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Original Research

The Acute Effect of *In Natura* Beetroot Juice Intake on Intra-Session Exercise Sequences During Concurrent Training

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ABSTRACT

International Journal of Exercise Science 15(2): 1075-1085, 2022. The purpose of this study was to analyze the acute effects of *in natura* beetroot juice intake on intra-session exercise sequences during concurrent training. Following a randomized double-blind placebo-controlled crossover design, 20 well-trained men (21.4 ± 2.9 years; 74.8 ± 6.3 kg; 175.7 ± 5.0 cm) performed two concurrent training sessions with different intra-session exercise sequences: CT1 (aerobic exercise + resistance exercises) and CT2 (resistance exercises + aerobic exercise). The resistance exercises were bench-press, lat-pull down, and shoulder-press (three sets to failure; 2 s cadence for the concentric and eccentric phases; 90 s rest interval between sets and exercises; 75% 1RM), and the aerobic exercise was 4-km running. Each concurrent training session was randomized to placebo, beetroot juice, and control (no substances), totaling six exercise sessions. The rate of perceived exertion (RPE) was reported at the end of each exercise in each session. The beetroot juice significantly increased plasma nitric oxide concentration from 14.5 ± 3.9 mmol/L to 140.2 ± 37.5 mmol/L ($P < 0.01$) and there was no significant change after placebo intake (13.8 ± 4.2 vs 15.1 ± 5.7 mmol/L). The 4-km running time was significantly less ($P < 0.05$) after beetroot juice intake in CT1 (17.0 ± 2.1 min) and CT2 (18.5 ± 1.9 min) than placebo (19.1 ± 3.2 and 22.2 ± 2.9 min, respectively) and control (19.4 ± 2.6 and 21.7 ± 3.0 min, respectively). No differences were identified in the total number of repetitions in resistance exercises and RPE. In conclusion, the acute intake of *in natura* beetroot juice decreased the 4-km running time independently of concurrent training exercise sequences. Our results may assist trainers in order to choose the supplement to increase performance.

KEY WORDS: Nitrates, resistance training, exercise.

INTRODUCTION

The simultaneous development of muscle strength and oxygen consumption is necessary for different sports modalities (8). In this context, athletes from different modalities perform their training routine following a model known as concurrent training – i.e., integrating both resistance training and aerobic training in successive or the same sessions (11). However, some scientific evidence suggests that concurrent training can reduce physiological adaptations, especially those related to resistance training (such as strength and hypertrophy) (8, 34). In this sense, the chronic results of concurrent training may depend on the manipulation of the variables involved in the training, such as the exercise sequence, volume, and intensity (8).

In addition to manipulating training variables, the use of ergogenic resources could be a strategy to increase performance in both resistance and aerobic exercises (17, 18). Among the different possibilities of using ergogenic resources, the effect of *in natura* products, such as beetroot juice (BJ) (7), is gaining popularity as a dietary supplement, due to their high nutritional power and low cost (6). BJ intake can improve blood perfusion, reduce the oxygen consumption cost, improve muscle recovery, and, consequently, physical performance (1). This can be explained by the effect of nitrate (NO3-), present in the beetroot, stimulating the synthesis of nitric oxide (NO) (29).

The relationship between BJ and physical performance has been more widely researched in relation to aerobic exercise (13). In this context, several meta-analyses have described the positive effects of BJ on the performance of cycling and running (4, 12, 19). However, knowledge about the effects of BJ on muscle strength performance remains scarce. Two studies have demonstrated the enhancing of performance in bench press exercise after BJ intake in resistance-trained males (21, 33), in contrast, Flanagan et al. (10) did not observe the effect of BJ intake on performance in three sets of the back squat exercise. The physiological effects of BJ intake are unclear, although some data show that BJ increases the efficiency of muscle contraction, due to the reduction in the cost of ATP and reduced creatine phosphate depletion (31).

