Investigating the efficacy of cocoa flavanols as an ergogenic aid for muscle recovery in males and females following exercise-induced muscle damage

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Abstract

Cocoa flavanols (CF) are bioactive compounds that exert antioxidant and anti-inflammatory properties and can aid overall health as a result. This PhD looked to investigate the efficacy of CF as an ergogenic aid for muscle recovery following exercise-induced muscle damage (EIMD). As strenuous exercise can elicit oxidative stress and cause the muscle to enter an inflammatory state, CF may aid recovery by blunting the overproduction of reactive oxygen species and limit the pro-inflammatory response. A systematic review of the literature was carried out, resulting in 14 studies, identifying that acute and sub-chronic consumption of CF blunts exercise-induced oxidative stress and, likely through a similar mechanism, may delay fatigue during exercise. It was identified that the most pertinent area of research related to CF and EIMD as only three studies currently existed, with evidence of the benefits unclear due to methodological issues, such as ineffective muscle damaging protocols. Not only that, but within sporting settings, optimal recovery is crucial for maintaining high levels of performance whether in training or during competition. A failure of full recovery can reduce athletic performance, e.g., reductions in force output or sprint ability, and can increase injury risk.

The first experimental study investigated the impact of an acute dose of CF (either 830mg or 1245mg) on muscle recovery following EIMD. Overall, 23 participants completed the study. Participants performed maximal voluntary isometric contractions (MVIC) of the knee flexors to assess muscle function and a visual analogue scale (VAS) and lower extremity functional scale (LEFS) to assess perceived soreness. To induce muscle damage five sets of 10 maximal concentric/eccentric hamstring curls were performed on each leg using an isokinetic dynamometer, with muscle function and soreness being measured immediately post, 24, 48 and 72 hr following EIMD. It was observed that the highest dose of CF (1245mg) may have a minimal effect on the recovery of MVIC and muscle soreness; although not statistically significant. The second experimental study was a sub-group inter and intra-sex analysis of the data gathered from the first study. Overall, no significant differences were observed between males and females for measures of muscle recovery. The third experimental study investigated the impact of daily consumption of 1245mg of CF on muscle recovery following repeated bouts of strenuous exercise, separated by 72 hours. In addition to MVIC, VAS, and LEFS, electromyography was included within the measures to assess muscle recovery. This study had 9 participants complete the seven-day protocol (one baseline and six consecutive days) ingesting 8 beverages throughout the period. No overall significant differences were observed between the groups, however at the final time point (48 hr post the second EIMD protocol) large effect sizes were observed and a statistically significant difference at that time point for MVIC data, VAS, and LEFS. The data contained within this thesis provides novel information on the potential of CF as an ergogenic aid for muscle recovery. It appears that CF does not offer a significant benefit for muscle recovery when compared to a recovery drink containing only carbohydrate and protein. However, the large effect sizes observed in all three studies imply there may be a small effect of CF on recovery, as such the data from this PhD needs to be corroborated by future research to further justify the use of CF as an ergogenic aid for recovery.

Publications

Corr, L. D., Field, A., Pufal, D., Clifford, T., Harper, L. D., & Naughton, R. J. (2021). The effects of cocoa flavanols on indices of muscle recovery and exercise performance: a narrative review. *BMC Sports Science, Medicine and Rehabilitation*, *13*(1), 1-16.

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Publications arising from collaborative work alongside this PhD

Khatri, M., Naughton, R. J., Clifford, T., Harper, L. D., & **Corr, L.** (2021). The effects of collagen peptide supplementation on body composition, collagen synthesis, and recovery from joint injury and exercise: a systematic review. *Amino Acids*, 1-14.

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Abbreviations

CF = Cocoa flavanols

EIMD = Exercise-induced muscle damage

SGLT-1 – Sodium-glucose transport protein 1

ATP = adenosine triphosphate

Ca^{2+} = Calcium ions

CK = Creatine kinase

DOMS = Delayed onset muscle soreness

MVIC = Maximal voluntary isometric contraction

MVC = Maximal voluntary contraction

CMJ = Countermovement jump

IL-6 = Interleukin-6

IL-1β = Interleukin-1 beta

IL-10 = Interleukin-10

TNF-α = Tumour necrosis factor-alpha

ROS = Reactive oxygen species

OCP = Oral contraceptive pill

NF-κβ = Nuclear foctor-kappa beta

Nrf2 = Nuclear factor erythroid 2-related factor 2

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Chapter 1 General Introduction

1.1 Introduction

Chocolate and cocoa products have long been a part of the global diet, from humble beginnings to worldwide consumption. To understand the roots of cocoa and how it became ingrained in society we must look back to the Spanish Empire in the 16th century as it approached its peak. This was a time of great exploration and rapid colonialization. The Spanish Empire was beginning to establish a foothold in Mesoamerica thanks to the efforts of various Conquistadors, explorers who conquered new territories in the name of their king. One of the most well-known Conquistadors is Hernan Cortes, attributed as not only the conqueror of the Aztec Empire but also commonly thought of as the person responsible for the integration of cocoa into Europe following his return from the New World, Mesoamerica (Lippi, 2013).

In 1518 Cortes and his men embarked on what would become an era defining conquest. By 1519 the Spaniards landed at what is now modern-day Mexico (specifically Chalchihuecan, Veracruz), then in a masterful stroke of strategy Cortes destroyed[1] almost the entirety of his fleet; supposedly in an attempt to root out the cowardly and treacherous members of his party (Reynolds, 1959). Emboldened in the wake of a speech that Cortes gave in which he spoke of the untold riches that awaited them, he and his men set out on a journey to the Aztec capital, Tenochtitlan. Their time in Mexico was well documented by both Cortes himself and an officer, Bernal Diaz del Castillo, both of whom describe cocoa in detail.

Upon arrival at Tenochtitlan, the Spaniards were welcomed by Aztec King Montezuma and housed within his court. According to certain texts Montezuma allegedly believed Cortes to be Quetzalcoatl, the Aztec god of creation, returned, although this is contested, especially considering the overly polite nature of Nahuatl (Aztec language) and its proclivity for misinterpretation (Townsend, 2003). During this period, the Spanish were exposed to a warm, bitter beverage made up from cocoa beans that the Aztecs referred to as ‘chikolatl’ in Nahuatl. Even though the Spanish did not take to the drink, deeming it not sweet enough, King Montezuma was observed to consume it 50 times a day (Dillinger et al., 2000). The Aztec people highly revered the cacao

1 Texts relating to this are conflicting. Stating that Cortes: *i*) had ships scuttled secretly *ii*) had a ship master publicly divulge that the ships were no longer safe *iii*) had them burned, although this is more of a poetic retelling than fact

tree, believing it was gifted to the Earth by Quetzalcoatl planting it in southern Mexico (Young 1994). Similarly, the Maya believed Kukulkan (a Mayan deity) brought the cacao tree to Earth from ‘paradise’. This is likely why Swedish botanist Carl Linnaeus named the plant *Theobroma cacao*, derived from the Ancient Greek words ‘theos’ meaning god and ‘broma’ meaning food, ergo ‘food of the gods’, presumably in reference to its heritage.

The Aztecs and Maya, another civilisation situated in the Yucatán Peninsula, utilised cocoa to a great extent, mostly as a way of treating various medical conditions, such as fever, diarrhoea, coughs and infections (Dillinger et al., 2000). There are numerous texts dating back to the 16th century that are of Spanish, Mayan and Aztec origin that denote the use of cacao (chocolate) as medicine in the form of a hot beverage with varying amounts of cacao beans (potentially mixed with maize or spices) recommended depending on the ailment. Cocoa and its derivatives were almost viewed as a panacea, finding use in the treatment of a surfeit of conditions, not only for the conditions previously mentioned but for angina, dental problems, dysentery, fatigue, indigestion and more (Lippi, 2013). It is possible that the Aztec and Mayan people believed cocoa had aphrodisiac properties, with it seeing use at wedding ceremonies and regularly consumed by King Montezuma before attending to one of his many wives (D. Lippi, 2015). It is believed that Montezuma may have fathered ~100 children (Sweet & Nash, 1981); whether cocoa was the key to his virility it is almost impossible to know. Modern research into the aphrodisiac properties of cocoa has deciphered that specific compounds found within cocoa, such as phenylethylamine, theobromine, and N-acylethanolamine, may improve sexual desire and pleasure as well as mood, *via* the stimulation of the hypothalamus (Afoakwa, 2008). However, even in the 16th century cocoa was not without drawbacks. Excessive intake of green cocoa (unroasted beans) made people who quaffed such amounts confused, whereas a moderate intake was considered ideal and stimulating (Lippi, 2013).

Returning from his conquests in Mexico to show King Charles his treasures, Cortes brought samples of the beverage ‘xocoatl’ and spread information about its potent effects (D. Lippi, 2015). Originally, the Aztec version of the beverage was considered unpalatable for the Spanish so the recipe was modified to include sugar, spices, and honey, whilst excluding chilli from the final version (Presilla, 2009). The drink became

widely popular amongst the Spanish population, with the Empire monopolising the production and cultivation of cocoa in its New World settlements for almost a century (Badrie, Bekele, Sikora, & Sikora, 2015). In the 17th century the notoriety of this drink quickly spread throughout other European nations, such as France and England. Chocolate consumption continued to increase in Europe and in North America with the development of eating chocolate. The use of cocoa was beginning to shift, the Aztec and Mayan people used it for symbolic ceremonies, healing, holding significant cultural and economic importance; now, it was considered only for the affluent as an indulgence. Nevertheless, by the 19th Century the enjoyment and ingestion of chocolate was established in the general population, figuratively taking over the world (Badrie 2014). Interestingly, by the early 20th century chocolate was still considered medicinal as a drink due to its therapeutic benefits (Lippi, 2009). As the century progressed chocolate even saw use as food rations for soldiers during World War II (Waterhouse, Shirley, & Donovan, 1996), mostly due to the nutrient density when consumed as a solid.

The reason behind the belief that cocoa was this ‘miracle drink’ or elixir by the Mesoamerican civilisations was perhaps due to their religious convictions, as their respective chief god gifted them the cocoa tree. Yet, the reason for its potency most likely owes to the high concentration of (poly)phenols found within natural cocoa as opposed to divine intervention. The word (poly)phenol is the umbrella term given to a vast array of dietary antioxidants, with reported intakes potentially being as high a 1g/day (Scalbert, Johnson, & Saltmarsh, 2005). A diverse and substantial collection of plant metabolites, (poly)phenols were sporadically researched throughout the early 20th century gathering a greater body of research as the decades progressed. Now, the amount of research performed investigating the effects of the various groups of (poly)phenols has grown exponentially. From this research, it has come to be understood that (poly)phenols have a multitude of potential health benefits, such as being cardioprotective, anti-cancer, anti-inflammatory, neuroprotective, as well as improving glycaemic control and more (Del Rio, Costa, Lean, & Crozier, 2010). This is a far step from the role of (poly)phenols in plants; the original role of these metabolites is to protect against ultraviolet radiation and pathogenic compounds (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004).

Different (poly)phenols are distinguished by their chemical structure, hydroxyl groups bonded to an aromatic ring creating a phenol ring (or multiple). The categories of (poly)phenols are phenolic acids, lignans, stilbenes and flavonoids, with the latter being the largest category. Flavonoids can be further separated into subgroups, these are as follows: flavonols, flavanols, flavones, isoflavones, flavanones and anthocyanidins. Within cocoa, flavanols form the bulk of the (poly)phenolic profile and is in fact the richest source of flavanols out of all other dietary sources, e.g., tea, apricots, and beans (Hackman et al., 2008). Flavanols are commonly found in monomeric forms, such as catechin, epicatechin, epigallocatehin and more, but can also be found in polymeric forms known as anthocyaninidins (Andres-Lacueva et al., 2008).

Even though cocoa is a rich source of (poly)phenols, specifically flavanols, there is considerable variability when it comes to the flavanol content in chocolate, mostly due to the manufacturing process from bean to bar. The manufacturing process has gone through centuries of development, from humble beginnings with the Aztecs and Maya, to the initial removal of excess fat *via* a hydraulic press, to the original chocolate bar, to milk chocolate and modern-day chocolatiers. An aspect that is important to consider is that although the process may have arguably improved palatability, it may have had a detrimental effect on what made cocoa so beneficial in the first place.

1.2 Changes to the manufacturing process – is that the problem?

In more recent times, chocolate is considered a functional food, rather than the catholicon of previous civilisations, due to the high concentration of (poly)phenols, specifically cocoa flavanols (CF). As previously mentioned, (poly)phenol is an umbrella term given to various plant metabolites; flavanols being a sub-class of the flavonoid (poly)phenol group. It should be noted that not all chocolate is created equal, the flavanol content drastically varies between cocoa products. Factors that influence flavanol content include the strain of cocoa bean, the origin of said bean, fermentation, and the manufacturing process, e.g., roasting and alkalisation.

During the fermentation, the monomers are catalysed by polyphenoloxidase to cause polymerisation of cocoa, giving it the distinct brown colorant it is known for (Hollman & Arts, 2000). Fermentation of the beans occurs early on in the whole process, commonly following harvesting resulting in the beans being wrapped within banana leaves and left for multiple days. Just this initial step in the process has an impact on

the (poly)phenol make up, potentially reducing up to 90% of the flavanol content (Elwers, Zambrano, Rohsius, & Lieberei, 2009).

The roasting of the bean also has an impact on the flavanol content but is an important step in the creation of what chocolate is today. Numerous reactions occur during this treatment such as protein degradation, changes in pH, colour and shape (García-Alamilla, Lagunes-Gálvez, Barajas-Fernández, & García-Alamilla, 2017). Additionally, since flavanols are unstable in high heats, roasting can reduce the total content as a result of thermal and oxidative degradation. Various studies have found that roasting at temperatures of between 150-250^{o}C for ≥45 minutes causes the greatest decrease of total (poly)phenol content (Ioannone et al., 2015; Payne, Hurst, Miller, Rank, & Stuart, 2010). Another aspect of roasting is the epimerisation of the different flavanols from dimers to simple monomers leading to an increase in the latter (amount dependent on the original content of the bean). Kothe, Zimmermann, and Galensa (2013) found that beans from the Ivory Coast had an 836% increase in catechin (a CF monomer) content, whereas Java beans rose by ~174%. Furthermore, they concluded that temperatures below 140^{o}C should preserve most of the flavanol content. Unfortunately, most chocolatiers do not take the CF content into consideration, as the reduction of flavanols is what lessens the bitterness of natural cocoa, creating the more well-known chocolate flavour.

The other main aspect of manufacturing is alkalisation of the cocoa, this is known as 'Dutching'. The term stems from a 19th Century Dutch chocolatier called Coenraad Van Houten who was one of the people responsible for the transition of cocoa from a beverage to an edible. Alkalisation leads to reductions in bitter and sour flavours, increases the solubility of powder and alters the colour (Kamphuis, 2017). Natural cocoa is slightly acidic with a pH of ~5.3 and an average flavanol content of 34.6 mg/g, whereas treated cocoa can range from 6.5 to 7.6 pH and have a flavanol content of 3.6 mg/g or lower (Miller et al., 2008). Therefore, the less processing cocoa is exposed to the greater the flavanol content. The higher the concentration the greater potential for beneficial effects post-consumption, although many factors can influence the bioavailability of flavanols.

1.3 Bioavailability

The bioavailability of CF is reportedly high following digestion and metabolism across the entire gastrointestinal tract. After ingestion, flavanols remain intact during transit

into the small intestine from the stomach following little degradation from the gastric acid (Kwik-Uribe & Bektash, 2008). Consequently, the monomers (catechin and epicatechin) and oligomers (proanthocyanidins) of flavanols reach the upper intestinal tract intact.

A percentage of the flavanols ingested are then absorbed into the enterocytes and undergo phase I (reduction, oxidation, or hydrolysis) and phase II (conjugation, glucuronidation, methylation, sulfation or a combination) biotransformation. Epicatechin is converted into a wide range of metabolites upon absorption into the enterocytes of the small intestine, such as (-)-epicatechin 3' –sulfate, 3' –O-methyl-(-)-epicatechin 5-sulfate and (-)-epicatechin-3'-β-D-glucuronide (Actis-Goretta et al., 2012). Much of the remaining flavanols that are not absorbed continue through the gastrointestinal tract to the colon and undergo phase II biotransformation by the gut microflora before absorption, with catechin monomers converted into simple phenolic acids (Rios et al., 2003). Post-absorption, the metabolites are transported via the hepatic portal vein to the liver for further metabolism before being transported into the systemic circulatory system to be distributed around the body. Resultantly, within approximately 30 minutes of ingestion, epicatechin is absorbed and has entered the blood plasma (Rusconi & Conti, 2010) reaching peak concentrations two hours (hr) post consumption (Decroix et al., 2017; Kwik-Uribe & Bektash, 2008). Flavanol concentrations in the blood return to baseline after ~8 hr, indicating rapid excretion via biliary and renal systems (Hackman et al., 2008). Other research has shown that the microbial metabolites remain in circulation at relevant amounts for up to 24 hrs post consumption (Gómez-Juaristi, Sarria, Martínez-López, Bravo Clemente, & Mateos, 2019). See Figure 1 for an outline of the digestion, absorption, and excretion pathway of CF.

Food matrix has been reported to have an effect on total and maximum concentrations of flavanols following ingestion of cocoa as a drink rather than as a solid (Neilson et al., 2009). The bioavailability and absorption of flavanols can be further modified via the simultaneous consumption of carbohydrates, consuming ~4 kcal·kg alongside CF increased flavanol concentrations in the plasma by 40% (Badrie et al., 2015; D. D. Schramm et al., 2003). Carbohydrates stimulate and activate sodium-glucose transport protein 1 (SGLT-1) and lactase phlorizin hydrolyase both of which are involved in flavanol absorption and metabolism (Bohn, 2014; D. D. Schramm et al.,

2003). The effects of mixing CF with another macronutrient, protein, has also been investigated. It has been reported that the presence of whey, a predominant milk protein, negatively affects the bioavailability of cocoa flavanols in chocolate confectionery, e.g., chocolate bars (Cifuentes-Gomez, Rodriguez-Mateos, Gonzalez-Salvador, Alanon, & Spencer, 2015; Serafini et al., 2003). However, Roura et al., (2007) stated that there is no significant detrimental effect on absorption when cocoa powder is mixed with milk proteins as a drink. Keogh, McInerney, and Clifton (2007) corroborate this and found that a mix of milk proteins slightly increased the rate of absorption, measured using plasma concentrations of catechins, but to no physiological significance. Therefore, it is possible CF combined with protein and carbohydrates in the form of a recovery drink may constitute an ideal beverage for athletes to consume after intense exercise to enhance recovery, should CF have such a benefit. This is the purpose of this thesis, investigating the possible benefit of CF on recovery from exercise.

Another factor that might influence the bioavailability and absorption of cocoa flavanols is human age. Age leads to noticeable differences in the bioavailability of certain micronutrients such as Vitamin A, Vitamin B12 and other fat-soluble Vitamins likely due to impaired uptake via chylomicrons upon digestion. However, this is not the case with cocoa flavanols as the absorption, distribution, metabolism and excretion of these compounds has been reported as not being significantly different, with intakes of up to 400 mg a day, between young and elderly Caucasian males (Cifuentes-Gomez et al., 2015).

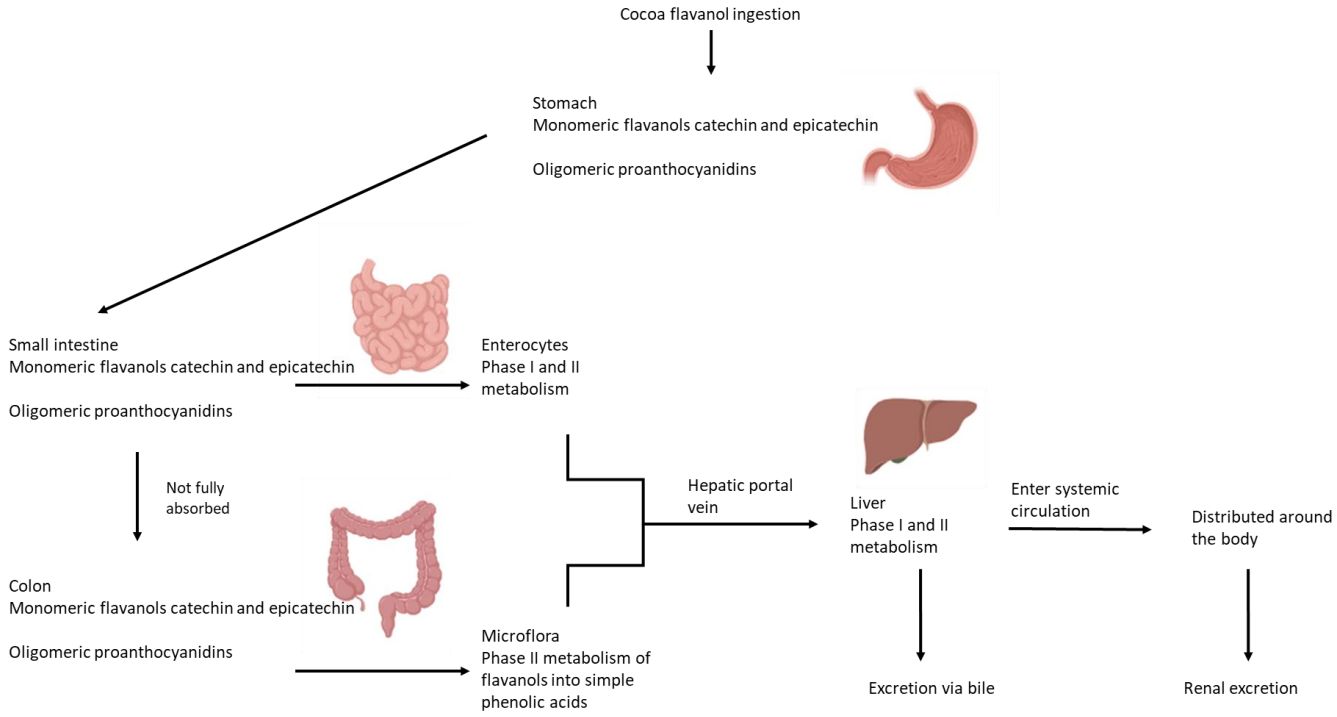

Figure 1.1 Cocoa flavanol metabolism in brief

Chapter 2 General Literature Review

2.1 Health Benefits

The understanding of the physiological effects of cocoa has deepened greatly over the previous few decades. So much so, that in 2013 the European Food Safety Authority approved a health claim for Barry Callebaut (a chocolate manufacturer) that dark chocolate and cocoa-based products that contain at least 200 mg CF improve and maintain the elasticity of blood vessels, aiding circulation (EFSA Panel on Dietetic Products, 2014).

The effects that CF exert on vascular health are well documented, one of the main reasons why the health claim was successful. Consumption of CF has beneficial effects on flow mediated dilation (the dilation of an artery in response to increased blood flow) following regular consumption of CF containing ~98 mg or more of epicatechin (Davison, Coates, Buckley, & Howe; Heiss et al., 2007; Monahan et al., 2011). Additionally, consumption of a high CF beverage increased bioactive nitric oxide (NO) production, increasing flow mediated dilation as a result (Fisher, Hughes, Gerhard-Herman, & Hollenberg). The importance of NO is due to its multiple roles in vascular health. It is antithrombotic, antiproliferative, anti-atherogenic and is a vasodilator (Huynh & Chin-Dusting, 2006). However, the exact role of CF on NO has not been fully elucidated. Cocoa has been shown to increase endothelial derived nitric oxide synthase (eNOS) and reduce the activity of vascular arginase, allowing for an increased concentration of L-arginine which is needed for the production of NO *via* eNOS (Huynh & Chin-Dusting, 2006). Corti, Flammer, Hollenberg, and Lüscher (2009) describe that the immediate impact CF may have is through the inhibition of NADPH oxidase which can inactivate NO, and long-term impact may be *via* increased eNOS expression. Additionally, CF may reduce the level of microparticles found within circulation; high levels of microparticles are correlated with reduced endothelial function (Singh et al., 2006). Commonly, people with high atherothrombotic risk have a high level of microparticles within circulation and it is possible that these microparticles attract the accumulation of inflammatory cells within the vascular wall (Angelillo-Scherrer, 2012). Horn et al., (2014) found that a twice daily dose of 375 mg CF significantly reduced endothelial microparticles and improved endothelial function measured by flow mediated dilation after one month.

The effects that CF have on vascular health seem to also have a positive influence on blood pressure, further adding to the suggestion that the regular consumption of CF

may be cardioprotective. Studies have demonstrated a blood pressure-reducing effect from consumption of cocoa, with greater benefit in younger and also hypertensive individuals (Reid et al., 2017). These effects are likely due to not only the role of CF on NO and eNOS but also the antioxidant properties provided by the flavanols which can potentially reduce atherosclerotic risk (Grassi et al., 2005). Even short-term consumption appears to be effective in reducing blood pressure by an average of 1.8 mmHg, albeit a fairly modest reduction (Reid et al., 2017). One study involved the daily consumption of 75 mg of catechin for 24 weeks, with this strategy proving effective in reducing systolic blood pressure (Matsuyama et al., 2008). To realise more benefits, it is likely that a large dose of CF is necessary for blood pressure-reducing effects. In a study comparing various doses, it was reported that only when the daily dosage was above 1000 mg of CF was there an antihypertensive effect (Davison et al., 2010). Potentially, this benefit at higher doses may come from the increased amounts of the monomers catechin and epicatechin, which are considered the most active *in vivo.* As such, Ellinger et al., (2012), reported that the epicatechin dose is more important than the overall CF dose, with a daily dose of ≥25 mg being effective in reduced blood pressure.

As a result, of all the CF, it is thought that epicatechin is the most important compound in regard to the beneficial effects derived from consumption. When consuming epicatechin alone, very similar vascular effects to cocoa are observed (Vlachojannis, Erne, Zimmermann, & Chrubasik-Hausmann, 2016). This indicates the importance of epicatechin content when determining a truly beneficial dose of CF. However, it is worth noting that when consuming cocoa, there are more bioactive compounds than just CF; there are unsaturated fatty acids, theobromine, methylxanthines and other flavonoids that are not found within the flavanol sub-class (Vlachojannis et al., 2016). It is considered likely that these other compounds contribute to the overall benefits of cocoa. The methylxanthines found within cocoa may even improve the absorption of flavanols as it appears that the presence of these compounds leads to an increased plasma concentration of epicatechin when ingested simultaneously (Sansone et al., 2017).

The improvements that have been observed for blood flow may in turn aid with cognitive function. This may be due to increased cerebral oxygenation as a result of increased cranial blood flow (Francis, Head, Morris, & Macdonald, 2006). In one study,

acute consumption of 720 mg of CF improved cognitive function across a number of different tasks, such as choice reaction time. The authors speculate that due to the wide range of overall improvements, it is possible that the increased blood flow may improve motivation or attention during the tasks (Field, Williams, & Butler, 2011). The same study also found improvements in visual performance during the tasks, with the potential mechanisms being improved retinal blood flow. Furthermore, doses of 520 and 994 mg of CF have shown to have varying effects on cognitive function as well as subjective measures, e.g., perceived mental fatigue. A dose of 520 mg attenuated perceived mental fatigue in comparison to a 0 mg control, whereas a 994 mg dose improved response time in a rapid visual information processing task (Scholey et al., 2010). However, other research has indicated that although CF may improve cerebral blood flow and oxygenation, they do not improve cognitive performance (Lieselot Decroix et al., 2016).

It is of note that as CF metabolites can cross the blood-brain barrier, the possibility that they can aid cognitive function and cerebral oxygenation may result in benefits for attenuating cognitive decline. The role of CF as neuroprotective agents may result from their antioxidant capacity, limiting neuronal death from apoptosis due to the presence of toxic radicals (Nehlig, 2013). Not only that but the increase in brain perfusion and oxygenation may lead to the stimulation of angiogenesis (the creation of new blood vessels) *via* an increase in the amount of mobilised angiogenic cells (Heiss et al., 2010). Therefore, alongside the increased synthesis of NO, the improvement of endothelial health, and possible benefits of CF for cardiovascular and neural health are becoming somewhat clearer.

2.2 Muscle damage

When muscle fibres are exposed to a significant amount of stress, usually as a result of strenuous exercise, the structures can become deformed and damaged. This stress can occur through mechanical and metabolic mechanisms, both of which will be discussed in the subsequent paragraphs. There are various exercise modalities attributed to instigating muscle damage. Exposure to unaccustomed exercise, the first time (or most recent time following an extended period of no training) a person is subjected to an exercise stimulus, commonly results in a significant level of muscle damage; however, repeat exposure leads to a lessened damage response (Brown, Child, Day, & Donnelly, 1997). Exercise that involves a substantial amount of high

effort eccentric contractions frequently leads to muscle damage; these contractions are known to place a significant amount of mechanical stress on the muscle fibres. An eccentric contraction is the action in which a muscle lengthens under tension. Eccentric-biased exercise protocols are used within research settings to experimentally induce muscle damage; these exercise protocols involve numerous maximal effort eccentric contractions (resisting an external force) to elicit sufficient mechanical stress required for damage (Chen, Lin, Chen, Lin, & Nosaka, 2011; Hesselink, Kuipers, Geurten, & Van Straaten, 1996).

Mechanical stress occurs mostly during the aforementioned eccentric contractions. The reason for this is that more force is produced during an eccentric contraction than concentric or isometric. Yet, fewer motor units are recruited during eccentric contractions, some may be uncoupled/'derecruited' following the concentric phase (Duchateau & Baudry, 2014), therefore, more force is placed upon fewer motor units leading to a greater chance of contractile failure (Clarkson & Hubal, 2002; Hesselink et al., 1996). Post damaging event, there is a disruption of the sarcomeres in the damaged fibres occurring from an over-stretching of sarcomeres resulting in structural deformation of myofibrils (Morgan & Proske, 2004). This is referred to as the 'popping sarcomere' hypothesis. The crux of this theory is that when a muscle is stretched beyond its optimal length, the point of optimum tension generation (Morgan & Allen, 1999), then the longer weaker sarcomeres are stretched more rapidly and possibly beyond the myofilament overlap, potentially leading to a shearing of the myofibrils (Morgan, 1990; Morgan & Proske, 2004). Subsequently, calcium ion (Ca^{2+}) homeostasis is disrupted leading to the stimulation of proteases and instigating protein breakdown and further damage (Gissel, 2006). This combined with excitation-contraction coupling failure (Byrne, Twist, & Eston, 2004) can lead to reduced muscle function and various other symptoms (Chapter 2 Section 3).

Muscle damage can also occur following exercise that involves lower intensity eccentric contractions, such as prolonged cycling and marathon running. This is thought to be a result of metabolic stress; that is muscle damage is potentially caused by metabolic deficiencies increasing the muscle's susceptibility to damage (Tee, Bosch, & Lambert, 2007). This theory proposes that energy levels within working muscle (adenosine triphosphate (ATP) concentrations) could decrease to an amount that leads to the inhibition of sarcoplasmic Ca^{2+} ATPase, in turn causing increased

concentrations of Ca^{2+} and as a result a rise in Ca^{2+} mediated proteases, initiating a protein degradation response (Duncan, 1978; Gissel, 2006; Tee et al., 2007). This deleterious reduction in ATP could potentially stem from glycogen depletion and fatigue-induced physiological changes within the muscle. Various studies have found that in prolonged sport there is localised damage to fibres that are depleted in glycogen such as in marathon running (Warhol, Siegel, Evans, & Silverman, 1985), soccer (Krustrup et al., 2006) and prolonged cycling (Lepers, Hausswirth, Maffiuletti, Brisswalter, & Van Hoecke, 2000).

Following exercise, a disruption to calcium homeostasis can occur, either due to structural damage from mechanical stress, e.g., sarcomere popping, a reduced action of calcium specific enzymes, e.g., Ca^{2+} ATPase, or increased permeability of the sarcolemma (Duncan, 1978). This leads to an influx of Ca^{2+} into the cytosol which, if the concentration rises to a substantial amount, can then trigger a cascade of calcium dependent proteolytic enzymes, such as calpains and phospholipases, alongside increased reactive oxygen species (ROS) production (Gissel, 2006). This can then result in potential ultrastructural damage to the sarcolemma (Armstrong, 1984) consequently resulting in myofilament degradation (Duncan, 1978).

The differences between mechanical stress and metabolic stress are somewhat highlighted in a study that compared concentric cycling to eccentric cycling. Both cycling activities were performed at 60% of maximal concentric power at 60 rpm for 30 minutes but resulted in different outcomes; the concentric trial had a higher oxygen consumption, perceived effort, blood lactate and heart rate during the trial. However, in the days following, the eccentric trial resulted in greater decrements in muscle function and jump height, as well as a far greater increase in quadriceps muscle soreness compared to the concentric trial (Penailillo, Blazevich, Numazawa, & Nosaka, 2013). Indeed, it appears the metabolic cost of concentric contractions is greater than that of eccentric, although the latter results in a more pronounced overall muscle damage response. It is likely that in most sporting scenarios muscle damage is induced by both mechanical and metabolic factors.

2.3 Consequences of muscle damage

Exercise performance during the recovery window, commonly 72 hr post muscle damaging exercise, is likely to be compromised. The consequences of muscle damage vary considerably, both in severity and between individuals (Baumert, Lake,

Stewart, Drust, & Erskine, 2016). The full extent of individual markers will not be explained within this thesis; however, an overview will be provided that details the important and relevant aspects of each marker/symptom and discussed within the context of this thesis.

2.3.1 Reduced muscle function and neuromuscular control

Force generation can be reduced as a result of acute fatigue following the exercise bout (Kellis 1999), structural damage resulting in a change in sarcomere distribution (Morgan & Proske, 2004) or even the oxidative environment of the muscle (Powers & Jackson, 2008). Normally, muscle function is measured using maximal voluntary isometric contractions (MVIC), defined as the amount of force that a muscle can produce at a specific angle. However, changes can also be observed through dynamic actions such as sprints, jumps and concentric/eccentric muscle contractions. The previous tests are most frequently measured pre- and post-EIMD to investigate the change resulting from the damage. This allows for an understanding of whole muscle status and how it has been affected by the exercise insult as well as a direct indicator for athletic performance. However, the use of isometric contractions alone may provide either an over- or under-estimation of muscle force output due to the selection of a single joint angle, which is normally the case in the available research. More dynamic activities may provide a more representative picture of muscle function due to the involvement of whole muscle contractions. This does not mean that isometric contractions are not useful markers.

The strength loss that occurs following the exercise can last between a few hours or as long as a week or more (Clarkson & Hubal, 2002). Notable muscle damage is considered when reductions in force-generating capacity are ~20% of pre-exercise data, with greater reductions of up to 50% also correlating with a greater accumulation of inflammatory molecules within the damaged tissue (Paulsen, Ramer Mikkelsen, Raastad, & Peake, 2012). The largest decrements were observed following exercise involving a considerable focus on repetitive, maximal eccentric muscle contractions, which in some cases resulted in a dampened force generating capacity for over 7 days post (Paulsen et al., 2012). More moderate reductions, ranging from 10-40% from pre-exercise values are observed following other exercise including team sports, e.g., soccer, and downhill running, with recovery of force generating capacity occurring in the days following EIMD (Paulsen et al., 2012). As discussed previously, these

eccentric contractions put a large amount of strain on recruited motor units which can lead to structural damage, one of the primary causes of reduced function (Paulsen et al., 2012). The most common muscle groups selected for EIMD research include biceps brachii, quadriceps and hamstrings.

