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

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Abstract

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 (EIMD). The study was a laboratory-based, 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 dose of 830mg CF (CF_{830}, n=8, 5 females) or supra dose of 1245mg CF (CF_{1245}, n=7, 4 females). The EIMD 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 analogue scale (VAS) and lower extremity function scale (LEFS) were taken at baseline, immediately, 24, 48 and 72-hr post-EIMD. There was a main effect for time for all variables ($P<0.05$). However, no significant differences were observed between groups for all measures ($P\geq0.17$). At 48 hr there were large effect sizes between CON and CF_{1245} for MVIC60 ($P=0.17$, $d=0.8$), MVIC30 ($P=0.26$, $d=0.8$), MVIC30 percentage change ($P=0.24$ $d=0.9$) and VAS ($P=0.25$, $d=0.9$). 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.

1. Introduction

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, Twist, Cobley, Howatson, & Close, 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). Optimising 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 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 matches (Ekstrand, Hägglund, & Waldén, 2011; Page, Marrin, Brogden, Greig, & Research, 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, 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

71 focused on the effects of cocoa flavanols (CF) on the cardiovascular system, with evidence
72 suggesting CF intake can reduce endothelial dysfunction by improving flow mediated dilation
73 (Hooper et al., 2012) and reducing blood pressure (Buitrago-Lopez et al., 2011). Furthermore,
74 CF have been shown to enhance endogenous antioxidant capacity (Serafini & Peluso, 2016),
75 limit oxidative stress (Allgrove et al., 2011), and influence the inflammatory process by
76 reducing both platelet aggregation and the stimulation of neutrophils (Ellinger & Stehle, 2016).

77 Regarding muscle recovery and exercise, research has shown that acute (single dose on day of
78 exercise stimulus) and sub-chronic (regular intake for ≥14 days) CF supplementation of ≥200
79 mg reduces exercise-induced oxidative stress (Allgrove et al., 2011; Davison, Callister,
80 Williamson, Cooper, & Gleeson, 2012). Furthermore, in relation to exercise, the ingestion of
81 CF may improve sprint performance by potentially preventing ROS-increased calcium
82 sensitivity of myofilaments within working muscles, therefore, delaying fatigue (de Carvalho
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
85 increase of perceived soreness. One such study used a CF dose too low (74 mg CF and 8 mg
86 epicatechin) to be effective (Morgan, Wollman, Jackman, & Bowtell, 2018). Benefits begin to
87 be observed at doses of ~700 mg CF; and more importantly, with >50 mg epicatechin, the most
88 biologically active flavanol (Schroeter et al., 2006). However, an optimal dose is not yet known
89 in addition to any potential dose response. Furthermore, previous research that investigated the
90 impact of CF on muscle recovery did not induce notable muscle damage using a drop jumps
91 protocol (de Carvalho et al., 2019) and a downhill running protocol (Peschek, Pritchett,
92 Bergman, & Pritchett, 2013). This can be defined as reductions in muscle force-generating
93 capability of ≥20% following EIMD (Paulsen, Ramer Mikkelsen, Raastad, & Peake, 2012).
94 Therefore, making conclusions about the impact of CF on markers of muscle damage is
95 difficult, indicating that more research is warranted. Furthermore, none of the previous studies

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

Therefore, the aims of this study were twofold; *i)* to investigate the impact of an acute dose of CF on indices of muscle recovery *ii*) 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.

2. Methods

2.1 Participants

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; 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 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 MVIC recovery at 48 hr (Bowtell, Sumners, Dyer, Fox, & Mileva, 2011). Baseline testing involved maximal voluntary isometric contractions (MVIC) of the knee flexors to assess muscle function and measures of perceived muscle soreness using a visual analogue scale (VAS) and lower extremity function scale (LEFS). All participants were classed as recreationally active and injury free for the previous six-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

120 participants involved to reliably estimate cycle phase. The luteal phase was selected for testing
121 or an equivalent period for participants who were on hormonal contraception, as to avoid peak
122 oestrogen concentrations observed during the follicular phase (Brown, 2017). Participants
123 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.
126 Participants were randomised into a control (CON), high (CF_{830}) or supra (CF_{1245}) group and
127 remained unaware of their allocation for the entirety of the study. Participants were required to
128 come to the laboratory for five days, the first being baseline testing and familiarisation of the
129 EIMD protocol (ten sub-maximal concentric-eccentric hamstring curls). The remaining four
130 days took place consecutively; as such, measures were taken in the following order: baseline,
131 immediately post-EIMD (0 hr), 24, 48 and 72 hr post-EIMD. For randomisation, participants
132 were assigned to separate strata, 'strong' and 'not strong', based on their baseline MVIC values
133 and randomised into matched and counterbalanced groups (using random.org). To decide what
134 could be classified as strong or not, a normative MVIC strength index was used [Risberg et al.
135 (2018) for females and Ruas, Minozzo, Pinto, Brown, and Pinto (2015) for males]. Following
136 this, eight participants were allocated to the control group (four females, four males), eight to
137 the CF_{830} group (five females, three males), and seven to the CF_{1245} group (four females, three
138 males).

