1	Acute consumption of varied doses of cocoa flavanols does not improve muscle recovery
2	following exercise-induced muscle damage in active males and females
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22 Abstract

Polyphenol consumption has become a popular method of trying to temper muscle damage. 23 24 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 25 following exercise induced muscle damage (EIMD). The study was a laboratory-based, 26 27 randomised, single-blind, nutrient-controlled trial involving 23 participants (13 females, 10 males). Participants were randomised into either control ~0mg CF (CON, n=8, 4 females), high 28 dose of 830mg CF (CF₈₃₀, n=8, 5 females) or supra dose of 1245mg CF (CF₁₂₄₅, n=7, 4 29 females). The EIMD protocol consisted of five sets of 10 maximal concentric/eccentric 30 hamstring curls and immediately consumed their assigned drink following completion. To 31 32 measure muscle recovery, maximal voluntary isometric contraction (MVIC) of the knee flexors at 60° and 30°, a visual analogue scale (VAS) and lower extremity function scale (LEFS) were 33 taken at baseline, immediately, 24, 48 and 72-hr post-EIMD. There was a main effect for time 34 35 for all variables (P < 0.05). However, no significant differences were observed between groups for all measures ($P \ge 0.17$). At 48 hr there were large effect sizes between CON and CF₁₂₄₅ for 36 MVIC60 (P=0.17, d=0.8), MVIC30 (P=0.26, d=0.8), MVIC30 percentage change (P=0.24) 37 d=0.9) and VAS (P=0.25, d=0.9). As no significant differences were observed following the 38 consumption of CF there is reason to believe that CF offer no benefit for muscle recovery when 39 40 ingested acutely.

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46 1. Introduction

Eccentric muscle contractions are typically responsible for the muscular disruption that leads 47 to exercise induced muscle damage (EIMD) (Nikolaidis et al., 2007). Therefore, resistance 48 training and intermittent high-intensity exercise often evoke EIMD (Owens, Twist, Cobley, 49 Howatson, & Close, 2019). Consequences of EIMD include inflammation and oxidative stress 50 51 (Kanda et al., 2013), impaired force generating capacity (Twist & Eston, 2009), and increased muscle soreness (Impellizzeri et al., 2008). Optimising the time course of recovery is now a 52 priority in modern sport, mainly due to the rapid turnaround of competitions and fixtures. 53 Contemporary examples include tennis players performing every other day at major 54 championships and congested fixture periods in soccer when players perform two 90 min 55 56 matches within three days. Notably, injury-risk and muscular fatigue may be increased during congested fixture periods in soccer, namely due to the insufficient recovery time between 57 58 matches (Ekstrand, Hägglund, & Waldén, 2011; Page, Marrin, Brogden, Greig, & Research, 59 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 60 capacity. To reduce fatigue and facilitate recovery, high carbohydrate protein meals or 61 beverages, as well as high polyphenolic foodstuffs (e.g., cocoa) have become a common feature 62 of an athlete's diet (Knapik et al., 2016). Polyphenol is an umbrella term for the different 63 classes of plant metabolites, including flavonoids, stilbenes, phenolic acids and lignans. 64

Flavonoids are the largest group of dietary polyphenols and the most common source of antioxidants within the diet (Scalbert, Johnson, & Saltmarsh, 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, teas, broad beans and cocoa (Williamson, 2017). Cocoa has the highest proportion of flavanols per serving than any other natural source (Lee, Kim, Lee, & Lee, 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 77 exercise stimulus) and sub-chronic (regular intake for ≥ 14 days) CF supplementation of ≥ 200 78 mg reduces exercise-induced oxidative stress (Allgrove et al., 2011; Davison, Callister, 79 Williamson, Cooper, & Gleeson, 2012). Furthermore, in relation to exercise, the ingestion of 80 81 CF may improve sprint performance by potentially preventing ROS-increased calcium sensitivity of myofilaments within working muscles, therefore, delaying fatigue (de Carvalho 82 83 et al., 2019; Patel, Brouner, & Spendiff, 2015). However, evidence is lacking regarding the 84 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 85 epicatechin) to be effective (Morgan, Wollman, Jackman, & Bowtell, 2018). Benefits begin to 86 be observed at doses of ~700 mg CF; and more importantly, with >50 mg epicatechin, the most 87 biologically active flavanol (Schroeter et al., 2006). However, an optimal dose is not yet known 88 in addition to any potential dose response. Furthermore, previous research that investigated the 89 impact of CF on muscle recovery did not induce notable muscle damage using a drop jumps 90 protocol (de Carvalho et al., 2019) and a downhill running protocol (Peschek, Pritchett, 91 92 Bergman, & Pritchett, 2013). This can be defined as reductions in muscle force-generating capability of $\geq 20\%$ following EIMD (Paulsen, Ramer Mikkelsen, Raastad, & Peake, 2012). 93 Therefore, making conclusions about the impact of CF on markers of muscle damage is 94 95 difficult, indicating that more research is warranted. Furthermore, none of the previous studies

