Ergogenic Effects of Creatine Monohydrate on Blood-Flow Restricted Resistance Exercise Performance: Double-Blind, Randomised, Crossover Trial.

by

Mikal G. Thrones

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University of Manitoba

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ABSTRACT

Creatine monohydrate (CM) is one of the most extensively researched and widely used dietary supplements for enhancing performance during high-intensity, short-duration resistance exercise. Its ergogenic effects are primarily attributed to increased intramuscular phosphocreatine (PCr) availability, facilitating ATP resynthesis during repeated bouts of muscular work. While CM has consistently demonstrated performance-enhancing effects in traditional resistance training, its efficacy during blood-flow restricted resistance exercise (BFR-RE) — a low-load, skeletal muscle hypertrophy-oriented training method — remained unexplored. This study evaluated the ergogenic effect of CM during BFR-RE, to expand understanding of its utility in emerging resistance-training modalities.

Thirteen participants (7 males, 6 females) completed a crossover protocol involving four sets of three lower-body BFR-RE exercises (leg press, knee flexion, knee extension) under both CM and placebo conditions. A 4-way mixed ANOVA was conducted to determine significant effects. A significant main effect of treatment showed participants completed more total repetitions under CM (277.38 ± 83.06) compared to placebo (251.62 ± 91.73), $p = .004$. A significant main effect of exercise was observed, with repetition performance highest during the leg press, followed by knee flexion, and lowest during knee extension ($p < .001$). A significant main effect of set was also found, with repetitions decreasing progressively from set 1 through set 4 ($p < .001$).

Significant two-way interactions included treatment × exercise ($p = .033$), where CM increased repetitions in the leg press ($p < .001$) and knee extension ($p = .015$), but not knee flexion ($p = .710$); and treatment × set ($p = .038$), where CM increased repetitions primarily in set 1 ($p = .02$). A three-way interaction between sex × treatment × exercise ($p = .038$) indicated that males experienced a greater benefit from CM in the leg press, while females showed no significant differences between

conditions across exercises. Additionally, a sex × treatment × set interaction ($p = .009$) showed CM improved male performance in sets 1 and 2, but not in later sets, with no significant effects observed in females.

In summary, CM supplementation acutely enhanced repetition performance during BFR-RE, particularly in multi-joint exercises and early sets, with more pronounced effects in males. These findings suggest CM may be an effective strategy for augmenting BFR-RE performance, potentially leading to greater adaptations with BFR-RE training.

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CHAPTER 1 – SCIENTIFIC FRAMEWORK

1.1 Introduction

Resistance exercise is a common exercise modality to improve skeletal muscle (hereinafter called muscle) mass and strength (Benito et al., 2020). Blood-flow restricted resistance exercise (BFR-RE) is a novel method of resistance exercise in which venous return of blood is restricted with a pressurized cuff while the participant completes low-intensity resistance exercise for the extremities. This restriction induces a hypoxic environment in the working muscles, promoting the accumulation of metabolites such as lactate, which is thought to enhance muscle activation and anabolic signaling. Despite using lower external loads, BFR-RE causes similar increases in muscle hypertrophy (an increase in skeletal muscle size) as traditional high-load resistance exercise (Centner et al., 2019; Lixandrão et al., 2018; Martín-Hernández et al., 2013). It is not yet clear what mechanisms account for the similarity in training responses between BFR-RE and traditional resistance exercise (Pearson & Hussain, 2015), although potential factors include increased cell swelling, heightened muscle fiber recruitment, and enhanced hormonal responses.

Creatine monohydrate (CM) is one of the most frequently used supplements to enhance muscular performance (Garthe & Maughan, 2018). CM improves exercise performance by increasing the availability of intramuscular phosphocreatine (PCr) (Casey et al., 1996; Greenhaff et al., 1994; Mesa et al., 2002), which serves as a rapid buffer for ATP resynthesis during high-intensity muscular work. Supplementation with CM typically follows a loading phase of 20 to 25 grams per day for 5 to 7 days, followed by a maintenance phase of 3 to 5 grams daily, which elevates intramuscular creatine stores by 10% to 40% and PCr by 8% to 13%. These increases help maintain higher ATP/ADP ratios during exercise, thereby enhancing short-duration, high-intensity performance. During resistance exercise, CM typically allows individuals to perform

more repetitions across a variety of loading ranges (Izquierdo et al., 2002; Mills et al., 2020) with improvements in training volume translating into greater fat-free mass and hypertrophic adaptations over time (Lanhers et al., 2015; Volek et al., 1997). However, a subset of individuals—approximately 20% to 30%—are classified as non-responders due to lower increases in intramuscular creatine following supplementation, often linked to high baseline creatine concentrations or lower proportions of type II muscle fibers. Females may respond differently to creatine supplementation than males. Females typically exhibit more characteristics associated with CM non-responders, indicating they may experience a less robust increase in performance. Despite the popularity of CM as an ergogenic aid, there is a lack of research investigating its effects with novel exercise modalities, including BFR-RE, and how CM may differentially improve performance across different sexes.

The purpose of this research study was to determine if CM was an effective supplement for improving performance during BFR-RE. It was hypothesized that there would be a significant increase in the number of repetitions performed until failure in the CM condition compared to the placebo condition. Given CM's known benefits in enhancing muscular endurance during traditional resistance exercise and its role in increasing overall training volume, it is plausible that CM may also augment performance during BFR-RE by supporting energy demands under restricted oxygen conditions and prolonging fatigue onset.

1.2 Review of Literature

1.2.1 Blood-Flow Restricted Resistance Exercise.

Resistance exercise is a common exercise modality to improve muscle mass and strength (Benito et al., 2020). BFR-RE is a type of resistance exercise in which venous return of blood is occluded using specialized inflatable cuffs or elastic wraps. BFR-RE typically involves the use of lower loads (20 – 50% 1-repetition Maximum (1RM)) than traditional, high-load resistance exercise (60 – 80% 1RM). This type of exercise has been shown to be effective for populations for which high-load resistance exercise may be undesirable or contraindicated, such as older adults (Centner et al., 2019) and those engaged in rehabilitation (Hughes et al., 2017).

BFR-RE has been studied extensively for its effect on skeletal muscle hypertrophy and strength while utilizing lower loads than traditional resistance exercise. This method of training has been shown to cause similar increases in hypertrophy as high-load resistance exercise (Centner et al., 2019; Libardi et al., 2015; Lixandrão et al., 2018; Martín-Hernández et al., 2013; Vechin et al., 2015; Yasuda et al., 2011), and causes greater increases in hypertrophy than similar low-load, volume-matched resistance exercise without blood-flow restriction (Centner et al., 2019; Lixandrão et al., 2018; Loenneke, et al., 2012a; Takarada et al., 2000). With respect to strength, BFR-RE has demonstrated greater strength improvements than low-load resistance exercise without blood-flow restriction (Centner et al., 2019; Patterson & Ferguson, 2011). Despite finding similarities in the hypertrophy responses, many studies did not find that BFR-RE increases strength to the same extent as high-load traditional resistance exercise (Lixandrão et al., 2015, 2018; Martín-Hernández et al., 2013; Vechin et al., 2015; Yasuda et al., 2011). Two hypotheses may explain the lower strength improvements resulting from BFR-RE. First, it is

hypothesized that a lack of training specificity to the strength tests utilized reduces the strength improvement following BFR-RE; indeed, Yasuda et al. (2011) tested a group that combined high-intensity traditional resistance exercise and BFR-RE, with groups doing traditional resistance exercise only or BFR-RE only. Adding some high-load resistance exercise to the BFR-RE protocol lead to similar strength gains as the high-load resistance only group, despite performing significantly less volume of high-load resistance exercise. Secondly, preferential fiber type hypertrophy may affect the strength differences following BFR-RE compared to traditional resistance exercise. BFR-RE has been demonstrated to cause more hypertrophy in type-I muscle fibers compared to type-II muscle fibers in resistance-trained participants (Bjørnsen et al., 2019). This may explain the differences in strength adaptation as type-II muscle fibers have been shown to produce more force than type-I fibers (Miller et al., 2015). Similar improvements in strength between high-load traditional resistance exercise and BFR-RE have also been observed (Ellefsen et al., 2015; Libardi et al., 2015; Takarada et al., 2000). Interestingly, each of the studies that found no additional benefit of high-load resistance exercise compared to BFR-RE on strength were carried out on untrained individuals, indicating that the novelty of resistance exercise in this population may be enough to overcome the benefits of practice with higher loads (Ellefsen et al., 2015; Libardi et al., 2015; Takarada et al., 2000).

It is not yet clear what mechanisms account for the similarity in training responses between BFR-RE and traditional resistance exercise (Pearson & Hussain, 2015). Most research focuses on the role of metabolic stress in mediating the effects of BFR-RE. Metabolic stress may increase hypertrophy through increased cell swelling, type-II muscle fiber recruitment, muscle damage, and/or proliferation of satellite cells.

1.2.2 Blood-Flow Restricted Resistance Exercise Mechanism of Action.

1.2.2.1 Cell swelling.

Loenneke et al. (2012a) propose that BFR-RE may mediate skeletal muscle hypertrophy through an increase in cellular swelling. Research on blood-flow restriction without a resistance exercise component has shown that restriction alone can cause some muscle hypertrophy (Abe et al., 2006; Kubota et al., 2008). Cellular swelling is a plausible mechanism which could explain the benefits of blood-flow restriction without resistance exercise.

An increase in cell swelling may be due to a combination of blood pooling, accumulation of metabolites, and reactive hyperemia. Blood pooling and reactive hyperemia would be present during restriction without resistance exercise and increase cellular fluid volume through an increase in pressure (Frigeri et al., 2004; Johnson et al., 1976). Metabolite accumulation increases cellular swelling by disrupting the osmotic gradient and driving more fluid into the cell through aquaporin 4, a water channel protein (Frigeri et al., 2004). Swelling threatens the structural integrity of the cell, which can be sensed by intracellular volume sensors. These sensors, in turn, activate mitogen activated protein kinase (MAPK) and mammalian target of rapamycin (mTOR) to increase the rate of skeletal muscular hypertrophy (Lang, 2007; Loenneke et al., 2012a; Schoenfeld, 2010). In addition to these mechanical responses, increased osmotic pressure and resulting swelling may stimulate anabolic pathways independent of mechanical load. Importantly, blood-flow restriction increases the accumulation of intramuscular metabolites, such as lactate, which has been implicated in the activation of anabolic signaling and muscle growth (Pearson & Hussain, 2015). Therefore, the swelling and associated

biochemical environment created by BFR may serve as important hypertrophic stimuli even in the absence of substantial mechanical tension.

1.2.2.2 Myogenic Pathways.

Muscle hypertrophy resulting from resistance exercise may be mediated through a variety of stimuli such as muscle damage, mechanical tension, and metabolic stress (Schoenfeld, 2010). These stimuli increase muscle hypertrophy through various pathways including activation of mTOR, myogenic stem cell proliferation, and alteration of the expression of signaling peptides and proteins (known as "myokines") (Schoenfeld, 2010).

Muscle damage resulting from BFR-RE has been minimal (soreness lasting <24 hours) when sets are not taken to failure (Thiebaud et al., 2013, 2014). More significant muscle damage lasting >48 hours has been observed when taking BFR-RE to failure (Umbel et al., 2009), indicating that the volume of BFR-RE performed may mediate its relationship with muscle damage and the potential effects on hypertrophy. Additionally, mechanical tension generated even at lower loads during BFR-RE appears sufficient to activate key anabolic pathways such as mTOR signaling (Fry et al., 2010). The hypoxic and acidic environment generated by BFR-RE may also contribute to the upregulation of genes involved in hypertrophic adaptations (Pearson & Hussain, 2015). Therefore, although muscle damage may not be the primary driver of growth in BFR-RE, mechanical tension and metabolic stress likely still initiate significant myogenic signaling leading to hypertrophy.

1.2.2.2.1 mTOR Signaling.

mTOR is a central regulator of skeletal muscle anabolism, integrating diverse signals from nutrients, growth factors, mechanical stimuli, and exercise. Activation of mTOR signaling plays a crucial role in promoting muscle protein synthesis and hypertrophy. Mechanical loading activates mTOR via phosphatidic acid (PA)-mediated recruitment to macropinosomes, with stretch-induced deformation contributing to this process (Lin & Liu, 2019). Both resistance and aerobic exercise increase mTOR signaling, although the response is typically more robust with resistance training, as indicated by increased phosphorylation of mTOR and p70S6K1 during resistance training compared to aerobic training (Mazo et al., 2021). Notably, BFR-RE—despite the use of low external loads—elicits mTOR activation comparable to high-load training, likely due to enhanced metabolic stress causing motor unit cycling, and cellular swelling (Fry et al., 2010; Loenneke et al., 2012b; Pearson & Hussain, 2015).

Importantly, the energy status of the muscle cell can modulate mTOR signaling, particularly through the actions of AMP-activated protein kinase (AMPK). Under conditions of energetic stress, AMPK is activated and typically inhibits mTOR signaling to conserve energy (Kazyken et al., 2019; Ramnanan et al., 2010). However, certain phosphorylation sites on mTOR, such as Ser1261, are AMPK-dependent and may respond to contractile activity, suggesting that AMPK can modulate mTOR in a context-dependent manner (Li et al., 2025). In the case of BFR-RE, the imposed hypoxic and ischemic conditions markedly elevate metabolic stress, increasing AMP concentrations and thereby activating AMPK (Freitas et al., 2020; Pope et al., 2013). Acute BFR sessions produce physiological responses comparable to high-load resistance training, including muscle swelling and lactate accumulation—both indicators of metabolic strain and potential AMPK activation (Freitas et al., 2020). Over time, chronic BFR training has been shown to

induce hypertrophic adaptations similar to those seen with high-load resistance exercise (Davids et al., 2021). Thus, while traditionally viewed as a catabolic regulator, AMPK may serve a more nuanced role in BFR-RE by helping to coordinate the cellular energy balance required for adaptive remodeling, potentially supporting muscle growth under metabolically stressful conditions.

1.2.2.2.2 Myokines.

Myokines play a significant role in skeletal muscle hypertrophy following resistance training. These myokines are secreted by muscle fibers in response to contraction and exert autocrine, paracrine, and endocrine effects that regulate muscle growth, repair, and metabolism (Cornish et al., 2020). Notably, interleukin-6 (IL-6) and irisin have been implicated in modulating key anabolic pathways such as the Akt-mTOR-p70S6K axis, which governs protein synthesis and contributes directly to hypertrophic adaptations (Nitzsche et al., 2018). IL-6, in particular, is rapidly elevated in response to acute resistance exercise and plays a dual role in both metabolic regulation and muscle remodeling, although its levels tend to decrease with chronic training adaptations (Cordingley et al., 2023). Beyond protein synthesis, myokines are crucial for facilitating satellite cell activation, muscle repair, and regeneration following exercise-induced damage (Cornish et al., 2020; Newmire & Willoughby, 2015).