139 ***INSERT TABLE 1 NEAR HERE***

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).

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).

2.5 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, Massachusetts). Participants 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 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 of 10 maximal concentric/eccentric contractions of the knee flexors with an interset rest of one minute; rotation speed was $60°\cdot s^{-1}$. Participants were verbally encouraged throughout and once all repetitions were completed, the participant immediately repeated the protocol on the opposite leg.

2.6 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 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 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 1245 mg CF (149.4 mg epicatechin; Table 2).

INSERT TABLE 2 NEAR HERE

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).

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

and MVIC30 was calculated as percentage change from baseline alongside absolute means. To calculate effect sizes, Cohen's d (*d*) was utilised, with the magnitude of effects considered small (0.2), moderate (0.5) and large (0.8). Significance was set at $P \leq 0.05$ pre-analysis. Descriptive statistics are reported as means (%) ± standard deviation (SD).

INSERT FIGURE 1 NEAR HERE

3. Results

There were no significant differences for participant characteristics or dietary intake between groups ($P \geq 0.33$). See Table 3 for dietary intake.

INSERT TABLE 3 NEAR HERE

3.1 Muscle function

Muscle function measured using MVIC at 60° and 30° found a main effect of time (P<0.05). There were no significant differences between groups for knee flexor peak torque at MVIC60 (P=0.99) or MVIC30 (P=0.95) 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<0.05). For MVIC60 there were no significant differences between groups (P≥0.17). For MVIC30 there were no significant differences between groups (P≥0.55).

3.2 Measures of Perceived soreness

Perceived soreness measured using a VAS and LEFS found a main effect of time for both measures (P=<0.05). There were no significant differences between groups for VAS scores (P≥0.39). There were no significant differences between groups for LEFS scores (P≥0.75).

INSERT FIGURE 2 NEAR HERE

INSERT TABLE 4 NEAR HERE

INSERT TABLE 5 NEAR HERE

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 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, Hume, & Maxwell, 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, Lin, Chen, Lin, & Nosaka, 2011). Thus, it may be more pertinent to investigate the hamstrings and recovery, espcially when considering the high injury rate of the knee flexors in sport, e.g., soccer (Ekstrand et al., 2011). These methodological differences make comparisons difficult to make 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 utilised in the current study (Nikolaidis 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 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). Additionally, it is unlikely that CF outcompete the existing antioxidant defence system. Instead, epicatechin and catechin metabolites may upregulate the endogenous antioxidant enzymes rather than act directly on ROS (Ruijters, Weseler, Kicken, 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≥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 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, Claxton, Winter, & Pockley, 2003). As the peak rate of absorption for CF is ~30 min post-ingestion, it is feasible that the acute dose of 1245mg 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, Peterson, Baas, & Koh, 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 tumour necrosis factor-α, a pro-inflammatory cytokine involved in muscle lysis (Liao, Zhou, Ji, & Zhang, 2010; Mao, van de Water, Keen, Schmitz, & Gershwin, 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, firstly, 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, Montgomery, Levine, Beynnon, & Shultz, 2013). However, hormone analysis was not feasible for the current research. Secondly, it is possible that the interindividual variability associated with muscle damage (Damas, Nosaka, Libardi, Chen, & Ugrinowitsch, 2016) and variability between sex responses to EIMD (Sewright, Hubal, Kearns, Holbrook, & Clarkson, 2008) reduced the power of this study when paired with relatively small groups. Thirdly, 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 seperated 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 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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Conflicts of Interest

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

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Tables

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: Data is presented as mean ± standard deviation. No significant differences observed between groups.

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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
CF_{830}	366/1531	63.3	21.4	2.9	830	20,000
CF_{1245}	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.

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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
MVIC 60 (Nm)	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
MVIC 30 (Nm)	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

