Nutrition 62 (2019) 47–51

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Brief report

Cocoa flavanol effects on markers of oxidative stress and recovery after muscle damage protocol in elite rugby players

Flávia Giolo de Carvalho Ph.D. [a,b,*], Mitchell Garant Fisher M.Sc. [c], Todd T. Thornley M.Sc. [c], Karen Roemer Ph.D. [c], Robert Pritchett Ph.D. [c], Ellen Cristini de Freitas Ph.D. [a,b], Kelly Pritchett Ph.D. [c]

[a] Postgraduate Program in Food and Nutrition. Department of Food and Nutrition. Faculty of Pharmaceutical Sciences, State University of Sao Paulo, Sao Paulo, Brazil
[b] School of Physical Education and Sport of Ribeirão Preto, University of Sao Paulo, Sao Paulo, Brazil
[c] Department of Health Science, Central Washington University, Ellensburg, Washington

ARTICLE INFO

Article History:
Received 26 February 2018
Received in revised form 14 July 2018
Accepted 29 October 2018

Keywords:
Rugby
chocolate milk
cocoa flavanols
muscle damage
recovery

ABSTRACT

Objectives: Strenuous exercise can impair athletic performance due to muscular inflammation and oxidative stress. Antioxidants such as cocoa flavanols have been used as a supplement to prevent oxidative stress; however, the benefits of dietary antioxidants for athletic performance after muscle soreness (MS) is unclear. The purpose of this study was to examine the effects of cocoa flavanols after a MS inducing protocol.
Methods: In a randomized, double-blinded design, 13 male collegiate rugby players consumed either chocolate milk (CHOC) or chocolate milk with additional cocoa flavanols (CocoaCHOC) during a 7-d loading phase. MS was induced by a drop jump protocol on day 5 of the intervention. Athlete performance was assessed with vertical-jump and yo-yo tests and subjective measures of soreness 5 d before and 2 d post-MS protocol. Urinary markers of oxidative stress (isoprostanes) were assessed before and 48 h post-MS.
Results: No changes were observed between the groups over time for isometric torque ($P = .63$), vertical jump performance ($P = .39$), and yo-yo testing ($P = .57$) between the trials. No interaction was found in isoprostanes levels between the trials (CocoaCHOC baseline: 88 ± 0.38 pg/mL and 48 h post-MS: 81 ± 0.53 pg/mL; $P = .82$; and CHOC baseline: 98 ± 0.96 pg/mL and 48 h post-MS: 96 ± 0.38 pg/mL; $P = .59$). No main effect (treatment × time; $P = .58$) was observed for isoprostanes. Although not significant, the CocoaCHOC group ran 97 meters further than the CHOC group in the yo-yo test.
Conclusions: Cocoa flavanols added to a post-exercise recovery beverage for 7 d has no oxidative stress or athletic performance benefits.

Introduction

Exercise naturally increases oxidative stress, and low-to-moderate levels of oxidants are essential to the regulation of cell signaling pathways and skeletal muscle modulation. However, higher rates of oxygen consumption consequently increase the production of free radicals and oxidative stress, which may compromise an athlete's performance [1,2].

Dietary flavanols have antioxidant properties and the potential to prevent oxidative stress; thus, favoring postexercise muscle recovery [3]. In particular, cocoa-based beverages, such as chocolate milk, have been found to be an effective post-exercise recovery aid [4,5]. Besides the antioxidant flavanol content, these foods contain carbohydrates and proteins that are similar to some fluid replacement drinks, and become effective to replenish depleted glycogens in the muscles; thus promoting recovery after high-intensity exercise. Also, chocolate milk tastes good and is well accepted among athletes [4,6].

Holt et al. [7] evaluated cocoa flavanol kinetics in human plasma after intake of flavanol-rich cocoa, and cocoa flavanols were detected 0.5 h post ingestion, reaching a maximal concentration at 2 h. Thus, considering the peak levels, could cocoa flavanol supplementation prevent free radical overproduction and oxidative damage immediately postexercise and not interrupt exercise adaptations induced by reactive oxygen species produced during exercise? Could a 1-wk loading phase with a cocoa-based drink be effective as a post-exercise recovery beverage?

