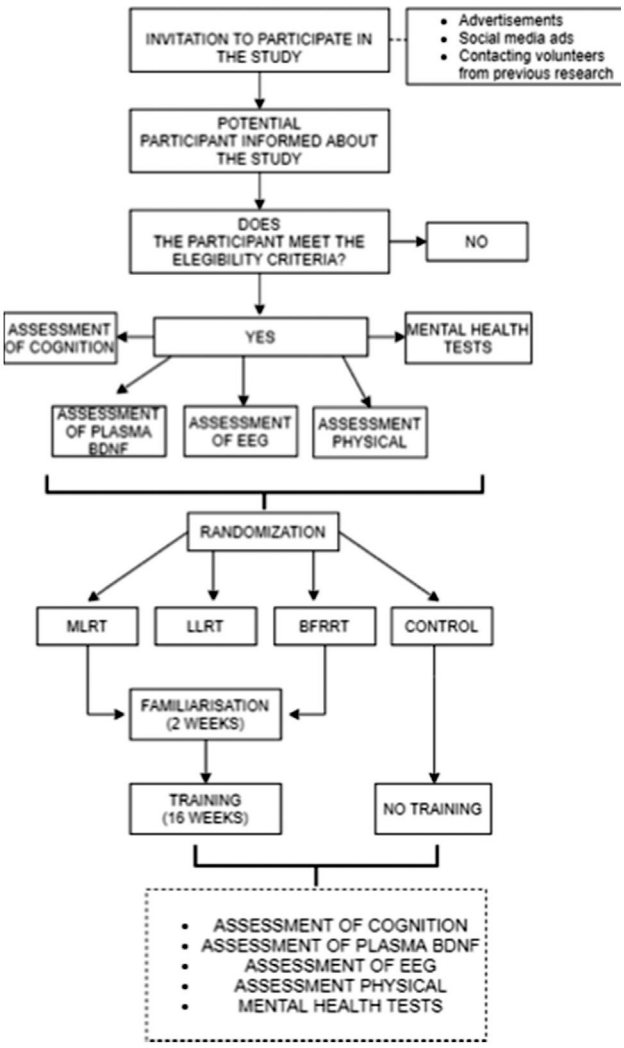

Figure 1 - Diagram of study design.

Leg press 45º, Supine and Biceps curl). Exercise prescription will be performed with volume equalization (sets x repetitions) and same rest interval, containing three fixed sets of 10 repetitions for all groups, differing only the intensity (load) between groups (BFRRT, LLRT versus MLRT). The recovery interval between sets and exercises will be 60 and 120 seconds, respectively. Training progression and adjustments of training loads will be performed according to the American College of Sports Medicine recommendations[15].

Moderate-load resistance training group

The determination of the training load for the MLRT group will be carried out through the test of 15 maximum repetition (MR)[16].

Blood flow restricted resistance training group

The training load for the BFRRT group will be determined through the 30 - RM test[16]. The training program for this group will be carried out using the sphygmomanometer equipment (tourniquet pneumatic komprimeter to hemostasis in extremities - Riester®) placed in the most proximal of both the arms (width 6 cm x length 47 cm) or legs (width 10 cm x length 54 cm).

The determination of total blood flow restriction pressure total (mmHg) will be assessed according to the procedures by Laurentino et al.[17] using a portable vascular doppler (MedPeg® DV-2001, Ribeirão Preto, SP, Brazil). With participants lying down in the supine position, a sphygmomanometer will be placed in the most proximal portion of the thighs and arms and inflated until the arterial auscultatory pulse is interrupted, which is considered the arterial occlusion point. The blood flow restriction pressure used during exercise will correspond to 50% of the arterial occlusion point. Cuff pressure will be maintained during the exercises (including rest between sets), will be deflated between exercises and will be constantly monitored to ensure that the pressure is maintained at ~50% of the arterial occlusion point.

Low-load resistance training group

The training load for the LLRT group will be obtained through the same test as the BFRRT group, however the training program will be carried out without the use of blood flow restriction.

Control group

Participants in the CON group will be instructed to continue with their daily routine until the end of the 16 weeks. For ethical reasons, the CON will have the possibility to undertake the resistance exercise intervention once the trial finalizes.

No diet intervention will be provided for the participants of any group, however participants will be asked to fill a feed reminder to follow up the food ingested during the study (with application before, after 8 and after 16 weeks of training).

Outcomes

Primary and secondary outcomes will be measured before and after the sixteenth week of intervention. Figure 2 summarizes the primary and secondary outcomes.