Recent evidence indicates that BFR-RE can modulate myokine expression, although the magnitude and direction of these responses are influenced by age, training status, and specific myokines measured. For example, IL-4 and IL-6 levels are elevated in untrained older males following BFR-RE, whereas these differences are not seen in younger males, suggesting an age-dependent modulation of inflammatory and regenerative pathways (Cordingley et al., 2023).

Additionally, irisin levels tend to be higher in younger males, and leukemia inhibitory factor (LIF) responses appear sensitive to training status, indicating that BFR-RE-induced myokine expression is not uniform across individuals (Cordingley et al., 2023). Interestingly, decorin and IL-15, myokines associated with muscle growth and satellite cell activity, do not appear to be significantly altered by BFR-RE, suggesting that its myokine profile may differ from traditional resistance exercise (Bugera et al., 2018). However, BFR-RE decreases myostatin expression while increasing follistatin, mirroring the anabolic signaling typically observed following high-load resistance training (Laurentino et al., 2012; Manini et al., 2011). These findings support the idea that BFR-RE promotes hypertrophy through both mechanical and biochemical pathways, including modulation of the myokine environment. However, the heterogeneity in responses across age and training background highlights the need for further research to clarify the regulatory roles of specific myokines in BFR-RE-mediated muscle adaptation.

1.2.2.2.3 Satellite cells.

Satellite cells are stem cells local to skeletal muscle and play a role in muscle regeneration (Ciciliot & Schiaffino, 2010). Satellite cells begin in a quiescent state, but activate in response to muscle injury or exercise-induced damage (Tatsumi et al., 2006). After damage has occurred, they are stimulated by various pathways and migrate to the location of the damage. Once at the site of damage, the satellite cells will proliferate and then differentiate into mature myocytes to fuse with damaged myofibers. The fusion donates a nucleus to the myofiber to increase transcription and repair the damaged myofiber (Dumont et al., 2015).

Given the minimal effects of BFR-RE on muscle damage (Thiebaud et al., 2013, 2014; Umbel et al., 2009), it seems unlikely that satellite cell proliferation would be increased because of BFR-

RE. Despite this, Nielsen et al. (2012) reported a significant increase in satellite cell proliferation, as well as myonuclei number following BFR-RE. This difference likely occurred due to the studies evaluating muscle damage terminating sets at a pre-determined number of repetitions, whereas subjects in the Nielsen et al. (2012) study performed every set to concentric failure. Ellefsen et al. (2015) also examined myonuclei number in BFR-RE and traditional resistance exercise but, interestingly, did not find significant increases in either group. The authors of Ellefsen et al. (2015) stated the lack of significant findings regarding satellite cell response were likely due to a lack of exercise volume. Indeed, subjects only performed five sets of knee extension twice per week whereas subjects in the study by Nielsen et al. (2017) performed up to 10 exercise sessions per week, each comprising of four sets of knee extensions.

Beyond satellite cell activation, emerging evidence suggests that the systemic hormonal environment following BFR-RE may contribute to the observed hypertrophic effects. Acute increases in growth hormone concentrations have been reported after BFR-RE (Abe et al., 2006), which may indirectly enhance satellite cell activity and muscle protein synthesis. Although the exact contribution of these hormonal changes to hypertrophy remains unclear, they likely work synergistically with local muscle adaptations to promote muscle growth in response to BFR-RE.

A better understanding of the mechanism(s) underlying the benefits from BFR-RE would lead to more appropriate interventions for populations in which traditional resistance exercise is unsuitable.

1.2.3 Creatine Monohydrate.

CM is a common nutritional supplement used to enhance performance of activities lasting 3 minutes or less (Garthe & Maughan, 2018; Lanhers et al., 2015). The most common protocol for

CM supplementation is to consume a loading phase of 20 to 25g (or 0.3g/kg of body mass) CM per day for 5 to 7 days (Casey et al., 1996; Ferguson & Syrotuik, 2006; Greenhaff et al., 1994; Harris et al., 1992; Hultman et al., 1996; Mesa et al., 2002; Stevenson & Dudley, 2001; Syrotuik & Bell, 2004). The elevation in intramuscular creatine can then be maintained with smaller doses of 3 to 5g (or 0.03g/kg of body mass) per day (Cribb et al., 2007; Ferguson & Syrotuik, 2006). Supplementation protocols like those described above have been shown to be effective at elevating intramuscular creatine stores by between 10% to 40% (Casey et al., 1996; Greenhaff et al., 1994; Harris et al., 1992; Hultman et al., 1996; Syrotuik & Bell, 2004), with an 8% - 13% increase in phosphocreatine (PCr) (Casey et al., 1996; Harris et al., 1992; Hultman et al., 1996). Following cessation of CM supplementation, intramuscular PCr returns to baseline levels after 4 weeks (Hultman et al., 1996; Vandenberghe et al., 1997).

Previous research has shown that individual responses to CM supplementation are variable, with 20 – 30% of the population being categorized as “non-responders” (increases in total creatine <6mmol/kg dry muscle) (Greenhaff et al., 1994; Lemon, 2002; Syrotuik & Bell, 2004). Some of the traits that have been associated with high response rates to CM include a higher proportion of type II muscle fibers, larger muscle cross-sectional area, lower baseline levels of creatine, and higher fat-free mass (Ferguson & Syrotuik, 2006; Forsberg et al., 1991; Harris et al., 1992; Syrotuik & Bell, 2004).

1.2.4 Creatine Monohydrate Supplementation Mechanisms of Action.

1.2.4.1 Energy Availability and Phosphocreatine System.

The two primary functions of PCr allowing it to facilitate exercise performance include maintaining a higher adenosine triphosphate/adenosine diphosphate (ATP/ADP) ratio during

exercise (Ellington, 1989; Kammermeier, 1987), and facilitating the transport of inorganic phosphates from the mitochondria to areas of ATP utilization in the muscle, where they can then be used to resynthesize ATP (Kammermeier, 1987).

During high-intensity exercise activity, ATP is utilized to fuel muscle contraction in myofibrils and is subsequently broken down into ADP and an inorganic phosphate. When concentrations of ATP decline, PCr is broken down and its phosphate is used to rapidly resynthesize ATP with the aid of the enzyme creatine kinase (CK). Indeed, (Casey et al., 1996) found that ATP losses were reduced by 30% in participants who supplemented with CM compared to those taking a placebo after 30 seconds of maximal cycling activity. As exercise duration increases, a larger proportion of ATP is derived from slower forms of energy production including glycolysis and oxidative phosphorylation (Cooper et al., 2012; Izquierdo et al., 2002; Lanhers et al., 2015; Reardon et al., 2006), limiting the benefits of CM supplementation for long duration exercise.

During resistance exercise, CM supplementation has been shown to improve repetitions to fatigue at intensities ranging from 50% to 80% of 1RM (Chrusch et al., 2001; Greenhaff et al., 1993; Izquierdo et al., 2002; Kreider, 2003; Lanhers et al., 2015; Mills et al., 2020; Volek et al., 1997, 1999). Some studies have failed to find any significant effect on repetition performance resulting from CM supplementation (Cornish et al., 2006; Kilduff et al., 2002; Syrotuik et al., 2001). Kilduff et al. (2002) did not find any significant effect of CM on peak force or total force during repeated maximal isometric contractions, but, after removing non-responders (<21mmol/kg dry muscle, n = 4), there was a significant effect for both peak force and total force among the responders. Since it is known that a significant portion of the population do not respond to CM supplementation, it should be expected that some research may find non-significant results due to sampling variance.

1.2.4.2 Muscle Protein Synthesis and Hypertrophy.

The improved ability to perform muscular work resulting from CM supplementation has been studied for its effect on long-term training adaptations. In adults ranging from 18 – 40 years of age, studies have found that CM supplementation results in greater fat-free mass following resistance-exercise programs lasting 8 – 16 weeks (Becque et al., 2000; Bemben et al., 2001; Bonilla et al., 2021; Cribb et al., 2007; Kaviani et al., 2019; Olsen et al., 2006; Vandenberghe et al., 1997; Volek et al., 1999). These results were observed across multiple training methods and muscles trained. Despite these results, Wilder et al. (2001) reported no benefit to fat-free mass from CM for division 1 college football athletes following a 10-week resistance-exercise program. This result was likely due to the resistance-exercise program prescribing exact volumes to be performed for each session. It is unlikely that CM would result in significantly different adaptations with static volume prescriptions since the main benefit of CM is the increase in volume performance. Wilborn et al. (2016) and Ferguson & Syrotuik (2006) also found no benefit to fat-free mass from CM supplementation. Both studies exclusively recruited women and had relatively short program durations, 8 and 10 weeks, respectively. Additionally, women typically have lower absolute rates of hypertrophy (Kojić et al., 2021), making it less likely that a short-term study would be able to identify significant differences in muscle growth. Additionally, as identified by Forsberg et al. (1991), women tend to have higher baseline creatine values relative to muscle mass, which is negatively associated with responsiveness to CM supplementation (Ferguson & Syrotuik, 2006; Greenhaff et al., 1994; Harris et al., 1992). The potential for higher rates of non-responders and the short duration of the studies could explain the nonsignificant results. CM has been shown to increase fat-free mass in older individuals when combined with resistance exercise (Aguiar et al., 2013; Brose et al., 2003; Candow et al.,

2008, 2015; Chilibeck et al., 2005; Chrusch et al., 2001; Forbes et al., 2021; Gualano et al., 2014; Pinto et al., 2016). Some studies have reported no significant differences because of CM supplementation in older adults (Bemben et al., 2010; Bermon et al., 1998; Chilibeck et al., 2015; Cooke et al., 2014; Eijnde et al., 2003; Eliot et al., 2008). Three of the studies reporting insignificant results supplemented with CM only on training days (Bemben et al., 2010; Cooke et al., 2014; Eliot et al., 2008). Due to older adults having a greater proportion of type I muscle fibers and lower fat-free mass, there may be higher rates of non-responders among this population and less frequent supplementation may exacerbate response issues. Furthermore, the training program in Bermon et al. (1998) was only 7-weeks in duration, which may be insufficient for significant changes in fat-free mass due to CM supplementation.

There is research to suggest that CM supplementation may preferentially support hypertrophy of type II muscle fibers (Olsen et al., 2006; Syrotuik & Bell, 2004; Volek et al., 1999; Willoughby & Rosene, 2003). It is believed that this preferential growth is due to these muscle fibers being able to store higher amounts of PCr. Since type II fibers are more reliant on anaerobic ATP production, these fibers will become more resistant to fatigue than without supplementation. During resistance training with CM supplementation, more of the additional volume performed is due to type II muscle fibers being able to continue producing force, since these muscles rely on anaerobic energy production more than type I fibers (Casey et al., 1996). This, in turn, causes these fibers to experience a greater volume of mechanical tension, leading to more growth. However, there are additional mechanisms that have been observed that may contribute to the preferential growth in Type II muscle fibers. Volek et al., (1999) and Willoughby & Rosene (2003) found that CM supplementation with resistance training increased satellite cell and myogenic regulatory factors in type II muscle fibers, specifically.

CM supplementation also exerts myogenic effects through multiple anabolic pathways that support hypertrophy. One of these myogenic mechanisms is the stimulation the Akt/mTOR pathway, which plays a central role in promoting muscle protein synthesis and hypertrophy (Mateus O et al., 2023). CM has been shown to modulate specific components within this pathway, including increasing the phosphorylation of p70S6K, a downstream target of mTOR that facilitates muscle cell growth (Mateus O et al., 2023). Beyond these intracellular signaling effects, CM may also influence the muscle regeneration process by altering the secretion of myokines such as myostatin and insulin-like growth factor-1 (IGF-1). These changes may enhance satellite cell activation and differentiation, thereby supporting the repair and hypertrophy of skeletal muscle fibers (Farshidfar et al., 2017).

1.2.4.3 Cellular Swelling.

CM supplementation leads to an increase in total intramuscular creatine content, comprising both free creatine and phosphocreatine (Casey et al., 1996; Greenhaff et al., 1994; Harris et al., 1992; Hultman et al., 1996; Syrotuik & Bell, 2004). This increase is osmotically active and is accompanied by a corresponding increase in intramuscular water content to preserve osmotic balance (Mendes & Tirapegui, 2002; Murphy et al., 2004; Saab et al., 2002; Sobolewski et al., 2011). The cellular environment becomes hyperosmotic with CM accumulation, resulting in water influx and a subsequent increase in cell volume.

This initial uptake of creatine and the associated water retention can contribute to observable muscle hypertrophy and increases in body mass, particularly in the early stages of supplementation (Bjelica et al., 2020; Kim et al., 2015; Mendes & Tirapegui, 2002; Saab et al., 2002; Sobolewski et al., 2011). While some of this mass gain is attributable to water retention,

the increase in fluid and thus volume of muscle cells itself is believed to support a hypertrophic environment.

Increased intramuscular water content may enhance muscle volume and function by improving cellular hydration status, which is critical for optimal biochemical and mechanical function during exercise (Häussinger, 1996; Murphy et al., 2004; Persky & Brazeau, 2001; Rawson et al., 2019). Proper hydration at the cellular level supports metabolic efficiency and may delay fatigue, indirectly supporting training quality and recovery.

Beyond passive hydration, the osmotic effect of creatine serves as a cellular stressor that may activate anabolic signaling pathways. Similar to the mechanism proposed to contribute to hypertrophy following BFR-RE, this threat to cellular integrity has been implicated in the stimulation of protein synthesis via pathways such as mTOR (Farshidfar et al., 2017; Schliess et al., 2006). Additionally, creatine may contribute to muscular adaptation by stabilizing lipid membranes and influencing molecular structure, which could enhance cellular integrity under stress (Rawson & Persky, 2007; Schliess et al., 2006).

Despite concerns raised anecdotally, there is no robust scientific evidence supporting claims that CM supplementation leads to muscle cramps. On the contrary, creatine may reduce the incidence of cramps and soft-tissue injuries by improving muscle hydration and function (Dalbo et al., 2008)

1.2.5 Creatine Monohydrate for Females.

Females tend to have lower total body creatine stores compared to males, although when normalized to skeletal muscle mass, women may exhibit relatively higher baseline creatine

concentrations (Forsberg et al., 1991; Smith-Ryan et al., 2021). This higher relative baseline could potentially attenuate the response to CM supplementation, as individuals with lower initial levels typically demonstrate greater responsiveness (Syrotuik & Bell, 2004). Another factor that may influence CM efficacy in females is muscle fiber composition. Women generally have a greater proportion of type I (slow-twitch) muscle fibers compared to men in the lower body musculature (Fournier et al., 2022; Nuzzo, 2024), which have a lower capacity for creatine storage and may not respond as robustly to supplementation. However, dietary intake is also a relevant consideration; on average, women consume less creatine through their diet, which may actually enhance responsiveness to supplementation due to a lower starting point in endogenous stores (Brosnan & Brosnan, 2007).