Additional research is necessary to evaluate the effect of the effects of cocoa on muscle recovery and oxidative stress levels. Therefore, our study aimed to evaluate the effects of cocoa flavanols on indices of muscle recovery and performance and oxidative stress markers in collegiate rugby players after a muscle-damage protocol.

* Corresponding author: Tel.: +55 16 3315-0529; fax: +55 16 3633-3949.
E-mail address: Flaviagiolo@gmail.com (F.G. de Carvalho).

https://doi.org/10.1016/j.nut.2018.10.035
0899-9007/

Methods

Participants

Thirteen male rugby players, who were members of the Central Washington University Rugby Team, participated in this study. The sample size was estimated by a priori power analysis and considering the data from Saunders et al. [8]. For both primary dependent measures with a power of 0.80, a minimally detectable difference could be detected in means of 350 U/L for creatine kinase and 3 cm for muscle soreness (MS) based on an estimated effect size of 1.0 SD units (SD between treatments: 200 U/L for creatine kinase, and 2 cm for MS). Finally, a two-tailed alpha-level of 0.05 power analysis indicated the need for six participants.

The group was homogenous, training for a preparatory period for the university championship season, and aged between 18 and 26 y old. The protocol was performed 1 mo before the start of the championship season, and all participants actively participated daily in the rugby-specific training sessions. Initially, 18 athletes were recruited, but only 13 finished the study protocol. Two players did not follow the supplementation protocol, and three athletes did not complete all steps of the protocol.

Human subjects review committee–approval was obtained from Central Washington University (approval number H15057). Written consent was obtained from the participants before participation. The exclusion criteria included lower extremity injury within the past 6 mo or currently taking nutritional supplements or anti-inflammatory medications [2].

Study design

A randomized, double-blinded study was conducted. Participants were designated to one of two independent groups: chocolate milk + cocoa flavanols (CocoaCHOC) or chocolate milk only (CHOC). The study consisted of a 7-d supplementation period with the assigned recovery beverage based on 1 g/carbohydrate/kg of body weight given daily to the athletes immediately post- and 2-h after rugby practice.

Athletes reported to the laboratory four times during the study. Anthropometric measurements were taken during the introductory laboratory visit, including height, weight, and body fat percentage, using a three-site skin-fold mensuration on the right side of the body in accordance with the Jackson and Pollock method [9].

Performance tests included the yo-yo test, vertical jump, muscle function protocol, and subjective measures of MS. All tests were performed during the introductory visit (baseline) and 48 h post-MS protocol (48 h-POST MS).

Participants started CocoaCHOC or CHOC treatment after baseline urine collection on day 1. Urine samples were collected to quantify oxidative stress markers. On day 5, participants reported to the laboratory for a blood draw (pre-) and then performed an MS protocol. At 24 h post-MS protocol, perceptual measures of MS were collected. The rugby players had practice daily during treatment, except for days 6 and 7, which was the recovery period, to isolate the effects of the MS protocol. On day 7, blood and urine samples were taken (48 h post-MS), and participants completed the performance and subjective measures of the MS tests (Fig. 1).

Treatment

Treatment consisted of two beverages: a cocoa-based carbohydrate + protein beverage (0 mg of flavanols) versus a carbohydrate + protein beverage with cocoa flavanols (308 mg). Participants consumed their assigned beverage immediately postexercise and again 2 h after exercise. The volume of chocolate milk per serving was calculated based on 1 g of CHO/kg of body weight daily for a 7-d period, and the total amount of cocoa flavanols provided was 616 mg/d [3,4]. The cocoa flavanols and placebo powder were provided by The Hershey Company (Hershey, PA). Considering that plasma peak levels are reached only 2 h post intake, the supplementation protocol did not interrupt exercise adaptations that are induced by reactive oxygen species and produced during exercise.