Primary outcomes: cognitive function

For cognitive function, neuropsychological tests will be performed to assess a range of aspects: global cognitive function, executive function, inhibitory control, memory and attention. CF tests are expected to last for about 30 minutes, and the following tests will be used:

a) Mini-Mental State Examination (MMSE) to assess the global cognitive state of the participants. The MMSE[18] consists of a cognitive screening tool composed of seven cognitive domains (temporal and spatial orientation, word localization, attention and

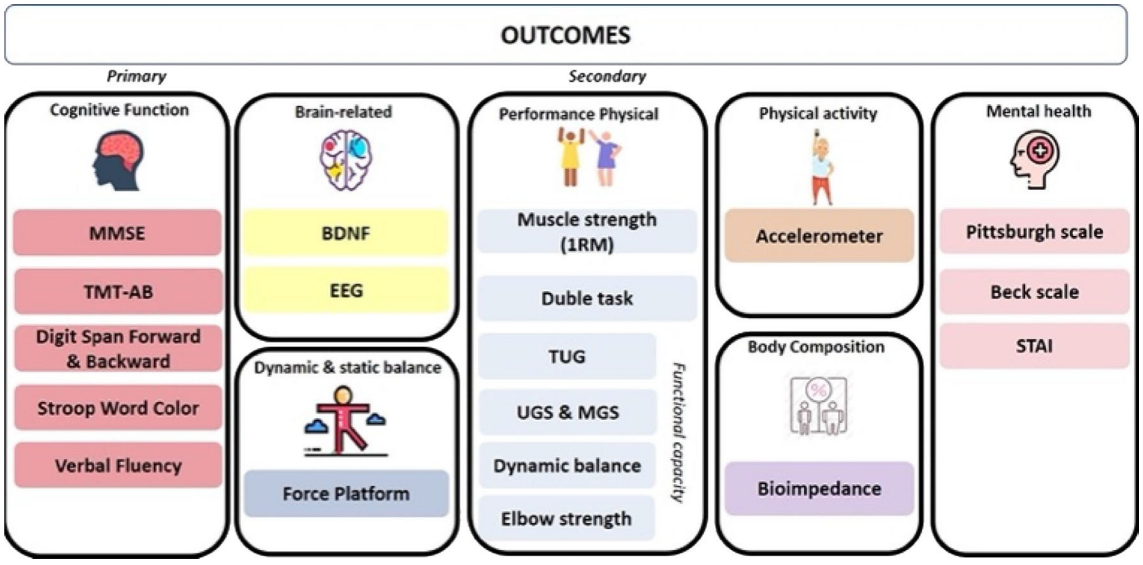

Figure 2 - Study outcomes summary. MMSE: Mini-Mental State Examination; TMT: Trial Making Tests; BDNF: Brain-derived neurotrophic factor; EEG: electroencephalogram; 1-RM: one maximal repetition test; TUG: timed up and go; UGS: usual gate speed; MGS: maximum gate speed; STAI: State-Trait Anxiety Inventory.

calculation, word recording, language and visual constructive capacity). Total score ranges from zero to a maximum of 30 points. Values equal to or above 24 points are considered 'normal', while values bellow 24 points are considered mild cognitive impairment (MCI) or 'dementia'. The average completion time for the MMSE is approximately 10 minutes.

b) Digit Span Forward & Backward scale to evaluate working memory. The test consists of random number sequences that begin with three digits and progressively increase to the maximum of nine digits. In the forward section, a list of numbers must be repeated correctly in the same order, while in the backward section the list of numbers must be repeated correctly in inverse order[19]. The test is completed when the participant cannot recall any of the two sequences of the same size. The final score is based on the number of successful sequences, with higher scores representing better performance.

c) Stroop Word Color (TESTINPACS®) to assess the selective process of attention, visual attention, and inhibitory control[20]. The test consists of 3 phases: the first (congruent) and second (neutral) phases are control conditions (i.e, indicating the correct name of the ink color of a rectangle on the screen; indicate), and the third phase is considered incongruent (indicating the correct name of the ink color of the colored words instead of the name of the written color). Selective attention and conflict resolution will be calculated as the mean difference in reaction time between incongruent and neutral phase responses[21].

d) Trial Making Tests (TMT AB), known as trail test, will evaluate processing velocity, mental flexibility, selective visual attention, visual search and changing capacity[22]. The TMT consists of two parts (A and B). Part A (with circles numbered 1 to 25) requires the participant to draw lines that connect sequentially surrounded numbers, in ascending order. In part B, the participant must draw a line that connects numbers and letters alternately and in ascending order. The amount of time (in seconds) spent to complete each task will be recorded.

e) Verbal Fluency test will investigate semantic and phonologic language[23]. This test evaluates the number of words spontaneously evoked orally for 1 min (by category). In the semantic category, the participants will be requested to say as many animal names as possible, while in the phonological category, they will say as many words as possible beginning with the letters F, A, and S.