Another reason why muscle function reduces is perhaps due to the decline in neuromuscular control following EIMD, which can be measured concurrently with force production using electromyography (EMG), providing an indicator of the electrical stimulation of the concerned muscle. A fatigue-induced reduction in neuromuscular control appears to be peripheral, rather than central in origin. This indicates that the change in control may be a consequence of excitation-contraction coupling failure within the motor unit, changes to the structural units of the muscle fibres or cellular disturbances (Byrne et al., 2004). It is possible these changes contribute to reductions in force output *via* an inability to fully stimulate motor units, this can result in increased motor unit recruitment for a reduced force output (Contessa, Adam, & De Luca, 2009; Stock, Beck, & Defreitas, 2012).

Additionally, it has been suggested that there is an acute change in sarcomere distribution, as mentioned earlier (see Chapter 2 Section 2). This can lead to greater non-uniformity amongst the sarcomeres and alterations to optimum angle and force output, as well as a loss of calcium homeostasis. A potential temporary adaptation is that a working muscle must be at a greater stretched position to optimally produce force following the damage (Byrne, Eston, & Edwards, 2001; Byrne et al., 2004). The recovery of 'normal' optimal angle and force output may take between 24 to 168 hr depending on the individual (Jones, Newham, & Torgan, 1989). It has also been speculated that in severe cases, immediately following EIMD certain parts of the myofibril may be too damaged to continue to function appropriately and unable to participate in force production, this would likely result in the week long recovery mentioned earlier (Gregory, Morgan, Allen, & Proske, 2007).

Furthermore, EIMD can impair glycogen resynthesis (Asp, Daugaard, Kristiansen, Kiens, & Richter, 1998). This may result in a diminished capacity for a muscle to cope with the energy demands of the subsequent exercise resulting in reduced performance. This is a result of reductions in glucose transporter 4 translocation and reduced glucose uptake due to impaired insulin action (Asp, Daugaard, Kristiansen,

Kiens, & Richter, 1996; Asp, Daugaard, & Richter, 1995). Additionally, it is possible that due to the close relationship between intramyofibrillar glycogen and Ca^{2+} release, that if a muscle is currently depleted or low in glycogen then Ca^{2+} release may be impaired resulting in possible contractile impairments and reduced force production (Ørtenblad, Westerblad, & Nielsen, 2013).

There exists a large inter-individual variation, in as much as some people are 'high responders' and some are 'low responders' (Mann, Lamberts, & Lambert, 2014). These terms refer to individuals that display either a large reduction in force-generating capacity (high responders) or a small reduction (low responders). Interestingly, it is unlikely that the root cause of this dichotomy is genetic, as a study by Gulbin and Gaffney (2002) found that identical twins had varying reductions in muscle function even though there were similarities pre-exercise.

Previously, Paulsen et al., (2012) discuss the idea that the response of an individual may be impacted by how recently they performed intense eccentric exercise involving the muscle group being targeted. This theory has credence due to the repeated bout effect. Various adaptations occur following a bout of intense exercise, it is possible that during subsequent performance following the initial bout a greater number of motor units are recruited to reduce the level of stress placed upon the muscle fibres (McHugh, Connolly, Eston, & Gleim, 1999). Furthermore, a remodelling process occurs during the recovery window involving the expansion in number of sarcomeres to reduce the chance of over-straining and loss of structural integrity (McHugh, 2003). Therefore, the activity level of an individual is a key factor to consider for research into EIMD, albeit very difficult to control for.

2.3.2 Increased soreness

Delayed onset muscle soreness (DOMS) is the term given to the feelings of pain that arise following exercise, the severity of this pain is widely variable. Paulsen et al., (2012) refers to DOMS as the most common symptom of muscle damage but may not reveal the true extent of the damage. One reason for this perhaps is that measuring DOMS is difficult due to the subjective nature of pain. However, there are various ways of quantifying an individual's soreness.

One of the most commonly implemented tools for assessing DOMS is a visual analogue scale (VAS). To quantify the soreness, an individual must mark a point on a

line that can be measured using a corresponding length (e.g., 45 mm), the lengths chosen are commonly 100 mm or 200 mm. The VAS presents itself as a low-burden, fast and simple measure to assess DOMS. However, a clear explanation of the anchor points and participants interpretation of them is beneficial for a more accurate result (Hjermstad et al., 2011). Another method to assess pain is the lower extremity functional scale (LEFS) (McBrier et al., 2010). The LEFS contains 20 hypothetical activities that are rated from 0 to 4, 0 indicating extreme difficulty and 4 indicating no difficulty to perform. It is considered a reliable measure of pain (Watson et al., 2005) and similarly to the VAS is easy to perform in research settings.

Aside from the previously mentioned scales, an algometer can be used to quantify pain, specifically pain pressure threshold (PPT). Algometry is used to identify the threshold of pain perception of an individual. To do so pressure is applied to a specific point using the algometer and an individual will state when there is a switch from 'pressure' to 'pain' (Hogeweg, Langereis, Bernards, Faber, & Helders, 1992). The use of PPT is considered reliable within and between sessions for assessing pain (Potter, McCarthy, & Oldham, 2006). However, care must be taken during assessment, with the first measurement to be disregarded and no more than two more measurements be taken immediately following to ensure a reliable estimate of pain (Lacourt, Houtveen, & van Doornen, 2012). This potentially creates an increased chance of technician-error during assessment, something that the VAS and LEFS do not; although if a trained researcher is able to accurately carry out the test, this chance is reduced. Furthermore, as the largest accumulation of nociceptors is at the distal aspect of a muscle, this is the area in which pain would be most intense (Mense, 2008); PPT however, is commonly performed at or around the muscle belly (Casanova et al., 2018) and as such may not reflect the full extent of the pain of the individual. It is likely that there is a need to involve multiple methods in an attempt to provide a better insight into perceptions of pain arising from muscle damage, as different measures may assess different aspects of pain (Kahl & Cleland, 2005). Notably due to the individual and subjective nature of pain perception, especially considering the notion that pain perception differs greatly between people participating in sport and those who are classified as 'active' (Tesarz, Schuster, Hartmann, Gerhardt, & Eich, 2012).

DOMS are known to peak around 48 hr post-exercise, building up over the initial 8-24 hr post exercise (Cheung, Hume, & Maxwell, 2003), hence the name.. The cause of

DOMS has been long debated, with multiple theories considered over time. The most likely possibility is that it is a mixture of various different physiological responses. Plausible theories include the connective tissue theory, the muscle damage theory, and the inflammation theory.

Connective tissue is what surrounds a bundle of muscle fibres. This tissue is less elastic than muscle fibres themselves and may be more susceptible to strain-related injury during excessive mechanical stress (Cleak & Eston, 1992). Because of this, it is thought that DOMS may not necessarily reflect the magnitude of muscle damage, as Nosaka, Newton, and Sacco (2002) found that DOMS poorly correlated with other markers of muscle damage, such as MVIC. Therefore, when measuring EIMD, soreness alone is likely insufficient but provides additional insight (Paulsen et al., 2012).

The muscle damage theory revolves around the idea that damage to the structural units and contractile components of muscle resulting in a disruption to the original muscle architecture leading to a stimulation of nociceptors within surrounding tissue (Cheung, Hume, & Maxwell, 2003). A common way of measuring the structural damage is through the identification of possible content leakage, e.g., release of creatine kinase (CK) into circulation. However, although CK is found within skeletal and cardiac muscle, it is not a wholly reliable marker of muscle damage. Not only is CK highly variable between individuals at rest and post-exercise but recently it has been suggested that the release of CK from the muscle may occur in an attempt to delay fatigue (Baird, Graham, Baker, & Bickerstaff, 2012).

Furthermore, inflammation may play a role in DOMS. Following EIMD neutrophils and macrophages are attracted to the damaged site to remove cellular debris (Butterfield, Best, & Merrick, 2006). This accumulation of various inflammatory molecules which secrete protein degrading enzymes and produce ROS may in turn stimulate various nociceptors causing the sensation of pain (Smith, 1991). Yet, it is possible to experience feelings of pain without any signs of intramuscular inflammation (Yu, Malm, & Thornell, 2002). The most likely answer is that it is a combination of the above theories, and the root cause of soreness is multifaceted, as well as being inherently individual.

2.3.3 Inflammation

Inflammation following EIMD is a complex and dynamic process that is now being seen as beneficial for remodelling, repair, and adaptation. Inflammation and oxidative stress are considered the cause of secondary muscle damage, in as much as these biochemical responses to the original exercise stimulus can result in further damage. The inflammatory response can be somewhat considered biphasic, an initial pro-inflammatory phase which can exacerbate the damage and an anti-inflammatory phase that is involved in repair and regeneration of the muscle (Toumi & Best, 2003).

The inflammatory process begins in the first few hours (1-4 hr) following EIMD if the mechanical stress or influx of Ca^{2+} is sufficient. This involves a rapid invasion of neutrophils, with macrophages accumulating sequentially thereafter (Butterfield et al., 2006). Around one hour post EIMD, as neutrophils begin to invade the damaged site, they then release proteases and cytotoxic molecules to help degrade and remove cellular debris as well as possible necrotic tissue. However, healthy surrounding bystander tissue may be damaged due to the increased cytolytic and cytotoxic environment created by neutrophils (Pizza, Peterson, Baas, & Koh, 2005; Tiidus, 1998). Furthermore, neutrophils actively secrete ROS that aid with muscle membrane lysis, but potentially increasing oxidative environment within the muscle, inciting oxidative stress (Halliwell, 2006).

Macrophages further the removal of debris and the inflammatory cascade, secreting cytokines, growth factors, and ROS, and through these they can modulate the cellular response to damage (Tidball, 2005). Interestingly, macrophages appear to perform muscle lysis *via* a NO dependent mechanism and muscle cells are observed to increase the release of NO from macrophages (Filippin, Moreira, Marroni, & Xavier, 2009). Pro-inflammatory cytokines, e.g., Interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α) and Interleukin-8, are secreted to aid with the initial removal of debris and along with neutrophils initiate an oxidative burst releasing ROS to aid with lysis (Fisher-Wellman & Bloomer, 2009). Within the extracellular space, ROS can initiate oxidative stress 24-48 hr post-exercise due to the increased concentration of possible reactants. One interesting note regarding the cytokine IL-6 is that the role it has is complex and multifaceted, it not only acts in a pro-inflammatory manner by stimulating IL-1β but it also increase the production of anti-inflammatory cytokines such as IL-10 (Peake,

Neubauer, Della Gatta, & Nosaka, 2017; Petersen & Pedersen, 2006; Woods, Vieira, & Keylock, 2009).

Macrophages not only secrete pro-inflammatory cytokines but also, anti-inflammatory cytokines, e.g., IL-10, and transforming growth factor-β, to aid the repair and regeneration of the muscle. More specifically, there are two phenotypes for macrophages; M1 are pro-inflammatory and M2 are anti-inflammatory, secreting different cytokines depending on phenotype (Mills, 2012). Deng, Wehling-Henricks, Villalta, Wang, and Tidball (2012) found that the cytokine IL-10 may instigate the phenotype switch in macrophages to promote regeneration. Therefore, the role of a macrophage is likely determined by the microenvironment in which it is present (Woods et al., 2009).

Interestingly, if the pro-inflammatory phase is blunted, potentially through exogenous administration of anti-inflammatory molecules, then the regenerative process may also be negatively affected (Deng et al., 2012). It has been speculated that the initial destructive effects of neutrophils allow for macrophages to begin regenerative processes sooner due to a more rapid removal of debris (Butterfield et al., 2006). This anti-inflammatory phase promotes repair *via* the increase of cellular proliferation and differentiation of satellite cells (stem cells that remain near muscle) and the synthesis of connective tissue that may have been damaged during exercise (Peake et al., 2017). Additionally, satellite cells are integral in the regeneration of damaged muscle fibres. Aiding with the growth of a myofiber during repair, satellite cells act to replace damaged tissue specific to the environment they are in and the needs of the muscle (Yin, Price, & Rudnicki, 2013).

2.3.4 Oxidative stress

During normal physiological functioning low levels of reactive nitrogen species and ROS are produced. They are known to have various mechanisms on a cellular level. These roles include cell signalling and the activation of various genes (Hancock, Desikan, & Neill, 2001), cell proliferation and differentiation (Napoli, De Nigris, & Palinski, 2001), and inducing apoptosis *via* a potential initiation of a caspase cascade (Simon, Haj-Yehia, & Levi-Schaffer, 2000). However, exercise can lead to an over production of ROS.

The production of free radicals and ROS is an immutable aspect of exercise metabolism, for example, muscle contractions can increase superoxide and hydroxyl radical production (McArdle et al., 2004; O'Neill, Stebbins, Bonigut, Halliwell, & Longhurst, 1996). Even though free radical production is a natural by-product of exercise, intense exercise and subsequently muscle damage, can lead to an imbalance between radical production and the endogenous antioxidant defence mechanisms within the muscle, e.g., antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and glutathione reductase (Ashton et al., 1998; Nikolaidis et al., 2007). This imbalance towards pro-oxidants can lead to oxidative stress and or damage, which can cause damage to DNA, proteins, lipid membranes and as a result exacerbate the damage in the days following exercise (Powers, Nelson, & Hudson, 2011).

Measuring the production of free radicals and various other ROS is difficult to do directly, mainly due to the reactive nature of these molecules resulting in a short half-life, e.g., superoxide 10^{-6} s, hydroxyl 10^{-10} s, and alkoxyl radicals 10^{-6} s (Phaniendra, Jestadi, & Periyasamy, 2015). Therefore, no obvious biomarker exists currently to accurately measure the production of these radicals, other than using immediate biological tissue or blood samples (Majewski et al., 2014). Because of this, most researchers look at indirect markers of radical activity as opposed to measuring the radicals themselves, instead measuring breakdown or oxidation products. Common measures include markers of lipid peroxidation, such as malondialdehyde, protein oxidation, such as dityrosine or protein carbonyls, and glutathione oxidation (Orhan et al., 2004; Vasankari, Kujala, Heinonen, Kapanen, & Ahotupa, 1995). It is outside the purview of this thesis to provide a critical discussion of these individual markers. The reason for this is due to markers of oxidative stress (and inflammation) not being included within this thesis due to a lack of funding and resources. Instead, the role of oxidative stress has on muscle damage will briefly be considered in the ensuing paragraphs.

During exercise, the mitochondria utilise oxygen and produce small amounts of superoxide radicals (Brand, 2010). Therefore, the increased oxygen requirements associated with intense exercise may be partially responsible for an increase in ROS during aerobic activity, however, evidence suggests that increased substrate availability may cause the mitochondrial production of ROS to decrease (Wong, Dighe,

Mezera, Monternier, & Brand, 2017). Furthermore, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase may contribute to the formation of ROS *via* the generation of superoxide which has a role in stimulating Ca^{2+} release from the sarcoplasmic reticulum, aiding with muscle contraction (Powers & Jackson, 2008; Powers et al., 2011).

Moreover, muscle contractions, as previously mentioned, instigate a rise in ROS. However, the type of contraction appears to influence the extent of the pro-oxidant status of the muscle, with eccentric contractions resulting in a greater level of oxidative stress compared to concentric contractions in the days following exercise (Kon et al., 2007). Both eccentric and concentric contractions resulted in marked increases in thiobarbituric acid reactive substances, likely as a result of lipid peroxidation from the increase in ROS.

Interestingly, ROS may influence force production in skeletal muscle. It is believed that redox balance is tightly controlled during exercise to promote an optimal state of being for force output; however, when exposed to high levels of ROS force output declines (Powers et al., 2011; Reid, 2001). This may be due to a ROS-induced reduction in calcium sensitivity of the myofibrils as well as a reduced activity of Ca^{2+} ATPase which may lead to contractile dysfunction through excessive Ca^{2+} accumulation (Siems, Capuozzo, Lucano, Salerno, & Crifo, 2003; Smith & Reid, 2006).

The second wave of oxidative stress that can occur following muscle damage is typically referred to as the respiratory burst, or oxidative burst, the process is driven by NADPH oxidase (Thomas, 2017). As discussed earlier, the invading inflammatory molecules, both neutrophils and macrophages, release free radicals to aid with the removal and degradation of cellular debris (Peake et al., 2017). This burst is further stimulated, or 'primed', by the presence of pro-inflammatory cytokines such as TNF-α (El-Benna et al., 2016), a molecule which is associated with protein lysis. If excessive, this can lead to further tissue damage, especially if within the extracellular space (Butterfield et al., 2006; El-Benna et al., 2016).

2.4 Impact of sex on muscle recovery

Everyone may experience muscle damage, albeit with a high degree of variability as discussed throughout the previous sections. However, one source of variability that should be considered is the impact that biological sex can have on the damage

response post-exercise. Research has found that females may exhibit a moderately reduced level of soreness in comparison to males following EIMD (Dannecker et al., 2012; Radaelli et al., 2014). Furthermore, following acute exercise females appear to be less fatigued and exhibit a more rapid recovery in torque output than males (Ansdell, Brownstein, Škarabot, Hicks, Howatson, et al., 2019; Senefeld, Pereira, Elliott, Yoon, & Hunter, 2018). However, this may not be the case following strenuous exercise, including EIMD, when differences appear to be minor (Lee et al., 2017). The key discrepancy between males and females that can have a theoretical difference on muscle is the variation in steroid hormones, most notably oestrogen.

It has been reported that oestrogen may have a protective role against inflammation and therefore, muscle damage. Oestrogen has the capacity to act as an antioxidant and in stabilising muscle membranes, although the role it may have in protecting skeletal muscle is complex and not well understood (Kendall & Eston, 2002). One study investigating the difference in inflammatory responses between males and females following muscle damage observed that damage response is similar between sex, however, the inflammatory response is greater in males than females (Stupka et al., 2000). The study identified that females had a reduced invasion of neutrophils and macrophages post-exercise compared to males. It is possible that oestrogen (or rather E2) reduces membrane fluidity and increases antioxidant defence to protect against lipid peroxidation, as such, it may protect the membranes from free radical damage during strenuous exercise, potentially limiting the inflammatory response attributed to oxidative stress (Kendall & Eston, 2002). The overall extent to which oestrogen can attenuate any level of damage is still relatively unclear. Some research has found that males experience more oxidative stress in muscle than females (Pansarasa et al., 2000). However, other work has found that females have higher levels of oxidative stress following sub-maximal eccentric running (Magdalena Wiecek, Maciejczyk, Szymura, & Szygula, 2017). A contributor to these conflicting findings may be the variation in hormone levels of females across the menstrual cycle.

The menstrual cycle is an important biological function in which an individual's hormonal profile fluctuates across various phases. The measurable change in hormones allows for a relatively straightforward identification of cycle phases, commonly referred to as the follicular and luteal phases. The follicular phase begins at the first day of menses and lasts till ovulation, commonly lasting between 10-16

days, whereas the luteal phase begins post-ovulation and lasts till the onset of menses, lasting around 14 days (Reed & Carr, 2018). Oestrogen concentrations begin to rise during the follicular phase, peaking around ovulation before a sharp drop-off, this then leads to a gradual increase and secondary, smaller peak during the luteal phase (Mihm, Gangooly, & Muttukrishna, 2011). These fluctuations in hormones could theoretically impact both exercise performance and muscle recovery. It appears the impact of cycle phase on performance is limited as previous studies have shown no difference for sprint performance (Tsampoukos, Peckham, James, & Nevill, 2010), $\dot{V}O_{2max}$ (Brutsaert et al., 2002) or anaerobic performance and endurance (Wiecek, Szymura, Maciejczyk, Cempla, & Szygula, 2016). Contrarily, maximal endurance performance has been observed to be reduced during the mid-luteal phase in female soccer players (Julian, Hecksteden, Fullagar, & Meyer, 2017) but not in female rowers (Vaiksaar et al., 2011). A recent meta-analysis concluded that the impact of cycle phase on exercise performance is relatively small or 'trivial' (McNulty et al., 2020). Stronger evidence in the form of high-quality studies is required to better inform future guidance.

As for the impact of cycle phase on recovery following muscle damage, it has been reported that there is a prolonged recovery from DOMS and a greater CK and IL-6 response during the follicular phase, and it is speculated that this may be due to the reduced levels of oestrogen during that phase (Carter, Dobridge, & Hackney, 2001; Hackney, Kallman, & Ağgön, 2019; Oosthuyse & Bosch, 2017). However, other research has found that there is no significant difference between menstrual cycle phase and IL-6 in healthy, eumenorrheic women (Chaffin et al., 2011). One study only observed a difference during the early follicular phase for DOMS but no other indirect markers of muscle damage such as countermovement jump or limb girth (Romero-Parra, Alfaro-Magallanes, et al., 2020).

It should be noted that it is difficult to compare results of previous studies mostly due to the variability that exists within the menstrual cycle between individuals. Briefly, the follicular phase has a high level of intra- and inter-variability, in that not each menstrual cycle a person experiences will be identical in length and also not necessarily generic between other females (Fehring, Schneider, & Raviele, 2006). Therefore, it is possible that even though some individuals may be studied during the follicular phase they may have high levels of circulating oestrogen as they approach the peak, pre-ovulation

period, compared to others at the early follicular phase. Indeed, it was recently identified that the largest difference in exercise performance was between the early and late follicular phase of the menstrual cycle (McNulty et al., 2020), albeit the difference was calculated as trivial. The reason for this difference is speculated to be due to the sharp rise in oestrogen during the late follicular following the period of low oestrogen during the early follicular phase. Progesterone also remains low during the late follicular rise, which may perhaps increase the bioactivity of oestrogen (Reed & Carr, 2018). This does at least advocate the need for researchers to identify the specific phase timing of individuals if the focus of the research is the impact of the menstrual cycle. Hormonal testing is likely required for accurate quantification of cycle phase rather than calendar-based testing (Wideman, Montgomery, Levine, Beynnon, & Shultz, 2013).

Furthermore, the relative inconsistency between research may also partially be due to the variety of contraceptives available to females, which may result in a large variety of contraceptive use within a single study, a potential confounding factor. Indeed, Oosthuyse and Bosch (2017) speculate that one of the reasons for the inconsistency between studies is in fact due to a lack control of contraceptive use in previous research.

Regarding contraceptives, one type of contraceptive that has been investigated is the oral contraceptive pill (OCP). A recent meta-analysis found that individuals on the OCP had suffered from slightly impaired exercise performance (both endurance and strength related activities) compared to naturally menstruating females (Elliott-Sale et al., 2020). This was however, considered a trivial difference. Research has found that females supplementing the OCP have lower circulating oestrogen levels compared to non-supplementing females (Hicks, Onambele-Pearson, Winwood, & Morse, 2017). As such it is feasible that due to the theoretical protective effect of oestrogen on muscle damage, individuals not taking the OCP may have an inherently improved recovery compared to OCP users.

Currently, evidence suggests that MVIC recovery is slower in OCP users than that of naturally menstruating females following EIMD (Mackay, González, Zbinden-Foncea, & Peñailillo, 2019; Minahan, Joyce, Bulmer, Cronin, & Sabapathy, 2015; Savage & Clarkson, 2002). Furthermore, the CK response may also be greater in OCP users,

potentially indicating a greater fatigue response (Hicks et al., 2017; Minahan et al., 2015). The reason for this is likely because the OCP users have lower oestrogen levels resulting in a greater risk of membrane disruption which may result in an increased creatine kinase response following EIMD. It should be noted that Hicks et al., (2017) found no other differences between OCP and naturally menstruating females for other markers of muscle damage. Indeed, there is evidence refuting the findings that OCP users suffer from EIMD more. In fact, one study found that OCP users had less muscular soreness following EIMD than non-OCP users (Thompson, Hyatt, De Souza, & Clarkson, 1997). More research is required to arrive at a consensus about the impact of oral contraceptives on recovery. Furthermore, there is a dearth of research into other forms of contraceptives that are available; this research is needed to better understand the effects that each may have on exercise recovery, and even exercise performance.

2.5 Importance of recovery within sport settings

Improving recovery has long been an area of great interest and within the purview of modern research as much as it was in ancient times. This is best exemplified through the art and application of massage therapy, with Chinese texts dating back to 2598 BC as well as the Ancient Greek scholar Hippocrates citing it as an effective method of aiding sports injuries (Goats, 1994). Current understanding suggests that massage therapy may assuage DOMS following exercise (Guo et al., 2017). This highlights how recovery from exercise was, is, and may always be a pertinent area of research.

In modern day sports, there is not always adequate time for full recovery after exercise (Page, Marrin, Brogden, & Greig, 2019; Rojas-Valverde et al., 2019). There are various Olympic sports that entail multiple bouts of exercise within the same day, e.g., judo. Additionally, in soccer, fixture congestion has become increasingly prevalent in recent times. In the 2020-21 season Manchester City FC played a total of 61 games between 21st September 2020 – 29th May 2021, ~36 weeks, averaging 1.7 matches each week or a game roughly every four days across the season. This does not account for International breaks when many of the first team squad will still be performing and it is very likely that many players were exposed to extended periods of two games per week. Impaired recovery can increase injury risk and impair athletic

performance (Killen, Gabbett, & Jenkins, 2010; Small, McNaughton, Greig, Lohkamp, & Lovell, 2009b). For this reason, attempting to optimise the recovery period and reduce the time frame has become a key area of research. However, there are times when optimising recovery may not be the key focus. These are periods of intense training, when inducing physiologic adaptations are the priority, e.g., improve the endogenous capacity of a muscle to cope with the demands of the exercise, e.g., in pre-season with team sports and training camps with combat and weightlifting sports (Burgomaster et al., 2008; Ebbeling & Clarkson, 1989; Gomez-Cabrera et al., 2006). Including the use of an external aid may be beneficial short term but may blunt possible adaptations from the exercise stimulus, although the evidence is equivocal (see review Merry and Ristow (2016)). Further, it has been observed that blunting the pro-inflammatory phase can impact the anti-inflammatory phase and as a result impair muscle regeneration (Deng et al., 2012). Other research has suggested that antioxidant supplementation may inhibit cellular adaptations that arise from exercise (Morrison et al., 2015; Strobel et al., 2011), although this is still debated (Mankowski, Anton, Buford, & Leeuwenburgh, 2015; Peternelj & Coombes, 2011). In elite sport, it is likely that recovery will commonly be the priority during competition phases, this is to enable optimal performance by the time of the next bout of exercise.

2.6 Hormesis

The theoretical driving force behind adaptation is a process known as hormesis and is perhaps the reason for the long-standing debate behind whether antioxidant supplements or other recovery methods that target the inflammatory response should or should not be used. The theory entails that when a biological system is exposed to a low or moderate stress (e.g., toxic molecules or ROS) then this may result in an adaptive response by said system. However, being subjected to a high level of stress may result in a negative outcome (Mattson, 2008; Radak, Chung, & Goto, 2008). It is possible that hormesis may explain the immediate benefits seen following exposure to strenuous exercise and as a result the repeated bout effect (Hubal, Chen, Thompson, & Clarkson, 2008; Nosaka, Sakamoto, Newton, & Sacco, 2001). Regular exercise training may lead to an upregulation of the genes involved in transcribing antioxidant enzymes as well as improving the inflammatory response through a faster phenotype switch to anti-inflammatory macrophages (Gordon et al., 2012). Therefore, it is possible that ROS and inflammatory molecules are drivers of cellular adaptations,

but long term or excessive exposure may be detrimental (Scheele, Nielsen, & Pedersen, 2009).

2.7 How cocoa could help recovery

The possibility that CF may aid muscle recovery following muscle damage is intriguing. Current nutritional interventions that are beneficial, albeit somewhat equivocally, for improving recovery, include Montmorency tart cherry juice (Bell, Stevenson, Davison, & Howatson, 2016; Bowtell, Sumners, Dyer, Fox, & Mileva, 2011; Connolly, McHugh, Padilla-Zakour, Carlson, & Sayers, 2006) and beetroot juice (Clifford, Bell, West, Howatson, & Stevenson, 2016; Clifford, Berntzen, et al., 2016; Clifford, Howatson, West, & Stevenson, 2017). However, the palatability of these interventions is debateable, with both cherry juice and beetroot juice sometimes being supplemented or mixed with another flavouring to improve taste (Dimitriou et al., 2015). Chocolate, on the other hand, is a highly palatable food also capable of acutely improving mood state following ingestion (Macht & Mueller, 2007). Therefore, CF may be a welcome addition to the cornucopia of nutritional interventions for recovery, should it prove efficacious.

Regarding muscle damage, CF may influence specific aspects of the damaging and recovery processes. More specifically, CF may act as an antioxidant to reduce the likelihood of oxidative stress and potentially modulate the inflammatory response post-exercise. As discussed in the previous section, exercise can induce a shift in redox homeostasis that leads to oxidative stress. It has been observed that the consumption of CF may increase the activity of glutathione peroxidase and glutathione reductase, two endogenous antioxidant enzymes (Martín et al., 2010), increase antioxidant capacity (Lotito & Frei, 2006; Wang et al., 2000), reduce ROS production (Ramiro-Puig et al., 2009; Rein et al., 2000),and protect cell membranes from ROS damage (Zhu, Holt, Lazarus, Orozco, & Keen); thus, supplementation may protect against oxidative stress.

The role that CF has on inflammation is complex. As discussed earlier, following muscle damage there is a localised inflammatory response at the damaged site. This involves the accumulation of different leukocytes initially, specifically neutrophils and macrophages, which in turn can exacerbate the damage through the secretion of pro-inflammatory cytokines, e.g., TNF-α, IL-2 and IL-6. It appears that CF may modulate this phase of inflammation, as CF monomers and dimers exert a slight inhibitory

potential on TNF-α secretion, whereas larger CF may stimulate increases of TNF-α (Mao, van de Water, Keen, Schmitz, & Gershwin, 2002). Other research has found that CF downregulates various other pro-inflammatory molecules, such as IL-1 α and IL-6, that are released by macrophages in periods of inflammation, potentially *via* the inhibition of specific transcription factors, e.g., nuclear factor-kappaβ (NF-kβ) or activated protein-1 (Ramiro et al., 2005; Selmi, Mao, Keen, Schmitz, & Eric Gershwin, 2006).

Furthermore, it is possible that CF may reduce the neutrophil induced oxidative burst that occurs, perhaps through a reduced activation of the NF-kβ pathway (Mackenzie et al., 2004). Similar effects have been observed in other flavonoid research with different interaction effects associated with the different chemical structures that make up the various flavonoid sub-classes (Ciz et al., 2012; Nam, 2006; Vázquez-Agell et al., 2013). The NF-kβ pathway is responsible for the regulation of the majority of inflammatory mediators, including cytokines, chemokines, and other transcription factors (Dorrington & Fraser, 2019). The inhibition of this pathway is speculated to offer a therapeutic effect on various inflammatory conditions that induce an abnormal production of cytokines (Yamamoto & Gaynor 2001).

Therefore, due to the potential CF have as antioxidants and immunomodulators, they may exert a beneficial effect on various aspects of the initial inflammatory response and oxidative stress elicited by an exercise insult. It should be noted that many investigations into the impact CF have on inflammation are commonly *in vitro*, in subjects with high levels of systemic inflammation or in animals and thus, their effect in humans remains unclear. Nonetheless, the application of CF on muscle recovery does pose an interesting area of research.

Chapter 3 The Effects of Cocoa Flavanols on Indices of Muscle Recovery and Exercise Performance: A Systematic review of the literature

This systematic review has in part been published as the following citation:

'Corr, L. D., Field, A., Pufal, D., Clifford, T., Harper, L. D., & Naughton, R. J. (2021). The effects of cocoa flavanols on indices of muscle recovery and exercise performance: a narrative review. *BMC Sports Science, Medicine and Rehabilitation*, *13*(1), 1-16.'

For the publication the review was condensed into a narrative review.

It has been amended to be consistent with the thesis. As lead author I wrote the article, performed the systematic search which was replicated by a co-author (AF) analysed the studies. The co-authors aided with study conceptualisation during the initial phases of the PhD and provided feedback on the writing before publication of this as a narrative review.

3.1. Background

Muscle damage is associated with various negative symptoms, such as delayed onset muscle soreness, impaired muscle function, and increased inflammation (Lee et al., 2002; Powers et al., 2011). Consequently, the use of recovery interventions purported to accelerate recovery has become increasingly prevalent. There is an emerging interest in the effects of the non-nutritive compounds (poly)phenols as recovery aids following strenuous exercise. As such their popularity as a nutritional aid has increased in athletes and recreational exercisers, likely because these plant-based bioactive compounds have numerous additional health benefits (Solheim et al., 2017).

The term (poly)phenol refers to a variety of bioactive compounds including flavonoids, stilbenes, phenolic acids and lignans (Tangney & Rasmussen, 2013). The largest subclass, flavonoids, can be further classified into flavonols, flavanols, flavanones, anthocyanins, flavones and isoflavones. Of these subclasses, the majority of research has focused on flavanols with particular attention on cocoa, not only because of the palatability of chocolate (Lima, Almeida, Nout, & Zwietering, 2011) but due to the high proportion of monomers catechin, epicatechin and gallocatechin; collectively referred to as CF, see Chapter 1 Section 1 for more information. These monomers are found in the largest quantities in cocoa when compared with other flavanol containing foodstuffs such as tea and fruits; however, the amounts vary considerably. See Chapter 1 Section 2 for information about how flavanol content can vary.

Cocoa flavanols (CF) have been shown to possess anti-inflammatory and antioxidant effects, with epicatechin the most potent monomer of the flavanol group (Andres-Lacueva et al., 2008). Cardiovascular benefits, such as improved flow mediated dilation and reduced blood pressure, have been observed following various doses of CF, such as, 918 mg (Heiss et al., 2007), 701 mg (Berry, Davison, Coates, Buckley, & Howe, 2010), 750 mg (Horn et al., 2014), and 917 mg (Schroeter et al., 2006) and epicatechin intakes as low as 25 mg (Ellinger, Reusch, Stehle, & Helfrich, 2012) and 46 mg (Heiss et al., 2003). Regarding epicatechin, greater efficacy has been reported at higher epicatechin doses (see review (Bernatova, 2018)). These benefits have been observed following supplementation periods ranging from the same day of testing (Berry et al., 2010; Schroeter et al., 2006), to seven days (Heiss et al., 2007), and 30 days (Horn et al., 2014). Additionally, CF may be beneficial for reducing markers of oxidative stress (defined as an imbalance in the generation of various

reactive species and antioxidants (Cobley, Close, Bailey, & Davison, 2017)) and inflammation (Decroix, Soares, Meeusen, Heyman, & Tonoli, 2018; Prince et al., 2016). The role of CF in modulating inflammation may stem from their capacity to influence signalling cascades, i.e., *via* an alteration to eicosanoid production (Derek D Schramm et al., 2001), and reducing the activation of certain inflammatory transcription factors, e.g., NF-kβ (Vázquez-Agell et al., 2013). Given that EIMD is thought to partly stem from inflammation and oxidative stress, CF may be able to attenuate functional symptoms that impede athlete recovery, such as muscular soreness and deficits in muscle function (Decroix et al., 2018; Vlachojannis et al., 2016).