1.2.5.2 Creatine and Hormonal Fluctuations.

Hormonal fluctuations throughout the female lifespan—particularly during the menstrual cycle, pregnancy, and menopause—can significantly influence creatine metabolism. These fluctuations may alter the activity of creatine kinase (CK), an enzyme crucial for creatine utilization. For example, research has shown that both pregnancy and menopause are associated with alterations in creatine synthesis and utilization, indicating that supplementation strategies may need to be tailored for these life stages (Ellery et al., 2016; Muccini et al., 2021; Smith-Ryan et al., 2021). Although studies have not shown significant sex differences in creatine transporter (CreaT) gene expression or total skeletal muscle creatine content (Murphy et al., 2003), women may still exhibit different creatine utilization patterns due to hormonal influences, which could impact energy metabolism and substrate selection during exercise.

Sex-specific dosing strategies to ensure increased intramuscular creatine levels have not been directly examined. Females, on average, exhibit approximately 80% of the creatinine excretion rate of males, which may reflect differences in muscle mass or lower habitual dietary creatine intake (Ellery et al., 2016). Additionally, creatinine excretion in females appears to fluctuate across the menstrual cycle, with elevated excretion observed during phases of increased estrogen levels (Muccini et al., 2021). These hormonal variations influence creatine metabolism, including utilization and turnover, by increasing the activity of CK (Muccini et al., 2021). Given these potential sex- and hormone-related differences, further research is needed to determine whether phase-specific creatine dosing strategies might be beneficial for optimizing and maintaining intramuscular creatine saturation in females.

Despite mechanistic support for the idea that hormonal status could influence the effectiveness of CM supplementation, these effects have not been observed in practical settings. For instance, Gordon et al. (2023) evaluated the effects of CM supplementation across different phases of the menstrual cycle and found no significant performance differences, suggesting that any hormonal modulation of CK activity may not have a meaningful impact on short-term exercise outcomes in practice.

1.2.5.3 Exercise Outcomes with Creatine Supplementation in Women.

CM supplementation has been shown to improve muscle performance in women, particularly when paired with resistance training. These improvements are likely due to enhanced CK system activity, which facilitates more rapid energy availability during high-intensity activity (Azevedo et al., 2022; Tam et al., 2025). Evidence suggests that CM can enhance strength and power outputs in both males and females. For example, (Tarnopolsky & MacLennan, 2000) reported

significant increases in peak anaerobic cycling power and dorsiflexion maximal voluntary contraction torque in both sexes following CM supplementation, with no sex-specific differences. Similarly, (Francaux et al., 2000) found that while maximal power output increased comparably in both sexes, females demonstrated a greater performance improvement under fatigue conditions.

With regard to body composition, CM supplementation has been linked to increases in fat-free mass and decreases in body fat percentage across both genders. (Dinan et al., 2022) observed similar improvements in male and female athletes, independent of the timing of CM intake. However, some sex-specific responses have been noted; (Francaux et al., 2000) reported an increase in total body mass in males but not in females, suggesting potential sex differences in the mechanisms driving creatine-related gains in body composition.

Nonetheless, the degree to which females benefit from CM supplementation appears to vary. Some studies report minimal improvements in performance outcomes among female participants, which may be due to differences in training background, hormonal status, or other physiological variables specific to females (Tam et al., 2025). These findings highlight the importance of accounting for sex-specific factors in the design and interpretation of CM supplementation studies.

Despite a multitude of research on CM's effects on resistance exercise repetition performance, there is a lack of research examining if these effects are consistent during novel resistance-exercise strategies, such as BFR-RE.

1.3 Statement of Problem.

CM supplementation strategies to improve performance during BFR-RE, to my knowledge, have not been examined. CM serves as a likely candidate to improve the performance and training response resulting from BFR-RE due to the multitude of research demonstrating it's benefit during traditional resistance exercise (Branch, 2003).

Research on CM supplementation in a female population is sparse. This study will perform an exploratory analysis of potential sex-based difference in the response to CM supplementation.

Therefore, the purpose of this research is to determine if CM supplementation can improve exercise performance during an acute bout of BFR-RE and whether this improvement is the same or differs between male and female participants.

1.4 Hypotheses.

The primary hypothesis for this study was that there would be a significant increase in the number of repetitions performed until failure in the CM supplemented condition compared to the placebo condition. CM has been shown to increase exercise performance in repetition tasks in intensities ranging from 50% - 80% 1RM as well as tasks up to 3 minutes in duration (Chrusch et al., 2001; Greenhaff et al., 1993; Izquierdo et al., 2002; Kreider, 2003; Lanhers et al., 2015; Mills et al., 2020; Volek et al., 1997, 1999).

Secondary hypotheses for this study included that CM supplementation would have a larger improvement in earlier sets and in earlier exercises. This is due to the short rest times used in BFR-RE not allowing for sufficient recovery of PCr stores (Balsom et al., 1995; Greenhaff et al., 1994)

An additional hypothesis for this study was that there would be differences in the response to CM supplementation between males and females. Due to females generally possessing more physiological attributes that correspond to being a non-responder to CM, it was anticipated that male participants would respond more strongly to CM supplementation.

CHAPTER 2 – METHODS

The study was a double-blind, randomized, counterbalanced, crossover trial conducted in Canada at the Fort Garry campus of the University of Manitoba located in Winnipeg, Manitoba. The study was designed to determine whether CM supplementation was superior to a placebo for increasing repetition performance during BFR-RE.

2.1 Participants

Participants were resistance-trained males and females between 18 and 30 years of age. Resistance trained was defined as training ≥2 days per week for ≥1 year. Participants were asked to wear athletic attire suitable for exercise for each laboratory visit. Participants age range was selected to minimize age-related effects on skeletal muscle hypertrophy as older individuals have been shown to have blunted responses to resistance exercise compared to younger populations (Kumar et al., 2009). Mixed sex participants were chosen to evaluate potential sex differences in acute performance effects due to CM supplementation. Participants were asked to report the sex which they were identified as at birth.

2.1.1 Sample size calculation. Based on previous research it was calculated that 8 participants would be required to reach statistical significance at $\alpha = 0.05$, $1-\beta = 0.9$ using means and standard deviations from (Aguiar et al., 2013) where there was a mean difference of 8 more repetitions to failure in the CM supplemented group compared to the placebo group. It was decided to double this sample size to include 8 biological males and 8 biological females to allow for preliminary analysis of potential sex differences in the repetitions to fatigue plus an additional 25% to account for participant dropouts (N = 20 total). The main question was whether CM supplementation would enhance muscular performance of three lower body resistance exercises to fatigue over 4 sets of exercise.

2.1.2 Inclusion and Exclusion Criteria. Participants were required to have at least one year of resistance exercise experience with a training frequency of ≥2 days per week.

Participants were excluded if they had participated in BFR-RE in the previous 6-months or had taken nutritional supplements in the previous 1-month. Participants were asked to refrain from alcohol and cannabis for at least 6 hours and caffeine, nicotine, and food for at least 2 hours before each testing session.

Participants were excluded if they had any lower body injuries or medical conditions that would impede their ability to complete the exercise protocol such as a diagnosis of heart disease or stroke, or pain or swelling that affected their ability to engage in physical activity. Participants were screened for contraindications to exercise via completion of a "Get Active Questionnaire (GAQ)". Lastly, participants were excluded if they were pregnant or planned to become pregnant during the study period, due to increased intra-abdominal pressure as a result of the Valsalva maneuver as well as significant changes in hormonal profiles of the participants affecting creatine metabolism (Ellery et al., 2016).

2.2 Procedures

2.2.1 Study Design. This study utilized a randomized, counterbalanced crossover design. Participants were randomly assigned to begin with either the creatine monohydrate or placebo condition before crossing over to the alternate condition following a washout period. Following BFR-RE performance testing, participants completed a 3-week washout period before completing the opposite intervention. Figure 2.1 depicts the flow of participants through the study, and figure 2.2 depicts the timeline of one study arm.

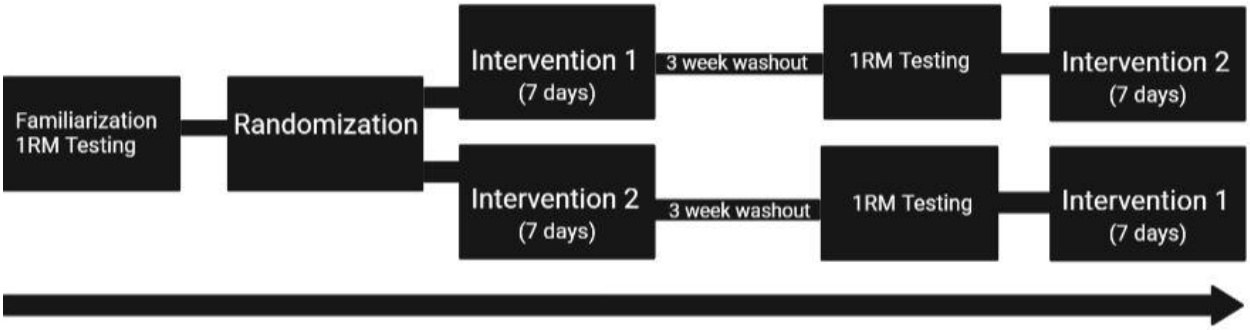

Figure 2.1 Timeline of Participant Intervention

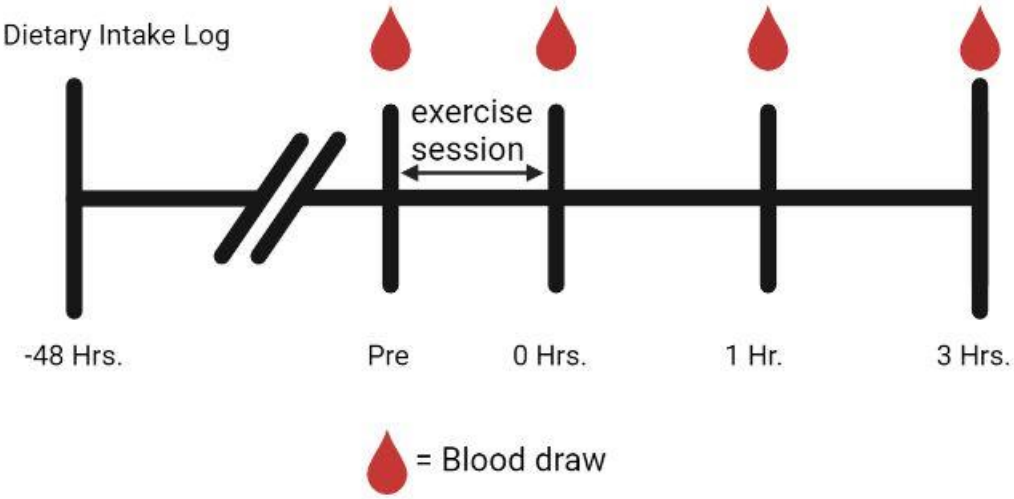

Figure 2.2 Timeline of One Study Arm

2.2.2 Randomization and Blinding. Randomization was performed using a computer-generated allocation sequence created via the sealed envelope randomization website by a research assistant within the laboratory who was not directly involved in data collection. Randomization was stratified by sex to ensure balanced allocation. The sequence was implemented using sequentially numbered, opaque, sealed containers that held either creatine monohydrate or placebo. Neither the participants nor the study personnel who enrolled participants, assigned interventions, or conducted data collection and analyses had access to the allocation sequence. Participants, exercise supervisors, data collectors, and data analysts were blinded to the supplement assignment. Blinding was achieved by providing creatine and placebo in identical

unmarked containers. Both supplements were similar in taste and appearance, although the placebo powder had a slightly different texture, which was not disclosed to participants.

2.2.3 Familiarization and 1 Repetition Maximum Testing. Participants completed a familiarization session in which they were introduced to the equipment used during the study, anthropometric data were taken, and one-repetition maximum (1RM) strength testing was performed for the leg press, knee flexion, and knee extension exercises. All anthropometric and strength measurements, as well as the exercises sessions were administered by a National Strength and Conditioning Association (NCSA) Certified Strength and Conditioning Specialist (CSCS). Anthropometric measures taken included participant height (Seca 213, Seca, Chino, CA, USA), body mass (Seca 813, Seca, Chino, CA, USA), and body fat percentage (InBody 270, InBody Canada, Ottawa, ON, Canada). Prior to 1RM testing, participants performed a 5-minute warm up on a cycle ergometer. Warm-up intensity was determined for each participant as 50% of the individuals heart rate reserve (HRR) using the participants age predicted heart rate maximum (APHRM) and their resting heart rate (RHR) and the Karvonen formula (Jeffreys, 2008).

Karvonen formula:

HRR = ((APHRM – RHR) * %Intensity) + RHR.

Participants then performed 5-10 repetitions at 40-60% of their estimated 1RM, rested for one minute, then completed 3-5 repetitions at 60-80% of their estimated 1RM. Following these warm-up sets, the load was progressively increased. After resting for 3–5 minutes, participants attempted a single repetition. If successful, they rested another 3–5 minutes before attempting the next load. This protocol was repeated until the participants were unable to successfully lift the

load through a full range of motion for a single repetition. The 1RM was the heaviest weight with which the participant successfully completed one full repetition.

Figure 2.3 Visual Depiction of Cuff Placement During Blood-Flow Restricted Lower Body Exercises using KAATSU.

2.2.4 Supplementation and Resistance Exercise Sessions. Upon completion of the familiarization session, participants were randomized to consume CM (20 g/day) or placebo (maltodextrin; 20 g/day) for 7 consecutive days prior to each BFR-RE testing session. CM was provided by Alzchem Trostberg GmbH, commercially available from the Creapure website (https://www.creapure.com/). Maltodextrin was provided by Canadian Protein, commercially available from the Canadian Protein website (https://canadianprotein.com/). Both supplements were provided in unmarked, sealed containers and were similar in appearance and taste, although slight differences in texture were noted. Participants were instructed to mix each dose with water and consume evenly across the day. Compliance was monitored by self-report and verified by weighing remaining contents of returned containers. During the 7-day period between the familiarization session and the first BFR-RE session, participants were asked to complete a 3-day food diary to evaluate their dietary intake. Participants were asked to not change their diet for the duration of the study. Participants were asked to consume the same 3-day food intake for both arms of this cross-over trial to mitigate the influence of dietary intake on the main outcome variables.

Following the 7-day supplement period, participants reported to the exercise laboratory for the first session of BFR-RE. A 6mL blood sample was obtained pre-exercise via venipuncture from the antecubital vein by a licensed primary care paramedic into vacutainer blood collection tubes containing ethylene diamine tetra-acetic acid (EDTA). After collection of the blood sample, participants performed a 5-minute warm-up on a cycle ergometer. Participants were then fitted with a 5 cm wide cuff (KAATSU Nano; KAATSU-Global, Huntington Beach, CA, USA) at the most proximal portion of the thigh. The cuff was be inflated until the pressure reaches

120mmHg, this pressure was held for 30 seconds before pressure was released. This process was repeated 4 more times with the pressure increasing by 20mmHg each time until the pressure reaches 200mmHg (Fry et al., 2010). Once pressure reached 200mmHg, it was maintained as the subject began the exercise protocol. The exercise protocol was 4 sets to failure with 30% 1RM on leg press, knee flexion, and knee extension exercises with 30 seconds of rest between sets and 2 minutes rest between exercises. Failure was defined as the inability to complete a repetition through a full range of motion. All sets of one exercise were completed before beginning the following exercise. The cuff remained inflated during the 30 second rest between exercise sets but was deflated during rest periods between exercises. 1RMs were reassessed after the wash-out period to account for any adaptations during the 3-week washout period. Participants were given the alternate supplement condition than they consumed for the first BFR-RE session. Another 7-day supplementation period was completed, thus having a total of 4 weeks between exercise sessions. Participants then performed an identical BFR-RE session as they previously did. In total, each participant completed four supervised exercise testing sessions: two familiarization and strength testing visits (one before each supplementation arm) and two blood-flow restricted resistance exercise testing sessions (one following creatine monohydrate supplementation and one following placebo supplementation). Each supplementation phase lasted 7 days and was separated by a 3-week washout period, resulting in a total study duration of approximately 5 weeks per participant.

