

18 **Abstract**

19

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

36 The first experimental study investigated the impact of an acute dose of CF (either
37 830mg or 1245mg) on muscle recovery following EIMD. Overall, 23 participants
38 completed the study. Participants performed maximal voluntary isometric contractions
39 (MVIC) of the knee flexors to assess muscle function and a visual analogue scale
40 (VAS) and lower extremity functional scale (LEFS) to assess perceived soreness. To
41 induce muscle damage five sets of 10 maximal concentric/eccentric hamstring curls
42 were performed on each leg using an isokinetic dynamometer, with muscle function
43 and soreness being measured immediately post, 24, 48 and 72 hr following EIMD. It
44 was observed that the highest dose of CF (1245mg) may have a minimal effect on the
45 recovery of MVIC and muscle soreness; although not statistically significant. The
46 second experimental study was a sub-group inter and intra-sex analysis of the data
47 gathered from the first study. Overall, no significant differences were observed
48 between males and females for measures of muscle recovery. The third experimental
49 study investigated the impact of daily consumption of 1245mg of CF on muscle
50 recovery following repeated bouts of strenuous exercise, separated by 72 hours. In
51 addition to MVIC, VAS, and LEFS, electromyography was included within the
52 measures to assess muscle recovery. This study had 9 participants complete the
53 seven-day protocol (one baseline and six consecutive days) ingesting 8 beverages
54 throughout the period. No overall significant differences were observed between the
55 groups, however at the final time point (48 hr post the second EIMD protocol) large
56 effect sizes were observed and a statistically significant difference at that time point
57 for MVIC data, VAS, and LEFS. The data contained within this thesis provides novel
58 information on the potential of CF as an ergogenic aid for muscle recovery. It appears
59 that CF does not offer a significant benefit for muscle recovery when compared to a
60 recovery drink containing only carbohydrate and protein. However, the large effect
61 sizes observed in all three studies imply there may be a small effect of CF on recovery,
62 as such the data from this PhD needs to be corroborated by future research to further
63 justify the use of CF as an ergogenic aid for recovery.

64 Publications

65

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

69 **Corr L.D.**, Field A, Pufal D, Killely J, Clifford T, Harper LD, Naughton R. Acute consumption
70 of varied doses of cocoa flavanols does not improve muscle recovery following exercise-
71 induced muscle damage in active males and females. *Int J Sports Nutr Exerc Metab* 2020
72 (<https://pubmed.ncbi.nlm.nih.gov/32663386/>).

73

74 Publications arising from collaborative work alongside this PhD

75

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

79 Field A, **Corr L. D.**, Sarmiento H, Naughton R, Clifford T, Haines M, Page R, Harper LD. The
80 impact of the extra-time period of soccer on recovery. (In Review)

81 Field, A., **Corr, L.D.**, Thompson, C., Gonzalez Lucena, J. C., Sarmiento, H., Naughton, R., ...
82 & Harper, L. (2021). Recovery following the extra-time period of soccer: Practitioner
83 perspectives and applied practices. *Biology of Sport*.

84 Field, A., Page, R. M., **Corr, L.D.**, Naughton, R., Haines, M., Harper, L. D., & Hudson, S.
85 (2020). Lower-Limb Muscle Excitation, Peak Torque, and External Load Responses to a
86 120-Minute Treadmill-Based Soccer-Specific Simulation. *Research Quarterly for Exercise*
87 *and Sport*, 1-11.

88 Field, A., Naughton, R. J., Haines, M., Lui, S., **Corr, L. D.**, Russell, M., ... & Harper, L. D.
89 (2020). The Demands of the Extra-Time Period of Soccer: A Systematic Review. *Journal of*
90 *Sport and Health Science*.

91 Field, A., **Corr, L. D.**, Haines, M., Lui, S., Naughton, R., Page, R. M., & Harper, L. D. (2020).
92 Biomechanical and Physiological Responses to 120 min of Soccer-Specific Exercise.
93 *Research quarterly for exercise and sport*, 1-13.

94 Harper, L. D., Field, A., **Corr, L. D.**, & Naughton, R. J. (2019). The physiological, physical,
95 and biomechanical demands of walking football: implications for exercise prescription and
96 future research in older adults. *Journal of aging and physical activity*, 1(aop), 1-11.

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116	Contents	
117	Abstract.....	1
118	Publications.....	2
119	Publications arising from collaborative work alongside this PhD.....	2
120	Copyright Statement.....	3
121	List of Figures.....	8
122	List of Tables.....	9
123	Abbreviations.....	9
124	Acknowledgements.....	11
125	Chapter 1 General Introduction.....	13
126	1.1 Introduction.....	14
127	1.2 Changes to the manufacturing process – is that the problem?.....	17
128	1.3 Bioavailability.....	18
129	Chapter 2 General Literature Review.....	22
130	2.1 Health Benefits.....	23
131	2.2 Muscle damage.....	25
132	2.3 Consequences of muscle damage.....	27
133	2.3.1 Reduced muscle function and neuromuscular control.....	28
134	2.3.2 Increased soreness.....	30
135	2.3.3 Inflammation.....	33
136	2.3.4 Oxidative stress.....	34
137	2.4 Impact of sex on muscle recovery.....	36
138	2.5 Importance of recovery within sport settings.....	40
139	2.6 Hormesis.....	41
140	2.7 How cocoa could help recovery.....	42
141	Chapter 3 The Effects of Cocoa Flavanols on Indices of Muscle Recovery and Exercise	
142	Performance: A Systematic review of the literature.....	44
143	3.1. Background.....	45
144	3.2. Methods.....	47
145	3.2.1 Information Sources and Search Strategies.....	47
146	3.2.2 Quality Assessment.....	48
147	3.2.3 Study Selection Process and Eligibility Criteria.....	50
148	3.3. Results.....	50
149	3.3.1 Study Selection and Screening.....	50
150	3.3.2 Study Characteristics.....	50
151	3.3.3 Summary of Studies.....	51

152	3.4. Discussion	53
153	3.4.1 Impact of Cocoa Flavanols on Exercise-induced Oxidative Stress.....	53
154	3.4.2 Impact of Cocoa Flavanols on Exercise-induced Inflammation	61
155	3.4.3 Impact of Cocoa Flavanols on the Recovery of Muscle Function.....	66
156	3.4.4 Impact of Cocoa Flavanols on Perceived Soreness.....	69
157	3.4.5 Impact of Cocoa Flavanols on Exercise Performance	71
158	3.4.6 Practical Recommendations and Future Research	77
159	3.5. Conclusion	78
160	Chapter 4 General methods	79
161	4.1 Participants.....	80
162	4.2 Dietary control and analysis.....	81
163	4.3 Cocoa flavanol intervention.....	81
164	4.3.1 Cocoa flavanol intervention in Chapters 5.....	82
165	4.3.2 Cocoa flavanol intervention in Chapter 6.....	82
166	4.4 Muscle damage protocol	83
167	4.5 Measuring muscle function	84
168	4.6 Muscle activation	84
169	4.7 Measuring muscle soreness	86
170	4.8 Assessing menstrual cycle.....	86
171	4.9 Statistical Analysis Approach.....	87
172	Chapter 5 Acute consumption of varied doses of cocoa flavanols on indices of muscle	
173	recovery following exercise-induced muscle damage in active males and females.....	88
174	5.1. Introduction.....	89
175	5.2. Methods	92
176	5.2.1 Participants.....	92
177	5.2.2 Study Design.....	92
178	5.2.3 Muscle Function	94
179	5.2.4 Subjective Soreness	94
180	5.2.5 Muscle Damaging Protocol.....	94
181	5.2.6 Nutritional Intervention.....	94
182	5.2.7 Dietary Measures	95
183	5.2.8 Statistical analyses.....	95
184	5a.3. Results	96
185	5a.3.1 Muscle function.....	96
186	5a.3.2 Measures of Perceived soreness	98
187	5a.4. Discussion	100

188	5b.3 Results	102
189	5b.3.1 Participant Characteristics and Nutritional Intake	102
190	5b.3.2 Sex differences for muscle function	103
191	5b.3.3 Sex differences for perceived soreness	106
192	5b.4 Discussion	108
193	5b.5 Limitations.....	111
194	Chapter 6 Investigating the effect of regular consumption of a high dose of cocoa flavanols	
195	on muscle recovery and repeat performance in males and females	113
196	6.1 Introduction.....	114
197	6.2 Methods	117
198	6.2.1 Study Design.....	117
199	6.2.2 Participants.....	117
200	6.2.3 Muscle Damaging Protocol.....	118
201	6.2.4 Nutritional Intervention.....	118
202	6.2.5 Exercise Performance	119
203	6.2.6 Muscle Function	119
204	6.2.7 Electromyography	119
205	6.2.8 Perceived Soreness.....	119
206	6.2.9 Dietary Analysis.....	119
207	6.2.10 Statistical Analysis	119
208	6.3 Results	120
209	6.3.1 Exercise Performance	120
210	6.3.2 Muscle function.....	121
211	6.3.3 Electromyography	124
212	6.3.4 Perceived soreness	125
213	6.4 Discussion.....	127
214	Chapter 7 General Discussion	132
215	7.1 Summary of experimental findings.....	133
216	7.2 Cocoa Flavanols impact on muscle function and perceived soreness	137
217	7.2.1 Muscle function.....	137
218	7.2.2 Perceived soreness	140
219	7.2.3 Sex Differences and Cocoa Flavanols.....	141
220	7.3 Practical applications	142
221	7.4 Future Directions	144
222	7.5 Limitations of the thesis	146
223	7.6 Reflections During a Global Pandemic	149

224	7.7 Conclusion	150
225	References	150
226	Appendices.....	172
227	Appendix 1 – Example of Consent Form.....	172
228	Appendix 2 – Example of Institutional Ethical Approval	173
229	Appendix 3 – Menstrual Cycle History Questionnaire	174
230		
231		

232	List of Figures
233	Figure 1.1 Cocoa flavanol metabolism in brief
234	Figure 3.1. PRISMA flow chart detailing the screening process
235	Figure 5.1 Study schematic detailing experimental timeline
236	Figure 5.2 Percentage change from baseline for MVIC following EIMD
237	Figure 5.3 Individual MVIC60 data
238	Figure 5.4 Individual MVIC30 data
239	Figure 5.5 Individual VAS data
240	Figure 5.6 Inter- and Intra-sex MVIC60% data
241	Figure 5.7 Inter- and Intra-sex MVIC30% data
242	Figure 5.8 Inter- and Intra-sex VAS data
243	Figure 6.1 Study timeline Schematic
244	Figure 6.2 MVIC60 percentage change.
245	Figure 6.3 MVIC30 percentage change.
246	Figure 6.4 MVIC60 Individual data across all time points
247	Figure 6.5 MVIC30 Individual data across all time points
248	

249 **List of Tables**

- 250 Table 3.1 Quality and bias assessment of included articles using the National
251 Institute for Health and Excellence checklist for randomised controlled trials
- 252 Table 3.2 The effect of CF supplementation on exercise-induced oxidative stress
- 253 Table 3.3 The effect of CF supplementation on exercise-induced inflammation
- 254 Table 3.4 The effect of CF supplementation on exercise-induced changes in muscle
255 function
- 256 Table 3.5 The effect of CF supplementation on exercise-induced changes in
257 perceived soreness
- 258 Table 3.6 The effect of CF supplementation on exercise performance
- 259 Table 4.1 Nutritional information of treatment beverages
- 260 Table 5.1 Participant characteristics
- 261 Table 5.2 Participant Characteristics separated by sex
- 262 Table 5.3 Dietary intake between groups
- 263 Table 5.4 Changes in MVIC following EIMD
- 264 Table 5.5 Changes in perceived soreness post-EIMD
- 265 Table 5.6 Nutritional Intake between groups
- 266 Table 5.7 Changes in MVIC Following EIMD as Percentage Change
- 267 Table 5.8 Changes in MVIC Following EIMD
- 268 Table 5.9 Changes in Perceived Soreness Following EIMD
- 269 Table 6.1 Participant Characteristics
- 270 Table 6.2 Dietary characteristics of the participants
- 271 Table 6.3 Percentage change from first EIMD protocol measured as concentric peak
272 torque
- 273 Table 6.4 Percentage change from first EIMD protocol measured as eccentric peak
274 torque
- 275 Table 6.5 Changes in muscle function measured using MVIC

276 **Abbreviations**

- 277 CF = Cocoa flavanols
- 278 EIMD = Exercise-induced muscle damage
- 279 SGLT-1 – Sodium-glucose transport protein 1
- 280 ATP = adenosine triphosphate

281 Ca^{2+} = Calcium ions
282 CK = Creatine kinase
283 DOMS = Delayed onset muscle soreness
284 MVIC = Maximal voluntary isometric contraction
285 MVC = Maximal voluntary contraction
286 CMJ = Countermovement jump
287 IL-6 = Interleukin-6
288 IL-1 β = Interleukin-1 beta
289 IL-10 = Interleukin-10
290 TNF- α = Tumour necrosis factor-alpha
291 ROS = Reactive oxygen species
292 OCP = Oral contraceptive pill
293 NF- $\kappa\beta$ = Nuclear factor-kappa beta
294 Nrf2 = Nuclear factor erythroid 2-related factor 2
295
296

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298

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343

Chapter 1 General Introduction

344 1.1 Introduction

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

355 In 1518 Cortes and his men embarked on what would become an era defining
356 conquest. By 1519 the Spaniards landed at what is now modern-day Mexico
357 (specifically Chalchihuecan, Veracruz), then in a masterful stroke of strategy Cortes
358 destroyed¹ almost the entirety of his fleet; supposedly in an attempt to root out the
359 cowardly and treacherous members of his party (Reynolds, 1959). Emboldened in the
360 wake of a speech that Cortes gave in which he spoke of the untold riches that awaited
361 them, he and his men set out on a journey to the Aztec capital, Tenochtitlan. Their
362 time in Mexico was well documented by both Cortes himself and an officer, Bernal
363 Diaz del Castillo, both of whom describe cocoa in detail.

364 Upon arrival at Tenochtitlan, the Spaniards were welcomed by Aztec King Montezuma
365 and housed within his court. According to certain texts Montezuma allegedly believed
366 Cortes to be Quetzalcoatl, the Aztec god of creation, returned, although this is
367 contested, especially considering the overly polite nature of Nahuatl (Aztec language)
368 and its proclivity for misinterpretation (Townsend, 2003). During this period, the
369 Spanish were exposed to a warm, bitter beverage made up from cocoa beans that the
370 Aztecs referred to as 'chikolatl' in Nahuatl. Even though the Spanish did not take to
371 the drink, deeming it not sweet enough, King Montezuma was observed to consume
372 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

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

379 The Aztecs and Maya, another civilisation situated in the Yucatán Peninsula, utilised
380 cocoa to a great extent, mostly as a way of treating various medical conditions, such
381 as fever, diarrhoea, coughs and infections (Dillinger et al., 2000). There are numerous
382 texts dating back to the 16th century that are of Spanish, Mayan and Aztec origin that
383 denote the use of cacao (chocolate) as medicine in the form of a hot beverage with
384 varying amounts of cacao beans (potentially mixed with maize or spices)
385 recommended depending on the ailment. Cocoa and its derivatives were almost
386 viewed as a panacea, finding use in the treatment of a surfeit of conditions, not only
387 for the conditions previously mentioned but for angina, dental problems, dysentery,
388 fatigue, indigestion and more (Lippi, 2013). It is possible that the Aztec and Mayan
389 people believed cocoa had aphrodisiac properties, with it seeing use at wedding
390 ceremonies and regularly consumed by King Montezuma before attending to one of
391 his many wives (D. Lippi, 2015). It is believed that Montezuma may have fathered
392 ~100 children (Sweet & Nash, 1981); whether cocoa was the key to his virility it is
393 almost impossible to know. Modern research into the aphrodisiac properties of cocoa
394 has deciphered that specific compounds found within cocoa, such as
395 phenylethylamine, theobromine, and N-acylethanolamine, may improve sexual desire
396 and pleasure as well as mood, *via* the stimulation of the hypothalamus (Afoakwa,
397 2008). However, even in the 16th century cocoa was not without drawbacks. Excessive
398 intake of green cocoa (unroasted beans) made people who quaffed such amounts
399 confused, whereas a moderate intake was considered ideal and stimulating (Lippi,
400 2013).

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

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

421 The reason behind the belief that cocoa was this 'miracle drink' or elixir by the
422 Mesoamerican civilisations was perhaps due to their religious convictions, as their
423 respective chief god gifted them the cocoa tree. Yet, the reason for its potency most
424 likely owes to the high concentration of (poly)phenols found within natural cocoa as
425 opposed to divine intervention. The word (poly)phenol is the umbrella term given to a
426 vast array of dietary antioxidants, with reported intakes potentially being as high a
427 1g/day (Scalbert, Johnson, & Saltmarsh, 2005). A diverse and substantial collection
428 of plant metabolites, (poly)phenols were sporadically researched throughout the early
429 20th century gathering a greater body of research as the decades progressed. Now,
430 the amount of research performed investigating the effects of the various groups of
431 (poly)phenols has grown exponentially. From this research, it has come to be
432 understood that (poly)phenols have a multitude of potential health benefits, such as
433 being cardioprotective, anti-cancer, anti-inflammatory, neuroprotective, as well as
434 improving glycaemic control and more (Del Rio, Costa, Lean, & Crozier, 2010). This is
435 a far step from the role of (poly)phenols in plants; the original role of these metabolites
436 is to protect against ultraviolet radiation and pathogenic compounds (Manach,
437 Scalbert, Morand, Remesy, & Jimenez, 2004).

438 Different (poly)phenols are distinguished by their chemical structure, hydroxyl groups
439 bonded to an aromatic ring creating a phenol ring (or multiple). The categories of
440 (poly)phenols are phenolic acids, lignans, stilbenes and flavonoids, with the latter
441 being the largest category. Flavonoids can be further separated into subgroups, these
442 are as follows: flavonols, flavanols, flavones, isoflavones, flavanones and
443 anthocyanidins. Within cocoa, flavanols form the bulk of the (poly)phenolic profile and
444 is in fact the richest source of flavanols out of all other dietary sources, e.g., tea,
445 apricots, and beans (Hackman et al., 2008). Flavanols are commonly found in
446 monomeric forms, such as catechin, epicatechin, epigallocatechin and more, but can
447 also be found in polymeric forms known as anthocyaninidins (Andres-Lacueva et al.,
448 2008).

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

457 1.2 Changes to the manufacturing process – is that the problem?

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

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

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

473 The roasting of the bean also has an impact on the flavanol content but is an important
474 step in the creation of what chocolate is today. Numerous reactions occur during this
475 treatment such as protein degradation, changes in pH, colour and shape (García-
476 Alamilla, Lagunes-Gálvez, Barajas-Fernández, & García-Alamilla, 2017). Additionally,
477 since flavanols are unstable in high heats, roasting can reduce the total content as a
478 result of thermal and oxidative degradation. Various studies have found that roasting
479 at temperatures of between 150-250°C for ≥45 minutes causes the greatest decrease
480 of total (poly)phenol content (Ioannone et al., 2015; Payne, Hurst, Miller, Rank, &
481 Stuart, 2010). Another aspect of roasting is the epimerisation of the different flavanols
482 from dimers to simple monomers leading to an increase in the latter (amount
483 dependent on the original content of the bean). Kothe, Zimmermann, and Galensa
484 (2013) found that beans from the Ivory Coast had an 836% increase in catechin (a CF
485 monomer) content, whereas Java beans rose by ~174%. Furthermore, they concluded
486 that temperatures below 140°C should preserve most of the flavanol content.
487 Unfortunately, most chocolatiers do not take the CF content into consideration, as the
488 reduction of flavanols is what lessens the bitterness of natural cocoa, creating the
489 more well-known chocolate flavour.

490 The other main aspect of manufacturing is alkalisation of the cocoa, this is known as
491 'Dutching'. The term stems from a 19th Century Dutch chocolatier called Coenraad
492 Van Houten who was one of the people responsible for the transition of cocoa from a
493 beverage to an edible. Alkalisation leads to reductions in bitter and sour flavours,
494 increases the solubility of powder and alters the colour (Kamphuis, 2017). Natural
495 cocoa is slightly acidic with a pH of ~5.3 and an average flavanol content of 34.6 mg/g,
496 whereas treated cocoa can range from 6.5 to 7.6 pH and have a flavanol content of
497 3.6 mg/g or lower (Miller et al., 2008). Therefore, the less processing cocoa is exposed
498 to the greater the flavanol content. The higher the concentration the greater potential
499 for beneficial effects post-consumption, although many factors can influence the
500 bioavailability of flavanols.

501 1.3 Bioavailability

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

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

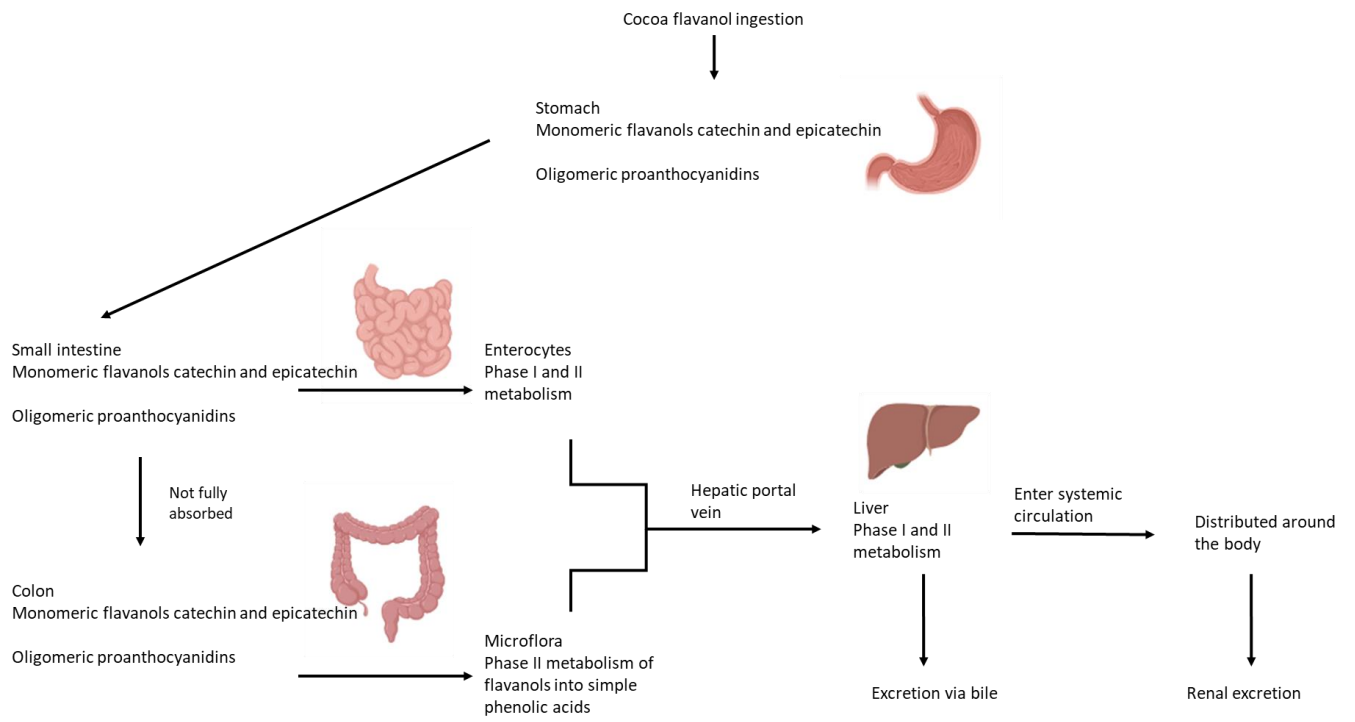
508 A percentage of the flavanols ingested are then absorbed into the enterocytes and
509 undergo phase I (reduction, oxidation, or hydrolysis) and phase II (conjugation,
510 glucuronidation, methylation, sulfation or a combination) biotransformation.
511 Epicatechin is converted into a wide range of metabolites upon absorption into the
512 enterocytes of the small intestine, such as (-)-epicatechin 3' –sulfate, 3' –O-methyl(-
513)-epicatechin 5-sulfate and (-)-epicatechin-3'-β-D-glucuronide (Actis-Goretta et al.,
514 2012). Much of the remaining flavanols that are not absorbed continue through the
515 gastrointestinal tract to the colon and undergo phase II biotransformation by the gut
516 microflora before absorption, with catechin monomers converted into simple phenolic
517 acids (Rios et al., 2003). Post-absorption, the metabolites are transported via the
518 hepatic portal vein to the liver for further metabolism before being transported into the
519 systemic circulatory system to be distributed around the body. Resultantly, within
520 approximately 30 minutes of ingestion, epicatechin is absorbed and has entered the
521 blood plasma (Rusconi & Conti, 2010) reaching peak concentrations two hours (hr)
522 post consumption (Decroix et al., 2017; Kwik-Urbe & Bektash, 2008). Flavanol
523 concentrations in the blood return to baseline after ~8 hr, indicating rapid excretion via
524 biliary and renal systems (Hackman et al., 2008). Other research has shown that the
525 microbial metabolites remain in circulation at relevant amounts for up to 24 hrs post
526 consumption (Gómez-Juaristi, Sarria, Martínez-López, Bravo Clemente, & Mateos,
527 2019). See Figure 1 for an outline of the digestion, absorption, and excretion pathway
528 of CF.

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

537 2003). The effects of mixing CF with another macronutrient, protein, has also been
538 investigated. It has been reported that the presence of whey, a predominant milk
539 protein, negatively affects the bioavailability of cocoa flavanols in chocolate
540 confectionery, e.g., chocolate bars (Cifuentes-Gomez, Rodriguez-Mateos, Gonzalez-
541 Salvador, Alanon, & Spencer, 2015; Serafini et al., 2003). However, Roura et al.,
542 (2007) stated that there is no significant detrimental effect on absorption when cocoa
543 powder is mixed with milk proteins as a drink. Keogh, McInerney, and Clifton (2007)
544 corroborate this and found that a mix of milk proteins slightly increased the rate of
545 absorption, measured using plasma concentrations of catechins, but to no
546 physiological significance. Therefore, it is possible CF combined with protein and
547 carbohydrates in the form of a recovery drink may constitute an ideal beverage for
548 athletes to consume after intense exercise to enhance recovery, should CF have such
549 a benefit. This is the purpose of this thesis, investigating the possible benefit of CF on
550 recovery from exercise.

551

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



560

561 Figure 1.1 Cocoa flavanol metabolism in brief

562

563

564

565

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568

Chapter 2 General Literature Review

569 2.1 Health Benefits

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

576 The effects that CF exert on vascular health are well documented, one of the main
577 reasons why the health claim was successful. Consumption of CF has beneficial
578 effects on flow mediated dilation (the dilation of an artery in response to increased
579 blood flow) following regular consumption of CF containing ~98 mg or more of
580 epicatechin (Davison, Coates, Buckley, & Howe; Heiss et al., 2007; Monahan et al.,
581 2011). Additionally, consumption of a high CF beverage increased bioactive nitric
582 oxide (NO) production, increasing flow mediated dilation as a result (Fisher, Hughes,
583 Gerhard-Herman, & Hollenberg). The importance of NO is due to its multiple roles in
584 vascular health. It is antithrombotic, antiproliferative, anti-atherogenic and is a
585 vasodilator (Huynh & Chin-Dusting, 2006). However, the exact role of CF on NO has
586 not been fully elucidated. Cocoa has been shown to increase endothelial derived nitric
587 oxide synthase (eNOS) and reduce the activity of vascular arginase, allowing for an
588 increased concentration of L-arginine which is needed for the production of NO *via*
589 eNOS (Huynh & Chin-Dusting, 2006). Corti, Flammer, Hollenberg, and Lüscher (2009)
590 describe that the immediate impact CF may have is through the inhibition of NADPH
591 oxidase which can inactivate NO, and long-term impact may be *via* increased eNOS
592 expression. Additionally, CF may reduce the level of microparticles found within
593 circulation; high levels of microparticles are correlated with reduced endothelial
594 function (Singh et al., 2006). Commonly, people with high atherothrombotic risk have
595 a high level of microparticles within circulation and it is possible that these
596 microparticles attract the accumulation of inflammatory cells within the vascular wall
597 (Angelillo-Scherrer, 2012). Horn et al., (2014) found that a twice daily dose of 375 mg
598 CF significantly reduced endothelial microparticles and improved endothelial function
599 measured by flow mediated dilation after one month.