ROS are produced as part of normal metabolic processes, such as cellular respiration, and in certain scenarios, such as exercise, are produced in high amounts (Powers et al., 2011). Various ROS molecules are involved in a plethora of functions at a cellular level, including, growth and proliferation (Hoidal, 2001), immune response (Halliwell, 2006) and apoptosis (Fuchs, Gruber, Uberall, & Wachter, 1994). Additionally, it is believed that ROS act as signalling molecules in various tissues; however, this is still not fully understood due to the numerous ROS produced at rest and during exercise (Powers, Duarte, Kavazis, & Talbert, 2010). Antioxidant defence systems maintain a balance between ROS production and neutralisation; if the production of ROS outweighs their neutralisation, then proteins, lipids and DNA may be oxidised altering their function (Betteridge, 2000). This process is typically referred to as oxidative stress. Alternatively, if cells are exposed to low levels of ROS, such as during moderate intensity exercise, they may act as signalling molecules for skeletal muscle adaptations (Mattson, 2008). Such adaptations include an increase in endogenous antioxidants such as superoxide dismutase, glutathione peroxidase and catalase, reduced oxidative damage from exercise and an improved resistance to oxidative stress (Radak et al., 2008). The mechanisms by which CF modulate redox metabolism and oxidative stress are not entirely clear, but activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) transcription pathway, which activates a battery of cytoprotective protein with antioxidant and anti-inflammatory functions is a potential candidate (Cheng, Wu, Ho, & Yen, 2013). For example, it has been observed that supplementation with catechin results in an increase in the expression of heme-oxygenase 1, an enzyme with antioxidant and anti-inflammatory functions (Paine, Eiz-

Vesper, Blasczyk, & Immenschuh, 2010), *via* upregulation of Nrf2 activity (Cheng et al., 2013). Moreover, cells treated with CF induced an increase in glutathione peroxidase and glutathione reductase, likely *via* Nrf2 activation (Cordero-Herrera, Martín, Goya, & Ramos, 2015). In addition, CF treatment has been shown to prevent a depletion in reduced glutathione and replenish glutathione peroxidase, as well as effectively limiting lipid and protein peroxidation (Martins et al., 2020). Collectively, these studies suggest CF may modulate oxidative stress, at least partly *via* redox sensitive pathways, e.g., stimulating Nrf2 which in turn leads to an increase in redox enzyme expression.

Strenuous exercise may generate large amounts of ROS that leads to oxidative stress. The ROS produced is thought to stem from the increase in cellular respiration, and/or immune cells like neutrophils (Lee et al., 2002; Souglis et al., 2018). Leukocytes that accumulate in the muscle after EIMD evoke a respiratory burst, whereby macrophages and neutrophils produce large amounts of ROS to lyse cellular debris and begin regeneration. However, it has been proposed that during this process ROS may also induce lipid peroxidation in nearby healthy tissues (Fisher-Wellman & Bloomer, 2009). It is thought that this damage to neighbouring cells might contribute to EIMD, and at least partly explain why decrements in muscle function and increased muscle soreness can persist for several days after strenuous exercise (Steinbacher & Eckl, 2015).

Therefore, the aim of this systematic review was to critically examine research on the effects of CF on oxidative stress, inflammation, muscle function, perceived soreness, and exercise performance. This review builds on previous work by Decroix et al., (2018) that reviewed the effects of CF on exercise performance. The present review includes research completed since the aforementioned article and unlike Decroix and colleagues focuses on CF and EIMD.

3.2. Methods

3.2.1 Information Sources and Search Strategies

This systematic review followed the guidelines outlined by the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA; http://www.prisma-statement.org). The goal of this systematic review was to collate and critique the current literature involving CF supplementation and exercise. The focus was on

muscle damage and recovery measured through various outcomes on intramuscular inflammation, oxidative stress, muscle function, perceived soreness, and performance. To accomplish this, five databases were searched: PubMed, Scopus, Web of Science, ScienceOpen and MEDLINE as well as bibliographies of potential articles were explored. Key terms for the search were as follows: 'cocoa flavanols,' OR 'dark chocolate,' AND 'muscle damage,' OR 'muscle recovery,' OR 'exercise recovery,' OR 'exercise-induced muscle damage,' OR 'exercise.' The latest search was carried out on 10th February 2020.

3.2.2 Quality Assessment

To assess the quality and potential risks of bias and quality of studies included, the National Institute for Health and Excellence checklist for randomised controlled trials was utilised (Popay, 2012). This checklist has been used in a previous systematic review by Decroix et al., (2018) about the impact of CF on vascular function, oxidative stress, and exercise performance. The tool is divided into four sections: section A – selection bias on the randomisation and allocation of participants. Section B – performance bias on care provided and blinding of participants and investigators. Section C – attrition bias on the differences between groups, including drop-out rate of participants. Section D – detection bias on the appropriateness of outcomes and measures used as well as the nature of blinding of investigators. A study can achieve a maximum score of 14 if all criteria are fulfilled within the article. If a study achieves a score ≥11 it is considered high quality with a low risk of potential bias, a score between 8-10 is considered good quality and has a low risk of potential bias. However, if a study achieves a score ≤7 the research is considered of poor quality and has a very high risk of bias (NICE, 2013). Table 3.1 shows the quality assessment scores of the included studies.

Table 3.1 Quality and bias assessment of included articles using the National Institute for Health and Excellence checklist for randomised controlled trials

References	Selection bias			Performance bias			Attrition bias			Detection bias					Score (/14)
	A1	A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	D3	D4	D5	
Allgrove et al., (2011)	✓	≠	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	×	×	10
Davison et al., (2012)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	13
de Carvalho et al., (2019)	✓	≠	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	13
Decroix et al., (2017)	✓	≠	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	13
Decroix et al., (2018)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Fraga et al., (2005)	✓	≠	✓	✓	×	×	✓	✓	✓	✓	✓	✓	×	×	9
Morgan et al., (2018)	✓	≠	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	13
Patel et al., (2015)	✓	≠	✓	✓	≠*	×	✓	✓	✓	✓	✓	✓	×	×	9
Patel et al., (2020)	≠	≠	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	12
Peschek et al., (2013)	✓	≠	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	×	11
Sadler et al., (2020)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	14
Stellingwerff et al., (2013)	✓	≠	✓	✓	≠*	×	✓	✓	✓	✓	✓	✓	×	×	9
Taub et al., (2016)	≠	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	13
Wiswedel et al., (2004)	≠	✓	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	×	✓	11

Note: ✓ indicates the study fulfils the criteria, ≠ indicates it is unclear if the study fulfils the criteria, ≠ indicates that study is described as single blind however the treatments were not blinded for participants only the study aims, × indicates the study does not fulfil the criteria.*

1386

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3.2.3 Study Selection Process and Eligibility Criteria

The results from all three databases were pooled and all duplicates were removed using either Zotero (https://www.zotero.org/) or manually by the thesis author and another independent researcher. For the studies to be deemed as viable for the review they had to satisfy the following inclusion criteria: research involving human participants, research studies involving acute, sub-chronic or chronic cocoa flavanol supplementation, an exercise protocol involved alongside supplementation, and explicit statement of cocoa flavanol use. Exclusion criteria were non-human participants, no CF supplementation (e.g., tea flavanols or wine (poly)phenols), review articles and case studies, no exercise involvement during the trial period and no disclosure CF dose in the methods. Articles were reviewed through titles and abstracts for initial screening and thereafter, full texts were screened to check eligibility until a consensus was reached between the thesis author and another independent researcher regarding the inclusion of studies. The PRISMA flow chart (Figure 3.1) outlines the identification, screening, and exclusion process.

3.3. Results

3.3.1 Study Selection and Screening

The preliminary screening using the aforementioned search terms resulted in an output of 491 articles. Following the collation of all articles the process of removing duplicates began, leading to a removal of 323 articles. Subsequently, all remaining articles titles were screened for relevance before an in-depth examination of abstracts and then full texts which led to the final 17 articles. A further three of these studies were then excluded from subsequent review due to no explicit CF amount stated in the text; Singh et al., (2006) expressed CF as total (poly)phenols, Gonzalez-Garrido, Garcia-Sanchez, Garrido-Llanos, and Olivares-Corichi (2017) only referred to total flavonoid content and Cavarretta et al., (2018) expressed flavanol content as gallic acid equivalents.

3.3.2 Study Characteristics

The fourteen studies totalled 213 participants (129 untrained and 84 classed as trained) with an average age of 28 ± 9 years old. The strata of ‘untrained’ was defined as participants who were referred to as untrained/sedentary or healthy/active individuals in the text (Allgrove et al., 2011; Davison, Callister, Williamson, Cooper, &

Gleeson, 2012; Morgan, Wollman, Jackman, & Bowtell, 2018; Patel, Brouner, Allgrove, & Spendiff, 2020; Sadler et al., 2020; Stellingwerff et al., 2013; Taub et al., 2016; Wiswedel et al., 2004), whereas 'trained' was defined as participants who were described as well-trained/professional athletes or as 'elite' athletes in the text (de Carvalho et al., 2019; Decroix et al., 2018; Decroix et al., 2017; Fraga et al., 2005; Patel, Brouner, & Spendiff, 2015; Peschek, Pritchett, Bergman, & Pritchett, 2013). All the included studies examined the effects of CF on one or more of the following: exercise-induced oxidative stress and inflammation, changes in muscle function, changes in levels of perceived soreness and impact of supplementation on exercise performance.

3.3.3 Summary of Studies

Of the 14 articles, nine examined the effects of CF consumption and exercise on oxidative stress response and five investigated the effects on inflammation. Three investigated the effects CF has on muscle function and measures of perceived soreness, and nine studied effects on exercise performance. Some of the included studies are involved in multiple categories. The studies utilised various methods of supplementing CF: 1) a sub-chronic (moderate length) supplementation period of up to 14 days, 2) a seven-day loading phase pre-exercise protocol and 3) an acute dose on the day of the exercise protocol, 4) a chronic three-month supplementation period. Three of the fourteen articles followed a sub-chronic CF supplementation period (Allgrove et al., 2011; Fraga et al., 2005; Patel et al., 2015), four utilised a seven-day loading phase in the build-up to an exercise protocol (de Carvalho et al., 2019; Decroix et al., 2018; Morgan et al., 2018; Sadler et al., 2020) and six used an acute dose on the day of the exercise protocol (Davison et al., 2012; Decroix et al., 2017; Patel et al., 2020; Peschek et al., 2013; Stellingwerff et al., 2013; Wiswedel et al., 2004). All studies used doses of CF that were categorised as low (≤ 250 mg), moderate (250 to 700 mg) or high (≥ 700 mg). Six studies measured flavanol concentrations in plasma following CF consumption, five found that epicatechin concentrations peaked between 90 and 190 min post exercise (Davison et al., 2012; Decroix et al., 2018; Decroix et al., 2017; Stellingwerff et al., 2013; Wiswedel et al., 2004), whereas (Fraga et al., 2005) did not find significant levels of CF, likely due participants being in a fasted state before blood sampling. Details of the included studies are reported in Tables 3.2, 3.3, 3.4, 3.5, and 3.6.

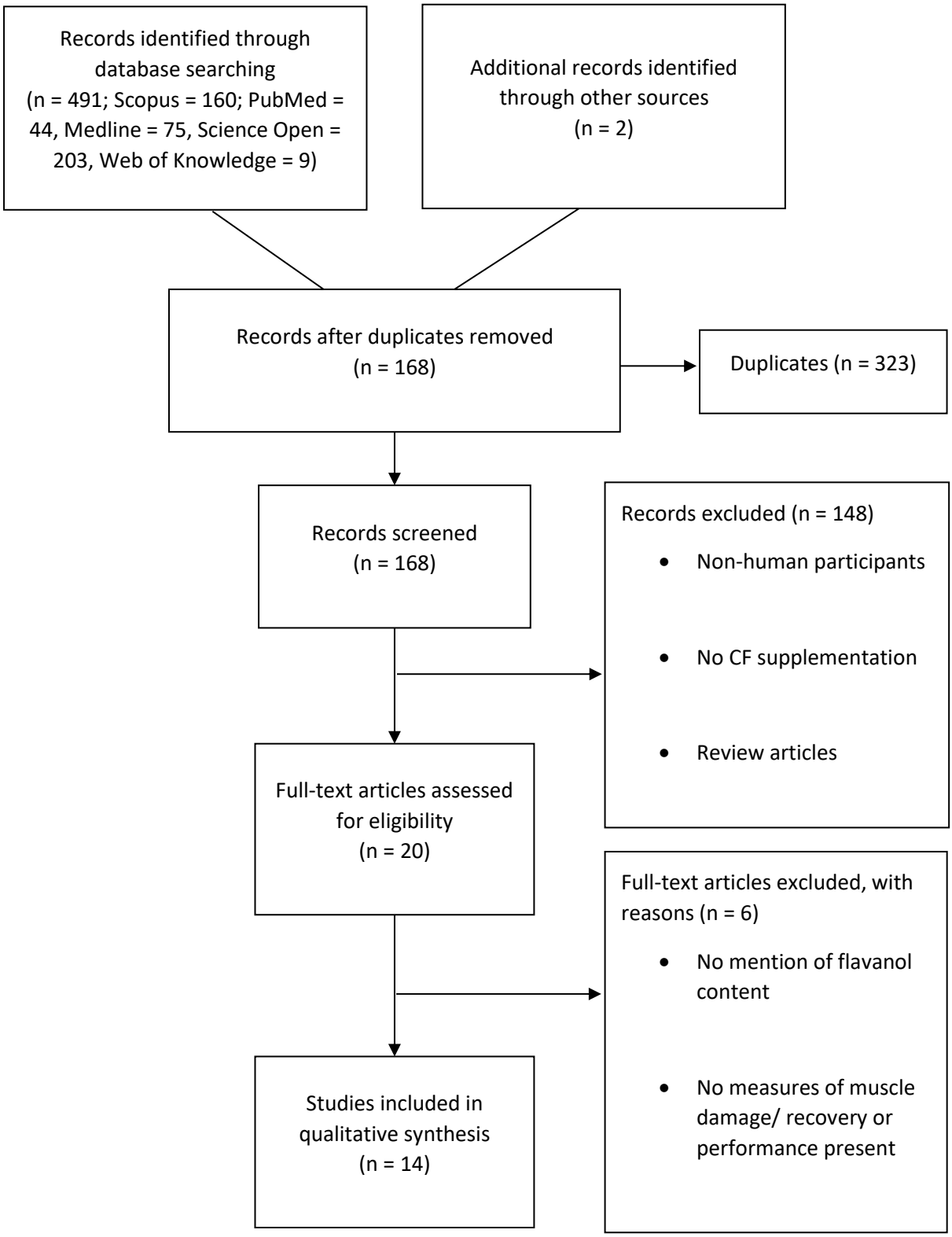

Figure 3.1. PRISMA flow chart detailing the screening

3.4. Discussion

3.4.1 Impact of Cocoa Flavanols on Exercise-induced Oxidative Stress

Antioxidants maintain redox status by neutralising ROS produced by metabolic reactions (Halliwell, 2007). However, as explained in Chapter 2 Section 3.4, the upregulation of ROS can lead to oxidative stress if cellular antioxidant capacity is overwhelmed. Oxidative stress in skeletal muscle decreases force output (Reid, 2001), likely through a reduction in Ca^{2+} sensitivity in the myofibrils and reduced activity of calcium ATPase, suggesting contractile dysfunction partly due to the accumulation of Ca^{2+} (Reid, 2008; Siems et al., 2003). Therefore, an increase in antioxidant capacity may lead to improvements in performance and recovery through reductions in fatigue associated with ROS during and after exercise.

Two studies that examined the effects of CF on markers of oxidative stress observed significant interaction effects following a 14-day sub-chronic supplementation period (Allgrove et al., 2011; Fraga et al., 2005). Allgrove and colleagues observed that F_2-isoprostanes and oxidised low density lipoprotein (markers of oxidative stress) were significantly lower in the treatment group, supplementing 197.4 mg CF and 77.4 mg epicatechin, versus placebo post 90 min of cycling at 60% $\dot{V}O_{2max}$, interspersed with 30 s efforts at 90% $\dot{V}O_{2max}$ every 10 min (Allgrove et al., 2011). Similarly, (Fraga et al., 2005) found that regular CF intake (168 mg) alongside soccer training and match play over a 14-day period resulted in a 12% decrease in malondialdehyde (MDA; a marker of lipid peroxidation), whereas in the placebo condition values increased by 10%, indicating a reduction in oxidative stress associated with training and match play. A study by Decroix et al., (2017) observed that although cycling time trial exercise increased MDA concentrations, CF had no significant impact compared to placebo. Wiswedel et al., (2004) also found no significant treatment effect of CF on MDA concentrations following cycling exercise. Interestingly, Wiswedel et al., (2004) included a no exercise control and found that the high flavanol group had a lesser increase in MDA than the low CF group four- and six- hr post-ingestion. In contrast, when supplementing 1,765 mg of cocoa extract (containing 530 mg CF) for six days in the lead up to exercise and once more immediately before, CF blunted the exercise-induced rise in MDA concentrations (Decroix et al., 2018). These changes imply that sub-chronic consumption of CF may reduce exercise-induced oxidative stress more effectively than an acute dose. The results suggest that CF may be a potent

antioxidant, with plasma MDA levels decreasing from baseline over a 14-day period of 168 mg of CF consumption a day (Fraga et al., 2005). These findings may have applicability to clinical populations as it has been reported previously that CF supplementation prevents systemic oxidative stress (measured via plasma MDA and urinary prostaglandin F2α) in type II diabetes and cancer (Abdulkhaleq et al., 2017). Notwithstanding, the other markers of oxidative stress and antioxidant activity were not affected by the treatment (8-oxo-2-deoxyguanosine and total relative antioxidant potency respectively), with a possible explanation being the relatively low amount of collective epicatechin and catechin in the treatment — only 39 mg per dose (Fraga et al., 2005), or the markers were not sensitive enough to detect changes in healthy, soccer players that trained at least twice and played a 90 min match each week.

Where Allgrove and colleagues found a significant difference for F_2-isoprostanes post-cycling exercise after a sub-chronic dosing protocol of CF, both Davison et al., (2012) (246.8 mg, 96.8 mg epicatechin) and Wiswedel et al., (2004) (187 mg) observed that even an acute dose of CF pre-cycling exercise elicited reductions in F_2-isoprostanes when compared to placebo in a crossover design. These were the only acute dose studies to observe any treatment effect on oxidative stress as the other two reported no differences between treatments (de Carvalho et al., 2019; Morgan et al., 2018). The only study to assess oxidative stress over a chronic supplementation period had participants consuming 175.2 mg daily for 30 days and found that CF significantly increased the reduced glutathione/oxidised glutathione ratio and reduced protein carbonylation (Taub et al., 2016). This again indicates that prolonged supplementation may be more beneficial than solely acute consumption.

Data regarding uric acid/urate is conflicting across studies. Decroix et al., (2017) reported that an acute dose of 900 mg CF increased uric acid following two 30 min time trials. In contrast, Fraga et al., (2005) found that sub-chronic dosing of 168 mg CF per day decreased urate levels by 11% compared to the beginning of supplementation, Decroix et al., (2018) also found that 1,765 mg cocoa extract (530 mg CF) per day over a seven day period did not influence uric acid concentrations at rest or post-exercise. However, the contrasting observations may be attributed to the fact that Fraga et al., (2005) collected blood samples on a rest day, while Decroix et al., (2017) took blood samples immediately post-exercise; which has been observed to increase uric acid concentrations 1-2 hr post intense exercise (Quindry, Stone, King,

& Broeder, 2003). As Decroix et al., (2018) took samples at rest and post-exercise whilst using the highest dose of CF and found no impact, this may imply that the mechanism that CF act as an antioxidant may be independent to the mechanism behind changes in uric acid concentrations. Uric acid can be used as a marker of oxidative stress due to its role in the conversion of xanthine dehydrogenase to xanthine oxidase, which then increases the production of ROS (Glantzounis, Tsimoyiannis, Kappas, & Galaris, 2005). Counterintuitively, uric acid is also one of the predominant antioxidants found within the plasma (El Ridi & Tallima, 2017; Ghezzi, 2020). The role of uric acid as a pro-oxidant within the cellular compartment, coupled with its role as an antioxidant in the plasma, make it difficult to draw practical conclusions from antioxidant based nutritional studies. Additionally, certain flavonoids, such as quercetin due to its chemical structure, may act as an inhibitor of the production of xanthine oxidase (an enzyme that increases ROS concentrations) and as such have a direct influence on uric acid concentrations (Mohos et al., 2019).

However, there are times during an athletes' training when reducing oxidative stress may not be desired, such as during pre-season when adaptations from exercise are the priority as opposed to accelerated recovery. The adaptations associated with oxidative stress during and following exercise include improved cellular repair systems and reduced production of damaging ROS (Radak, Taylor, Ohno, & Goto, 2001). However, these exercise related training adaptations may be hindered by regular high doses of antioxidant compounds and prevent or obstruct key cellular functions associated with ROS (Peternelj & Coombes, 2011). Nevertheless, a recent meta-analysis identified that the evidence for a blunting effect of (poly)phenol supplementation on exercise adaptations is equivocal, more research is needed to fully understand how (poly)phenols may augment exercise adaptations (Martinez-Negrin, Acton, Cocksedge, Bailey, & Clifford, 2020).

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Table 3.2 The effect of CF supplementation on exercise-induced oxidative stress

Reference	Participants	Nutritional Intervention	Supplementation period	Exercise stimulus	Measure(s)	Key outcome(s)
Allgrove et al., (2011)	20 healthy males Age 22 ± 4 years Mass 74.6 ± 8 kg $\dot{V}O_{2max}$ 53.1 ± 7.0 ml·kg^{-1} Power output 300 ± 30 W	CF: 80 g dark chocolate a day for 14 days, 197.4 mg CF per dose (EPI: 77.4 mg, CAT: 31.2 mg) CON: 56.8 g iso-CHO-fat control chocolate, 0 mg CF	Each day for 14 days, with a half dose 2 hr pre-exercise	Cycling at 60% $\dot{V}O_{2max}$ for 1.5 hr, intensity raised to 90% every 10 min for 30 s. 5 min post cycling there was a time to exhaustion trial at 90% $\dot{V}O_{2max}$	i) F_2-isoprostanes ii) Oxidised LDLs iii) Plasma uric acid iv) TEAC v) Plasma Vitamin C	*i*) significantly ↓ in CF group. *ii*) significantly ↓ across each time point in CF group *iii*) ↑ post-exercise in both treatments iv) ↔ between groups v) ↔ between groups
Davison et al., (2012)	14 healthy males Age 22 ± 1 years Mass 71.6 ± 1.6 kg $\dot{V}O_{2max}$ 53.1 ± 1.9 ml·kg^{-1} min^{-1} Power output 300 ± 12 W	CF: 100 g dark chocolate 246.8 mg CF (EPI: 96.8 mg, CAT: 39.1 mg) CON: isomacronutrient control, 0 mg CF None: water	Acute dose 2 hr pre-exercise	Cycling at ~60% $\dot{V}O_{2max}$ for 2.5 hr	i) F_2-isoprostanes ii) Plasma Vitamin C iii) TEAC	i) ↓ CF group vs CON ii) ↔ between groups iii) ↑ pre-exercise CF vs CON

de Carvalho et al., (2019)	13 trained males Age 21 ± 2 years Stature 180 ± 0.05 cm Mass 87.02 ± 8.03 kg	CF: CHO + protein cocoa beverage, 306 mg CF per beverage CON: cocoa based CHO + protein beverage, 0 mg CF	7 days, beverage consumed twice daily	Five sets of 20 drop jumps from 0.6 m, 10 s between jumps and 2 min interset rest.	Urinary F_2-isoprostanes	↔ between groups
Decroix et al., (2017)	12 well-trained males Age 30 ± 3 years Stature 177.9 ± 8.8 cm Mass 72.8 ± 7.8 kg $\dot{V}O_{2max}$ 63.0 ± 3.5 $ml \cdot kg^{-1} min^{-1}$	CF: cocoa drink, 900 mg CF (EPI: 185 mg, CAT: 20 mg) CON: placebo, 15 mg CF (EPI: 0 mg, CAT: 0 mg)	Acute dose 1.5 pre-exercise	Two 30 min time trials 60 min apart, performed at a ~75% peak power output.	i) Uric acid ii) MDA iii) TEAC	*i*) ↑ in CF vs CON *ii*) ↔ between group iii) ↑ in CF vs CON

Decroix et al., (2018)	14 well-trained males Age 31 ± 3 years Stature 180 ± 5 cm Mass 73 ± 7 kg $\dot{V}O_{2max}$ 62.9 ± 5.8 ml·kg^{-1} min^{-1} Peak Power Output 366 ± 45 W	CF: Capsule, 530 mg CF (EPI: 100 mg, CAT: 21 mg) CON: 1,764 mg maltodextrin	Consumed daily for six days and then a seventh on the day of testing	20 min steady state cycling at 45% peak power output 20 min time trial beginning at 75% peak power output Completed in normoxic and hypoxic environments	i) TEAC ii) Uric acid iii) MDA	*i)* ↔ *ii)* ↔ *iii)* CF blunted ↑ in both N and H
Fraga et al., (2005)	28 trained males Age 18 ± 1 years Mass 74 ± 1 kg	CF: 105 g chocolate confectionery, 168 mg CF (EPI + CAT: 39 mg) CON: 105 g cocoa butter chocolate, <5 mg CF	Sub-chronic, 14 day consumption	Soccer training sessions twice per week and one match per week.	i) MDA ii) Urate iii) Oxo^{8}dG iv) TRAP v) α-tocopherol vi) lycopene vii) β-carotene viii) coenzyme Q-10	*i)* Post CF ↓ by 12% CON ↑ by 10% *ii*) ↓ by 11% in CF *iii-viii)* ↔ between groups

Morgan et al., (2018)	10 active males Age 23 ± 3 years Stature 184 ± 59 cm Mass 85.3 ± 12.0 kg Single leg 1RM 90.4 ± 19.0 kg	CF: 330 ml cacao juice, 74 mg CF (EPI: 8 mg, CAT: 43 mg) CON: 330 ml CHO and flavour matched placebo, 0 mg CF	10 days supplementation (7 days pre-exercise, 3 days post)	10 sets of 10 single leg knee extensions at ~80% 1RM.	Protein carbonylation	Protein carbonylation not elevated following exercise protocol
Taub et al., (2016)	17 sedentary (9 males 8 females) participants CF: Age 50 ± 3 Stature 168 ± 3 Mass 78.8 ± 5.6 $\dot{V}O_{2max}$ 22.9 ± 1.9 ml·kg^{-1} min^{-1} CON: Age 50 ± 2 Stature 175 ± 5 Mass 92.2 ± 9.7 $\dot{V}O_{2max}$ 24 ± 1.7 ml·kg^{-1} min^{-1}	CF: 20g dark chocolate, 175.2 mg CF (EPI: 26 mg, CAT: 4.6) CON: 20g placebo chocolate	Chronic (3 months daily intake)	Cycling exercise including $\dot{V}O_{2max}$	i) GSH:GSSG ratio ii) Protein carbonylation	i) significant ↑ in CF group vs CON ii) significant ↓ in CF group vs CON

Wiswedel et al., (2004)	20 untrained males Age ~20-25	CF: cocoa drink, 185 mg CF CON: cocoa drink 14 mg CF	Acute, 2 hr pre cycling exercise	Cycling at 75W increasing to 150W for 10 min	i) F2-isoprostanes ii) MDA iii) α-tocopherol iv) ascorbate v) TAC	i) CON small ↑ 2 and 4 hr post-consumption, CF did not Significant difference CF vs CON 2 and 4 hr post-intake following exercise ii-v) ↔ between groups

Note: CF = cocoa flavanols, EPI = epicatechin, CAT = catechin, CON = control, CHO = carbohydrate, TEAC = Trolox equivalent antioxidant capacity, MDA = malonaldehyde, LDL =low density lipoprotein, Oxo^{8}dG = 8-Oxo-2'-deoxyguanosine, 1RM = one rep max, GSH:GSSG = reduced glutathione: oxidised glutathione ratio, TAC = total antioxidant capacity, TRAP = total relative antioxidant potency, ↑ = increase, ↓ = decrease ↔ = no significant effect/change

3.4.2 Impact of Cocoa Flavanols on Exercise-induced Inflammation

Strenuous exercise resulting in muscle damage evokes an acute inflammatory response (Peake et al., 2017). Several studies have observed systemic increases in markers such as IL-6, c-reactive protein (CRP) and TNF- α (Kanda et al., 2013; Kasapis & Thompson, 2005) following intense exercise. These markers are typically increased for several hours following exercise, but may persist for several days depending on the severity of the damage (Peake, Nosaka, & Suzuki, 2005). Inflammation, particularly the increase in neutrophils, has been associated with muscle function loss following exercise, suggesting the acute inflammatory response plays a role in recovery after exercise (Paulsen et al., 2010).

In vitro studies have shown that CF have anti-inflammatory properties and can reduce TNF-α from inducing an upregulation of vascular endothelial growth factor activity (Kim et al., 2010) and inhibit nuclear factor-kappa beta activation (Rodríguez-Ramiro et al., 2013). In humans, CF supplementation has been shown to decrease Interleukin-1β and Interleukin-10 levels (Sarriá et al., 2014), four weeks of dark chocolate consumption reduced leukocyte accumulation, soluble adhesion molecules, and the expression of adhesion markers on leukocytes (Esser et al., 2014) (see review by Goya et al., (2016) for more detail as it was beyond the scope of the thesis to fully review every aspect of CF on inflammation). This may indicate that dark chocolate or cocoa powder with a high proportion of CF would perhaps be viable as a therapeutic, anti-inflammatory intervention.

Studies by Allgrove et al., (2011) and Davison et al., (2012) found that prolonged cycling at 60% $\dot{V}O_{2max}$ increased inflammatory markers (IL-6, IL-10 and IL-1ra and IL-6, blood leucocyte count and neutrophil count, respectively) but found no difference between CF supplementation or placebo. Decroix et al., (2017) used two 30 min time trials separated by 90 min; the first time trial starting 100 min post ingestion of a 900 mg CF beverage. This resulted in no treatment or time effect on inflammatory markers (TNF-α, IL-1 and IL-6), perhaps implying the stimulus was not intense enough to induce inflammation in a cohort of well-trained cyclists. However, as both Allgrove et al., (2011) and Davison et al., (2012) used relatively low doses of CF (197.4 mg and 246.8 mg respectively), a higher dose of both total flavanols and epicatechin is perhaps necessary to evoke the purported anti-inflammatory effects of CF (Ellinger & Stehle, 2016), in situations that induce an increase in inflammatory markers. These

effects include the modulation of particular aspects of the inflammatory cascade, such as, inhibiting platelet aggregation (Murphy et al., 2003) and altering cytokine production *via* stimulation or inhibition of certain interleukins and growth factors (Selmi et al., (2006). Therefore, it is possible that for CF to confer anti-inflammatory benefits, the inflammation must be pronounced and/or prolonged. Furthermore, cycling exercise does not include a significant eccentric action; the type of contraction that is most associated with EIMD and as a result may not cause systemic inflammation to reach the same level of studies that involve eccentric biased exercise (Malm & Yu, 2012).

Currently, the only EIMD study with CF that measured inflammation was by Morgan et al., (2018), in this study no differences between treatment groups for IL-6 or CRP, following 100 maximal leg extensions with an elongated eccentric phase (three seconds). However, the researchers utilised a low dose (74 mg) of CF which is potentially why no effect was observed. The lack of studies showing robust changes in inflammation following exercise suggests that the anti-inflammatory effects of CF observed in *in vitro* studies may not translate to the *in vivo* environment. It is pertinent that future research investigates the impact of CF on markers of inflammation following EIMD (e.g., TNF-α), potentially including muscle biopsies to provide measured changes of inflammation in the muscle. It should be noted that the inflammatory process is necessary for skeletal muscle adaptation, and by blunting the initial pro-inflammatory phase, it is possible that the muscle regenerative phase can be impaired (Deng et al., 2012). Indeed, an adaptation to exercise is the increased activity of peroxisome proliferator-activated receptor γ co-activator 1α, which may aid the phenotype switch of macrophages from pro- to anti-inflammatory and reduce the expression of genes associated with oxidative stress (Kang & Ji, 2012; Metsios, Moe, & Kitas, 2020). Therefore, forgoing an anti-inflammatory intervention may be effective when adaptations to exercise are the priority, akin to adaptations related to ROS and oxidative stress. However, the evidence that long term supplementation of CF, (poly)phenols, or other antioxidant supplements (e.g., Vitamin C and E) can inhibit training adaptations is equivocal (Beyer et al., 2017; Clifford, Jeffries, Stevenson, & Davies, 2020; Myburgh, 2014); as such, more research is warranted to better understand how these compounds may influence exercise adaptations.

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Table 3.3 The effect of CF supplementation on exercise-induced inflammation

Reference	Participants	Nutritional Intervention	Supplementation period	Exercise stimulus	Measure(s)	Key outcome(s)
Allgrove et al., (2011)	20 healthy males Age 22 ± 4 years Mass 74.6 ± 8 kg $\dot{V}O_{2max}$ 53.1 ± 7.0 ml·kg^{-1} Power output 300 ± 30 W	CF: 80 g dark chocolate a day for 14 days, 197.4 mg CF per dose (EPI: 77.4 mg, CAT: 31.2 mg) CON: 56.8 g iso-CHO-fat control chocolate, 0 mg CF	Each day for 14 days, with a half dose 2 hr pre-exercise	Cycling at 60% $\dot{V}O_{2max}$ for 1.5 hr, intensity raised to 90% every 10 min for 30 s. 5 min post cycling there was a time to exhaustion trial at 90% $\dot{V}O_{2max}$	i) Circulating leukocytes ii) Neutrophils iii) IL-10 iv) IL-6 v) IL-1ra	i-v) ↔ between groups
Davison et al., (2012)	14 healthy males Age 22 ± 1 years Mass 71.6 ± 1.6 kg $\dot{V}O_{2max}$ 53.1 ± 1.9 ml·kg^{-1} min^{-1} Power output 300 ± 12 W	CF: 100 g dark chocolate 246.8 mg CF (EPI: 96.8 mg, CAT: 39.1 mg) CON: isomacronutrient control, 0 mg CF None: water	Acute dose 2 hr pre-exercise	Cycling at ~60% $\dot{V}O_{2max}$ for 2.5 hr	IL-6	↔ between groups

Decroix et al., (2017)	12 well-trained males Age 30 ± 3 years Stature 177.9 ± 8.8 cm Mass 72.8 ± 7.8 kg $\dot{V}O_{2max}$ 63.0 ± 3.5 ml·kg^{-1} min^{-1}	CF: cocoa drink, 900 mg CF (EPI: 185 mg, CAT: 20 mg) CON: placebo, 15 mg CF (EPI: 0 mg, CAT: 0 mg)	Acute dose 1.5 pre-exercise	Two 30 min time trials 60 min apart, performed at a ~75% peak power output.	i) TNF-α ii) IL-1 iii) IL-6	*i*) ↔ between groups. *ii*) ↔ between groups
Morgan et al., (2018)	10 active males Age 23 ± 3 years Stature 184 ± 59 cm Mass 85.3 ± 12.0 kg Single leg 1RM 90.4 ± 19.0 kg	CF: 330 ml cacao juice, 74 mg CF (EPI: 8 mg, CAT: 43 mg) CON: 330 ml CHO and flavour matched placebo, 0 mg CF	10 days supplementation (7 days pre-exercise, 3 days post)	10 sets of 10 single leg knee extensions at ~80% 1RM	i) CRP ii) IL-6	*i*) ↔ between groups. *ii*) ↔ between groups
Taub et al., (2016)	17 sedentary (9 males 8 females) participants CF: Age 50 ± 3 Stature 168 ± 3 Mass 78.8 ± 5.6 $\dot{V}O_{2max}$ 22.9 ± 1.9 ml·kg^{-1} min^{-1}	CF: 20g dark chocolate, 175.2 mg CF (EPI: 26 mg, CAT: 4.6) CON: 20g placebo chocolate	Chronic (3 months daily intake)	Cycling exercise including $\dot{V}O_{2max}$	CRP	↔ between groups.