2.2.5 Blood Sampling and Analysis. A licensed primary care paramedic with clinical experience in venipuncture drew 6 mL blood samples before the exercise sessions, immediately following exercise, and at 1- and 3- hours post-exercise via venipuncture from the antecubital vein into vacutainer tubes coated with EDTA. Blood samples were inverted several times and

then centrifuged at 1500 x gravity for 15 minutes at 4°C. The resulting plasma was aliquoted using a transfer pipette into microtubes and stored in a -80°C freezer until analysis was completed. Analysis was carried out to determine myokine concentrations of apelin, BDNF, myostatin, irisin, and IL-6.

Myokine analysis was conducted with a Human Myokine Multiplex Magnetic Bead Panel (Milliplex Map Kit, EMD Millipore Corp, Billerica, MA, USA). Luminex multiplex technology utilizes antibodies to identify proteins of interest. Colour-coded microscopic beads are added to the wells of the plate, allowing for the evaluation of multiple myokines at once. These protein-specific beads react with the plasma samples during a period of incubation. Detection antibodies are then added followed by a treatment of phycoerythrin-streptavidin, which binds to the antibodies and is fluorescent for the analysis. The microplates are then placed in a microplate reader (Luminex MagPix, Luminex Corp., Toronto, Canada) which determines the colour hue of the beads (specific to the analyte) and the fluorescence (the concentration of the analyte).

Following the first BFR-RE session, participants discontinued supplementation and completed a 3-week washout period before strength testing was repeated. After this washout, participants began the alternate supplement condition, which included another 7-day loading period before the second BFR-RE trial. Thus, the total time without supplementation between intervention arms was approximately 4 weeks. Immediately before, and following this exercise session, blood samples were collected again following the same procedures as the first intervention.

Potential harms or adverse events were monitored non-systematically during all supplementation and exercise sessions. Participants were instructed to report any gastrointestinal discomfort,

muscle cramping, dizziness, or other symptoms. Some participants experienced light-headedness and/or feelings of nausea during blood draws, but no serious adverse events were reported.

2.3 Statistical Analysis.

Descriptive statistics were used to report all participants baseline anthropometric data. All data is presented as mean ± standard deviation. The primary outcome was the total number of repetitions completed to failure across four sets of each resistance exercise (leg press, knee flexion, and knee extension). The analysis metric was the number of successful repetitions, aggregated as mean repetitions per set and across all sets for each exercise.

The secondary outcomes were changes in circulating myokines (apelin, BDNF, myostatin, irisin, and IL-6), measured from plasma at baseline (pre-exercise), immediately post-exercise, 1 h post, and 3 h post. The method of aggregation was mean plasma concentration at each time point. A 4-way repeated measure analysis of variance (ANOVA) was conducted with condition (CM versus placebo), exercise (leg press, knee flexion, and knee extension), and sets (exercise sets 1 through 4 for each exercise) as within-subject factors, and biological sex (male vs. female) as a between-subjects factor. A p-value of ≤ 0.05 was used to determine significance. If a significant effect was found, simple main effects were analyzed using Bonferroni-adjusted post hoc comparisons to determine specific differences.

A sensitivity analysis was conducted for the 4-way mixed ANOVA to evaluate the robustness of the findings. No additional subgroup analyses were performed beyond the stratification by sex described above. All statistical analyses were made using SPSS version 30.0.0.0 (IBM SPSS Statistics for Windows, Version 30.0.0.0, IBM Corp., Armonk, NY, USA) statistical analysis software.

CHAPTER 3 – RESULTS

3.1 Participant Characteristics

Recruitment occurred from September 2023 to August 2024. A total of 19 participants (9 males, 10 females) were initially recruited for this study. Of these, 13 (7 males, 6 females) completed all testing and training sessions, resulting in a dropout rate of approximately 32%. Reasons for dropout included injury unrelated to the exercise intervention (n = 2), time constraints (n = 1), and loss of follow-up due to unresponsiveness (n = 3). No participants withdrew due to adverse events experienced as a result of the intervention. It is likely that the demanding nature of the BFR-RE protocol contributed to the attrition rate. All 13 participants were included in the final analyses for the primary outcomes. No participants discontinued one arm of the crossover while continuing in the other.

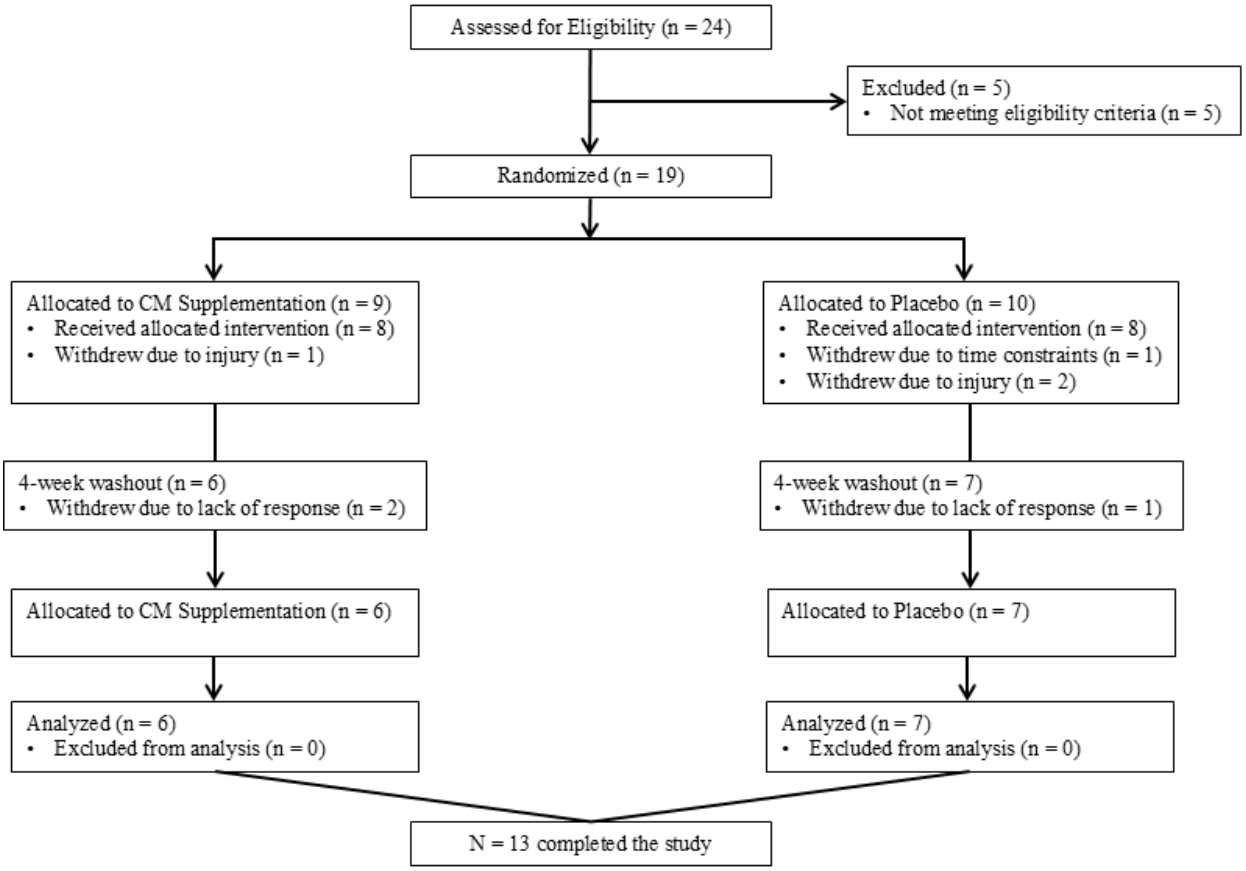

Figure 3.1 Participant Flow Diagram. The diagram illustrates the number of participants at each stage of the trial, including those screened for eligibility, randomized to each intervention sequence, lost to follow-up with reasons, and included in the final analysis.

Participants completed a familiarization and 1RM testing session during which, their resting heart rate, resting blood pressure, height, body mass, body fat percentage, and 1RMs for leg press, knee flexion, and knee extension exercises were measured. A summary of participant characteristic can be seen in table 3.1.

Table 3.1 Participant Characteristics

Characteristic	Mean ± SD			Range		
	Male	Female	All	Male	Female	All
Age (years)	26.86	27	26.92	20 – 29	24 – 30	20 – 30
Height (m)	1.78 ± 0.09	1.67 ± 0.09	1.73 ± 0.1	1.66 – 1.93	1.5 – 1.74	1.5 – 1.93
BM (kg)	105.36 ± 22.74	67.18 ± 8.38	87.73 ± 26.08	76.1 – 135.6	52.5 – 74.5	52.5 – 135.6
BMI (kg/m^2)	33.1 ± 6.74	24.1 ± 1.11	28.93 ± 6.69	26.42 – 42.84	22.66 – 25.78	22.66 – 42.84
%BF	18.37 ± 10.99	22.98 ± 5.49	20.5 ± 8.87	6.7 – 39	18 – 32.9	6.7 – 39
RHR (bpm)	66.86	64.67	65.85	60 – 76	53 – 71	53 – 76
SBP (mmHg)	122.14	114	118.38	111 – 134	106 – 120	106 – 134
DBP (mmHg)	74.86	68.83	72.08	67 – 86	62 – 79	62 – 86
LP1RM	286.2 ± 48.53	171.61 ± 26.05	243.43 ± 70.76	244.94 – 353.8	140.61 – 199.58	140.61 – 353.8
LF1RM	81.48 ± 13.15	51.6 ± 14.8	67.69 ± 20.45	61.24 – 94.12	34.02 – 77.11	34.02 – 94.12
LE1RM	165.56 ± 32.02	105.84 ± 13.35	138 ± 39.34	113.4 – 208.65	86.18 – 127.01	86.18 – 208.65

Note: BM = body mass, BMI = body mass index, %BF = percentage body fat, RHR = resting heart rate, SBP = systolic blood pressure, DBP = diastolic blood pressure, LP1RM = leg press 1RM, LF1RM = knee flexion I-repetition maximum, LE1RM = knee extension 1RM.

3.2 Exercise Intervention

All participants adhered to the supplementation and training protocol as prescribed. Both CM and placebo supplements were provided as powders that were similar in appearance and taste, though not in texture, and were consumed at home as instructed. Compliance was monitored by weighing the remaining contents of each participant's supplement container at the end of the intervention. Exercise sessions were supervised by a NSCA CSCS to ensure fidelity of the blood flow restriction protocol. Adherence to the exercise protocol was 100% among the 13

participants who completed the study. Adherence to the supplementation protocol was estimated based on the weight of the remaining supplement after each supplementation period. While there was variance in the amount of supplement remaining, none of the participants who completed the protocol differed significantly from the group average. Additionally, no participants reported any difficulty in adhering to the supplementation protocol.

A 4-way mixed ANOVA was conducted to examine the effects of treatment (CM vs placebo), exercise type (leg press, knee flexion, knee extension), and set number (1–4) on the number of repetitions performed, with sex included as a between-subjects factor.

3.2.1 Main Effects

A significant main effect of treatment was observed, with participants completing more total repetitions in the CM supplementation condition (277.38 ± 83.06) than in the placebo condition (251.62 ± 91.73), $F(1, 11) = 12.84$, $p = .004$.

A significant main effect of exercise was also observed, $F(2, 22) = 35.61$, $p < .001$. Average repetitions were highest for the leg press (31.53 ± 2.88), followed by knee flexion (24.83 ± 3.11), and lowest for the knee extension (10.18 ± 1.01).

A significant main effect of set was found, $F(3, 33) = 106.14$, $p < .001$. Repetitions decreased across sets in the following pattern: set 1 (42.71 ± 3.73) > set 2 (18.47 ± 1.87) > set 3 (14.75 ± 1.54) > set 4 (12.78 ± 1.45).

The main effect of sex was not analyzed as a standalone effect due to its role as a between-subjects moderator, but relevant interactions are reported below.

