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ORIGINAL RESEARCH

Acute Consumption of Varied Doses of Cocoa Flavanols Does Not Influence Exercise-Induced Muscle Damage

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Polyphenol consumption has become a popular method of trying to temper muscle damage. Cocoa flavanols (CF) have attracted attention due to their high polyphenol content and palatability. As such, this study will investigate whether an acute dose of CF can aid recovery following exercise-induced muscle damage. The study was a laboratory-based, randomized, single-blind, nutrient-controlled trial involving 23 participants (13 females and 10 males). Participants were randomized into either control ~0 mg CF ($n = 8$, four females); high dose of 830 mg CF (CF_{830}, $n = 8$, five females); or supra dose of 1,245 mg CF (CF_{1245}, $n = 7$, four females). The exercise-induced muscle damage protocol consisted of five sets of 10 maximal concentric/eccentric hamstring curls and immediately consumed their assigned drink following completion. To measure muscle recovery, maximal voluntary isometric contraction (MVIC) of the knee flexors at 60° and 30°, a visual analog scale (VAS), and lower-extremity function scale were taken at baseline, immediately, 24-, 48-, and 72-hr postexercise-induced muscle damage. There was a main effect for time for all variables ($p < .05$). However, no significant differences were observed between groups for all measures ($p \geq .17$). At 48 hr, there were large effect sizes between control and CF_{1245} for MVIC60 ($p = .17$, $d = 0.8$); MVIC30 ($p = .26$, $d = 0.8$); MVIC30 percentage change ($p = .24$ $d = 0.9$); and visual analog scale ($p = .25$, $d = 0.9$). As no significant differences were observed following the consumption of CF, there is reason to believe that CF offer no benefit for muscle recovery when ingested acutely.

Keywords: antioxidants, muscle function, perceived soreness, polyphenols

Eccentric muscle contractions are typically responsible for the muscular disruption that leads to exercise-induced muscle damage (EIMD; Nikolaidis et al., 2007). Therefore, resistance training and intermittent high-intensity exercise often evoke EIMD (Owens et al., 2019). Consequences of EIMD include inflammation and oxidative stress (Kanda et al., 2013); impaired force-generating capacity (Twist & Eston, 2009); and increased muscle soreness (Impellizzeri et al., 2008). Optimizing the time course of recovery is now a priority in modern sport, mainly due to the rapid turnaround of competitions and fixtures. Contemporary examples include tennis players performing every other day at major championships and congested fixture periods in soccer when players perform two 90-min matches within 3 days. Notably, injury-risk and muscular fatigue may be increased during congested fixture periods in soccer, namely due to the insufficient recovery time between matches (Ekstrand et al., 2011; Page et al., 2019). Therefore, the aim of recovery is to restore normative values for an individual following exercise by reducing neuromuscular fatigue, soreness, and restoring contractile functional capacity. To reduce fatigue and facilitate recovery, high carbohydrate protein meals or beverages, as well as high polyphenolic foodstuffs (e.g., cocoa) have become a common feature of an athlete's diet (Knapik et al., 2016). Polyphenol is an umbrella term for the different classes of plant metabolites, including flavonoids, stilbenes, phenolic acids, and lignans.

Flavonoids are the largest group of dietary polyphenols and the most common source of antioxidants within the diet (Scalbert et al., 2005). In recent years, a subclass of flavonoids, known as flavanols, such as catechin and epicatechin, have attracted much attention as health-promoting nutrients. Sources of flavanols include lychees, apples, tea, broad beans, and cocoa (Williamson, 2017). Cocoa has the highest proportion of flavanols per serving than any other natural source (Lee et al., 2003). Previous research has focused on the effects of cocoa flavanols (CF) on the cardiovascular system, with evidence suggesting CF intake can reduce endothelial dysfunction by improving flow-mediated dilation (Hooper et al., 2012) and reducing blood pressure (Buitrago-Lopez et al., 2011). Furthermore, CF have been shown to enhance endogenous antioxidant capacity (Serafini & Peluso, 2016); limit oxidative stress (Allgrove et al., 2011); and influence the inflammatory process by reducing both platelet aggregation and the stimulation of neutrophils (Ellinger & Stehle, 2016).