The chocolate milk (low-fat; Darigold, Seattle, WA) contained 246 Kcal, 36 g of CHO, 11 g of proteins, and 12 g of fat per 240 mL. The beverages were packaged in unmarked bottles. During the recovery period, participants were permitted to take part in simple daily activities and were instructed to eat a similar diet during each trial.

Muscle soreness protocol

MS was induced by a drop-jump protocol. The athletes performed the MS protocol, including a total of 100 drop jumps from a height of 0.6 m, which has previously been suggested to induce muscle damage in rugby players and other similar sports [10,11]. Five sets of 20 drop jumps were completed with maximal effort, with a 10 s break between each jump and 2 min of recovery between each set.

Measurements

Muscle function protocol (isometric contractions)

Muscle function was evaluated in the dominant-limb knee extensors against the lever arm of the isokinetic dynamometer (Biodex Medical Systems, Shirley, NY). The athletes performed three maximum voluntary contractions with the knee at an angle of 90°, lasting 3 s, with 60 s rest in between. The average peak torque value was recorded as Newton meters (Nm) [12].

Vertical jump

Performance was evaluated by a vertical jump test using a 60 × 40 cm force plate (AMTI, Watertown, MA). Participants were required to flex their knees to approximately 90° and then jump vertically as high as possible (three attempts with minute rest in between each jump) in accordance with the method described by Linthorn [13].

Rugby performance test: Yo-yo test

Participants performed the yo-yo test, consisting of repeated 2 × 20 m shuttle runs (i.e., shuttle pairs) at a gradually increased speed, set by audio beeps from a prerecorded compact disk [14]. The test was completed when the athlete failed to reach the finishing line in time. The distance run (in m) was recorded and represented the test result. The yo-yo test was completed indoors on running lanes and was done during the introductory visit (baseline) and 48 h post-MS protocol. This performance test is familiar to the rugby players because the coaches use this test during the season.

Subjective measurements of muscle soreness

Self-perceived MS was evaluated using a visual analog scale (VAS) with the following anchor points: no pain at all (left side) and unbearable pain (right side). MS was measured using a lower extremity functional scale (LEFS) [2] at the time of the introductory visit (baseline), 24 h post-, and 48 h post-MS protocol to evaluate

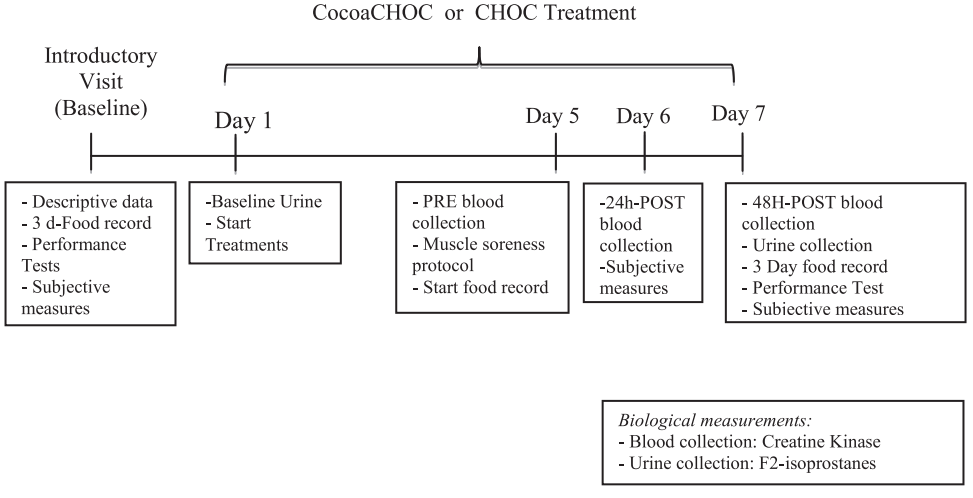

Fig. 1. Study design. CHOC, chocolate milk; CocoaCHOC, chocolate milk with additional cocoa flavanols.