Notes: Group 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

Notes: Group mean ± SD

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454 **Figures**

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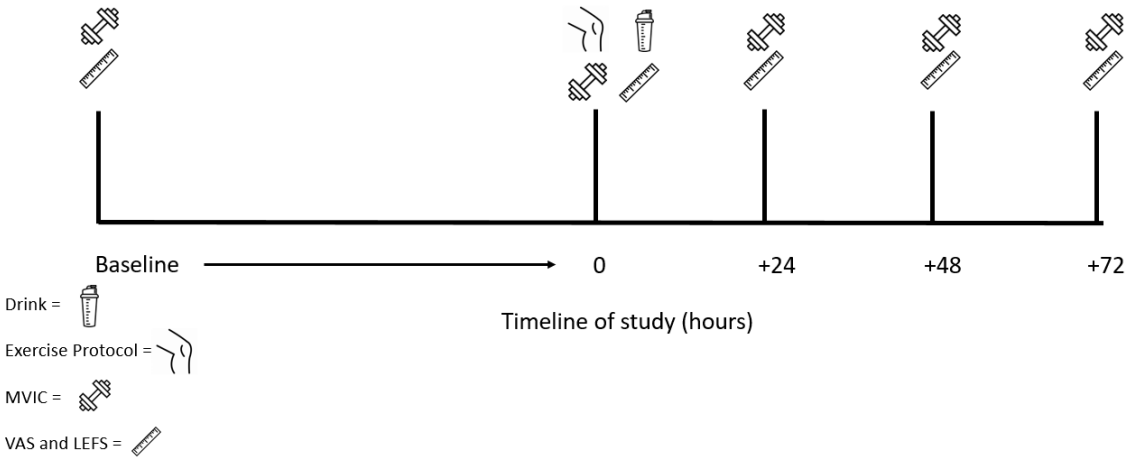

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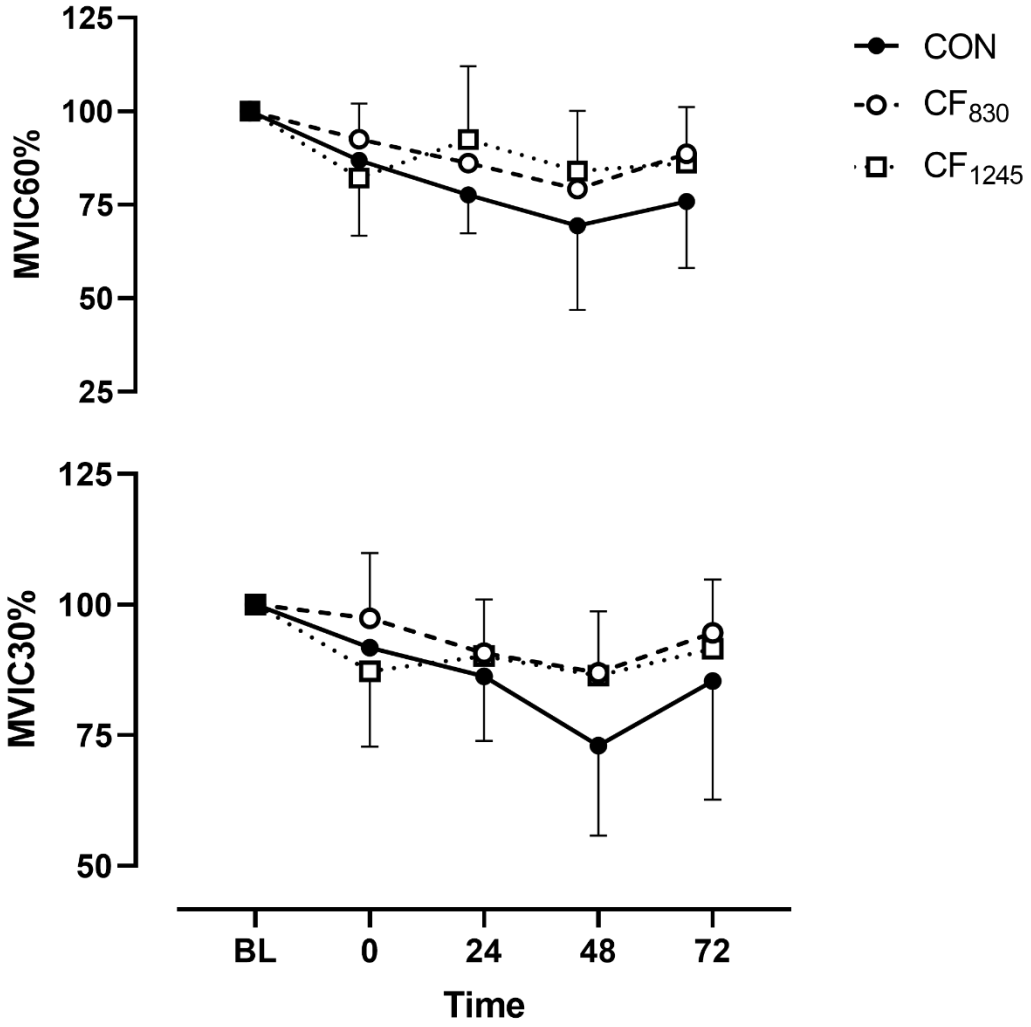
457 **Figure 1**. Study schematic detailing experimental timeline



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459 **Figure 2**. Percentage change from baseline for MVIC following EIMD

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