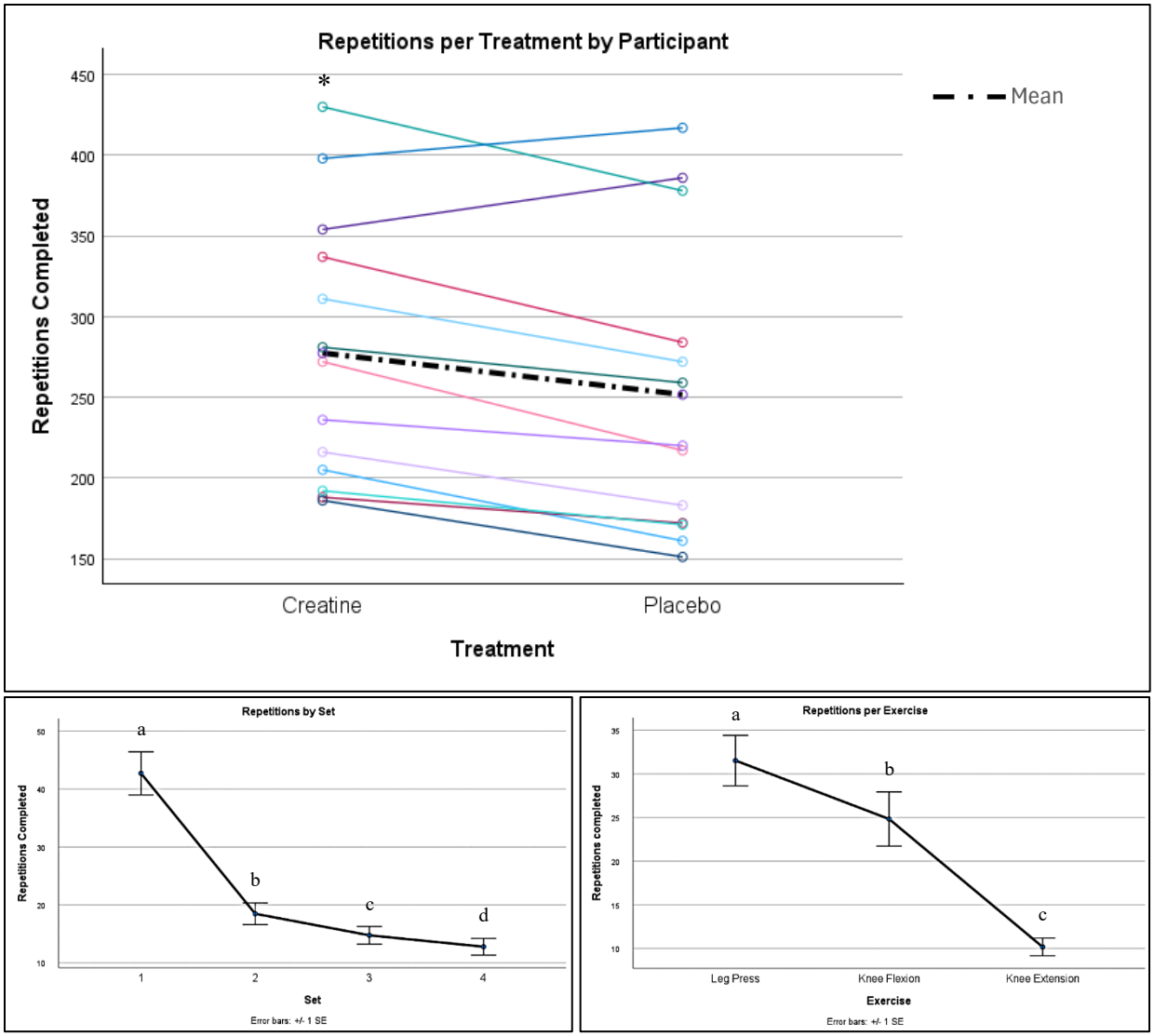

Figure 3.2 Repetition Performance Across Treatments, Sets, and Exercises.
Top panel: Total repetitions completed by each participant (thin colored lines) and group mean (thick dashed line) under CM supplementation and placebo conditions. An asterisk (*) indicates significant difference between conditions.
Bottom left panel: Mean repetitions completed (±1 SE) across successive sets, collapsed across treatments. Different letters (a–d) denote significant differences between all sets ($p < 0.05$).
Bottom right panel: Mean repetitions completed (±1 SE) across different exercises, collapsed across treatments. Different letters (a–c) denote significant differences between all exercises ($p < 0.05$).
Note: SE = Standard Error.

3.2.2 Two-Way Interactions

A significant treatment × exercise interaction was found, $F(2, 22) = 4.004$, $p = .033$, $\eta^2 = .267$, with CM supplementation leading to higher repetitions in the leg press and knee extension but not in knee flexion. Specifically, participants performed significantly more repetitions in the CM supplementation condition compared to placebo for the leg press ($p < .001$) and knee extension ($p = .015$), while no significant difference was observed for the knee flexion ($p = .710$).

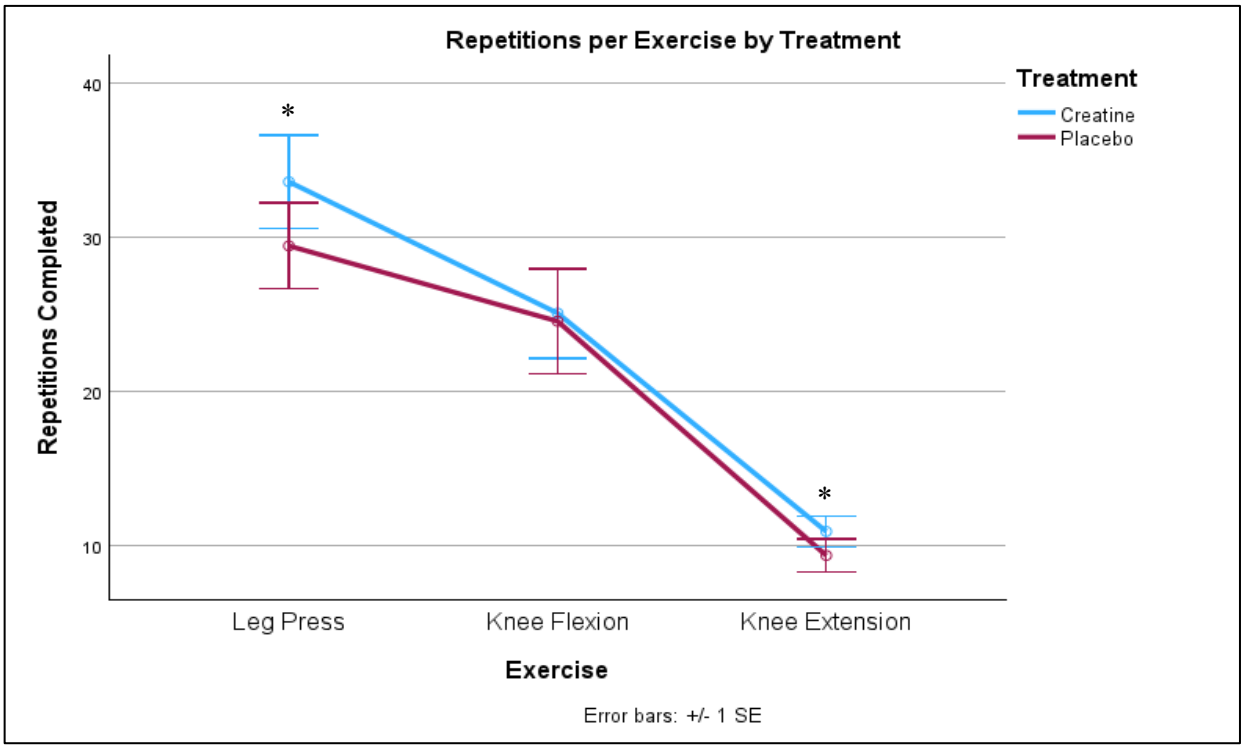

Figure 3.3 Treatment Comparison Between Exercises. Mean ± SE of total repetitions performed during each exercise by treatment. CM supplementation is indicated by the blue line and placebo is indicated by the red line. (*) significant difference between supplementation and placebo.

A significant treatment × set interaction was also observed, $F(3, 33) = 3.137$, $p = .038$, $\eta^2 = .222$, with CM supplementation leading to higher repetitions primarily in the earlier stages of the exercise bout. Pairwise comparisons revealed that CM supplementation significantly increased

repetitions compared to placebo during set 1 ($p = .02$), while differences in sets 2 ($p = .053$), 3 ($p = .114$), and 4 ($p = .087$) did not reach statistical significance.

A significant exercise × set interaction was observed, $F(6, 66) = 5.597$, $p < .001$, $\eta^2 = .337$. Across all exercises, repetitions declined with each successive set; however, the magnitude of decline differed across exercises. For the leg press, repetitions significantly declined between each successive set (all $p < .01$), with the largest drop observed between Set 1 and Set 2. A similar pattern was found for knee flexion, with significant declines between Sets 1 and 2 ($p < .001$), Sets 1 and 3 ($p < .001$), and Sets 1 and 4 ($p < .001$), though the decline between Sets 3 and 4 was not statistically significant ($p = .183$). For the knee extension, significant declines occurred between Sets 1 and 2 ($p < .001$), 1 and 3 ($p < .001$), 1 and 4 ($p < .001$), 2 and 3 ($p < .001$), and 2 and 4 ($p < .001$), while performance between Sets 3 and 4 remained stable ($p = 1.000$).

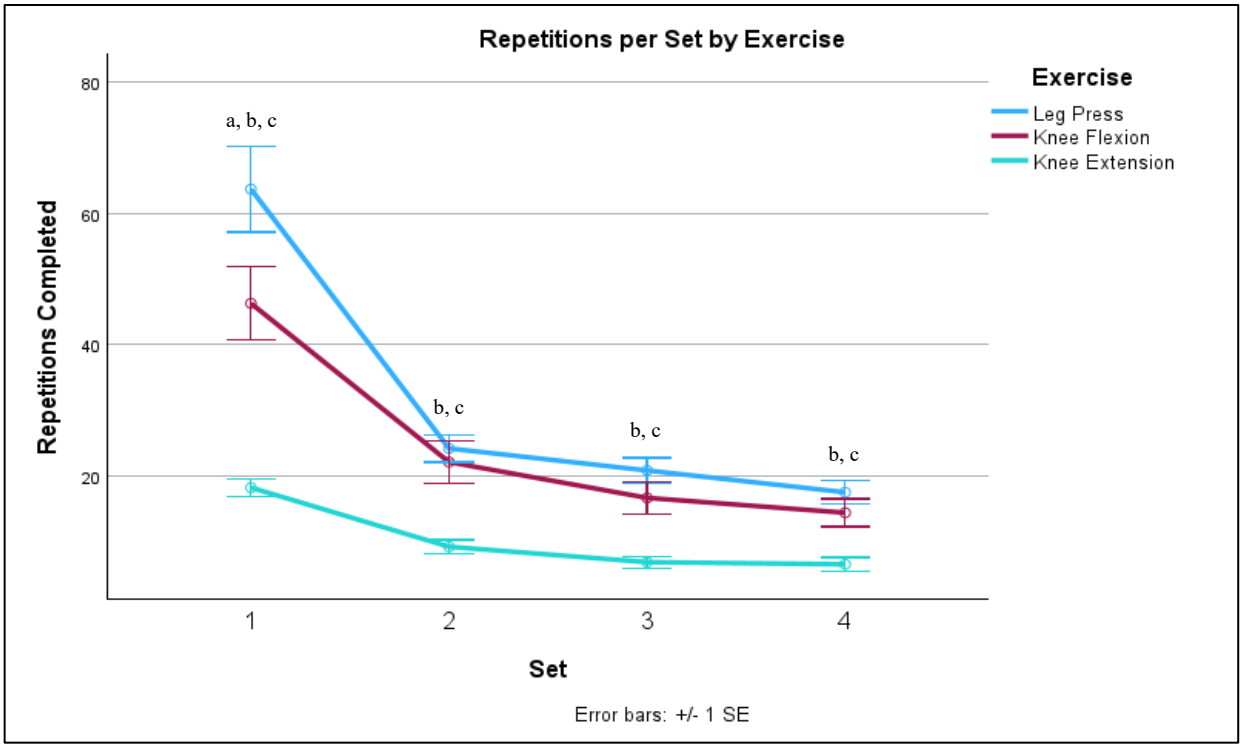

Figure 3.4 Repetitions per Set Between Exercises. Mean ± SE of repetitions completed per set during BFR-RE for the leg press, knee flexion, and knee extension exercises. Data are presented as mean ± 1 standard error (SE). Different letters indicate significant differences within a given set ($p < 0.05$), where (a) denotes significant differences between leg press and knee flexion, (b) denotes significant differences between leg press and knee extension, and (c) denotes significant differences between knee extension and knee flexion.

3.2.3 Three-Way Interactions

A significant sex × treatment × exercise interaction was observed, $F(2, 22) = 3.798$, $p = .038$, $\eta^2 = .257$. Males in the CM supplementation group showed the most pronounced increase in leg press repetitions (male CM: 34.25 ± 4.09 vs male placebo: 27.07 ± 3.79; $p < .001$), while no significant differences between CM supplementation and placebo were observed for females across any exercise (all $p > .25$).

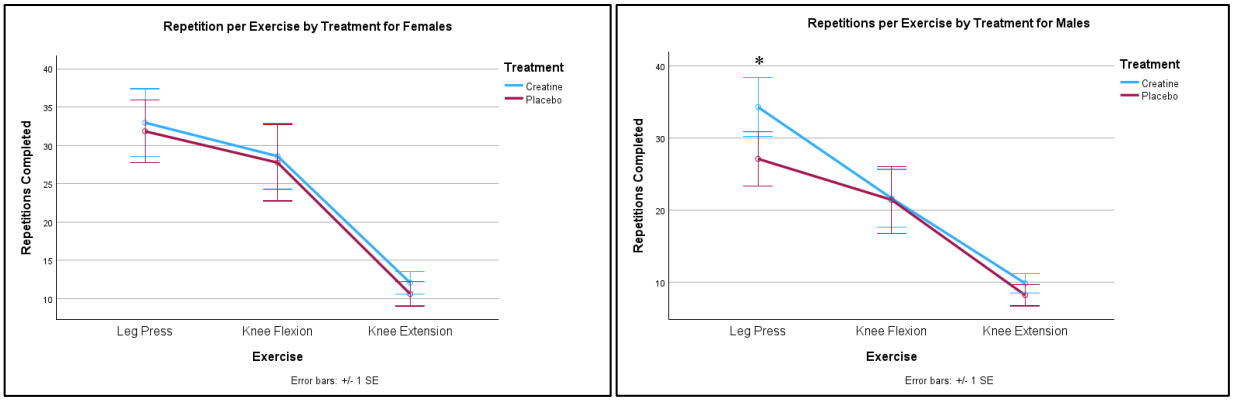

Figure 3.5 Repetitions Completed Per Exercise for Females (left panel) and Males (right panel). Mean ± SE of repetitions completed per exercise during BFR-RE following CM or placebo supplementation. In females, no significant differences were observed between CM and placebo treatments across any of the exercises. In males, CM supplementation significantly increased repetition performance compared to placebo during the leg press exercise, as indicated by the asterisk ($p < 0.05$). No significant differences were observed between treatments for knee flexion or knee extension in either sex.

A significant sex × treatment × set interaction was found, $F(3, 33) = 4.513$, $p = .009$, $\eta^2 = .291$, indicating that CM supplementation improved performance differently across sexes and sets. In males, CM supplementation significantly enhanced repetitions during Set 1 ($p = .003$) and Set 2 ($p = .010$) compared to placebo, with no differences in Sets 3 ($p = .523$) or 4 ($p = .442$). Among females, no significant differences between treatments were observed across any set (all $p > .05$).

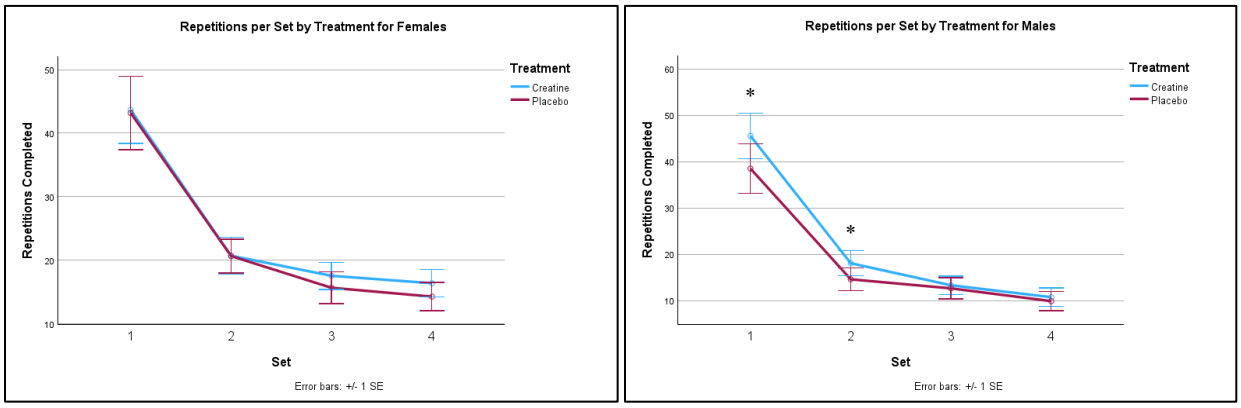

Figure 3.6 Repetitions Completed Per Set for Females (left panel) and Males (right panel). Mean ± SE of repetitions completed per set during BFR-RE following CM or placebo supplementation. In females, no significant differences were observed between CM and placebo treatments across any of the sets. In males, CM supplementation significantly increased

repetition performance compared to placebo in sets 1 and 2, as indicated by the asterisk ($p < 0.05$).