Secondary outcomes

Brain-related outcomes: Assessment of plasma BDNF

Brain-derived neurotrophic factor (BDNF) will be measured. Plasma samples from participants will be collected before and post-intervention for all groups. Blood samples will be drawn into tubes containing EDTA, and then centrifuged immediately after at 3,000g for 10 min. Plasma will be isolated and stored at minus eighty degrees until analyses. Plasma concentrations of BDNF will be measured through enzyme-linked immunosorbent assays (ELISA) (SIGMA- ALDRICH, St. Louis, USA). After thawing, the samples will be centrifuged at 10,000g for 10 min at 4 °C for complete platelet removal. After the steps indicated by the manufacturer, the reaction will be

read in a spectrophotometer at 450 nm. BDNF levels will be detection in ng mL^{-1}. All samples will be analyzed in triplicates.

Assessment of EEG

The brain activation will be assessed using Muse®, a headband electroencephalogram (EEG) which represents cortical electrical activity and is composed by four channels: temporoparietal (TP9 e TP10) e frontal (AF7 e AF8) e o reference electrode (Fpz).

Physical performance outcomes

a) Muscle strength: The 1RM test assessment will follow the recommendations from the American College of Sports Medicine[24] and will be performed for all four exercises. Participants will undergo a specific warm-up composed of two series of 1) eight repetitions with approximately 50% of 1RM estimated load perceived and 2) three repetitions of the 70% of 1RM estimated load. After warm-up, participants will attempt to perform one complete repetition with a load that will be increased by 5% at each attempt until the volunteer is unable to perform the movement and the previous load will be considered as 1RM. The rest between attempts will be 3 minutes. The maximum number of attempts should be no more than five, otherwise the test will be considered invalid and rescheduled. The test will be performed again after 72 hours for accuracy of the 1RM load.
b) Functional capacity will be assessed through tests of agility, dynamic balance and lower limbs strength.
    1) The Sitting-Rising test (SRT) assess mucle strength of lower limbs and consists of the number of times an individual sit and rise from a 43 cm chair in a period of 30 seconds[25]. The Intraclass Correlation Coefficient (ICC) this test is of 0.92[25].
    2) The Timed up and Go test (TUG) assess the time a participant takes to rise from a chair, walk until a line there is 3 meters away, turn around (180), return and sit again in the chair[26]. Participants will be orientated to walk as fast as possible, safely and comfortably. TUGT is simple and evaluates mobility, static and dynamic balance. The Intraclass Correlation Coefficient (ICC) this test is of 0.99[26].
    3) For the usual (UGS) and maximum (MGS) tests gate speed, participants will be asked to walk a 4-meter distance in their usual and maximum (but without running) speed. To minimize acceleration and deacceleration effects, the participants will begin to walk 1 meter before the starting point and will deaccelerate 1 meter after the end. Volunteers will walk three times for each test (UGS and MGS). Van Loo, Moseley, Bosma, de Bie & Hassett[27] reported an Intraclass Correlation Coefficient (ICC) of 0.95 for this test.
    4) The Dynamic Balance test (DB) consists in the participants to stand (back foot toes touching front foot heel) at the beginning of a straight line. When command to start test is given, participant will walk on the line for 6 meters. This test has an excellent test-retest correlation coefficient (r = 0.94 to 1.00). After familiarization, the test will be performed three times[28].
    5) For the Elbow Strength test, the participant will be asked to sit in a chair and perform the maximum number of elbow flexion and extension for 30 seconds[25]. The Intraclass Correlation Coefficient (ICC) this test is of 0.80[25].
    6) Double task: this test will be used to quantify the individual's ability to simultaneously execute two tasks (motor and cognitive; motor-motor; double motor-cognitive). Simple motor tasks will be TUG. Motor-cognitive tasks will be to perform the simple motor tasks while saying backwards the days of the week, for the motor-motor participants will hold a glass of water while performing the simple motor tasks. As for the double motor-cognitive they will perform simple motor tasks while holding a glass of water and stating backwards the days of the week.