96 involved female participants, likely due to the purported protective effects of oestrogen against
97 muscle damage (Tiidus, 2003) and physiological variations across the menstrual cycle
98 (Hayashida, Shimura, Sugama, Kanda, & Suzuki, 2016). Therefore, investigating the effect of
99 CF supplementation on muscle recovery in females is required.

100 Therefore, the aims of this study were twofold; *i*) to investigate the impact of an acute dose of 101 CF on indices of muscle recovery *ii*) to compare two different doses of CF on indices of muscle 102 recovery. The hypothesis for this study was that EIMD might be attenuated following acute 103 consumption of CF, with the highest dose offering the most benefit.

104 2. Methods

105 2.1 Participants

106 Following institutional ethical approval and in agreement with the Declaration of Helsinki, 30 participants consented to take part between the months of April 2019 to October 2019; 107 108 however, only 23 completed the study (13 females, 10 males) due to the following reasons; two due to injury and five due to unforeseen circumstances following baseline testing. An a 109 priori power calculation determined that a sample size of 21 was required for 80% power and 110 111 to detect significance, based on the effect size from previous research regarding MVIC recovery at 48 hr (Bowtell, Sumners, Dyer, Fox, & Mileva, 2011). Baseline testing involved 112 maximal voluntary isometric contractions (MVIC) of the knee flexors to assess muscle function 113 and measures of perceived muscle soreness using a visual analogue scale (VAS) and lower 114 extremity function scale (LEFS). All participants were classed as recreationally active and 115 injury free for the previous six-months (both informed *via* self-report) and were not taking any 116 dietary supplements (e.g., vitamin C, glutamine, or branched-chain amino acids). Participants 117 were asked to avoid anti-inflammatory medications and resistance training during 118 participation. A menstrual cycle questionnaire (Brown, 2017) was completed by the female 119

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 oestrogen concentrations observed during the follicular phase (Brown, 2017). Participants completed each day within 26±2 hr of original participation to account for diurnal influence.

124 2.2 Study Design

125 The study was a laboratory-based, randomised, single-blind, nutrient-controlled trial. Participants were randomised into a control (CON), high (CF₈₃₀) or supra (CF₁₂₄₅) group and 126 127 remained unaware of their allocation for the entirety of the study. Participants were required to come to the laboratory for five days, the first being baseline testing and familiarisation of the 128 129 EIMD protocol (ten sub-maximal concentric-eccentric hamstring curls). The remaining four days took place consecutively; as such, measures were taken in the following order: baseline, 130 immediately post-EIMD (0 hr), 24, 48 and 72 hr post-EIMD. For randomisation, participants 131 were assigned to separate strata, 'strong' and 'not strong', based on their baseline MVIC values 132 and randomised into matched and counterbalanced groups (using random.org). To decide what 133 could be classified as strong or not, a normative MVIC strength index was used [Risberg et al. 134 (2018) for females and Ruas, Minozzo, Pinto, Brown, and Pinto (2015) for males]. Following 135 this, eight participants were allocated to the control group (four females, four males), eight to 136 the CF_{830} group (five females, three males), and seven to the CF_{1245} group (four females, three 137 138 males).

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140 2.3 Muscle Function

141 Values were recorded for knee flexor MVIC using the isokinetic dynamometer (Cybex
142 NORM®, Model 770, CA, USA), providing a reliable quantification of decrements in muscle
143 function for assessing EIMD (Warren, Lowe, & Armstrong, 1999). Knee angles of 60°

(MVIC60) and 30° (MVIC30) of the anatomical zero (full knee flexion) were selected due to
the differences in muscle activation at various knee angles; biceps femoris has increased
activation at reduced angles, whilst semitendinosus and semimembranosus at greater knee
angles (Onishi et al., 2002).