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

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

619 As a result, of all the CF, it is thought that epicatechin is the most important compound
620 in regard to the beneficial effects derived from consumption. When consuming
621 epicatechin alone, very similar vascular effects to cocoa are observed (Vlachojannis,
622 Erne, Zimmermann, & Chrubasik-Hausmann, 2016). This indicates the importance of
623 epicatechin content when determining a truly beneficial dose of CF. However, it is
624 worth noting that when consuming cocoa, there are more bioactive compounds than
625 just CF; there are unsaturated fatty acids, theobromine, methylxanthines and other
626 flavonoids that are not found within the flavanol sub-class (Vlachojannis et al., 2016).
627 It is considered likely that these other compounds contribute to the overall benefits of
628 cocoa. The methylxanthines found within cocoa may even improve the absorption of
629 flavanols as it appears that the presence of these compounds leads to an increased
630 plasma concentration of epicatechin when ingested simultaneously (Sansone et al.,
631 2017).

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

635 acute consumption of 720 mg of CF improved cognitive function across a number of
636 different tasks, such as choice reaction time. The authors speculate that due to the
637 wide range of overall improvements, it is possible that the increased blood flow may
638 improve motivation or attention during the tasks (Field, Williams, & Butler, 2011). The
639 same study also found improvements in visual performance during the tasks, with the
640 potential mechanisms being improved retinal blood flow. Furthermore, doses of 520
641 and 994 mg of CF have shown to have varying effects on cognitive function as well as
642 subjective measures, e.g., perceived mental fatigue. A dose of 520 mg attenuated
643 perceived mental fatigue in comparison to a 0 mg control, whereas a 994 mg dose
644 improved response time in a rapid visual information processing task (Scholey et al.,
645 2010). However, other research has indicated that although CF may improve cerebral
646 blood flow and oxygenation, they do not improve cognitive performance (Lieselot
647 Decroix et al., 2016).

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

658 2.2 Muscle damage

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

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

676 Mechanical stress occurs mostly during the aforementioned eccentric contractions.
677 The reason for this is that more force is produced during an eccentric contraction than
678 concentric or isometric. Yet, fewer motor units are recruited during eccentric
679 contractions, some may be uncoupled/'derecruited' following the concentric phase
680 (Duchateau & Baudry, 2014), therefore, more force is placed upon fewer motor units
681 leading to a greater chance of contractile failure (Clarkson & Hubal, 2002; Hesselink
682 et al., 1996). Post damaging event, there is a disruption of the sarcomeres in the
683 damaged fibres occurring from an over-stretching of sarcomeres resulting in structural
684 deformation of myofibrils (Morgan & Proske, 2004). This is referred to as the 'popping
685 sarcomere' hypothesis. The crux of this theory is that when a muscle is stretched
686 beyond its optimal length, the point of optimum tension generation (Morgan & Allen,
687 1999), then the longer weaker sarcomeres are stretched more rapidly and possibly
688 beyond the myofilament overlap, potentially leading to a shearing of the myofibrils
689 (Morgan, 1990; Morgan & Proske, 2004). Subsequently, calcium ion (Ca^{2+})
690 homeostasis is disrupted leading to the stimulation of proteases and instigating protein
691 breakdown and further damage (Gissel, 2006). This combined with excitation-
692 contraction coupling failure (Byrne, Twist, & Eston, 2004) can lead to reduced muscle
693 function and various other symptoms (Chapter 2 Section 3).

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

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

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

718 The differences between mechanical stress and metabolic stress are somewhat
719 highlighted in a study that compared concentric cycling to eccentric cycling. Both
720 cycling activities were performed at 60% of maximal concentric power at 60 rpm for
721 30 minutes but resulted in different outcomes; the concentric trial had a higher oxygen
722 consumption, perceived effort, blood lactate and heart rate during the trial. However,
723 in the days following, the eccentric trial resulted in greater decrements in muscle
724 function and jump height, as well as a far greater increase in quadriceps muscle
725 soreness compared to the concentric trial (Penailillo, Blazevich, Numazawa, &
726 Nosaka, 2013). Indeed, it appears the metabolic cost of concentric contractions is
727 greater than that of eccentric, although the latter results in a more pronounced overall
728 muscle damage response. It is likely that in most sporting scenarios muscle damage
729 is induced by both mechanical and metabolic factors.

730 2.3 Consequences of muscle damage

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

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

738 2.3.1 Reduced muscle function and neuromuscular control

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

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

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

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

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

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

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

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

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

822 2.3.2 Increased soreness

823 Delayed onset muscle soreness (DOMS) is the term given to the feelings of pain that
824 arise following exercise, the severity of this pain is widely variable. Paulsen et al.,
825 (2012) refers to DOMS as the most common symptom of muscle damage but may not
826 reveal the true extent of the damage. One reason for this perhaps is that measuring
827 DOMS is difficult due to the subjective nature of pain. However, there are various ways
828 of quantifying an individual's soreness.

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

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

840 Aside from the previously mentioned scales, an algometer can be used to quantify
841 pain, specifically pain pressure threshold (PPT). Algometry is used to identify the
842 threshold of pain perception of an individual. To do so pressure is applied to a specific
843 point using the algometer and an individual will state when there is a switch from
844 'pressure' to 'pain' (Hogeweg, Langereis, Bernards, Faber, & Helders, 1992). The use
845 of PPT is considered reliable within and between sessions for assessing pain (Potter,
846 McCarthy, & Oldham, 2006). However, care must be taken during assessment, with
847 the first measurement to be disregarded and no more than two more measurements
848 be taken immediately following to ensure a reliable estimate of pain (Lacourt,
849 Houtveen, & van Doornen, 2012). This potentially creates an increased chance of
850 technician-error during assessment, something that the VAS and LEFS do not;
851 although if a trained researcher is able to accurately carry out the test, this chance is
852 reduced. Furthermore, as the largest accumulation of nociceptors is at the distal
853 aspect of a muscle, this is the area in which pain would be most intense (Mense, 2008);
854 PPT however, is commonly performed at or around the muscle belly (Casanova et al.,
855 2018) and as such may not reflect the full extent of the pain of the individual. It is likely
856 that there is a need to involve multiple methods in an attempt to provide a better insight
857 into perceptions of pain arising from muscle damage, as different measures may
858 assess different aspects of pain (Kahl & Cleland, 2005). Notably due to the individual
859 and subjective nature of pain perception, especially considering the notion that pain
860 perception differs greatly between people participating in sport and those who are
861 classified as 'active' (Tesarz, Schuster, Hartmann, Gerhardt, & Eich, 2012).

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

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

868 Connective tissue is what surrounds a bundle of muscle fibres. This tissue is less
869 elastic than muscle fibres themselves and may be more susceptible to strain-related
870 injury during excessive mechanical stress (Cleak & Eston, 1992). Because of this, it is
871 thought that DOMS may not necessarily reflect the magnitude of muscle damage, as
872 Nosaka, Newton, and Sacco (2002) found that DOMS poorly correlated with other
873 markers of muscle damage, such as MVIC. Therefore, when measuring EIMD,
874 soreness alone is likely insufficient but provides additional insight (Paulsen et al.,
875 2012).

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

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

895 2.3.3 Inflammation

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

903 The inflammatory process begins in the first few hours (1-4 hr) following EIMD if the
904 mechanical stress or influx of Ca^{2+} is sufficient. This involves a rapid invasion of
905 neutrophils, with macrophages accumulating sequentially thereafter (Butterfield et al.,
906 2006). Around one hour post EIMD, as neutrophils begin to invade the damaged site,
907 they then release proteases and cytotoxic molecules to help degrade and remove
908 cellular debris as well as possible necrotic tissue. However, healthy surrounding
909 bystander tissue may be damaged due to the increased cytolytic and cytotoxic
910 environment created by neutrophils (Pizza, Peterson, Baas, & Koh, 2005; Tiidus,
911 1998). Furthermore, neutrophils actively secrete ROS that aid with muscle membrane
912 lysis, but potentially increasing oxidative environment within the muscle, inciting
913 oxidative stress (Halliwell, 2006).

914 Macrophages further the removal of debris and the inflammatory cascade, secreting
915 cytokines, growth factors, and ROS, and through these they can modulate the cellular
916 response to damage (Tidball, 2005). Interestingly, macrophages appear to perform
917 muscle lysis *via* a NO dependent mechanism and muscle cells are observed to
918 increase the release of NO from macrophages (Filippin, Moreira, Marroni, & Xavier,
919 2009). Pro-inflammatory cytokines, e.g., Interleukin-6 (IL-6), tumour necrosis factor- α
920 (TNF- α) and Interleukin-8, are secreted to aid with the initial removal of debris and
921 along with neutrophils initiate an oxidative burst releasing ROS to aid with lysis (Fisher-
922 Wellman & Bloomer, 2009). Within the extracellular space, ROS can initiate oxidative
923 stress 24-48 hr post-exercise due to the increased concentration of possible reactants.
924 One interesting note regarding the cytokine IL-6 is that the role it has is complex and
925 multifaceted, it not only acts in a pro-inflammatory manner by stimulating IL-1 β but it
926 also increase the production of anti-inflammatory cytokines such as IL-10 (Peake,

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

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

938 Interestingly, if the pro-inflammatory phase is blunted, potentially through exogenous
939 administration of anti-inflammatory molecules, then the regenerative process may also
940 be negatively affected (Deng et al., 2012). It has been speculated that the initial
941 destructive effects of neutrophils allow for macrophages to begin regenerative
942 processes sooner due to a more rapid removal of debris (Butterfield et al., 2006). This
943 anti-inflammatory phase promotes repair *via* the increase of cellular proliferation and
944 differentiation of satellite cells (stem cells that remain near muscle) and the synthesis
945 of connective tissue that may have been damaged during exercise (Peake et al.,
946 2017). Additionally, satellite cells are integral in the regeneration of damaged muscle
947 fibres. Aiding with the growth of a myofiber during repair, satellite cells act to replace
948 damaged tissue specific to the environment they are in and the needs of the muscle
949 (Yin, Price, & Rudnicki, 2013).

950 2.3.4 Oxidative stress

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

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

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

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

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

996 Moreover, muscle contractions, as previously mentioned, instigate a rise in ROS.
997 However, the type of contraction appears to influence the extent of the pro-oxidant
998 status of the muscle, with eccentric contractions resulting in a greater level of oxidative
999 stress compared to concentric contractions in the days following exercise (Kon et al.,
1000 2007). Both eccentric and concentric contractions resulted in marked increases in
1001 thiobarbituric acid reactive substances, likely as a result of lipid peroxidation from the
1002 increase in ROS.

1003 Interestingly, ROS may influence force production in skeletal muscle. It is believed that
1004 redox balance is tightly controlled during exercise to promote an optimal state of being
1005 for force output; however, when exposed to high levels of ROS force output declines
1006 (Powers et al., 2011; Reid, 2001). This may be due to a ROS-induced reduction in
1007 calcium sensitivity of the myofibrils as well as a reduced activity of Ca^{2+} ATPase which
1008 may lead to contractile dysfunction through excessive Ca^{2+} accumulation (Siems,
1009 Capuozzo, Lucano, Salerno, & Crifo, 2003; Smith & Reid, 2006).

1010 The second wave of oxidative stress that can occur following muscle damage is
1011 typically referred to as the respiratory burst, or oxidative burst, the process is driven
1012 by NADPH oxidase (Thomas, 2017). As discussed earlier, the invading inflammatory
1013 molecules, both neutrophils and macrophages, release free radicals to aid with the
1014 removal and degradation of cellular debris (Peake et al., 2017). This burst is further
1015 stimulated, or 'primed', by the presence of pro-inflammatory cytokines such as TNF- α
1016 (El-Benna et al., 2016), a molecule which is associated with protein lysis. If excessive,
1017 this can lead to further tissue damage, especially if within the extracellular space
1018 (Butterfield et al., 2006; El-Benna et al., 2016).

1019 2.4 Impact of sex on muscle recovery

1020 Everyone may experience muscle damage, albeit with a high degree of variability as
1021 discussed throughout the previous sections. However, one source of variability that
1022 should be considered is the impact that biological sex can have on the damage

1023 response post-exercise. Research has found that females may exhibit a moderately
1024 reduced level of soreness in comparison to males following EIMD (Dannecker et al.,
1025 2012; Radaelli et al., 2014). Furthermore, following acute exercise females appear to
1026 be less fatigued and exhibit a more rapid recovery in torque output than males
1027 (Ansdell, Brownstein, Škarabot, Hicks, Howatson, et al., 2019; Senefeld, Pereira,
1028 Elliott, Yoon, & Hunter, 2018). However, this may not be the case following strenuous
1029 exercise, including EIMD, when differences appear to be minor (Lee et al., 2017). The
1030 key discrepancy between males and females that can have a theoretical difference on
1031 muscle is the variation in steroid hormones, most notably oestrogen.

1032 It has been reported that oestrogen may have a protective role against inflammation
1033 and therefore, muscle damage. Oestrogen has the capacity to act as an antioxidant
1034 and in stabilising muscle membranes, although the role it may have in protecting
1035 skeletal muscle is complex and not well understood (Kendall & Eston, 2002). One
1036 study investigating the difference in inflammatory responses between males and
1037 females following muscle damage observed that damage response is similar between
1038 sex, however, the inflammatory response is greater in males than females (Stupka et
1039 al., 2000). The study identified that females had a reduced invasion of neutrophils and
1040 macrophages post-exercise compared to males. It is possible that oestrogen (or rather
1041 E2) reduces membrane fluidity and increases antioxidant defence to protect against
1042 lipid peroxidation, as such, it may protect the membranes from free radical damage
1043 during strenuous exercise, potentially limiting the inflammatory response attributed to
1044 oxidative stress (Kendall & Eston, 2002). The overall extent to which oestrogen can
1045 attenuate any level of damage is still relatively unclear. Some research has found that
1046 males experience more oxidative stress in muscle than females (Pansarasa et al.,
1047 2000). However, other work has found that females have higher levels of oxidative
1048 stress following sub-maximal eccentric running (Magdalena Wiecek, Maciejczyk,
1049 Szymura, & Szygula, 2017). A contributor to these conflicting findings may be the
1050 variation in hormone levels of females across the menstrual cycle.

1051 The menstrual cycle is an important biological function in which an individual's
1052 hormonal profile fluctuates across various phases. The measurable change in
1053 hormones allows for a relatively straightforward identification of cycle phases,
1054 commonly referred to as the follicular and luteal phases. The follicular phase begins
1055 at the first day of menses and lasts till ovulation, commonly lasting between 10-16

1056 days, whereas the luteal phase begins post-ovulation and lasts till the onset of
1057 menses, lasting around 14 days (Reed & Carr, 2018). Oestrogen concentrations begin
1058 to rise during the follicular phase, peaking around ovulation before a sharp drop-off,
1059 this then leads to a gradual increase and secondary, smaller peak during the luteal
1060 phase (Mihm, Gangooly, & Muttukrishna, 2011). These fluctuations in hormones could
1061 theoretically impact both exercise performance and muscle recovery. It appears the
1062 impact of cycle phase on performance is limited as previous studies have shown no
1063 difference for sprint performance (Tsampoukos, Peckham, James, & Nevill, 2010),
1064 $\dot{V}O_{2max}$ (Brutsaert et al., 2002) or anaerobic performance and endurance (Wiecek,
1065 Szymura, Maciejczyk, Cempla, & Szygula, 2016). Contrarily, maximal endurance
1066 performance has been observed to be reduced during the mid-luteal phase in female
1067 soccer players (Julian, Hecksteden, Fullagar, & Meyer, 2017) but not in female rowers
1068 (Vaiksaar et al., 2011). A recent meta-analysis concluded that the impact of cycle
1069 phase on exercise performance is relatively small or 'trivial' (McNulty et al., 2020).
1070 Stronger evidence in the form of high-quality studies is required to better inform future
1071 guidance.

1072 As for the impact of cycle phase on recovery following muscle damage, it has been
1073 reported that there is a prolonged recovery from DOMS and a greater CK and IL-6
1074 response during the follicular phase, and it is speculated that this may be due to the
1075 reduced levels of oestrogen during that phase (Carter, Dobridge, & Hackney, 2001;
1076 Hackney, Kallman, & Ağgön, 2019; Oosthuysen & Bosch, 2017). However, other
1077 research has found that there is no significant difference between menstrual cycle
1078 phase and IL-6 in healthy, eumenorrhic women (Chaffin et al., 2011). One study only
1079 observed a difference during the early follicular phase for DOMS but no other indirect
1080 markers of muscle damage such as countermovement jump or limb girth (Romero-
1081 Parra, Alfaro-Magallanes, et al., 2020).

1082 It should be noted that it is difficult to compare results of previous studies mostly due
1083 to the variability that exists within the menstrual cycle between individuals. Briefly, the
1084 follicular phase has a high level of intra- and inter-variability, in that not each menstrual
1085 cycle a person experiences will be identical in length and also not necessarily generic
1086 between other females (Fehring, Schneider, & Raviele, 2006). Therefore, it is possible
1087 that even though some individuals may be studied during the follicular phase they may
1088 have high levels of circulating oestrogen as they approach the peak, pre-ovulation

1089 period, compared to others at the early follicular phase. Indeed, it was recently
1090 identified that the largest difference in exercise performance was between the early
1091 and late follicular phase of the menstrual cycle (McNulty et al., 2020), albeit the
1092 difference was calculated as trivial. The reason for this difference is speculated to be
1093 due to the sharp rise in oestrogen during the late follicular following the period of low
1094 oestrogen during the early follicular phase. Progesterone also remains low during the
1095 late follicular rise, which may perhaps increase the bioactivity of oestrogen (Reed &
1096 Carr, 2018). This does at least advocate the need for researchers to identify the
1097 specific phase timing of individuals if the focus of the research is the impact of the
1098 menstrual cycle. Hormonal testing is likely required for accurate quantification of cycle
1099 phase rather than calendar-based testing (Wideman, Montgomery, Levine, Beynon,
1100 & Shultz, 2013).

1101 Furthermore, the relative inconsistency between research may also partially be due to
1102 the variety of contraceptives available to females, which may result in a large variety
1103 of contraceptive use within a single study, a potential confounding factor. Indeed,
1104 Oosthuyse and Bosch (2017) speculate that one of the reasons for the inconsistency
1105 between studies is in fact due to a lack control of contraceptive use in previous
1106 research.

1107 Regarding contraceptives, one type of contraceptive that has been investigated is the
1108 oral contraceptive pill (OCP). A recent meta-analysis found that individuals on the OCP
1109 had suffered from slightly impaired exercise performance (both endurance and
1110 strength related activities) compared to naturally menstruating females (Elliott-Sale et
1111 al., 2020). This was however, considered a trivial difference. Research has found that
1112 females supplementing the OCP have lower circulating oestrogen levels compared to
1113 non-supplementing females (Hicks, Onambele-Pearson, Winwood, & Morse, 2017).
1114 As such it is feasible that due to the theoretical protective effect of oestrogen on muscle
1115 damage, individuals not taking the OCP may have an inherently improved recovery
1116 compared to OCP users.

1117 Currently, evidence suggests that MVIC recovery is slower in OCP users than that of
1118 naturally menstruating females following EIMD (Mackay, González, Zbinden-Foncea,
1119 & Peñailillo, 2019; Minahan, Joyce, Bulmer, Cronin, & Sabapathy, 2015; Savage &
1120 Clarkson, 2002). Furthermore, the CK response may also be greater in OCP users,

1121 potentially indicating a greater fatigue response (Hicks et al., 2017; Minahan et al.,
1122 2015). The reason for this is likely because the OCP users have lower oestrogen levels
1123 resulting in a greater risk of membrane disruption which may result in an increased
1124 creatine kinase response following EIMD. It should be noted that Hicks et al., (2017)
1125 found no other differences between OCP and naturally menstruating females for other
1126 markers of muscle damage. Indeed, there is evidence refuting the findings that OCP
1127 users suffer from EIMD more. In fact, one study found that OCP users had less
1128 muscular soreness following EIMD than non-OCP users (Thompson, Hyatt, De Souza,
1129 & Clarkson, 1997). More research is required to arrive at a consensus about the impact
1130 of oral contraceptives on recovery. Furthermore, there is a dearth of research into
1131 other forms of contraceptives that are available; this research is needed to better
1132 understand the effects that each may have on exercise recovery, and even exercise
1133 performance.

1134

1135 2.5 Importance of recovery within sport settings

1136 Improving recovery has long been an area of great interest and within the purview of
1137 modern research as much as it was in ancient times. This is best exemplified through
1138 the art and application of massage therapy, with Chinese texts dating back to 2598
1139 BC as well as the Ancient Greek scholar Hippocrates citing it as an effective method
1140 of aiding sports injuries (Goats, 1994). Current understanding suggests that massage
1141 therapy may assuage DOMS following exercise (Guo et al., 2017). This highlights how
1142 recovery from exercise was, is, and may always be a pertinent area of research.

1143 In modern day sports, there is not always adequate time for full recovery after exercise
1144 (Page, Marrin, Brogden, & Greig, 2019; Rojas-Valverde et al., 2019). There are
1145 various Olympic sports that entail multiple bouts of exercise within the same day, e.g.,
1146 judo. Additionally, in soccer, fixture congestion has become increasingly prevalent in
1147 recent times. In the 2020-21 season Manchester City FC played a total of 61 games
1148 between 21st September 2020 – 29th May 2021, ~36 weeks, averaging 1.7 matches
1149 each week or a game roughly every four days across the season. This does not
1150 account for International breaks when many of the first team squad will still be
1151 performing and it is very likely that many players were exposed to extended periods
1152 of two games per week. Impaired recovery can increase injury risk and impair athletic

1153 performance (Killen, Gabbett, & Jenkins, 2010; Small, McNaughton, Greig, Lohkamp,
1154 & Lovell, 2009b). For this reason, attempting to optimise the recovery period and
1155 reduce the time frame has become a key area of research. However, there are times
1156 when optimising recovery may not be the key focus. These are periods of intense
1157 training, when inducing physiologic adaptations are the priority, e.g., improve the
1158 endogenous capacity of a muscle to cope with the demands of the exercise, e.g., in
1159 pre-season with team sports and training camps with combat and weightlifting sports
1160 (Burgomaster et al., 2008; Ebbeling & Clarkson, 1989; Gomez-Cabrera et al., 2006).
1161 Including the use of an external aid may be beneficial short term but may blunt possible
1162 adaptations from the exercise stimulus, although the evidence is equivocal (see review
1163 Merry and Ristow (2016)). Further, it has been observed that blunting the pro-
1164 inflammatory phase can impact the anti-inflammatory phase and as a result impair
1165 muscle regeneration (Deng et al., 2012). Other research has suggested that
1166 antioxidant supplementation may inhibit cellular adaptations that arise from exercise
1167 (Morrison et al., 2015; Strobel et al., 2011), although this is still debated (Mankowski,
1168 Anton, Buford, & Leeuwenburgh, 2015; Peternej & Coombes, 2011). In elite sport, it
1169 is likely that recovery will commonly be the priority during competition phases, this is
1170 to enable optimal performance by the time of the next bout of exercise.

1171 2.6 Hormesis

1172 The theoretical driving force behind adaptation is a process known as hormesis and
1173 is perhaps the reason for the long-standing debate behind whether antioxidant
1174 supplements or other recovery methods that target the inflammatory response should
1175 or should not be used. The theory entails that when a biological system is exposed to
1176 a low or moderate stress (e.g., toxic molecules or ROS) then this may result in an
1177 adaptive response by said system. However, being subjected to a high level of stress
1178 may result in a negative outcome (Mattson, 2008; Radak, Chung, & Goto, 2008). It is
1179 possible that hormesis may explain the immediate benefits seen following exposure
1180 to strenuous exercise and as a result the repeated bout effect (Hubal, Chen,
1181 Thompson, & Clarkson, 2008; Nosaka, Sakamoto, Newton, & Sacco, 2001). Regular
1182 exercise training may lead to an upregulation of the genes involved in transcribing
1183 antioxidant enzymes as well as improving the inflammatory response through a faster
1184 phenotype switch to anti-inflammatory macrophages (Gordon et al., 2012). Therefore,
1185 it is possible that ROS and inflammatory molecules are drivers of cellular adaptations,

1186 but long term or excessive exposure may be detrimental (Scheele, Nielsen, &
1187 Pedersen, 2009).

1188 2.7 How cocoa could help recovery

1189 The possibility that CF may aid muscle recovery following muscle damage is intriguing.
1190 Current nutritional interventions that are beneficial, albeit somewhat equivocally, for
1191 improving recovery, include Montmorency tart cherry juice (Bell, Stevenson, Davison,
1192 & Howatson, 2016; Bowtell, Sumners, Dyer, Fox, & Mileva, 2011; Connolly, McHugh,
1193 Padilla-Zakour, Carlson, & Sayers, 2006) and beetroot juice (Clifford, Bell, West,
1194 Howatson, & Stevenson, 2016; Clifford, Berntzen, et al., 2016; Clifford, Howatson,
1195 West, & Stevenson, 2017). However, the palatability of these interventions is
1196 debateable, with both cherry juice and beetroot juice sometimes being supplemented
1197 or mixed with another flavouring to improve taste (Dimitriou et al., 2015). Chocolate,
1198 on the other hand, is a highly palatable food also capable of acutely improving mood
1199 state following ingestion (Macht & Mueller, 2007). Therefore, CF may be a welcome
1200 addition to the cornucopia of nutritional interventions for recovery, should it prove
1201 efficacious.

1202 Regarding muscle damage, CF may influence specific aspects of the damaging and
1203 recovery processes. More specifically, CF may act as an antioxidant to reduce the
1204 likelihood of oxidative stress and potentially modulate the inflammatory response post-
1205 exercise. As discussed in the previous section, exercise can induce a shift in redox
1206 homeostasis that leads to oxidative stress. It has been observed that the consumption
1207 of CF may increase the activity of glutathione peroxidase and glutathione reductase,
1208 two endogenous antioxidant enzymes (Martín et al., 2010), increase antioxidant
1209 capacity (Lotito & Frei, 2006; Wang et al., 2000), reduce ROS production (Ramiro-
1210 Puig et al., 2009; Rein et al., 2000), and protect cell membranes from ROS damage
1211 (Zhu, Holt, Lazarus, Orozco, & Keen); thus, supplementation may protect against
1212 oxidative stress.

1213 The role that CF has on inflammation is complex. As discussed earlier, following
1214 muscle damage there is a localised inflammatory response at the damaged site. This
1215 involves the accumulation of different leukocytes initially, specifically neutrophils and
1216 macrophages, which in turn can exacerbate the damage through the secretion of pro-
1217 inflammatory cytokines, e.g., TNF- α , IL-2 and IL-6. It appears that CF may modulate
1218 this phase of inflammation, as CF monomers and dimers exert a slight inhibitory

1219 potential on TNF- α secretion, whereas larger CF may stimulate increases of TNF- α
1220 (Mao, van de Water, Keen, Schmitz, & Gershwin, 2002). Other research has found
1221 that CF downregulates various other pro-inflammatory molecules, such as IL-1 α and
1222 IL-6, that are released by macrophages in periods of inflammation, potentially *via* the
1223 inhibition of specific transcription factors, e.g., nuclear factor-kappa β (NF-k β) or
1224 activated protein-1 (Ramiro et al., 2005; Selmi, Mao, Keen, Schmitz, & Eric Gershwin,
1225 2006).

1226 Furthermore, it is possible that CF may reduce the neutrophil induced oxidative burst
1227 that occurs, perhaps through a reduced activation of the NF-k β pathway (Mackenzie
1228 et al., 2004). Similar effects have been observed in other flavonoid research with
1229 different interaction effects associated with the different chemical structures that make
1230 up the various flavonoid sub-classes (Ciz et al., 2012; Nam, 2006; Vázquez-Agell et
1231 al., 2013). The NF-k β pathway is responsible for the regulation of the majority of
1232 inflammatory mediators, including cytokines, chemokines, and other transcription
1233 factors (Dorrington & Fraser, 2019). The inhibition of this pathway is speculated to
1234 offer a therapeutic effect on various inflammatory conditions that induce an abnormal
1235 production of cytokines (Yamamoto & Gaynor 2001).

1236 Therefore, due to the potential CF have as antioxidants and immunomodulators, they
1237 may exert a beneficial effect on various aspects of the initial inflammatory response
1238 and oxidative stress elicited by an exercise insult. It should be noted that many
1239 investigations into the impact CF have on inflammation are commonly *in vitro*, in
1240 subjects with high levels of systemic inflammation or in animals and thus, their effect
1241 in humans remains unclear. Nonetheless, the application of CF on muscle recovery
1242 does pose an interesting area of research.

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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.

1258 3.1. Background

1259 Muscle damage is associated with various negative symptoms, such as delayed onset
1260 muscle soreness, impaired muscle function, and increased inflammation (Lee et al.,
1261 2002; Powers et al., 2011). Consequently, the use of recovery interventions purported
1262 to accelerate recovery has become increasingly prevalent. There is an emerging
1263 interest in the effects of the non-nutritive compounds (poly)phenols as recovery aids
1264 following strenuous exercise. As such their popularity as a nutritional aid has increased
1265 in athletes and recreational exercisers, likely because these plant-based bioactive
1266 compounds have numerous additional health benefits (Solheim et al., 2017).