The following equations will be used[29]:

$$\text{CDT[\%] TUG motor} - \text{cognitive} = \frac{100 \times (\text{Time of timed up and go test (TUG) single} - \text{Time of TUG in dual task})}{\text{Time of TUG in single task}}$$

$$\text{CDT [\%] TUG motor} - \text{motor} = \frac{100 \times (\text{Time of timed up and go test (TUG) single} - \text{time of TUG motor in dual task})}{\text{Time of TUG in single task}}$$

$$\text{CDT [\%] TUG double motor} - \text{cognitive} = \frac{100 \times (\text{Time of timed up and go test (TUG) single} - \text{time of TUG double motor} - \text{cognitive in dual task})}{\text{Time of TUG in single task}}$$

where CDT = Cost Dual Task.

c) Dynamic and static balance will be analyzed using the balance platform Biodex® Balance System (BBS). Subjects will adjust their feet in the platform aiming to achieve the most possible stability. Then the grid

parameters will be recorded (closest letter and number to the heel and closest angle between second and third toe) while in bipedal support. Participants will be familiarized with the test. For the bipedal test, participants will remain with the upper limbs free without touching the equipment and will be instructed to look at the BBS monitor.

d) Physical activity and sedentary behavior: participants will receive an 'Actigraph GT3X+' triaxial accelerometer (Actigraph®, Pensacola, FL, USA). They will be instructed to use the equipment for seven consecutive days, on the right side of the hip, fixated with an elastic belt and remove it only for sleeping, bathing or performing activities in the water in addition to register in a diary the time and reasons why they removed the equipment. This measured will be assessed before the intervention and after 16 weeks. Subjects will receive a flyer with instructions, and they will be phone-called three times during the week (every two days) to encourage the use of the equipment. ActiLife 6.12 software (Actigraph®, Pensacola, FL, USA) will be used to upload and analyze the data with intervals of 60 seconds. Physical Activity data will only be included if the participant accumulates a minimum of 10h/day of recording for at least 4 days, including 1 weekend day. The cutoff point used will be[11]: light physical activity as 100-1041 counts/min and moderate to vigorous physical activity as ≥ 1041 counts/min. Total daily physical activity levels will be calculated and expressed as minutes of physical activity per day. Sedentary behavior will be defined by the cutoff <200 counts/min[30].

e) Body composition: total body mass, body mass index, lean mass and fat percentage will be assessed using a multi-frequency, tetrapolar bioimpedance equipment with eight electrodes (InBody 570 Biospace®, San Francisco - Califórnia, EUA).

Mental health

a) Pittsburgh scale will be used to assed sleep quality. It is a self-administered tool, with good Kappa score (*Kappa* = 0,75)[31]. This test has 19 questions structured in seven components that can be scored from zero (No difficulty) to three (Severe difficulty): subjective sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, use of sleeping drugs and daytime dysfunction.

b) Beck scale will assess subjects' depression levels. The questionnaire has 21 items, each with four statements as options. The final score will be calculated by the sum of the answer where a score of 0-9 is considered normal, 10-15 mild depression, 16-23 moderate depression and more than 24 severe depression[32].

c) Anxiety will be assed using the State-Trait Anxiety Inventory (STAI)[33]. It is a 40-item self-administered tool divided into two sections for anxiety as a personality trail and/or a state. The two sections are scored separately, with a minimum score of 20 and maximum score of 80, respectively, with higher scores indicating more intense levels of anxiety. The proposed cut-off point will be: < 33, which is equivalent to the absence of symptoms of anxiety or mild anxiety, between 33 and 49, equivalent to average anxiety, and > 49, equivalent to a high level of anxiety.

Data analysis

Data will be analyzed using the Statistical Package for the Social Sciences (SPSS version 22.0). An exploratory analysis of the variables will be conducted, with graphic representation (histograms and box plot) and frequency distribution, aiming to identify outliers and possible typing errors. Deviances from normal distribution in each variable will be analyzed using the Shapiro-Wilk test. The intraclass correlation coefficient will be used (ICC) to verify reproducibility for 1RM tests and retests. One-way analysis of variance will be used to explore baseline differences between groups. To analyze the training effects on dependent variables Mixed Generalized Linear Models (GMM), considering the groups (MLRT x LLRT x BFRRT x Control) and time (pre- and post-training) as fixed factors and the individuals as random factors. When the tested variables present statistically significant differences, post-hoc Bonferroni analysis will be performed for multiple comparisons. In addition, intention-to-treat analysis (ITT) will be performed. Percentage changes (Δ%) will be calculated to express possible mean differences of the dependent variables. The significance level will be set at 5%.

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