CON:	
Age 50 ± 2	
Stature 175 ± 5	
Mass 92.2 ± 9.7	
$\dot{V}O_{2max}$ 24 ± 1.7 ml·kg^{-1} min^{-1}	

Note: CF = cocoa flavanols, EPI = epicatechin, CAT = catechin, CON = control, CHO = carbohydrate, IL = interleukin, CRP = c-reactive protein, TNF-α = Tumour-necrosis factor-, ↔ =no significant effect/change

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3.4.3 Impact of Cocoa Flavanols on the Recovery of Muscle Function

Muscle function is negatively impacted by EIMD, with reductions in muscle force and power capacity evident for several days following strenuous exercise. However, based on the current evidence it seems that CF supplementation has minimal, if not any, impact on MVC; as measured using peak torque with no effect observed on knee extensor strength recovery (de Carvalho et al., 2019; Morgan et al., 2018; Peschek et al., 2013). Currently, for (poly)phenols it has been suggested that >3 days of supplementation above 1000 mg may be required to observe an ergogenic benefit (Bowtell & Kelly, 2019), however no CF research has been performed fulfilling that criteria, highlighting a key area of research.

It is noteworthy that only Morgan and colleagues (2018) observed notable muscle damage based on decrements in muscle function across groups (Paulsen et al., 2012). To best understand the mechanisms behind CFs role in muscle damage recovery, it would be prudent to ensure symptoms of EIMD such as a decrease in muscle function are pronounced. In fact, it is noteworthy that the participants in de Carvalho et al., (2019) had fully recovered muscle function (based on peak torque data) 48 h post-exercise, indicating that the 100 drop-jump protocol did not elicit significant damage in a group of elite rugby players. Therefore, in populations with high baseline strength and power, protocols designed to induce EIMD need to be of a sufficient magnitude. Similarly, Peschek et al., (2013) observed 2-5% decrements in the control group and 10-22% in the CF group from pre to 24 hr post, indicating that perhaps CF ingestion exacerbated muscle damage or only the CF group suffered the deleterious effects of the EIMD protocol. Interestingly, from 24 hr to 48 hr post-exercise the CF groups muscle function improved, whereas no changes occurred in the control group. Nevertheless, as the control group did not experience pronounced levels of muscle damage, it is possible that the protocol was not sufficient to adequately study the effects of CF on muscle function. Nevertheless, if the protocol is not representative of the training loads regularly experienced by those individuals, the functional relevance of investigating EIMD and CF supplementation becomes questionable. A further measure of muscle function used was vertical jump height, in which they found no significant differences between groups (de Carvalho et al., 2019).

In contrast, Morgan et al., (2018) found that an acute dose of CF (74 mg) aided CMJ height recovery as participants returned to 95% of baseline at 48 hr in the CF group

compared to 87% in the placebo group. However, in this study they consumed a much lower dose than used previously in the literature, especially the epicatechin content (8 mg). Furthermore, the researchers utilised a unilateral EIMD protocol, yet the CMJ is a bilateral test, which could have influenced the findings. Instead, a more appropriate test could have been implemented, e.g., a single leg CMJ, as differences between participants' dominant and non-dominant legs may have been a confounding variable for jump height.

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Table 3.4 The effect of CF supplementation on exercise-induced changes in muscle function

Reference	Participants	Nutritional Intervention	Supplementation period	Exercise stimulus	Measure(s)	Key outcome(s)
de Carvalho et al., (2019)	13 trained males Age 21 ± 2 years Stature 180 ± 0.05 cm Mass 87.02 ± 8.03 kg	CF: CHO + protein cocoa beverage, 306 mg CF per beverage CON: cocoa based CHO + protein beverage, 0 mg CF	7 days, beverage consumed twice daily	Five sets of 20 drop jumps from 0.6 m, 10 s between jumps and 2 min interset rest.	i) MVC ii) Vertical jump	i) ↔ between groups ii) ↔ between groups
Morgan et al., (2018)	10 active males Age 23 ± 3 years Stature 184 ± 59 cm Mass 85.3 ± 12.0 kg Single leg 1RM 90.4 ± 19.0 kg	CF: 330 ml cacao juice, 74 mg CF (EPI: 8 mg, CAT: 43 mg) CON: 330 ml CHO and flavour matched placebo, 0 mg CF	10 days supplementation (7 days pre-exercise, 3 days post)	10 sets of 10 single leg knee extensions at ~80% 1RM	i) MVC ii) CMJ	*i*) ↔ between groups *ii*) ↑ recovery of CMJ
Peschek et al., (2013)	8 well-trained males Age 25 ± 6 years Stature 182.1 ± 6.3 cm Mass 73.4 ± 7.0 $\dot{V}O_{2max}$ 64.4 ± 7.6 ml·kg^{-1} min^{-1}	CF: Cocoa based CHO protein beverage with added cocoa, 350 mg CF CON: cocoa based CHO protein beverage, 0 mg CF	Acute ingestion of two beverages separated by 2 hr post-exercise protocol	30 min downhill running at a -10% gradient at 70% $\dot{V}O_{2max}$	MVC	↔ between groups.

Note: CF = cocoa flavanols, EPI = epicatechin, CAT = catechin, CON = control, CHO = carbohydrate, 1RM = one rep max, MVC = maximal voluntary contraction, CMJ = countermovement jump, ↑ = increase, ↔ = no significant effect/change

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3.4.4 Impact of Cocoa Flavanols on Perceived Soreness

Measures of perceived soreness are predominantly subjective in nature, typically measured using validated scales to quantify subjective pain, soreness and discomfort such as a VAS (Hjermstad et al., 2011) or LEFS (Yeung, Wessel, Stratford, & Macdermid, 2009). As muscular soreness is ubiquitous with EIMD, most studies investigating muscle damage utilised these measures of perceived soreness as a way of tracking recovery (de Carvalho et al., 2019; Morgan et al., 2018; Peschek et al., 2013). Peschek et al., (2013) administered two doses of 350 mg CF post EIMD which were separated by two hours and found no effect of treatment on VAS or LEFS scores. Interestingly, the increase in soreness from baseline to 24 and 48 hr post was not significant. This suggests that the protocol used (downhill running at a -10% gradient for 30 min) may not have induced significant levels of muscle damage in a cohort of well-trained endurance athletes.

Similarly, de Carvalho et al., (2019) did not find any interaction effect of the treatment following the EIMD protocol with only minor changes from baseline at 48 hr, even though this is when DOMS is known to peak (Kanda et al., 2013). Out of the three studies only Morgan et al., (2018) found a main effect of time on VAS scores following their respective protocols (100 knee extensions and 100 isokinetic hamstring curls respectively). Finding no significant difference between conditions; although as mentioned previously Morgan et al., (2018) used a small dose of 74 mg CF and a very low dose of 8 mg epicatechin. This amount is unlikely to exert any benefit as the required amounts to have a physiological influence are reported to begin around 400 - 700 mg (Schroeter et al., 2006) and at an epicatechin intake of 50 mg (Ellinger et al., 2012).

Table 3.5 The effect of CF supplementation on exercise-induced changes in perceived soreness

Reference	Participants	Nutritional Intervention	Supplementation period	Exercise stimulus	Measure(s)	Key outcome(s)
de Carvalho et al., (2019)	13 trained males Age 21 ± 2 years Stature 180 ± 0.05 cm Mass 87.02 ± 8.03 kg	CF: CHO + protein cocoa beverage, 306 mg CF per beverage CON: cocoa based CHO + protein beverage, 0 mg CF	7 days, beverage consumed twice daily	Five sets of 20 drop jumps from 0.6 m, 10 s between jumps and 2 min interset rest	i) VAS ii) LEFS	i) ↔ between groups. ii) ↔ between groups.
Morgan et al., (2018)	10 active males Age 23 ± 3 years Stature 184 ± 59 cm Mass 85.3 ± 12.0 kg Single leg 1RM 90.4 ± 19.0 kg	CF: 330 ml cacao juice, 74 mg CF (EPI: 8 mg, CAT: 43 mg) CON: 330 ml CHO and flavour matched placebo, 0 mg CF	10 days supplementation (7 days pre-exercise, 3 days post)	10 sets of 10 single leg knee extensions at ~80% 1RM	VAS	↔ between groups.
Peschek et al., (2013)	8 well-trained males Age 25 ± 6 years Stature 182.1 ± 6.3 cm Mass 73.4 ± 7.0 $\dot{V}O_{2max}$ 64.4 ± 7.6 ml·kg^{-1} min^{-1}	CF: Cocoa based CHO protein beverage with added cocoa, 350 mg CF CON: cocoa based CHO protein beverage, 0 mg CF	Acute ingestion of two beverages separated by 2 hr post-exercise protocol	30 min downhill running at a -10% gradient at 70% $\dot{V}O_{2max}$	i) VAS ii) LEFS	i) ↔ between groups. ii) ↔ between groups.

Note: CF = cocoa flavanols, EPI = epicatechin, CAT = catechin, CON = control, CHO = carbohydrate, VAS = visual analogue scale, LEFS = lower extremity functional scale, 1RM = one rep max, ↔ = no significant effect/change

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3.4.5 Impact of Cocoa Flavanols on Exercise Performance

The impact that CF may have on performance is likely through the antioxidant potential of the cocoa and delayed ROS-induced fatigue. Patel et al., (2015) measured performance using maximal distance timed sprint trial, which was completed after 20 min of cycling. It was found that 259 mg CF consumed daily for 14 days resulted in participants covering 17% more distance than baseline and 13% more distance than a white chocolate control. The mechanism for this increase may be due to CF decreasing ROS production and thereby attenuating fatigue (Allgrove et al., 2011; Fraga et al., 2005). An acute dosing strategy with higher flavanol products did not elicit any cycling performance benefit, only inducing slightly higher nitric oxide levels during exercise, which could aid muscle blood flow (Patel et al., 2020).

Many sports have limited recovery time between competitions. For example, in field hockey tournaments, matches are often played 48 hr apart; similar recovery times are evident in soccer and handball. As a result, it may be pertinent to accelerate recovery and attenuate symptoms of EIMD in these sports (Julian, Page, & Harper, 2020). In one study, supplementation of CF (616 mg CF for 7 days) increased distance covered during the Yo-Yo Intermittent test 1 of 9.85% from baseline to 48 hr post a 100 drop jump EIMD protocol. Whereas the placebo group covered 5.8% less distance (de Carvalho et al., 2019). In this study CF may have reduced any potential oxidative stress that would be associated with training, exercise or the EIMD protocol, which may subsequently delay fatigue.

Even though CF supplementation may improve distance covered in a set amount of time, it may not improve performance related to completing a set amount of work or distance in a time trial setting. Decroix et al., (2017), Decroix et al., (2018) and Stellingwerff et al., (2013) found no significant differences between groups (CF vs placebo) for time trial performance. However, Decroix and colleagues (2017) observed that in a crossover design the CF group tended to complete the first of the two time trials faster (29:47 min placebo vs 29:13 min cocoa), although statistical significance was not reached. It is difficult to ascertain whether the 34 s difference between groups is meaningful, as the trial involved participants completing a set amount of work equivalent to cycling at 75% peak power output for 30 min as fast as possible. As each time trial would have been individualised to each participant any practical conclusions are difficult to make other than that CF may have allowed participants to maintain a

slightly higher power output than a placebo (Decroix et al., 2017). The CF group also produced a higher power output after 25 min (for the final ~5 min of the first time trial) compared to placebo (PLA 73.09% vs CF 76.75% of maximal power output). Decroix et al., (2018) found no differences for rating of perceived exertion, heart rate, lactate or work performed (kilojoules) within the 20-minute time trial between groups in normoxic or hypoxic environments. Interestingly, Stellingwerff et al., (2013) found that performance increased for seven participants following CF supplementation whereas another seven had improved performance following ingestion of the placebo. This may suggest that some individuals are potential 'non-responders' to CF supplementation, or that the differences seen were due to chance and not the allocated treatments. Other studies that investigated performance and CF supplementation found no significant differences between groups for 5 km time trial performance or $\dot{V}O_{2max}$ (Fraga et al., 2005; Patel et al., 2020; Peschek et al., 2013). However, recent work by Sadler et al., (2020) suggests that 400 mg daily CF supplementation for seven days improves oxygen uptake during moderate-intensity exercise, but this benefit was not observed during high-intensity exercise. Additionally, after three-months of supplementing 175.2 mg/day of CF, Taub et al., (2016) observed an increase in participants' $\dot{V}O_{2max}$ by 2.8 ± 1.2 ml kg^{-1} min^{-1} and power values (140.7 ± 11.6 to 148.3 ± 11 watts), whereas there were no significant differences in the placebo group.

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Table 3.6 The effect of CF supplementation on exercise performance

Reference	Participants	Nutritional Intervention	Supplementation period	Exercise stimulus	Measure(s)	Key outcome(s)
de Carvalho et al., (2019)	13 trained males Age 21 ± 2 years Stature 180 ± 0.05 cm Mass 87.02 ± 8.03 kg	CF: CHO + protein cocoa beverage, 306 mg CF per beverage CON: cocoa based CHO + protein beverage, 0 mg CF	7 days, beverage consumed twice daily	Five sets of 20 drop jumps from 0.6 m, 10 s between jumps and 2 min interset rest	Yo-Yo intermittent test	↔ between groups CF group ↑ 9.85% compared to baseline CON ↓ 5.8% compared to baseline.
Decroix et al., (2017)	12 well-trained males Age 30 ± 3 years Stature 177.9 ± 8.8 cm Mass 72.8 ± 7.8 kg $\dot{V}O_{2max}$ 63.0 ± 3.5 ml·kg^{-1} min^{-1}	CF: cocoa drink, 900 mg CF (EPI: 185 mg, CAT: 20 mg) CON: placebo, 15 mg CF (EPI: 0 mg, CAT: 0 mg)	Acute dose 1.5 pre-exercise	Two 30 min time trials 60 min apart, performed at a ~75% peak power output.	i) Time trial ii) PPO	i) ↔ between groups. ii) PPO ↑ after 25 min in the 1st time trial for CF
Decroix et al., (2018)	14 well-trained males Age 31 ± 3 years Stature 180 ± 5 cm Mass 73 ± 7 kg $\dot{V}O_{2max}$ 62.9 ± 5.8 ml·kg^{-1} min^{-1}	CF: Capsule, 530 mg CF (EPI: 100 mg, CAT: 21 mg) CON: 1,764 mg maltodextrin	Consumed daily for six days and then a seventh on the day of testing	20 min steady state cycling at 45% peak power output 20 min time trial beginning at 75% peak power output	Time trial	↔ between groups

	Peak Power Output 366 ± 45 W			Completed in normoxic and hypoxic environments		
Fraga et al., (2005)	28 trained males Age 18 ± 1 years Mass 74 ± 1 kg	CF: 105 g chocolate confectionery, 168 mg CF (EPI + CAT: 39 mg) CON: 105 g cocoa butter chocolate, <5 mg CF	Sub-chronic, 14 day consumption	Soccer training sessions twice per week and one match per week	$\dot{V}O_{2max}$ shuttle run	↔ between groups
Patel et al., (2015)	9 trained males Age 21 ± 1 years Stature 177 ± 9.4 cm Mass 76.0 ± 9.3 kg $\dot{V}O_{2max}$ 41.89 ± 5.4 $ml \cdot kg^{-1} min^{-1}$	CF: 40 g dark chocolate, 259 mg CF CON: 40 g white chocolate	Sub-chronic, 14 days consumption	20 min cycling at 80% of gas exchange threshold followed by a 2 min maximal sprint time trial	Time trial	A 17% ↑ in distance covered was observed following CF supplementation.

Patel et al., (2020)	15 healthy (10 males, 5 females) participants Age 30 ± 7 years Stature 176.8 ± 8.6 cm Mass 80.3 ± 8.4 kg $\dot{V}O_{2max}$ Males: 51.1 ± 3.5 Females: 41.6 ± 5.5 ml·kg^{-1} min^{-1}	CF: dark chocolate, 1060 mg CF, 764 mg CF, or 406 mg CF CON: 88 mg CF	Acute ingestion, 2 hr pre-exercise	2-min incremental cycling warm-up until 80% of GET then maintained for 40 min. Followed by an incremental test to failure	*i)* $\dot{V}O_2$ *ii*) PPO	*i)* ↔ between treatment *ii)* ↔ between treatment
Peschek et al., (2013)	8 well-trained males Age 25 ± 6 years Stature 182.1 ± 6.3 cm Mass 73.4 ± 7.0 $\dot{V}O_{2max}$ 64.4 ± 7.6 ml·kg^{-1} min^{-1}	CF: Cocoa based CHO protein beverage with added cocoa, 350 mg CF CON: cocoa based CHO protein beverage, 0 mg CF	Acute ingestion of two beverages separated by 2 hr post-exercise protocol	30 min downhill running at a -10% gradient at 70% $\dot{V}O_{2max}$	5 km time trial	↔ between treatments.
Sadler et al., (2020)	17 healthy (11 males, 6 females) participants Age 45 ± 6 years Stature 162 ± 0.1 cm Mass 68.2 ± 17.7 kg	CF: capsule containing 100 mg CF (EPI+CAT: 22 mg)	Four capsules taken daily (two in the morning and two in the evening) for seven consecutive days Four capsules consumed 45 min prior to arrival at the	6 min cycling at 80% GET threshold x 3 and 1 bout of cycling at 60% of the difference between GET and $\dot{V}O_{2peak}$ until exhaustion	*i*) t$\dot{V}O_2$ *ii*) ET	*i*) 15% ↓ in CF group than CON *ii*) ↔ between treatments for ET

			lab on the day of the protocol (7th day)			
Stellingwerff et al., (2013)	16 healthy males Age 30 ± 6 years Stature 179.9 ± 7.8 cm Mass 72.8 ± 6.0 kg $\dot{V}O_{2peak}$ 56.3 ± 5.7 ml·kg^{-1} min^{-1}	CF: 561 Kcal dark chocolate, 240 mg CF (EPI: 89 mg, CAT: 24 mg) CON: chocolate ~0 mg CF	Acute ingestion 2 hr pre-exercise	Cycled for 2.5 hr at ~45% $\dot{V}O_{2max}$, followed by 15 min time trial	Time trial	↔ between treatments.
Taub et al., (2016)	17 sedentary (9 males 8 females) participants CF: Age 50 ± 3 Stature 168 ± 3 Mass 78.8 ± 5.6 $\dot{V}O_{2max}$ 22.9 ± 1.9 ml·kg^{-1} min^{-1} CON: Age 50 ± 2 Stature 175 ± 5 Mass 92.2 ± 9.7 $\dot{V}O_{2max}$ 24 ± 1.7 ml·kg^{-1} min^{-1}	CF: 20g dark chocolate, 175.2 mg CF (EPI: 26 mg, CAT: 4.6) CON: 20g placebo chocolate	Chronic (3 months daily intake)	Cycling exercise including $\dot{V}O_{2max}$	*i)* $\dot{V}O_{2max}$ ii) Power	i) Significant ↑ in CF vs CON ii) CF significant ↑, CON ↔

Note: CF = cocoa flavanols, EPI = epicatechin, CAT = catechin, CON = control, CHO = carbohydrate, PPO = peak power output, t$\dot{V}O_2$ = time constant of the fundamental phase of $\dot{V}O_2$ kinetics, ET = exercise tolerance, ↑= increase, ↓= decrease, ↔ = no significant effect/change

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3.4.6 Practical Recommendations and Future Research

The available data suggests it may be beneficial to ingest a moderate dose of CF pre-exercise, with benefits effects on oxidative stress observed at doses ~200 mg acutely and if taken more longer term in the lead up to exercise. Higher doses of (poly)phenols may elicit greater physiological effects *in vivo* (Bowtell & Kelly, 2019) and for CF dosage the amount of epicatechin is an important factor when considering supplementation (≥50 mg).

To maximise absorption and bioavailability, CF can be ingested as part of a beverage as opposed to a solid (e.g., high flavanol powder dissolved into a beverage instead of solid dark chocolate), potentially due to the faster gastric emptying associated with liquids (Cifuentes-Gomez et al., 2015). The bioavailability and absorption of flavanols can be further improved *via* the simultaneous consumption of carbohydrates, as consuming ~4 $kcal \cdot kg^{-1}$ body mass alongside CF increases flavanol concentrations in the plasma by 40% (Badrie et al., 2015; Schramm et al., 2003). Carbohydrates stimulate and activate SGLT-1 and lactase phlorizin hydrolyase both of which are involved in flavanol absorption and metabolism (Bohn, 2014; D. D. Schramm et al., 2003). From a practical perspective, the consumption of CF concurrently with carbohydrates post-exercise may lead to the benefits of both replenishing glycogen stores and accelerating recovery following muscle damaging exercise.

Future studies should look to investigate the muscle recovery process post EIMD alongside the supplementation of CF. A focus should be placed on whether regular (daily) supplementation of high doses of CF (>750 mg) can affect perceived soreness, oxidative stress, and inflammation post EIMD, and whether it can influence repeat performance, fatigue, and perceived effort. Comparisons between different doses and thus establishing of an optimal dose to elicit benefits is needed before concrete recommendations can be made. It is also important that studies investigating EIMD should use protocols that evoke sufficient muscle damage (e.g., inflammation, muscle soreness). Although, such protocols may not be applicable to real world sport, they will be useful for determining the potential mechanisms by which CF might alter physiology and enhance exercise performance and recovery. Nevertheless, studies should also investigate the effect of CF supplementation on recovery following real world exercise or movements that can induce muscle damage (e.g., repeated sprint protocols) instead of solely laboratory-based protocols that may not replicate the

demands or damage response that follows sporting performances. This may lead to greater practical application within sport settings. Utilising both variants of EIMD protocol approaches will aid understanding of the potential ergogenic value of supplementing CF in an athlete's diet. Furthermore, females participants should be included in more research to better understand any inter-sex differences between males and females for muscle recovery following EIMD, within this review only three studies included females (Patel et al., 2020; Sadler et al., 2020; Taub et al., 2016)

It may be pertinent to investigate prolonged flavanol supplementation on repeated bouts of exercise, with a focus on performance and recovery. Moreover, investigating the impact that CF may have on exercising muscle is required to develop greater understanding of the mechanisms in which CF exert any effects, such as their influence on endogenous antioxidant enzymes and survival signalling proteins. Indeed, future research should also look to further the knowledge of CF and their role in signalling pathways such as NF-kβ and Nrf2, and how the regulation of these pathways may attenuate muscle damage.

3.5. Conclusion

Few studies have examined the effects of CF on recovery following EIMD. Of the available data acute and sub-chronic (~7-14 day) supplementation of CF *via* dark chocolate solids or in the form of a high flavanol beverage reduces exercise-induced oxidative stress and has potential for delaying fatigue during exercise allowing for prolonged performance. However, data on recovery of muscle function, and the analgesic and anti-inflammatory effects of CF is limited. Research should look to investigate these areas further to identify if CF are viable as an ergogenic aid used for recovery and potentially performance.

Chapter 4 General methods

This Chapter will enumerate the methods utilised in the studies that form the basis of this PhD thesis. It will cover the participants and recruitment strategies employed, as well as dietary assessment and nutritional intervention, muscle damage protocol and measures used to measure recovery. Before any recruitment began, institutional ethical approval was obtained. Following this, participants were informed of the study protocols and provided written consent before any testing was performed.

4.1 Participants

Prior to each study a power calculation was performed using G*Power (Version 3.1.9.7, Universität Dusseldorf, Germany; (Faul, Erdfelder, Buchner, & Lang, 2009)) using data from previous research (Bowtell et al., 2011) in an attempt to allow for discernible conclusions that are demonstrable based on the findings. Each investigation utilised healthy, male, and female University students between 18 and 38 years old. For the studies in Chapters 5, 6, and 7, the participants had to be recreationally active, this was defined as performing regular physical activity or exercise at least two days a week, e.g., running or resistance training.

Participants were recruited in various ways, these included: posters, recruitment talks within lectures or seminars, and *via* email. To be eligible to take part in the studies, further inclusion criteria existed as follows: *i)* no lower limb injury within the previous six months, *ii)* the individual did not perform regular (>5 days a week) heavy resistance training, *iii)* was not currently taking anti-inflammatory or pain medication, *iv)* had no other health contraindications that would prevent them from performing exercise, and for females specifically *v)* was not pregnant. During involvement in a study, the participant was required to refrain from exercise (including 48 hr before a laboratory visit), pain medication, nutritional supplements (e.g., Vitamin C), recovery treatments (e.g., massage) and avoid taking anti-inflammatory drugs.

Randomisation was performed using a stratified randomisation procedure based on baseline MVIC values, assigning participants into ‘strong’ or ‘not strong’ strata, before randomisation into a group. To determine the thresholds for the strata, a normative MVIC strength index was consulted [Risberg et al., (2018) for females and Ruas, Minozzo, Pinto, Brown, and Pinto (2015) for males]. Following this, participants were allocated into groups using online randomisation software (random.org). For Chapters 5 this was performed by the thesis author and for Chapter 6 this was performed by an

independent laboratory technician and the thesis author remained unaware of assignment until data analysis.

4.2 Dietary control and analysis

Throughout each of the exercise studies (Chapters 5, 6, and 7) participants completed a 24-hr dietary recall with a trained researcher at the end of every laboratory visit. A dietary recall was chosen to reduce the participant burden that comes with other alternative methods, for example a weighed food diary (Louise M Burke, 2015). A single pass method was selected alongside a full review of the completed recall during each day of testing to ensure the participant had not forgotten any foods/drinks. To further accuracy, time and detail of the meal was taken to 'walk participants through their day' during the review. This method is not without any limitations as it relies on participant memory and food knowledge, as well as having an increased risk of selection bias when recalling foods. For the duration of the studies, participants were asked to consume their typical diet whilst refraining from food and drinks high in (poly)phenols. These included: chocolate, various berries, tea and green tea, red wine, cherries, and lychees. Participants were provided a list of foods to be excluded during the time frame of the study. This was to limit any confounding effects of other dietary (poly)phenols on muscle recovery. This method of tracking dietary information is not without limitations and participant diets could not be controlled entirely. Indeed, on one occasion a participant within Chapter 5 consumed coffee on one of the testing days and were reminded to refrain from breaching protocol again.

Participant diets were analysed for macronutrient content and compared between groups to examine any potential differences in energy, carbohydrate, protein, or fat intake throughout each study. This was to ensure that the groups did not differ significantly, therefore not strongly influencing the outcome of the studies. As such, potential conclusions inferred from the data could be made with the knowledge that dietary intake was not a confounding influence. Dietary analysis was performed using online nutritional analysis software called Nutrimen (Dark Green Media Ltd, ©2016).

4.3 Cocoa flavanol intervention

The nutritional intervention that forms the basis of this thesis involved CF. The cocoa powder used was commercially available known as Chococru Extraordinary Flavanol Cocoa (Chococru© Extraordinary Flavanol Cocoa), containing ~8.3% flavanols and a total (poly)phenol content of ~12% (unpublished data from Chococru©). All drinks

involved in this thesis contained a base amount of 25 g whey protein and 60 g maltodextrin (both Myprotein, Manchester, UK) and water. As the control beverage contained whey that was chocolate flavoured (Chocolate Smooth, Impact Whey Protein Concentrate, Myprotein) it maintained both a similar taste and appearance to the test beverages. It must be noted that due to the contents of the control it cannot be referred to as a placebo, however, it does allow the drinks to represent a more realistic recovery drink from an athletic scenario (Burke, 1997). Furthermore, the results of the studies in Chapters 5, 6, and 7 provide an insight as to whether CF provide additional recovery benefits to a standard exercise recovery drink. The control used throughout this thesis contained whey protein, maltodextrin, and water. It should be noted that the products used within this thesis are not Informed Sport tested and therefore, may not be appropriate for athletes who are tested for banned substances.

4.3.1 Cocoa flavanol intervention in Chapters 5

In Chapter 5, two flavanol drinks were used with the only difference being 5g of cocoa powder (drink 2 contained 10g and drink 3 contained 15g; see Table 4.1 for a nutritional breakdown of the beverages). Drink 1 indicates control. As these Chapters were single-blind none of the participants were aware of the group they were assigned to and only informed that they would receive a chocolate flavoured beverage post-exercise. Furthermore, due to the independent groups design of the studies, each participant only received one of the beverages and therefore, had no frame of reference as to which they may have received.

4.3.2 Cocoa flavanol intervention in Chapter 6

For Chapter 6, the study was double blinded and as such the bottles containing the ingredients to each drink was wrapped in opaque duct tape and powder mixed together to prevent the contents being easily identified. An independent laboratory technician was responsible for randomising the groups and assembling the drinks.

Table 4.1 Nutritional information of treatment beverages

Drink	kcal/kj	CHO (g)	Pro (g)	Fat (g)	Flavanol (mg)	ORAC
1	340/1427	61.9	19	1.9	Nil	Nil
2	366/1531	63.3	21.4	2.9	830	20,000
3	379/1589	64	22.6	3.4	1245	30,000

Note: All drinks contain 60 g of maltodextrin and 25 g chocolate smooth whey protein powder, drinks 2 & 3 contain 10 g and 15 g of Chococru powder respectively; ORAC = oxygen radical absorbance capacity. Drinks 1, 2, and 3 were used within Chapters 5. Drinks 1 and 3 were used within Chapter 6

4.4 Muscle damage protocol

To induce muscle damage in Chapter 5 and 6, a validated protocol was adapted from previous research (White et al., 2008). The protocol targeted the knee flexors (hamstrings) muscle group to induce EIMD. The protocol consisted of five sets of ten maximal concentric and eccentric unilateral hamstring curls, repeated on each leg, using a HUMAC Cybex Norm isokinetic dynamometer (CSMi, Boston, Massachusetts). Participants were seated at a hip angle of 85 degrees, then secured into the dynamometer using a torso seatbelt and thigh strap to limit any hip involvement during the contraction. The lateral femoral condyle was positioned parallel to the dynamometer's centre axis of rotation. Following this, the rotational arm was strapped to the ankle of the working limb roughly one inch above the lateral malleolus. The participant then actively extended and flexed the knee to demonstrate an appropriate alignment of the dynamometer. This is an important step when assessing knee flexion as it has been shown that a deviation, whether vertically or horizontally, from appropriate alignment can impact peak torque output leading to an error of measurement (Houweling & Hamzeh, 2010). All measurements were noted on a participants' first visit to the laboratory so that the position could be replicated on each day of testing.

In an attempt to familiarise a participant with the protocol and the uniqueness of an eccentric contraction on the dynamometer, each individual performed a set of 15 concentric eccentric hamstring curls at a self-perceived low effort level. This is considered an adequate method for familiarising an individual to the protocol (Impellizzeri, Bizzini, Rampinini, Cereda, & Maffiuletti, 2008). To warm up, each participant performed a series of dynamic hamstring stretches and a sub-maximal warm up set of ten concentric eccentric hamstring curls. During the protocol participants were verbally encouraged during each repetition, as well as being reminded to maintain maximum effort throughout. To monitor effort levels, participants were asked to rate their perceived level of exertion using a Borg 6-20 scale (Borg, 1982), following individual sets. To reduce the chance of an order effect influencing

results, participants were asked to rate their exertion spontaneously and not following the completion of each set.

4.5 Measuring muscle function

To measure changes in muscle function, MVIC of the non-dominant leg were used in Chapters 5 and 6. To measure MVIC of the knee flexors the same isokinetic dynamometer (CSMi, Boston, Massachusetts) was utilised. Participants took up a seated position and the same procedure discussed in Section 4.4 for aligning the dynamometer was appropriately followed.

Participants then set their full range of motion from full knee extension (anatomical zero) to individual full knee flexion, commonly between 95 to 115 degrees. MVIC was performed at two separate joint angles, 60 degrees and 30 degrees knee flexion; 0 degrees being anatomical zero. The multiple joint angles were chosen for two reasons; *i)* the hamstrings individual activation varies at different angles, biceps femoris is more effective at decreased angles (e.g., 60-90) whereas semitendinosus and semimembranosus have optimum angle ranges between 40-30 degrees and *ii)* selecting only one angle could potentially over or under-estimate changes in peak torque (Paulsen et al., 2012). Therefore, incorporating multiple angles may allow for a greater insight into the functional changes of the hamstrings. Each angle for MVIC involved three, five second contractions with each being separated by 30 seconds rest, totalling six MVICs. Participants were instructed to 'pull' as hard as they could once instructed to begin. Verbal encouragement was provided during each contraction to aid with maximal exertion and was consistent between participants. Only the peak torque values were used for data analysis.

4.6 Muscle activation

Surface electromyography (EMG) was utilised within this thesis to measure hamstring muscle activation and recorded using wireless surface EMG sensors (Inter-electrode distance 10mm; Trigno™, Delsys Inc, USA) The biceps femoris long head was selected for data analysis, both semitendinosus and semimembranosus were omitted. This was due to the location of the muscles and potential error regarding surface EMG placement on the individual muscles increasing the chance of crosstalk and as a result, measurement error. To identify the biceps femoris muscle a participant lay prone on a plinth, with a researcher then locating the distal portion of the muscle tendon connecting to the proximal aspect of the fibula. From there the researcher

began to raise the lower leg of the participant, following the tendon *via* lateral palpation towards the ischial tuberosity and eventually stopping at the muscle belly, with the participant actively flexing the hamstrings to aid with identification. This was followed by continued lateral palpation as the participant lowered the limb slowly and the placement of the EMG device. To aid with repeat identical placement on subsequent testing days a semi-permanent marker was used to outline the EMG device, this outline remained visible on subsequent visits and reapplied if beginning to fade. To prepare for EMG device placement the following was performed: shaving foam was applied to the posterior of the thigh and a moderate portion, roughly $10cm^2$, of hair was shaved from the back of the participants leg (if required). The area was then cleaned with an alcohol wipe to remove any debris and sanitise the site. This was to reduce any potential noise that hair or debris could elicit and interfere with data collection. The EMG device was then attached to the muscle belly using specialist sticky tape made specifically for the devices provided by Delsys Inc.

The exercise task selected for the measurement of muscle activation was a glute-hamstring bridge beginning at a knee angle of 60 degrees. The exercise involves the participant extending at the hips to raise them off the floor, creating a diagonal line from the knees, hips, and shoulders and were instructed to maintain this peak position for five seconds, timed independently by a laboratory technician. EMG data collection began two seconds before contraction and ceased two seconds after allowing for an obvious beginning and end for data analysis. Participants performed this three times each testing session during Chapter 6.

Data analysis for EMG consisted of muscle activation and median frequency data. To perform this analysis EMGworks analysis software (EMGworks®, Version 4.7.9, Delsys, USA) was utilised, following the methods used by (Starbuck & Eston, 2012). In brief, each individual EMG graph was first filtered *via* a band pass filter set at 12-450 Hz and applied to the raw data, following this the data was rectified using root mean squared with a 0.1 s time constant. The peak and mean values were recorded for each exercise bout. Additionally, after filtering each graph was assessed for median frequency using a 0.1 s time constant. The median frequency and peak median frequency were recorded.