1267 The term (poly)phenol refers to a variety of bioactive compounds including flavonoids,
1268 stilbenes, phenolic acids and lignans (Tangney & Rasmussen, 2013). The largest
1269 subclass, flavonoids, can be further classified into flavonols, flavanols, flavanones,
1270 anthocyanins, flavones and isoflavones. Of these subclasses, the majority of research
1271 has focused on flavanols with particular attention on cocoa, not only because of the
1272 palatability of chocolate (Lima, Almeida, Nout, & Zwietering, 2011) but due to the high
1273 proportion of monomers catechin, epicatechin and gallic acid; collectively referred
1274 to as CF, see Chapter 1 Section 1 for more information. These monomers are found
1275 in the largest quantities in cocoa when compared with other flavanol containing
1276 foodstuffs such as tea and fruits; however, the amounts vary considerably. See
1277 Chapter 1 Section 2 for information about how flavanol content can vary.

1278 Cocoa flavanols (CF) have been shown to possess anti-inflammatory and antioxidant
1279 effects, with epicatechin the most potent monomer of the flavanol group (Andres-
1280 Lacueva et al., 2008). Cardiovascular benefits, such as improved flow mediated
1281 dilation and reduced blood pressure, have been observed following various doses of
1282 CF, such as, 918 mg (Heiss et al., 2007), 701 mg (Berry, Davison, Coates, Buckley,
1283 & Howe, 2010), 750 mg (Horn et al., 2014), and 917 mg (Schroeter et al., 2006) and
1284 epicatechin intakes as low as 25 mg (Ellinger, Reusch, Stehle, & Helfrich, 2012) and
1285 46 mg (Heiss et al., 2003). Regarding epicatechin, greater efficacy has been reported
1286 at higher epicatechin doses (see review (Bernatova, 2018)). These benefits have
1287 been observed following supplementation periods ranging from the same day of
1288 testing (Berry et al., 2010; Schroeter et al., 2006), to seven days (Heiss et al., 2007),
1289 and 30 days (Horn et al., 2014). Additionally, CF may be beneficial for reducing
1290 markers of oxidative stress (defined as an imbalance in the generation of various

1291 reactive species and antioxidants (Cobley, Close, Bailey, & Davison, 2017)) and
1292 inflammation (Decroix, Soares, Meeusen, Heyman, & Tonoli, 2018; Prince et al.,
1293 2016). The role of CF in modulating inflammation may stem from their capacity to
1294 influence signalling cascades, i.e., *via* an alteration to eicosanoid production (Derek D
1295 Schramm et al., 2001), and reducing the activation of certain inflammatory
1296 transcription factors, e.g., NF- κ B (Vázquez-Agell et al., 2013). Given that EIMD is
1297 thought to partly stem from inflammation and oxidative stress, CF may be able to
1298 attenuate functional symptoms that impede athlete recovery, such as muscular
1299 soreness and deficits in muscle function (Decroix et al., 2018; Vlachoianis et al.,
1300 2016).

1301 ROS are produced as part of normal metabolic processes, such as cellular respiration,
1302 and in certain scenarios, such as exercise, are produced in high amounts (Powers et
1303 al., 2011). Various ROS molecules are involved in a plethora of functions at a cellular
1304 level, including, growth and proliferation (Hoidal, 2001), immune response (Halliwell,
1305 2006) and apoptosis (Fuchs, Gruber, Uberall, & Wachter, 1994). Additionally, it is
1306 believed that ROS act as signalling molecules in various tissues; however, this is still
1307 not fully understood due to the numerous ROS produced at rest and during exercise
1308 (Powers, Duarte, Kavazis, & Talbert, 2010). Antioxidant defence systems maintain a
1309 balance between ROS production and neutralisation; if the production of ROS
1310 outweighs their neutralisation, then proteins, lipids and DNA may be oxidised altering
1311 their function (Betteridge, 2000). This process is typically referred to as oxidative
1312 stress. Alternatively, if cells are exposed to low levels of ROS, such as during
1313 moderate intensity exercise, they may act as signalling molecules for skeletal muscle
1314 adaptations (Mattson, 2008). Such adaptations include an increase in endogenous
1315 antioxidants such as superoxide dismutase, glutathione peroxidase and catalase,
1316 reduced oxidative damage from exercise and an improved resistance to oxidative
1317 stress (Radak et al., 2008). The mechanisms by which CF modulate redox metabolism
1318 and oxidative stress are not entirely clear, but activation of the nuclear factor erythroid
1319 2-related factor 2 (Nrf2) transcription pathway, which activates a battery of
1320 cytoprotective protein with antioxidant and anti-inflammatory functions is a potential
1321 candidate (Cheng, Wu, Ho, & Yen, 2013). For example, it has been observed that
1322 supplementation with catechin results in an increase in the expression of heme-
1323 oxygenase 1, an enzyme with antioxidant and anti-inflammatory functions (Paine, Eiz-

1324 Vesper, Blasczyk, & Immenschuh, 2010), *via* upregulation of Nrf2 activity (Cheng et
1325 al., 2013). Moreover, cells treated with CF induced an increase in glutathione
1326 peroxidase and glutathione reductase, likely *via* Nrf2 activation (Cordero-Herrera,
1327 Martín, Goya, & Ramos, 2015). In addition, CF treatment has been shown to prevent
1328 a depletion in reduced glutathione and replenish glutathione peroxidase, as well as
1329 effectively limiting lipid and protein peroxidation (Martins et al., 2020). Collectively,
1330 these studies suggest CF may modulate oxidative stress, at least partly *via* redox
1331 sensitive pathways, e.g., stimulating Nrf2 which in turn leads to an increase in redox
1332 enzyme expression.

1333 Strenuous exercise may generate large amounts of ROS that leads to oxidative stress.
1334 The ROS produced is thought to stem from the increase in cellular respiration, and/or
1335 immune cells like neutrophils (Lee et al., 2002; Souglis et al., 2018). Leukocytes that
1336 accumulate in the muscle after EIMD evoke a respiratory burst, whereby macrophages
1337 and neutrophils produce large amounts of ROS to lyse cellular debris and begin
1338 regeneration. However, it has been proposed that during this process ROS may also
1339 induce lipid peroxidation in nearby healthy tissues (Fisher-Wellman & Bloomer, 2009).
1340 It is thought that this damage to neighbouring cells might contribute to EIMD, and at
1341 least partly explain why decrements in muscle function and increased muscle
1342 soreness can persist for several days after strenuous exercise (Steinbacher & Eckl,
1343 2015).

1344 Therefore, the aim of this systematic review was to critically examine research on the
1345 effects of CF on oxidative stress, inflammation, muscle function, perceived soreness,
1346 and exercise performance. This review builds on previous work by Decroix et al.,
1347 (2018) that reviewed the effects of CF on exercise performance. The present review
1348 includes research completed since the aforementioned article and unlike Decroix and
1349 colleagues focuses on CF and EIMD.

1350 3.2. Methods

1351 3.2.1 Information Sources and Search Strategies

1352 This systematic review followed the guidelines outlined by the Preferred Reporting
1353 Items for Systematic Reviews and Meta-analyses (PRISMA; [http://www.prisma-](http://www.prisma-statement.org)
1354 [statement.org](http://www.prisma-statement.org)). The goal of this systematic review was to collate and critique the
1355 current literature involving CF supplementation and exercise. The focus was on

1356 muscle damage and recovery measured through various outcomes on intramuscular
1357 inflammation, oxidative stress, muscle function, perceived soreness, and
1358 performance. To accomplish this, five databases were searched: PubMed, Scopus,
1359 Web of Science, ScienceOpen and MEDLINE as well as bibliographies of potential
1360 articles were explored. Key terms for the search were as follows: 'cocoa flavanols,'
1361 OR 'dark chocolate,' AND 'muscle damage,' OR 'muscle recovery,' OR 'exercise
1362 recovery,' OR 'exercise-induced muscle damage,' OR 'exercise.' The latest search
1363 was carried out on 10th February 2020.

1364 3.2.2 Quality Assessment

1365 To assess the quality and potential risks of bias and quality of studies included, the
1366 National Institute for Health and Excellence checklist for randomised controlled trials
1367 was utilised (Popay, 2012). This checklist has been used in a previous systematic
1368 review by Decroix et al., (2018) about the impact of CF on vascular function, oxidative
1369 stress, and exercise performance. The tool is divided into four sections: section A –
1370 selection bias on the randomisation and allocation of participants. Section B –
1371 performance bias on care provided and blinding of participants and investigators.
1372 Section C – attrition bias on the differences between groups, including drop-out rate
1373 of participants. Section D – detection bias on the appropriateness of outcomes and
1374 measures used as well as the nature of blinding of investigators. A study can achieve
1375 a maximum score of 14 if all criteria are fulfilled within the article. If a study achieves
1376 a score ≥ 11 it is considered high quality with a low risk of potential bias, a score
1377 between 8-10 is considered good quality and has a low risk of potential bias. However,
1378 if a study achieves a score ≤ 7 the research is considered of poor quality and has a
1379 very high risk of bias (NICE, 2013). Table 3.1 shows the quality assessment scores of
1380 the included studies.

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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.

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1388 3.2.3 Study Selection Process and Eligibility Criteria

1389 The results from all three databases were pooled and all duplicates were removed
1390 using either Zotero (<https://www.zotero.org/>) or manually by the thesis author and
1391 another independent researcher. For the studies to be deemed as viable for the review
1392 they had to satisfy the following inclusion criteria: research involving human
1393 participants, research studies involving acute, sub-chronic or chronic cocoa flavanol
1394 supplementation, an exercise protocol involved alongside supplementation, and
1395 explicit statement of cocoa flavanol use. Exclusion criteria were non-human
1396 participants, no CF supplementation (e.g., tea flavanols or wine (poly)phenols), review
1397 articles and case studies, no exercise involvement during the trial period and no
1398 disclosure CF dose in the methods. Articles were reviewed through titles and abstracts
1399 for initial screening and thereafter, full texts were screened to check eligibility until a
1400 consensus was reached between the thesis author and another independent
1401 researcher regarding the inclusion of studies. The PRISMA flow chart (Figure 3.1)
1402 outlines the identification, screening, and exclusion process.

1403 3.3. Results

1404 3.3.1 Study Selection and Screening

1405 The preliminary screening using the aforementioned search terms resulted in an
1406 output of 491 articles. Following the collation of all articles the process of removing
1407 duplicates began, leading to a removal of 323 articles. Subsequently, all remaining
1408 articles titles were screened for relevance before an in-depth examination of abstracts
1409 and then full texts which led to the final 17 articles. A further three of these studies
1410 were then excluded from subsequent review due to no explicit CF amount stated in
1411 the text; Singh et al., (2006) expressed CF as total (poly)phenols, Gonzalez-Garrido,
1412 Garcia-Sanchez, Garrido-Llanos, and Olivares-Corichi (2017) only referred to total
1413 flavonoid content and Cavarretta et al., (2018) expressed flavanol content as gallic
1414 acid equivalents.

1415 3.3.2 Study Characteristics

1416 The fourteen studies totalled 213 participants (129 untrained and 84 classed as
1417 trained) with an average age of 28 ± 9 years old. The strata of 'untrained' was defined
1418 as participants who were referred to as untrained/sedentary or healthy/active
1419 individuals in the text (Allgrove et al., 2011; Davison, Callister, Williamson, Cooper, &

1420 Gleeson, 2012; Morgan, Wollman, Jackman, & Bowtell, 2018; Patel, Brouner,
1421 Allgrove, & Spendiff, 2020; Sadler et al., 2020; Stellingwerff et al., 2013; Taub et al.,
1422 2016; Wiswedel et al., 2004), whereas 'trained' was defined as participants who were
1423 described as well-trained/professional athletes or as 'elite' athletes in the text (de
1424 Carvalho et al., 2019; Decroix et al., 2018; Decroix et al., 2017; Fraga et al., 2005;
1425 Patel, Brouner, & Spendiff, 2015; Peschek, Pritchett, Bergman, & Pritchett, 2013). All
1426 the included studies examined the effects of CF on one or more of the following:
1427 exercise-induced oxidative stress and inflammation, changes in muscle function,
1428 changes in levels of perceived soreness and impact of supplementation on exercise
1429 performance.

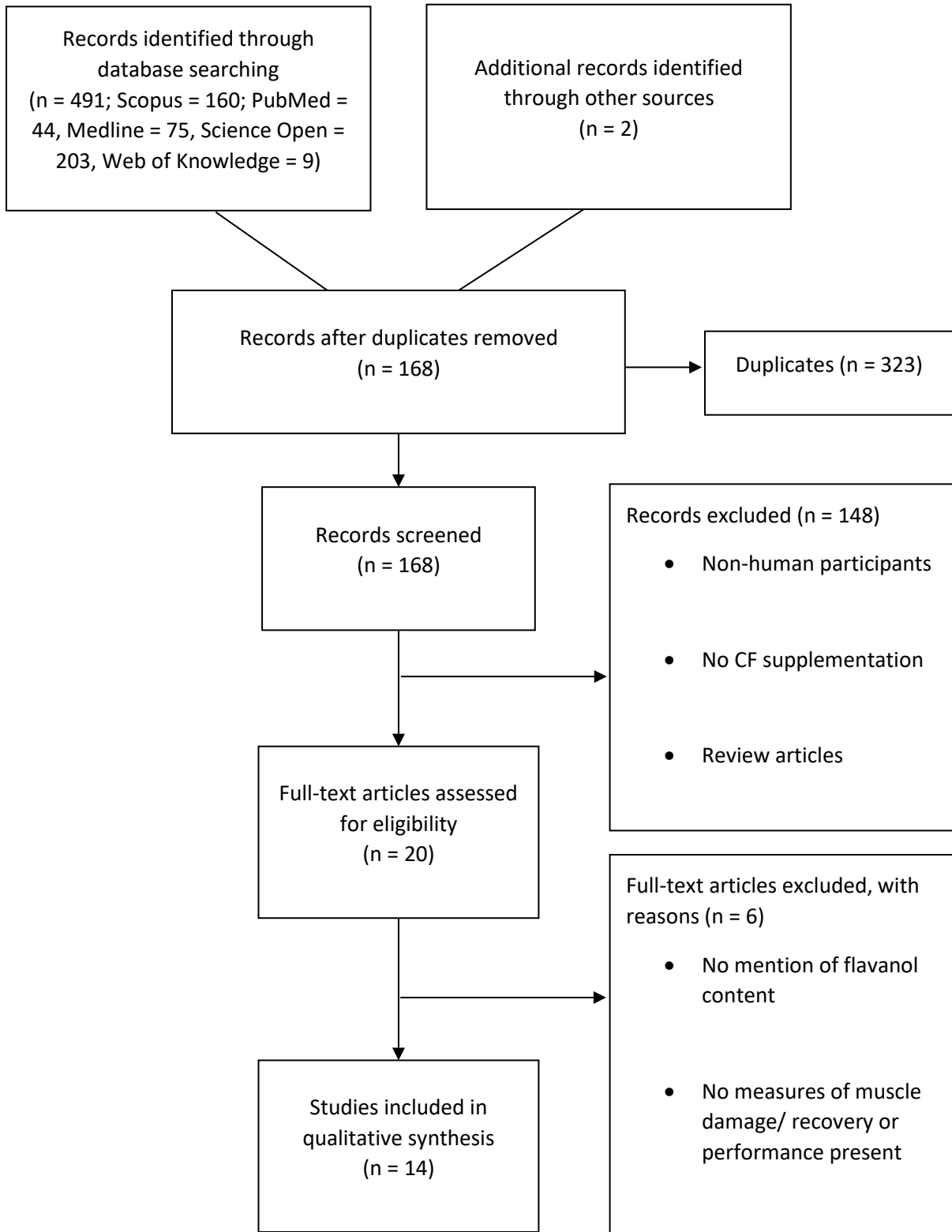
1430 3.3.3 Summary of Studies

1431 Of the 14 articles, nine examined the effects of CF consumption and exercise on
1432 oxidative stress response and five investigated the effects on inflammation. Three
1433 investigated the effects CF has on muscle function and measures of perceived
1434 soreness, and nine studied effects on exercise performance. Some of the included
1435 studies are involved in multiple categories. The studies utilised various methods of
1436 supplementing CF: 1) a sub-chronic (moderate length) supplementation period of up
1437 to 14 days, 2) a seven-day loading phase pre-exercise protocol and 3) an acute dose
1438 on the day of the exercise protocol, 4) a chronic three-month supplementation period.
1439 Three of the fourteen articles followed a sub-chronic CF supplementation period
1440 (Allgrove et al., 2011; Fraga et al., 2005; Patel et al., 2015), four utilised a seven-day
1441 loading phase in the build-up to an exercise protocol (de Carvalho et al., 2019; Decroix
1442 et al., 2018; Morgan et al., 2018; Sadler et al., 2020) and six used an acute dose on
1443 the day of the exercise protocol (Davison et al., 2012; Decroix et al., 2017; Patel et al.,
1444 2020; Peschek et al., 2013; Stellingwerff et al., 2013; Wiswedel et al., 2004). All studies
1445 used doses of CF that were categorised as low (≤ 250 mg), moderate (250 to 700 mg)
1446 or high (≥ 700 mg). Six studies measured flavanol concentrations in plasma following
1447 CF consumption, five found that epicatechin concentrations peaked between 90 and
1448 190 min post exercise (Davison et al., 2012; Decroix et al., 2018; Decroix et al., 2017;
1449 Stellingwerff et al., 2013; Wiswedel et al., 2004), whereas (Fraga et al., 2005) did not
1450 find significant levels of CF, likely due participants being in a fasted state before blood
1451 sampling. Details of the included studies are reported in Tables 3.2, 3.3, 3.4, 3.5, and
1452 3.6.

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1456 Figure 3.1. PRISMA flow chart detailing the screening

1457 3.4. Discussion

1458 3.4.1 Impact of Cocoa Flavanols on Exercise-induced Oxidative Stress

1459 Antioxidants maintain redox status by neutralising ROS produced by metabolic
1460 reactions (Halliwell, 2007). However, as explained in Chapter 2 Section 3.4, the
1461 upregulation of ROS can lead to oxidative stress if cellular antioxidant capacity is
1462 overwhelmed. Oxidative stress in skeletal muscle decreases force output (Reid, 2001),
1463 likely through a reduction in Ca^{2+} sensitivity in the myofibrils and reduced activity of
1464 calcium ATPase, suggesting contractile dysfunction partly due to the accumulation of
1465 Ca^{2+} (Reid, 2008; Siems et al., 2003). Therefore, an increase in antioxidant capacity
1466 may lead to improvements in performance and recovery through reductions in fatigue
1467 associated with ROS during and after exercise.

1468 Two studies that examined the effects of CF on markers of oxidative stress observed
1469 significant interaction effects following a 14-day sub-chronic supplementation period
1470 (Allgrove et al., 2011; Fraga et al., 2005). Allgrove and colleagues observed that F₂-
1471 isoprostanes and oxidised low density lipoprotein (markers of oxidative stress) were
1472 significantly lower in the treatment group, supplementing 197.4 mg CF and 77.4 mg
1473 epicatechin, versus placebo post 90 min of cycling at 60% $\dot{V}\text{O}_{2\text{max}}$, interspersed with
1474 30 s efforts at 90% $\dot{V}\text{O}_{2\text{max}}$ every 10 min (Allgrove et al., 2011). Similarly, (Fraga et al.,
1475 2005) found that regular CF intake (168 mg) alongside soccer training and match play
1476 over a 14-day period resulted in a 12% decrease in malondialdehyde (MDA; a marker
1477 of lipid peroxidation), whereas in the placebo condition values increased by 10%,
1478 indicating a reduction in oxidative stress associated with training and match play. A
1479 study by Decroix et al., (2017) observed that although cycling time trial exercise
1480 increased MDA concentrations, CF had no significant impact compared to placebo.
1481 Wiswedel et al., (2004) also found no significant treatment effect of CF on MDA
1482 concentrations following cycling exercise. Interestingly, Wiswedel et al., (2004)
1483 included a no exercise control and found that the high flavanol group had a lesser
1484 increase in MDA than the low CF group four- and six- hr post-ingestion. In contrast,
1485 when supplementing 1,765 mg of cocoa extract (containing 530 mg CF) for six days
1486 in the lead up to exercise and once more immediately before, CF blunted the exercise-
1487 induced rise in MDA concentrations (Decroix et al., 2018). These changes imply that
1488 sub-chronic consumption of CF may reduce exercise-induced oxidative stress more
1489 effectively than an acute dose. The results suggest that CF may be a potent

1490 antioxidant, with plasma MDA levels decreasing from baseline over a 14-day period
1491 of 168 mg of CF consumption a day (Fraga et al., 2005). These findings may have
1492 applicability to clinical populations as it has been reported previously that CF
1493 supplementation prevents systemic oxidative stress (measured via plasma MDA and
1494 urinary prostaglandin F₂α) in type II diabetes and cancer (Abdulkhaleq et al., 2017).
1495 Notwithstanding, the other markers of oxidative stress and antioxidant activity were
1496 not affected by the treatment (8-oxo-2-deoxyguanosine and total relative antioxidant
1497 potency respectively), with a possible explanation being the relatively low amount of
1498 collective epicatechin and catechin in the treatment — only 39 mg per dose (Fraga et
1499 al., 2005), or the markers were not sensitive enough to detect changes in healthy,
1500 soccer players that trained at least twice and played a 90 min match each week.

1501 Where Allgrove and colleagues found a significant difference for F₂-isoprostanes post-
1502 cycling exercise after a sub-chronic dosing protocol of CF, both Davison et al., (2012)
1503 (246.8 mg, 96.8 mg epicatechin) and Wiswedel et al., (2004) (187 mg) observed that
1504 even an acute dose of CF pre-cycling exercise elicited reductions in F₂-isoprostanes
1505 when compared to placebo in a crossover design. These were the only acute dose
1506 studies to observe any treatment effect on oxidative stress as the other two reported
1507 no differences between treatments (de Carvalho et al., 2019; Morgan et al., 2018).
1508 The only study to assess oxidative stress over a chronic supplementation period had
1509 participants consuming 175.2 mg daily for 30 days and found that CF significantly
1510 increased the reduced glutathione/oxidised glutathione ratio and reduced protein
1511 carbonylation (Taub et al., 2016). This again indicates that prolonged supplementation
1512 may be more beneficial than solely acute consumption.

1513 Data regarding uric acid/urate is conflicting across studies. Decroix et al., (2017)
1514 reported that an acute dose of 900 mg CF increased uric acid following two 30 min
1515 time trials. In contrast, Fraga et al., (2005) found that sub-chronic dosing of 168 mg
1516 CF per day decreased urate levels by 11% compared to the beginning of
1517 supplementation, Decroix et al., (2018) also found that 1,765 mg cocoa extract (530
1518 mg CF) per day over a seven day period did not influence uric acid concentrations at
1519 rest or post-exercise. However, the contrasting observations may be attributed to the
1520 fact that Fraga et al., (2005) collected blood samples on a rest day, while Decroix et
1521 al., (2017) took blood samples immediately post-exercise; which has been observed
1522 to increase uric acid concentrations 1-2 hr post intense exercise (Quindry, Stone, King,

1523 & Broeder, 2003). As Decroix et al., (2018) took samples at rest and post-exercise
1524 whilst using the highest dose of CF and found no impact, this may imply that the
1525 mechanism that CF act as an antioxidant may be independent to the mechanism
1526 behind changes in uric acid concentrations. Uric acid can be used as a marker of
1527 oxidative stress due to its role in the conversion of xanthine dehydrogenase to
1528 xanthine oxidase, which then increases the production of ROS (Glantzounis,
1529 Tsimoyiannis, Kappas, & Galaris, 2005). Counterintuitively, uric acid is also one of the
1530 predominant antioxidants found within the plasma (El Ridi & Tallima, 2017; Ghezzi,
1531 2020). The role of uric acid as a pro-oxidant within the cellular compartment, coupled
1532 with its role as an antioxidant in the plasma, make it difficult to draw practical
1533 conclusions from antioxidant based nutritional studies. Additionally, certain flavonoids,
1534 such as quercetin due to its chemical structure, may act as an inhibitor of the
1535 production of xanthine oxidase (an enzyme that increases ROS concentrations) and
1536 as such have a direct influence on uric acid concentrations (Mohos et al., 2019).

1537 However, there are times during an athletes' training when reducing oxidative stress
1538 may not be desired, such as during pre-season when adaptations from exercise are
1539 the priority as opposed to accelerated recovery. The adaptations associated with
1540 oxidative stress during and following exercise include improved cellular repair systems
1541 and reduced production of damaging ROS (Radak, Taylor, Ohno, & Goto, 2001).
1542 However, these exercise related training adaptations may be hindered by regular high
1543 doses of antioxidant compounds and prevent or obstruct key cellular functions
1544 associated with ROS (Peternelj & Coombes, 2011). Nevertheless, a recent meta-
1545 analysis identified that the evidence for a blunting effect of (poly)phenol
1546 supplementation on exercise adaptations is equivocal, more research is needed to
1547 fully understand how (poly)phenols may augment exercise adaptations (Martinez-
1548 Negrin, Acton, Cocksedge, Bailey, & Clifford, 2020).

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)	<p>13 trained males</p> <p>Age 21 ± 2 years</p> <p>Stature 180 ± 0.05 cm</p> <p>Mass 87.02 ± 8.03 kg</p>	<p>CF: CHO + protein cocoa beverage, 306 mg CF per beverage</p> <p>CON: cocoa based CHO + protein beverage, 0 mg CF</p>	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)	<p>12 well-trained males</p> <p>Age 30 ± 3 years</p> <p>Stature 177.9 ± 8.8 cm</p> <p>Mass 72.8 ± 7.8 kg</p> <p>$\dot{V}O_{2max}$ 63.0 ± 3.5 ml·kg⁻¹ min⁻¹</p>	<p>CF: cocoa drink, 900 mg CF (EPI: 185 mg, CAT: 20 mg)</p> <p>CON: placebo, 15 mg CF (EPI: 0 mg, CAT: 0 mg)</p>	Acute dose 1.5 pre-exercise	Two 30 min time trials 60 min apart, performed at a ~75% peak power output.	<p>i) Uric acid</p> <p>ii) MDA</p> <p>iii) TEAC</p>	<p>i) ↑ in CF vs CON</p> <p>ii) ↔ between group</p> <p>iii) ↑ in CF vs CON</p>

Decroix et al., (2018)	<p>14 well-trained males</p> <p>Age 31 ± 3 years</p> <p>Stature 180 ± 5 cm</p> <p>Mass 73 ± 7 kg</p> <p>$\dot{V}O_{2max}$ 62.9 ± 5.8 ml·kg⁻¹ min⁻¹</p> <p>Peak Power Output 366 ± 45 W</p>	<p>CF: Capsule, 530 mg CF (EPI: 100 mg, CAT: 21 mg)</p> <p>CON: 1,764 mg maltodextrin</p>	<p>Consumed daily for six days and then a seventh on the day of testing</p>	<p>20 min steady state cycling at 45% peak power output</p> <p>20 min time trial beginning at 75% peak power output</p> <p>Completed in normoxic and hypoxic environments</p>	<p>i) TEAC</p> <p>ii) Uric acid</p> <p>iii) MDA</p>	<p>i) ↔</p> <p>ii) ↔</p> <p>iii) CF blunted ↑ in both N and H</p>
Fraga et al., (2005)	<p>28 trained males</p> <p>Age 18 ± 1 years</p> <p>Mass 74 ± 1 kg</p>	<p>CF: 105 g chocolate confectionery, 168 mg CF (EPI + CAT: 39 mg)</p> <p>CON: 105 g cocoa butter chocolate, <5 mg CF</p>	<p>Sub-chronic, 14 day consumption</p>	<p>Soccer training sessions twice per week and one match per week.</p>	<p>i) MDA</p> <p>ii) Urate</p> <p>iii) Oxo⁸dG</p> <p>iv) TRAP</p> <p>v) α-tocopherol</p> <p>vi) lycopene</p> <p>vii) β-carotene</p> <p>viii) coenzyme Q-10</p>	<p>i) Post CF ↓ by 12%</p> <p>CON ↑ by 10%</p> <p>ii) ↓ by 11% in CF</p> <p>iii-viii) ↔ between groups</p>

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

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 \uparrow 2 and 4 hr post-consumption, CF did not Significant difference CF vs CON 2 and 4 hr post-intake following exercise ii-v) \leftrightarrow between groups
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Note: CF = cocoa flavanols, EPI = epicatechin, CAT = catechin, CON = control, CHO = carbohydrate, TEAC = Trolox equivalent antioxidant capacity, MDA = malonaldehyde, LDL = low density lipoprotein, Oxo⁸dG = 8-Oxo-2'-deoxyguanosine, 1RM = one rep max, GSH:GSSG = reduced glutathione: oxidised glutathione ratio, TAC = total antioxidant capacity, TRAP = total relative antioxidant potency, \uparrow = increase, \downarrow = decrease \leftrightarrow = no significant effect/change

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1557 3.4.2 Impact of Cocoa Flavanols on Exercise-induced Inflammation

1558 Strenuous exercise resulting in muscle damage evokes an acute inflammatory
1559 response (Peake et al., 2017). Several studies have observed systemic increases in
1560 markers such as IL-6, c-reactive protein (CRP) and TNF- α (Kanda et al., 2013;
1561 Kasapis & Thompson, 2005) following intense exercise. These markers are typically
1562 increased for several hours following exercise, but may persist for several days
1563 depending on the severity of the damage (Peake, Nosaka, & Suzuki, 2005).
1564 Inflammation, particularly the increase in neutrophils, has been associated with muscle
1565 function loss following exercise, suggesting the acute inflammatory response plays a
1566 role in recovery after exercise (Paulsen et al., 2010).