Regarding muscle recovery and exercise, research has shown that acute (single dose on day of exercise stimulus) and subchronic (regular intake for ≥14 days) CF supplementation of ≥200 mg reduces exercise-induced oxidative stress (Allgrove et al., 2011; Davison et al., 2012). Furthermore, in relation to exercise, the ingestion of CF may improve sprint performance by potentially preventing reactive oxygen species (ROS) increased calcium

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sensitivity of myofilaments within working muscles therefore delaying fatigue (de Carvalho et al., 2019; Patel et al., 2015). However, evidence is lacking regarding the impact of CF on markers of muscle recovery, such as perturbations in muscle function and an increase of perceived soreness. One such study used a CF dose too low (74-mg CF and 8 mg epicatechin) to be effective (Morgan et al., 2018). Benefits begin to be observed at doses of ~700 mg CF; and more importantly, with >50-mg epicatechin, the most biologically active flavanol (Schroeter et al., 2006). However, an optimal dose is not yet known in addition to any potential dose–response. Furthermore, previous research that investigated the impact of CF on muscle recovery did not induce notable muscle damage using a drop-jumps protocol (de Carvalho et al., 2019) and a downhill running protocol (Peschek et al., 2013). This can be defined as reductions in muscle force-generating capability of ≥20% following EIMD (Paulsen et al., 2012). Therefore, making conclusions about the impact of CF on markers of muscle damage is difficult, indicating that more research is warranted. Furthermore, none of the previous studies involved female participants, likely due to the purported protective effects of estrogen against muscle damage (Tiidus, 2003) and physiological variations across the menstrual cycle (Hayashida et al., 2016). Therefore, investigating the effect of CF supplementation on muscle recovery in females is required.

Therefore, the aims of this study were twofold; (a) to investigate the impact of an acute dose of CF on indices of muscle recovery and (b) to compare two different doses of CF on indices of muscle recovery. The hypothesis for this study was that EIMD might be attenuated following acute consumption of CF, with the highest dose offering the most benefit.

Methods

Participants

Following institutional ethical approval from the University of Huddersfield and in agreement with the Declaration of Helsinki, 30 participants consented to take part between the months of April 2019 to October 2019. However, only 23 completed the study (13 females and 10 males) due to the following reasons: two due to injury and five due to unforeseen circumstances following baseline testing. For participant characteristics see Table 1. An a priori power calculation determined that a sample size of 21 was required for 80% power and to detect significance, based on the effect size from previous research regarding maximal voluntary isometric contraction (MVIC) recovery at 48 hr (Bowtell et al., 2011). Baseline testing involved MVIC of the knee flexors to assess muscle function and measures of perceived muscle soreness using a visual analog scale (VAS) and lower-extremity function scale (LEFS). All participants were classed as recreationally active and injury free for the previous 6-months (both informed via self-report) and were not taking any dietary supplements (e.g., vitamin C, glutamine, or branched-chain amino acids). Participants were asked to avoid anti-inflammatory medications and resistance training during participation. A menstrual cycle questionnaire (Brown, 2017) was completed by the female participants involved to reliably estimate cycle phase. The luteal phase was selected for testing or an equivalent period for participants who were on hormonal contraception, as to avoid peak estrogen concentrations observed during the follicular phase (Brown, 2017). Participants completed each day within 26 ± 2 hr of original participation to account for diurnal influence.

Study Design

The study was a laboratory-based, randomized, single-blind, nutrient-controlled trial. Participants were randomized into a control (CON), high (CF_{830}), or supra (CF_{1245}) group and remained unaware of their allocation for the entirety of the study. Participants were required to come to the laboratory for 5 days, the first being baseline testing and familiarization of the EIMD protocol (10 submaximal concentric–eccentric hamstring curls). The remaining 4 days took place consecutively; as such, measures were taken in the following order: baseline, immediately post-EIMD (0 hr), 24-, 48-, and 72-hr post-EIMD. For full study timeline, see Figure 1. For randomization, participants were assigned to separate strata, "strong" and "not strong," based on their baseline MVIC values and randomized into matched and counterbalanced groups (using random.org). To decide what could be classified as strong or not, a normative MVIC strength index was used (Risberg et al., 2018 for females and Ruas et al., 2015 for males). Following this, eight participants were allocated to the control group (four females and four males), eight to the CF_{830} group (five females and three males), and seven to the CF_{1245} group (four females and three males).