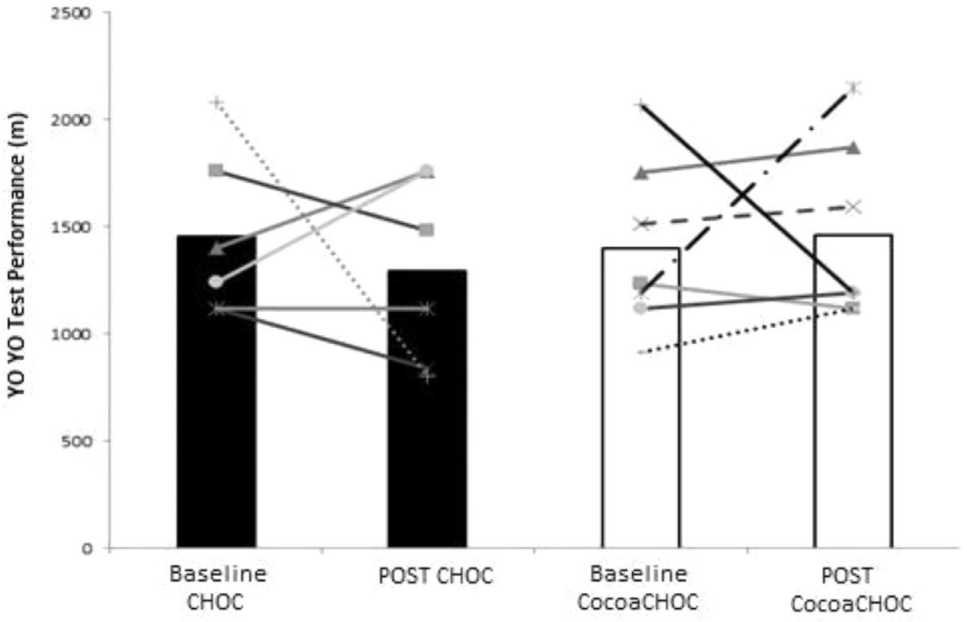

Fig. 2. Yo-Yo test performance of trained rugby players (n = 13) on two treatments (CocoaCHOC and CHOC). Individual and mean data are presented. No significant differences were observed between the trials. CHOC, chocolate milk; CocoaCHOC, chocolate milk with additional cocoa flavanols.

the athlete's level of exertion with daily living activities using a scale to classify the perceived level of difficulty in a 0 to 4 scale (0 = unable to; and 4 = having no difficulty performing the activity) [15].

Isoprostanes

Urine samples were collected into sterilized 100 mL cups to determine urinary F2-isoprostanes, which are oxidative stress markers, and quantified by immunoassay using commercially available enzyme-linked immunosorbent assay kits (Oxford Biomedical Research, Oxford, MI) [16].

Statistical analysis

A one-way, repeated-measures analysis of variance (treatment × time) was used to compare creatine kinase (CK), VAS, and LEFS. A *t* test was used to compare isoprostane levels, and performance test between baseline and 48 h post-trials, using the SPSS software, version 15.0 for all statistical analyses. The results were reported as means ± standard deviation, and statistical significance was set at $P < .05$ for all analyses.

Results

The descriptive characteristics of the athletes were reported as means ± standard deviation as follows: age: 20.69 ± 1.49 y, height: 180.0 ± 0.05 cm, weight: 87.02 ± 8.03 kg, and body fat percentage: 12.91% ± 4.20%.

Performance measures and muscle soreness

Performance was measured using accumulated shuttle distance (m) by the participants in the yo-yo test. There was no significant difference ($P = .57$) between the baseline and 48 h post-MS protocol (CocoaCHOC baseline: 1405.71 ± 373.52 m and 48 h post-MS: 1468 ± 378.25 m; CHOC pre-MS: 1453.33 ± 355.28 m and 48 h post-MS: 1293.33 ± 397.77 m; Fig. 2). However, total distance increased by 9.85% (62.86 ± 539.48 m) in the CocoaCHOC trial and decreased by 5.8% (160.0 ± 583.48 m) in the CHOC trial from baseline to 48 h post-MS in the yo-yo test performance.