1567 In vitro studies have shown that CF have anti-inflammatory properties and can reduce
1568 TNF- α from inducing an upregulation of vascular endothelial growth factor activity (Kim
1569 et al., 2010) and inhibit nuclear factor-kappa beta activation (Rodríguez-Ramiro et al.,
1570 2013). In humans, CF supplementation has been shown to decrease Interleukin-1 β
1571 and Interleukin-10 levels (Sarriá et al., 2014), four weeks of dark chocolate
1572 consumption reduced leukocyte accumulation, soluble adhesion molecules, and the
1573 expression of adhesion markers on leukocytes (Esser et al., 2014) (see review by
1574 Goya et al., (2016) for more detail as it was beyond the scope of the thesis to fully
1575 review every aspect of CF on inflammation). This may indicate that dark chocolate or
1576 cocoa powder with a high proportion of CF would perhaps be viable as a therapeutic,
1577 anti-inflammatory intervention.

1578 Studies by Allgrove et al., (2011) and Davison et al., (2012) found that prolonged
1579 cycling at 60% $\dot{V}O_{2max}$ increased inflammatory markers (IL-6, IL-10 and IL-1ra and IL-
1580 6, blood leucocyte count and neutrophil count, respectively) but found no difference
1581 between CF supplementation or placebo. Decroix et al., (2017) used two 30 min time
1582 trials separated by 90 min; the first time trial starting 100 min post ingestion of a 900
1583 mg CF beverage. This resulted in no treatment or time effect on inflammatory markers
1584 (TNF- α , IL-1 and IL-6), perhaps implying the stimulus was not intense enough to
1585 induce inflammation in a cohort of well-trained cyclists. However, as both Allgrove et
1586 al., (2011) and Davison et al., (2012) used relatively low doses of CF (197.4 mg and
1587 246.8 mg respectively), a higher dose of both total flavanols and epicatechin is
1588 perhaps necessary to evoke the purported anti-inflammatory effects of CF (Ellinger &
1589 Stehle, 2016), in situations that induce an increase in inflammatory markers. These

1590 effects include the modulation of particular aspects of the inflammatory cascade, such
1591 as, inhibiting platelet aggregation (Murphy et al., 2003) and altering cytokine
1592 production *via* stimulation or inhibition of certain interleukins and growth factors (Selmi
1593 et al., (2006). Therefore, it is possible that for CF to confer anti-inflammatory benefits,
1594 the inflammation must be pronounced and/or prolonged. Furthermore, cycling
1595 exercise does not include a significant eccentric action; the type of contraction that is
1596 most associated with EIMD and as a result may not cause systemic inflammation to
1597 reach the same level of studies that involve eccentric biased exercise (Malm & Yu,
1598 2012).

1599 Currently, the only EIMD study with CF that measured inflammation was by Morgan
1600 et al., (2018), in this study no differences between treatment groups for IL-6 or CRP,
1601 following 100 maximal leg extensions with an elongated eccentric phase (three
1602 seconds). However, the researchers utilised a low dose (74 mg) of CF which is
1603 potentially why no effect was observed. The lack of studies showing robust changes
1604 in inflammation following exercise suggests that the anti-inflammatory effects of CF
1605 observed in *in vitro* studies may not translate to the *in vivo* environment. It is pertinent
1606 that future research investigates the impact of CF on markers of inflammation following
1607 EIMD (e.g., TNF- α), potentially including muscle biopsies to provide measured
1608 changes of inflammation in the muscle. It should be noted that the inflammatory
1609 process is necessary for skeletal muscle adaptation, and by blunting the initial pro-
1610 inflammatory phase, it is possible that the muscle regenerative phase can be impaired
1611 (Deng et al., 2012). Indeed, an adaptation to exercise is the increased activity of
1612 peroxisome proliferator-activated receptor γ co-activator 1 α , which may aid the
1613 phenotype switch of macrophages from pro- to anti-inflammatory and reduce the
1614 expression of genes associated with oxidative stress (Kang & Ji, 2012; Metsios, Moe,
1615 & Kitas, 2020). Therefore, forgoing an anti-inflammatory intervention may be effective
1616 when adaptations to exercise are the priority, akin to adaptations related to ROS and
1617 oxidative stress. However, the evidence that long term supplementation of CF,
1618 (poly)phenols, or other antioxidant supplements (e.g., Vitamin C and E) can inhibit
1619 training adaptations is equivocal (Beyer et al., 2017; Clifford, Jeffries, Stevenson, &
1620 Davies, 2020; Myburgh, 2014); as such, more research is warranted to better
1621 understand how these compounds may influence exercise adaptations.

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

CON:

Age 50 ± 2

Stature 175 ± 5

Mass 92.2 ± 9.7

$\dot{V}O_{2\max}$ 24 ± 1.7
 $\text{ml}\cdot\text{kg}^{-1}\text{ min}^{-1}$

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

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1627 3.4.3 Impact of Cocoa Flavanols on the Recovery of Muscle Function

1628 Muscle function is negatively impacted by EIMD, with reductions in muscle force and
1629 power capacity evident for several days following strenuous exercise. However, based
1630 on the current evidence it seems that CF supplementation has minimal, if not any,
1631 impact on MVC; as measured using peak torque with no effect observed on knee
1632 extensor strength recovery (de Carvalho et al., 2019; Morgan et al., 2018; Peschek et
1633 al., 2013). Currently, for (poly)phenols it has been suggested that >3 days of
1634 supplementation above 1000 mg may be required to observe an ergogenic benefit
1635 (Bowtell & Kelly, 2019), however no CF research has been performed fulfilling that
1636 criteria, highlighting a key area of research.

1637 It is noteworthy that only Morgan and colleagues (2018) observed notable muscle
1638 damage based on decrements in muscle function across groups (Paulsen et al., 2012).
1639 To best understand the mechanisms behind CFs role in muscle damage recovery, it
1640 would be prudent to ensure symptoms of EIMD such as a decrease in muscle function
1641 are pronounced. In fact, it is noteworthy that the participants in de Carvalho et al.,
1642 (2019) had fully recovered muscle function (based on peak torque data) 48 h post-
1643 exercise, indicating that the 100 drop-jump protocol did not elicit significant damage in
1644 a group of elite rugby players. Therefore, in populations with high baseline strength
1645 and power, protocols designed to induce EIMD need to be of a sufficient magnitude.
1646 Similarly, Peschek et al., (2013) observed 2-5% decrements in the control group and
1647 10-22% in the CF group from pre to 24 hr post, indicating that perhaps CF ingestion
1648 exacerbated muscle damage or only the CF group suffered the deleterious effects of
1649 the EIMD protocol. Interestingly, from 24 hr to 48 hr post-exercise the CF groups
1650 muscle function improved, whereas no changes occurred in the control group.
1651 Nevertheless, as the control group did not experience pronounced levels of muscle
1652 damage, it is possible that the protocol was not sufficient to adequately study the
1653 effects of CF on muscle function. Nevertheless, if the protocol is not representative of
1654 the training loads regularly experienced by those individuals, the functional relevance
1655 of investigating EIMD and CF supplementation becomes questionable. A further
1656 measure of muscle function used was vertical jump height, in which they found no
1657 significant differences between groups (de Carvalho et al., 2019).

1658 In contrast, Morgan et al., (2018) found that an acute dose of CF (74 mg) aided CMJ
1659 height recovery as participants returned to 95% of baseline at 48 hr in the CF group

1660 compared to 87% in the placebo group. However, in this study they consumed a much
1661 lower dose than used previously in the literature, especially the epicatechin content (8
1662 mg). Furthermore, the researchers utilised a unilateral EIMD protocol, yet the CMJ is
1663 a bilateral test, which could have influenced the findings. Instead, a more appropriate
1664 test could have been implemented, e.g., a single leg CMJ, as differences between
1665 participants' dominant and non-dominant legs may have been a confounding variable
1666 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

1677 3.4.4 Impact of Cocoa Flavanols on Perceived Soreness

1678 Measures of perceived soreness are predominantly subjective in nature, typically
1679 measured using validated scales to quantify subjective pain, soreness and discomfort
1680 such as a VAS (Hjermstad et al., 2011) or LEFS (Yeung, Wessel, Stratford, &
1681 Macdermid, 2009). As muscular soreness is ubiquitous with EIMD, most studies
1682 investigating muscle damage utilised these measures of perceived soreness as a way
1683 of tracking recovery (de Carvalho et al., 2019; Morgan et al., 2018; Peschek et al.,
1684 2013). Peschek et al., (2013) administered two doses of 350 mg CF post EIMD which
1685 were separated by two hours and found no effect of treatment on VAS or LEFS scores.
1686 Interestingly, the increase in soreness from baseline to 24 and 48 hr post was not
1687 significant. This suggests that the protocol used (downhill running at a -10% gradient
1688 for 30 min) may not have induced significant levels of muscle damage in a cohort of
1689 well-trained endurance athletes.

1690 Similarly, de Carvalho et al., (2019) did not find any interaction effect of the treatment
1691 following the EIMD protocol with only minor changes from baseline at 48 hr, even
1692 though this is when DOMS is known to peak (Kanda et al., 2013). Out of the three
1693 studies only Morgan et al., (2018) found a main effect of time on VAS scores following
1694 their respective protocols (100 knee extensions and 100 isokinetic hamstring curls
1695 respectively). Finding no significant difference between conditions; although as
1696 mentioned previously Morgan et al., (2018) used a small dose of 74 mg CF and a very
1697 low dose of 8 mg epicatechin. This amount is unlikely to exert any benefit as the
1698 required amounts to have a physiological influence are reported to begin around 400
1699 - 700 mg (Schroeter et al., 2006) and at an epicatechin intake of 50 mg (Ellinger et al.,
1700 2012).

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

<u>Reference</u>	<u>Participants</u>	<u>Nutritional Intervention</u>	<u>Supplementation period</u>	<u>Exercise stimulus</u>	<u>Measure(s)</u>	<u>Key outcome(s)</u>
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

1703 3.4.5 Impact of Cocoa Flavanols on Exercise Performance

1704 The impact that CF may have on performance is likely through the antioxidant potential
1705 of the cocoa and delayed ROS-induced fatigue. Patel et al., (2015) measured
1706 performance using maximal distance timed sprint trial, which was completed after 20
1707 min of cycling. It was found that 259 mg CF consumed daily for 14 days resulted in
1708 participants covering 17% more distance than baseline and 13% more distance than
1709 a white chocolate control. The mechanism for this increase may be due to CF
1710 decreasing ROS production and thereby attenuating fatigue (Allgrove et al., 2011;
1711 Fraga et al., 2005). An acute dosing strategy with higher flavanol products did not elicit
1712 any cycling performance benefit, only inducing slightly higher nitric oxide levels during
1713 exercise, which could aid muscle blood flow (Patel et al., 2020).

1714 Many sports have limited recovery time between competitions. For example, in field
1715 hockey tournaments, matches are often played 48 hr apart; similar recovery times are
1716 evident in soccer and handball. As a result, it may be pertinent to accelerate recovery
1717 and attenuate symptoms of EIMD in these sports (Julian, Page, & Harper, 2020). In
1718 one study, supplementation of CF (616 mg CF for 7 days) increased distance covered
1719 during the Yo-Yo Intermittent test 1 of 9.85% from baseline to 48 hr post a 100 drop
1720 jump EIMD protocol. Whereas the placebo group covered 5.8% less distance (de
1721 Carvalho et al., 2019). In this study CF may have reduced any potential oxidative
1722 stress that would be associated with training, exercise or the EIMD protocol, which
1723 may subsequently delay fatigue.

1724 Even though CF supplementation may improve distance covered in a set amount of
1725 time, it may not improve performance related to completing a set amount of work or
1726 distance in a time trial setting. Decroix et al., (2017), Decroix et al., (2018) and
1727 Stellingwerff et al., (2013) found no significant differences between groups (CF vs
1728 placebo) for time trial performance. However, Decroix and colleagues (2017) observed
1729 that in a crossover design the CF group tended to complete the first of the two time
1730 trials faster (29:47 min placebo vs 29:13 min cocoa), although statistical significance
1731 was not reached. It is difficult to ascertain whether the 34 s difference between groups
1732 is meaningful, as the trial involved participants completing a set amount of work
1733 equivalent to cycling at 75% peak power output for 30 min as fast as possible. As each
1734 time trial would have been individualised to each participant any practical conclusions
1735 are difficult to make other than that CF may have allowed participants to maintain a

1736 slightly higher power output than a placebo (Decroix et al., 2017). The CF group also
1737 produced a higher power output after 25 min (for the final ~5 min of the first time trial)
1738 compared to placebo (PLA 73.09% vs CF 76.75% of maximal power output). Decroix
1739 et al., (2018) found no differences for rating of perceived exertion, heart rate, lactate
1740 or work performed (kilojoules) within the 20-minute time trial between groups in
1741 normoxic or hypoxic environments. Interestingly, Stellingwerff et al., (2013) found that
1742 performance increased for seven participants following CF supplementation whereas
1743 another seven had improved performance following ingestion of the placebo. This may
1744 suggest that some individuals are potential 'non-responders' to CF supplementation,
1745 or that the differences seen were due to chance and not the allocated treatments.
1746 Other studies that investigated performance and CF supplementation found no
1747 significant differences between groups for 5 km time trial performance or $\dot{V}O_{2max}$
1748 (Fraga et al., 2005; Patel et al., 2020; Peschek et al., 2013). However, recent work by
1749 Sadler et al., (2020) suggests that 400 mg daily CF supplementation for seven days
1750 improves oxygen uptake during moderate-intensity exercise, but this benefit was not
1751 observed during high-intensity exercise. Additionally, after three-months of
1752 supplementing 175.2 mg/day of CF, Taub et al., (2016) observed an increase in
1753 participants' $\dot{V}O_{2max}$ by $2.8 \pm 1.2 \text{ ml kg}^{-1} \text{ min}^{-1}$ and power values (140.7 ± 11.6 to 148.3
1754 ± 11 watts), whereas there were no significant differences in the placebo group.

Table 3.6 The effect of CF supplementation on exercise performance

<u>Reference</u>	<u>Participants</u>	<u>Nutritional Intervention</u>	<u>Supplementation period</u>	<u>Exercise stimulus</u>	<u>Measure(s)</u>	<u>Key outcome(s)</u>
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	\leftrightarrow between groups CF group \uparrow 9.85% compared to baseline CON \downarrow 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) \leftrightarrow between groups. ii) PPO \uparrow after 25 min in the 1 st 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	\leftrightarrow 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)	Sub-chronic, 14 day consumption	Soccer training sessions twice per week and one match per week	$\dot{V}O_{2max}$ shuttle run	↔ between groups
		CON: 105 g cocoa butter chocolate, <5 mg CF				
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·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 (7 th 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

1757 3.4.6 Practical Recommendations and Future Research

1758 The available data suggests it may be beneficial to ingest a moderate dose of CF pre-
1759 exercise, with benefits effects on oxidative stress observed at doses ~200 mg acutely
1760 and if taken more longer term in the lead up to exercise. Higher doses of (poly)phenols
1761 may elicit greater physiological effects *in vivo* (Bowtell & Kelly, 2019) and for CF
1762 dosage the amount of epicatechin is an important factor when considering
1763 supplementation (≥ 50 mg).

1764 To maximise absorption and bioavailability, CF can be ingested as part of a beverage
1765 as opposed to a solid (e.g., high flavanol powder dissolved into a beverage instead of
1766 solid dark chocolate), potentially due to the faster gastric emptying associated with
1767 liquids (Cifuentes-Gomez et al., 2015). The bioavailability and absorption of flavanols
1768 can be further improved *via* the simultaneous consumption of carbohydrates, as
1769 consuming ~ 4 kcal·kg⁻¹ body mass alongside CF increases flavanol concentrations in
1770 the plasma by 40% (Badrie et al., 2015; Schramm et al., 2003). Carbohydrates
1771 stimulate and activate SGLT-1 and lactase phlorizin hydrolyase both of which are
1772 involved in flavanol absorption and metabolism (Bohn, 2014; D. D. Schramm et al.,
1773 2003). From a practical perspective, the consumption of CF concurrently with
1774 carbohydrates post-exercise may lead to the benefits of both replenishing glycogen
1775 stores and accelerating recovery following muscle damaging exercise.

1776 Future studies should look to investigate the muscle recovery process post EIMD
1777 alongside the supplementation of CF. A focus should be placed on whether regular
1778 (daily) supplementation of high doses of CF (>750 mg) can affect perceived soreness,
1779 oxidative stress, and inflammation post EIMD, and whether it can influence repeat
1780 performance, fatigue, and perceived effort. Comparisons between different doses and
1781 thus establishing of an optimal dose to elicit benefits is needed before concrete
1782 recommendations can be made. It is also important that studies investigating EIMD
1783 should use protocols that evoke sufficient muscle damage (e.g., inflammation, muscle
1784 soreness). Although, such protocols may not be applicable to real world sport, they
1785 will be useful for determining the potential mechanisms by which CF might alter
1786 physiology and enhance exercise performance and recovery. Nevertheless, studies
1787 should also investigate the effect of CF supplementation on recovery following real
1788 world exercise or movements that can induce muscle damage (e.g., repeated sprint
1789 protocols) instead of solely laboratory-based protocols that may not replicate the

1790 demands or damage response that follows sporting performances. This may lead to
1791 greater practical application within sport settings. Utilising both variants of EIMD
1792 protocol approaches will aid understanding of the potential ergogenic value of
1793 supplementing CF in an athlete's diet. Furthermore, females participants should be
1794 included in more research to better understand any inter-sex differences between
1795 males and females for muscle recovery following EIMD, within this review only three
1796 studies included females (Patel et al., 2020; Sadler et al., 2020; Taub et al., 2016)

1797 It may be pertinent to investigate prolonged flavanol supplementation on repeated
1798 bouts of exercise, with a focus on performance and recovery. Moreover, investigating
1799 the impact that CF may have on exercising muscle is required to develop greater
1800 understanding of the mechanisms in which CF exert any effects, such as their
1801 influence on endogenous antioxidant enzymes and survival signalling proteins.
1802 Indeed, future research should also look to further the knowledge of CF and their role
1803 in signalling pathways such as NF- κ B and Nrf2, and how the regulation of these
1804 pathways may attenuate muscle damage.

1805 3.5. Conclusion

1806 Few studies have examined the effects of CF on recovery following EIMD. Of the
1807 available data acute and sub-chronic (~7-14 day) supplementation of CF *via* dark
1808 chocolate solids or in the form of a high flavanol beverage reduces exercise-induced
1809 oxidative stress and has potential for delaying fatigue during exercise allowing for
1810 prolonged performance. However, data on recovery of muscle function, and the
1811 analgesic and anti-inflammatory effects of CF is limited. Research should look to
1812 investigate these areas further to identify if CF are viable as an ergogenic aid used for
1813 recovery and potentially performance.

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Chapter 4 General methods

1818 This Chapter will enumerate the methods utilised in the studies that form the basis of
1819 this PhD thesis. It will cover the participants and recruitment strategies employed, as
1820 well as dietary assessment and nutritional intervention, muscle damage protocol and
1821 measures used to measure recovery. Before any recruitment began, institutional
1822 ethical approval was obtained. Following this, participants were informed of the study
1823 protocols and provided written consent before any testing was performed.

1824 4.1 Participants

1825 Prior to each study a power calculation was performed using G*Power (Version
1826 3.1.9.7, Universität Dusseldorf, Germany; (Faul, Erdfelder, Buchner, & Lang, 2009))
1827 using data from previous research (Bowtell et al., 2011) in an attempt to allow for
1828 discernible conclusions that are demonstrable based on the findings. Each
1829 investigation utilised healthy, male, and female University students between 18 and
1830 38 years old. For the studies in Chapters 5, 6, and 7, the participants had to be
1831 recreationally active, this was defined as performing regular physical activity or
1832 exercise at least two days a week, e.g., running or resistance training.

1833 Participants were recruited in various ways, these included: posters, recruitment talks
1834 within lectures or seminars, and *via* email. To be eligible to take part in the studies,
1835 further inclusion criteria existed as follows: *i*) no lower limb injury within the previous
1836 six months, *ii*) the individual did not perform regular (>5 days a week) heavy resistance
1837 training, *iii*) was not currently taking anti-inflammatory or pain medication, *iv*) had no
1838 other health contraindications that would prevent them from performing exercise, and
1839 for females specifically *v*) was not pregnant. During involvement in a study, the
1840 participant was required to refrain from exercise (including 48 hr before a laboratory
1841 visit), pain medication, nutritional supplements (e.g., Vitamin C), recovery treatments
1842 (e.g., massage) and avoid taking anti-inflammatory drugs.

1843 Randomisation was performed using a stratified randomisation procedure based on
1844 baseline MVIC values, assigning participants into 'strong' or 'not strong' strata, before
1845 randomisation into a group. To determine the thresholds for the strata, a normative
1846 MVIC strength index was consulted [Risberg et al., (2018) for females and Ruas,
1847 Minozzo, Pinto, Brown, and Pinto (2015) for males]. Following this, participants were
1848 allocated into groups using online randomisation software (random.org). For Chapters
1849 5 this was performed by the thesis author and for Chapter 6 this was performed by an

1850 independent laboratory technician and the thesis author remained unaware of
1851 assignment until data analysis.

1852 4.2 Dietary control and analysis

1853 Throughout each of the exercise studies (Chapters 5, 6, and 7) participants completed
1854 a 24-hr dietary recall with a trained researcher at the end of every laboratory visit. A
1855 dietary recall was chosen to reduce the participant burden that comes with other
1856 alternative methods, for example a weighed food diary (Louise M Burke, 2015). A
1857 single pass method was selected alongside a full review of the completed recall during
1858 each day of testing to ensure the participant had not forgotten any foods/drinks. To
1859 further accuracy, time and detail of the meal was taken to 'walk participants through
1860 their day' during the review. This method is not without any limitations as it relies on
1861 participant memory and food knowledge, as well as having an increased risk of
1862 selection bias when recalling foods. For the duration of the studies, participants were
1863 asked to consume their typical diet whilst refraining from food and drinks high in
1864 (poly)phenols. These included: chocolate, various berries, tea and green tea, red wine,
1865 cherries, and lychees. Participants were provided a list of foods to be excluded during
1866 the time frame of the study. This was to limit any confounding effects of other dietary
1867 (poly)phenols on muscle recovery. This method of tracking dietary information is not
1868 without limitations and participant diets could not be controlled entirely. Indeed, on one
1869 occasion a participant within Chapter 5 consumed coffee on one of the testing days
1870 and were reminded to refrain from breaching protocol again.

1871 Participant diets were analysed for macronutrient content and compared between
1872 groups to examine any potential differences in energy, carbohydrate, protein, or fat
1873 intake throughout each study. This was to ensure that the groups did not differ
1874 significantly, therefore not strongly influencing the outcome of the studies. As such,
1875 potential conclusions inferred from the data could be made with the knowledge that
1876 dietary intake was not a confounding influence. Dietary analysis was performed using
1877 online nutritional analysis software called Nutrimen (Dark Green Media Ltd, ©2016).

1878 4.3 Cocoa flavanol intervention

1879 The nutritional intervention that forms the basis of this thesis involved CF. The cocoa
1880 powder used was commercially available known as Chococru Extraordinary Flavanol
1881 Cocoa (Chococru© Extraordinary Flavanol Cocoa), containing ~8.3% flavanols and a
1882 total (poly)phenol content of ~12% (unpublished data from Chococru©). All drinks

1883 involved in this thesis contained a base amount of 25 g whey protein and 60 g
 1884 maltodextrin (both Myprotein, Manchester, UK) and water. As the control beverage
 1885 contained whey that was chocolate flavoured (Chocolate Smooth, Impact Whey
 1886 Protein Concentrate, Myprotein) it maintained both a similar taste and appearance to
 1887 the test beverages. It must be noted that due to the contents of the control it cannot
 1888 be referred to as a placebo, however, it does allow the drinks to represent a more
 1889 realistic recovery drink from an athletic scenario (Burke, 1997). Furthermore, the
 1890 results of the studies in Chapters 5, 6, and 7 provide an insight as to whether CF
 1891 provide additional recovery benefits to a standard exercise recovery drink. The control
 1892 used throughout this thesis contained whey protein, maltodextrin, and water. It should
 1893 be noted that the products used within this thesis are not Informed Sport tested and
 1894 therefore, may not be appropriate for athletes who are tested for banned substances.

1895 4.3.1 Cocoa flavanol intervention in Chapters 5

1896 In Chapter 5, two flavanol drinks were used with the only difference being 5g of cocoa
 1897 powder (drink 2 contained 10g and drink 3 contained 15g; see Table 4.1 for a
 1898 nutritional breakdown of the beverages). Drink 1 indicates control. As these Chapters
 1899 were single-blind none of the participants were aware of the group they were assigned
 1900 to and only informed that they would receive a chocolate flavoured beverage post-
 1901 exercise. Furthermore, due to the independent groups design of the studies, each
 1902 participant only received one of the beverages and therefore, had no frame of
 1903 reference as to which they may have received.

1904 4.3.2 Cocoa flavanol intervention in Chapter 6

1905 For Chapter 6, the study was double blinded and as such the bottles containing the
 1906 ingredients to each drink was wrapped in opaque duct tape and powder mixed
 1907 together to prevent the contents being easily identified. An independent laboratory
 1908 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

1909

1910 4.4 Muscle damage protocol

1911 To induce muscle damage in Chapter 5 and 6, a validated protocol was adapted from
1912 previous research (White et al., 2008). The protocol targeted the knee flexors
1913 (hamstrings) muscle group to induce EIMD. The protocol consisted of five sets of ten
1914 maximal concentric and eccentric unilateral hamstring curls, repeated on each leg,
1915 using a HUMAC Cybex Norm isokinetic dynamometer (CSMi, Boston,
1916 Massachusetts). Participants were seated at a hip angle of 85 degrees, then secured
1917 into the dynamometer using a torso seatbelt and thigh strap to limit any hip
1918 involvement during the contraction. The lateral femoral condyle was positioned parallel
1919 to the dynamometer's centre axis of rotation. Following this, the rotational arm was
1920 strapped to the ankle of the working limb roughly one inch above the lateral malleolus.
1921 The participant then actively extended and flexed the knee to demonstrate an
1922 appropriate alignment of the dynamometer. This is an important step when assessing
1923 knee flexion as it has been shown that a deviation, whether vertically or horizontally,
1924 from appropriate alignment can impact peak torque output leading to an error of
1925 measurement (Houweling & Hamzeh, 2010). All measurements were noted on a
1926 participants' first visit to the laboratory so that the position could be replicated on each
1927 day of testing.

1928 In an attempt to familiarise a participant with the protocol and the uniqueness of an
1929 eccentric contraction on the dynamometer, each individual performed a set of 15
1930 concentric eccentric hamstring curls at a self-perceived low effort level. This is
1931 considered an adequate method for familiarising an individual to the protocol
1932 (Impellizzeri, Bizzini, Rampinini, Cereda, & Maffiuletti, 2008). To warm up, each
1933 participant performed a series of dynamic hamstring stretches and a sub-maximal
1934 warm up set of ten concentric eccentric hamstring curls. During the protocol
1935 participants were verbally encouraged during each repetition, as well as being
1936 reminded to maintain maximum effort throughout. To monitor effort levels, participants
1937 were asked to rate their perceived level of exertion using a Borg 6-20 scale (Borg,
1938 1982), following individual sets. To reduce the chance of an order effect influencing

1939 results, participants were asked to rate their exertion spontaneously and not following
1940 the completion of each set.

1941 4.5 Measuring muscle function

1942 To measure changes in muscle function, MVIC of the non-dominant leg were used in
1943 Chapters 5 and 6. To measure MVIC of the knee flexors the same isokinetic
1944 dynamometer (CSMi, Boston, Massachusetts) was utilised. Participants took up a
1945 seated position and the same procedure discussed in Section 4.4 for aligning the
1946 dynamometer was appropriately followed.