Muscle Function

Values were recorded for knee flexor MVIC using the isokinetic dynamometer (model 770; Cybex NORM®, CA), providing a reliable quantification of decrements in muscle function for assessing EIMD (Warren et al., 1999). Knee angles of 60° (MVIC60) and 30° (MVIC30) of the anatomical zero (full knee extension) were selected due to the differences in muscle activation at various knee angles; biceps femoris has increased activation at angles between 15–30°, while semitendinosus and semimembranosus at knee angles between 60–105° (Onishi et al., 2002).

Subjective Soreness

Soreness was recorded using a 200-mm VAS, which has been previously included as a validated measure of subjective soreness (Peschek et al., 2013). The LEFS is a validated questionnaire which quantifies an individual's perceived level of muscle function using 20 hypothetical activities that are scored from 0 to 4; 0 = *extreme difficulty*; 4 = *no difficulty* (de Carvalho et al., 2019).

Muscle Damaging Protocol

The exercise protocol used to induce muscle damage was adapted from White et al. (2008) using the Cybex Norm isokinetic dynamometer (CSMi, Boston, MA). Participants were then secured into the dynamometer at 85° hip flexion using straps to isolate the knee and to remove hip flexor involvement. Body position was noted during baseline testing and replicated throughout. A specific warm-up consisting of 10 concentric/eccentric contractions of the knee flexors at a self-perceived low effort level was performed pre-exercise. Following the warm-up, participants performed five sets

Table 1 Participant Characteristics

Group	Age (years)	Stature (cm)	Mass (kg)
CON	24 ± 4	175 ± 8	74 ± 15
CF_{830}	25 ± 5	168 ± 9	68 ± 10
CF_{1245}	24 ± 5	168 ± 11	65 ± 12

Note. CON = control; CF = cocoa flavanols. Data are presented as mean ± *SD*. No significant differences observed between groups.

of 10 maximal concentric/eccentric contractions of the knee flexors with an interset rest of 1 min; rotation speed was 60°/s. Participants were verbally encouraged throughout, and once all repetitions were completed, the participant immediately repeated the protocol on the opposite leg.

Nutritional Intervention

Participants were blinded to which group they were assigned, with only the lead researcher being aware of the contents of each drink. Participants consumed their assigned beverage within 5 min following the protocol. Each beverage consisted of 300 ml water, 60 g maltodextrin, and 25 g whey protein powder (20 g protein). The cocoa powder used was a commercially available high flavanol powder (Chococru© Extraordinary Flavanol Cocoa), containing ~8.3% flavanols and a total polyphenol content of ~12% (unpublished data from Chococru©). The beverage for CF_{830} included an additional 10 g of Chococru© cocoa powder which contained 830 mg CF (98.6 mg epicatechin), and for CF_{1245}, 15 g of Chococru© cocoa powder was added, containing 1,245 mg CF (149.4 mg epicatechin; Table 2).

Dietary Measures

Participants completed a 24-hr dietary recall each day of testing, totaling five food recalls, and were asked to continue eating their usual diet throughout testing. During baseline testing, participants were provided a list of high polyphenolic food and drink (cherries, blueberries, dark chocolate, green and black tea, wine, apples, lychees, pomegranates, and fruit juices) to refrain from consuming 3 days before and during the testing period, reducing the confounding influence of other dietary polyphenols on recovery (Scalbert et al., 2005). Dietary analysis was carried out using Nutrimen (Dark Green Media Ltd., Flemingston, Wales, ©2016).

Statistical Analyses

Statistical analysis was performed using IBM SPSS Statistics (version 24.0; IBM Corp., Armonk, NY). All data was assessed for normality, a Greenhouse–Geisser correction was used if sphericity was violated. A repeated-measures analysis of variance was used to determine interaction and time effects for the recovery variables. If any significance was observed, Fisher's Least Significant Difference post hoc testing was performed to identify the point of significance. Data for MVIC60 and MVIC30 was calculated as percentage change from baseline alongside absolute means. To calculate effect sizes, Cohen's *d* was utilized, with the magnitude of effects considered small (0.2), moderate (0.5), and large (0.8). Significance was set at $p \leq .05$ preanalysis. Descriptive statistics are reported as mean (%) ± *SD*.

Results

There were no significant differences for participant characteristics or dietary intake between groups ($p \geq .33$). See Table 3 for dietary intake.