Regarding isometric torque over time, no significant differences ($P = .63$) were found between the trials (CocoaCHOC baseline: 311.71 ± 48.91 Nm and 48 h post-MS: 321.53 ± 56.45 Nm; CHOC baseline: 299.34 ± 43.97 Nm and 48 h post-MS: 332.29 ± 60.25 Nm). In addition, no significant changes ($P = .39$) were achieved for vertical jump performance between the trials (CocoaCHOC baseline: 0.47 ± 0.11 m and 48 h post-MS: 0.41 ± 0.89 m; CHOC baseline: 0.52 ± 0.55 m and 48 h post-MS: 0.45 ± 0.05 m).

No interaction (treatment × time) for MS was observed using the VAS ($P = .23$; Fig. 3A) and LEFS ($P = .82$; Fig. 3B) scales at baseline, 24 h post-MS, and 48 h post-MS.

Creatine kinase and isoprostanes

Regarding CK levels, a one-way analysis of variance showed no significant changes between the groups for CK pre-MS ($P = .36$; CocoaCHOC: 112.94 ± 64.82 U/L; CHOC: 77.95 ± 56.09 U/L), for CK 24 h POST-MS ($P = .74$; CocoaCHOC: 92.06 ± 57.89 U/L; CHOC: 97.06 ± 58.81 U/L), and for CK 48 h post-MS ($P = .24$; CocoaCHOC: 41.97 ± 19.25 U/L; CHOC: 56.41 ± 28.27 U/L). Furthermore, there was no effect (treatment × time; $P = .95$) for CK when comparing the groups.

No significant difference was found for isoprostanes between the trials (CocoaCHOC baseline: 88 ± 0.38 pg/mL and 48 h post-MS: 81 ± 0.53 pg/mL; $P = .82$; and CHOC baseline: 98 ± 0.96 pg/mL and 48 h post-MS: 96 ± 0.38 pg/mL; $P = .59$). No main effect (treatment × time; $P = .58$) was observed for isoprostanes.

Discussion

The main objective of this research was to evaluate the effects of CocoaCHOC versus CHOC on oxidative stress and muscle-damage markers. Exercise performance was assessed using a yo-yo test, isometric torque, and vertical jump height to verify the efficacy of the recovery beverages. Our results suggest that the addition of cocoa flavanols or placebo to chocolate milk for a period of a