1947 Participants then set their full range of motion from full knee extension (anatomical
1948 zero) to individual full knee flexion, commonly between 95 to 115 degrees. MVIC was
1949 performed at two separate joint angles, 60 degrees and 30 degrees knee flexion; 0
1950 degrees being anatomical zero. The multiple joint angles were chosen for two reasons;
1951 *i)* the hamstrings individual activation varies at different angles, biceps femoris is more
1952 effective at decreased angles (e.g., 60-90) whereas semitendinosus and
1953 semimembranosus have optimum angle ranges between 40-30 degrees and *ii)*
1954 selecting only one angle could potentially over or under-estimate changes in peak
1955 torque (Paulsen et al., 2012). Therefore, incorporating multiple angles may allow for a
1956 greater insight into the functional changes of the hamstrings. Each angle for MVIC
1957 involved three, five second contractions with each being separated by 30 seconds
1958 rest, totalling six MVICs. Participants were instructed to 'pull' as hard as they could
1959 once instructed to begin. Verbal encouragement was provided during each contraction
1960 to aid with maximal exertion and was consistent between participants. Only the peak
1961 torque values were used for data analysis.

1962 4.6 Muscle activation

1963 Surface electromyography (EMG) was utilised within this thesis to measure hamstring
1964 muscle activation and recorded using wireless surface EMG sensors (Inter-electrode
1965 distance 10mm; Trigno™, Delsys Inc, USA) The biceps femoris long head was
1966 selected for data analysis, both semitendinosus and semimembranosus were omitted.
1967 This was due to the location of the muscles and potential error regarding surface EMG
1968 placement on the individual muscles increasing the chance of crosstalk and as a
1969 result, measurement error. To identify the biceps femoris muscle a participant lay
1970 prone on a plinth, with a researcher then locating the distal portion of the muscle
1971 tendon connecting to the proximal aspect of the fibula. From there the researcher

1972 began to raise the lower leg of the participant, following the tendon *via* lateral palpation
1973 towards the ischial tuberosity and eventually stopping at the muscle belly, with the
1974 participant actively flexing the hamstrings to aid with identification. This was followed
1975 by continued lateral palpation as the participant lowered the limb slowly and the
1976 placement of the EMG device. To aid with repeat identical placement on subsequent
1977 testing days a semi-permanent marker was used to outline the EMG device, this
1978 outline remained visible on subsequent visits and reapplied if beginning to fade. To
1979 prepare for EMG device placement the following was performed: shaving foam was
1980 applied to the posterior of the thigh and a moderate portion, roughly 10cm², of hair
1981 was shaved from the back of the participants leg (if required). The area was then
1982 cleaned with an alcohol wipe to remove any debris and sanitise the site. This was to
1983 reduce any potential noise that hair or debris could elicit and interfere with data
1984 collection. The EMG device was then attached to the muscle belly using specialist
1985 sticky tape made specifically for the devices provided by Delsys Inc.

1986 The exercise task selected for the measurement of muscle activation was a glute-
1987 hamstring bridge beginning at a knee angle of 60 degrees. The exercise involves the
1988 participant extending at the hips to raise them off the floor, creating a diagonal line
1989 from the knees, hips, and shoulders and were instructed to maintain this peak position
1990 for five seconds, timed independently by a laboratory technician. EMG data collection
1991 began two seconds before contraction and ceased two seconds after allowing for an
1992 obvious beginning and end for data analysis. Participants performed this three times
1993 each testing session during Chapter 6.

1994 Data analysis for EMG consisted of muscle activation and median frequency data. To
1995 perform this analysis EMGworks analysis software (EMGworks®, Version 4.7.9,
1996 Delsys, USA) was utilised, following the methods used by (Starbuck & Eston, 2012).
1997 In brief, each individual EMG graph was first filtered *via* a band pass filter set at 12-
1998 450 Hz and applied to the raw data, following this the data was rectified using root
1999 mean squared with a 0.1 s time constant. The peak and mean values were recorded
2000 for each exercise bout. Additionally, after filtering each graph was assessed for median
2001 frequency using a 0.1 s time constant. The median frequency and peak median
2002 frequency were recorded.

2003 4.7 Measuring muscle soreness

2004 To measure changes in muscle soreness a VAS and LEFS were implemented in
2005 Chapters 5 and 6. The VAS utilised within this thesis was a 200mm ruled line with
2006 three anchor points across it. The far left at 0mm had the anchor point 'no pain', at
2007 100mm the anchor point 'moderate pain' was included and on the far right, at 200mm,
2008 the anchor point 'extreme pain'. The inclusion of a mid-point for pain identification and
2009 the avoidance of absolute anchor points such as 'worst possible pain' have recently
2010 been recommended for research that involves the measurement of pain using VAS
2011 (Reed & Van Nostran, 2014). When making a mark on the line participants were
2012 instructed to contract the hamstrings by flexing at the knee, raising their foot off the
2013 floor towards the hips. This allowed participants to better judge the soreness in the
2014 hamstrings, otherwise they remain in a passive state when standing stationary and
2015 may underestimate soreness. Furthermore, the use of three anchor points allowed for
2016 a more considered response regarding subjective pain.

2017 The LEFS involved 20 hypothetical activities that range from everyday activities, e.g.,
2018 rolling over in bed, to more athletic tasks such as making sharp changes of direction.
2019 Each activity is rated from 0 to 4 with the following ratings: 0 = extreme difficulty or
2020 unable to perform the activity, 1 = quite a bit of difficulty 2 = moderate difficulty, 3 = a
2021 little bit of difficulty and 4 = no difficulty. The use of these two methods allowed for a
2022 greater insight into the soreness of the participants involved in the study, as discussed
2023 in Chapter 2 Section 3.2. A score of 80 indicates there is no issue regarding perceived
2024 muscle function or muscular discomfort, whereas reductions from 80 indicate a decline
2025 in perceived muscle function or increase in muscular discomfort.

2026 4.8 Assessing menstrual cycle

2027 The assessment of the menstrual cycle is an important aspect to consider for exercise
2028 research. Testing for females was carried out during the luteal phase in participants
2029 who followed the common menstrual cycle hormonal phases. The luteal phase was
2030 selected for various reasons: *i)* it is the longest phase within the cycle, *ii)* the phase is
2031 similarly constant in length amongst women and *iii)* to avoid the peak in oestrogen
2032 observed pre-ovulation (Reed & Carr, 2018). To identify this timepoint each female
2033 participant completed a menstrual cycle questionnaire, using this data a prediction
2034 could be made to schedule testing to coincide with the mid-luteal phase for the
2035 individual participant. To estimate the mid luteal phase, eight days were added on to

2036 day 10 from the beginning of each participant's menstrual cycle (Wideman et al.,
2037 2013). This method is easily implemented and has a relatively high level of accuracy,
2038 however, in an ideal setting hormonal testing would have been carried to track the
2039 phase of each individual participant due to its greater precision (Wideman et al., 2013).
2040 For individuals on different contraceptives that disrupt the natural hormonal profile of
2041 the menstrual cycle an equivalent time point was selected. For example, a participant
2042 supplementing the combined pill would take part during the 21 days of
2043 supplementation to avoid the drop off in exogenous hormones that occurs following
2044 cessation of the pill for six days.

2045

2046 4.9 Statistical Analysis Approach

2047

2048 For statistical analysis, an *a priori* decision was made on the statistical tests that would
2049 be selected for the prospective data of the studies and the structure of the data that
2050 would be collected. To this end, repeated measures designs were the most
2051 appropriate using mixed analysis of variance to assess for within and between subject
2052 differences across multiple time points. Once collected, all data was assessed for
2053 normality to determine whether parametric or nonparametric analysis would be
2054 performed. For post hoc analysis Fisher's least significant difference was selected to
2055 locate the differences in the event of a significant time and interaction effect.
2056 Additionally, an *a priori* power analysis was carried out for both studies to determine
2057 the appropriate sample size for both Chapters 5 and 6, see Chapter 5 Section 2.8 and
2058 Chapter 6 Section 2.10 for more details.

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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

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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.

2075 5.1. Introduction

2076 Eccentric muscle contractions are typically responsible for the muscular disruption that
2077 leads to EIMD (Nikolaidis et al., 2007). Therefore, resistance training and intermittent
2078 high-intensity exercise often evoke EIMD (Owens, Twist, Cobley, Howatson, & Close,
2079 2019). Consequences of EIMD include inflammation and oxidative stress (Kanda et
2080 al., 2013), impaired force generating capacity (Twist & Eston, 2009), and increased
2081 muscle soreness (Impellizzeri et al., 2008). Optimising the time course of recovery is
2082 now a priority in modern sport, mainly due to the rapid turnaround of competitions and
2083 fixtures. Contemporary examples include tennis players performing every other day at
2084 major championships and congested fixture periods in soccer when players perform
2085 two 90 min matches within three days. Notably, injury-risk and muscular fatigue may
2086 be increased during congested fixture periods in soccer, namely due to the insufficient
2087 recovery time between matches (Ekstrand, Hägglund, & Waldén, 2011; Page et al.,
2088 2019). Therefore, the aim of recovery is to restore normative values for an individual
2089 following exercise by reducing neuromuscular fatigue, soreness and restoring
2090 contractile functional capacity. To reduce fatigue and facilitate recovery, high
2091 carbohydrate protein meals or beverages, as well as high (poly)phenolic foodstuffs
2092 (e.g., cocoa) have become a common feature of an athlete's diet (Knapik et al., 2016).

2093 Flavonoids are the largest group of dietary (poly)phenols and the most common
2094 source of antioxidants within the diet (Scalbert et al., 2005). In recent years, a subclass
2095 of flavonoids, known as flavanols, such as catechin and epicatechin, have attracted
2096 much attention as health promoting nutrients. Sources of flavanols include lychees,
2097 apples, teas, broad beans and cocoa (Williamson, 2017). Cocoa has the highest
2098 proportion of flavanols per serving than any other natural source (Lee, Kim, Lee, &
2099 Lee, 2003). Previous research has focused on the effects of CF on the cardiovascular
2100 system, with evidence suggesting CF intake can reduce endothelial dysfunction by
2101 improving flow mediated dilation (Hooper et al., 2012) and reducing blood pressure
2102 (Buitrago-Lopez et al., 2011). Furthermore, CF have been shown to enhance
2103 endogenous antioxidant capacity (Mauro Serafini & Peluso, 2016), limit oxidative
2104 stress (Allgrove et al., 2011), and influence the inflammatory process by reducing both
2105 platelet aggregation and the stimulation of neutrophils (Ellinger & Stehle, 2016).

2106 Regarding muscle recovery and exercise, research has shown that acute (single dose
2107 on day of exercise stimulus) and sub-chronic (regular intake for ≥ 14 days) CF

2108 supplementation of ≥ 200 mg reduces exercise-induced oxidative stress (Allgrove et
2109 al., 2011; Davison et al., 2012), see Chapter 4 Section 4.1 for more details.
2110 Furthermore, in relation to exercise, the ingestion of CF may improve sprint
2111 performance by potentially preventing ROS-increased calcium sensitivity of
2112 myofilaments within working muscles, therefore, delaying fatigue (de Carvalho et al.,
2113 2019; Patel et al., 2015). However, evidence is lacking regarding the impact of CF on
2114 markers of muscle recovery, such as perturbations in muscle function and an increase
2115 of perceived soreness (see Chapter 4 Sections 4.3 and 4.4). One such study used a
2116 CF dose too low to be effective - 74 mg CF and 8 mg epicatechin (Morgan et al., 2018).
2117 Benefits begin to be observed at doses of ~ 700 mg CF; and more importantly, with
2118 > 50 mg epicatechin, the most biologically active flavanol (Schroeter et al., 2006).
2119 However, an optimal dose is not yet known in addition to any potential dose response.
2120 Furthermore, previous research that investigated the impact of CF on muscle recovery
2121 did not induce notable muscle damage using a drop jumps protocol (de Carvalho et
2122 al., 2019) and a downhill running protocol (Peschek, Pritchett, Bergman, & Pritchett,
2123 2013). This can be defined as reductions in muscle force-generating capability of
2124 $\geq 20\%$ following EIMD (Paulsen et al., 2012). Therefore, making conclusions about the
2125 impact of CF on markers of muscle damage is difficult, indicating that more research
2126 is warranted. Furthermore, none of the previous studies involved female participants,
2127 likely due to the purported protective effects of oestrogen against muscle damage
2128 (Tiidus, 2003) and physiological variations across the menstrual cycle (Hayashida,
2129 Shimura, Sugama, Kanda, & Suzuki, 2016). Therefore, investigating the effect of CF
2130 supplementation on muscle recovery in females is required.

2131 Females experience a menstrual cycle leading to hormonal fluctuations over the
2132 course of ~ 28 days, split into the follicular phase and the luteal phase. The follicular
2133 phase can be further divided into the early follicular (onset of menses) which is known
2134 to have low levels of oestrogen and progesterone, and late follicular phase (following
2135 menses until ovulation), which is known for a rapid rise and peak in oestrogen
2136 concentrations and continued low levels of progesterone. The luteal phase begins
2137 post-ovulation and lasts until the onset of the subsequent cycle, this phase is known
2138 for a secondary peak in oestrogen around day 20 (day one is considered the first day
2139 of menstruation) and a rise in progesterone also, these concentrations are relatively
2140 consistent until menses (Mihm et al., 2011).

2141 Oestrogen is known to have an anabolic effect on skeletal muscle (Enns & Tiidus,
2142 2010), aid with the maintenance of muscle function (Kitajima & Ono, 2016), have a
2143 neuroexcitatory effect which may lead to increased contractile capability (Ansdell,
2144 Brownstein, Škarabot, Hicks, Simoes, et al., 2019), influence substrate utilisation
2145 (Lundsgaard & Kiens, 2014) and may have a role in affecting mood state (Birkhaeuser,
2146 2018). In the context of exercise recovery oestrogen can act as an antioxidant and aid
2147 with the stabilisation of muscle membranes (Kendall & Eston, 2002) potentially
2148 reducing the impact that ROS may have and as such limiting the level of lipid
2149 peroxidation. This is likely *via* the presence of a free phenolic group on the molecule,
2150 providing oestrogen the capacity to quench ROS (Chainy & Sahoo, 2020). The
2151 stabilisation of muscle membranes may also lead to a reduction in the leakage of
2152 intracellular proteins following the mechanical stress to the muscle fibres and as such
2153 may limit the inflammatory response post-exercise (Enns & Tiidus, 2010).

2154 Therefore, the aims of this study were twofold; *i*) to investigate the impact of an acute
2155 dose of CF on indices of muscle recovery *ii*) to compare two different doses of CF on
2156 indices of muscle recovery. The hypothesis for this study was that EIMD might be
2157 attenuated following acute consumption of CF, with the highest dose offering the most
2158 benefit.

2159

2160 5.2. Methods

2161 Main overview of methods is contained within Chapter 4, as such this section will
2162 report methods in brief.

2163 5.2.1 Participants

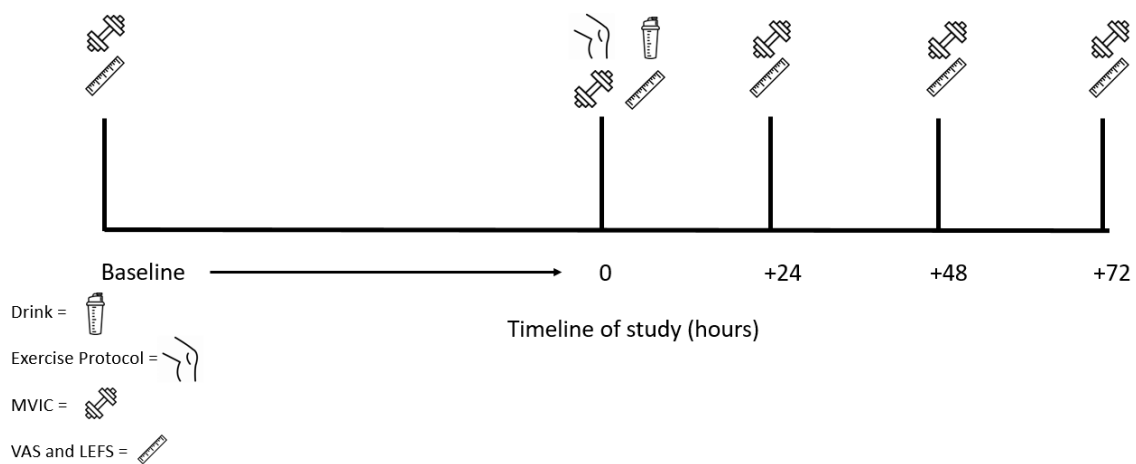
2164 Following institutional ethical approval and in agreement with the Declaration of
2165 Helsinki, 30 participants consented to take part between the months of April 2019 to
2166 October 2019; however, only 23 completed the study (13 females, 10 males) due to
2167 the following reasons: two due to injury and five due to unforeseen circumstances
2168 following baseline testing. An *a priori* power calculation determined that a sample size
2169 of 21 was required for 80% power and to detect significance, based on the effect size
2170 from previous research regarding MVIC recovery at 48 hr (Bowtell et al., 2011).
2171 Baseline testing involved MVIC of the knee flexors to assess muscle function and
2172 measures of perceived muscle soreness using a VAS and LEFS. All participants were
2173 classed as recreationally active and injury free for the previous six-months (both
2174 informed *via* self-report) and were not taking any dietary supplements (e.g., Vitamin
2175 C, glutamine, or branched-chain amino acids). Participants were asked to avoid anti-
2176 inflammatory medications and resistance training during participation. An adapted
2177 menstrual cycle questionnaire (Brown, 2017) was completed by the female
2178 participants involved to reliably estimate cycle phase. The luteal phase was selected
2179 for testing or an equivalent period for participants who were on hormonal
2180 contraception, as to avoid peak oestrogen concentrations observed during the late
2181 follicular phase (Brown, 2017). Participants completed each day at the same time of
2182 original participation, ± 2 hr, to account for diurnal influence.

2183 5.2.2 Study Design

2184 The study was a laboratory-based, randomised, single-blind, nutrient-controlled trial.
2185 Participants were randomised into a control (CON), high (CF₈₃₀ = 830 mg CF) or supra
2186 (CF₁₂₄₅ = 1245 mg CF) group and remained unaware of their allocation for the entirety
2187 of the study. Participants were required to visit the laboratory for five days; the first
2188 visit was to conduct baseline testing and familiarisation of the EIMD protocol (ten sub-
2189 maximal concentric-eccentric hamstring curls). The remaining four days took place
2190 consecutively; as such, measures were taken in the following order: baseline,
2191 immediately post-EIMD (0 hr), 24, 48 and 72 hr post-EIMD. For a visual representation
2192 of the study design, see Figure 5.1. For randomisation, participants were assigned to

2193 separate strata, 'strong' and 'not strong', based on their baseline MVIC values and
 2194 randomised into matched and counterbalanced groups (using random.org). To decide
 2195 what could be classified as strong or not, a normative MVIC strength index was used
 2196 [Risberg et al., (2018) for females and Ruas et al., (2015) for males]. Following this,
 2197 eight participants were allocated to the control group (four females, four males), eight
 2198 to the CF₈₃₀ group (five females, three males), and seven to the CF₁₂₄₅ group (four
 2199 females, three males). For participant characteristics see Table 5.1. Participants were
 2200 also compared as separate groups based on sex, creating two subgroups within each
 2201 treatment group. For sex specific participant characteristics see Table 5.2.

2202



2204 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.

2205

2206

2207

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

2208

2209

2210 5.2.3 Muscle Function

2211 Values were recorded for knee flexor MVIC using the isokinetic dynamometer (Cybex
 2212 NORM®, Model 770, CA, USA), providing a reliable quantification of decrements in
 2213 muscle function for assessing EIMD (Warren, Lowe, & Armstrong, 1999). Knee angles
 2214 of 60° (MVIC60) and 30° (MVIC30) of the anatomical zero (full knee flexion) were
 2215 selected due to the differences in muscle activation at various knee angles; biceps
 2216 femoris has increased activation at reduced angles, whilst semitendinosus and
 2217 semimembranosus at greater knee angles (Onishi et al., 2002). For more detail see
 2218 Chapter 4 Section 5.

2219 5.2.4 Subjective Soreness

2220 Soreness was recorded using a 200 mm VAS, which has been previously included as
 2221 a validated measure of subjective soreness (Peschek et al., 2013). The LEFS is a
 2222 validated questionnaire which quantifies an individuals perceived level of muscle
 2223 function using 20 hypothetical activities that are scored from 0 to 4; 0 = extreme
 2224 difficulty; 4 = no difficulty. For more detail see Chapter 4 Section 7

2225 5.2.5 Muscle Damaging Protocol

2226 The exercise protocol used to induce muscle damage was adapted from White et al.,
 2227 (2008) using the Cybex Norm Isokinetic Dynamometer (CSMi, Boston,
 2228 Massachusetts). For more detail see Chapter 4 Section 4.

2229 5.2.6 Nutritional Intervention

2230 Participants were blinded to which group they were assigned, with only the lead
 2231 researcher being aware of the contents of each drink. Participants consumed their

2232 assigned beverage within five minutes following the protocol. Each beverage
2233 consisted of 300 ml water, 60 g maltodextrin and 25 g whey protein powder (20 g
2234 protein). The cocoa powder used was a commercially available high flavanol powder
2235 (Chococru© Extraordinary Flavanol Cocoa), containing ~8.3% flavanols and a total
2236 (poly)phenol content of ~12% (unpublished data from Chococru©). The beverage for
2237 CF₈₃₀ included an additional 10 g of Chococru© cocoa powder which contained 830
2238 mg CF (98.6 mg epicatechin) and for CF₁₂₄₅ 15 g of Chococru© cocoa powder was
2239 added, containing 1245 mg CF (149.4 mg epicatechin). See Chapter 4 Section 3
2240 (Table 4.1) for nutritional breakdown of the test beverages.

2241 5.2.7 Dietary Measures

2242 Participants completed a 24-hr dietary recall each day of testing, totalling five food
2243 recalls, and were asked to continue eating their usual diet throughout testing. During
2244 baseline testing, participants were provided a list of high (poly)phenolic food and drink
2245 (cherries, blueberries, dark chocolate, green and black tea, wine, apples, lychees,
2246 pomegranates and fruit juices) to refrain from consuming three days before and during
2247 the testing period, reducing the confounding influence of other dietary (poly)phenols
2248 on recovery (Scalbert et al., 2005). Dietary analysis was carried out using Nutrimen
2249 (Dark Green Media Ltd, ©2016). For more detail see Chapter 4 Section 2.

2250 5.2.8 Statistical analyses

2251 Statistical analysis was performed using IBM SPSS Statistics (version 24, IBM Corp.,
2252 Armonk, N.Y., USA). All data was assessed for normality using a Shapiro-Wilk test
2253 and quantile-quantile plots were examined to establish whether the data was normally
2254 distributed. A Greenhouse-Geisser correction was used if sphericity was violated. A
2255 mixed analysis of variance was used to determine interaction and time effects for the
2256 recovery variables. Furthermore, sub-group analysis of intra and inter-sex differences
2257 were performed for Chapter 5b. If any significance was observed, Fisher LSD post hoc
2258 testing was performed to identify the point of significance. Data for MVIC60 and
2259 MVIC30 was calculated as percentage change from baseline alongside absolute
2260 means. To calculate effect sizes, Cohen's *d* (*d*) was utilised, with the magnitude of
2261 effects considered small (0.2), moderate (0.5) and large (0.8). Significance was set at
2262 $p \leq .05$ pre-analysis. Descriptive statistics are reported as means (MVIC also displayed
2263 as percentage change %) \pm standard deviation (SD).

2264

2265 **5a.3. Results**

2266 There were no significant differences for participant characteristics; height ($p = .33$),
 2267 weight ($p = .46$) and age ($p = .88$) or dietary intake; energy ($p = .98$), protein ($p = .99$),
 2268 CHO ($p = .60$), or fat ($p = .57$) between groups. See Table 5.3 for dietary intake.

Table 5.3. Dietary intake between groups

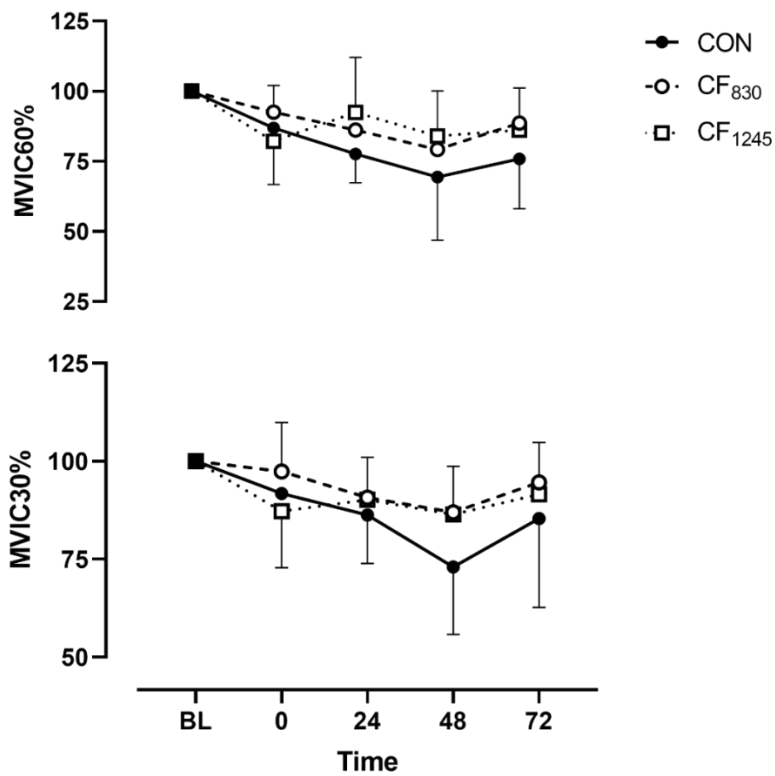
	CON	CF ₈₃₀	CF ₁₂₄₅
Energy (kcal)	2137 ± 559	2101 ± 394	2164 ± 591
Protein (g)	109 ± 49	106 ± 47	106 ± 43
CHO (g)	227 ± 46	253 ± 41	265 ± 106
Fat (g)	93 ± 32	81 ± 19	79 ± 21

Note: Group mean ± SD

2269

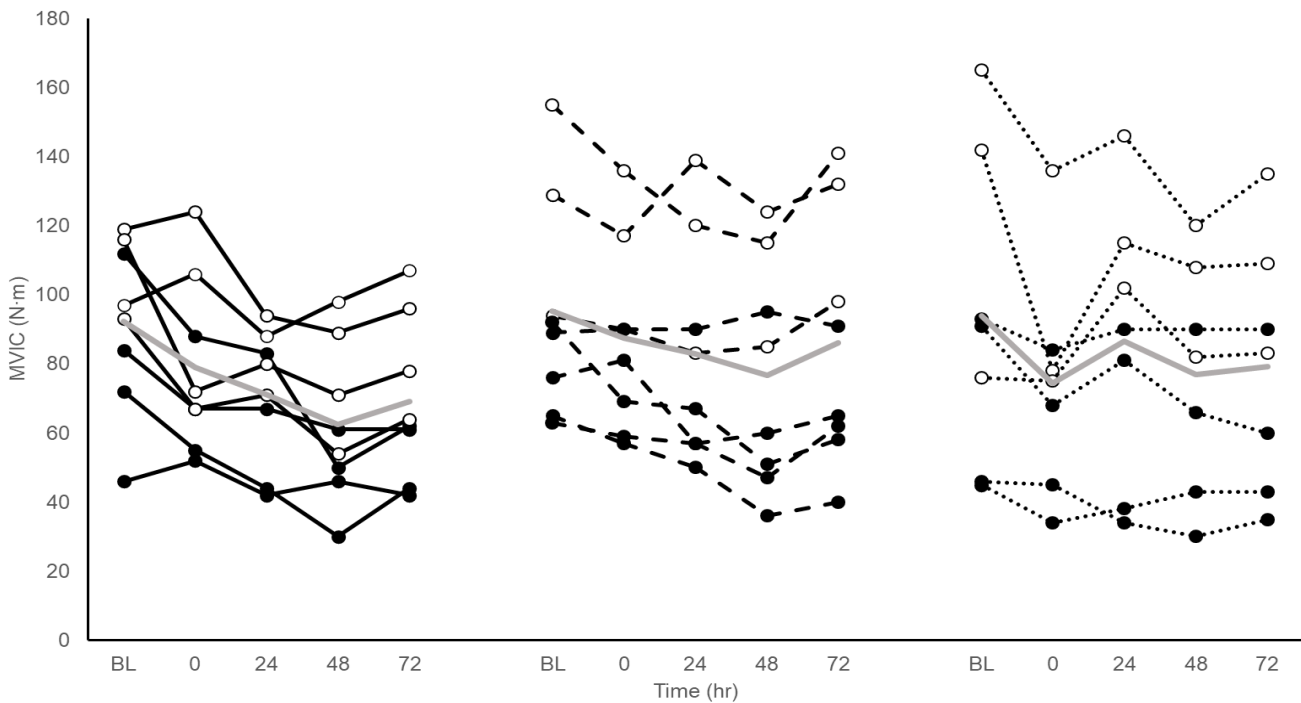
2270 **5a.3.1 Muscle function**

2271 Muscle function measured using MVIC at 60° and 30° found a main effect of time for
 2272 MVIC60 ($p = .002$) and MVIC30 ($p = .002$), both data were normally distributed. For
 2273 MVIC60 significant differences between baseline and 0, 24, 48, 72 hr ($p \leq .001$), 0 and
 2274 48 ($p = .03$), 24 and 48 ($p = .002$), and 48 and 72 ($p \leq .001$) were observed. For
 2275 MVIC30 significant differences between baseline and 0, 24, 48, 72 hr ($p \leq .04$), 0 and
 2276 48 ($p = .01$), 24 and 48 ($p = .01$), and 48 and 72 ($p = .001$). There were no significant
 2277 differences between groups for knee flexor peak torque at MVIC60 ($p = .99$) or MVIC30
 2278 ($p = .95$) at baseline. Following the exercise protocol, overall mean knee flexor peak
 2279 torque reduced to 79% of baseline. There were no significant differences between
 2280 groups for MVIC60 ($F(2,20) = 1.415$, $p = .27$), MVIC30 ($F(2,20) = .189$, $p = .83$),
 2281 MVIC60% ($F(2,20) = 1.015$, $p = .38$) or MVIC30% ($F(2,20) = .960$, $p = .40$). See Figure
 2282 5.2 for MVIC data as percentage change and Table 5.4 for absolute values. See Figure
 2283 5.3 and 5.4 for individual MVIC data spread for MVIC60 and MVIC30, respectively.