Regarding the effects of ergogenic resources on the performance of concurrent training, we identified only one study with caffeine (26). To our knowledge, no studies have investigated the effect of BJ on the performance of aerobic and strength exercises performed in the same session. In this context, we hypothesized that during a concurrent training session: 1) if there is a drop in performance in the subsequent exercise, the intake of BJ could reduce or prevent this drop; 2) if there is no drop in performance in the subsequent exercise, the intake of BJ could increase performance.

Thus, the purpose of this study was to verify the effects of acute BJ ingestion on performance in two concurrent training sessions following different sequences.

METHODS

Participants

The sample size calculation was performed with data from a pilot study considering the mean of 20.5 min in the 4-km running time, standard deviation of 5 min and minimum detectable difference of 3 min (G*power V 3.1.9.4). The results suggested a minimum of 18 subjects. Thus, 20 healthy, non-smoking men (age = 21.4 ± 2.9 years; weight = 74.8 ± 6.3 kg; height = 175.7 ± 5.0

cm; VO_{2max} = 42.2 ml.kg^{-1}.min^{-1}) with at least 12 months experience in both aerobic and resistance training (minimum of 3 times a week) participated in the study. Exclusion criteria were users of pure caffeine, anabolic steroids, energy drinks, drugs, and/or ergogenic substances; a diagnosis of cardiovascular, respiratory, metabolic, infectious, bone, articular, muscular, neurological, neoplastic, and psychiatric diseases; and psychological/emotional/behavioral impairment. Informed consent was obtained from each participant before data collection and all experimental procedures were approved by the Research Ethics Committee Involving Humans of the State University of Londrina. This research was carried out in accordance with the ethical standards of the International Journal of Exercise Science (22).

The subjects were instructed to arrive at the laboratory rested; not perform any training or leisure physical activity for 24 h before the tests; be in a hydrated state and eat a light meal 2 h before the procedure; not to drink alcohol, eat caffeinated foods, or use mouthwash or chewing gum for 48 h before the test; and to maintain eating habits during data collection. Subjects were also informed about nitrate-rich *in natura* foods and were instructed to avoid these foods during data collection, as well as any nutritional supplements with nitrate.

Protocol

Data collection was carried out on seven non-consecutive days (interval of 48 h) at the same time (8-10 am) and with controlled temperature (22-24º C).

On the first day, anthropometric measurements (body weight and height) and maximum repetition tests (1RM) were performed in the bench-press, lat pull-down, and shoulder-press. The 1RM tests were started after a warm-up with a subjectively light load. The warm-up consisted of a single set of 15-20 repetitions for each exercise. After the warm-up, the subjects were given up to 5 attempts to obtain the highest load that allowed only one single correct repetition. The interval between attempts was up to five minutes. The participants were also familiarized about the rating of perceived exertion (RPE) scale CR-10 (3).

On the other days, following a randomized double-blind and cross-over design, the subjects were allocated to perform concurrent training sessions starting with aerobic exercise followed by resistance exercise (CT1) or starting with resistance exercise followed by aerobic exercise (CT2). A passive interval of 5 min was established between aerobic and resistance exercise for each session. In each session, the subjects were randomized to a control session (without any substance), placebo, or BJ, totaling six exercise sessions. The intake of placebo or BJ occurred 60 min before the training. Each exercise session was preceded by the same warm-up pattern, consisting of 10 min on a cycle ergometer with subjectively light/moderate intensity and upper limb joint movements. At the end of each exercise in each session, the subjects reported their RPE (3). Figure 1 illustrates the distribution of the subjects about the allocation in the exercise groups (CT1, CT2, and control).