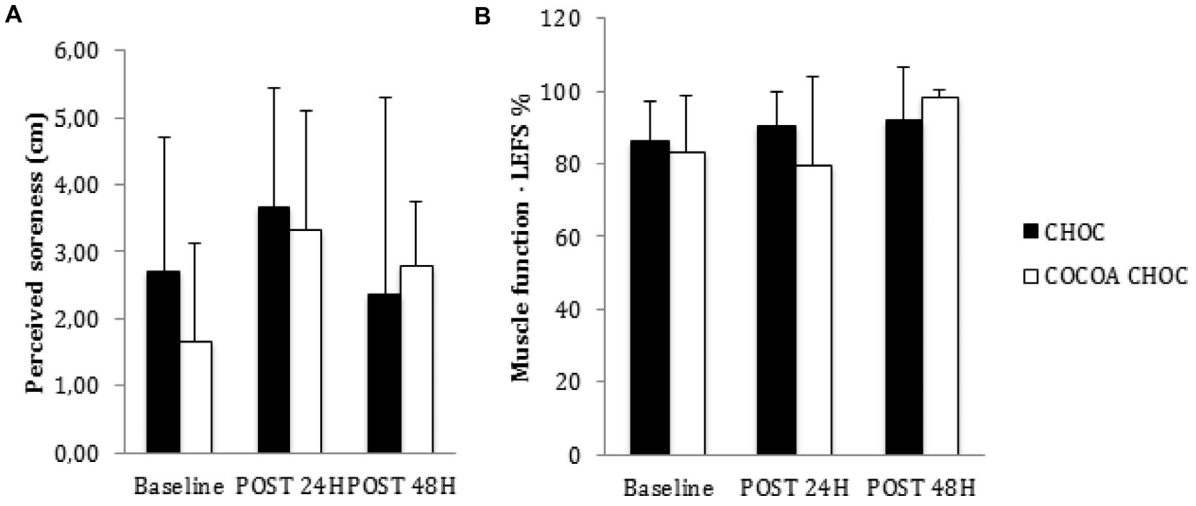

Fig. 3. Subjective measurements of muscle soreness on day 1, baseline, 24 h post MS, and 48 h post-MS protocol for each beverage (CHOC and CocoaCHOC). (A) mean ± standard deviation percent muscle function (LEFS); and (B) mean ± standard deviation perceived MS using the visual analog scale. No significant differences were observed between the trials. CHOC, chocolate milk; CocoaCHOC, chocolate milk with additional cocoa flavanols; LEFS, lower extremity functional scale; MS, muscle soreness.

week provides no additional benefits on indices of perceived soreness and muscle recovery. Furthermore, there were no benefits for exercise performance after muscle damage between the two treatments.

Benefits were expected with the intervention because cocoa flavanols are composed of phytochemicals such as epicatechin, proanthocyanidins, and theobromine. These components present functional proprieties that could prevent muscle damage with its anti-inflammatory and antioxidant properties [17], including metal chelation and scavenging of free radicals.

Cocoa flavanols may also decrease the expression of inflammatory signaling by the direct scavenging of nitric oxide, inhibiting the synthesis or excessive production of inducible nitric oxide, and decreasing some enzymes such as xanthine oxidase, NADPH oxidase, and lipoxygenases [18,19]. However, regarding oxidative stress and muscle damage, no benefits were observed in our study.

Intense training is well known to increase oxidative metabolism and exacerbate free radical production, thereby negatively affecting performance [20]. Therefore, the utilization of antioxidant nutrients after an MS protocol aimed to prevent oxidative stress and muscle damage [21]. Using a quadriceps, strenuous, contractions MS protocol, McLeay et al. [22] examined the effect of a blueberry smoothie before, immediately after, 12 h after, and 36 h after an MS protocol on oxidative stress markers. In contradiction with the results of the present study, an increase was found in oxidative stress biomarkers after the damage protocol, and a quicker decrease in oxidative stress markers 36 h after the MS protocol was observed in the blueberry group.

In the present study, a drop-jump MS protocol was performed 5 d after CocoaCHOC or CHOC treatment was started, and no significant differences were found in relation to oxidative stress between the treatments. Of note, increased isoprostane levels were observed in the rugby players in either trial, and these levels were similar those of patients with lung diseases [16,23]. Therefore, the chocolate milk (i.e., source of carbohydrates and proteins) may have prevented an overproduction of isoprostanes in both groups. Goodall and Howatson [10] and Nosaka et al. [11] used the same MS protocol, and muscle damage was induced. However, our study was the first to investigate the effect of the drop-jump MS protocol on oxidative stress markers specifically, and its association with a loading phase with the ingestion of a carbohydrate and protein recovery beverage that included additional cocoa flavanols.

Although no significant treatment effects were observed for the yo-yo test, our data indicate that there may be potential performance benefits attributed to cocoa flavanols. From a practical perspective, even performing the MS protocol that could induce muscle damage and impar performance, 71% of athletes in the CocoaCHOC group had a better yo-yo performance compared with the test performed in the introductory visit and 48 h post-MS protocol, but only 28% of participants performed better in the CHOC group (Fig. 2). When examining the percentage of change over time (baseline to 48 h post-MS protocol), an improvement of 9.85% was found in the accumulated shuttle distance by the CocoaCHOC group, which means approximately 97 m more than the CHOC group, considering that the CHOC group presented a decrease of 5.8% in the yo-yo test performed 48 h post-MS protocol. Therefore, cocoa flavanols combined with low-fat chocolate milk may offer some practical performance benefits after an MS protocol.

Conclusions

The current research results suggests that the combination of cocoa flavanols with low-fat chocolate milk as a post-exercise recovery beverage resulted in no additional advantage in performance and oxidative stress (Fig. 4). Additional research is necessary to evaluate the potential benefits of the addition of cocoa flavanols for longer loading phases on markers of exercise recovery and oxidative stress because some possible benefits were observed when considering individual athletes performance.