148 2.4 Subjective Soreness

Soreness was recorded using a 200 mm VAS, which has been previously included as a validated measure of subjective soreness (Peschek, Pritchett, Bergman, & Pritchett, 2013). The LEFS is a validated questionnaire which quantifies an individuals 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).

154 2.5 Muscle Damaging Protocol

The exercise protocol used to induce muscle damage was adapted from White et al. (2008) 155 using the Cybex Norm Isokinetic Dynamometer (CSMi, Boston, Massachusetts). Participants 156 157 were then secured into the dynamometer at 85° hip flexion using straps to isolate the knee and remove hip flexor involvement. Body position was noted during baseline testing and replicated 158 159 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-160 161 up, participants performed five sets of 10 maximal concentric/eccentric contractions of the knee flexors with an interset rest of one minute; rotation speed was $60^{\circ} \cdot s^{-1}$. Participants were verbally 162 encouraged throughout and once all repetitions were completed, the participant immediately 163 repeated the protocol on the opposite leg. 164

165 2.6 Nutritional Intervention

Participants were blinded to which group they were assigned, with only the lead researcher 166 being aware of the contents of each drink. Participants consumed their assigned beverage 167 168 within five minutes 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 169 commercially available high flavanol powder (Chococru© Extraordinary Flavanol Cocoa), 170 containing ~8.3% flavanols and a total polyphenol content of ~12% (unpublished data from 171 172 Chococru©). The beverage for CF₈₃₀ included an additional 10 g of Chococru© cocoa powder which contained 830 mg CF (98.6 mg epicatechin) and for CF₁₂₄₅ 15 g of Chococru© cocoa 173 174 powder was added, containing 1245 mg CF (149.4 mg epicatechin; Table 2).

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176 2.7 Dietary Measures

Participants completed a 24-hour dietary recall each day of testing, totalling 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 three 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, ©2016).

184 2.8 Statistical analyses

Statistical analysis was performed using IBM SPSS Statistics (version 24, IBM Corp., Armonk, N.Y., USA). 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 LSD post hoc testing was performed to identify the point of significance. Data for MVIC60

190	and MVIC30 was calculated as percentage change from baseline alongside absolute means. To
191	calculate effect sizes, Cohen's d (d) was utilised, with the magnitude of effects considered
192	small (0.2), moderate (0.5) and large (0.8). Significance was set at $P \leq 0.05$ pre-analysis.
193	Descriptive statistics are reported as means (%) \pm standard deviation (SD).
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198	***INSERT FIGURE 1 NEAR HERE***
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203	3. Results
204	There were no significant differences for participant characteristics or dietary intake between
205	groups ($P \ge 0.33$). See Table 3 for dietary intake.
206	***INSERT TABLE 3 NEAR HERE***
207	
208	3.1 Muscle function

209	Muscle function measured using MVIC at 60° and 30° found a main effect of time ($P < 0.05$).
210	There were no significant differences between groups for knee flexor peak torque at MVIC60
211	(P=0.99) or MVIC30 $(P=0.95)$ at baseline. Following the exercise protocol, overall mean knee
212	flexor peak torque reduced to 79% of baseline. There was a significant effect of time across all
213	groups ($P < 0.05$). For MVIC60 there were no significant differences between groups ($P \ge 0.17$).
214	For MVIC30 there were no significant differences between groups ($P \ge 0.55$).
215	3.2 Measures of Perceived soreness
216	Perceived soreness measured using a VAS and LEFS found a main effect of time for both
217	measures ($P = < 0.05$). There were no significant differences between groups for VAS scores
218	($P \ge 0.39$). There were no significant differences between groups for LEFS scores ($P \ge 0.75$).
219	***INSERT FIGURE 2 NEAR HERE***
220	***INSERT TABLE 4 NEAR HERE***
221	***INSERT TABLE 5 NEAR HERE***