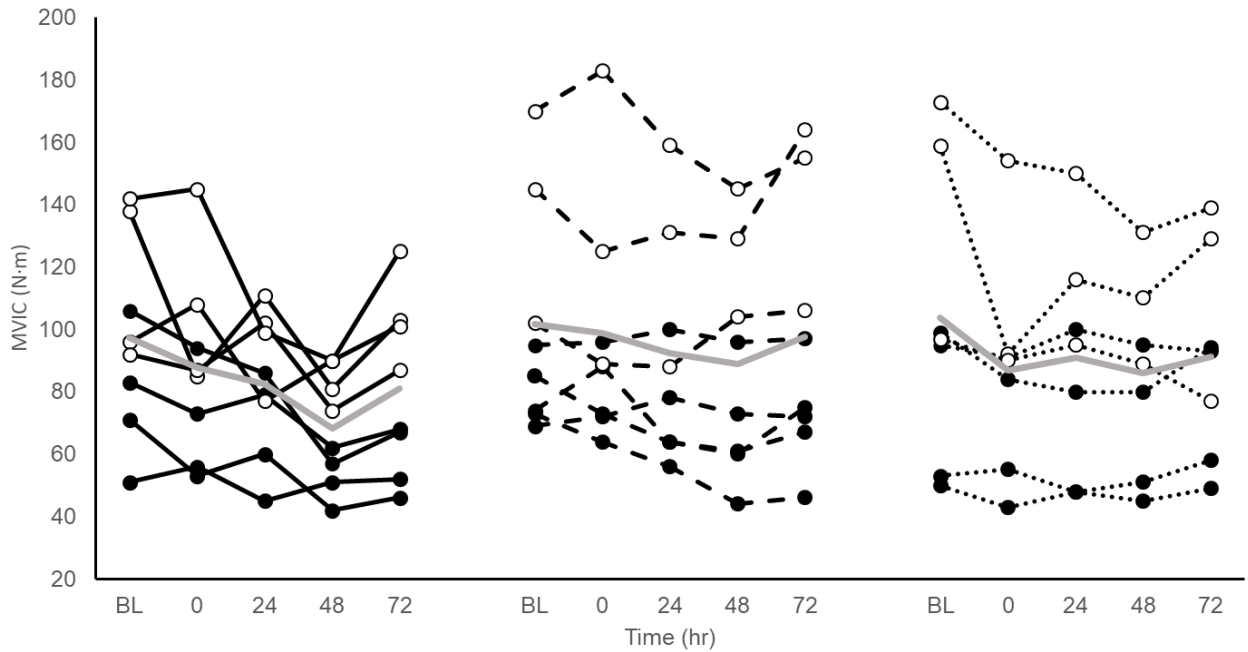


2284

2285 Figure 5.2. Percentage change from baseline for MVIC following EIMD



2286 Figure 5.3 Individual MVIC60 data; CON = solid lines, CF₈₃₀ = dashed lines, CF₁₂₄₅ =
 2287 dotted lines, grey lines = group averages, black circles = female participants, white
 2288 circles = males



2289

2290 Figure 5.4 Individual MVIC30 data; CON = solid lines, CF₈₃₀ = dashed lines, CF₁₂₄₅ =
 2291 dotted lines, grey lines = group averages, black circles = females, white circles = males

Table 5.4. Changes in MVIC following EIMD

Measure	Group	Time post-EIMD (hr)				
		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

2292

2293 5a.3.2 Measures of Perceived soreness

2294 For measures of perceived soreness, a significant main effect for time was observed
 2295 for VAS ($p \leq .001$) and LEFS ($p \leq .001$), both data were normally distributed. For VAS
 2296 significant differences were observed between baseline and 0, 24, 48, and 72 hr ($p \leq$
 2297 $.001$), 0 and 48 ($p \leq .001$), 24 and 48 ($p \leq .001$), and 48 and 72 ($p \leq .001$). For LEFS
 2298 significant differences were observed between baseline and 0, 24, 48, and 72 hr ($p \leq$

2299 .001), 0 and 48 ($p = .001$), 24 and 48 ($p \leq .001$), and 48 and 72 ($p = .001$) There were
 2300 no significant differences between groups for VAS scores ($F(2,20) = .39, p = .68$).
 2301 There were no significant differences between groups for LEFS scores ($F(2,20) = .059,$
 2302 $p = .94$). See Table 5.5 for perceived soreness data and Figure 5.5 for individual VAS
 2303 data.

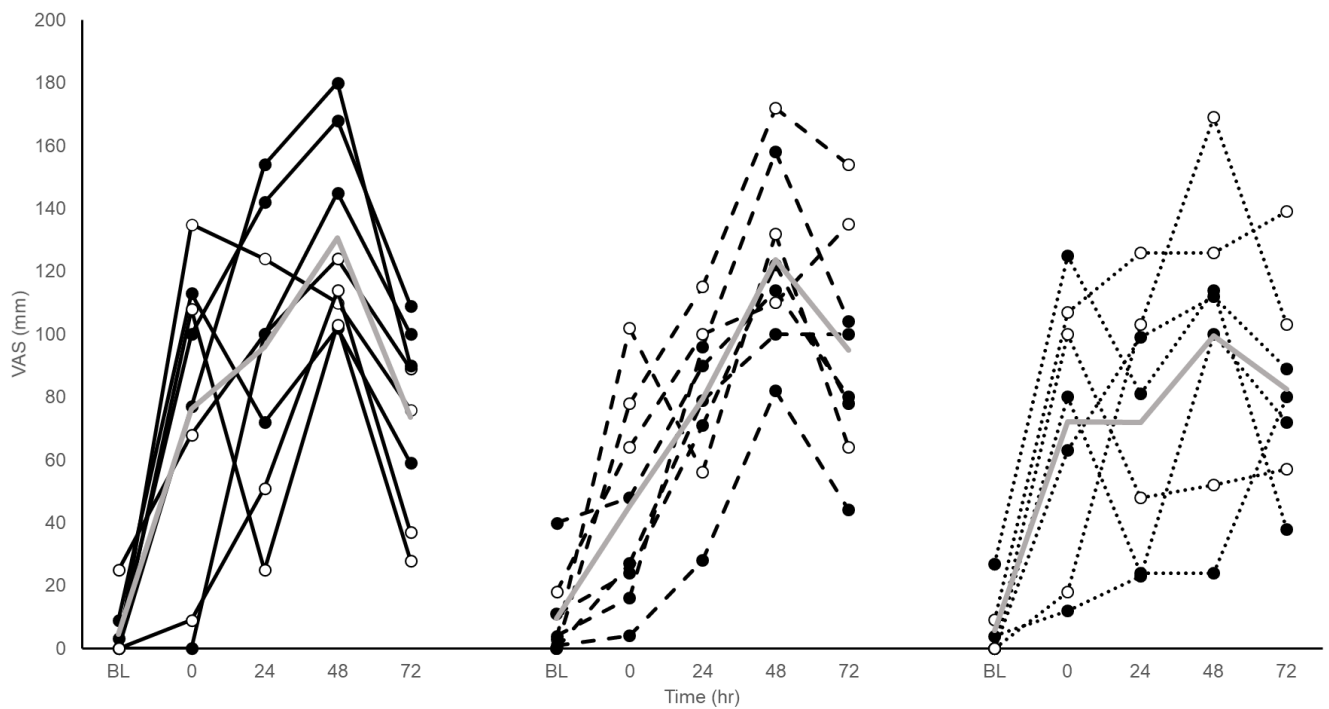
2304

Table 5.5. Changes in perceived soreness post-EIMD

Measure	Group	Time post-EIMD (hr)				
		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

2305



2306 Figure 5.5 Individual VAS data; CON = solid lines, CF₈₃₀ = dashed lines, CF₁₂₄₅ =
 2307 dotted lines, grey lines = group averages, black circles = females, white circles =
 2308 males

2309 5a.4. Discussion

2310 The main aim of this study was to investigate whether various doses of CF have any
2311 impact on indices of muscle recovery following EIMD. Based on the results of the
2312 current research, no significant differences were found following the addition of CF.
2313 This study corroborates previous findings that suggest an acute dose of CF has no
2314 significant impact on measures of muscle function, or measures of perceived soreness
2315 (de Carvalho et al., 2019; Morgan et al., 2018; Peschek et al., 2013).

2316 Differences between this study and previous studies should be noted, in that both de
2317 Carvalho et al., (2019) and Peschek et al., (2013) used EIMD protocols that did not
2318 elicit muscle soreness or deficits in muscle function in the populations they used. By
2319 contrast, the protocol used in this study elicited muscle damage as evidenced by a
2320 ~21% reduction in muscle function alongside a reduction of ~27% for perceived
2321 muscle function measured using the LEFS and a 17-fold increase in perceived
2322 soreness at 48 hr post-protocol (see Tables 11 and 12), at which the negative effects
2323 of muscle damage are known to peak (Cheung et al., 2003). Furthermore, this study
2324 targeted the hamstring muscle group as the location for inducing muscle damage
2325 when previous studies targeted the quadriceps (de Carvalho et al., 2019; Morgan et
2326 al., 2018; Peschek et al., 2013). The knee flexors are ostensibly more susceptible to
2327 muscle damage than the knee extensors following eccentric exercise (Chen et al.,
2328 2011). Thus, it may be more pertinent to investigate the hamstrings and recovery,
2329 especially when considering the high injury rate of the knee flexors in sport, e.g., soccer
2330 (Ekstrand et al., 2011). These methodological differences make comparisons difficult
2331 to make between this current study and the previous literature.

2332 The reductions in peak torque in the present research that were observed in the days
2333 post-EIMD are likely due to a combination of the mechanical disruptions and
2334 subsequent oxidative stress elicited by the exercise protocol. The high levels of
2335 oxidative stress typically observed following EIMD, including similar protocols to the
2336 one utilised in the current study (Nikolaidis et al., 2007), can cause the muscle to enter
2337 an oxidised state, limiting contractile capability (Powers & Jackson, 2008). However,
2338 although CF have been shown to blunt exercise-induced oxidative stress (Davison et
2339 al., 2012), the high variability between individuals in regard to the level of oxidative
2340 stress seen in response to exercise must be considered when interpreting these
2341 findings (Mullins et al., 2013). Additionally, it is unlikely that CF outcompete the existing

2342 antioxidant defence system. Instead, epicatechin and catechin metabolites may
2343 upregulate the endogenous antioxidant enzymes rather than act directly on ROS
2344 (Ruijters, Weseler, Kicken, Haenen, & Bast). Nonetheless, such effects require
2345 confirmation with future research. Therefore, with the previous in mind, and as no
2346 markers of oxidative stress were taken, it is difficult to conclude that the large effect
2347 sizes seen between CF₁₂₄₅ and CON for MVIC60%, MVIC30 and MVIC30% at 24 and
2348 48 hr post-EIMD ($d \geq 0.8$) are a result of CF reducing oxidative damage. Hence, more
2349 research is required to understand the potential benefits of CF as a recovery aid.

2350 For subjective measures of muscle soreness it was hypothesised that CF consumption
2351 may reduce muscular soreness *via* the inhibition of pro-inflammatory cytokines, which
2352 are associated with neuropathic pain (Zhang & An, 2007). This was not the case in the
2353 present study, as subjective measures did not differ between groups. However, a large
2354 effect size was observed between CF₁₂₄₅ and CON for VAS at 48 hr post-EIMD
2355 (difference of 31 mm, $d=0.9$). The inflammatory process begins immediately following
2356 muscle damaging exercise, further developing in the subsequent 24-48 hr if the
2357 disruption is significant (Saxton, Claxton, Winter, & Pockley, 2003). As the peak rate
2358 of absorption for CF is ~30 min post-ingestion, it is feasible that the acute dose of 1245
2359 mg CF could reduce the immediate increase in cytokines and other inflammatory
2360 mediators (e.g., neutrophils) that propagate following exercise. Because these
2361 mediators have the capacity to exacerbate muscle damage (Paulsen et al., 2012;
2362 Pizza et al., 2005; Toumi & Best, 2003) and delay recovery in the subsequent days,
2363 an early reduction in this response could lead to an enhanced recovery. This effect
2364 may result from the inhibitory potential of CF monomers on tumour necrosis factor- α ,
2365 a pro-inflammatory cytokine involved in muscle lysis (Liao, Zhou, Ji, & Zhang, 2010;
2366 Mao et al., 2002). Nonetheless, these are speculative mechanisms that require
2367 confirmation from further research that includes a comprehensive array of
2368 inflammation mediators. The inability to measure these in the present study is
2369 acknowledged as a limitation of the work.

2370 This study is not without its limitations, firstly, even though menstrual cycle was
2371 accounted for through the use of self-report questionnaires; they are not as accurate
2372 as hormonal tests to appropriately determine cycle phase (Wideman et al., 2013).
2373 However, hormone analysis was not feasible for the current research. Secondly, it is
2374 possible that the interindividual variability associated with muscle damage (Damas,

2375 Nosaka, Libardi, Chen, & Ugrinowitsch, 2016) and variability between sex responses
2376 to EIMD (Sewright, Hubal, Kearns, Holbrook, & Clarkson, 2008) reduced the power of
2377 this study when paired with relatively small groups. Thirdly, no inflammatory or
2378 oxidative stress markers were taken, thus it was not possible to ascertain whether the
2379 intervention did in fact reduce these markers. Future research should look to include
2380 these measures and investigate the effect of CF supplementation on repeated bouts
2381 of high-intensity exercise separated by short recovery times to better reflect
2382 competition patterns typical of team-sport athletes.

2383 In conclusion, there is no significant benefit for muscle recovery when comparing an
2384 acute dose of either 830 and 1245 mg CF to a nutrient controlled carbohydrate-protein
2385 control. However, this needs to be confirmed with future research, whilst addressing
2386 the limitations above, to confirm or refute any benefits CF supplementation may have
2387 following a dose >1000 mg. Research should focus on CF impact on repeat
2388 performance and a more comprehensive study investigating sex differences following
2389 CF supplementation should be conducted.

2390

2391 5b.3 Results

2392 5b.3.1 Participant Characteristics and Nutritional Intake

2393 No significant differences were observed for intra sex differences between groups for
2394 height (males $p = .45$ females $p = .84$), weight (males $p = .15$ females $p = .23$), or age
2395 (males $p = .30$ females $p = .49$). However, there were significant differences for some
2396 inter sex comparisons: CON for height ($p = .008$) and weight ($p = .01$) and CF₁₂₄₅ for
2397 height ($p = .008$). Otherwise, no significant differences were observed ($p \geq .51$). No
2398 significant differences for dietary intake between groups were observed when
2399 compared for intra sex differences for energy (males $p = .72$ females $p = .61$), protein
2400 (males $p = .62$ females $p = .66$), CHO (males $p = .08$ females $p = .11$), or fat (males p
2401 $= .51$ females $p = .75$). For inter-sex differences, statistical significance was observed
2402 for CON for protein intake ($p = .03$), CF₈₃₀ for protein intake ($p = .001$) and CF₁₂₄₅ for
2403 energy intake ($p = .008$) and carbohydrate intake ($p = .01$). See Table 5.6 for nutritional
2404 intake data.

2405

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 CF₈₃₀ and CF₁₂₄₅

2406

2407 5b.3.2 Sex differences for muscle function

2408 Muscle function was measured using MVIC60 and MVIC30 absolute values and
 2409 MVIC60% and MVIC30% to assess for relative changes. From the protocol a
 2410 significant time effect was observed for males and females for MVIC60, MVIC30,
 2411 MVIC60% and MVIC30% ($p \leq .004$) indicating that muscle function was significantly
 2412 impaired following the EIMD protocol. For inter-sex comparisons of MVIC60 significant
 2413 differences were observed for CON ($p = .04$) and CF₈₃₀ ($p = .01$) but not CF₁₂₄₅ ($p =$
 2414 $.06$). Further significant intra sex differences were observed for MVIC30 for CON ($p =$
 2415 $.008$) and CF₈₃₀ ($p = .01$) but not for CF₁₂₄₅ ($p = .06$). For intra-sex comparison, no
 2416 significant differences were observed for MVIC60 and MVIC30 ($p \geq .07$). Additionally,
 2417 no significant inter- or intra-sex differences were observed for MVIC60% or MVIC30%
 2418 ($p \geq .09$). Post-hoc analysis between the males and females of CF₁₂₄₅ found a
 2419 significant difference at 72 hr post-EIMD when assessing for MVIC30% ($p = .03$). See
 2420 Table 5.7 for MVIC data as percentage change and Table 5.8 for absolute values.
 2421 Figure 5.6 and 5.7 display group MVIC60 and MVIC30 data as percentage change.

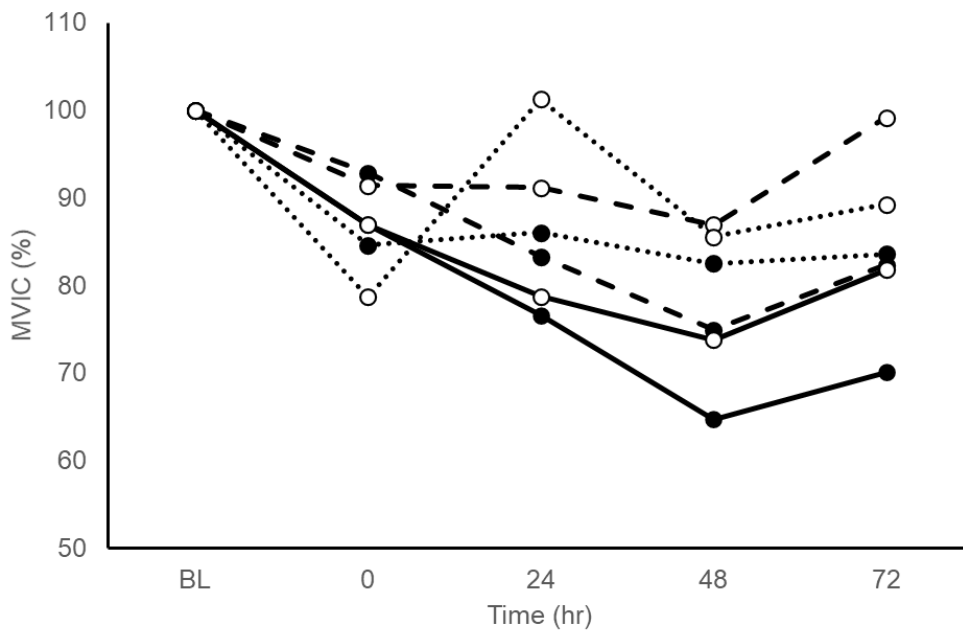
2422

Table 5.7 Changes in MVIC Following EIMD as Percentage Change

Measure	Group	Sex	Time post-EIMD (hr)				
			Baseline	0	24	48	72
	CON	F	100 ± 0	87 ± 17	77 ± 13	65 ± 27	70 ± 16
		M	100 ± 0	87 ± 23	79 ± 9	74 ± 20	82 ± 20
MVIC60%	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
	CON	F	100 ± 0	90 ± 15	87 ± 6	72 ± 21	78 ± 18
		M	100 ± 0	93 ± 22	85 ± 18	74 ± 16	93 ± 27
MVIC30%	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*

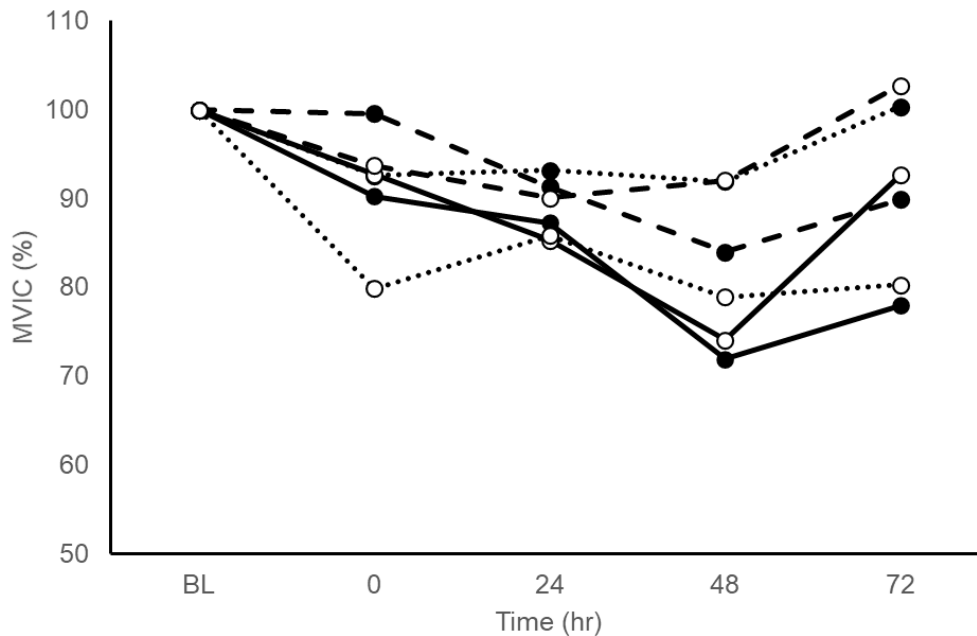
Notes: Means ± standard deviations, F = females, M = males, MVIC = maximal voluntary isometric contraction, EIMD = exercise-induced muscle damage, * = denotes a significant difference within the group



2423

2424 Figure 5.6 Inter- and Intra-sex MVIC60% data; CON = solid lines, CF₈₃₀ = dashed
 2425 lines, CF₁₂₄₅ = dotted lines, grey lines = group averages, black circles = female
 2426 participants, white circles = males; data reported as means, for SD see Table 6.

2427



2428 Figure 5.7 Inter- and Intra-sex MVIC30% data; CON = solid lines, CF₈₃₀ = dashed
 2429 lines, CF₁₂₄₅ = dotted lines, grey lines = group averages, black circles = female
 2430 participants, white circles = males; data reported as means, for SD see Table 6.3

2431

Table 5.8 Changes in MVIC Following EIMD

Measure	Group	Sex	Time post-EIMD (hr)				
			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 \pm 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

2432

2433 5b.3.3 Sex differences for perceived soreness

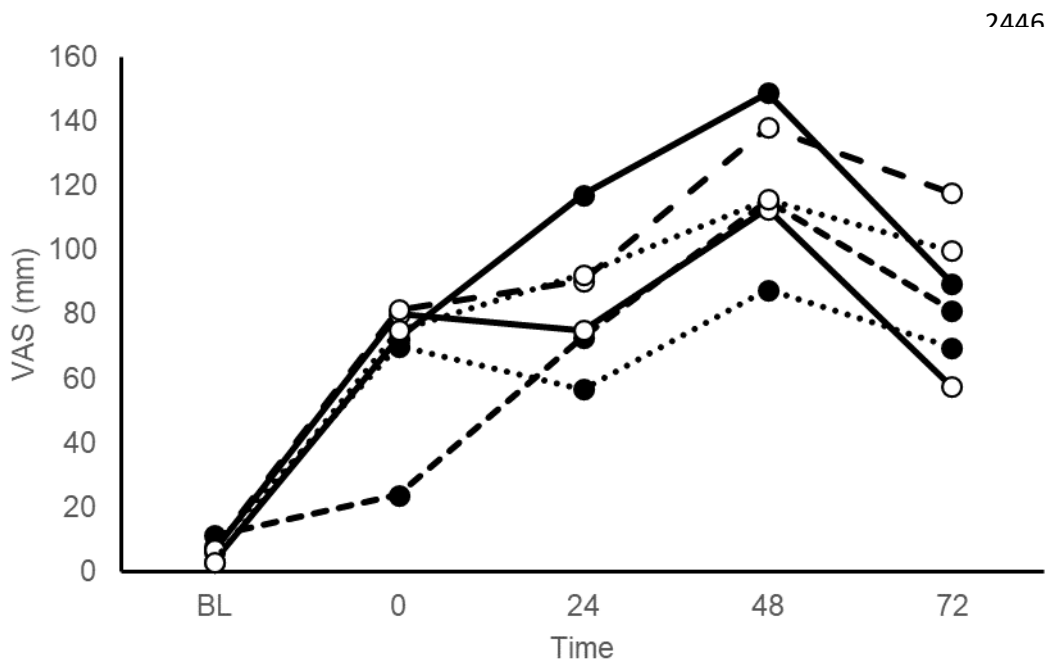
2434 Measures of perceived soreness assessed using a VAS and LEFS found a significant
2435 main time effect for males and females ($p \leq .006$), indicating that the EIMD protocol
2436 was effective in inducing muscle soreness. For VAS scores, no significant differences
2437 were observed when analysing for inter-sex comparisons ($p \geq .08$) or intra-sex
2438 comparisons ($p \geq .06$). For LEFS scores, no significant differences were observed
2439 when analysing for inter-sex comparisons ($p \geq .60$) or intra-sex comparisons ($p \geq .62$).
2440 Post-hoc analysis for intra-sex differences for VAS scores between CON and CF₁₂₄₅
2441 found significant differences 24 hr ($p = .03$) and 48 hr ($p = .03$) post-EIMD. Additionally,
2442 a significant difference was observed immediately post-EIMD when comparing the
2443 males and females of CF₈₃₀ for VAS scores ($p = .004$). See Table 5.9 for perceived
2444 soreness data and Figure 5.8 for VAS data.

2445

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



2447 Figure 5.8 Inter- and Intra-sex VAS data; CON = solid lines, CF₈₃₀ = dashed lines,
 2448 CF₁₂₄₅ = dotted lines, grey lines = group averages, black circles = female
 2449 participants, white circles = males; data reported as means, for SD see Table 6.5

2450 5b.4 Discussion

2451 The main aim of this study was to investigate whether any sex differences for muscle
2452 recovery are present following the administration of an acute CF recovery beverage
2453 of varying amounts post-EIMD. Based on the current research there are no significant
2454 differences between sexes regarding the impact of CF on muscle recovery.

2455 Based on the current data it appears that there are no significant differences for muscle
2456 function recovery between males and females whilst supplementing CF. For MVIC
2457 percentage change at both 60 and 30 degrees there were no significant differences
2458 for inter or intra-sex comparisons. However, a p value of .092 was observed between
2459 males and females within the CF₁₂₄₅ group for MVIC30%, with a significant difference
2460 identified at 72 hr post-EIMD ($p = .03$). The data at 72 hr shows that females had
2461 reached 100% of baseline MVIC whereas males only reached 80%, potentially
2462 indicating that the males within CF₁₂₄₅ were still in an impaired contractile state 72 hr
2463 following the EIMD protocol. Interestingly, females on average had greater reductions
2464 in MVIC than males (based on relative changes not absolute values). Furthermore,
2465 males have been observed to demonstrate greater neuromuscular fatigue and slower
2466 acute recovery than females following strenuous exercise (Häkkinen, 1993). This is
2467 somewhat evidenced within the data as immediately post-EIMD females achieved a
2468 higher percentage of MVIC than males across all data, excluding MVIC60% and
2469 MVIC30% for the CON group. Other research has indicated that females may be less
2470 fatigable than males following intermittent, MVIC exercise (Ansdell, Brownstein,
2471 Škarabot, Hicks, Howatson, et al., 2019; Ansdell, Thomas, Howatson, Hunter, &
2472 Goodall, 2017). However, the exercise utilised within this Chapter was maximal
2473 eccentric knee flexor exercise, therefore comparisons are limited with further research
2474 required using separate muscle groups or comparisons between muscle groups.