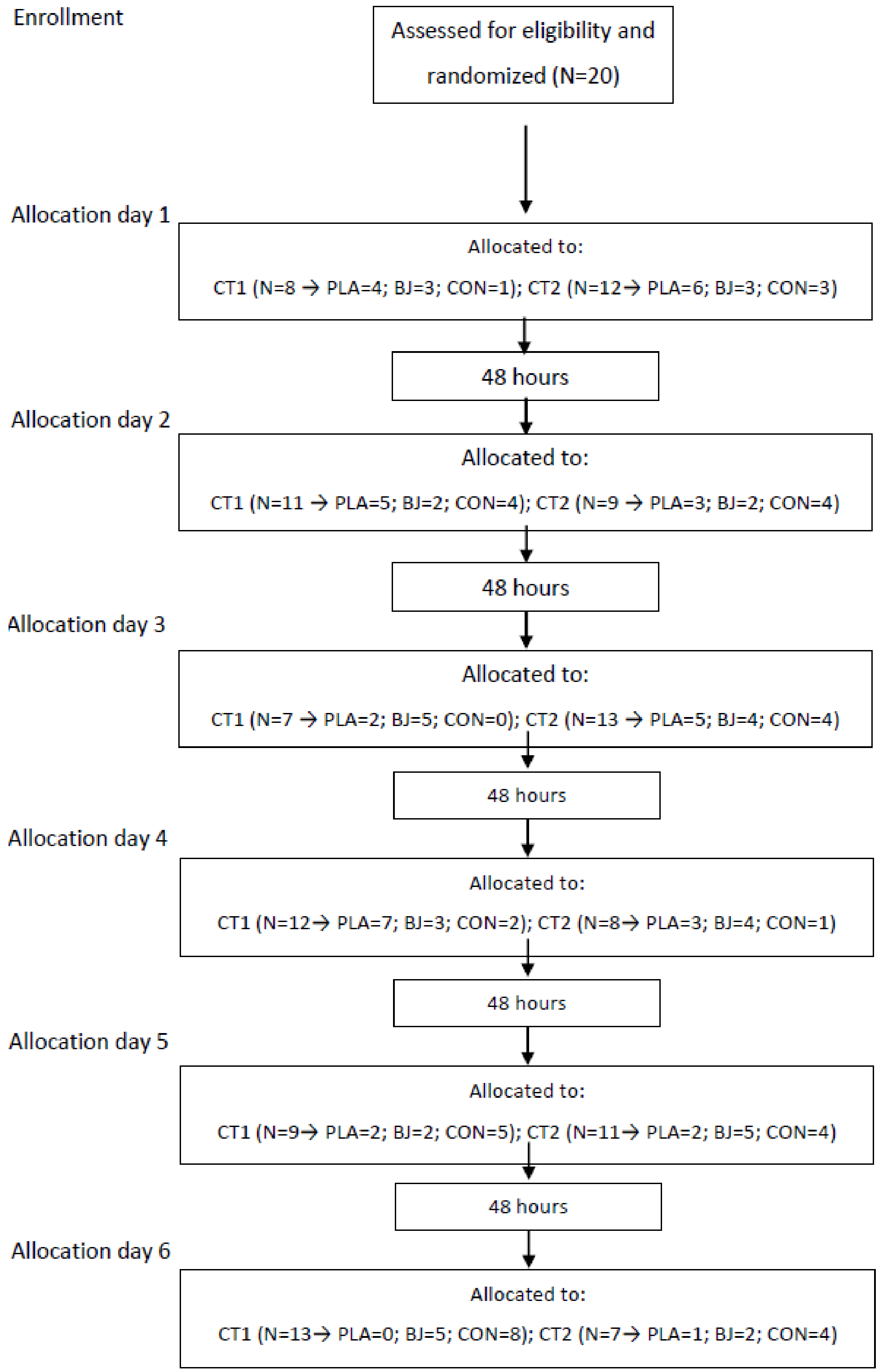

Figure 1. CONSORT diagram showing the flow of participants through each day of the randomized crossover design. CT1 = concurrent training 1 (aerobic + resistance exercises); CT2 = concurrent training 2 (resistance + aerobic exercises); PLA = placebo; BJ = beetroot juice; CON = control

4-km running test: The 4-km running test was performed on an electric treadmill (Inbramed KT - 10200 ATL, Brazil) without inclination. The subjects were instructed to cover the distance in the shortest possible time. The subjects had the autonomy to control the speed and had access to the distance and time.