3.2.4 Four-Way Interaction

The treatment × exercise × set × sex interaction did not reach significance, $F(6, 66) = 1.83$, $p = .108$.

3.2.5 Non-Significant Effects

There were no significant main effects of sex, nor were there significant interactions for sex × treatment, sex × set, or sex × exercise.

3.3 Blood Sample Analysis.

Although blood samples were successfully collected as part of the study protocol, analysis of these samples could not be completed due to unforeseen technical difficulties with the assay procedures. Despite repeated attempts, the issues could not be resolved within the timeframe of the current project. As such, while blood collection was an integral part of the research methodology and is documented accordingly, no data or results from blood sample analyses can be presented or discussed at the current time.

3.4 Sensitivity Analysis

Given the relatively small sample size, a series of post-hoc sensitivity analyses were conducted to assess the minimum detectable effect sizes (Cohen's *f*) at 90% statistical power ($1–\beta = 0.90$) and $\alpha = 0.05$. Using G*Power version 3.1.9.7 (Faul et al., 2007), sensitivity analyses were performed separately for each primary main effect and interaction effect.

Specifically, the minimum detectable effect sizes were as follows:

- **Main effect of treatment:** $\eta^2_p \geq 0.02$
- **Main effect of exercise:** $\eta^2_p \geq 0.15$

- **Main effect of set:** $\eta^2_p \geq 0.04$
- **Two-way interaction of treatment × exercise:** $\eta^2_p \geq 0.08$
- **Two-way interaction of treatment × set:** $\eta^2_p \geq 0.03$
- **Two-way interaction of exercise × set:** $\eta^2_p \geq 0.06$
- **Two-way interaction of sex × exercise:** $\eta^2_p \geq 0.15$
- **Two-way interaction of sex × set:** $\eta^2_p \geq 0.04$
- **Two-way interaction of sex × treatment:** $\eta^2_p \geq 0.02$
- **Three-way interaction of treatment × exercise × set:** $\eta^2_p \geq 0.04$
- **Three-way interaction of sex × treatment × set:** $\eta^2_p \geq 0.03$
- **Three-way interaction of sex × treatment × exercise:** $\eta^2_p \geq 0.08$
- **Three-way interaction of sex × exercise × set:** $\eta^2_p \geq 0.06$
- **Four-way interaction of sex × treatment × exercise × set:** $\eta^2_p \geq 0.04$

Collectively, these sensitivity thresholds span from small to large effect sizes according to conventional benchmarks for partial eta squared in behavioral sciences (Richardson, 2011). Thus, the study was adequately powered to detect effects of practical and theoretical importance, including small-to-moderate main effects and moderate-to-large interaction effects. Nevertheless, it is important to acknowledge that smaller interaction effects may not have been reliably detected, and the possibility of Type II error remains, particularly for higher-order interactions. Despite this limitation, the sensitivity analysis supports the overall findings within the context of exercise science research (Archer et al., 1997).

It is important to note that for several higher-order interactions (e.g., sex × exercise × set; sex × set; sex × exercise), the minimum detectable effect sizes were larger than the calculated sensitivity. As such, the study may have been underpowered to detect smaller but potentially meaningful interaction effects.

CHAPTER 4 – DISCUSSION

The primary purpose of this study was to investigate the effects of CM supplementation on muscular performance during BFR-RE. A total of 13 participants (6 females, 7 males) completed both BFR-RE sessions and supplement conditions of the study. Participants completed a double-blind randomised crossover trial in which they completed a familiarization and 1RM testing session. 1RMs were found for the leg press, knee flexion, and knee extension exercises. Following this session, participants were provided with CM or a placebo and instructed to take 20g per day for 7 days. After the supplementation period, participants completed BFR-RE at 30% of their 1RMs previously tested. A KAATSU air band cuff was placed on the most proximal region of the participants thigh and inflated to 200mmHg. The cuff remained inflated during all four sets of each exercise but was deflated during rest periods between exercises. Rest times were 30-seconds between sets, and 120-seconds between exercises. All sets were taken to momentary failure. Repetition performance was recorded for each set during the BFR-RE session. Following the first BFR-RE session, participants completed a 3-week washout period to allow PCr levels to return to baseline. After this washout period, participants re-tested their 1RMs on each exercise and were provided with the opposite supplement condition as their initial trial and the process was repeated.

This project had three hypotheses.

1) CM supplementation will improve repetition performance during BFR-RE more than placebo.
2) CM supplementation will improve performance more in earlier sets and exercises than later sets and exercises due to short rest times utilized during BFR-RE not allowing for significant replenishment of PCr stores between sets.

3) CM supplementation may affect females and males differently with males experiencing a larger benefit from supplementation due to having fewer characteristic typically associated with non-responders to CM supplementation.

4.1 Exercise Outcomes

Our findings indicate that CM supplementation significantly enhanced overall performance, as evidenced by a main effect of treatment. Specifically, participants who received CM supplementation performed a greater number of total repetitions compared to those who received a placebo. Given that greater training volume has been associated with more robust hypertrophic and strength adaptations (Baz-Valle et al., 2022; Pelland et al., 2024), these findings suggest that CM supplementation may amplify the anabolic and performance benefits of BFR resistance training. Importantly, participants in the current study performed each set to momentary failure. Previous research indicates that CM's impact on hypertrophy and strength outcomes is most pronounced when training is performed to failure as opposed to protocols where intensity and volume are equated between treatments (Volek et al., 1999; Wilder et al., 2001). Thus, the ability of the CM group to complete more repetitions under failure-based conditions would likely contribute to more robust hypertrophic and strength adaptations for individuals looking to benefit from BFR-RE.

In addition to the treatment effect, significant main effects for exercise and set were observed. Repetition performance decreased across exercises (leg press > knee flexion > knee extension) and across successive sets. These patterns are consistent with expected fatigue accumulation due to the static exercise order, a phenomenon commonly referred to as an "order effect" (Perreault, Jr., 1975). Participants could perform a higher number of repetitions early in the session when

they were least fatigued but experienced a gradual decline in performance as muscular fatigue accumulated.

The exercise by set interaction was also significant and supports this interpretation. As exercises were performed in a fixed sequence, later exercises (such as knee extension) started from a lower baseline of repetitions, which may have attenuated the rate of decline in performance across sets. In contrast, the leg press, performed first when participants were least fatigued, displayed the greatest initial repetition performance but also the most pronounced decrease across sets.

Several significant interaction effects provide deeper insight into the context of the main findings. Notably, a significant treatment × exercise interaction revealed that CM supplementation enhanced performance during the leg press and knee extension exercises but did not significantly affect performance during the knee flexion. One possible explanation for this pattern relates to the nature of the exercises: the leg press and knee extension involve larger muscle groups and higher overall force production, as well as the leg press being a multi-joint movement, which may be more responsive to CM's known effects on phosphocreatine resynthesis (Lanhers et al., 2015; Urbanski et al., 1999). In contrast, knee flexion, being a single-joint isolation movement targeting a smaller muscle group (hamstrings), may not have presented a sufficient energetic demand to reveal CM’s ergogenic properties. Psychological factors may also have contributed; participants may have been more motivated to exert maximal effort during the leg press at the beginning of the session and during knee extension when nearing the conclusion of the protocol, whereas motivation may have waned during the intermediate knee flexion exercise.

A significant sex × treatment × set three-way interaction revealed nuanced sex-based differences in response to CM supplementation. Males appeared to benefit from CM supplementation during

the earlier sets (sets 1 and 2), whereas females showed no appreciable differences across sets. One possible explanation is that males, due to having greater skeletal muscle mass and a higher proportion of type II muscle fibers, may have had a larger difference in creatine storage between the supplement and placebo conditions compared to females. This greater relative increase in storage could have enhanced performance in the initial sets for males. However, the short rest periods between sets may not have allowed sufficient time for phosphocreatine (PCr) resynthesis, which may explain why no significant differences were observed in the later sets.

Additionally, a significant treatment × exercise × sex interaction suggests that CM supplementation significantly improved leg press performance in males to a greater extent than females. This may be due to sex-based differences in muscle fiber composition, baseline creatine stores, or psychological drive toward multi-joint lower-body movements.

Notably, several interaction effects were not significant, including sex × treatment, sex × exercise, sex × set, treatment × set interactions, exercise × set × sex, treatment × exercise × sex, and treatment × exercise × set × sex. These findings suggest that although CM supplementation consistently improved overall performance, its relative benefit across sexes, exercises, and sets was generally uniform outside of the specific three-way interactions identified.

4.2 Limitations

Several limitations of this study should be acknowledged. The relatively small sample size (n = 13) limits the generalizability of the findings and increases the risk of Type II error, particularly for detecting smaller effects. Although the study was sufficiently powered to detect small-to-moderate main effects and moderate-to-large interaction effects, the sensitivity analysis revealed that smaller interaction effects may have gone undetected. In particular, some non-significant two- and three-way interactions may reflect insufficient statistical power rather than a true

absence of effect. Consequently, findings related to higher-order interactions should be interpreted with caution. Future research utilizing larger sample sizes may be necessary to more fully characterize nuanced moderating effects, such as potential sex-specific adaptations or differential responses across exercises and sets. sex-based differences observed in three-way interactions were exploratory in nature, and the study was not specifically powered to detect sex-specific responses.

The exercise sequence (leg press, knee flexion, knee extension) was fixed for all participants, introducing a potential order effect that could confound interpretations of exercise-specific performance.

Dietary intake, hydration status, and habitual physical activity outside of the study sessions were not tightly controlled, which could have influenced baseline creatine stores and exercise performance.

The placebo was not an exact match for the CM supplement. While the placebo powder and supplement powder were well matched for taste and appearance, they differed noticeably in texture. Participants were encouraged to ensue that the powders were well mixed before ingestion, but discussions with participants did find that some participants noticed this difference in textures. Despite this, no participant stated that they were familiar with CM texture and so this did not appear to affect their perception of which condition they were taking for each exercise session. Future studies should use encapsulated supplements to eliminate the need for matching taste and textures.

Lastly, the washout period was determined based on urinary creatinine excretion observed in previous literature (Hultman et al., 1996). However, a more recent case study using muscle

biopsies found that the time for PCr stores to return to baseline may take longer than urinary creatinine excretion indicated (Rawson et al., 2004). This indicates that longer washout periods may be required.

CHAPTER 5 – CONCLUSIONS

The results of this research indicate that CM supplementation significantly improves repetition performance during BFR-RE. This effect appears to differ between males and females in terms of both impacting performance on different exercises and performance across sets, with males appearing to benefit disproportionately more in earlier sets and exercises, whereas females benefited similarly throughout the training session.

5.1 Future Directions

Future research should seek to replicate these findings with larger, more diverse samples to better characterize potential sex differences and interindividual variability in CM supplementation responsiveness during BFR resistance training. Randomization of exercise order could help isolate the true effects of supplementation across different movements. Additionally, mechanistic studies evaluating muscle biopsy samples, intramuscular creatine content, or real-time muscle energetics via magnetic resonance spectroscopy could provide deeper insight into the physiological underpinnings of the observed performance benefits. Finally, longitudinal training studies combining CM supplementation with BFR resistance programs would help determine whether acute improvements in repetition performance translate into superior gains in muscle hypertrophy, strength, and endurance over time.

Beyond these directions, further work is warranted to clarify creatine kinetics in females, particularly across different phases of the menstrual cycle where fluctuations in estrogen may alter creatine kinase activity and intramuscular creatine handling. Closely related, research should examine whether CM supplementation differentially impacts exercise performance throughout the menstrual cycle. At present, only a single study (Gordon et al., 2023) has addressed this question and found no effect, but replication in larger, well-controlled samples is

necessary before firm conclusions can be drawn. Another important avenue involves characterizing the time course of creatine washout following cessation of supplementation. While urinary creatinine data suggest a return to baseline within approximately four weeks, case study evidence using muscle biopsies indicates that intramuscular creatine content may remain elevated beyond this timeframe. More well-controlled studies utilizing more direct measures and larger sample sizes are needed to resolve these discrepancies and refine our understanding of creatine retention and clearance.

5.3 Data Availability Statement

The datasets generated and analyzed during the current study are not publicly available at this time due to participant confidentiality. Aggregated participant data may be made available upon reasonable request to the corresponding author.

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Creatine monohydrate was supplied by Creapure®, who had no role in the study design, data collection, analysis, interpretation of results, or manuscript preparation. No additional funding was received for this project.

5.4 Conflicts of Interest

The author declares no financial or personal conflicts of interest relevant to the conduct of this study.

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APPENDICES

APPENDIX A – GET ACTIVE QUESTIONNAIRE

Get Active Questionnaire

CANADIAN SOCIETY FOR EXERCISE PHYSIOLOGY –
PHYSICAL ACTIVITY TRAINING FOR HEALTH (CSEP-PATH®)

Physical activity improves your physical and mental health. Even small amounts of physical activity are good, and more is better.

For almost everyone, the benefits of physical activity far outweigh any risks. For some individuals, specific advice from a Qualified Exercise Professional (QEP – has post-secondary education in exercise sciences and an advanced certification in the area – see csep.ca/certifications) or health care provider is advisable. **This questionnaire is intended for all ages – to help move you along the path to becoming more physically active.**

- ☐ I am completing this questionnaire for myself.
- ☐ I am completing this questionnaire for my child/dependent as parent/guardian.

PREPARE TO BECOME MORE ACTIVE

The following questions will help to ensure that you have a safe physical activity experience. Please answer **YES** or **NO** to each question before you become more physically active. If you are unsure about any question, answer **YES**.

YES	NO	
		1 Have you experienced **ANY** of the following (A to F) **within the past six months**?
○	○	**A** A diagnosis of/treatment for heart disease or stroke, or pain/discomfort/pressure in your chest during activities of daily living or during physical activity?
○	○	**B** A diagnosis of/treatment for high blood pressure (BP), or a resting BP of 160/90 mmHg or higher?
○	○	**C** Dizziness or lightheadedness during physical activity?
○	○	**D** Shortness of breath at rest?
○	○	**E** Loss of consciousness/fainting for any reason?
○	○	**F** Concussion?
○	○	**2** Do you currently have pain or swelling in any part of your body (such as from an injury, acute flare-up of arthritis, or back pain) that affects your ability to be physically active?
○	○	**3** Has a health care provider told you that you should avoid or modify certain types of physical activity?
○	○	**4** Do you have any other medical or physical condition (such as diabetes, cancer, osteoporosis, asthma, spinal cord injury) that may affect your ability to be physically active?