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4. 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 Carvalhoet 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 232 this study elicited muscle damage as evidenced by a ~21% reduction in muscle function 233 234 alongside a reduction of $\sim 27\%$ 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 235 negative effects of muscle damage are known to peak (Cheung, Hume, & Maxwell, 2003). 236 Furthermore, this study targeted the hamstring muscle group as the location for inducing 237 238 muscle damage when previous studies targeted the quadriceps (de Carvalho et al., 2019; 239 Morgan et al., 2018; Peschek et al., 2013). The knee flexors are ostensibly more susceptible to 240 muscle damage than the knee extensors following eccentric exercise (Chen, Lin, Chen, Lin, & Nosaka, 2011). Thus, it may be more pertinent to investigate the hamstrings and recovery, 241 espcially when considering the high injury rate of the knee flexors in sport, e.g., soccer 242 (Ekstrand et al., 2011). These methodological differences make comparisons difficult to make 243 between this current study and the previous literature. 244

The reductions in peak torque in the present research that were observed in the days post-EIMD 245 are likely due to a combination of the mechanical disruptions and subsequent oxidative stress 246 elicited by the exercise protocol. The high levels of oxidative stress typically observed 247 following EIMD, including similar protocols to the one utilised in the current study (Nikolaidis 248 249 et al., 2007), can cause the muscle to enter an oxidised state, limiting contractile capability (Powers & Jackson, 2008). However, although CF have been shown to blunt exercise-induced 250 oxidative stress (Davison et al., 2012), the high variability between individuals in regard to the 251 level of oxidative stress seen in response to exercise must be considered when interpreting 252 these findings (Mullins et al., 2013). Additionally, it is unlikely that CF outcompete the existing 253 254 antioxidant defence system. Instead, epicatechin and catechin metabolites may upregulate the endogenous antioxidant enzymes rather than act directly on ROS (Ruijters, Weseler, Kicken, 255 256 Haenen, & Bast). 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 ($d \ge 0.8$) 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 hypothesised that CF consumption may 262 reduce muscular soreness via the inhibition of pro-inflammatory cytokines, which are 263 associated with neuropathic pain (Zhang & An, 2007). This was not the case in the present 264 study, as subjective measures did not differ between groups. However, a large effect size was 265 266 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 267 developing in the subsequent 24-48 hr if the disruption is significant (Saxton, Claxton, Winter, 268 & Pockley, 2003). As the peak rate of absorption for CF is ~30 min post-ingestion, it is feasible 269 that the acute dose of 1245mg CF could reduce the immediate increase in cytokines and other 270 271 inflammatory mediators (e.g., neutrophils) that propagate following exercise. Because these mediators have the capacity to exacerbate muscle damage (Paulsen et al., 2012; Pizza, Peterson, 272 Baas, & Koh, 2005; Toumi & Best, 2003) and delay recovery in the subsequent days, an early 273 274 reduction in this response could lead to an enhanced recovery. This effect may result from the inhibitory potential of CF monomers on tumour necrosis factor- α , a pro-inflammatory cytokine 275 involved in muscle lysis (Liao, Zhou, Ji, & Zhang, 2010; Mao, van de Water, Keen, Schmitz, 276 & Gershwin, 2002). Nonetheless, these are speculative mechanisms that require confirmation 277 from further research that includes a comprehensive array of inflammation mediators. Our 278 279 inability to measure these in the present study is acknowledged as a limitation of the work.

This study is not without its limitations, firstly, even though menstrual cycle was accounted forthrough the use of self-report questionnaires; they are not as accurate as hormonal tests to

appropriately determine cycle phase (Wideman, Montgomery, Levine, Beynnon, & Shultz, 282 2013). However, hormone analysis was not feasible for the current research. Secondly, it is 283 284 possible that the interindividual variability associated with muscle damage (Damas, Nosaka, Libardi, Chen, & Ugrinowitsch, 2016) and variability between sex responses to EIMD 285 (Sewright, Hubal, Kearns, Holbrook, & Clarkson, 2008) reduced the power of this study when 286 paired with relatively small groups. Thirdly, no inflammatory or oxidative stress markers were 287 288 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 289 290 CF supplementation on repeated bouts of high-intensity exercise seperated by short recovery times to better reflect competition patterns typical of team-sport athletes. 291

In conclusion, there is no significant benefit for muscle recovery when comparing an acute dose of either 830 and 1245 mg CF to a nutrient matched carbohydrate-protein control. However, this needs to be confirmed with future research, whilst addressing the limitations above, to confirm or refute any benefits CF supplementation may have following a dose >1000 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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306 Conflicts of Interest

307 No conflicts of interest exist with any of the authors and no funding was received

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444 Tables

Table 1. Participant characteristics

Group	Age \pm years	Stature \pm cm	$Mass \pm kg$
CON	24 ± 4	175 ± 8	74 ± 15
CF ₈₃₀	25 ± 5	168 ± 9	68 ± 10
CF ₁₂₄₅	24 ± 5	168 ± 11	65 ± 12

Note: Data is presented as mean ± *standard deviation. No significant differences observed*

between groups.