4.7 Measuring muscle soreness

To measure changes in muscle soreness a VAS and LEFS were implemented in Chapters 5 and 6. The VAS utilised within this thesis was a 200mm ruled line with three anchor points across it. The far left at 0mm had the anchor point 'no pain', at 100mm the anchor point 'moderate pain' was included and on the far right, at 200mm, the anchor point 'extreme pain'. The inclusion of a mid-point for pain identification and the avoidance of absolute anchor points such as 'worst possible pain' have recently been recommended for research that involves the measurement of pain using VAS (Reed & Van Nostran, 2014). When making a mark on the line participants were instructed to contract the hamstrings by flexing at the knee, raising their foot off the floor towards the hips. This allowed participants to better judge the soreness in the hamstrings, otherwise they remain in a passive state when standing stationary and may underestimate soreness. Furthermore, the use of three anchor points allowed for a more considered response regarding subjective pain.

The LEFS involved 20 hypothetical activities that range from everyday activities, e.g., rolling over in bed, to more athletic tasks such as making sharp changes of direction. Each activity is rated from 0 to 4 with the following ratings: 0 = extreme difficulty or unable to perform the activity, 1 = quite a bit of difficulty 2 = moderate difficulty, 3 = a little bit of difficulty and 4 = no difficulty. The use of these two methods allowed for a greater insight into the soreness of the participants involved in the study, as discussed in Chapter 2 Section 3.2. A score of 80 indicates there is no issue regarding perceived muscle function or muscular discomfort, whereas reductions from 80 indicate a decline in perceived muscle function or increase in muscular discomfort.

4.8 Assessing menstrual cycle

The assessment of the menstrual cycle is an important aspect to consider for exercise research. Testing for females was carried out during the luteal phase in participants who followed the common menstrual cycle hormonal phases. The luteal phase was selected for various reasons: *i)* it is the longest phase within the cycle, *ii)* the phase is similarly constant in length amongst women and *iii)* to avoid the peak in oestrogen observed pre-ovulation (Reed & Carr, 2018). To identify this timepoint each female participant completed a menstrual cycle questionnaire, using this data a prediction could be made to schedule testing to coincide with the mid-luteal phase for the individual participant. To estimate the mid luteal phase, eight days were added on to

day 10 from the beginning of each participant's menstrual cycle (Wideman et al., 2013). This method is easily implemented and has a relatively high level of accuracy, however, in an ideal setting hormonal testing would have been carried to track the phase of each individual participant due to its greater precision (Wideman et al., 2013). For individuals on different contraceptives that disrupt the natural hormonal profile of the menstrual cycle an equivalent time point was selected. For example, a participant supplementing the combined pill would take part during the 21 days of supplementation to avoid the drop off in exogenous hormones that occurs following cessation of the pill for six days.

4.9 Statistical Analysis Approach

For statistical analysis, an *a priori* decision was made on the statistical tests that would be selected for the prospective data of the studies and the structure of the data that would be collected. To this end, repeated measures designs were the most appropriate using mixed analysis of variance to assess for within and between subject differences across multiple time points. Once collected, all data was assessed for normality to determine whether parametric or nonparametric analysis would be performed. For post hoc analysis Fisher's least significant difference was selected to locate the differences in the event of a significant time and interaction effect. Additionally, an *a priori* power analysis was carried out for both studies to determine the appropriate sample size for both Chapters 5 and 6, see Chapter 5 Section 2.8 and Chapter 6 Section 2.10 for more details.

Chapter 5 Acute consumption of varied doses of cocoa flavanols on indices of muscle recovery following exercise-induced muscle damage in active males and females

This Chapter has been published as an individual paper, reference

'Corr, L. D., Field, A., Pufal, D., Killey, J., Clifford, T., Harper, L. D., & Naughton, R. J. (2020). Acute consumption of varied doses of cocoa flavanols does not influence exercise-induced muscle damage. International journal of sport nutrition and exercise metabolism, 30(5), 338-344.'

It has been amended to be consistent with the thesis. As lead author I wrote the article, as well as conducted the data collection and analysis. The co-authors aided with study conceptualisation during the initial phases of the PhD and provided feedback on the writing.

5.1. Introduction

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

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

Regarding muscle recovery and exercise, research has shown that acute (single dose on day of exercise stimulus) and sub-chronic (regular intake for ≥14 days) CF

supplementation of ≥200 mg reduces exercise-induced oxidative stress (Allgrove et al., 2011; Davison et al., 2012), see Chapter 4 Section 4.1 for more details. Furthermore, in relation to exercise, the ingestion of CF may improve sprint performance by potentially preventing ROS-increased calcium sensitivity of myofilaments within working muscles, therefore, delaying fatigue (de Carvalho et al., 2019; Patel et al., 2015). However, evidence is lacking regarding the impact of CF on markers of muscle recovery, such as perturbations in muscle function and an increase of perceived soreness (see Chapter 4 Sections 4.3 and 4.4). One such study used a CF dose too low to be effective - 74 mg CF and 8 mg epicatechin (Morgan et al., 2018). Benefits begin to be observed at doses of ~700 mg CF; and more importantly, with >50 mg epicatechin, the most biologically active flavanol (Schroeter et al., 2006). However, an optimal dose is not yet known in addition to any potential dose response. Furthermore, previous research that investigated the impact of CF on muscle recovery did not induce notable muscle damage using a drop jumps protocol (de Carvalho et al., 2019) and a downhill running protocol (Peschek, Pritchett, Bergman, & Pritchett, 2013). This can be defined as reductions in muscle force-generating capability of ≥20% following EIMD (Paulsen et al., 2012). Therefore, making conclusions about the impact of CF on markers of muscle damage is difficult, indicating that more research is warranted. Furthermore, none of the previous studies involved female participants, likely due to the purported protective effects of oestrogen against muscle damage (Tiidus, 2003) and physiological variations across the menstrual cycle (Hayashida, Shimura, Sugama, Kanda, & Suzuki, 2016). Therefore, investigating the effect of CF supplementation on muscle recovery in females is required.

Females experience a menstrual cycle leading to hormonal fluctuations over the course of ~28 days, split into the follicular phase and the luteal phase. The follicular phase can be further divided into the early follicular (onset of menses) which is known to have low levels of oestrogen and progesterone, and late follicular phase (following menses until ovulation), which is known for a rapid rise and peak in oestrogen concentrations and continued low levels of progesterone. The luteal phase begins post-ovulation and lasts until the onset of the subsequent cycle, this phase is known for a secondary peak in oestrogen around day 20 (day one is considered the first day of menstruation) and a rise in progesterone also, these concentrations are relatively consistent until menses (Mihm et al., 2011).

Oestrogen is known to have an anabolic effect on skeletal muscle (Enns & Tiidus, 2010), aid with the maintenance of muscle function (Kitajima & Ono, 2016), have a neuroexcitatory effect which may lead to increased contractile capability (Ansdell, Brownstein, Škarabot, Hicks, Simoes, et al., 2019), influence substrate utilisation (Lundsgaard & Kiens, 2014) and may have a role in affecting mood state (Birkhaeuser, 2018). In the context of exercise recovery oestrogen can act as an antioxidant and aid with the stabilisation of muscle membranes (Kendall & Eston, 2002) potentially reducing the impact that ROS may have and as such limiting the level of lipid peroxidation. This is likely *via* the presence of a free phenolic group on the molecule, providing oestrogen the capacity to quench ROS (Chainy & Sahoo, 2020). The stabilisation of muscle membranes may also lead to a reduction in the leakage of intracellular proteins following the mechanical stress to the muscle fibres and as such may limit the inflammatory response post-exercise (Enns & Tiidus, 2010).

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

5.2. Methods

Main overview of methods is contained within Chapter 4, as such this section will report methods in brief.

5.2.1 Participants

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

5.2.2 Study Design

The study was a laboratory-based, randomised, single-blind, nutrient-controlled trial. Participants were randomised into a control (CON), high (CF_{830} = 830 mg CF) or supra (CF_{1245} = 1245 mg CF) group and remained unaware of their allocation for the entirety of the study. Participants were required to visit the laboratory for five days; the first visit was to conduct baseline testing and familiarisation of the EIMD protocol (ten sub-maximal concentric-eccentric hamstring curls). The remaining four days took place consecutively; as such, measures were taken in the following order: baseline, immediately post-EIMD (0 hr), 24, 48 and 72 hr post-EIMD. For a visual representation of the study design, see Figure 5.1. For randomisation, participants were assigned to

separate strata, ‘strong’ and ‘not strong’, based on their baseline MVIC values and randomised into matched and counterbalanced groups (using random.org). To decide what could be classified as strong or not, a normative MVIC strength index was used [Risberg et al., (2018) for females and Ruas et al., (2015) for males]. Following this, eight participants were allocated to the control group (four females, four males), eight to the CF_{830} group (five females, three males), and seven to the CF_{1245} group (four females, three males). For participant characteristics see Table 5.1. Participants were also compared as separate groups based on sex, creating two subgroups within each treatment group. For sex specific participant characteristics see Table 5.2.

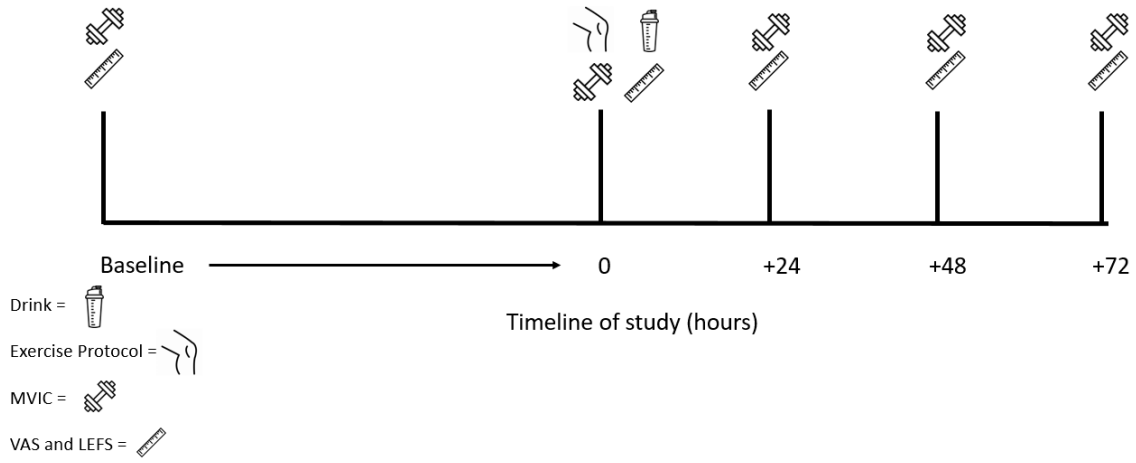

Figure 5.1. Study schematic detailing experimental timeline

Table 5.1 Participant characteristics

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

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

Table 5.2 Participant Characteristics separated by sex

Group	Sex (N)	Age (years)	Height (cm)	Weight (kg)
CON	F (4)	22 ± 5	168 ± 6	61 ± 13
	M (4)	26 ± 3	181 ± 2	86 ± 6
CF_{830}	F (5)	27 ± 6	164 ± 7	62 ± 10
	M (3)	22 ± 3	176 ± 7	77 ± 5
CF_{1245}	F (4)	24 ± 7	159 ± 6	58 ± 11
	M (3)	23 ± 3	179 ± 5	74 ± 10

Notes: Means ± standard deviations, F = females, M = males

5.2.3 Muscle Function

Values were recorded for knee flexor MVIC using the isokinetic dynamometer (Cybex NORM®, Model 770, CA, USA), providing a reliable quantification of decrements in muscle function for assessing EIMD (Warren, Lowe, & Armstrong, 1999). Knee angles of 60° (MVIC60) and 30° (MVIC30) of the anatomical zero (full knee flexion) were selected due to the differences in muscle activation at various knee angles; biceps femoris has increased activation at reduced angles, whilst semitendinosus and semimembranosus at greater knee angles (Onishi et al., 2002). For more detail see Chapter 4 Section 5.

5.2.4 Subjective Soreness

Soreness was recorded using a 200 mm VAS, which has been previously included as a validated measure of subjective soreness (Peschek et al., 2013). The LEFS is a validated questionnaire which quantifies an individuals perceived level of muscle function using 20 hypothetical activities that are scored from 0 to 4; 0 = extreme difficulty; 4 = no difficulty. For more detail see Chapter 4 Section 7

5.2.5 Muscle Damaging Protocol

The exercise protocol used to induce muscle damage was adapted from White et al., (2008) using the Cybex Norm Isokinetic Dynamometer (CSMi, Boston, Massachusetts). For more detail see Chapter 4 Section 4.

5.2.6 Nutritional Intervention

Participants were blinded to which group they were assigned, with only the lead researcher being aware of the contents of each drink. Participants consumed their

assigned beverage within five minutes following the protocol. Each beverage consisted of 300 ml water, 60 g maltodextrin and 25 g whey protein powder (20 g protein). The cocoa powder used was a commercially available high flavanol powder (Chococru© Extraordinary Flavanol Cocoa), containing ~8.3% flavanols and a total (poly)phenol content of ~12% (unpublished data from Chococru©). The beverage for CF_{830} included an additional 10 g of Chococru© cocoa powder which contained 830 mg CF (98.6 mg epicatechin) and for CF_{1245} 15 g of Chococru© cocoa powder was added, containing 1245 mg CF (149.4 mg epicatechin). See Chapter 4 Section 3 (Table 4.1) for nutritional breakdown of the test beverages.

5.2.7 Dietary Measures

Participants completed a 24-hr dietary recall each day of testing, totalling five food recalls, and were asked to continue eating their usual diet throughout testing. During baseline testing, participants were provided a list of high (poly)phenolic food and drink (cherries, blueberries, dark chocolate, green and black tea, wine, apples, lychees, pomegranates and fruit juices) to refrain from consuming three days before and during the testing period, reducing the confounding influence of other dietary (poly)phenols on recovery (Scalbert et al., 2005). Dietary analysis was carried out using Nutrimen (Dark Green Media Ltd, ©2016). For more detail see Chapter 4 Section 2.

5.2.8 Statistical analyses

Statistical analysis was performed using IBM SPSS Statistics (version 24, IBM Corp., Armonk, N.Y., USA). All data was assessed for normality using a Shapiro-Wilk test and quantile-quantile plots were examined to establish whether the data was normally distributed. A Greenhouse-Geisser correction was used if sphericity was violated. A mixed analysis of variance was used to determine interaction and time effects for the recovery variables. Furthermore, sub-group analysis of intra and inter-sex differences were performed for Chapter 5b. If any significance was observed, Fisher LSD post hoc testing was performed to identify the point of significance. Data for MVIC60 and MVIC30 was calculated as percentage change from baseline alongside absolute means. To calculate effect sizes, Cohen's d (*d*) was utilised, with the magnitude of effects considered small (0.2), moderate (0.5) and large (0.8). Significance was set at $p \leq .05$ pre-analysis. Descriptive statistics are reported as means (MVIC also displayed as percentage change %) ± standard deviation (SD).

5a.3. Results

There were no significant differences for participant characteristics; height (p = .33), weight (p = .46) and age (p = .88) or dietary intake; energy (p = .98), protein (p = .99), CHO (p = .60), or fat (p = .57) between groups. See Table 5.3 for dietary intake.

Table 5.3. Dietary intake between groups

	CON	CF_{830}	CF_{1245}
Energy (kcal)	2137 ± 559	2101 ± 394	2164 ± 591
Protein (g)	109 ± 49	106 ± 47	106 ± 43
CHO (g)	227 ± 46	253 ± 41	265 ± 106
Fat (g)	93 ± 32	81 ± 19	79 ± 21

Note: Group mean ± SD

5a.3.1 Muscle function

Muscle function measured using MVIC at 60° and 30° found a main effect of time for MVIC60 (p = .002) and MVIC30 (p = .002), both data were normally distributed. For MVIC60 significant differences between baseline and 0, 24, 48, 72 hr (p ≤ .001), 0 and 48 (p = .03), 24 and 48 (p = .002), and 48 and 72 (p ≤ .001) were observed. For MVIC30 significant differences between baseline and 0, 24, 48, 72 hr (p ≤ .04), 0 and 48 (p =.01), 24 and 48 (p = .01), and 48 and 72 (p = .001). There were no significant differences between groups for knee flexor peak torque at MVIC60 (p = .99) or MVIC30 (p = .95) at baseline. Following the exercise protocol, overall mean knee flexor peak torque reduced to 79% of baseline. There were no significant differences between groups for MVIC60 ($F(2,20) = 1.415$, p =.27), MVIC30 ($F(2,20) = .189$, p = .83), MVIC60% ($F(2,20) = 1.015$, p = .38) or MVIC30% ($F(2,20) = .960$, p = .40). See Figure 5.2 for MVIC data as percentage change and Table 5.4 for absolute values. See Figure 5.3 and 5.4 for individual MVIC data spread for MVIC60 and MVIC30, respectively.

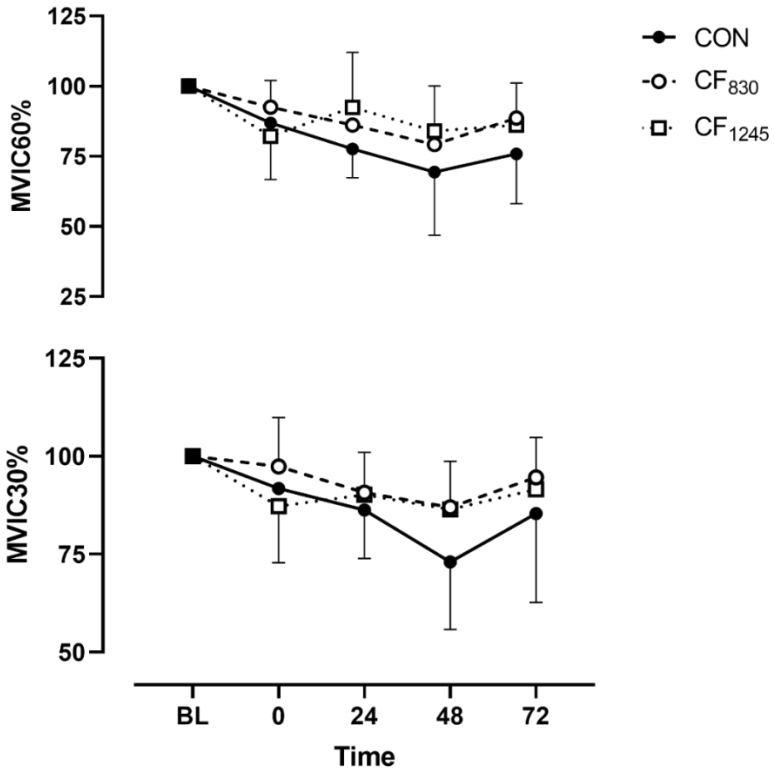

Figure 5.2. Percentage change from baseline for MVIC following EIMD

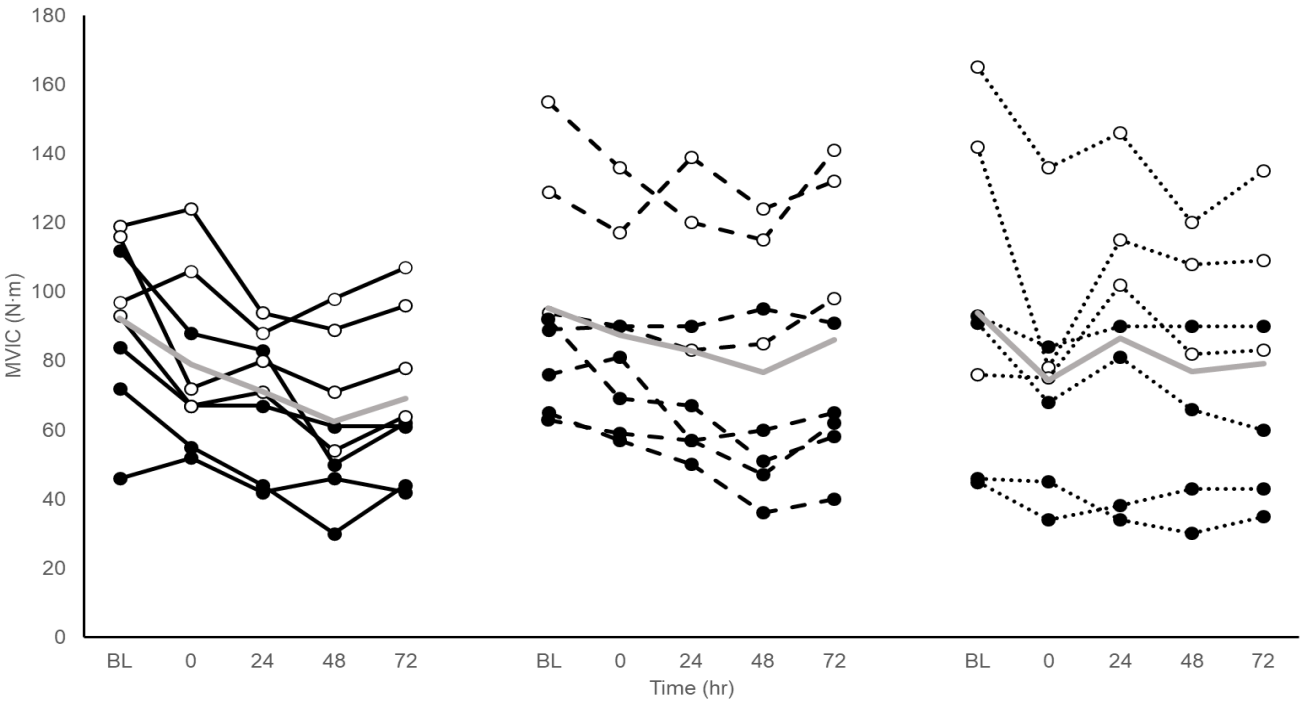

Figure 5.3 Individual MVIC60 data; *CON = solid lines, CF_{830} = dashed lines, CF_{1245} = dotted lines, grey lines = group averages, black circles = female participants, white circles = males*

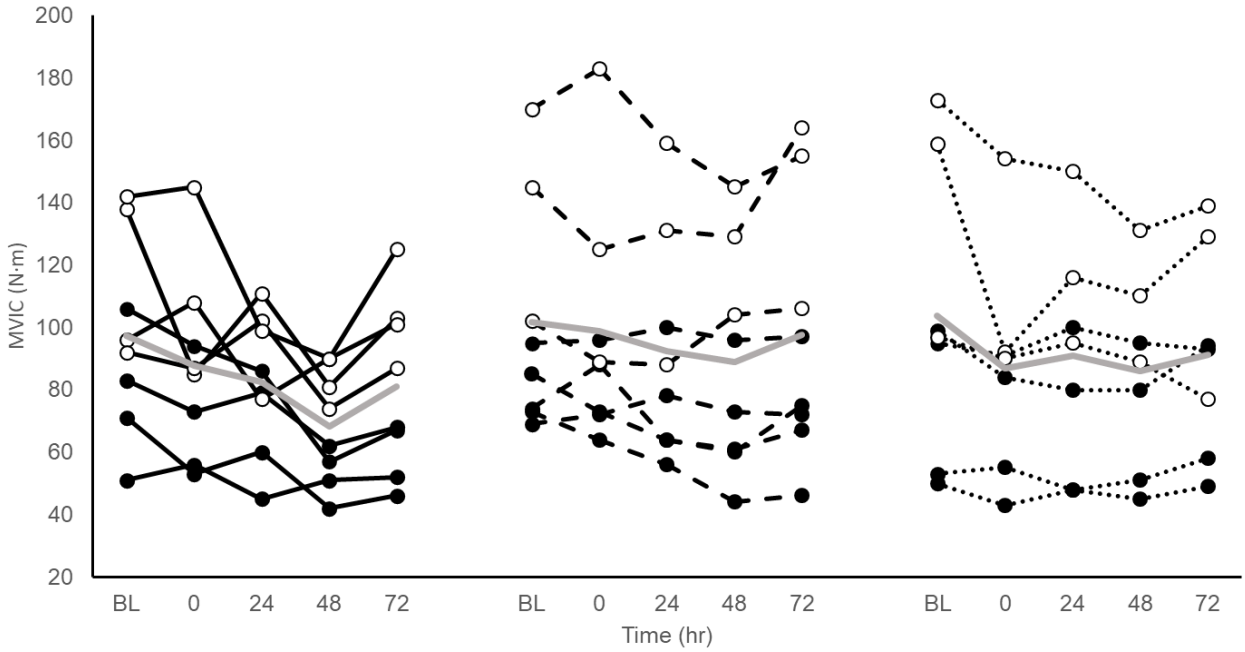

Figure 5.4 Individual MVIC30 data; *CON = solid lines, CF_{830} = dashed lines, CF_{1245} = dotted lines, grey lines = group averages, black circles = females, white circles = males*

Table 5.4. Changes in MVIC following EIMD

		Time post-EIMD (hr)				
Measure	Group	Baseline	0	24	48	72
MVIC 60 (Nm)	CON	92 ± 23	79 ± 24	71 ± 18	62 ± 21	69 ± 22
	CF_{830}	95 ± 30	87 ± 26	83 ± 30	77 ± 31	86 ± 34
	CF_{1245}	94 ± 42	74 ± 30	87 ± 37	77 ± 30	79 ± 33
MVIC 30 (Nm)	CON	97 ± 29	88 ± 28	82 ± 21	68 ± 17	81 ± 26
	CF_{830}	102 ± 35	99 ± 36	93 ± 34	89 ± 33	98 ± 40
	CF_{1245}	104 ± 44	87 ± 33	91 ± 34	86 ± 28	91 ± 31

Notes: Group mean ± SD

5a.3.2 Measures of Perceived soreness

For measures of perceived soreness, a significant main effect for time was observed for VAS ($p \leq .001$) and LEFS ($p \leq .001$), both data were normally distributed. For VAS significant differences were observed between baseline and 0, 24, 48, and 72 hr ($p \leq .001$), 0 and 48 ($p \leq .001$), 24 and 48 ($p \leq .001$), and 48 and 72 ($p \leq .001$). For LEFS significant differences were observed between baseline and 0, 24, 48, and 72 hr ($p \leq$

.001), 0 and 48 (p = .001), 24 and 48 (p ≤ .001), and 48 and 72 (p = .001) There were no significant differences between groups for VAS scores (F(2,20) = .39, p = .68). There were no significant differences between groups for LEFS scores (F(2,20) = .059, p = .94). See Table 5.5 for perceived soreness data and Figure 5.5 for individual VAS data.

Table 5.5. Changes in perceived soreness post-EIMD

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

Notes: Group mean ± SD

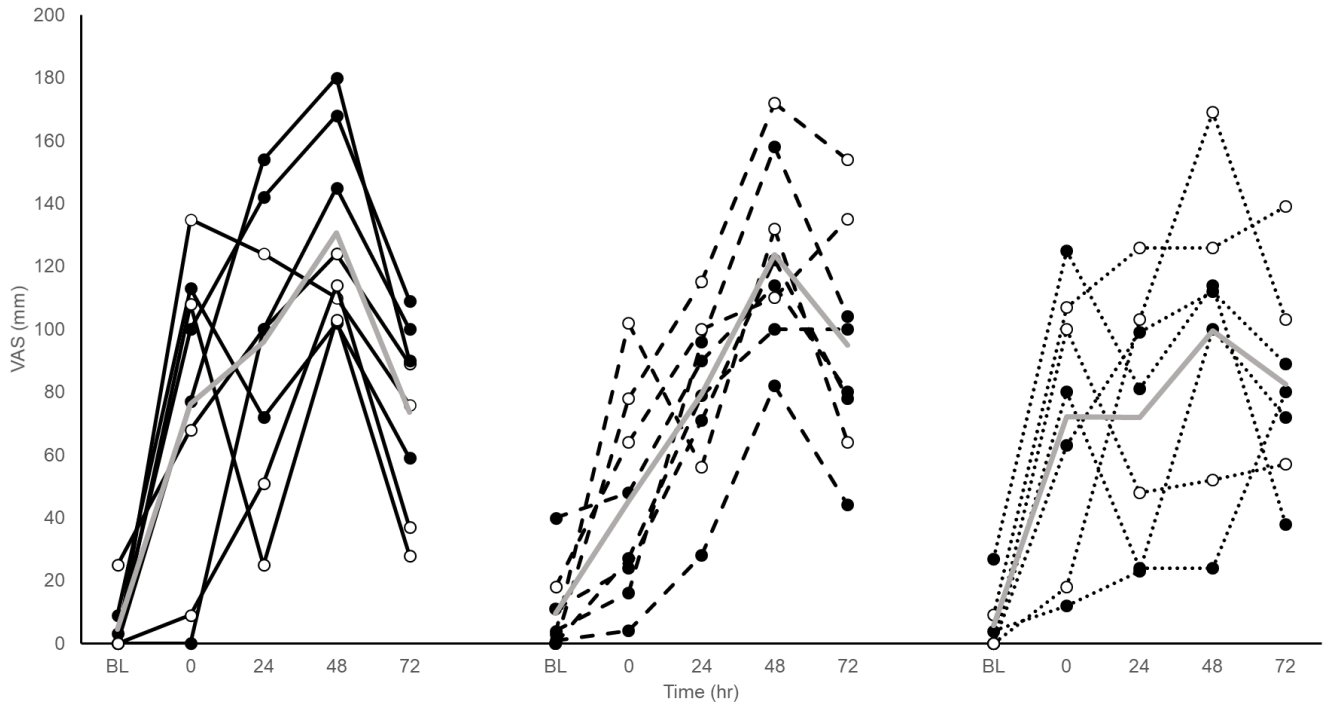

Figure 5.5 Individual VAS data; *CON = solid lines, CF_{830} = dashed lines, CF_{1245} = dotted lines, grey lines = group averages, black circles = females, white circles = males*

5a.4. Discussion

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

Differences between this study and previous studies should be noted, in that both de Carvalho et al., (2019) and Peschek et al., (2013) used EIMD protocols that did not elicit muscle soreness or deficits in muscle function in the populations they used. By contrast, the protocol used in this study elicited muscle damage as evidenced by a ~21% reduction in muscle function alongside a reduction of ~27% for perceived muscle function measured using the LEFS and a 17-fold increase in perceived soreness at 48 hr post-protocol (see Tables 11 and 12), at which the negative effects of muscle damage are known to peak (Cheung et al., 2003). Furthermore, this study targeted the hamstring muscle group as the location for inducing muscle damage when previous studies targeted the quadriceps (de Carvalho et al., 2019; Morgan et al., 2018; Peschek et al., 2013). The knee flexors are ostensibly more susceptible to muscle damage than the knee extensors following eccentric exercise (Chen et al., 2011). Thus, it may be more pertinent to investigate the hamstrings and recovery, espcially when considering the high injury rate of the knee flexors in sport, e.g., soccer (Ekstrand et al., 2011). These methodological differences make comparisons difficult to make between this current study and the previous literature.

The reductions in peak torque in the present research that were observed in the days post-EIMD are likely due to a combination of the mechanical disruptions and subsequent oxidative stress elicited by the exercise protocol. The high levels of oxidative stress typically observed following EIMD, including similar protocols to the one utilised in the current study (Nikolaidis et al., 2007), can cause the muscle to enter an oxidised state, limiting contractile capability (Powers & Jackson, 2008). However, although CF have been shown to blunt exercise-induced oxidative stress (Davison et al., 2012), the high variability between individuals in regard to the level of oxidative stress seen in response to exercise must be considered when interpreting these findings (Mullins et al., 2013). Additionally, it is unlikely that CF outcompete the existing

antioxidant defence system. Instead, epicatechin and catechin metabolites may upregulate the endogenous antioxidant enzymes rather than act directly on ROS (Ruijters, Weseler, Kicken, Haenen, & Bast). Nonetheless, such effects require confirmation with future research. Therefore, with the previous in mind, and as no markers of oxidative stress were taken, it is difficult to conclude that the large effect sizes seen between CF_{1245} and CON for MVIC60%, MVIC30 and MVIC30% at 24 and 48 hr post-EIMD ($d \geq 0.8$) are a result of CF reducing oxidative damage. Hence, more research is required to understand the potential benefits of CF as a recovery aid.

For subjective measures of muscle soreness it was hypothesised that CF consumption may reduce muscular soreness *via* the inhibition of pro-inflammatory cytokines, which are associated with neuropathic pain (Zhang & An, 2007). This was not the case in the present study, as subjective measures did not differ between groups. However, a large effect size was observed between CF_{1245} and CON for VAS at 48 hr post-EIMD (difference of 31 mm, d=0.9). The inflammatory process begins immediately following muscle damaging exercise, further developing in the subsequent 24-48 hr if the disruption is significant (Saxton, Claxton, Winter, & Pockley, 2003). As the peak rate of absorption for CF is ~30 min post-ingestion, it is feasible that the acute dose of 1245 mg CF could reduce the immediate increase in cytokines and other inflammatory mediators (e.g., neutrophils) that propagate following exercise. Because these mediators have the capacity to exacerbate muscle damage (Paulsen et al., 2012; Pizza et al., 2005; Toumi & Best, 2003) and delay recovery in the subsequent days, an early reduction in this response could lead to an enhanced recovery. This effect may result from the inhibitory potential of CF monomers on tumour necrosis factor-α, a pro-inflammatory cytokine involved in muscle lysis (Liao, Zhou, Ji, & Zhang, 2010; Mao et al., 2002). Nonetheless, these are speculative mechanisms that require confirmation from further research that includes a comprehensive array of inflammation mediators. The inability to measure these in the present study is acknowledged as a limitation of the work.

This study is not without its limitations, firstly, even though menstrual cycle was accounted for through the use of self-report questionnaires; they are not as accurate as hormonal tests to appropriately determine cycle phase (Wideman et al., 2013). However, hormone analysis was not feasible for the current research. Secondly, it is possible that the interindividual variability associated with muscle damage (Damas,

Nosaka, Libardi, Chen, & Ugrinowitsch, 2016) and variability between sex responses to EIMD (Sewright, Hubal, Kearns, Holbrook, & Clarkson, 2008) reduced the power of this study when paired with relatively small groups. Thirdly, no inflammatory or oxidative stress markers were taken, thus it was not possible to ascertain whether the intervention did in fact reduce these markers. Future research should look to include these measures and investigate the effect of CF supplementation on repeated bouts of high-intensity exercise seperated by short recovery times to better reflect competition patterns typical of team-sport athletes.

In conclusion, there is no significant benefit for muscle recovery when comparing an acute dose of either 830 and 1245 mg CF to a nutrient controlled carbohydrate-protein control. However, this needs to be confirmed with future research, whilst addressing the limitations above, to confirm or refute any benefits CF supplementation may have following a dose >1000 mg. Research should focus on CF impact on repeat performance and a more comprehensive study investigating sex differences following CF supplementation should be conducted.

5b.3 Results

5b.3.1 Participant Characteristics and Nutritional Intake

No significant differences were observed for intra sex differences between groups for height (males $p = .45$ females $p = .84$), weight (males $p = .15$ females $p = .23$), or age (males $p = .30$ females $p = .49$). However, there were significant differences for some inter sex comparisons: CON for height ($p = .008$) and weight ($p = .01$) and CF_{1245} for height ($p = .008$). Otherwise, no significant differences were observed ($p \geq .51$). No significant differences for dietary intake between groups were observed when compared for intra sex differences for energy (males $p = .72$ females $p = .61$), protein (males $p = .62$ females $p = .66$), CHO (males $p = .08$ females $p = .11$), or fat (males $p = .51$ females $p = .75$). For inter-sex differences, statistical significance was observed for CON for protein intake ($p = .03$), CF_{830} for protein intake ($p = .001$) and CF_{1245} for energy intake ($p = .008$) and carbohydrate intake ($p = .01$). See Table 5.6 for nutritional intake data.