Resistance exercises: Three sets were performed until exhaustion at 75% of 1RM and 2-s cadence for each of the concentric and eccentric phases, controlled by a metronome. The rest interval between sets and exercises was 90 s. The order of execution of the exercises was bench-press, lat-pull down, and shoulder press. The criterion for interrupting execution was the inability to maintain the execution rate for two consecutive repetitions.

Beetroot juice and placebo intake: Beetroot from the same production lot and stored in a refrigerator were sanitized, peeled, and processed for pure juice extraction before each data collection. The placebo consisted of açaí-flavored maltodextrin, to equalize the caloric content of the beetroot juice. To give flavor, consistency, and color to the placebo, 20 ml of beetroot juice was heated and added to the placebo. On test days, both beetroot juice and placebo were delivered to participants in disposable bottles.

Samples of BJ and placebo were analyzed in an independent food laboratory to quantify the BJ dosage to be ingested. Blood collection for NO dosing from NO2- and NO3- quantification was performed before and 60 min after ingestion of 500 ml of BJ or placebo (1, 2, 4, 14). The subjects were also asked if they were able to differentiate which of the drinks was BJ and placebo.

Nitric oxide analysis: For NO analysis, blood samples before and 60 min after ingestion of beetroot juice or placebo were collected from the antecubital region and placed in tubes containing EDTA as an anticoagulant. After centrifugation at 3,000 rpm for 5 min, the plasma was separated and stored in a freezer at -80° C until examination. NO metabolite levels were determined from NO2- and NO3- quantification using cadmium granules to reduce nitrate to nitrite. Subsequently, the concentrations of these metabolites were determined according to the Griess method (32).

Statistical Analysis

The Shapiro-Wilk test was used to observe the data distribution and the Levene test to verify the homogeneity of the variances. The Student's *t*-test was used to test the isolated effect of the concurrent training session on the performance of each exercise (control session). One-way ANOVA was used to compare NO metabolites after ingestion of BJ or placebo. The two-way ANOVA was used to compare the performance of each exercise and the RPE in the two concurrent training sessions in relation to the intake of a placebo, BJ, or the control condition. Tukey's *post-hoc* test was used when necessary to identify differences.

The data were analyzed with the software Statistica 10 (Statsoft, Tulsa, OK, USA) and $P < 0.05$ was adopted as the level of statistical significance.

RESULTS

All data are presented as means and standard deviations. The biochemical analysis showed a concentration of 16 mmol of nitrate in 500 ml of BJ, while the concentration in the placebo was < 1 mmol. Intake of BJ significantly increased plasma NO concentration from 14.5 ± 3.9 mmol/L to 140.2 ± 37.5 mmol/L ($P < 0.01$). There was no significant change after placebo intake (13.8 ± 4.2 vs 15.1 ± 5.7 mmol/L).

Regarding the isolated effect of the concurrent training session on performance, no changes were identified in the 4-km running time or total repetitions performed in each exercise. The inter-group comparison showed a significant difference between the session with BJ intake and the placebo and control sessions only for the 4-km running time, in both CT1 and CT2 (Table 1).

There were no differences in RPE between the groups in the different exercise sessions (Table 2).

Table 1. Total number of repetitions and 4-km running time on different concurrent training sessions

	Control	Placebo	Beetroot juice	Inter-group comparison
Concurrent training 1				
4-km running (min)	19.4 ± 2.6	19.1 ± 3.2	17.0 ± 2.1	BJ x placebo: $P = 0.02$ BJ x control: $P = 0.03$
Bench-press (total n of repetitions)	28.7 ± 3.5	31.2 ± 4.4	30.9 ± 3.0	NS
Lat pull-down (total n of repetitions)	25.6 ± 4.1	26.9 ± 3.6	27.1 ± 3.2	NS
Shoulder-press (total n of repetitions)	22.8 ± 2.9	23.1 ± 3.2	22.8 ± 2.8	NS
Concurrent training 2				
Bench-press (total n of repetitions)	29.5 ± 3.7	30.8 ± 3.5	31.1 ± 2.6	NS
Lat pull-down (total n of repetitions)	27.2 ± 3.9	28.0 ± 2.7	26.9 ± 2.2	NS
Shoulder-press (total n of repetitions)	24.4 ± 3.3	25.3 ± 2.9	24.2 ± 3.6	NS
4-km running (min)	21.7 ± 3.0	22.2 ± 2.9	18.5 ± 1.9	BJ x placebo: $P = 0.01$ BJ x control: $P = 0.01$