2475 The differences observed here for the relative differences between males and females
2476 for MVIC may be due to individual variation and limited sample size as opposed to sex
2477 differences as no other data was statistically significant for MVIC30% or MVIC60%.
2478 There are a number of reasons this may be apparent. It could be due to individual sex-
2479 differences relating to lean body mass and strength, as males demonstrated
2480 significantly higher MVIC values. Following repeat exposure to exercise stimuli that
2481 includes high force eccentric contractions there are various physiological adaptations
2482 that occur to protect the muscle from future damage. One such adaptation is an

2483 increase in the number of motor units recruited during maximal eccentric contractions
2484 thereby reducing the stress placed on individual muscle fibres (McHugh, 2003). This
2485 may be the reason why individuals with higher training status may be at a reduced risk
2486 of severe muscle damage following an EIMD protocol. Not only that but training status
2487 appears to correlate with the inflammatory response associated with intense exercise,
2488 the greater an individual's training status the lower the response (Martín-Sánchez et
2489 al., 2011). However, participants were classed as recreationally active to be eligible
2490 for the study and participants who partook in extensive, regular eccentric training were
2491 excluded from participation. Nevertheless, it is also possible that due to the reduced
2492 number of males who completed the study than females the differences observed
2493 could relate to individual variation.

2494 For subjective measures of muscle soreness there were no significant differences
2495 between groups for VAS or LEFS. Interestingly, although not significant there were
2496 data that was approaching statistical significance for the VAS, specifically males vs
2497 females for CF₈₃₀ ($p = .08$), females vs females for CON vs CF₈₃₀ ($p = .07$) and CON
2498 vs CF₁₂₄₅ ($p = .06$). For the comparisons between females for CON vs CF₁₂₄₅,
2499 significant differences were observed at 24 hr ($p = .03$) and 48 hr ($p = .03$). This
2500 indicates that the females in the CON experienced significantly higher levels of
2501 perceived muscular soreness than those within the CF₁₂₄₅ group. Large effect sizes
2502 were observed between the females within the CON and both the CF₈₃₀ and CF₁₂₄₅
2503 groups at 24 and 48 hr post-EIMD ($d \geq 1.2$). At 24 hr the average score for the females
2504 within the CON were 60 mm higher and at 48 hr 61 mm higher. Similarly, the females
2505 within the CF₈₃₀ group consistently scored lower for muscle soreness than the CON
2506 group throughout the testing period, with CF₁₂₄₅ having the lowest average scores,
2507 save for immediately post-EIMD when CF₈₃₀ scored lower. It is possible that the CF
2508 provided some level of analgesic benefit for females more so than in males. Males
2509 scores remained similar between the groups for VAS.

2510 The effects of the menstrual cycle on muscle recovery are still being elucidated;
2511 however, menstrual phase may influence feelings of perceived soreness. A recent
2512 review by Romero-Parra et al., (2020) found that female athletes experienced higher
2513 levels of perceived soreness post-EIMD during the early follicular phase of their cycles,
2514 when oestrogen concentrations are low. Within this study, all female participants were
2515 tested within the luteal phase or a phase equivalent depending on contraceptive use,

2516 as the luteal phase is known for having relatively consistent levels of oestrogen
2517 throughout following the second peak of oestrogen at the beginning of the phase
2518 (Mihm et al., 2011; Reed & Carr, 2018). The increased oestrogen in the females
2519 alongside the high intake of CF may partially explain the reason that the female
2520 participants within the CF groups had lower levels of perceived soreness than their
2521 male counterparts. However, (poly)phenols have been shown to have both anti-
2522 oestrogenic and oestrogenic effects and may impact the bioactivity of oestrogen *via*
2523 the binding to and/or blocking of oestrogen receptors (Kiyama, 2020).

2524 Furthermore, within the current data set it was noted that on average males recorded
2525 higher VAS scores in the two CF groups than the females, however in the CON males
2526 scored lower than females. It is possible that due to the reported effects of CF
2527 upregulation of various pro- and anti-inflammatory molecules and redox enzymes
2528 combined with the potential benefits of oestrogen could explain these slight
2529 differences. Evidence has suggested that females may have a reduced inflammatory
2530 response in comparison to males following EIMD, with current evidence indicating a
2531 reduced invasion count of neutrophils and macrophages (Stupka et al., 2000).
2532 However, evidence is still equivocal, especially when considering variation due to the
2533 menstrual cycle as higher levels of inflammation following EIMD have been observed
2534 during the follicular phase (Carter et al., 2001; Hackney et al., 2019; Oosthuyse &
2535 Bosch, 2017). CF modulation of inflammatory markers alongside the role of oestrogen
2536 may present an explanation of these findings. It has been observed that *in vitro* CF
2537 administration downregulates various inflammatory molecules such as monocyte
2538 chemoattractant protein-1, TNF- α , IL-1 α , and IL-6, with *in vivo* evidence suggesting
2539 similar effects (Goya et al., 2016; Selmi et al., 2006). Oestrogen has also shown to
2540 have potential anti-inflammatory properties, such as through the inhibition of leucocyte
2541 infiltration following unaccustomed exercise (Stupka et al., 2000). Possibly, the
2542 combined benefit may elicit greater reductions in inflammation compared to males and
2543 partially explain the reduced soreness noted in females. This may be due to the role
2544 of inflammation on soreness as many inflammatory molecules sensitise nociceptors in
2545 the muscle, e.g., TNF- α , IL-1 β , and IL-6, as well as by macrophages and neutrophils
2546 (Pinho-Ribeiro, Verri Jr, & Chiu, 2017). However, future studies including greater
2547 participant numbers and the inclusion of inflammatory markers are required. Not only
2548 that but, studies should look to investigate any potential interaction effects of CF and

2549 oestrogen. By comparing menstrual cycle phase on markers of muscle recovery whilst
2550 supplementing CF during it may be possible to investigate any potential interaction
2551 effects.

2552 5b.5 Limitations

2553 The main limitation is the reduced number of individuals within each group when
2554 separated by sex, leading to a reduced ability to make any meaningful conclusions
2555 based on the data. Indeed, any differences that have been noted are likely due to inter-
2556 individual variation as opposed to inter-sex differences. The observed post-hoc power,
2557 calculated using G*Power further indicated the underpowered nature of the sex
2558 specific analysis, with data ranging from 78% to 30% power depending on the
2559 measure. This is to be expected with the nature of Part B of this study due to the
2560 reduced participant numbers when analysing for sex differences. It has been reported
2561 that observed post hoc power varies from true power significantly, this is due to the
2562 completion of data collection having already occurred. Furthermore, observed power
2563 reduces as a function of a p value increasing, and as this study had no significant
2564 differences it is very likely to provide low observed power (Hoenig & Heisey, 2001).

2565 The significant differences observed between males and females in the CON and
2566 CF₈₃₀ for MVIC60 and MVIC30 are due to overall differences in absolute values for
2567 force output, rather than treatment, as there were no significant differences when the
2568 values were analysed as percentage change from baseline. Future studies
2569 investigating the potential sex differences that may exist regarding CF consumption
2570 and EIMD are warranted. It is recommended that for a study that has the main focus
2571 of this research question, investigators should look to implement further methods to
2572 track the menstrual cycle in females controlling it as a variable as accurately as
2573 possible. Methods may include hormonal testing, basal body temperature testing or
2574 utilising ovulation kits and should be performed alongside calendar-based testing to
2575 account for the variation that exists around the menstrual cycle (Fehring et al., 2006;
2576 Wideman et al., 2013).

2577 Other areas in which future research should look to address regarding sex differences
2578 and CF are as follows. Potential variations in oxidative stress and antioxidant capacity
2579 between males and females following EIMD and CF consumption, as differences have
2580 been observed following the consumption of other (poly)phenols (Burton-Freeman et
2581 al., 2010); albeit this was not noted following EIMD. There may be differences in the

2582 absorption or metabolism of CF between sexes also, as such studies may utilise
2583 markers of epicatechin metabolites (or other CF metabolites) when investigating sex
2584 differences to measure availability of these compounds within circulation or in
2585 excretion. Investigating the potential affinity of CF monomers, such as epicatechin, to
2586 bind to oestrogen receptors will also provide a greater insight into the mechanistic
2587 action of these compounds. It has been noted already that certain (poly)phenols, e.g.,
2588 ellagic acid, have a greater affinity for oestrogen-receptor beta (Landete, 2011) and
2589 certain flavanols activating oestrogen receptor alpha (Kiyama, 2020). The expression
2590 of the former receptor is 30% lower during the early follicular phase of the menstrual
2591 cycle and as such may impact (poly)phenol action (Najjar, Turner, Wong, & Feresin,
2592 2021).

2593 It is pertinent that more research includes female participants, including sedentary
2594 individuals, recreational athletes, and elite athletes alike, allowing for more accurate
2595 practical applications to be made from the research without generalising from male
2596 cohorts. Recent methodological considerations for sport science research have been
2597 published with the intent to improve research on females, not only to improve quality
2598 but increase implementation (Elliott-Sale et al., 2021). At present only five studies have
2599 included female participants within CF investigations in relation to exercise (Garcia-
2600 Yu et al., 2021; Patel et al., 2020; Sadler et al., 2020; Shaw, Singh, Sirant, Neary, &
2601 Chilibeck, 2020; Taub et al., 2016) indicating a dearth of research in this area.

2602 In summary, from the present data there is no significant difference between males
2603 and females for indices of muscle recovery following CF supplementation of varying
2604 doses. It is possible that there is an increased analgesic effect of CF in females than
2605 males, however this requires further research and is currently speculation based on
2606 the data. It is possible that a greater number of male and female participants would be
2607 required to identify a statistically significant difference, and as such data should be
2608 interpreted cautiously.

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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

2621 6.1 Introduction

2622 Currently, sport and exercise science practitioners make use of numerous ergogenic
2623 aids to accelerate or optimise recovery, such as (poly)phenol supplementation. The
2624 use of these high (poly)phenol foods has become widespread (Maughan, Depiesse,
2625 & Geyer, 2007; Solheim et al., 2017; Teixeira, 2013), including outside of the athletic
2626 domain, with the intention of maximising any reported health improving benefits
2627 (Williamson, 2017). This thesis has focussed on CF and as discussed within this thesis
2628 (Chapter 2 Section 1) CF supplementation can aid with numerous health benefits
2629 relating to oxidative stress, cardiovascular health, and cognitive health. For exercise,
2630 research has sought to investigate the impact of CF on aspects of muscle recovery,
2631 such as oxidative stress, soreness, inflammation, perceived soreness, and muscle
2632 function (see Chapter 3). Currently, the evidence within this area of research is limited
2633 and equivocal. However, based on that research, CF supplementation appears to
2634 beneficially reduce exercise-induced oxidative stress (Allgrove et al., 2011; Davison
2635 et al., 2012) and within this thesis (Chapter 5), an acute, high dose of CF (1245 mg)
2636 may have some influence on the recovery of muscle function following exercise.
2637 Interestingly, one study found that regular supplementation of CF (616 mg daily)
2638 improved distance covered in a Yo-Yo test following a muscle damaging exercise
2639 protocol (de Carvalho et al., 2019). However, this is still a burgeoning area of research,
2640 with the current available literature still sparse.

2641 The deleterious effects of muscle damage can persist over a period of four or more
2642 days following exercise (Gibala, MacDougall, Tarnopolsky, Stauber, & Elorriaga,
2643 1995). This understanding of the longevity of EIMD can be applied to practically, in as
2644 much as resistance training, weightlifting sports, and sports that have a proclivity for
2645 rapid performance turnarounds. Furthermore, team sports, e.g., soccer, may not have
2646 adequate time between exercise bouts to fully recover, especially during fixture
2647 congested periods (Page et al., 2019). These periods involve repeat performance
2648 within 72 hr of the initial event, commonly observed in soccer and other tournament-
2649 based sports, e.g., hockey. It is possible that during these periods recovery is
2650 insufficient to allow for maximal/optimal performance during the second bout of
2651 exercise and an inability to train at appropriate intensities. For example, muscle
2652 soreness has been reported to still be present 72 hr post-match in soccer and can be
2653 exacerbated during fixture congested schedules (Lundberg & Weckström, 2017).

2654 The impact that muscle damage has on performance can be detrimental, for example,
2655 it can impede sprinting ability and explosive power (Khan et al., 2016), and reduce
2656 contractile muscle force (Magaudda, Di Mauro, Trimarchi, & Anastasi, 2004). These
2657 consequences may result from ultrastructural damage of the muscle fibres *via* the
2658 mechanical stress of intense exercise, most notably eccentric muscle contractions, as
2659 more strain is placed on fewer motor units than during concentric contractions
2660 (McKune, Semple, & Peters-Futre, 2012). Exercise-induced oxidative stress within
2661 muscle tissue can impact contractile capability; muscle fibres in oxidised states have
2662 been shown to have significantly reduced force generating capacity (Reid, 2008;
2663 Siems et al., 2003; Steinbacher & Eckl, 2015). The level of oxidative stress is
2664 dependent on the intensity of the exercise and the oxygen demands of it, as well as
2665 the inflammatory response in the days following completion (Uchiyama, Tsukamoto,
2666 Yoshimura, & Tamaki, 2006). As such exogenous antioxidants may be helpful in
2667 limiting the upsurge of ROS (Zhang & Tsao, 2016).

2668 In relation to feelings of soreness, pain during exercise can impact pacing strategies
2669 by making an athlete aware of fatigue (Stevens, Mauger, Hassmèn, & Taylor, 2018),
2670 therefore, beginning an event already in a damaged state may negatively impact
2671 athletic performance. Fatigue is a common component of intense and prolonged
2672 exercise (Nybo, 2003), these physiological responses signal the brain and other
2673 organs to initiate the reduction of exercise intensity or cease it entirely (Keller et al.,
2674 2001). Fatigue can accumulate if recovery is insufficient, e.g., a reduced recovery
2675 window before subsequent exercise, following the original bout of exercise (Page et
2676 al., 2019), leading to an increased injury risk and elongate the time course of recovery
2677 (Small et al., 2009b).

2678 Understanding the impact of CF on functional recovery, e.g., force recovery, may
2679 provide practitioners a better understanding of when to utilise CF as a recovery
2680 intervention during various sporting scenarios. Especially so, as functional markers of
2681 recovery are commonly the most impaired following EIMD, e.g., force output (Child,
2682 Saxton, & Donnelly, 1998; Howatson & Milak, 2009), jump height (West et al., 2014),
2683 and sprint performance (Keane, Salicki, Goodall, Thomas, & Howatson, 2015; Twist
2684 & Eston, 2005). Even still, changes in muscle function may also relate to leucocyte
2685 accumulation in a damaged muscle, myofibrillar disruption, and necrosis (Paulsen et
2686 al., 2012). Therefore, during critical sporting competition phases that require rapid

2687 turnarounds for repeated bouts of exercise, accelerating recovery is important. This
2688 also applies to resistance-based exercise and weightlifting sports, not just team sports,
2689 as training will likely be programmed for use of the same muscle group within a 48-72
2690 hr window, optimising recovery from the first session may aid performance during the
2691 next.

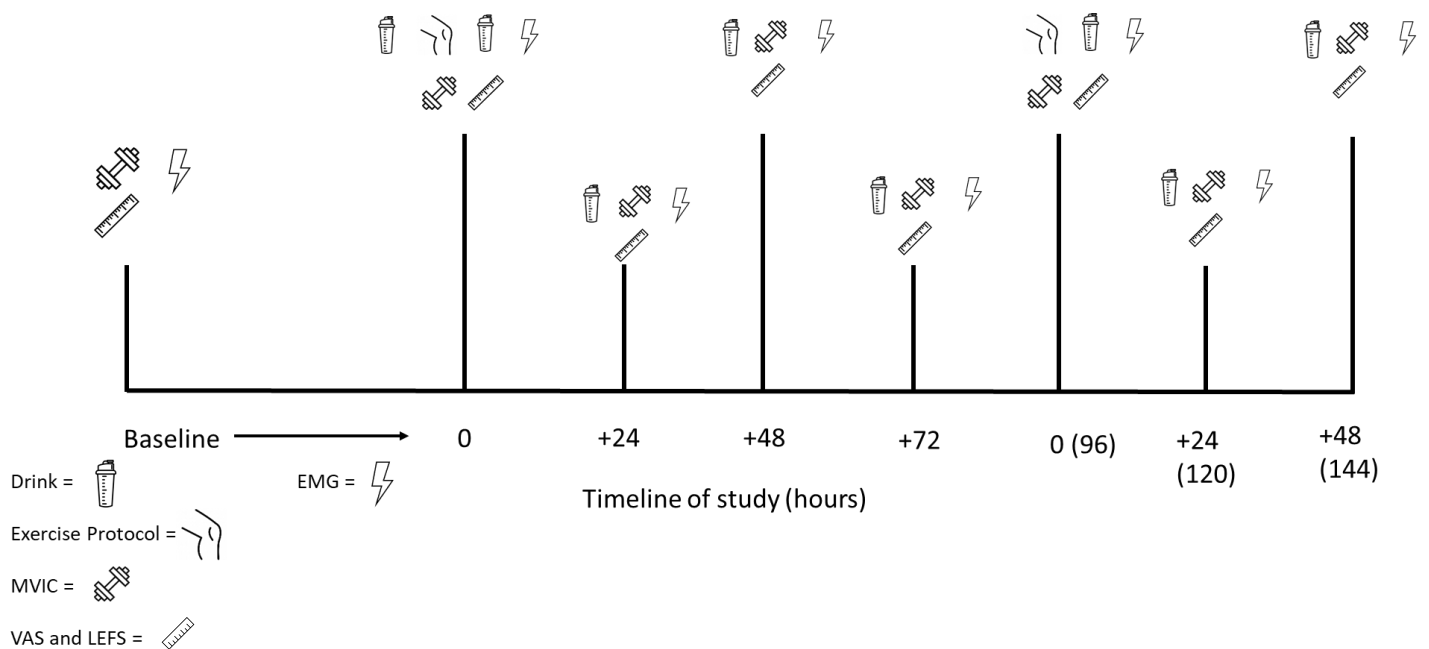
2692 Therefore, further investigations into the impact of regular CF supplementation on
2693 repeat performance are warranted, which is the aim of this Chapter, building on the
2694 previous work within this thesis. As Chapter 5 found no significant effect of an acute
2695 dose of varied doses of CF on muscle recovery. However, as the highest dose of 1245
2696 mg CF had notable effect sizes for the recovery of muscle function and soreness
2697 further research utilising repeated doses of CF is necessary. As such, this study aims
2698 to carry out such an investigation, following a time-frame not dissimilar to a congested
2699 fixture period in soccer. Ergo, the aims of this study are threefold; *i*) to investigate
2700 whether regular CF supplementation beneficially influences markers of recovery
2701 following EIMD, *ii*) if CF can aid repeat performance following EIMD, separated by 72
2702 hr, and *iii*) if CF can reduce the increased neuromuscular fatigue associated with
2703 repeat performance.

2704

2705 6.2 Methods

2706 6.2.1 Study Design

2707 This study was a laboratory-based, randomised, double-blind, nutrient controlled trial.
2708 Participants were randomised into either control (CON) or CF supplementation group.
2709 Participants were required to attend the laboratory for seven days. Day one of the
2710 study involved baseline testing and familiarisation of the EIMD protocol using the
2711 isokinetic dynamometer (one set of ten sub-maximal hamstring curls). The other six
2712 days took place consecutively, therefore, the testing schedule ran as follows: baseline,
2713 immediately post-EIMD (0hr), 24, 48, 72 hr post-EIMD, immediately post second EIMD
2714 protocol (2nd 0hr), 24 and 48 hr post-EIMD (see Figure 7.1 for a study schematic).
2715 Randomisation was performed by an independent laboratory technician, see Chapter
2716 4 Section 1 for further details.



2717 Figure 6.1 Study timeline Schematic

2718

2719 6.2.2 Participants

2720 Following ethical approval from the University of Huddersfield and in agreement with
2721 the Declaration of Helsinki, a total of ten participants were recruited for this study.
2722 However, only nine individuals (seven males, two females) completed the entire
2723 testing period, see Table 7.1 for participant characteristics. The only drop out cited 'an
2724 injury concern' as the reason for withdrawing from the study before the second EIMD
2725 protocol. An *a priori* power calculation determined that a sample size of 16 was

2726 required for 80% power and to detect significance based on the effect size of previous
2727 research regarding MVIC recovery at 48 hr post-EIMD. Unfortunately, due to the
2728 coronavirus pandemic data collection was postponed indefinitely from the 17th of
2729 March 2020. Data collection was due to recommence in November 2020, however a
2730 second national lockdown within the United Kingdom resulted in another cancellation
2731 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

2732

2733 6.2.3 Muscle Damaging Protocol

2734 The protocol to induce muscle damage consisted of five sets of ten maximal
2735 concentric-eccentric contractions of the knee flexors (each leg) using an isokinetic
2736 dynamometer. This protocol was completed twice over the testing period, on the first
2737 day of the testing period (0 hr) and 72 hr after the first protocol. See Chapter 4 Section
2738 4 for further details of the muscle damaging protocol.

2739 6.2.4 Nutritional Intervention

2740 Both the participants and researchers were blinded to the allocated beverage of each
2741 participant. To do so an independent laboratory technician randomised the participants
2742 and put together the beverage contents into an opaque bottle. Participants were
2743 provided with a bottle containing a pre-mixed powder of the ingredients (60 g
2744 maltodextrin and 25 g whey protein, plus 15 g Chococru© cocoa powder if assigned
2745 to the treatment group) and were instructed to add 300 ml of water to the bottle and
2746 shake vigorously until dissolved. Participants were instructed to drink their beverage
2747 ~60 min before arrival at the laboratory each day during the testing period and
2748 consumed another immediately post-EIMD protocols, totalling eight beverages. The
2749 test beverage contained 15 g Chococru© cocoa powder, totalling 1245 mg of CF, see
2750 Chapter 4 Section 3.2 for more details.

2751 **6.2.5 Exercise Performance**

2752 Exercise performance was measured *via* peak torque per set completed during both
2753 exercise protocols. Peak torques were collected per set for both concentric and
2754 eccentric contractions of the knee flexors for both the dominant and non-dominant
2755 legs. This data was collected to compare participant exercise performance from the
2756 first EIMD protocol with the second EIMD protocol. Additionally, data was compared
2757 as a percentage change from the first protocol, this was to account for percentage
2758 drop off from the first to second protocol and standardise data between participants.

2759 **6.2.6 Muscle Function**

2760 MVIC was measured at 30- and 60-degrees knee flexion from anatomical zero using
2761 an isokinetic dynamometer. Please refer to Chapter 4 Section 5 for further information
2762 on MVIC measures.

2763 **6.2.7 Electromyography**

2764 Neuromuscular activation of the of the biceps femoris was taken at 60 degrees of knee
2765 flexion from anatomical zero. See Chapter 4 Section 6 for further detail.

2766 **6.2.8 Perceived Soreness**

2767 Muscle soreness was measured using a VAS and LEFS. See Chapter 4 Section 7 for
2768 further information.

2769 **6.2.9 Dietary Analysis**

2770 Participants completed a 24-hr dietary recall each day of testing, excluding baseline
2771 testing. See Chapter 4 Section 2 for further detail.

2772 **6.2.10 Statistical Analysis**

2773 Statistical analysis was performed using IBM SPSS Statistics (version 26.0; IBM
2774 Corp., Armonk, NY). All data was assessed for normality using a Shapiro-Wilk test and
2775 quantile-quantile plots were examined to establish whether the data was normally
2776 distributed. A Greenhouse-Geisser correction was used if sphericity was violated. A
2777 mixed analysis of variance was used to determine interaction and time effects for the
2778 recovery variables. If any significant differences were observed for the data a Fisher's
2779 least significant difference post hoc test was performed to identify the point of
2780 significance. Data for MVIC and isokinetic peak torques were calculated as percentage
2781 changes from baseline alongside data reported as absolute values. Effect sizes were
2782 calculated using Cohen's *d*, with the magnitude of effects considered small (0.2),

2783 moderate (0.5), and large (0.8). Significance was set at $p \leq .05$ pre-analysis.
2784 Descriptive statistics are reported as means, percentage change (%) \pm SD.

2785 6.3 Results

2786 There were no significant differences for participant age ($p = .91$), height ($p = .74$),
2787 weight ($p = .30$) or dietary intake between groups for energy ($p = .88$), CHO ($p = .49$),
2788 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 \pm 429	268 \pm 62	89 \pm 17	85 \pm 28
CF	2217 \pm 487	237 \pm 69	99 \pm 40	97 \pm 27

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

2789

2790 6.3.1 Exercise Performance

2791 No significant differences were observed for peak torque within groups when
2792 comparing protocol one to protocol two for concentric contractions of the dominant leg
2793 ($p \geq .42$) and non-dominant leg ($p \geq .07$) as well as eccentric contractions of the
2794 dominant ($p \geq .11$) and non-dominant leg ($p \geq .10$). There were also no significant
2795 differences between the groups for exercise performance, measured as peak torque,
2796 during the first protocol for non-dominant ($p \geq .53$) and dominant leg ($p \geq .21$) or during
2797 the second protocol for non-dominant ($p \geq .82$) or dominant leg ($p \geq .59$). Interestingly,
2798 the CON group managed to achieve a greater percentage of their original peak torques
2799 from the first protocol in the second for concentric contractions (100 \pm 19 vs 88 \pm 15%)
2800 and eccentric contractions (97 \pm 21 vs 85 \pm 15%) of the dominant leg. However, the
2801 CF group managed to achieve a greater percentage of their original peak torques for
2802 concentric (80 \pm 17 vs 87 \pm 11%) and eccentric contractions (81 \pm 23 vs 85 \pm 11%) for
2803 the non-dominant leg. See Table 6.3 for concentric peak torques and 6.4 for eccentric
2804 peak torques displayed as a percentage of the first protocol.

2805

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

2806

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

2807

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

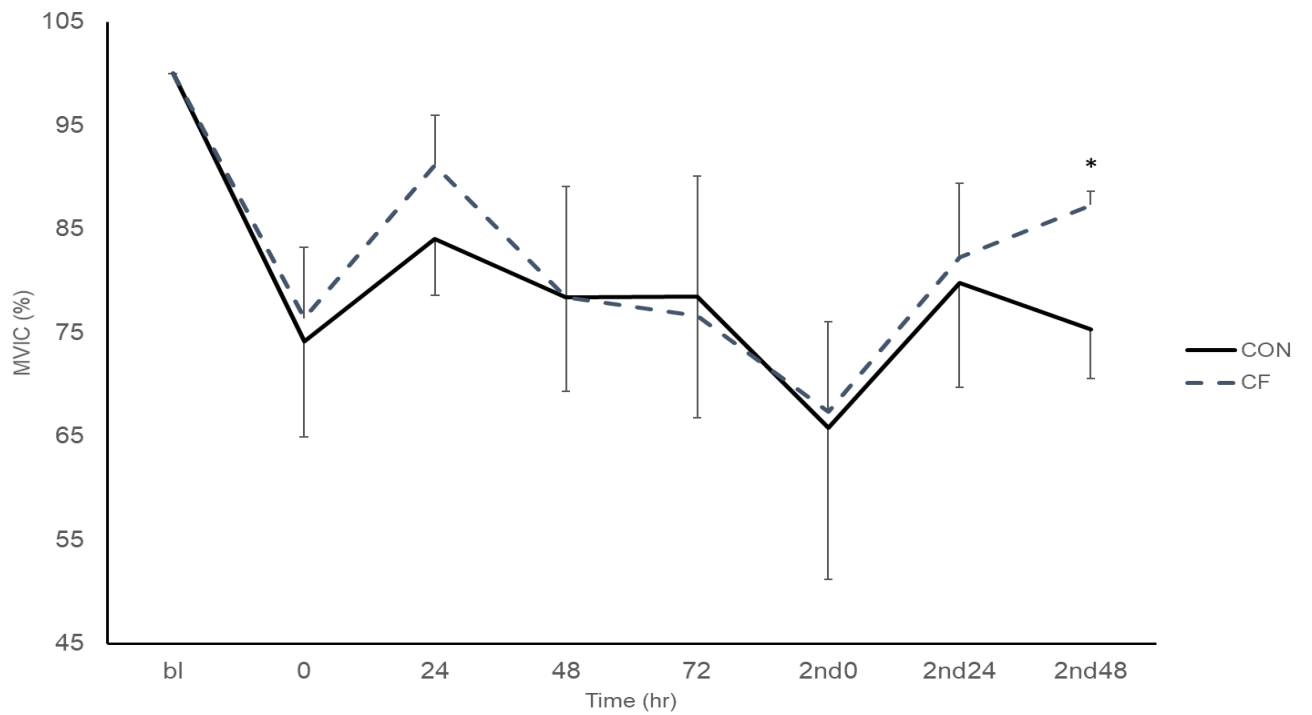
2811 all other time points ($p \leq .006$), 0 and 24 and 2nd0 ($p \leq .05$), 24 and 48, 72, 2nd0 and
 2812 2nd48 ($p \leq .03$), 48 and 2nd0 ($p = .03$), and between 2nd0 and all time points except
 2813 72 ($p \leq .05$). For MVIC30 there were significant differences observed between baseline
 2814 and all other time points ($p \leq .01$), 0 and 2nd0 ($p = .01$), 24 and 2nd0 ($p = .01$), 48 and
 2815 2nd0 ($p = .05$), and between 2nd0 and all other time points except 72 ($p \leq .05$). No
 2816 significant differences were observed for MVIC60 ($F(1,7) = .083$, $p = .78$), MVIC60%
 2817 ($F(1,7) = .429$, $p = .53$), MVIC30 ($F(1,7) = .080$, $p = .79$), or MVIC30% ($F(1,7) = 1.715$,
 2818 $p = .23$). However, significant differences and large effect sizes were observed at 110
 2819 hr post-EIMD (48 hr following the second protocol) for both MVIC60% ($t = -4.276$, $p =$
 2820 $.004$, $d = 3.1$) and MVIC30% ($t = -4.032$, $p = .005$, $d = 3.2$). Large effect sizes were
 2821 also observed for MVIC60 ($t = -1.049$, $p = .33$, $d = 0.8$) and MVIC30 ($t = -1.194$, $p =$
 2822 $.27$, $d = 0.9$) at the same time point. Muscle function loss was greatest immediately
 2823 following the second EIMD protocol. See Table 6.5 for MVIC data (both percentage
 2824 and absolute values) and Figures 6.2 and 6.3 for a visual representation of MVIC
 2825 percentage change data. Figures 6.4 and 6.5 show individual MVIC data for MVIC60
 2826 and MVIC30 respectively.