NO to all questions: go to Page 2 – ASSESS YOUR CURRENT PHYSICAL ACTIVITY

YES to any question: go to Reference Document – ADVICE ON WHAT TO DO IF YOU HAVE A YES RESPONSE

Get Active Questionnaire

ASSESS YOUR CURRENT PHYSICAL ACTIVITY

Answer the following questions to assess how active you are now.

1 During a typical week, on how many days do you do moderate- to vigorous-intensity aerobic physical activity (such as brisk walking, cycling or jogging)? ☐ DAYS/WEEK

2 On days that you do at least moderate-intensity aerobic physical activity (e.g., brisk walking), for how many minutes do you do this activity? ☐ MINUTES/DAY

For **adults**, please multiply your average number of days/week by the average number of minutes/day: ☐ MINUTES/WEEK

Canadian 24-Hour Movement Guidelines recommend that adults accumulate at least 150 minutes of moderate- to vigorous-intensity physical activity per week. For children and youth, at least 60 minutes daily is recommended. Strengthening muscles and bones at least two times per week for adults, and three times per week for children and youth, is also recommended (see csep.ca/guidelines).

GENERAL ADVICE FOR BECOMING MORE ACTIVE

Increase your physical activity gradually so that you have a positive experience. Build physical activities that you enjoy into your day (e.g., take a walk with a friend, ride your bike to school or work) and reduce your sedentary behaviour (e.g., prolonged sitting).

If you want to do **vigorous-intensity physical activity** (i.e., physical activity at an intensity that makes it hard to carry on a conversation), and you do not meet minimum physical activity recommendations noted above, consult a Qualified Exercise Professional (QEP) beforehand. This can help ensure that your physical activity is safe and suitable for your circumstances.

Physical activity is also an important part of a healthy pregnancy.

Delay becoming more active if you are not feeling well because of a temporary illness.

DECLARATION

To the best of my knowledge, all of the information I have supplied on this questionnaire is correct.
If my health changes, I will complete this questionnaire again.

I answered NO to all questions on Page 1

Sign and date the Declaration below

I answered YES to any question on Page 1

Check the box below that applies to you:

☐ I have consulted a health care provider or Qualified Exercise Professional (QEP) who has recommended that I become more physically active.

☐ I am comfortable with becoming more physically active on my own without consulting a health care provider or QEP.

Name (+ Name of Parent/Guardian if applicable) [Please print] | Signature (or Signature of Parent/Guardian if applicable) | Date of Birth

Date | Email (optional) | Telephone (optional)

With planning and support you can enjoy the benefits of becoming more physically active. A QEP can help.

☐ Check this box if you would like to consult a QEP about becoming more physically active.
(This completed questionnaire will help the QEP get to know you and understand your needs.)

APPENDIX B – NSCA 1RM TESTING PROTOCOL

1) Instruct the athlete to warm up with a light resistance that allows 5-10 repetitions.
2) Provide a one-minute rest period.
3) Estimate a warm-up load that will allow the athlete to complete three to five repetitions by adding:
    a. 10-20 pounds (4-9 kg) or 5%-10% for upper body exercise or
    b. 30-40 pounds (14-18 kg) or 10%-20% for lower body exercise.
4) Provide a two-minute rest period.
5) Estimate a conservative, near-maximal load that will allow the athlete to complete two to three repetitions by adding:
    a. 10-20 pounds (4-9 kg) or 5%-10% for upper body exercise or
    b. 30-40 pounds (14-18 kg) or 10%-20% for lower body exercise.
6) Provide a two- to four-minute rest period.
7) Make a load increase:
    a. 10-20 pounds (4-9 kg) or 5%-10% for upper body exercise or
    b. 30-40 pounds (14-18 kg) or 10%-20% for lower body exercise.
8) Instruct the athlete to attempt a 1RM.
9) If the athlete was successful, provide a two- to four-minute rest period and go back to step 7.

If the athlete failed, provide a two- to four-minute rest period, and then decrease the load by subtracting:

a) 5-10 pounds (2-4 kg) or 2.5% to 5% for upper body exercise or
b) 15-20 pounds (7-9 kg) or 5% to 10% for lower body exercise.

AND then go back to step 8.

Continue increasing or decreasing the load until the athlete can complete one repetition with proper exercise technique. Ideally, the athlete's 1RM will be measured within three to five testing sets (Baechle et al., 2008).

APPENDIX C – DIETARY INTAKE LOG

EXAMPLE FOOD RECORD

Study ID: BRC-000 **Day Number: ONE** **Day MONDAY** **Date: 10/21/15**

Record ALL food and drink consumed during the date including sweets, snacks, nibbles, sauces and dressings, water and supplements (vitamins, etc.).

Please remember to record:

- METHOD OF COOKING (eg. boiled pasta)
- TYPE OF FOOD (eg. wholemeal bread)
- AMOUNT OF FOOD (eg. 6 heaped tsp white sugar or weight (g))
- BRAND NAMES (eg Nestle diet lite) if possible

Time of Day	AMOUNT EATEN	DETAILS OF FOOD & DRINK
6:00 am	250ml	Apple Juice Fruitbox (Minutemaid)
	1	Multivitamin tablet
8:00 am	1 ½ cups	Bran Flakes
	½ cup	Lucerne Skim milk (fat free) (for Bran Flakes)
	2 tsp	White Sugar (for Bran Flakes)
	1 large glass (~400ml)	Tropicana orange juice with pulp
	1 toast slice	Safeway Multigrain toast
	1 tablespoon	IXL Apricot Jam
10:30 am	1 Large Cup (450ml)	Cappuccino from café
	31g	Quaker chocolate chip granola bar
	250ml	Water
	1 large	Apple – Granny Smith
1:00 pm	2 slices	Dempster's 12 grain bread
	1 tablespoon	Butter (enough to cover surface of 1 slice of bread)
	2 slices (20g)	Tomato
	1 leaf (5g)	Lettuce
	2 slices (40g)	Kraft Process Cheese – regular fat
	1 cup	Fries from cafeteria
	300ml	Water

EXAMPLE CONTINUED FOOD RECORD

Study ID: BRC-000 **Day Number: ONE** **Day MONDAY** **Date: 10/21/15**

FOOD CONSUMED	QUANTITY EATEN	DETAILS OF FOOD & DRINK
DURING AFTERNOON	1 small handful	Mixed Salted Nuts
EVENING MEAL	261g (2)	small lamb chops, fat cut off, grilled
	1 (med size)	Baked potato
	1tbs	Butter
	1cup	Mixed frozen Heinz vegetables (including corn, carrot, beans) – boiled
	200g (1 tub)	Strawberry yogurt 99% fat free (Danone)
	2 glasses (200ml)	Red wine
DURING EVENING / SUPPER	1 cup (250ml)	Whole fat plain milk
	5	Home made chocolate chip cookies ~5cm diameter
	200ml	Water

DAY ONE

FOOD RECORD

Study ID:_____ **Date:** _____ **Day of the week:** _____

Record ALL food and drink consumed during the date including sweets, snacks, nibbles, sauces and dressings, water and supplements (vitamins).

Please remember to record:

- METHOD OF COOKING (eg. boiled pasta)
- TYPE OF FOOD (eg. wholemeal bread)
- QUANTITY OF FOOD (eg. 6 heaped tsp white sugar or weight (g))
- BRAND NAMES (eg Nestle diet lite) if possible

FOOD CONSUMED	QUANTITY EATEN	DETAILS OF FOOD & DRINK
EARLY MORNING		
BREAKFAST		
DURING MORNING		
MIDDAY		

DAY ONE CONTINUED

FOOD RECORD

Study ID:_____ Date: _____ Day of the week: _____

FOOD CONSUMED	QUANTITY EATEN	DETAILS OF FOOD & DRINK
DURING AFTERNOON		
EVENING MEAL		
DURING EVENING / SUPPER		

DAY TWO

FOOD RECORD

Study ID:_____ **Date:** _____ **Day of the week:** _____

Record ALL food and drink consumed during the date including sweets, snacks, nibbles, sauces and dressings, water and supplements (vitamins).

Please remember to record:

- METHOD OF COOKING (eg. boiled pasta)
- TYPE OF FOOD (eg. wholemeal bread)
- QUANTITY OF FOOD (eg. 6 heaped tsp white sugar or weight (g))
- BRAND NAMES (eg Nestle diet lite) if possible

FOOD CONSUMED	QUANTITY EATEN	DETAILS OF FOOD & DRINK
EARLY MORNING		
BREAKFAST		
DURING MORNING		
MIDDAY		

DAY TWO CONTINUED

FOOD RECORD

Study ID:_____ Date: _____ Day of the week: _____

FOOD CONSUMED	QUANTITY EATEN	DETAILS OF FOOD & DRINK
DURING AFTERNOON		
EVENING MEAL		
DURING EVENING / SUPPER		

DAY THREE

FOOD RECORD

Study ID:_____Date: _____ Day of the week: _____

Record ALL food and drink consumed during the date including sweets, snacks, nibbles, sauces and dressings, water and supplements (vitamins).

Please remember to record:

- METHOD OF COOKING (eg. boiled pasta)
- TYPE OF FOOD (eg. wholemeal bread)
- QUANTITY OF FOOD (eg. 6 heaped tsp white sugar or weight (g))
- BRAND NAMES (eg Nestle diet lite) if possible

FOOD CONSUMED	QUANTITY EATEN	DETAILS OF FOOD & DRINK
EARLY MORNING		
BREAKFAST		
DURING MORNING		
MIDDAY		

DAY THREE CONTINUED

FOOD RECORD

Study ID:_____ Date: _____ Day of the week: _____

FOOD CONSUMED	QUANTITY EATEN	DETAILS OF FOOD & DRINK
DURING AFTERNOON		
EVENING MEAL		
DURING EVENING / SUPPER		

ADDITIONAL INFORMATION

If you have made any major changes to your eating habits during the past few months, please describe them here:

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

On completion of this record, please return it to the study staff at your next visit.

THANK YOU FOR YOUR CO-OPERATION IN COMPLETING SUCH A DETAILED FORM. WE ARE VERY GRATEFUL FOR YOUR TIME AND EFFORT.

If you have any comments or suggestions, please write them below.

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

APPENDIX D – INFORMED CONSENT

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Informed Consent

Research Project Title: Potential Ergogenic Effects of Creatine Monohydrate Supplementation on Blood Flow Restricted Resistance-Exercise Performance.

Principal Investigator and Contact Information: Mr. Mikal Thrones, MSc Student, Faculty of Kinesiology and Recreation Management, University of Manitoba, phone: [redacted], email: [redacted]

Advisor: Dr. Stephen Cornish, PhD., Associate Professor / Associate Dean (Undergraduate), Faculty of Kinesiology and Recreation Management, University of Manitoba, phone: [redacted] or [redacted], email: [redacted]

Research Assistants:
Mr. Thomas Rawliuk, MSc student, Dept. of Psychology, University of Manitoba; Email: [redacted]

This consent form, a copy of which will be left with you for your records and reference, is only part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. If you would like more detail about something mentioned here, or information not included here, you should feel free to ask. Please take the time to read this carefully and to understand any accompanying information.

Purpose of the Study: The purpose of this study is to evaluate if creatine monohydrate supplementation increases exercise performance during blood flow restricted resistance-exercise as well as creatine on its own or in combination with blood flow restricted resistance-exercise produce different myokine responses. Myokines are protein-based molecules that are released from skeletal muscle and are involved in several metabolic processes. Thus far, a complete explanation of how myokines may influence the response to blood flow restriction resistance-exercise has not been fully demonstrated. Additionally, the effects of creatine on exercise performance during blood flow restricted resistance-exercise have never been investigated.

Procedures: All participants will be asked to complete the same protocol for this study. After being enrolled in the study you will be measured on a number of parameters including body composition via bioelectrical impedance analysis (BIA), height, weight, resting blood pressure, and resting heart rate. Following these measurements, we will introduce you to the KAATSU blood flow restriction system, including cuff placement and pressure to be used during the exercise sessions. We will then measure your maximum strength (using a 1-repetition maximum test) in 3 different resistance exercise machines including: leg press, leg flexion, and leg

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extension. This will help us determine the intensity you will use for the resistance exercise session you will complete.

You will be asked to perform 2 acute sessions of exercise following 2 different supplementation interventions. Each supplementation intervention will be randomized regarding which order you will complete them in. The 2 supplementation interventions will consist of 5 grams of creatine monohydrate taken 4 times per day for 7 days as well as an equivalent dose of a placebo (maltodextrin). Following the first supplementation period, you will perform a blood flow restricted resistance-exercise session consisting of 4 sets to failure with 30% or your one-repetition maximum on 3 exercises (leg press, leg flexion, and leg extension) with 30 seconds of rest between sets and 2-minutes between exercises. During these exercises, we will place a cuff on both legs and inflate to 200mmHg. This cuff will remain inflated during and between sets, but will be deflated between exercises. Before and after each exercise session you will be asked to allow us to collect ~6 mL (1 teaspoon) of blood from a forearm vein. We will do this 4 times (immediately before exercise, immediately after exercise and 1-hour and 3-hours post-exercise) for each of the exercise sessions. This will allow us to measure the myokines of interest in your blood.

In between your exercise sessions there will be three-weeks of recovery before you complete a second 1 repetition-maximum test and blood flow restricted resistance exercise session. You will also be asked to avoid moderate-vigorous intensity exercise for 72 hours (3 days) before each of the exercise sessions and for 3 hours following each exercise session. You will also be asked to refrain from alcohol and cannabis for at least 6 hours and caffeine, nicotine, and food for at least 2 hours before each testing session.

You will also be asked to fill in a 3-day food log for the two days before the initial exercise session and the day of the exercise session. We will then ask you to repeat your dietary intake for the remaining exercise session. This is being collected so that you can repeat your food and beverage consumption the same way as before.

Individual parking passes will be available to participants who require them for each study visit.

This study will take approximately 10-hours of your time over 5 weeks.

Benefits of Participation: There are potential health benefits associated with participation in this research trial although these benefits are not guaranteed. You may feel more energetic and capable of accomplishing a variety of exercise tasks with this study.

Risks of Participation: There are physical risks associated with this study. Physical risks associated with this study include: the possibility of heart attack or death and an increased risk of muscle strain with the exercise testing or training. This will be minimized by using certified exercise professionals as research assistants in the fitness testing and exercise intervention. Proper warm-up and cool-down procedures will be utilized with all participants which will mitigate the risk of developing an increase in physical risks associated with the testing or exercise intervention.

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Also, all participants will be screened before any fitness testing or exercise training by the Get Active Questionnaire (GAQ) which will indicate if there are any potential contraindications to exercise testing or training in this population. Exercise leaders as part of this study are trained to deal with potential emergencies associated with exercise testing or exercise training.
There will be slight pain experienced as a consequence of the blood collection procedures. The pain will not be any greater than the pain experienced in collecting blood for normal health screening procedures. There is a chance of infection as a result of the blood collection procedure. This risk will be minimized by using completely sterile equipment for the blood draw and disinfecting the area of the arm where blood will be drawn by using isopropyl alcohol. There is a risk of bruising at the area of the blood draw. This will be minimized by using certified phlebotomists to perform the blood draw.