BJ = beetroot juice; NS = non-significant difference

Table 2. Rate of perceived exertion after each exercise in each session

	Control	Placebo	Beetroot juice
Concurrent training 1			
4-km running (min)	9.0 ± 2.0	9.2 ± 1.5	9.3 ± 1.8
Bench-press (total n of repetitions)	7.8 ± 1.4	7.9 ± 2.0	8.0 ± 1.4
Lat pull-down (total n of repetitions)	8.6 ± 0.9	9.0 ± 1.8	8.7 ± 1.2
Shoulder-press (total n of repetitions)	8.7 ± 1.7	9.0 ± 1.0	9.1 ± 1.2
Concurrent training 2			
Bench-press (total n of repetitions)	8.1 ± 1.3	8.6 ± 0.8	8.3 ± 1.9
Lat pull-down (total n of repetitions)	8.8 ± 2.0	9.0 ± 2.5	8.7 ± 1.5
Shoulder-press (total n of repetitions)	8.3 ± 1.1	8.4 ± 0.8	8.5 ± 1.6
4-km running (min)	9.1 ± 1.6	9.3 ± 1.7	9.3 ± 2.1

Regarding the identification of the taste of the drinks, of the 13 subjects who participated in the pilot study, 9 subjects were not sure which drink they had ingested; 3 subjects reported false-positive, and only 1 subject correctly identified the drink.

DISCUSSION

The present study aimed to verify the effects of acute BJ intake on the intra-session exercise sequences during concurrent training. The results showed that: 1) there were no differences between the aerobic and resistance exercise performances in either session; 2) BJ intake improved performance only during aerobic exercise, when compared to the control session and the placebo session, regardless of the intra-session exercise sequence; 3) there were no differences in resistance exercise and RPE.

Although some data show interference in performance during concurrent training conducted in the same session (20), the present study found no differences. This issue may be related to differences in the design of concurrent training sessions (intensity and volume) of the resistance and/or aerobic exercises. For example, Panissa et al. (23) concluded that aerobic exercise reduces performance in resistance exercise. However, the authors used the squat exercise (the same muscle group required for aerobic activity). In the present study, we used resistance exercises for the upper limbs, in order to avoid direct interference in relation to muscle mass.

However, the purpose of this study was to analyze the ergogenic effects of BJ on concurrent training performance. Based on the initial hypothesis, we thought that BJ could increase performance in concurrent sessions, either by improving the number of repetitions performed in resistance exercises or reducing the 4-km running time. However, the increase in performance only occurred in the 4-km running. According to previous studies, the ergogenic effect of BJ in modalities with aerobic characteristics seems to be associated with oxygen consumption economy (1, 14). In this sense, systematic reviews and meta-analyzes of clinical studies on the subject identified a positive effect size for BJ intake on some physical requirements, especially the time to exhaustion, which, indirectly, may be associated with greater speed or optimization of the effort (4, 12, 19). The subjects selected for the present study, although physically active, were not athletes. Recently, an investigation was found that the aerobic fitness level can affect the performance after BJ intake (24). They demonstrated that especially low-moderate aerobic fitness levels present a reduction of oxygen cost in moderate intensity exercise and an improvement in a 3 km time trial. In this context, the results could be different if experienced athletes performed this training model with BJ supplementation (5). In the present study, as the time to complete the 4 km distance was significantly shorter after ingesting BJ compared to placebo and control, the subjects were able to achieve higher speeds. This can be explained by the relationship between the ergogenic effect of BJ and the role of its precursor NO. After ingesting BJ, the nitrate present in the beetroot is absorbed and subsequently reduced to nitrite by the anaerobic bacteria in the oral cavity. Finally, nitrite is reduced to nitric oxide in the stomach acid environment (16). In this context, during the practice of exercises or modalities with aerobic demand, the intake of BJ has been associated with greater efficiency and mitochondrial biogenesis (15), lower oxygen consumption (2), vasodilation, and angiogenesis (9). In the case of the present study, these physiological alterations were manifested regardless of the order in which aerobic exercise was performed, suggesting that performance improvement may occur even when another activity is performed previously.