Muscle Function

Muscle function measured using MVIC at 60° and 30° found a main effect of time ($p < .05$). There were no significant differences between groups for knee flexor peak torque at MVIC60 ($p = .99$) or MVIC30 ($p = .95$) at baseline. Following the exercise protocol, overall mean knee flexor peak torque reduced to 79% of baseline. There was a significant effect of time across all groups ($p < .05$). For MVIC60, there were no significant differences between groups ($p \geq .17$). For MVIC30, there were no significant differences between groups ($p \geq .55$) (Figure 2).

Table 2 Nutritional Information of Beverages

Beverage	kcal/kJ	CHO (g)	Pro (g)	Fat (g)	Flavanol (mg)	ORAC
CON	340/1,427	61.9	19	1.9	Nil	Nil
CF_{830}	366/1,531	63.3	21.4	2.9	830	20,000
CF_{1245}	379/1,589	64	22.6	3.4	1,245	30,000

Note. ORAC = oxygen radical absorbance capacity; CON = control; CF = cocoa flavanols; CHO = carbohydrate. All drinks contain 60 g of maltodextrin and 25-g chocolate smooth whey protein powder, drinks 2 and 3 contain 10 and 15 g of Chococru powder, respectively.

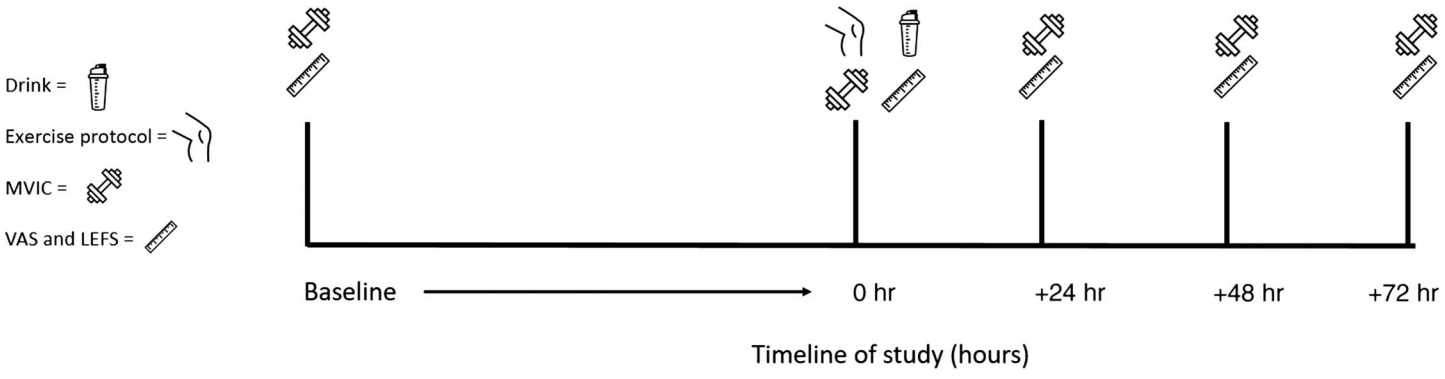

Figure 1 — Study schematic detailing experimental timeline. MVIC indicates maximal voluntary isometric contraction; VAS = visual analog scale; LEFS = lower-extremity function scale.

Table 3 Dietary Intake Between Groups

Nutrients	CON	CF_{830}	CF_{1245}	Significance (*p*)
Energy (kcal)	2,137 ± 559	2,101 ± 394	2,164 ± 591	.98
Protein (g)	109 ± 49	106 ± 47	106 ± 43	.99
CHO (g)	227 ± 46	253 ± 41	265 ± 106	.60
Fat (g)	93 ± 32	81 ± 19	79 ± 21	.57

Note. CON = control; CF = cocoa flavanols; CHO = carbohydrate. Values are presented as mean ± *SD*.