Confidentiality: Every effort will be made to keep personal information completely confidential. The participant ID key will be stored on the PI's password protected computer. All data collected will be held securely in a locked office and locked cabinet for storage. We will also be assigning all participants with an encoding scheme to replace their name. This will be used for all documents and data collection forms. Furthermore, all participant names and identifying traits will be removed from study reports and will not be made available to anyone outside of the research team.

Voluntary Withdrawal: Participation in this study is completely voluntary. Participants are free to withdraw from this study at any time point for any reason without explanation (including during exercise or blood drawing procedures). Withdrawing from the research trial will not result in the withdrawal of any health services or programs that are part of your regular health care regime. As a participant, you are free to withdraw from the research trial at any time during the trial by advising the primary investigator (Mikal Thrones) or one of the Research Assistants involved in this study in person or by email. You will not be able to withdraw your data from the study after March 2024 as this is when we anticipate the study will be completed by.

Research Dissemination: It is the intention to have the results of this study published in a scientific journal and/or presented at workshops or scientific conferences. The results will be published in the University of Manitoba institutional repository as part of the PI's thesis. In this case, all information will again be represented completely anonymized and appear as statistics.

Summary of Results: Participants are entitled to a complete summary and brief explanation (approximately 1-3 pages) of the research results. Interested participants may attach their physical mailing address or email address to this consent form to receive either a physical or electronic copy of this information upon completion of the project. An estimated completion time is currently set to September 2024. Participants can receive individual results as specified in the protocol.

Destruction of Confidential Information: All data will remain encoded and stored in a locked filing cabinet of a locked office building until such a time that all potential data analysis has been completed. Upon completion of this study, all physical copies of confidential data will be

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destroyed via an anonymous paper shredding company and all electronic copies of confidential data will be destroyed by formatting the storage hard drive. This process will occur no later than three years after the completion of the project. Estimated date: December 2026.

Your signature on this form indicates that you have understood to your satisfaction the information regarding participation in the research project and agree to participate as a subject. In no way does this waive your legal rights nor release the researchers, sponsors, or involved institutions from their legal and professional responsibilities. You are free to withdraw from the study at any time, and /or refrain from answering any questions you prefer to omit, without prejudice or consequence. Your continued participation should be as informed as your initial consent, so you should feel free to ask for clarification or new information throughout your participation.

The University of Manitoba may look at your research records to see that the research is being done in a safe and proper way.

This research has been approved by the Research Ethics Board at the University of Manitoba, Fort Garry campus. If you have any concerns or complaints about this project, you may contact any of the above-named persons or the Human Ethics Coordinator at 204-474-7122 or humanethics@umanitoba.ca. A copy of this consent form has been given to you to keep for your records and reference.

Participant's Signature: _____ Date: _____

Researcher and/or Delegate's Signature: _____ Date: _____

Mailing Address for Research Dissemination

Physical Address: _____

_____

Email Address: _____

APPENDIX E – ETHICS APPROVAL

University of Manitoba | Research Ethics and Compliance

Human Ethics - Fort Garry
208-194 Dafoe Road
Winnipeg, MB R3T 2N2
T: 204 474 8872
humanethics@umanitoba.ca

PROTOCOL APPROVAL

Effective: September 21, 2023 Expiry: September 19, 2024

Principal Investigator:	Mikal Thrones
Advisor(s):	Stephen Cornish
Protocol Number:	HE2023-0236
Protocol Title:	*Potential Ergogenic Effects of Creatine Monohydrate Supplementation on Blood-Flow Restricted Resistance Exercise*

Merissa Daborn, Acting Chair, REB1

Research Ethics Board 1 has reviewed and approved the above research. The Human Ethics Office (HEO) is constituted and operates in accordance with the current *Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans*- TCPS 2 (2022).

This approval is subject to the following conditions:

i. Approval is granted for the research and purposes described in the protocol only.

ii. Any changes to the protocol or research materials must be approved by the HEO before implementation.

iii. Any deviations to the research or adverse events must be reported to the HEO immediately through an REB Event.

iv. This approval is valid for one year only. A Renewal Request must be submitted and approved prior to the above expiry date.

v. A Protocol Closure must be submitted to the HEO when the research is complete or if the research is terminated.

vi. The University of Manitoba may request to audit your research documentation to confirm compliance with this approved protocol, and with the UM *Ethics of Research Involving Humans*Ethics of Research Involving Humans policies and procedures.

APPENDIX F – EXERCISE PERFORMANCE DATA

Leg Press Data

		Creatine				Placebo			
ID	**Sex**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 1**	**Set 2**	**Set 3**	**Set 4**
002	F	36	10	14	12	34	20	14	9
004	F	41	14	17	12	38	15	10	11
005	F	44	21	19	20	46	21	16	14
007	F	106	31	26	25	102	35	27	24
009	F	80	42	35	34	80	32	39	31
010	F	62	36	28	26	68	26	28	24
011	M	47	24	19	13	41	18	11	9
012	M	64	33	22	10	51	24	24	21
014	M	84	14	20	19	57	18	11	11
016	M	42	26	24	10	42	12	9	11
017	M	72	22	17	11	57	23	15	12
018	M	102	39	23	19	81	22	24	18
019	M	110	26	20	27	73	22	25	16

Knee Flexion Data

		Creatine				Placebo			
ID	**Sex**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 1**	**Set 2**	**Set 3**	**Set 4**
002	F	38	16	16	20	28	12	11	12
004	F	32	15	9	9	29	17	5	10
005	F	26	10	9	8	30	13	5	2
007	F	50	35	25	21	65	40	31	21
009	F	64	36	25	26	50	21	20	22
010	F	83	43	39	31	107	56	30	29
011	M	30	18	11	10	29	16	14	12
012	M	50	32	20	14	40	25	24	21
014	M	48	20	17	8	37	17	15	8
016	M	24	4	7	5	22	3	11	9
017	M	56	25	1	2	60	10	11	1
018	M	61	20	20	20	61	22	15	14
019	M	34	17	16	15	41	23	21	17

Knee Extension Data

		Creatine				Placebo			
ID	**Sex**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 1**	**Set 2**	**Set 3**	**Set 4**
002	F	23	8	6	6	12	4	3	2
004	F	16	7	7	9	17	9	5	6
005	F	14	8	7	6	8	6	5	5
007	F	15	10	6	4	19	9	7	6
009	F	34	18	18	18	27	23	15	18
010	F	22	12	9	7	17	12	10	10
011	M	17	11	9	7	16	6	6	5
012	M	16	9	5	6	15	8	4	2
014	M	20	9	5	8	21	10	6	6
016	M	27	7	5	5	14	6	7	5
017	M	21	5	3	1	19	5	5	2
018	M	18	6	5	4	18	7	1	1
019	M	13	12	10	11	13	9	6	6

APPENDIX G – CONSORT CHECKLIST

Section/topic	No	CONSORT 2025 checklist item description	Reported on page no.
Title and abstract			
Title and structured abstract	1a	Identification as a randomised trial	i
	1b	Structured summary of the trial design, methods, results, and conclusions	ii - iii
Open science			
Trial registration	2	Name of trial registry, identifying number (with URL) and date of registration	N/A
Protocol and statistical analysis plan	3	Where the trial protocol and statistical analysis plan can be accessed	N/A
Data sharing	4	Where and how the individual de-identified participant data (including data dictionary), statistical code and any other materials can be accessed	45
Funding and conflicts of interest	5a	Sources of funding and other support (eg, supply of drugs), and role of funders in the design, conduct, analysis and reporting of the trial	45
	5b	Financial and other conflicts of interest of the manuscript authors	45
Introduction			
Background and rationale	6	Scientific background and rationale	1 – 3
Objectives	7	Specific objectives related to benefits and harms	3
Methods			
Patient and public involvement	8	Details of patient or public involvement in the design, conduct and reporting of the trial	N/A
Trial design	9	Description of trial design including type of trial (eg, parallel group, crossover), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	22
Changes to trial protocol	10	Important changes to the trial after it commenced including any outcomes or analyses that were not prespecified, with reason	N/A
Trial setting	11	Settings (eg, community, hospital) and locations (eg, countries, sites) where the trial was conducted	22
Eligibility criteria	12a	Eligibility criteria for participants	23
	12b	If applicable, eligibility criteria for sites and for individuals delivering the interventions (eg, surgeons, physiotherapists)	N/A
Intervention and comparator	13	Intervention and comparator with sufficient details to allow replication. If relevant, where additional materials describing the intervention and comparator (eg, intervention manual) can be accessed	26 - 28
Outcomes	14	Prespecified primary and secondary outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome	29
Harms	15	How harms were defined and assessed (eg, systematically, non-systematically)	29
Sample size	16a	How sample size was determined, including all assumptions supporting the sample size calculation	22
	16b	Explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	17a	Who generated the random allocation sequence and the method used	24 – 25
	17b	Type of randomisation and details of any restriction (eg, stratification, blocking and block size)	24 - 25

APPENDIX H – TIDieR CHECKLIST

TIDieR
Template for Intervention Description and Replication

The TIDieR (Template for Intervention Description and Replication) Checklist*:

Information to include when describing an intervention and the location of the information

Item number	Item	Where located **	
		Primary paper (page or appendix number)	Other † (details)
	BRIEF NAME		
1.	Provide the name or a phrase that describes the intervention.	i	
	WHY		
2.	Describe any rationale, theory, or goal of the elements essential to the intervention.	1 - 2	
	WHAT		
3.	Materials: Describe any physical or informational materials used in the intervention, including those provided to participants or used in intervention delivery or in training of intervention providers. Provide information on where the materials can be accessed (e.g. online appendix, URL).	27 - 29	
4.	Procedures: Describe each of the procedures, activities, and/or processes used in the intervention, including any enabling or support activities.	25 - 29	
	WHO PROVIDED		
5.	For each category of intervention provider (e.g. psychologist, nursing assistant), describe their expertise, background and any specific training given.	25, 28	
	HOW		
6.	Describe the modes of delivery (e.g. face-to-face or by some other mechanism, such as internet or telephone) of the intervention and whether it was provided individually or in a group.	25 - 28	
	WHERE		
7.	Describe the type(s) of location(s) where the intervention occurred, including any necessary infrastructure or relevant features.	22	

	WHEN and HOW MUCH		
8.	Describe the number of times the intervention was delivered and over what period of time including the number of sessions, their schedule, and their duration, intensity or dose.	28	
	TAILORING		
9.	If the intervention was planned to be personalised, titrated or adapted, then describe what, why, when, and how.	N/A	
	MODIFICATIONS		
10.♦	If the intervention was modified during the course of the study, describe the changes (what, why, when, and how).	N/A	
	HOW WELL		
11.	Planned: If intervention adherence or fidelity was assessed, describe how and by whom, and if any strategies were used to maintain or improve fidelity, describe them.	27	
12.♦	Actual: If intervention adherence or fidelity was assessed, describe the extent to which the intervention was delivered as planned.	33	

** Authors - use N/A if an item is not applicable for the intervention being described. Reviewers – use '?' if information about the element is not reported/not sufficiently reported.

† If the information is not provided in the primary paper, give details of where this information is available. This may include locations such as a published protocol or other published papers (provide citation details) or a website (provide the URL).

♦ If completing the TIDieR checklist for a protocol, these items are not relevant to the protocol and cannot be described until the study is complete.

* We strongly recommend using this checklist in conjunction with the TIDieR guide (see *BMJ* 2014;348:g1687) which contains an explanation and elaboration for each item.

* The focus of TIDieR is on reporting details of the intervention elements (and where relevant, comparison elements) of a study. Other elements and methodological features of studies are covered by other reporting statements and checklists and have not been duplicated as part of the TIDieR checklist. When a **randomised trial** is being reported, the TIDieR checklist should be used in conjunction with the CONSORT statement (see www.consort-statement.org) as an extension of **item 5 of the CONSORT 2010 Statement**. When a **clinical trial protocol** is being reported, the TIDieR checklist should be used in conjunction with the SPIRIT statement as an extension of **item 11 of the SPIRIT 2013 Statement** (see www.spirit-statement.org). For alternate study designs, TIDieR can be used in conjunction with the appropriate checklist for that study design (see www.equator-network.org).

APPENDIX I – SAGER CHECKLIST

Table 1. SAGER guidelines checklist
Studies with human participants

Section / topic	Item number	Checklist item	Reported on page number
General			
	1	The terms sex/gender used appropriately	Yes
Title			
	2	Title specifies the sex/gender of participants if only one included	N/A
Abstract			
	3a	Abstract specifies the sex/gender of participants if only one included	N/A
	3b	Study population described with sex/gender breakdown*	ii
Introduction			
	4a	If relevant, previous studies that show presence or lack of sex/gender differences or similarities are cited	2
	4b	Mention of whether sex/gender might be an important variant and if differences might be expected	2
	4c	The demographics of the study population with regard to sex/gender (eg, disease prevalence among male/female study participants) are outlined*	N/A
Methods			
	5a	Method of definition of sex/gender (eg, self-report, genetic testing)	22
	5b	Description of how sex/gender was considered in the design, whether authors ensured adequate representation of male and female study participants, justification of the reasons for any exclusion of male or female participants, or explanation if not considered. Justification of other sex/gender-specific interventions of study designs (eg, mandating contraception for women).* Explicit reporting of the scientific rationale for	23

		contraception requirements and exclusions for pregnancy and lactation should be required*	
Results			
	6a	Study population description with complete gender/sex breakdown for all categories considered*	31 - 32
	6b	Where appropriate, data presented disaggregated by sex/gender, and sex/gender differences and similarities are described	32
	6c	Sex- and gender-based analyses reported regardless of outcome (in main paper if pre-specified; otherwise in appendix)*	37 - 39
	6d	For clinical trials, adverse event data disaggregated by sex/gender (in main paper if pre-specified; otherwise in appendix)*	N/A
	6e	Patient-reported outcome data disaggregated by sex/gender (in main paper if pre-specified; otherwise in appendix)*	N/A
	6f	For epidemiological studies, the effects of other exposures on health problems examined for all genders and analysed critically from a gender perspective	N/A
	6g	Table 1 includes separate rows for male sex/gender, female sex/gender and other categories if collected*	32
Discussion			
	7a	Potential implications of sex/gender on the study results and analyses, including the extent to which the findings can be generalized to all sexes/genders in a population	43 - 44
	7b	If a sex/gender analysis not done, a rationale is given and implications of the lack of such analysis on the interpretation of the results are discussed	N/A

Adapted from SAGER guidelines. Sex and Gender Equity in Research: rationale for the SAGER guidelines and recommended use. Research Integrity and Peer Review 1, Article number: 2 (2016) https://researchintegrityjournal.biomedcentral.com/articles/10.1186/s41073-016-0007-6.
* These points extend beyond the original SAGER table