On the other hand, the BJ did not change the resistance exercise performance. These data corroborate, at least in part, other information in the literature. For example, Trexler et al. (30) did not observe ergogenic effects associated with BJ on leg-press performance. However, Williams et al. (33) found that the intake of 70 ml of BJ increased power, speed, and repetition volume in the bench-press exercise. Thus, the intake of BJ as an ergogenic resource for resistance exercises remains inconclusive in the scientific literature. This is mainly due to the diversity of methodological protocols used. In the present study, we used three exercises for upper limbs, each one performed in 3 sets until exhaustion, totaling 9 sets. We chose to perform the statistical analysis considering the sum of the repetitions in all sets of each exercise since the analysis in each set would reduce the degrees of freedom and could compromise the statistical power. Thus, specific intra-set differences would be difficult to explain, since we would not have enough data to state that the difference occurred due to the ergogenic effect of BJ or if it occurred due to some uncontrolled intervening variable. In addition, it could be expected that the number of repetitions would significantly reduce during the sets in the same exercise since the repetitions were performed until exhaustion. Our initial hypothesis was that BJ would increase performance due to the relatively high volume and with repetitions until exhaustion. One explanation could be a reduction in the cost of phosphocreatine and an increase in contractile efficiency[7]. However, research involving BJ and resistance exercises still needs further investigation to establish a concrete position on a significant ergogenic effect.

Regarding RPE, we did not identify differences between the values reported after each exercise in each session. This can be explained by the fact that the exercises were performed until exhaustion (in the case of resistance exercises) and at the highest possible speed (in the case of the 4km running). Other experiments using ergogenic resources reported the same results as the present study. For example, Souza et al. (28) investigated the effect of caffeine and placebo on knee extension with blood flow restriction performed until exhaustion and did not identify differences for RPE. Furthermore, in an investigation with BJ, the results in performance were similar, with the increase of performance and no significant differences in RPE (27). Interestingly, this aspect demonstrates the potential of these substances in maintaining the RPE values even in a greater mechanical effort compared to placebo conditions.

Regardless of the results presented, some limitations need to be mentioned. Different from the previous studies (12, 25), which describe that BJ should be ingested between 2 and 2.5 h before the exercise due to the time of conversion of nitrate to NO, we used a pre-workout BJ intake time. However, even with the ingestion of BJ occurring 60 min before the exercise protocol, we identified a high plasma NO concentration. Still, on BJ supplementation, we chose an acute intake design. For this reason, we cannot discuss the chronic effect of BJ intake on performance in concurrent training; and for the same reason, we cannot conclude that continued training under BJ supplementation provides a significant increase in maximum oxygen consumption after several weeks or months. The subjects' diet was not strictly controlled and, therefore, we cannot state whether other sources of nitrate were added to the diet. Similarly, we did not control psycho-emotional variables that could interfere (positively or negatively) in the results. Finally, concurrent training can be carried out following different combinations of volume, intensity, and form of execution, both for aerobic exercise and resistance exercise. Thus, our results should not be generalized to other concurrent training models, and further experiments are needed to clarify the effect of BJ on this training model.

In conclusion, BJ improved performance in the 4 km running in concurrent training sessions regardless of the intra-session exercise sequence. However, BJ did not change the total repetitions performed in the resistance exercises. Our results can help coaches and practitioners on the acute effect of BJ related to performance enhancement or health-related training.

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