Table 5.6 Nutritional Intake between groups

Group	Sex	Energy (kcal)	CHO (g)	PRO (g)	FAT (g)
CON	F	1800 ± 282*	226 ± 37	73 ± 25*	73 ± 10
	M	2474 ± 672*	228 ± 66	146 ± 47*	114 ± 39
CF_{830}	F	1897 ± 329*	238 ± 42’	73 ± 20	78 ± 22
	M	2442 ± 358*	279 ± 41	162 ± 24*	86 ± 23
CF_{1245}	F	1711 ± 167*	185 ± 18’	90 ± 45	70 ± 15
	M	2769 ± 468*	373 ± 94*	128 ± 45	93 ± 27

*Notes: Means ± standard deviations, F = females, M = males, CHO = carbohydrate, PRO = protein, * = denotes a significance difference between males and females within the group, ‘ = significant difference between CF_{830} and CF_{1245}*

5b.3.2 Sex differences for muscle function

Muscle function was measured using MVIC60 and MVIC30 absolute values and MVIC60% and MVIC30% to assess for relative changes. From the protocol a significant time effect was observed for males and females for MVIC60, MVIC30, MVIC60% and MVIC30% ($p \leq .004$) indicating that muscle function was significantly impaired following the EIMD protocol. For inter-sex comparisons of MVIC60 significant differences were observed for CON ($p = .04$) and CF_{830} ($p = .01$) but not CF_{1245} ($p = .06$). Further significant intra sex differences were observed for MVIC30 for CON ($p = .008$) and CF_{830} ($p = .01$) but not for CF_{1245} ($p = .06$). For intra-sex comparison, no significant differences were observed for MVIC60 and MVIC30 ($p \geq .07$). Additionally, no significant inter- or intra-sex differences were observed for MVIC60% or MVIC30% ($p \geq .09$). Post-hoc analysis between the males and females of CF_{1245} found a significant difference at 72 hr post-EIMD when assessing for MVIC30% ($p = .03$). See Table 5.7 for MVIC data as percentage change and Table 5.8 for absolute values. Figure 5.6 and 5.7 display group MVIC60 and MVIC30 data as percentage change.

Table 5.7 Changes in MVIC Following EIMD as Percentage Change

			Time post-EIMD (hr)				
Measure	Group	Sex	Baseline	0	24	48	72
MVIC60%	CON	F	100 ± 0	87 ± 17	77 ± 13	65 ± 27	70 ± 16
		M	100 ± 0	87 ± 23	79 ± 9	74 ± 20	82 ± 20
	CF_{830}	F	100 ± 0	93 ± 12	83 ± 12	75 ± 24	82 ± 20
		M	100 ± 0	91 ± 4	91 ± 15	87 ± 11	99 ± 7
	CF_{1245}	F	100 ± 0	85 ± 11	86 ± 10	83 ± 16	84 ± 15

		M	100 ± 0	79 ± 22	101 ± 29	86 ± 19	89 ± 17
MVIC30%	CON	F	100 ± 0	90 ± 15	87 ± 6	72 ± 21	78 ± 18
		M	100 ± 0	93 ± 22	85 ± 18	74 ± 16	93 ± 27
	CF_{830}	F	100 ± 0	100 ± 13	91 ± 17	84 ± 19	90 ± 18
		M	100 ± 0	94 ± 12	90 ± 4	92 ± 9	103 ± 11
	CF_{1245}	F	100 ± 0	93 ± 9	93 ± 10	92 ± 11	100 ± 11*
		M	100 ± 0	80 ± 19	86 ± 13	79 ± 12	80 ± 1*

*Notes: Means ± standard deviations, F = females, M = males, MVIC = maximal voluntary isometric contraction, EIMD = exercise-induced muscle damage, * = denotes a significant difference within the group*

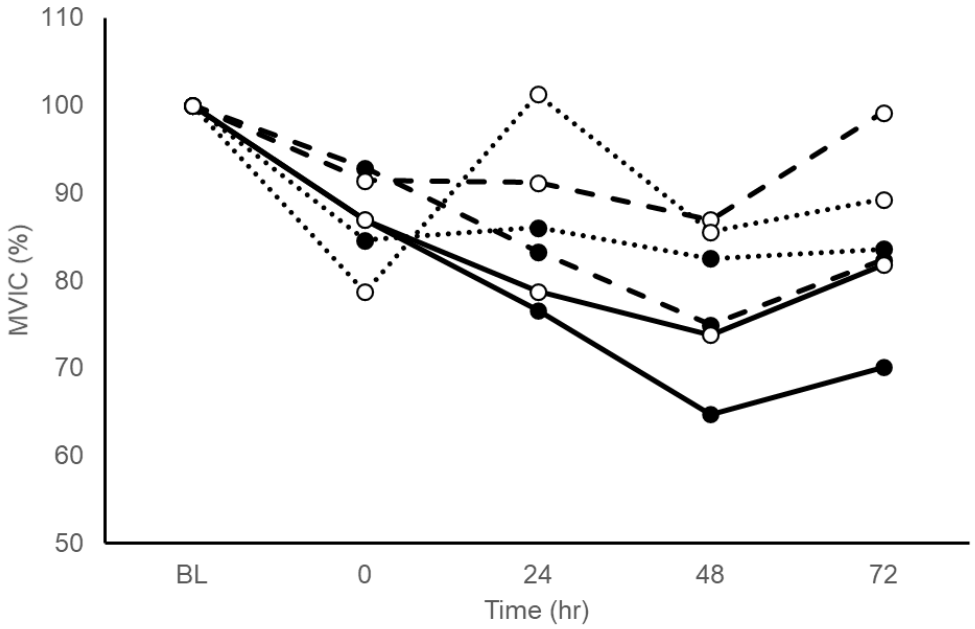

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Figure 5.6 Inter- and Intra-sex MVIC60% data; *CON = solid lines, CF_{830} = dashed lines, CF_{1245} = dotted lines, grey lines = group averages, black circles = female participants, white circles = males; data reported as means, for SD see Table 6.*

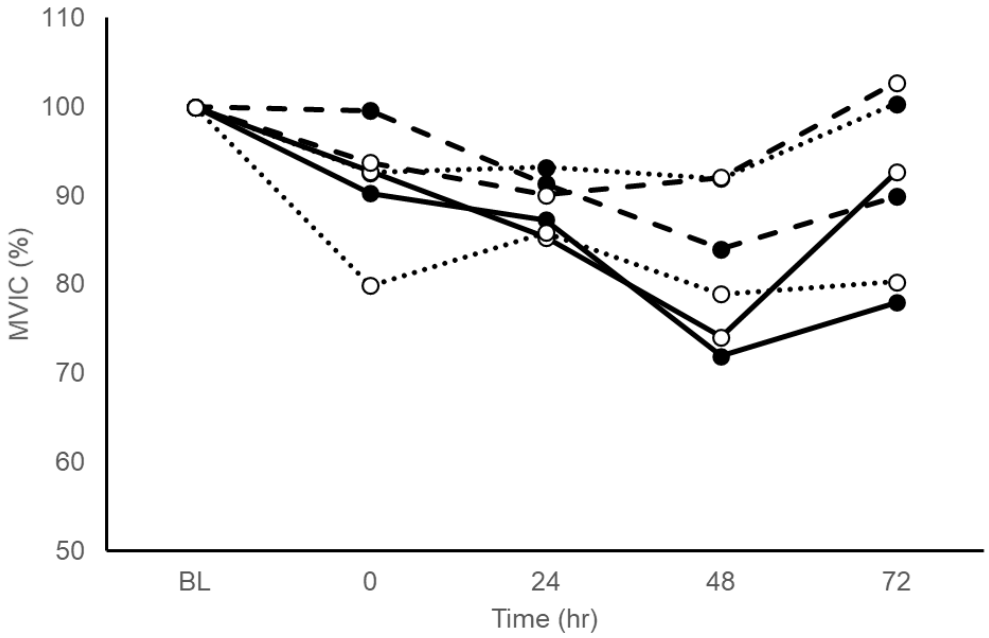

Figure 5.7 Inter- and Intra-sex MVIC30% data; *CON = solid lines, CF_{830} = dashed lines, CF_{1245} = dotted lines, grey lines = group averages, black circles = female participants, white circles = males; data reported as means, for SD see Table 6.3*

Table 5.8 Changes in MVIC Following EIMD

			Time post-EIMD (hr)				
Measure	Group	Sex	Baseline	0	24	48	72
MVIC60 (Nm)	CON	F^+	79 ± 27	66 ± 16	59 ± 20	47 ± 13*	52 ± 11*
		M^+	106 ± 13	92 ± 27	83 ± 10	78 ± 20*	86 ± 19*
	CF_{830}	F^+	77 ± 13*	71 ± 14*	64 ± 16*	58 ± 23*	63 ± 18*
		M^+	126 ± 31*	114 ± 23*	114 ± 28 *	108 ± 20*	124 ± 23*
	CF_{1245}	F	69 ± 27	58 ± 23	61 ± 29*	57 ± 26	57 ± 24*
		M	128 ± 46	96 ± 34	121 ± 23*	103 ± 19	109 ± 26*
MVIC30 (Nm)	CON	F^+	78 ± 23	69 ± 19	68 ± 19*	53 ± 9*	58 ± 11*
		M^+	117 ± 27	106 ± 28	97 ± 14*	84 ± 8*	104 ± 16*
	CF_{830}	F^+	79 ± 11*	79 ± 13*	72 ± 17*	67 ± 19*	71 ± 18*
		M^+	139 ± 34*	132 ± 47*	126 ± 36*	126 ± 21*	142 ± 31*
	CF_{1245}	F	74 ± 26*	68 ± 23	69 ± 26	68 ± 24	74 ± 23
		M	143 ± 40*	112 ± 36	120 ± 28	110 ± 21	115 ± 33

*Notes: Means ± standard deviations, F = females, M = males, MVIC = maximal voluntary isometric contraction, EIMD = exercise-induced muscle damage, ⁺ = significant difference for inter-sex comparisons, * = denotes a significant difference within the group at the specific timepoint*

5b.3.3 Sex differences for perceived soreness

Measures of perceived soreness assessed using a VAS and LEFS found a significant main time effect for males and females ($p \leq .006$), indicating that the EIMD protocol was effective in inducing muscle soreness. For VAS scores, no significant differences were observed when analysing for inter-sex comparisons ($p \geq .08$) or intra-sex comparisons ($p \geq .06$). For LEFS scores, no significant differences were observed when analysing for inter-sex comparisons ($p \geq .60$) or intra-sex comparisons ($p \geq .62$). Post-hoc analysis for intra-sex differences for VAS scores between CON and CF_{1245} found significant differences 24 hr ($p = .03$) and 48 hr ($p = .03$) post-EIMD. Additionally, a significant difference was observed immediately post-EIMD when comparing the males and females of CF_{830} for VAS scores ($p = .004$). See Table 5.9 for perceived soreness data and Figure 5.8 for VAS data.

Table 5.9 Changes in Perceived Soreness Following EIMD

Measure	Group	Sex	Time post-EIMD (hr) BL	0	24	48	72
VAS (mm)	CON	F	3 ± 4	73 ± 51	117±38/	149±34/	90 ± 22
		M	6 ± 13	80 ± 55	75 ± 45	113 ± 9	58 ± 30
	CF_{830}	F	11 ± 17	24±16*	73 ± 27	115± 28	80 ± 24
		M	7 ± 10	81± 19*	90 ± 31	138± 31	118± 47
	CF_{1245}	F	8 ± 13	70 ± 47	57 ± 39/	88 ± 43/	70 ± 22
		M	3 ± 5	75 ± 49	92 ± 40	116± 59	100± 41
LEFS (a.u.)	CON	F	79 ± 2	74 ± 4	61 ± 21	54 ± 21	65 ± 7
		M	79 ± 1	61 ± 16	65 ± 10	56 ± 11	68 ± 6
	CF_{830}	F	76 ± 2	73 ± 3	64 ± 10	54 ± 13	66 ± 10
		M	79 ± 2	70 ± 2	67 ± 8	55 ± 7	59 ± 2
	CF_{1245}	F	77 ± 4	67 ± 11	69 ± 8	62 ± 10	70 ± 8
		M	76 ± 6	61 ± 11	64 ± 16	61 ± 18	66 ± 8

*Notes: Means ± standard deviations, F = females, M = males, VAS = visual analogue scale, LEFS = lower extremity functional scale, EIMD = exercise-induced muscle damage, * = denotes a significant difference within the group at the specific time point, /= significant difference between females*

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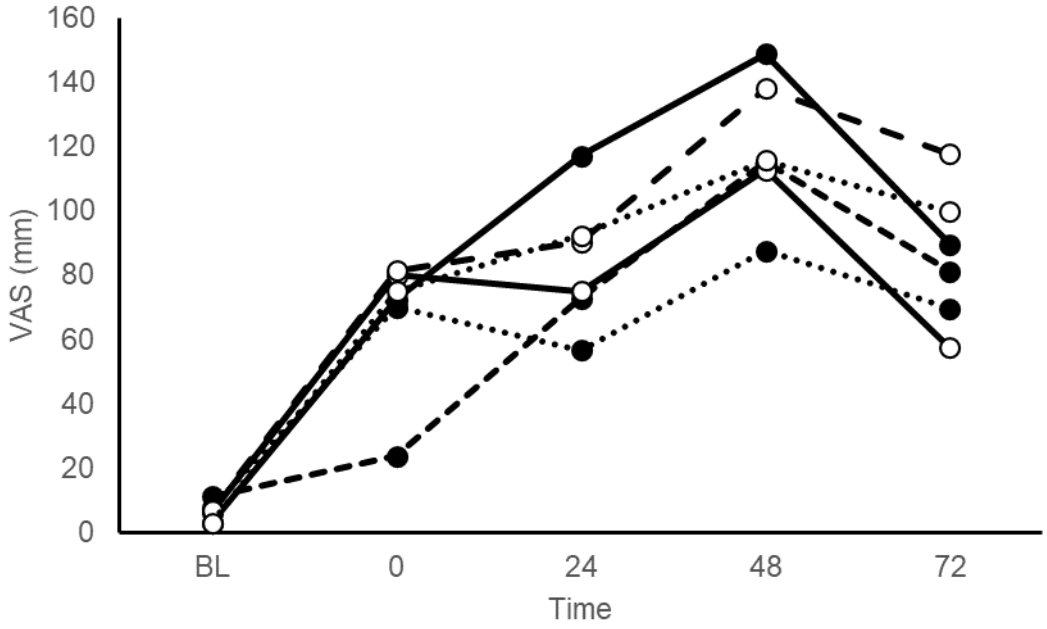

2447 Figure 5.8 Inter- and Intra-sex VAS data; *CON = solid lines, CF_{830} = dashed lines,*
2448 *CF_{1245} = dotted lines, grey lines = group averages, black circles = female*
2449 *participants, white circles = males; data reported as means, for SD see Table 6.5*

5b.4 Discussion

The main aim of this study was to investigate whether any sex differences for muscle recovery are present following the administration of an acute CF recovery beverage of varying amounts post-EIMD. Based on the current research there are no significant differences between sexes regarding the impact of CF on muscle recovery.

Based on the current data it appears that there are no significant differences for muscle function recovery between males and females whilst supplementing CF. For MVIC percentage change at both 60 and 30 degrees there were no significant differences for inter or intra-sex comparisons. However, a p value of .092 was observed between males and females within the CF_{1245} group for MVIC30%, with a significant difference identified at 72 hr post-EIMD ($p = .03$). The data at 72 hr shows that females had reached 100% of baseline MVIC whereas males only reached 80%, potentially indicating that the males within CF_{1245} were still in an impaired contractile state 72 hr following the EIMD protocol. Interestingly, females on average had greater reductions in MVIC than males (based on relative changes not absolute values). Furthermore, males have been observed to demonstrate greater neuromuscular fatigue and slower acute recovery than females following strenuous exercise (Häkkinen, 1993). This is somewhat evidenced within the data as immediately post-EIMD females achieved a higher percentage of MVIC than males across all data, excluding MVIC60% and MVIC30% for the CON group. Other research has indicated that females may be less fatigable than males following intermittent, MVIC exercise (Ansdell, Brownstein, Škarabot, Hicks, Howatson, et al., 2019; Ansdell, Thomas, Howatson, Hunter, & Goodall, 2017). However, the exercise utilised within this Chapter was maximal eccentric knee flexor exercise, therefore comparisons are limited with further research required using separate muscle groups or comparisons between muscle groups.

The differences observed here for the relative differences between males and females for MVIC may be due to individual variation and limited sample size as opposed to sex differences as no other data was statistically significant for MVIC30% or MVIC60%. There are a number of reasons this may be apparent. It could be due to individual sex-differences relating to lean body mass and strength, as males demonstrated significantly higher MVIC values. Following repeat exposure to exercise stimuli that includes high force eccentric contractions there are various physiological adaptations that occur to protect the muscle from future damage. One such adaptation is an

increase in the number of motor units recruited during maximal eccentric contractions thereby reducing the stress placed on individual muscle fibres (McHugh, 2003). This may be the reason why individuals with higher training status may be at a reduced risk of severe muscle damage following an EIMD protocol. Not only that but training status appears to correlate with the inflammatory response associated with intense exercise, the greater an individual's training status the lower the response (Martín-Sánchez et al., 2011). However, participants were classed as recreationally active to be eligible for the study and participants who partook in extensive, regular eccentric training were excluded from participation. Nevertheless, it is also possible that due to the reduced number of males who completed the study than females the differences observed could relate to individual variation.

For subjective measures of muscle soreness there were no significant differences between groups for VAS or LEFS. Interestingly, although not significant there were data that was approaching statistical significance for the VAS, specifically males vs females for CF_{830} ($p = .08$), females vs females for CON vs CF_{830} ($p = .07$) and CON vs CF_{1245} ($p = .06$). For the comparisons between females for CON vs CF_{1245}, significant differences were observed at 24 hr ($p = .03$) and 48 hr ($p = .03$). This indicates that the females in the CON experienced significantly higher levels of perceived muscular soreness than those within the CF_{1245} group. Large effect sizes were observed between the females within the CON and both the CF_{830} and CF_{1245} groups at 24 and 48 hr post-EIMD ($d \geq 1.2$). At 24 hr the average score for the females within the CON were 60 mm higher and at 48 hr 61 mm higher. Similarly, the females within the CF_{830} group consistently scored lower for muscle soreness than the CON group throughout the testing period, with CF_{1245} having the lowest average scores, save for immediately post-EIMD when CF_{830} scored lower. It is possible that the CF provided some level of analgesic benefit for females more so than in males. Males scores remained similar between the groups for VAS.

The effects of the menstrual cycle on muscle recovery are still being elucidated; however, menstrual phase may influence feelings of perceived soreness. A recent review by Romero-Parra et al., (2020) found that female athletes experienced higher levels of perceived soreness post-EIMD during the early follicular phase of their cycles, when oestrogen concentrations are low. Within this study, all female participants were tested within the luteal phase or a phase equivalent depending on contraceptive use,

as the luteal phase is known for having relatively consistent levels of oestrogen throughout following the second peak of oestrogen at the beginning of the phase (Mihm et al., 2011; Reed & Carr, 2018). The increased oestrogen in the females alongside the high intake of CF may partially explain the reason that the female participants within the CF groups had lower levels of perceived soreness than their male counterparts. However, (poly)phenols have been shown to have both anti-oestrogenic and oestrogenic effects and may impact the bioactivity of oestrogen *via* the binding to and/or blocking of oestrogen receptors (Kiyama, 2020).

Furthermore, within the current data set it was noted that on average males recorded higher VAS scores in the two CF groups than the females, however in the CON males scored lower than females. It is possible that due to the reported effects of CF upregulation of various pro- and anti-inflammatory molecules and redox enzymes combined with the potential benefits of oestrogen could explain these slight differences. Evidence has suggested that females may have a reduced inflammatory response in comparison to males following EIMD, with current evidence indicating a reduced invasion count of neutrophils and macrophages (Stupka et al., 2000). However, evidence is still equivocal, especially when considering variation due to the menstrual cycle as higher levels of inflammation following EIMD have been observed during the follicular phase (Carter et al., 2001; Hackney et al., 2019; Oosthuyse & Bosch, 2017). CF modulation of inflammatory markers alongside the role of oestrogen may present an explanation of these findings. It has been observed that *in vitro* CF administration downregulates various inflammatory molecules such as monocyte chemoattractant protein-1, TNF-α, IL-1α, and IL-6, with *in vivo* evidence suggesting similar effects (Goya et al., 2016; Selmi et al., 2006). Oestrogen has also shown to have potential anti-inflammatory properties, such as through the inhibition of leucocyte infiltration following unaccustomed exercise (Stupka et al., 2000). Possibly, the combined benefit may elicit greater reductions in inflammation compared to males and partially explain the reduced soreness noted in females. This may be due to the role of inflammation on soreness as many inflammatory molecules sensitise nociceptors in the muscle, e.g., TNF-α, IL-1β, and IL-6, as well as by macrophages and neutrophils (Pinho-Ribeiro, Verri Jr, & Chiu, 2017). However, future studies including greater participant numbers and the inclusion of inflammatory markers are required. Not only that but, studies should look to investigate any potential interaction effects of CF and

oestrogen. By comparing menstrual cycle phase on markers of muscle recovery whilst supplementing CF during it may be possible to investigate any potential interaction effects.

5b.5 Limitations

The main limitation is the reduced number of individuals within each group when separated by sex, leading to a reduced ability to make any meaningful conclusions based on the data. Indeed, any differences that have been noted are likely due to inter-individual variation as opposed to inter-sex differences. The observed post-hoc power, calculated using G*Power further indicated the underpowered nature of the sex specific analysis, with data ranging from 78% to 30% power depending on the measure. This is to be expected with the nature of Part B of this study due to the reduced participant numbers when analysing for sex differences. It has been reported that observed post hoc power varies from true power significantly, this is due to the completion of data collection having already occurred. Furthermore, observed power reduces as a function of a p value increasing, and as this study had no significant differences it is very likely to provide low observed power (Hoenig & Heisey, 2001).

The significant differences observed between males and females in the CON and CF_{830} for MVIC60 and MVIC30 are due to overall differences in absolute values for force output, rather than treatment, as there were no significant differences when the values were analysed as percentage change from baseline. Future studies investigating the potential sex differences that may exist regarding CF consumption and EIMD are warranted. It is recommended that for a study that has the main focus of this research question, investigators should look to implement further methods to track the menstrual cycle in females controlling it as a variable as accurately as possible. Methods may include hormonal testing, basal body temperature testing or utilising ovulation kits and should be performed alongside calendar-based testing to account for the variation that exists around the menstrual cycle (Fehring et al., 2006; Wideman et al., 2013).

Other areas in which future research should look to address regarding sex differences and CF are as follows. Potential variations in oxidative stress and antioxidant capacity between males and females following EIMD and CF consumption, as differences have been observed following the consumption of other (poly)phenols (Burton-Freeman et al., 2010); albeit this was not noted following EIMD. There may be differences in the

absorption or metabolism of CF between sexes also, as such studies may utilise markers of epicatechin metabolites (or other CF metabolites) when investigating sex differences to measure availability of these compounds within circulation or in excretion. Investigating the potential affinity of CF monomers, such as epicatechin, to bind to oestrogen receptors will also provide a greater insight into the mechanistic action of these compounds. It has been noted already that certain (poly)phenols, e.g., ellagic acid, have a greater affinity for oestrogen-receptor beta (Landete, 2011) and certain flavanols activating oestrogen receptor alpha (Kiyama, 2020). The expression of the former receptor is 30% lower during the early follicular phase of the menstrual cycle and as such may impact (poly)phenol action (Najjar, Turner, Wong, & Feresin, 2021).

It is pertinent that more research includes female participants, including sedentary individuals, recreational athletes, and elite athletes alike, allowing for more accurate practical applications to made from the research without generalising from male cohorts. Recent methodological considerations for sport science research have been published with the intent to improve research on females, not only to improve quality but increase implementation (Elliott-Sale et al., 2021). At present only five studies have included female participants within CF investigations in relation to exercise (Garcia-Yu et al., 2021; Patel et al., 2020; Sadler et al., 2020; Shaw, Singh, Sirant, Neary, & Chilibeck, 2020; Taub et al., 2016) indicating a dearth of research in this area.

In summary, from the present data there is no significant difference between males and females for indices of muscle recovery following CF supplementation of varying doses. It is possible that there is an increased analgesic effect of CF in females than males, however this requires further research and is currently speculation based on the data. It is possible that a greater number of male and female participants would be required to identify a statistically significant difference, and as such data should be interpreted cautiously.

Chapter 6 Investigating the effect of regular consumption of a high dose of cocoa flavanols on muscle recovery and repeat performance in males and females

6.1 Introduction

Currently, sport and exercise science practitioners make use of numerous ergogenic aids to accelerate or optimise recovery, such as (poly)phenol supplementation. The use of these high (poly)phenol foods has become widespread (Maughan, Depiesse, & Geyer, 2007; Solheim et al., 2017; Teixeira, 2013), including outside of the athletic domain, with the intention of maximising any reported health improving benefits (Williamson, 2017). This thesis has focussed on CF and as discussed within this thesis (Chapter 2 Section 1) CF supplementation can aid with numerous health benefits relating to oxidative stress, cardiovascular health, and cognitive health. For exercise, research has sought to investigate the impact of CF on aspects of muscle recovery, such as oxidative stress, soreness, inflammation, perceived soreness, and muscle function (see Chapter 3). Currently, the evidence within this area of research is limited and equivocal. However, based on that research, CF supplementation appears to beneficially reduce exercise-induced oxidative stress (Allgrove et al., 2011; Davison et al., 2012) and within this thesis (Chapter 5), an acute, high dose of CF (1245 mg) may have some influence on the recovery of muscle function following exercise. Interestingly, one study found that regular supplementation of CF (616 mg daily) improved distance covered in a Yo-Yo test following a muscle damaging exercise protocol (de Carvalho et al., 2019). However, this is still a burgeoning area of research, with the current available literature still sparse.

The deleterious effects of muscle damage can persist over a period of four or more days following exercise (Gibala, MacDougall, Tarnopolsky, Stauber, & Elorriaga, 1995). This understanding of the longevity of EIMD can be applied to practically, in as much as resistance training, weightlifting sports, and sports that have a proclivity for rapid performance turnarounds. Furthermore, team sports, e.g., soccer, may not have adequate time between exercise bouts to fully recover, especially during fixture congested periods (Page et al., 2019). These periods involve repeat performance within 72 hr of the initial event, commonly observed in soccer and other tournament-based sports, e.g., hockey. It is possible that during these periods recovery is insufficient to allow for maximal/optimal performance during the second bout of exercise and an inability to train at appropriate intensities. For example, muscle soreness has been reported to still be present 72 hr post-match in soccer and can be exacerbated during fixture congested schedules (Lundberg & Weckström, 2017).

The impact that muscle damage has on performance can be detrimental, for example, it can impede sprinting ability and explosive power (Khan et al., 2016), and reduce contractile muscle force (Magaudda, Di Mauro, Trimarchi, & Anastasi, 2004). These consequences may result from ultrastructural damage of the muscle fibres *via* the mechanical stress of intense exercise, most notably eccentric muscle contractions, as more strain is placed on fewer motor units than during concentric contractions (McKune, Semple, & Peters-Futre, 2012). Exercise-induced oxidative stress within muscle tissue can impact contractile capability; muscle fibres in oxidised states have been shown to have significantly reduced force generating capacity (Reid, 2008; Siems et al., 2003; Steinbacher & Eckl, 2015). The level of oxidative stress is dependent on the intensity of the exercise and the oxygen demands of it, as well as the inflammatory response in the days following completion (Uchiyama, Tsukamoto, Yoshimura, & Tamaki, 2006). As such exogenous antioxidants may be helpful in limiting the upsurge of ROS (Zhang & Tsao, 2016).

In relation to feelings of soreness, pain during exercise can impact pacing strategies by making an athlete aware of fatigue (Stevens, Mauger, Hassmèn, & Taylor, 2018), therefore, beginning an event already in a damaged state may negatively impact athletic performance. Fatigue is a common component of intense and prolonged exercise (Nybo, 2003), these physiological responses signal the brain and other organs to initiate the reduction of exercise intensity or cease it entirely (Keller et al., 2001). Fatigue can accumulate if recovery is insufficient, e.g., a reduced recovery window before subsequent exercise, following the original bout of exercise (Page et al., 2019), leading to an increased injury risk and elongate the time course of recovery (Small et al., 2009b).

Understanding the impact of CF on functional recovery, e.g., force recovery, may provide practitioners a better understanding of when to utilise CF as a recovery intervention during various sporting scenarios. Especially so, as functional markers of recovery are commonly the most impaired following EIMD, e.g., force output (Child, Saxton, & Donnelly, 1998; Howatson & Milak, 2009), jump height (West et al., 2014), and sprint performance (Keane, Salicki, Goodall, Thomas, & Howatson, 2015; Twist & Eston, 2005). Even still, changes in muscle function may also relate to leucocyte accumulation in a damaged muscle, myofibrillar disruption, and necrosis (Paulsen et al., 2012). Therefore, during critical sporting competition phases that require rapid

turnarounds for repeated bouts of exercise, accelerating recovery is important. This also applies to resistance-based exercise and weightlifting sports, not just team sports, as training will likely be programmed for use of the same muscle group within a 48-72 hr window, optimising recovery from the first session may aid performance during the next.

Therefore, further investigations into the impact of regular CF supplementation on repeat performance are warranted, which is the aim of this Chapter, building on the previous work within this thesis. As Chapter 5 found no significant effect of an acute dose of varied doses of CF on muscle recovery. However, as the highest dose of 1245 mg CF had notable effect sizes for the recovery of muscle function and soreness further research utilising repeated doses of CF is necessary. As such, this study aims to carry out such an investigation, following a time-frame not dissimilar to a congested fixture period in soccer. Ergo, the aims of this study are threefold; *i*) to investigate whether regular CF supplementation beneficially influences markers of recovery following EIMD, *ii*) if CF can aid repeat performance following EIMD, separated by 72 hr, and *iii*) if CF can reduce the increased neuromuscular fatigue associated with repeat performance.

6.2 Methods

6.2.1 Study Design

This study was a laboratory-based, randomised, double-blind, nutrient controlled trial. Participants were randomised into either control (CON) or CF supplementation group. Participants were required to attend the laboratory for seven days. Day one of the study involved baseline testing and familiarisation of the EIMD protocol using the isokinetic dynamometer (one set of ten sub-maximal hamstring curls). The other six days took place consecutively, therefore, the testing schedule ran as follows: baseline, immediately post-EIMD (0hr), 24, 48, 72 hr post-EIMD, immediately post second EIMD protocol (2nd 0hr), 24 and 48 hr post-EIMD (see Figure 7.1 for a study schematic). Randomisation was performed by an independent laboratory technician, see Chapter 4 Section 1 for further details.

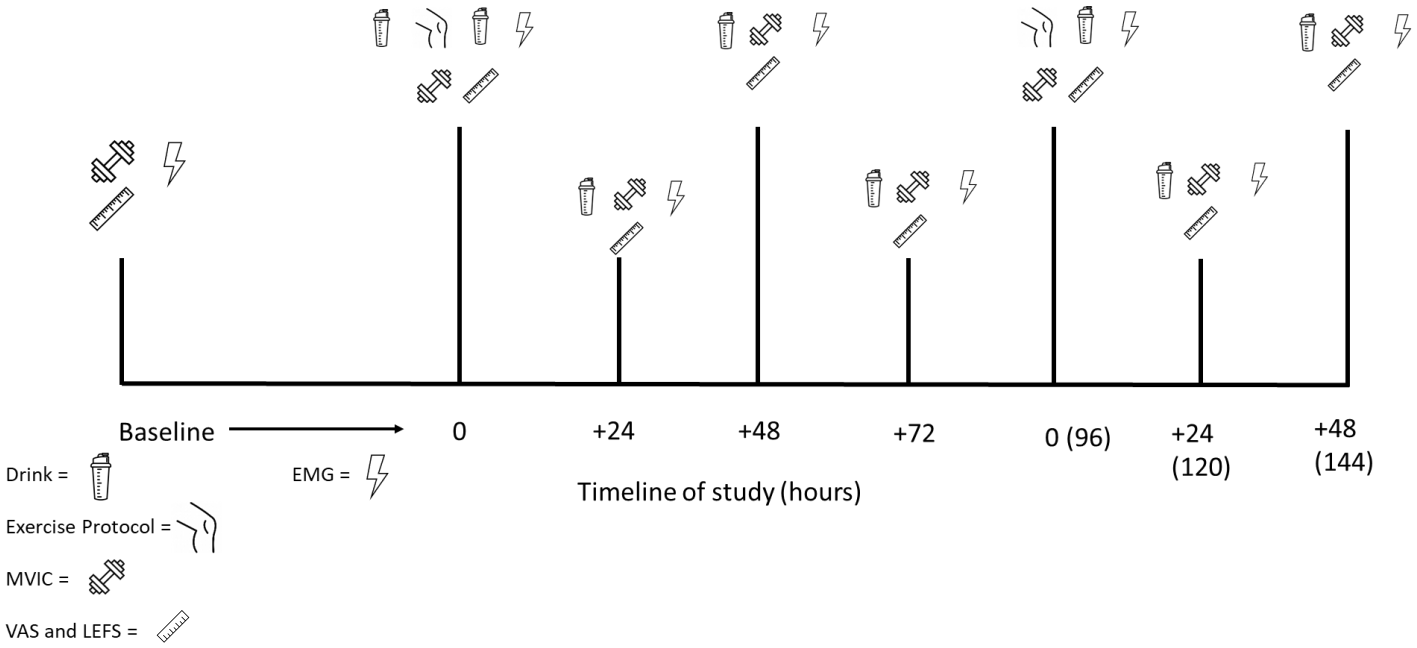

Figure 6.1 Study timeline Schematic

6.2.2 Participants

Following ethical approval from the University of Huddersfield and in agreement with the Declaration of Helsinki, a total of ten participants were recruited for this study. However, only nine individuals (seven males, two females) completed the entire testing period, see Table 7.1 for participant characteristics. The only drop out cited ‘an injury concern’ as the reason for withdrawing from the study before the second EIMD protocol. An *a priori* power calculation determined that a sample size of 16 was

required for 80% power and to detect significance based on the effect size of previous research regarding MVIC recovery at 48 hr post-EIMD. Unfortunately, due to the coronavirus pandemic data collection was postponed indefinitely from the 17th of March 2020. Data collection was due to recommence in November 2020, however a second national lockdown within the United Kingdom resulted in another cancellation of data collection.

Table 6.1 Participant Characteristics

Group (n)	Age (years)	Height (cm)	Weight (kg)
CON (5)	23 ± 3	176 ± 7	79 ± 5
CF (4)	24 ± 5	178 ± 7	71 ± 11

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

6.2.3 Muscle Damaging Protocol

The protocol to induce muscle damage consisted of five sets of ten maximal concentric-eccentric contractions of the knee flexors (each leg) using an isokinetic dynamometer. This protocol was completed twice over the testing period, on the first day of the testing period (0 hr) and 72 hr after the first protocol. See Chapter 4 Section 4 for further details of the muscle damaging protocol.