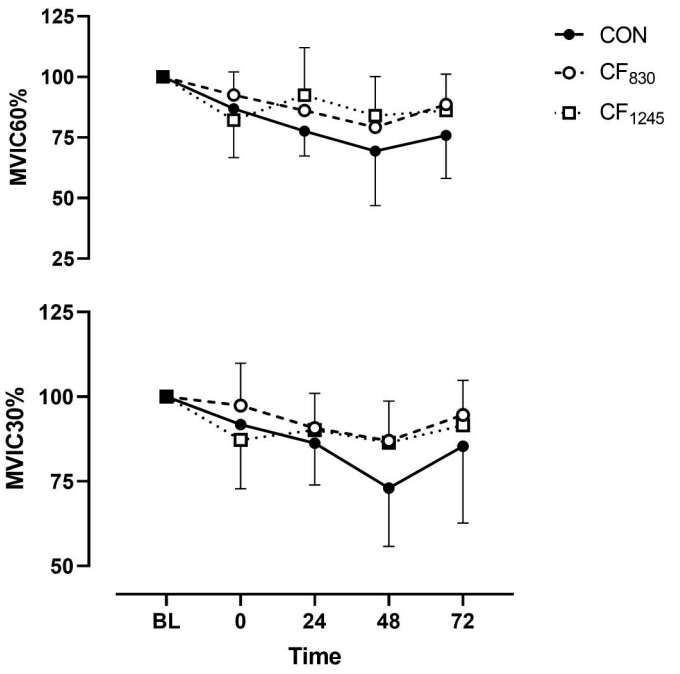

Figure 2 — Percentage change from baseline for MVIC following EIMD. MVIC indicates maximal voluntary isometric contraction; CON = control; CF = cocoa flavanols; BL = baseline; EIMD = exercise-induced muscle damage.

Measures of Perceived Soreness

Perceived soreness measured using a VAS and LEFS found a main effect of time for both measures ($p \leq .05$). There were no significant differences between groups for VAS scores ($p \geq .39$). There were no significant differences between groups for LEFS scores ($p \geq .75$).

Discussion

The main aim of this study was to investigate whether various doses of CF have any impact on indices of muscle recovery following EIMD. Based on the results of the current research, no significant differences were found following the addition of CF. This study corroborates previous findings that suggest an acute dose of CF has no significant impact on measures of muscle function, or measures of perceived soreness (de Carvalho et al., 2019; Morgan et al., 2018; Peschek et al., 2013).

Differences between this study and previous studies should be noted, in that both de Carvalho et al. (2019) and Peschek et al. (2013) used EIMD protocols that did not elicit muscle soreness or deficits in muscle function in the populations they used. By contrast, the protocol used in this study elicited muscle damage as evidenced by a ~21% reduction in muscle function alongside a reduction of ~27% for perceived muscle function measured using the LEFS and a 17-fold increase in perceived soreness at 48-hr post protocol (see Tables 4 and 5), at which the negative effects of muscle damage are known to peak (Cheung et al., 2003). Furthermore, this study targeted the hamstring muscle group as the location for inducing muscle damage when previous studies targeted the quadriceps (de Carvalho et al., 2019; Morgan et al., 2018; Peschek et al., 2013). The knee flexors are ostensibly more susceptible to muscle damage than the knee extensors following eccentric exercise (Chen et al., 2011). Thus, it may be more pertinent to investigate the hamstrings and recovery, especially when considering the high injury rate of the knee flexors in sport, for example, soccer (Ekstrand et al., 2011). These methodological differences make comparisons difficult between this current study and the previous literature.

The reductions in peak torque in the present research that were observed in the days post-EIMD are likely due to a combination of the mechanical disruptions and subsequent oxidative stress elicited by the exercise protocol. The high levels of oxidative stress typically observed following EIMD, including similar protocols to the one utilized in the current study (Nikolaidis et al., 2007), can cause the muscle to enter an oxidized state, limiting contractile capability (Powers & Jackson, 2008). However, although CF have been shown to blunt exercise-induced oxidative stress (Davison et al., 2012), the high variability between individuals in regard to the level of oxidative stress seen in response to exercise must be considered when interpreting these findings (Mullins et al., 2013). In addition, it is unlikely that CF outcompete the existing antioxidant defense system. Instead, epicatechin and catechin metabolites may upregulate the endogenous antioxidant enzymes rather than act directly on ROS (Ruijters et al., 2013). Nonetheless, such effects require confirmation with future research. Therefore, with the previous in mind, and as no markers of oxidative stress were taken, it is difficult to conclude that the large effect sizes seen between CF1245 and CON for MVIC60%, MVIC30, and MVIC30% at 24- and 48-hr post-EIMD ($d \geq 0.8$) are a result of CF reducing oxidative damage. Hence, more research is required to understand the potential benefits of CF as a recovery aid.