Table 2. Nutritional information of beverages

	kcal/kj	CHO (g)	Pro (g)	Fat (g)	Flavanol (mg)	ORAC
CON	340/1427	61.9	19	1.9	Nil	Nil
CF830	366/1531	63.3	21.4	2.9	830	20,000
CF1245	379/1589	64	22.6	3.4	1245	30,000

Note: All drinks contain 60 g of maltodextrin and 25 g chocolate smooth whey protein powder, drinks

2 & 3 contain 10 g and 15 g of Chococru powder respectively; ORAC = oxygen radical absorbance

capacity.

448

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Table 3. Dietary intake between groups

	CON	CF ₈₃₀	CF ₁₂₄₅	Significance (P)
Energy (kcal)	2137 ± 559	2101 ± 394	2164 ± 591	0.98
Protein (g)	109 ± 49	106 ± 47	106 ± 43	0.99
CHO (g)	227 ± 46	253 ± 41	265 ± 106	0.60
Fat (g)	93 ± 32	81 ± 19	79 ± 21	0.57

Note: Group mean ± *SD*

Table 4. Changes in MVIC following EIMD

			Time post-EIMD (hr)				
Measure	Group	Baseline	0	24	48	72	
	CON	92 ± 23	79 ± 24	71 ± 18	62 ± 21	69 ± 22	
MVIC 60 (Nm)	CF830	95 ± 30	87 ± 26	83 ± 30	77 ± 31	86 ± 34	
	CF ₁₂₄₅	94 ± 42	74 ± 30	87 ± 37	77 ± 30	79 ± 33	
MVIC 20 (Nm)	CON	97 ± 29	88 ± 28	82 ± 21	68 ± 17	81 ± 26	
MVIC 30 (Nm)	CF830	102 ± 35	99 ± 36	93 ± 34	89 ± 33	98 ± 40	
	CF ₁₂₄₅	104 ± 44	87 ± 33	91 ± 34	86 ± 28	91 ± 31	

Table 5. Changes in perceived soreness post-EIMD

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

Notes: Group mean \pm *SD*

454 Figures



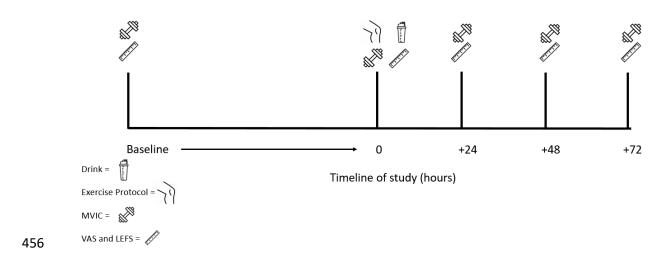


Figure 1. Study schematic detailing experimental timeline

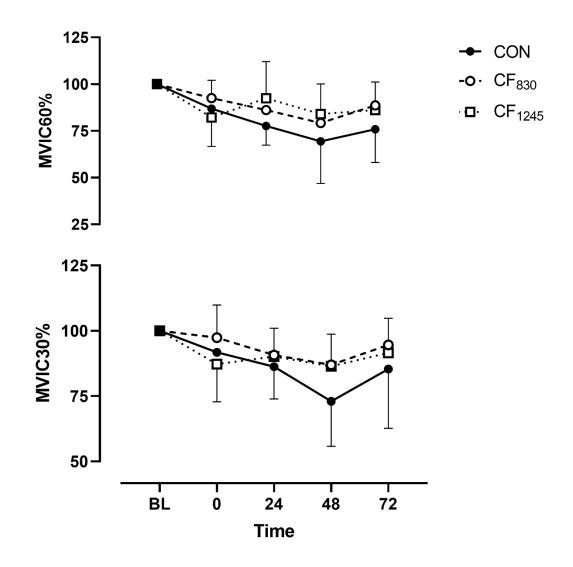


Figure 2. Percentage change from baseline for MVIC following EIMD