6.2.4 Nutritional Intervention

Both the participants and researchers were blinded to the allocated beverage of each participant. To do so an independent laboratory technician randomised the participants and put together the beverage contents into an opaque bottle. Participants were provided with a bottle containing a pre-mixed powder of the ingredients (60 g maltodextrin and 25 g whey protein, plus 15 g Chococru© cocoa powder if assigned to the treatment group) and were instructed to add 300 ml of water to the bottle and shake vigorously until dissolved. Participants were instructed to drink their beverage ~60 min before arrival at the laboratory each day during the testing period and consumed another immediately post-EIMD protocols, totalling eight beverages. The test beverage contained 15 g Chococru© cocoa powder, totalling 1245 mg of CF, see Chapter 4 Section 3.2 for more details.

6.2.5 Exercise Performance

Exercise performance was measured *via* peak torque per set completed during both exercise protocols. Peak torques were collected per set for both concentric and eccentric contractions of the knee flexors for both the dominant and non-dominant legs. This data was collected to compare participant exercise performance from the first EIMD protocol with the second EIMD protocol. Additionally, data was compared as a percentage change from the first protocol, this was to account for percentage drop off from the first to second protocol and standardise data between participants.

6.2.6 Muscle Function

MVIC was measured at 30- and 60-degrees knee flexion from anatomical zero using an isokinetic dynamometer. Please refer to Chapter 4 Section 5 for further information on MVIC measures.

6.2.7 Electromyography

Neuromuscular activation of the of the biceps femoris was taken at 60 degrees of knee flexion from anatomical zero. See Chapter 4 Section 6 for further detail.

6.2.8 Perceived Soreness

Muscle soreness was measured using a VAS and LEFS. See Chapter 4 Section 7 for further information.

6.2.9 Dietary Analysis

Participants completed a 24-hr dietary recall each day of testing, excluding baseline testing. See Chapter 4 Section 2 for further detail.

6.2.10 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics (version 26.0; IBM Corp., Armonk, NY). All data was assessed for normality using a Shapiro-Wilk test and quantile-quantile plots were examined to establish whether the data was normally distributed. A Greenhouse-Geisser correction was used if sphericity was violated. A mixed analysis of variance was used to determine interaction and time effects for the recovery variables. If any significant differences were observed for the data a Fisher's least significant difference post hoc test was performed to identify the point of significance. Data for MVIC and isokinetic peak torques were calculated as percentage changes from baseline alongside data reported as absolute values. Effect sizes were calculated using Cohen's *d,* with the magnitude of effects considered small (0.2),

moderate (0.5), and large (0.8). Significance was set at $p \leq .05$ pre-analysis. Descriptive statistics are reported as means, percentage change (%) ± SD.

6.3 Results

There were no significant differences for participant age ($p = .91$), height ($p = .74$), weight ($p = .30$) or dietary intake between groups for energy ($p = .88$), CHO ($p = .49$), protein ($p = .62$) and fat ($p = .55$). See Table 6.2 for details of dietary intake.

Table 6.2 Dietary characteristics of the participants

Group	Energy (kcal)	CHO (g)	PRO (g)	FAT (g)
CON	2171 ± 429	268 ± 62	89 ± 17	85 ± 28
CF	2217 ± 487	237 ± 69	99 ± 40	97 ± 27

Note CON = control, CF = cocoa flavanols, data displayed as means ± standard deviations

6.3.1 Exercise Performance

No significant differences were observed for peak torque within groups when comparing protocol one to protocol two for concentric contractions of the dominant leg ($p \geq .42$) and non-dominant leg ($p \geq .07$) as well as eccentric contractions of the dominant ($p \geq .11$) and non-dominant leg ($p \geq .10$). There were also no significant differences between the groups for exercise performance, measured as peak torque, during the first protocol for non-dominant ($p \geq .53$) and dominant leg ($p \geq 21$) or during the second protocol for non-dominant ($p \geq .82$) or dominant leg ($p \geq .59$). Interestingly, the CON group managed to achieve a greater percentage of their original peak torques from the first protocol in the second for concentric contractions (100 ± 19 vs 88 ± 15%) and eccentric contractions (97 ± 21 vs 85 ± 15%) of the dominant leg. However, the CF group managed to achieve a greater percentage of their original peak torques for concentric (80 ± 17 vs 87 ± 11%) and eccentric contractions (81 ± 23 vs 85 ± 11%) for the non-dominant leg. See Table 6.3 for concentric peak torques and 6.4 for eccentric peak torques displayed as a percentage of the first protocol.

Table 6.3 Percentage change from first EIMD protocol measured as concentric peak torque

Measure	Group	Leg	Set				
			1	2	3	4	5
Concentric Peak torque (%)	CON	Dominant	102±19	103±23	93±14	100±22	104±12
		Non-dominant	76±17	78±20	79±14	83±16	85±16
	CF	Dominant	89±15	89±15	88±14	86±17	87±15
		Non-dominant	84±4	83±12	86±7	93±8	90±16

Notes: CON = control, CF = cocoa flavanols, data displayed as means ±SD

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Table 6.4 Percentage change from first EIMD protocol measured as eccentric peak torque

Measure	Group	Leg	Set				
			1	2	3	4	5
Eccentric peak torque (%)	CON	Dominant	99 ± 18	104±23	92± 17	88± 19	104±23
		Non-dominant	85±10	76± 22	84± 28	83± 27	78± 25
	CF	Dominant	84±12	87± 16	81± 18	86± 15	90± 12
		Non-dominant	83±10	84± 11	83± 8	90± 12	84± 14

Note CON = control, CF = cocoa flavanols, data displayed as means ± SD

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2808 ## 6.3.2 Muscle function

2809 There was a significant main effect for time for MVIC60 ($p < .001$) and MVIC30 ($p <$

2810 .001). For MVIC60 there were significant differences observed between baseline and

all other time points (p ≤ .006), 0 and 24 and 2nd0 (p ≤ .05), 24 and 48, 72, 2nd0 and 2nd48 (p ≤ .03), 48 and 2nd0 (p = .03), and between 2nd0 and all time points except 72 (p ≤ .05). For MVIC30 there were significant differences observed between baseline and all other time points (p ≤ .01), 0 and 2nd0 (p = .01), 24 and 2nd0 (p = .01), 48 and 2nd0 (p = .05), and between 2nd0 and all other time points except 72 (p ≤ .05). No significant differences were observed for MVIC60 ($F(1,7) = .083$, $p = .78$), MVIC60% ($F(1,7) = .429$, $p = .53$), MVIC30 ($F(1,7) = .080$, $p = .79$), or MVIC30% ($F(1,7) = 1.715$, $p = .23$). However, significant differences and large effect sizes were observed at 110 hr post-EIMD (48 hr following the second protocol) for both MVIC60% ($t = -4.276$, $p = .004$, $d = 3.1$) and MVIC30% ($t = -4.032$, $p = .005$, $d = 3.2$). Large effect sizes were also observed for MVIC60 ($t = -1.049$, $p = .33$, $d = 0.8$) and MVIC30 ($t = -1.194$, $p = .27$, $d = 0.9$) at the same time point. Muscle function loss was greatest immediately following the second EIMD protocol. See Table 6.5 for MVIC data (both percentage and absolute values) and Figures 6.2 and 6.3 for a visual representation of MVIC percentage change data. Figures 6.4 and 6.5 show individual MVIC data for MVIC60 and MVIC30 respectively.

Table 6.5 Changes in muscle function measured using MVIC

Measure	Group	BL	0	24	48	72	0	24	48
MVIC60 (Nm)	CON	130±22	97 ± 24	109 ±17	103 ±25	102±24	86± 26	104 ±24	98±17
	CF	132± 30	101± 24	120 ±23	102 ±15	100±18	90± 27	109 ±26	116±27
MVIC60%	CON	100 ± 0	74 ± 9	84 ± 5	78±9	78±12	66±15	80 ±10	75± 5
	CF	100 ± 0	76 ± 7	91 ± 5	78±11	77±13	67±9	82± 7	87± 1
MVIC30 (Nm)	CON	144 ±33	117±29	121±27	122±39	121±42	106±28	118±37	113± 30
	CF	144± 26	126±25	126±17	121±15	121± 13	113±25	123± 29	136± 20
MVIC30%	CON	100 ± 0	81±3	85±6	83±10	82±14	74± 10	81 ± 10	78± 6
	CF	100 ± 0	87±5	88±6	84±6	85±9	78± 5	84±5	95± 4

Notes: MVIC = maximal voluntary isometric contraction, N = Newtons, CON = control, CF = cocoa flavanols, data displayed as means ± SD

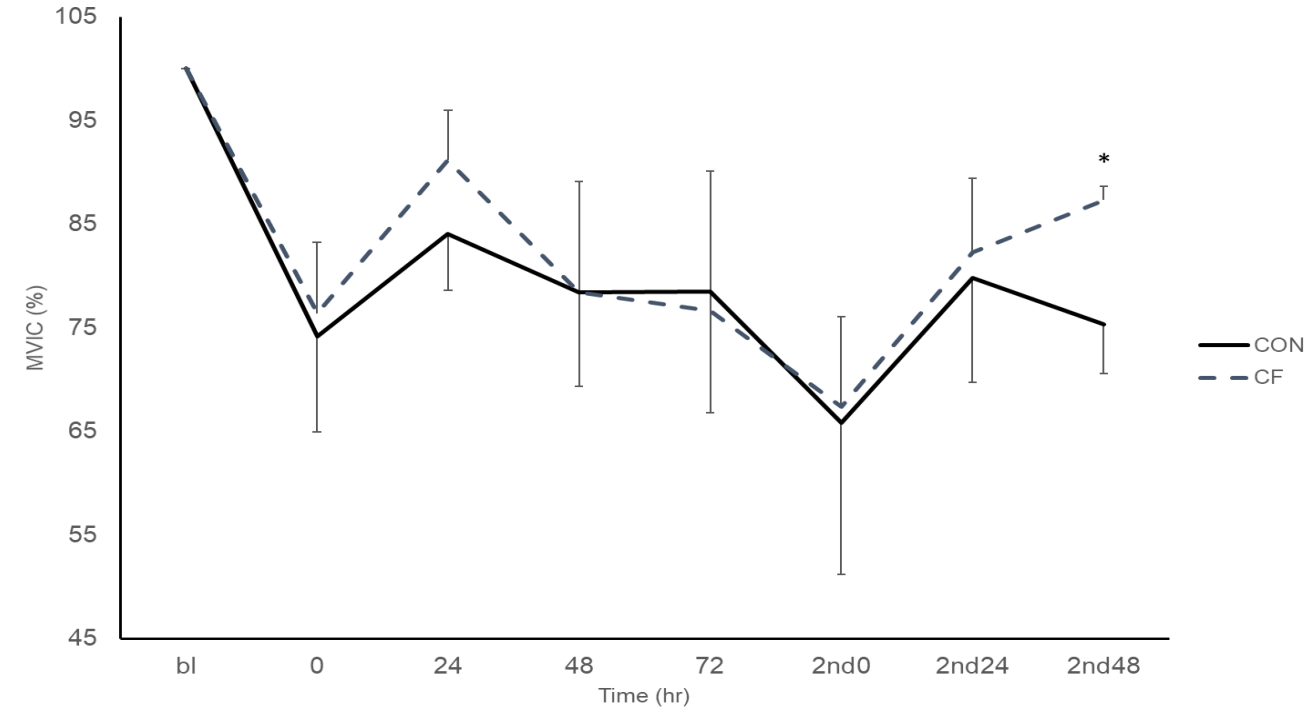

Figure 6.2. MVIC60 percentage change. * *denotes significant difference between groups*

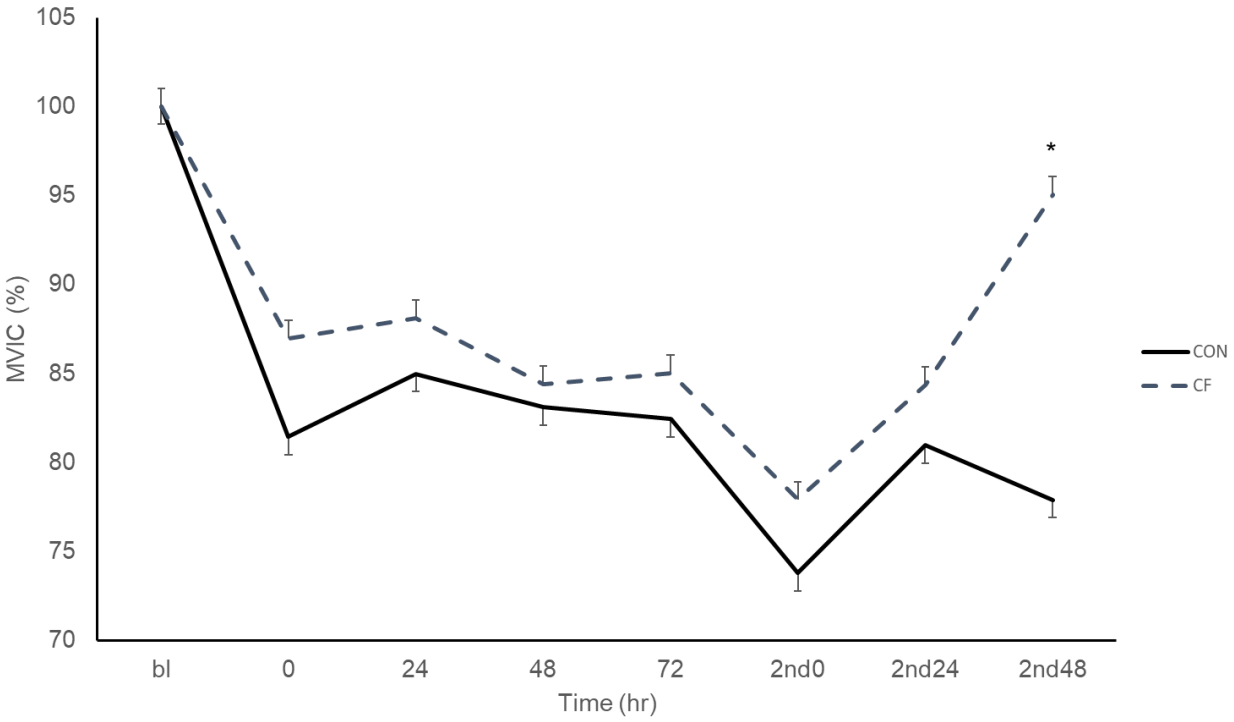

Figure 6.3. MVIC30 percentage change. * *denotes significant difference between groups*

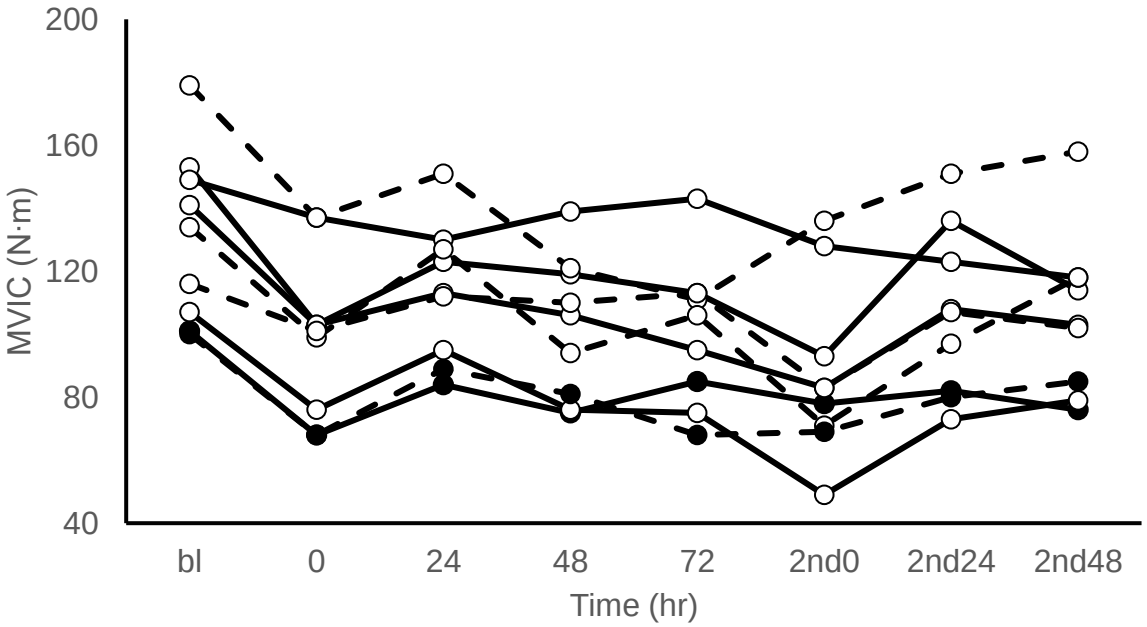

Figure 6.4 MVIC60 Individual data across all time points; *CON = solid lines, CF = dashed lines, black circles = females, white circles = males*

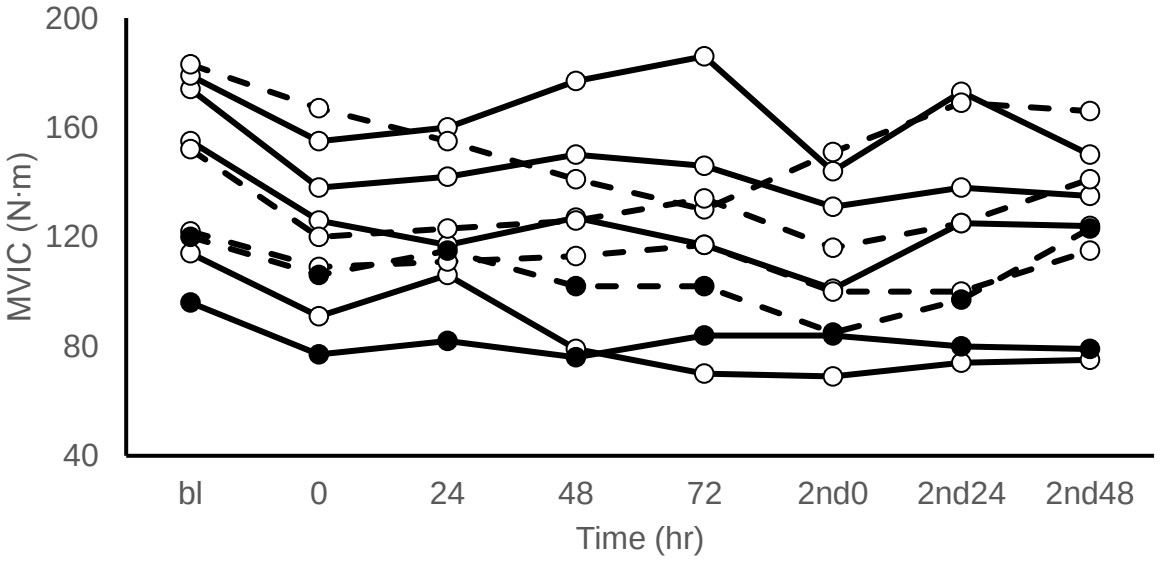

Figure 6.5 MVIC30 Individual data across all time points; *CON = solid lines, CF = dashed lines, black circles = females, white circles = males*

6.3.3 Electromyography

A significant main effect for time was observed for all EMG data ($p \leq .05$), except for median frequency as raw data. For normalised EMG data the greatest number of significant differences occurred between 0 and all other time points ($p \leq .03$). For median frequency (%) differences were observed between baseline and 0, 72, 2nd0, 2nd24, and 2nd48 ($p \leq .04$). For median frequency peak, significant differences were

observed between baseline and 0, 2nd0, 2nd24, and 2nd48 ($p \leq .04$). Significant differences were observed for peak median frequency (%) between baseline and 0, 2nd0, 2nd24, and 2nd 48 ($p \leq .03$). For normalised EMG amplitude data, no significant differences were observed between the CON and CF groups ($F(1,7) = .028$, $p = .87$). Data was similar between every time point for normalised EMG values ($p \geq .31$). Furthermore, no significant differences were observed for median frequency ($F(1,7) = .288$, $p = .61$, % $F(1,7) = 1.075$, $p = .33$) and peak median frequency ($F(1,7) = .227$, $p = .65$, % $F(1,7) = .024$, $p = .88$).

6.3.4 Perceived soreness

For measures of perceived muscle soreness, there was a significant main effect for time for VAS ($p < .001$) and LEFS ($p = .002$). For the VAS significant differences were observed between baseline and all other time points ($p \leq .02$), 0 and 48 and 2nd48 ($p \leq .04$), 24 and 48, 2nd24, and 2nd48 ($p \leq .04$), 48 and all other time points except 72 ($p \leq .05$), 72 and 2nd24 and 2nd48 ($p \leq .003$), 2nd0 and 2nd48 ($p = .004$). For LEFS significant differences were observed between baseline and 24, 48, 72, and 2nd0 ($p \leq .02$), 0 and 48 ($p = .03$), 24 and 48, 2nd24, and 2nd48 ($p \leq .05$), 48 and 2nd24, and 2nd48 ($p \leq .01$), 72 and 2nd24, and 2nd48 ($p \leq .002$), and 2nd0 and 2nd24, and 2nd48 ($p \leq .02$). There were no significant differences observed for VAS ($F(1,7) = 1.262$, $p = .30$) or LEFS ($F(1,7) = .278$, $p = .61$). However, a significant difference and large effect size was observed between groups at 110 hr post-EIMD (48 hr following the second protocol) for VAS ($t = 2.484$, $p = .04$, $d = 1.9$). A large effect size was also observed for LEFS at the same time point ($t = -1.886$, $p = .10$, $d = 1.3$) as well as 24 hr post initial protocol ($t = -1.614$, $p = .19$, $d = 1.1$) and 24 hr post the second protocol ($t = -.949$, $p = .37$, $d = 0.8$). Perceived muscle soreness was greatest at 48 hr post initial EIMD protocol. See Table 6.6 for perceived soreness data.

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Table 6.6 Changes in perceived soreness

Measure	Group	BL	0	24	48	72	0	24	48
VAS (mm)	CON	6 ± 11	101±58	107±36	143±35	98±51	103±49	60±37	67±35
	CF	1± 1	72±53	78± 21	118±32	92±16	85±50	49±27	16±11
LEFS (a.u)	CON	76± 5	63±17	54± 18	50± 19	57±15	52±16	69±10	71± 6
	CF	80± 0	63±15	69± 5	48± 22	58± 9	55±22	75±3	77± 2

Note VAS = visual analogue scale, LEFS = lower extremity functional scale, CON = control, CF = cocoa flavanols, data displayed as means ± SD

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6.4 Discussion

The purpose of this study was to investigate the efficacy of six days of CF supplementation on recovery following two muscle damaging protocols separated by 72 hr. This is the first CF study to investigate such a protocol and it was hypothesised that repeated high doses of CF would aid recovery and repeat performance during strenuous exercise. The data from this study indicate that supplementation did not offer a significant benefit over a control (matched closely for energy, carbohydrates, and protein) although, large effect sizes were noted between groups 48 hr post the second EIMD protocol for both objective and subjective markers of muscle damage (MVIC60 (d = 0.8), MVIC60% (d = 3.1), MVIC30 (d = 0.9), MVIC30% (d = 3.2), VAS (d = 1.9), and LEFS (d = 1.3)). This data suggests that there is no significant benefit of regular CF supplementation following EIMD, although more research is warranted due to the previously mentioned effect sizes that accounts for the limitations associated with this study.

Immediately following the first EIMD protocol there were reductions of MVIC of ~20% immediately and 48 hr post-EIMD in the CON and CF groups; indicating muscle damage was induced (Paulsen et al., 2012). Even though no significant difference was observed between CON and CF groups for overall recovery and repeat performance, by the end of the testing period the CF group showed a greater recovery of muscle function 48 hr following the second EIMD protocol ($p \leq .005$, $d \geq 3.1$). In fact, the CON showed a mean change of +12 N (+9%) for MVIC60(%) and +7 N (+4%) for MVIC30(%) from immediately post-protocol to 48 hr after compared to the CF group that showed improvements of +26 N (+20%) for MVIC60(%) and +23 N (17%) for MVIC30(%). Not only that, but the CON group also showed a negative mean change from 24 hr to 48 hr after the second protocol, whereas the CF group continued to recover peak torque values. Overall, however, the lack of statistically significant findings for recovery of muscle function is in line with previous research (de Carvalho et al., 2019; Morgan et al., 2018; Peschek et al., 2013). However, as this study had a longer supplementation period and a dose higher than what has been previously seen in CF and EIMD studies (~545 mg more than the than next highest total dose (Peschek et al., 2013)) it is possible that a dose above 1000 mg is required to provide any benefit, as seen with the large effect sizes observed within this Chapter and Chapter 5. This speculation is lent further credence by the review performed by Bowtell and

Kelly (2019) who suggest that (poly)phenol supplementation above 1000 mg for three days may be required to confer the proposed physiological benefits associated with their intake. Based on the current evidence it is possible that CF do not provide any significant benefit for the recovery of muscle function, however there is still a paucity of literature available to compare results between and more data is needed to provide a consensus on the possible benefits of CF when supplemented for multiple days. Furthermore, the differences between studies regarding EIMD protocols, measure of muscle function, and in CF dose and supplementation period further indicate the need for future research. The use of a high dose of CF is perhaps required to elicit an ergogenic benefit due to CF absorption being reportedly around 35% of the initial dose (regardless of amount); indicating that a greater dose will result in greater CF absorption (Gómez-Juaristi et al., 2019). However, more research is required to better understand if there is a ceiling to CF absorption.

Throughout the entire testing period perceived muscle soreness was lower in the CF group than the CON for VAS, and LEFS (excluding 48 hr post initial EIMD), although no significant difference was observed ($p \geq .30$). However, a large effect was noted for both VAS ($p = .04$, $d = 1.9$) and LEFS ($p = .10$, $d = 1.3$) at 110 hr (48 hr post second EIMD protocol). Interestingly, the CF group had greater reductions of perceived soreness, measured *via* VAS, following the second EIMD protocol than the CON group. During this time period (immediately following the second protocol to 48 hr post) the CF group showed consistent reductions in VAS scores, with a mean reduction of 69 mm, whereas the CON had a mean reduction of 36 mm. This indicates that the CF treatment may have assuaged feelings of perceived soreness that may arise from a repeated bout of strenuous exercise. Following the second bout, the CON group VAS scores increased from 24 hr to 48 hr post, whereas the CF continued to reduce and was nearly at baseline levels by 48 hr after the second bout. It is possible that the explanation for this is due to the antioxidant and anti-inflammatory properties of CF, inflammatory molecules are known to stimulate nociceptors *via* the secretion of protein degrading enzymes and ROS leading to feelings of pain (Pinho-Ribeiro et al., 2017).

It has been reported that following a secondary bout of soccer match-play and/or soccer simulation in a fixture congested format, akin to the format of this research, increase muscle soreness and inflammation (Page et al., 2019). As such, the use of a high dose of CF may provide a protective effect from potential negative consequences,

such as increased soreness and reduced markers of performance, that may arise from the residual fatigue associated with repeated bouts of strenuous exercise. However, the practical applicability of this data may be more suited to repeated bouts of intense resistance training type exercise due to the nature of the protocol. Interestingly, previous research investigating the specific impact of two repeated bouts of EIMD separated by three days has shown that the second bout of exercise does not impede recovery, with MVIC recovery only impacted immediately following a second bout of exercise but continuing to recover in the days following (Chen, 2003; Chen & Nosaka, 2006). Indeed, these data differ from Chen (2003) and Chen and Nosaka (2006) as only the CF groups recovery was not delayed by the second bout of exercise. The CON group suffered MVIC decrements and an increase in VAS scores from 24 hr post the second EIMD protocol to 48 hr post, whereas the CF did not. It should be noted that both the previous studies targeted the elbow flexors whereas the knee flexors were targeted within the present study. It has been reported previously that the elbow flexors are more susceptible to muscle damage than the knee flexors which could partly explain the differences between the studies (Chen et al., 2011). Within team sports such as soccer, knee flexor injuries are among the most frequent (Engebretsen, Myklebust, Holme, Engebretsen, & Bahr, 2010; Waldén, Hägglund, & Ekstrand, 2005), with many of these injuries a result of various factors including accumulated fatigue, strength imbalances, and previous injury (Opar, Williams, & Shield, 2012). Therefore, future research could look to investigate nutritional preventative methods for reducing muscular fatigue, with a specific look at the knee flexors and other posterior thigh muscle groups.

It is well known that a repeated bout of eccentric exercise leads to skeletal muscle adaptation that reduces subsequent muscle damage (Hyldahl, Chen, & Nosaka, 2017; McHugh et al., 1999; Starbuck & Eston, 2012). These adaptations normally lead to a reduction in the extent of post-exercise strength losses, muscle soreness, expulsion of myocellular proteins, and potentially a reduced inflammatory response. It is possible that various neural adaptations occur following the completion of a muscle damaging exercise bout. The central nervous system may cause an increase in motor unit synchronisation and alteration in muscle activation patterns as a way of protecting the fatigued/ damaged muscle and maintain task success (Kellis, Zafeiridis, & Amiridis, 2011; Missenard, Mottet, & Perrey, 2009). This is likely through an increase in the

coordination of synergist muscle to further distribute the mechanical load placed on the working muscles. The EMG data suggests that this may be the case, as the median frequency was reduced in both groups but decreased to a greater extent in the CON group compared to the CF following the second EIMD protocol during the EMG exercise activity (glute-hamstring bridge) (75 ± 3% vs 93 ± 22%, p = .160, *d* = 1.2). In fact, throughout the entire testing period the CON consistently had lower median frequency values than the CF group. Indeed, other research has shown reductions in median frequency following EIMD ranging from 20-30% (Chen, 2003; Starbuck & Eston, 2012; Warren, Hermann, Ingalls, Masselli, & Armstrong, 2000) similar to the CON group showing a reduction of 25 ± 3% following the second protocol.

The EMG exercise activity involved other muscle groups, not isolating the knee flexors, which may explain why EMG amplitude did not show an increase above baseline; an increase would imply a greater level of motor unit recruitment to perform the action. Reinforcing the idea that other muscles, e.g., other hip extensors, may have been recruited to a greater extent to compensate for the fatigued knee flexors. The EMG amplitude increased from baseline to immediately post-EIMD and 24 hr following in both groups, which does indicate that there is increased motor unit recruitment of the knee flexors to perform the exercise task. Whereas immediately post the second protocol only the CON group amplitude is above baseline (114 ± 39 vs 87 ± 21%, p = .31, *d* = 0.8) indicating there may be a change in the activation of surrounding muscle to perform the task in a fatigued state. This involuntary activity may be protective but is unlikely to compensate fully for the fatigued muscle. Future research should look to collect data from synergist muscle groups to account for any changes in their activation when a specific muscle group is in a fatigued/compromised state.

As EMG acquisition was used to assess to impact of EIMD on the change in muscle efforts measured *via* EMG amplitude the inclusion of median frequency analysis for muscle fatigue was considered as a secondary measure for fatigue. To develop a greater insight into the role of CF on fatigue, longer muscular contractions, and more frequent data points across each time points (e.g., separated by 30 min at each time point) would be valuable in future studies.

This study is not without its limitations, namely due to the COVID-19 pandemic. The pandemic resulted in the cessation of data collection and participant recruitment

leading to only nine participants completing the study. As such, the *a priori* power calculation was not satisfied, meaning any significant differences and findings should be interpreted with caution. It is highly likely, that differences observed could be due to individual variability and perhaps not entirely due to the CF supplementation. Another limitation is that due to the nature of the muscle damaging protocols used, the findings may not translate to team sports with short recovery periods, such as soccer. This is due to the lack of ecological validity associated with the protocol as it does not reflect realistic sport. Instead, the findings may have more application to repeated bouts of resistance training and have practical applications for weightlifters, power lifters, bodybuilders and so on.

Due to the current findings future replication of this study is warranted to better understand the possible benefits that regular CF consumption may elicit on muscle recovery. Further limitations are due to constraints regarding the feasibility of specific measures such as muscle biopsies, inflammatory and oxidative stress markers. However, this does not detract from the quality of the measures chosen instead rather the inclusion of biomarkers would have provided greater mechanistic insight into recovery.

In summary, CF provide no significant benefits for muscle recovery or repeat exercise performance compared to a carbohydrate protein control. Even though large effect sizes were observed at the final time point following the second EIMD protocol, it is possible that this is due to individual variability. As such, further research is required to better understand the potential beneficial nature of CF supplementation.

Chapter 7 General Discussion

Athletes desire to improve exercise recovery has resulted in an increase in the interest of nutritional interventions purported to expedite the recovery process. This thesis sought to increase the knowledge around one such intervention, CF. The rationale is that cocoa contains large amounts of bioactive compounds that may provide a protective effect against muscle damage, most likely against the inflammation and oxidative stress associated with EIMD. Due to the high concentration of (poly)phenols, specifically CF, it was hypothesised that a high dose of CF post-EIMD may assuage the negative consequences associated with muscle damage such as impaired muscle function, muscle soreness and changes to neuromuscular activation. As such, the objective of this thesis was to investigate whether CF could be efficacious as an ergogenic aid for muscle recovery *via* the attenuation of the aforementioned symptoms of muscle damage (Chapter 2 Section 3). Specifically, this thesis aimed to *i*) investigate whether a single acute dose of CF impacted muscle damage, *ii*) whether a 1245 mg dose conferred a greater benefit than 830 mg, *iii*) whether sex had any influence on the potential effects of CF following EIMD, *iv*) the efficacy of regular consumption of CF on attenuating EIMD and *v*) whether regular CF consumption aided repeat performance 72 hr post-EIMD. This Chapter will synthesise the findings of the investigations of the thesis and their contributions to the existing literature as well as include a discussion of possible practical applications and future research ideas before addressing the limitations of the thesis.

7.1 Summary of experimental findings

The first and second experimental studies (Chapter 5 and 6) had the purpose of addressing the aims *i*, *ii* and *iii*, investigating whether different doses of acute CF supplementation aided muscle recovery following EIMD and whether there was any variation between sex. Before experimental testing began, a systematic review (Chapter 3) was carried out to identify gaps within the literature and areas in need of development regarding CF and muscle damage. The key findings of the systematic review (Chapter 3) within the thesis, suggest that CF supplementation blunts exercise-induced oxidative stress. The exact mechanisms for this are still not clear, it is possible that supplementation may improve the cellular redox environment making it more capable to effectively quench ROS, perhaps *via* the upregulation of the Nrf2 pathway and endogenous antioxidants (Cheng et al., 2013; Cordero-Herrera et al., 2015;

Martins et al., 2020). Additionally, it was noted that CF may delay fatigue and improve performance during exercise, potentially by limiting ROS induced fatigue.