For subjective measures of muscle soreness, it was hypothesized that CF consumption may reduce muscular soreness via the inhibition of pro-inflammatory cytokines, which are associated with neuropathic pain (Zhang & An, 2007). This was not the case in the present study, as subjective measures did not differ between groups. However, a large effect size was observed between CF_{1245} and CON for VAS at 48-hr post-EIMD (difference of 31 mm, $d = 0.9$). The inflammatory process begins immediately following muscle damaging exercise, further developing in the subsequent 24–48 hr if the disruption is significant (Saxton et al., 2003). As the peak rate of absorption for CF is approximately 30-min post ingestion, it is feasible that the acute dose of 1,245 mg

Table 4 Changes in MVIC Following EIMD

		Time post-EIMD (hr)				
Measure	**Group**	**Baseline**	**0**	**24**	**48**	**72**
MVIC60 (N·m)	CON	92 ± 23	79 ± 24	71 ± 18	62 ± 21	69 ± 22
	CF_{830}	95 ± 30	87 ± 26	83 ± 30	77 ± 31	86 ± 34
	CF_{1245}	94 ± 42	74 ± 30	87 ± 37	77 ± 30	79 ± 33
MVIC30 (N·m)	CON	97 ± 29	88 ± 28	82 ± 21	68 ± 17	81 ± 26
	CF_{830}	102 ± 35	99 ± 36	93 ± 34	89 ± 33	98 ± 40
	CF_{1245}	104 ± 44	87 ± 33	91 ± 34	86 ± 28	91 ± 31

Note. CON = control; CF = cocoa flavanols; MVIC = maximal voluntary isometric contraction; EIMD = exercise-induced muscle damage. Values are presented as mean ± SD.

Table 5 Changes in Perceived Soreness Post-EIMD

		Time post-EIMD (hr)				
Measure	**Group**	**Baseline**	**0**	**24**	**48**	**72**
VAS (mm)	CON	5 ± 8	76 ± 46	96 ± 42	131 ± 28	74 ± 28
	CF_{830}	10 ± 13	45 ± 32	79 ± 26	124 ± 28	95 ± 34
	CF_{1245}	6 ± 9	72 ± 40	72 ± 38	100 ± 44	83 ± 57
LEFS (a.u.)	CON	79 ± 1	67 ± 12	63 ± 15	55 ± 14	66 ± 6
	CF_{830}	77 ± 2	72 ± 3	66 ± 8	54 ± 10	63 ± 8
	CF_{1245}	77 ± 4	65 ± 10	67 ± 10	62 ± 12	68 ± 7

Note. CON = control; CF = cocoa flavanols; VAS = visual analog scale; LEFS = lower-extremity function scale; EIMD = exercise-induced muscle damage. Values are presented as mean ± *SD*.

CF could reduce the immediate increase in cytokines and other inflammatory mediators (e.g., neutrophils) that propagate following exercise. Because these mediators have the capacity to exacerbate muscle damage (Paulsen et al., 2012; Pizza et al., 2005; Toumi & Best, 2003) and delay recovery in the subsequent days, an early reduction in this response could lead to an enhanced recovery. This effect may result from the inhibitory potential of CF monomers on tumor necrosis factor-α, a pro-inflammatory cytokine involved in muscle lysis (Liao et al., 2010; Mao et al., 2002). Nonetheless, these are speculative mechanisms that require confirmation from further research that includes a comprehensive array of inflammation mediators. Our inability to measure these in the present study is acknowledged as a limitation of the work.

This study is not without its limitations. First, even though menstrual cycle was accounted for through the use of self-report questionnaires, they are not as accurate as hormonal tests to appropriately determine cycle phase (Wideman et al., 2013). However, hormone analysis was not feasible for the current research. Second, it is possible that the interindividual variability associated with muscle damage (Damas et al., 2016) and variability between sex responses to EIMD (Sewright et al., 2008) reduced the power of this study when paired with relatively small groups. Third, no inflammatory or oxidative stress markers were taken; thus, it was not possible to ascertain whether the intervention did in fact reduce these markers. Future research should look to include these measures and investigate the effect of CF supplementation on repeated bouts of high-intensity exercise separated by short recovery times to better reflect competition patterns typical of team-sport athletes.

In conclusion, there is no significant benefit for muscle recovery when comparing an acute dose of either 830 or 1,245 mg CF with a nutrient-matched carbohydrate–protein control. However, this needs to be confirmed with future research, while addressing the previously mentioned limitations, to confirm or refute any benefits CF supplementation may have following a dose >1,000 mg. Research should focus on CF impact on repeat performance, and a more comprehensive study investigating sex differences following CF supplementation should be conducted.

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