Acknowledgment

The authors would like to thank Darigold (Seattle, WA) for providing the chocolate milk and The Hershey Company (Hershey, PA) for providing the cocoa powder. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (PDSE-CAPES) - Finance Code 001.

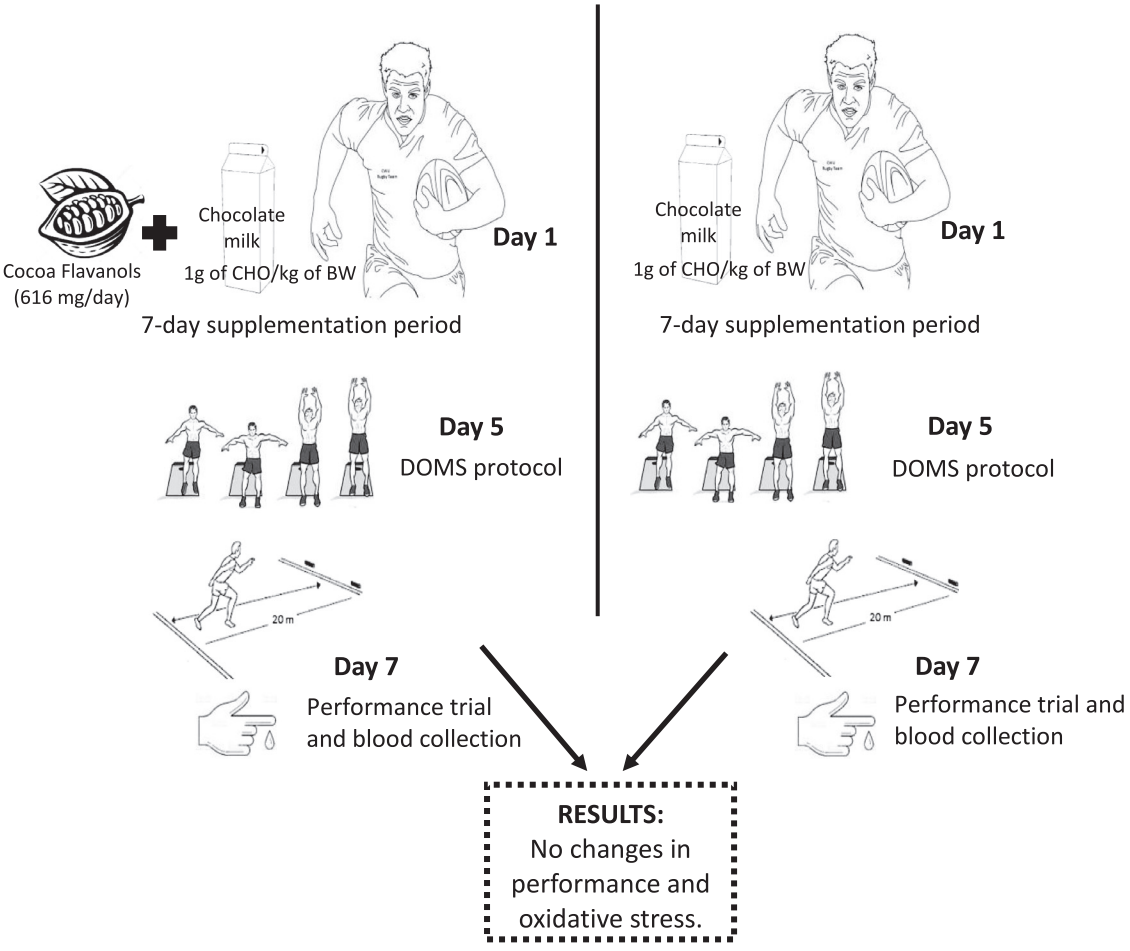

Fig. 4. Graphic abstract. BW, body weight; DOMS, delayed-onset muscle soreness.

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