From the systematic review of Chapter 3, it was identified that evidence for the anti-inflammatory effects of CF following exercise were limited, along with evidence for reducing soreness and improving muscle function recovery following EIMD. With only three previous studies investigating CF supplementation on EIMD (de Carvalho et al., 2019; Morgan, Wollman, Jackman, & Bowtell, 2018; Peschek, Pritchett, Bergman, & Pritchett, 2013) it was believed pertinent to contribute to this area of research. Furthermore, these three studies had utilised relatively low to moderate CF doses that may not be efficacious based on the surrounding literature regarding the oxidative stress and possible anti-inflammatory benefits (outside of exercise). Indeed, Peschek et al., (2013) used two acute doses of 350 mg, de Carvalho et al., (2019) used 616 mg daily for seven days, and Morgan et al., (2018) used a cacao mucilage drink containing 74 mg CF. This was something I looked to address within this thesis, utilising high dose CF supplementation. Furthermore, within two of the aforementioned studies it is possible that the EIMD protocols utilised were perhaps insufficient to elicit notable muscle damage within their respective cohorts (de Carvalho et al., 2019; Peschek et al., 2013). Consequently, I selected a validated laboratory based EIMD protocol (see Chapter 4 Section 4) to induce a desired muscle damage response. Furthermore, I selected the knee flexors as the muscle group to examine due to their propensity for injury and the fact that the other CF studies had not yet investigated this muscle group.

Based on the findings of Chapter 3, it was established that as the available literature was limited at the time, and for all intents and purpose still is, it was appropriate to establish whether a high dose of CF had an impact on muscle recovery when consumed acutely, e.g., immediately post EIMD. It was hypothesised that an acute dose of CF would attenuate the deleterious symptoms of muscle damage and a higher (1245 mg vs 830 mg) dose would have a greater effect when compared to a control. Based on the resultant findings an acute dose had no significant effect on recovery of muscle function or on measures of perceived soreness. However, large effect sizes were observed for a number of findings when comparing the highest dose to the control. When comparing the data as percentage change large effect sizes were observed between the 1245 mg CF group and the CON for MVIC60% and MVIC30%

at 24 and 48 hr post-EIMD. No significant differences were observed for VAS and LEFS, however a large effect size was observed between 1245 mg CF and CON at 48 hr for VAS data. Furthermore, moderate effect sizes were observed between 1245 mg CF and 830 mg CF in favour of the higher dose for VAS at 48 hr, and LEFS at 48, and 72 hr. The findings from Chapter 5a provided valuable information, allowing for an informed decision to be made on which dose to select for the future study involving repeated doses of CF (Chapter 6). This study provided evidence that *i*) an acute dose may be insufficient and *ii*) 1245 mg of CF may be more effective than 830 mg for assuaging feelings of soreness, albeit not significantly.

For the second experimental study (Chapter 5b) a subgroup analysis was performed splitting the participants by sex to compare for both inter and intra-sex differences. No significant differences were observed for any measures for inter- or intra-sex differences apart from MVIC data analysed as the raw values, when converted to percentage change no differences were observed. Interestingly however, females within the CF groups consistently scored lower for levels of perceived soreness than the CON group, whereas males scored similar values across groups. Specifically for female VAS scores, when comparing between the treatment groups and control the data was approaching significance (830 mg vs CON $p = .068$ and 1245 mg vs CON $p = .059$). Additionally, when comparing soreness between the sexes, females within the CF groups scored lower than that of the males within the same groups, whereas in the control group males scored lower than females. For muscle function, MVIC30 percentage change data showed a significant difference between the males and females in the CF_{1245} group at 72 hr ($p = .03$), females had returned to baseline values whereas males only achieved 80%. From this data, it almost appears that the females gained the greater benefit from CF supplementation when comparing within group differences for muscle soreness. However, no significant differences were observed between the groups other than for absolute MVIC data, of which these differences did not exist when expressed at percentage change. Indeed, this highlights an interesting area of future research, especially when considering the limited data set and the need for a fully powered study comparing any sex differences.

For the final experimental study (Chapter 6), the highest dose from the previous research was selected based off the data that was gathered from Chapter 5. Not only that, but instead of an acute dose, participants supplemented the CF drink each day

during the experimental protocol and an additional time post-EIMD protocol, of which there were two separated by 72 hr. This timeline was selected to mimic training structure for weightlifting sports and somewhat replicate fixture congestion and tournament settings in team sport, as these times require rapid recovery to ensure optimal performance and to limit injury risk. For this study it was hypothesised that based on the data from Chapter 5, indicating that an acute dose was insufficient to confer a significant benefit for attenuating the symptoms of muscle damage and as such regular supplementation may be required throughout the recovery period, as seen with other (poly)phenol research (Bell et al., 2016; Quinlan & Hill, 2020; Tanabe et al., 2019). Within Chapter 6, no overall significant differences were observed based on the data, likely due to the limited sample size of the study. However, even though participant numbers for this study were limited, significant differences and large effect sizes were observed when analysing the final time point between the CF and CON group for muscle function as percentage change, as absolute values, and for VAS data (which was also significantly different). Additionally, immediately post the second EIMD protocol a large effect size was observed for EMG data expressed as median frequency percentage change (75 ± 3% vs 93 ± 22%, p = .16, *d* = 1.2). Again, immediately post the second protocol only the CON group had a higher EMG amplitude compared to baseline when expressed as a percentage change (114 ± 39 vs 87 ± 21%, p = .31, *d* = 0.8). Therefore, it is possible that following the repeated bout of strenuous exercise the CON group was in a greater fatigued state than the CF group due to the possible protective effect of CF allowing for continued recovery as opposed to impeded recovery. For isokinetic data, the CON group had greater reductions in isokinetic concentric and eccentric peak torques than the CF group for the non-dominant limb, the one involved for MVIC. This may imply that the consumption of CF may aid with the maintenance of maximal performance during a repeated bout of exercise in a fatigued state.

Collectively, the results provided from the experimental studies conducted for this thesis add interesting and novel insights to the current body of knowledge and indicate a pertinent need for further research. Even though there was a lack of statistical significance within the research the large effect sizes provide some evidence that there may be a beneficial effect of CF supplementation. These data warrant future studies

including prolonged supplementation of high dose of CF and the potential effects they may elicit on muscle recovery.

7.2 Cocoa Flavanols impact on muscle function and perceived soreness

The growing demands of sport, such as the increase in fixture congestion observed in soccer, in turn propagates a potential increase in EIMD and for this reason ergogenic aids to improve recovery are becoming an integral part of many athlete's and general individual's training regimen. This thesis was to examine the efficacy of such an aid in a scenario that elicited muscle damage. From the data it was observed that the consumption of CF whether acute or regular resulted in no significant advantage over a carbohydrate-protein control recovery beverage for muscle recovery measured *via* muscle function and muscle soreness.

7.2.1 Muscle function

Specifically for the recovery of muscle function, assessed *via* MVIC and in Chapter 6 EMG, no overall significant effects were observed. This is in line with previous research that has used MVIC as a recovery marker (de Carvalho et al., 2019; Morgan et al., 2018; Peschek et al., 2013). A key difference between those studies and the ones included within the thesis is the selection of muscle group targeted for muscle damage. The other studies investigated the impact of CF on knee extensor recovery as opposed to knee flexors. Knee flexors are more susceptible than the knee extensors to muscle damage (Chen et al., 2011); thus, the knee flexors may be a more pertinent muscle group to investigate. Even more so when considering the evidence for knee flexor injuries being amongst the most common in sport (Chumanov, Schache, Heiderscheit, & Thelen, 2012; Opar et al., 2012; Small, McNaughton, Greig, Lohkamp, & Lovell, 2009a)

For measures of muscle function other than MVIC, Morgan et al., (2018) found that supplementation of cacao mucilage provided a significant benefit for the recovery of countermovement jump height. Indicating there may be some benefit for explosive power compared to strength. However, as a measure, countermovement jump height was not included within this thesis, this choice was made due to the difference in muscle contribution when comparing the role of the knee extensors to the knee flexors. The biceps femoris has been shown to maintain low level activation during the entire movement of a countermovement jump, however it only reaches around 40% of maximal activation, whereas the rectus femoris reaches ~100% of maximal activation

(Mackala, Stodólka, Siemienski, & Coh, 2013). This means that any changes in muscle fatigue will be more evident in muscles with greater levels of activation during the exercise.

Within Chapter 5a multiple large effect sizes were observed at 48 hr between the CON and CF group supplementing 1245 mg acutely for MVIC measures. However, as no significant difference was observed it is likely that an acute dose was insufficient in conferring a beneficial effect for the recovery of force generating capability. Peschek et al., (2013) also found no significant benefit of CF on MVIC following two acute doses of 350 mg CF, in fact the CF group had a greater loss in MVIC than the control group (~5% decrease in the control vs 11 and 22% decrease in CF from pre – 48 hr post in right and left legs, respectively). What is interesting from Peschek et al., (2013) data, however, is that the CF group MVIC increased from 24 to 48 hr post-EIMD whereas the control group did not. As the control group did not show signs of impaired muscle function it is possible that the EIMD protocol (downhill running) was insufficient for the well-trained endurance athletes used within the study. From a mechanistic perspective it is essential to elicit a muscle damage response when investigating the effects of an intervention on muscle damage, something this thesis aimed to do whilst utilising the laboratory-based protocol. As such, future research is warranted to reach a consensus about the potential ergogenic effects on recovery an acute dose of CF may have as there is a dearth of research in the area.

In Chapter 6 large effect sizes were observed 48 hr post the second protocol when comparing regular supplementation of 1245 mg CF to a CON. These data were accompanied this time by a statistically significant difference at the final time point for MVIC60 percentage change ($p = .004$, $d = 3.1$) and MVIC30 percentage change ($p = .005$, $d = 3.2$) with large effect sizes alone noted for the absolute values at the same time point (MVIC60 $d = 0.8$ and MVIC30 $d = 0.9$). Other studies that utilised repeated dosing of CF have previously found no benefit for MVIC recovery (de Carvalho et al., 2019; Morgan et al., 2018). Both of the previous studies incorporated MVIC of the knee extensors as a measure, however both only included a single angle of measurement. It is possible that following EIMD with a high amount of eccentric muscle contractions the optimum angle changes to longer muscle lengths, evidenced in the knee extensors (Bowers, Morgan, & Proske, 2004) and knee flexors (Brockett, Morgan, & Proske, 2001). As such, a single angle may over or underestimate changes

in contractile capability following EIMD (Paulsen et al., 2012). Participants within the study by de Carvalho and colleagues (2019) appear to not have suffered from the deleterious effects associated with muscle damaging exercise as at 48 hr post-EIMD both the control and CF group had MVIC scores ≥103% of baseline. Conversely, P. Morgan et al., (2018) found significant reductions in MVIC following the EIMD protocol, interestingly they found that at the final time point (48 hr post-EIMD) the treatment group had recovered to 90.8 ± 14% of baseline, whereas the control group only 85.1 ± 15.6% however this difference was non-significant. Similar to Chapter 6, Morgan and colleagues (2018) supplemented each day of recovery (as well as seven days before the protocol). In Chapter 6 participants supplemented every day of the testing period, twice on protocol days, totalling six days of supplementation. Although both studies saw that the CF group had a greater MVIC percentage on the final day of testing, only the data within Chapter 6 observed a large effect size between the groups. It is possible that the reason for this is the large difference between CF dose used in the two studies, Morgan and colleagues (2018) supplemented with a cacao juice drink containing 74 mg CF whereas Chapter 6 utilised a dose of 1245 mg. Indicating that perhaps regular supplementation of a high dose of CF is necessary to gain an additional benefit for MVIC recovery compared to a carbohydrate protein control.

Regarding the EMG data gathered in Chapter 6 for median frequency, it was found that the CON group had a similar reduction in median frequency as what has been observed in other research investigating EMG activity of muscle groups following two EIMD protocols (Chen, 2003; Starbuck & Eston, 2012; Warren et al., 2000). However, the CF group within the Chapter did not follow the same pattern, instead the reductions observed were smaller, albeit not significantly (75 ± 3% vs 93 ± 22%, d = 1.3). Other research has observed that supplementation using green tea extract prevented a decrease in median frequency of the left vastus lateralis during a period of cumulative fatigue (Machado, da Silva, Souza, & Carpes, 2018). Furthermore, the EMG amplitude post second EIMD protocol was far greater in the CON group than the CF (114 ± 39% vs 87 ± 21%, d = 0.8) indicating that although the frequency is reduced the biceps femoris is still at a greater level of motor unit recruitment than baseline in the CON group. This may suggest that regular CF, or other (poly)phenol supplementation provides a protective effect against muscle related fatigue and its impact on task completion. Therefore, to garner a greater understanding of this proposed mechanism

future research should gather data from other synergist muscles during the exercise task to assess change in involvement.

7.2.2 Perceived soreness

For measures of perceived soreness, the experimental investigations conducted for this thesis did not observe any significant differences between CON or CF groups for either VAS or LEFS. However, similar to muscle function, multiple large effect sizes were observed in Chapter 5a for VAS at 48 hr post-EIMD (*d* = 0.9) and in Chapter 5b, 48-hr post second EIMD protocol for VAS (*d* = 1.9). For LEFS, large effect sizes were observed in Chapter 6 at 24 hr post-EIMD (*d* = 1.1), then at 24 hr (*d* = 0.8) and 48 hr (*d* = 1.3) post second EIMD protocol. This indicates that CF may provide some analgesic benefit over carbohydrates and protein alone, assuaging feelings of perceived muscle soreness following a second bout of strenuous exercise. Although these large effect sizes were observed within this thesis, no significant differences were found. This is in line with other data from previous CF EIMD research (de Carvalho et al., 2019; Morgan et al., 2018; Peschek et al., 2013). It is possible that as the data in this thesis highlights large effect sizes between the groups it may be that the previous studies did not provide sufficient amounts of CF to induce any analgesic benefit. Indeed, this thesis included the largest CF dose seen within muscle damage research at 1245 mg, providing novel insights into CF dosing strategies. Furthermore, as De Carvalho and colleagues (2019) and Pescheck and colleagues (2013) did not appear to induce notable muscle damage based on the data, it is possible that the addition of CF would not signficiantly influence soreness due to the protocols only causing moderate increases in VAS scores.

As muscle tissue samples and markers of inflammation were not measured in this thesis, the underlying mechanisms by which CF may impact feelings of soreness remains elusive. Speculatively, it is likely due to the anti-inflammatory and antioxidant properties of CF. Inflammatory molecules are known to stimulate nociceptors which are responsible for ‘pain signals’ indicating that an upregulated anti-inflammatory response at the site of muscle damage may in turn reduce overall feelings of soreness, evidenced by the benefits observed following ibuprofen (Tokmakidis, Kokkinidis, Smilios, & Douda, 2003) and various (poly)phenol compounds (Herrlinger, Chirouzes, & Ceddia, 2015; Zhang & Tsao, 2016).

Furthermore, the role that CF may have on inflammation and oxidative stress may be the driving mechanism by which it aids both muscle function and soreness. The ingestion of CF immediately post-EIMD whether just acutely or as part of a supplementation period may reduce the acute rise in cytokines and inflammatory mediators post-EIMD (Paulsen et al., 2012; Pizza et al., 2005). These molecules can further damage the response by propagating an increase in the accumulation of pro-inflammatory molecules, e.g., TNF-α and ROS, which can damage healthy bystander tissues (Paulsen et al., 2012). Reducing or limiting this increase may enhance recovery by expediting the recovery of muscle function, which may be influenced by the redox state of the muscle and level of inflammation present (Powers, Ji, Kavazis, & Jackson, 2011), and reduce soreness, which can increase perceived effort during exercise and potentially – depending on severity, inhibit performance to avoid pain (Staiano, Bosio, de Morree, Rampinini, & Marcora, 2018).

Therefore, when combining the evidence from Chapters 5 and 6, it is likely that regular supplementation is required to confer the benefits that have been observed with other (poly)phenol nutritional aids like beetroot juice (Clifford, Bell, et al., 2016; Clifford et al., 2017), curcumin (Ms et al., 2020; Tanabe et al., 2019), and Montmorency tart cherries (Connolly et al., 2006; Kuehl, Perrier, Elliot, & Chesnutt, 2010). However, for each article evidencing the benefits of these functional foods there is another that does not observe any protective effect or instead showcases limited efficacy compared to a placebo (Abbott, Brashill, Brett, & Clifford, 2020; Costello et al., 2020; Lamb et al., 2019).

7.2.3 Sex Differences and Cocoa Flavanols

Another novel aspect of this thesis is the intra- and inter-sex comparisons of Chapter 5b. There is potential for sex differences to exist regarding reductions in oxidative stress following the consumption of different (poly)phenol blends (Burton-Freeman et al., 2010), and oxidative stress is a key contributor to muscle damage. However, to date, no research has looked to compare sex differences for the muscle damage response following CF consumption. Based on the data in Chapter 5b there are no significant differences, although further research utilising oxidative stress markers is warranted. However, when comparing female VAS scores from the CON group to the two CF groups the data was approaching significance (CON vs CF_{830} $p = .068$ and CON vs CF_{1245} $p = .059$) with both scoring large effect sizes at 24 and 48 hr post EIMD

(*d* ≥ 1.2). Specifically, the CF_{1245} females had significantly lower VAS scores than the CON females at 24 hr (p = .03 *d* = 1.6) and 48 hr (p = .03 *d* = 1.6). It is possible that there is a compounding element to the antioxidant and anti-inflammatory effects of CF and oestrogen which may in turn reduce feelings of soreness. This is perhaps further evidenced as females scored lower VAS scores than males did apart from in the CON group.

As all females were tested within the luteal phase of their menstrual cycle it is likely that oestrogen will play a role. Evidence has recently shown that females suffer higher levels of soreness following EIMD when performing in the follicular phase, characterised by low levels of circulating oestrogen (Romero-Parra, Alfaro-Magallanes, et al., 2020; Romero-Parra, Barba-Moreno, et al., 2020). Therefore, a reduction in pro-inflammatory molecules such as TNF-α and lL-1β would likely coincide with reduced feelings of soreness as these molecules are known to sensitise nociceptors within the muscle (Pinho-Ribeiro et al., 2017). Not only that but the females within CF_{1245} returned to baseline MVIC levels for MVIC30 at 72 hr whereas the males within the same group remained at 80 ± 1% of baseline (p = .03 *d* = 2.3). It is possible that when combining the findings for soreness and muscle function it seems more evident that females supplementing the highest dose of CF may have had the greatest benefit. However, more research is needed to elucidate differences in menstrual cycle phase and (poly)phenol metabolism as well as the inclusion of a battery of tests to measure inflammation to better understand the mechanisms involved in recovery. Furthermore, a larger cohort of participants should be included to reduce the chance of individual variation impacting results.

7.3 Practical applications

Based on the findings of this thesis, there are some practical applications that could be considered for the use of CF as an ergogenic aid for muscle recovery. However, no statistically significant findings were observed overall and only at specific time points during the experimental studies. Therefore, these should be considered cautiously and trialled before implementation in a practical setting to judge the effectiveness.

Firstly, CF consumption at high doses immediately post-EIMD may attenuate the deleterious effects that muscle damage has on muscle function, specifically with muscle force production, albeit not significantly compared to a carbohydrate-protein

control. These benefits were noted at the 48-hr post-EIMD in Chapter 5 and then at the 48-hr point post a second protocol in Chapter 6. Furthermore, the protocol used within this thesis induced notable muscle damage, characterized *via* strength losses of ≥ 20% with reductions existing for ≥ 48-hr (Paulsen et al., 2012). Thus, for athletes entering a period of performance with multiple strenuous bouts of exercise or periods that have a need for expedited recovery such as a fixture congested period, tournament setting in multiple team sports such as hockey, or Olympic athletes that compete daily or multiple times a day, e.g., judo, may find use of CF or (poly)phenols to aid recovery.

Secondly, similar results were noted for reductions in perceived pain as CF appeared to assuage feelings of soreness 48-hr post EIMD protocol in Chapter 5 and 48-hr post second EIMD protocol in Chapter 6. As muscle soreness can increase the perception of effort during exercise and reduce exercise performance (Pageaux, 2016) it may be beneficial during times when soreness is prevalent, e.g., fixture congestion, pre-season, long lasting competitions such as the tour de France or various tournaments such as in hockey and tennis. Furthermore, perception of recovery, e.g., reduced soreness, may be of benefit to athletes in understanding their own recovery and performance readiness.

From this thesis however, no recommendation can be given with certainty regarding an effective dose of CF other than the greatest benefit was observed following a dose of 1245 mg. Pairing this with other research recommendations for (poly)phenol doses, regular supplementation of ≥ 3 days may be the most efficacious in achieving the desired effects. Future research should look to further investigate the efficacy of a high dose of CF for recovery in sporting settings including a wide range of participants across the athletic pyramid, e.g., elite and/or recreational individuals. For a wide implementation of CF in athletic settings there is a requirement for more evidence of their benefits and an increase in the commercial availability of high flavanol cocoa powder.

Finally, a potentially interesting measure to include in future research regarding recovery drinks is a palatability scale or short questionnaire regarding the taste, texture, and enjoyment of the drink. This could allow for increased athlete usage if the drink is widely considered enjoyable, as one element that detracts from other recovery

aids, such as beetroot juice, is the taste. The development of a comparative index of nutritional ergogenic aids with reference to the benefits and taste may be worthwhile for applying their use into a practical setting. Additionally, as taste preferences vary on individual basis and are influenced at a genetic level (Eriksson, Esberg, Haworth, Holgerson, & Johansson, 2019), the use of any nutritional aid should be trialled on an individual basis.

7.4 Future Directions

The series of experimental studies compiled within this thesis have provided some interesting findings and have also uncovered avenues for future research relating to CF, muscle recovery and wider nutritional interventions. This section will cover some of these ideas.

The findings from both Chapter 5 and Chapter 6 have raised a pertinent question, can a very high dose of CF be efficacious as an ergogenic aid for muscle recovery. Neither Chapter observed significant differences for treatment however large effect sizes were found between the groups. As Chapter 6 was underpowered as discussed earlier in the thesis (see Chapter 6 Section 5) it would be of great interest for future research to follow a similar loading protocol with a sample size that satisfies a power calculation. This would allow for a continuation of the research and increase the understanding of the potential benefits of CF when consumed as a high dose (e.g., >1000 mg).

A study investigating the fate of the various flavanols and their metabolites following the consumption of a high dose would also provide a greater insight into the possible bioactivity of CF. This would also aid with the creation of a recommended amount if perhaps there is a ceiling for the absorption of CF, so a comparative study with various doses may provide insight into that. Furthermore, as it has been recently identified that the metabolites of CF from the gut microflora may still remain in circulation >24-hr post-consumption (Gómez-Juaristi et al., 2019; Spencer, Schroeter, Rechner, & Rice-Evans, 2001), any future study should look to include numerous timepoints that span over a 24-hr period. Not only that but these studies may benefit from including both plasma and urinary samples to identify the rate of appearance in circulation but also the rate of excretion. To allow for possible practical applications of research investigating the bioavailability of CF, a commercial high flavanol cocoa powder may be the best option. This way other investigations can take place using the same product and it would be accessible for the general public to acquire and consume.

Another important question for future studies is whether CF exert any benefit on EIMD when elicited by an ecologically valid protocol, i.e., repeated sprints, soccer simulation or real-world sporting scenarios, i.e., match play. These are the scenarios that may require expedited recovery the most, especially in soccer due to the increased prevalence of fixture congestion and reduced recovery time (Julian et al., 2020). Even though female soccer has fewer fixtures than male soccer, there are still times when recovery may be crucial for team success, specifically during tournament scenarios. Throughout the thesis the muscle damaging protocol used did not replicate the demands of any specific exercise other than maximal contractions of the knee flexors. However, Chapter 6 from within this thesis followed a structure akin to a fixture congested period albeit with muscle damaging protocols as opposed to soccer simulation or match play. Based on the findings of that investigation it may be possible that CF would exert a benefit for recovery following the second bout of soccer exercise 72 hr post the initial. The study uncovered that the individuals within the CF group were unimpeded by the second bout of exercise for recovery of muscle function and soreness, whereas the CON had their recovery impeded by the second bout, evidenced by drop-offs in MVIC and increased soreness. Large effect sizes were observed for all MVIC measures and VAS at 48 hr post the second protocol between the groups. This improved recovery could be pertinent in an actual sporting scenario in which rapid recovery could be a determining factor for success. As such, future studies should look to supplement CF alongside exercise protocols that mimic the demands of match play within a fixture congested schedule, possibly including an extra-time period during the second bout of exercise due to the added physiological and biomechanical load and exacerbated fatigue (Field, Corr, et al., 2020; Field, Page, et al., 2020). The inclusion of performance markers to assess recovery such as countermovement jump height, reactive stress index, muscle force output, and sprint time will greatly enhance the knowledge base for the impact of CF on muscle recovery and exercise performance.

One of the interesting findings observed in Chapter 6 related to the EMG data obtained immediately post the second exercise bout that week was related to the median EMG frequency. The CON group had a reduced median frequency to 75 ± 3% of baseline, indeed this reduction implies that other synergist muscles may have been recruited to aid with exercise task completion. The CF group however, reduced to 91 ± 14% of

baseline which indicates a greater usage of the knee flexors during the exercise task. Perhaps the difference observed came as a result of the supplementation of CF attenuating muscle damage allowing for a greater level of direct hamstring muscle usage. To better understand the role of CF on muscle fatigue future studies should look to collect EMG data from other contributing muscle groups for the chosen exercise. In the case of this thesis for the chosen exercise (glute-hamstring bridge) other hip extensors such as the gluteus maximus may have been preferentially recruited to aid with task completion. It has been previously reported that when in a fatigued condition the role of the hamstring muscle group appears to be different compared to non-fatigued (Edouard et al., 2018). Indeed, other muscles perform compensatory mechanisms to maintain performance and protect the fatigued muscle. Gathering more EMG data for various contributing muscle groups will allow for greater insight into the fatigue mechanics elicited from EIMD and whether CF or other (poly)phenol treatment may reduce muscle fatigue evidenced by the data.

7.5 Limitations of the thesis

The main limitation of this thesis is the lack of direct muscle biopsies, inflammatory markers, and oxidative stress markers. These markers would have provided a greater understanding of the mechanisms by which CF may act on a damaged muscle to aid recovery.

The reason that such markers were not included is due to cost restrictions and the limited facilities available, instead it was opted to only measure for non-invasive, accessible, and tangible measures of muscle damage such as changes in muscle function and perceived soreness. As discussed in Chapter 2, section 5, changes in muscle function, such as reductions in force generating capabilities, is considered one of the best measures of muscle damage (Paulsen et al., 2012; Warren et al., 1999), whilst also providing practical evidence of an individual's capacity to perform exercise. Importantly, as the choice of measure was maximal voluntary isometric contraction multiple angles were chosen as to not over or underestimate changes in force generating capability of the knee flexors. Changes in perceived muscle soreness are a more subjective marker however and inherently individual as ways of measuring pain, e.g., *via* a VAS, are reliant on what a participant considers a great deal of pain as an anchor point. For this reason, two methods of measuring soreness were included (VAS and LEFS) and recent relevant literature was utilised to best inform the

optimal use of a VAS and its anchor points (Reed & Van Nostran, 2014). Mechanistic markers would have provided valuable insight and future studies should look to incorporate inflammatory markers and oxidative stress markers if feasible.

During Chapter 5 there was a split of males and females. Females completed the experiment during the luteal phase of the menstrual cycle, and to calculate this, a calendar method was employed alongside a menstrual cycle history questionnaire to account for >2 previous cycles. Although this method can be relatively accurate and has been validated in the field, it is not as accurate as hormonal testing (Wideman et al., 2013). Ideally, regular blood sampling would take place to accurately define the menstrual cycle phase alongside regular individual tracking of the menstrual cycle, including >2 previous cycles. Recently, it has been recommended that to reduce possible variability in the data, as each menstrual cycle is relatively variable within and between individuals, outcome measures should be repeated in another cycle (Elliott-Sale et al., 2021). This undertaking would be extremely difficult in EIMD research, namely due to the repeated bout effect and as such is not something that could be achieved within the time frame of this PhD. Additionally, it was difficult to balance the groups for contraceptives used by each female participant, of the 13 females within the study eight were classed as naturally menstruating, three were on the oral combined pill and two were on the Depo-Provera injection. For the most accurate comparisons all participants would have either been naturally menstruating or all on one specific contraceptive. However, this would reduce the generalisability of the cohort as not everyone is on one type of contraceptive.

Another limitation of the thesis is the sole use of a laboratory-based exercise protocol to induce muscle damage. Even though the protocol induced notable EIMD it is not an ecological valid method of doing so due to the isolation of the knee flexors. Utilising a more ecologically valid protocol such as a repeated sprint protocol, one that replicates the demands and movement patterns of real-world exercise, make findings more generalisable to sporting settings. Additionally, team sports such as soccer commonly include changes of direction, physical contact with other players, jumping, kicking and other movement patterns, something most EIMD protocols lack. Therefore, for research to better translate to the practical setting selecting the protocol is a pertinent aspect to consider. It is worth noting that laboratory-based protocols are still an

important feature of muscle damage research due to their established reliability in eliciting the desired muscle damage response.

Another limitation of the thesis is the reduced data set for the final experimental study (Chapter 6), due to the coronavirus pandemic data collection was hindered drastically. The repeat national lockdowns that occurred three times from March 2020 to March 2021 meant that further data collection was not possible. From the end of the final lockdown, it was decided that there would be no return to the laboratory to continue with the study due to the time intensive nature of the protocol and the high probability of frequent dropouts due to possibility of a positive covid-19 test result. Not only that but University policy was reactionary to the ever-changing pandemic environment making forward planning almost impossible from a research perspective, especially when having to factor in six consecutive testing days, the University itself put all research endeavours ‘on hold’ during each national lockdown and during the local lockdowns also. Furthermore, it was also considered that many potential participants would be in a state of detraining and suffer a large muscle damage response, as most gyms were closed many individuals were unable to perform regular resistance training. This could result in skewed data from an exaggerated response from ‘untrained’ individuals. Not only that but participant injury risk was considered also. As the testing period incorporated two bouts of muscle damaging protocols it was possible that this may also increase the withdrawal rate of participants due to fear of injury. Due to the final study being underpowered the findings should be approached cautiously as reduced cohort numbers can increase the chance of large effect sizes, which were noted within the study.

As with all research, ethical considerations arose throughout this thesis, especially due to the nature of the maximal, strenuous exercise that participants volunteered to perform. Within both Chapter 5 and Chapter 6, a total of three participants withdrew due to ‘injury related reasons’, indicating that at times for a few individuals the muscle damage response experienced was too severe for them to feel comfortable continuing with the research. Participant health and wellbeing remained the utmost importance throughout this PhD. Indeed, all participants were reminded to continue to rest following the completion of the study before recommencing any training and ensure they were fully recovered following the protocol.

7.6 Reflections During a Global Pandemic

Conducting the final 18 months of this PhD during a global pandemic created difficulties that could not have been predicted in the months leading up to the beginning of the covid-19 period. The main issue was the cessation of data collection due to the closure of the University, this period of 'no data' has continued till now in the summer of 2021. Measures were put in place to return to data collection in October – December 2020, however the second national lockdown immediately ended that endeavour. Thankfully, enough data was collected prior to the University closure to allow for statistical analysis for a third and final study, albeit an underpowered one.

The pandemic also provided time to reflect on the PhD experience and just how much it has changed over the previous 18 months. The greatest shift was in the environment (not just for the obvious reasons), from conducting research, meetings, conferences, and teaching in person it was now performed remotely. Not only that but the PhD research community that had been ever present during the first half of the PhD had now all but vanished along with virtually all in-person social interactions. This along with living alone magnified the isolated feelings that had begun to brew over the initial lockdown. As time went on, I adjusted to the 'new world' and the new normal.

As data collection could not be continued, progress had to be made elsewhere. In this regard, the first publication of the thesis occurred in June/July 2020 (what is now Chapter 5 in the thesis) and in July 2021 published what is now Chapter 3. Alongside this I wrote an article for The Conversation in August 2020 (translated into French in December 2020) about the benefits of CF and a brief history of chocolate. This article was among the top read from those published by academics at the University of Huddersfield in 2020. Authoring the article improved my ability to write for a lay audience whilst still managing to get key information across to the reader. Additionally, in 2020 and 2021 I was part of the organising committee for the internal Engage conference at the University of Huddersfield aimed at postgraduate research students within the School of Human and Health Sciences. Due to the pandemic the conference was held online on both occasions. In doing so, the committee's main aim was recreating the supportive, community feeling that was present at previous in-person versions. To summarise, despite the circumstances I believe I have made progress in key aspects of being a researcher and academic.

A similarity between the Conquistadors and I is that we both had our own New Worlds to find our footing in. All in all, although the coronavirus pandemic impacted the final study, preventing it from reaching statistical power, it does not detract from the interesting and novel findings of this thesis.

7.7 Conclusion

To summarise, the three experimental studies that complete this thesis indicate that *i*) an acute dose of CF has no significant impact on muscle damage over a carbohydrate-protein control, *ii*) there are no significant differences between sex regarding an acute dose of CF on markers of muscle damage with a small benefit observed for soreness in females when consuming 1245 mg CF, *iii*) regular supplementation of 1245 mg CF may reduce accumulated fatigue associated with a repeated bout of strenuous exercise indicated by the recovery of muscle function, maintenance of knee flexor performance during a multi-joint exercise task whilst in a fatigued state, and the reduction of perceived soreness following the second bout.

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Appendices

Appendix 1 – Example of Consent Form

CONSENT FORM

Title of Research Project: Investigating the effect of varying doses of cocoa flavanol beverages on muscle recovery following exercise induced muscle damage in active females and males
It is important that you read, understand and sign the consent form. Your contribution to this research is entirely voluntary and you are not obliged in any way to participate, if you require any further details please contact Liam Corr (Researcher) at Liam.Corr@hud.ac.uk.

I have read the participant information sheet and understand what will be asked of me during the research period and have had the opportunity to ask any questions I may have. □

I understand that I can withdraw my data during the study and for two months after I complete the study. □

I understand that information about me will be stored securely and will be kept anonymous via coding (name will be replaced by a number after baseline measures are taken) to maintain participant privacy. □

I give my consent for the lead researcher to have access to my data and use it for scientific publication and further research. □

I give my consent for the researcher and research team to store my data at the university for up to 10 years following the research knowing it will be stored securely □

I give my consent to take part in this study □

Signature of Participant: Name of Participant: Date:	Signature of Researcher: Name of Researcher: Date:

(one copy to be retained by Participant / one copy to be retained by Researcher)

Appendix 2 – Example of Institutional Ethical Approval

SHUM Research Ethics
Thu 22/08/2019 13:42

To: Liam Corr (Researcher)

Cc: Robert Naughton

Dear Liam,

Apologies for the delay in getting back to you in connection with your amended SREP Application.

The panel reviewers have confirmed that you have addressed the issues raised to their satisfaction and your application has now been **approved outright.**

With best wishes for the success of your research project.

Regards,

Kirsty
(on behalf of SREP)

Appendix 3 – Menstrual Cycle History Questionnaire

MENSTRUAL CYCLE QUESTIONNAIRE

Participant number: _____

Please answer the following questions:

1. Do you have periods? YES NO

- **If YES how regular are they? Every month 4-9 times a year**

2. How long does your menstrual cycle usually last, from day 1 of bleeding to day 1 of the next bleed?____Days.

3. How long were your previous two menstrual cycles?

4. How many days does your menstrual flow usually last?____Days.

5. When was the approximate start date of your most recent cycle (i.e first day of bleeding)?

6. What date to you expect your next cycle will begin approximately?

7. Do you use contraceptive pills or any other form of female contraception? YES NO

- **If YES please answer the following:**
- **Brand:**
- **Duration (years/months):**
- **How often do you take a contraceptive pill? Everyday Every month Other, please state: _____**
- **Any additional details:**