2827

Table 6.5 Changes in muscle function measured using MVIC

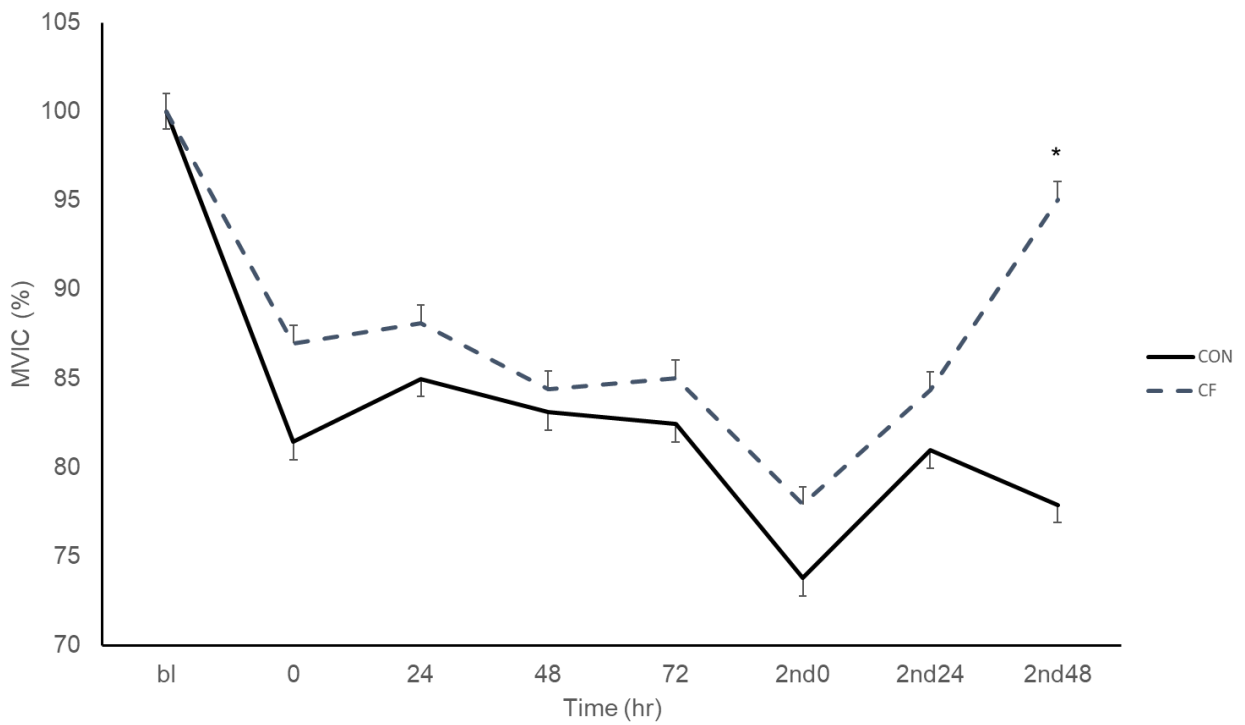
Measure	Group	BL	0	24	48	72	0	24	48
MVIC60 (Nm)	CON	130±22	97 ± 24	109 ±17	103 ±25	102±24	86± 26	104 ±24	98±17
	CF	132± 30	101± 24	120 ±23	102 ±15	100±18	90± 27	109 ±26	116±27
MVIC60%	CON	100 ± 0	74 ± 9	84 ± 5	78±9	78±12	66±15	80 ±10	75± 5
	CF	100 ± 0	76 ± 7	91 ± 5	78±11	77±13	67±9	82± 7	87± 1
MVIC30 (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



2828

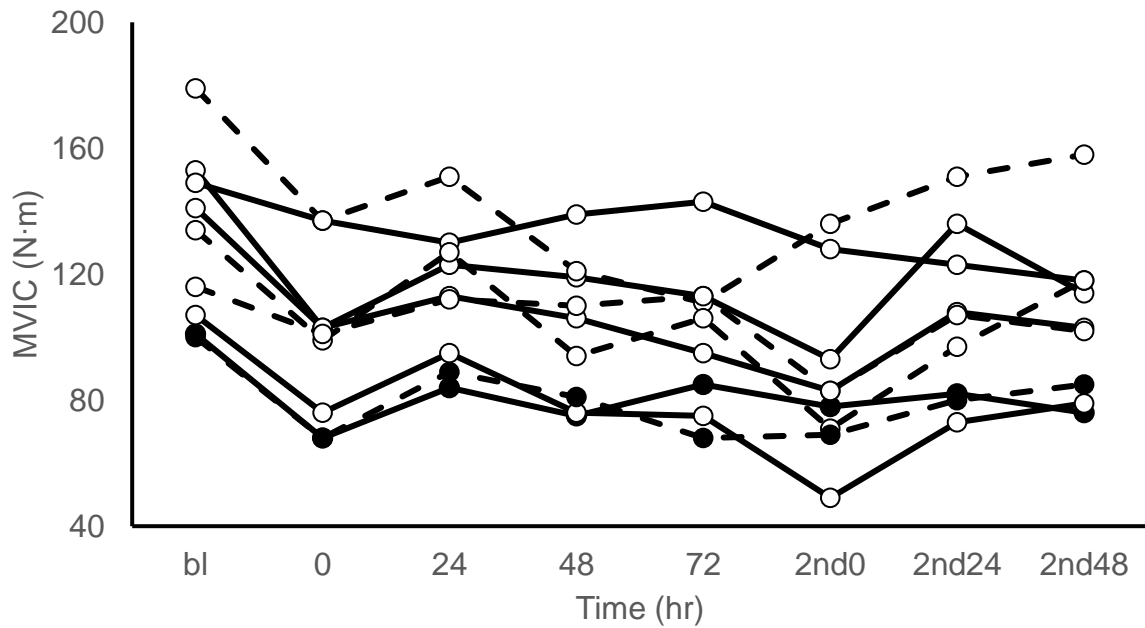
2829 Figure 6.2. MVIC60 percentage change. * denotes significant difference between groups



2830

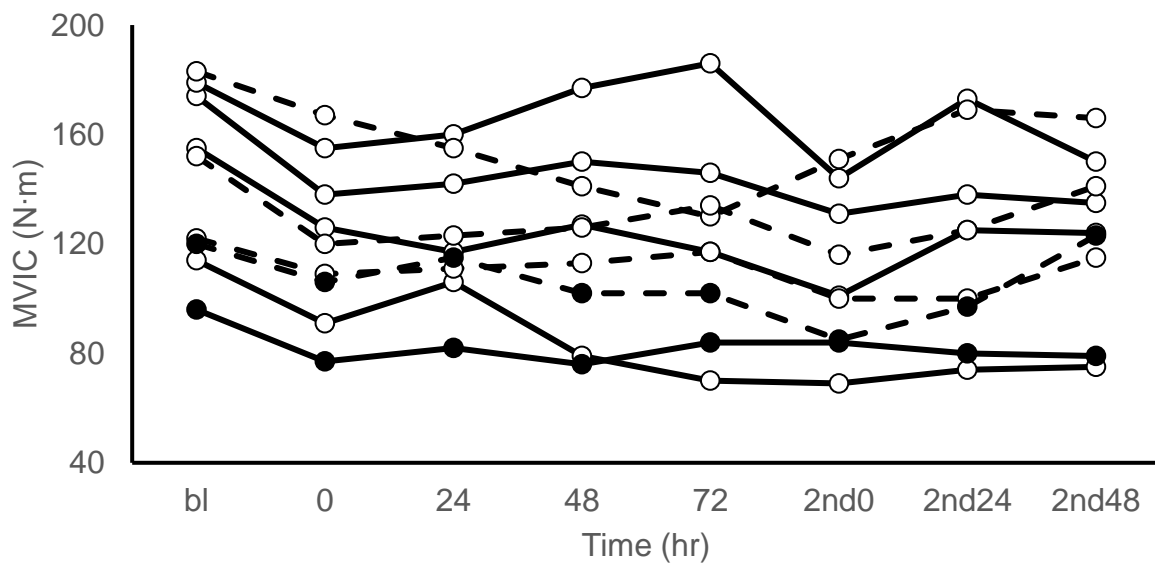
2831 Figure 6.3. MVIC30 percentage change. * denotes significant difference between groups

2832



2833

2834 Figure 6.4 MVIC60 Individual data across all time points; CON = solid lines, CF = dashed
 2835 lines, black circles = females, white circles = males



2836

2837 Figure 6.5 MVIC30 Individual data across all time points; CON = solid lines, CF = dashed
 2838 lines, black circles = females, white circles = males

2839 6.3.3 Electromyography

2840 A significant main effect for time was observed for all EMG data ($p \leq .05$), except for
 2841 median frequency as raw data. For normalised EMG data the greatest number of
 2842 significant differences occurred between 0 and all other time points ($p \leq .03$). For
 2843 median frequency (%) differences were observed between baseline and 0, 72, 2nd0,
 2844 2nd24, and 2nd48 ($p \leq .04$). For median frequency peak, significant differences were

2845 observed between baseline and 0, 2nd0, 2nd24, and 2nd48 ($p \leq .04$). Significant
2846 differences were observed for peak median frequency (%) between baseline and 0,
2847 2nd0, 2nd24, and 2nd 48 ($p \leq .03$). For normalised EMG amplitude data, no significant
2848 differences were observed between the CON and CF groups ($F(1,7) = .028$, $p = .87$).
2849 Data was similar between every time point for normalised EMG values ($p \geq .31$).
2850 Furthermore, no significant differences were observed for median frequency ($F(1,7) =$
2851 $.288$, $p = .61$, % $F(1,7) = 1.075$, $p = .33$) and peak median frequency ($F(1,7) = .227$, p
2852 $= .65$, % $F(1,7) = .024$, $p = .88$).

2853 6.3.4 Perceived soreness

2854 For measures of perceived muscle soreness, there was a significant main effect for
2855 time for VAS ($p < .001$) and LEFS ($p = .002$). For the VAS significant differences were
2856 observed between baseline and all other time points ($p \leq .02$), 0 and 48 and 2nd48 (p
2857 $\leq .04$), 24 and 48, 2nd24, and 2nd48 ($p \leq .04$), 48 and all other time points except 72
2858 ($p \leq .05$), 72 and 2nd24 and 2nd48 ($p \leq .003$), 2nd0 and 2nd48 ($p = .004$). For LEFS
2859 significant differences were observed between baseline and 24, 48, 72, and 2nd0 (p
2860 $\leq .02$), 0 and 48 ($p = .03$), 24 and 48, 2nd24, and 2nd48 ($p \leq .05$), 48 and 2nd24, and
2861 2nd48 ($p \leq .01$), 72 and 2nd24, and 2nd48 ($p \leq .002$), and 2nd0 and 2nd24, and 2nd48
2862 ($p \leq .02$). There were no significant differences observed for VAS ($F(1,7) = 1.262$, $p =$
2863 $.30$) or LEFS ($F(1,7) = .278$, $p = .61$). However, a significant difference and large effect
2864 size was observed between groups at 110 hr post-EIMD (48 hr following the second
2865 protocol) for VAS ($t = 2.484$, $p = .04$, $d = 1.9$). A large effect size was also observed
2866 for LEFS at the same time point ($t = -1.886$, $p = .10$, $d = 1.3$) as well as 24 hr post
2867 initial protocol ($t = -1.614$, $p = .19$, $d = 1.1$) and 24 hr post the second protocol ($t = -$
2868 $.949$, $p = .37$, $d = 0.8$). Perceived muscle soreness was greatest at 48 hr post initial
2869 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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2878

2879 6.4 Discussion

2880 The purpose of this study was to investigate the efficacy of six days of CF
2881 supplementation on recovery following two muscle damaging protocols separated by
2882 72 hr. This is the first CF study to investigate such a protocol and it was hypothesised
2883 that repeated high doses of CF would aid recovery and repeat performance during
2884 strenuous exercise. The data from this study indicate that supplementation did not
2885 offer a significant benefit over a control (matched closely for energy, carbohydrates,
2886 and protein) although, large effect sizes were noted between groups 48 hr post the
2887 second EIMD protocol for both objective and subjective markers of muscle damage
2888 (MVIC60 ($d = 0.8$), MVIC60% ($d = 3.1$), MVIC30 ($d = 0.9$), MVIC30% ($d = 3.2$), VAS
2889 ($d = 1.9$), and LEFS ($d = 1.3$)). This data suggests that there is no significant benefit
2890 of regular CF supplementation following EIMD, although more research is warranted
2891 due to the previously mentioned effect sizes that accounts for the limitations
2892 associated with this study.

2893 Immediately following the first EIMD protocol there were reductions of MVIC of ~20%
2894 immediately and 48 hr post-EIMD in the CON and CF groups; indicating muscle
2895 damage was induced (Paulsen et al., 2012). Even though no significant difference was
2896 observed between CON and CF groups for overall recovery and repeat performance,
2897 by the end of the testing period the CF group showed a greater recovery of muscle
2898 function 48 hr following the second EIMD protocol ($p \leq .005$, $d \geq 3.1$). In fact, the CON
2899 showed a mean change of +12 N (+9%) for MVIC60(%) and +7 N (+4%) for
2900 MVIC30(%) from immediately post-protocol to 48 hr after compared to the CF group
2901 that showed improvements of +26 N (+20%) for MVIC60(%) and +23 N (17%) for
2902 MVIC30(%). Not only that, but the CON group also showed a negative mean change
2903 from 24 hr to 48 hr after the second protocol, whereas the CF group continued to
2904 recover peak torque values. Overall, however, the lack of statistically significant
2905 findings for recovery of muscle function is in line with previous research (de Carvalho
2906 et al., 2019; Morgan et al., 2018; Peschek et al., 2013). However, as this study had a
2907 longer supplementation period and a dose higher than what has been previously seen
2908 in CF and EIMD studies (~545 mg more than the than next highest total dose (Peschek
2909 et al., 2013)) it is possible that a dose above 1000 mg is required to provide any
2910 benefit, as seen with the large effect sizes observed within this Chapter and Chapter
2911 5. This speculation is lent further credence by the review performed by Bowtell and

2912 Kelly (2019) who suggest that (poly)phenol supplementation above 1000 mg for three
2913 days may be required to confer the proposed physiological benefits associated with
2914 their intake. Based on the current evidence it is possible that CF do not provide any
2915 significant benefit for the recovery of muscle function, however there is still a paucity
2916 of literature available to compare results between and more data is needed to provide
2917 a consensus on the possible benefits of CF when supplemented for multiple days.
2918 Furthermore, the differences between studies regarding EIMD protocols, measure of
2919 muscle function, and in CF dose and supplementation period further indicate the need
2920 for future research. The use of a high dose of CF is perhaps required to elicit an
2921 ergogenic benefit due to CF absorption being reportedly around 35% of the initial dose
2922 (regardless of amount); indicating that a greater dose will result in greater CF
2923 absorption (Gómez-Juaristi et al., 2019). However, more research is required to better
2924 understand if there is a ceiling to CF absorption.

2925 Throughout the entire testing period perceived muscle soreness was lower in the CF
2926 group than the CON for VAS, and LEFS (excluding 48 hr post initial EIMD), although
2927 no significant difference was observed ($p \geq .30$). However, a large effect was noted for
2928 both VAS ($p = .04$, $d = 1.9$) and LEFS ($p = .10$, $d = 1.3$) at 110 hr (48 hr post second
2929 EIMD protocol). Interestingly, the CF group had greater reductions of perceived
2930 soreness, measured *via* VAS, following the second EIMD protocol than the CON
2931 group. During this time period (immediately following the second protocol to 48 hr post)
2932 the CF group showed consistent reductions in VAS scores, with a mean reduction of
2933 69 mm, whereas the CON had a mean reduction of 36 mm. This indicates that the CF
2934 treatment may have assuaged feelings of perceived soreness that may arise from a
2935 repeated bout of strenuous exercise. Following the second bout, the CON group VAS
2936 scores increased from 24 hr to 48 hr post, whereas the CF continued to reduce and
2937 was nearly at baseline levels by 48 hr after the second bout. It is possible that the
2938 explanation for this is due to the antioxidant and anti-inflammatory properties of CF,
2939 inflammatory molecules are known to stimulate nociceptors *via* the secretion of protein
2940 degrading enzymes and ROS leading to feelings of pain (Pinho-Ribeiro et al., 2017).

2941 It has been reported that following a secondary bout of soccer match-play and/or
2942 soccer simulation in a fixture congested format, akin to the format of this research,
2943 increase muscle soreness and inflammation (Page et al., 2019). As such, the use of a
2944 high dose of CF may provide a protective effect from potential negative consequences,

2945 such as increased soreness and reduced markers of performance, that may arise from
2946 the residual fatigue associated with repeated bouts of strenuous exercise. However,
2947 the practical applicability of this data may be more suited to repeated bouts of intense
2948 resistance training type exercise due to the nature of the protocol. Interestingly,
2949 previous research investigating the specific impact of two repeated bouts of EIMD
2950 separated by three days has shown that the second bout of exercise does not impede
2951 recovery, with MVIC recovery only impacted immediately following a second bout of
2952 exercise but continuing to recover in the days following (Chen, 2003; Chen & Nosaka,
2953 2006). Indeed, these data differ from Chen (2003) and Chen and Nosaka (2006) as
2954 only the CF groups recovery was not delayed by the second bout of exercise. The
2955 CON group suffered MVIC decrements and an increase in VAS scores from 24 hr post
2956 the second EIMD protocol to 48 hr post, whereas the CF did not. It should be noted
2957 that both the previous studies targeted the elbow flexors whereas the knee flexors
2958 were targeted within the present study. It has been reported previously that the elbow
2959 flexors are more susceptible to muscle damage than the knee flexors which could
2960 partly explain the differences between the studies (Chen et al., 2011). Within team
2961 sports such as soccer, knee flexor injuries are among the most frequent (Engebretsen,
2962 Myklebust, Holme, Engebretsen, & Bahr, 2010; Waldén, Hägglund, & Ekstrand, 2005),
2963 with many of these injuries a result of various factors including accumulated fatigue,
2964 strength imbalances, and previous injury (Opar, Williams, & Shield, 2012). Therefore,
2965 future research could look to investigate nutritional preventative methods for reducing
2966 muscular fatigue, with a specific look at the knee flexors and other posterior thigh
2967 muscle groups.

2968 It is well known that a repeated bout of eccentric exercise leads to skeletal muscle
2969 adaptation that reduces subsequent muscle damage (Hlydahl, Chen, & Nosaka, 2017;
2970 McHugh et al., 1999; Starbuck & Eston, 2012). These adaptations normally lead to a
2971 reduction in the extent of post-exercise strength losses, muscle soreness, expulsion
2972 of myocellular proteins, and potentially a reduced inflammatory response. It is possible
2973 that various neural adaptations occur following the completion of a muscle damaging
2974 exercise bout. The central nervous system may cause an increase in motor unit
2975 synchronisation and alteration in muscle activation patterns as a way of protecting the
2976 fatigued/ damaged muscle and maintain task success (Kellis, Zafeiridis, & Amiridis,
2977 2011; Missenard, Mottet, & Perrey, 2009). This is likely through an increase in the

2978 coordination of synergist muscle to further distribute the mechanical load placed on
2979 the working muscles. The EMG data suggests that this may be the case, as the median
2980 frequency was reduced in both groups but decreased to a greater extent in the CON
2981 group compared to the CF following the second EIMD protocol during the EMG
2982 exercise activity (glute-hamstring bridge) ($75 \pm 3\%$ vs $93 \pm 22\%$, $p = .160$, $d = 1.2$). In
2983 fact, throughout the entire testing period the CON consistently had lower median
2984 frequency values than the CF group. Indeed, other research has shown reductions in
2985 median frequency following EIMD ranging from 20-30% (Chen, 2003; Starbuck &
2986 Eston, 2012; Warren, Hermann, Ingalls, Masselli, & Armstrong, 2000) similar to the
2987 CON group showing a reduction of $25 \pm 3\%$ following the second protocol.

2988 The EMG exercise activity involved other muscle groups, not isolating the knee flexors,
2989 which may explain why EMG amplitude did not show an increase above baseline; an
2990 increase would imply a greater level of motor unit recruitment to perform the action.
2991 Reinforcing the idea that other muscles, e.g., other hip extensors, may have been
2992 recruited to a greater extent to compensate for the fatigued knee flexors. The EMG
2993 amplitude increased from baseline to immediately post-EIMD and 24 hr following in
2994 both groups, which does indicate that there is increased motor unit recruitment of the
2995 knee flexors to perform the exercise task. Whereas immediately post the second
2996 protocol only the CON group amplitude is above baseline (114 ± 39 vs $87 \pm 21\%$, $p =$
2997 $.31$, $d = 0.8$) indicating there may be a change in the activation of surrounding muscle
2998 to perform the task in a fatigued state. This involuntary activity may be protective but
2999 is unlikely to compensate fully for the fatigued muscle. Future research should look to
3000 collect data from synergist muscle groups to account for any changes in their activation
3001 when a specific muscle group is in a fatigued/compromised state.

3002 As EMG acquisition was used to assess to impact of EIMD on the change in muscle
3003 efforts measured *via* EMG amplitude the inclusion of median frequency analysis for
3004 muscle fatigue was considered as a secondary measure for fatigue. To develop a
3005 greater insight into the role of CF on fatigue, longer muscular contractions, and more
3006 frequent data points across each time points (e.g., separated by 30 min at each time
3007 point) would be valuable in future studies.

3008 This study is not without its limitations, namely due to the COVID-19 pandemic. The
3009 pandemic resulted in the cessation of data collection and participant recruitment

3010 leading to only nine participants completing the study. As such, the *a priori* power
3011 calculation was not satisfied, meaning any significant differences and findings should
3012 be interpreted with caution. It is highly likely, that differences observed could be due
3013 to individual variability and perhaps not entirely due to the CF supplementation.
3014 Another limitation is that due to the nature of the muscle damaging protocols used, the
3015 findings may not translate to team sports with short recovery periods, such as soccer.
3016 This is due to the lack of ecological validity associated with the protocol as it does not
3017 reflect realistic sport. Instead, the findings may have more application to repeated
3018 bouts of resistance training and have practical applications for weightlifters, power
3019 lifters, bodybuilders and so on.

3020 Due to the current findings future replication of this study is warranted to better
3021 understand the possible benefits that regular CF consumption may elicit on muscle
3022 recovery. Further limitations are due to constraints regarding the feasibility of specific
3023 measures such as muscle biopsies, inflammatory and oxidative stress markers.
3024 However, this does not detract from the quality of the measures chosen instead rather
3025 the inclusion of biomarkers would have provided greater mechanistic insight into
3026 recovery.

3027 In summary, CF provide no significant benefits for muscle recovery or repeat exercise
3028 performance compared to a carbohydrate protein control. Even though large effect
3029 sizes were observed at the final time point following the second EIMD protocol, it is
3030 possible that this is due to individual variability. As such, further research is required
3031 to better understand the potential beneficial nature of CF supplementation.

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Chapter 7 General Discussion

3037 Athletes desire to improve exercise recovery has resulted in an increase in the interest
3038 of nutritional interventions purported to expedite the recovery process. This thesis
3039 sought to increase the knowledge around one such intervention, CF. The rationale is
3040 that cocoa contains large amounts of bioactive compounds that may provide a
3041 protective effect against muscle damage, most likely against the inflammation and
3042 oxidative stress associated with EIMD. Due to the high concentration of (poly)phenols,
3043 specifically CF, it was hypothesised that a high dose of CF post-EIMD may assuage
3044 the negative consequences associated with muscle damage such as impaired muscle
3045 function, muscle soreness and changes to neuromuscular activation. As such, the
3046 objective of this thesis was to investigate whether CF could be efficacious as an
3047 ergogenic aid for muscle recovery *via* the attenuation of the aforementioned symptoms
3048 of muscle damage (Chapter 2 Section 3). Specifically, this thesis aimed to *i*)
3049 investigate whether a single acute dose of CF impacted muscle damage, *ii*) whether
3050 a 1245 mg dose conferred a greater benefit than 830 mg, *iii*) whether sex had any
3051 influence on the potential effects of CF following EIMD, *iv*) the efficacy of regular
3052 consumption of CF on attenuating EIMD and *v*) whether regular CF consumption aided
3053 repeat performance 72 hr post-EIMD. This Chapter will synthesise the findings of the
3054 investigations of the thesis and their contributions to the existing literature as well as
3055 include a discussion of possible practical applications and future research ideas before
3056 addressing the limitations of the thesis.

3057 7.1 Summary of experimental findings

3058 The first and second experimental studies (Chapter 5 and 6) had the purpose of
3059 addressing the aims *i*, *ii* and *iii*, investigating whether different doses of acute CF
3060 supplementation aided muscle recovery following EIMD and whether there was any
3061 variation between sex. Before experimental testing began, a systematic review
3062 (Chapter 3) was carried out to identify gaps within the literature and areas in need of
3063 development regarding CF and muscle damage. The key findings of the systematic
3064 review (Chapter 3) within the thesis, suggest that CF supplementation blunts exercise-
3065 induced oxidative stress. The exact mechanisms for this are still not clear, it is possible
3066 that supplementation may improve the cellular redox environment making it more
3067 capable to effectively quench ROS, perhaps *via* the upregulation of the Nrf2 pathway
3068 and endogenous antioxidants (Cheng et al., 2013; Cordero-Herrera et al., 2015;

3069 Martins et al., 2020). Additionally, it was noted that CF may delay fatigue and improve
3070 performance during exercise, potentially by limiting ROS induced fatigue.

3071 From the systematic review of Chapter 3, it was identified that evidence for the anti-
3072 inflammatory effects of CF following exercise were limited, along with evidence for
3073 reducing soreness and improving muscle function recovery following EIMD. With only
3074 three previous studies investigating CF supplementation on EIMD (de Carvalho et al.,
3075 2019; Morgan, Wollman, Jackman, & Bowtell, 2018; Peschek, Pritchett, Bergman, &
3076 Pritchett, 2013) it was believed pertinent to contribute to this area of research.
3077 Furthermore, these three studies had utilised relatively low to moderate CF doses that
3078 may not be efficacious based on the surrounding literature regarding the oxidative
3079 stress and possible anti-inflammatory benefits (outside of exercise). Indeed, Peschek
3080 et al., (2013) used two acute doses of 350 mg, de Carvalho et al., (2019) used 616
3081 mg daily for seven days, and Morgan et al., (2018) used a cacao mucilage drink
3082 containing 74 mg CF. This was something I looked to address within this thesis,
3083 utilising high dose CF supplementation. Furthermore, within two of the aforementioned
3084 studies it is possible that the EIMD protocols utilised were perhaps insufficient to elicit
3085 notable muscle damage within their respective cohorts (de Carvalho et al., 2019;
3086 Peschek et al., 2013). Consequently, I selected a validated laboratory based EIMD
3087 protocol (see Chapter 4 Section 4) to induce a desired muscle damage response.
3088 Furthermore, I selected the knee flexors as the muscle group to examine due to their
3089 propensity for injury and the fact that the other CF studies had not yet investigated this
3090 muscle group.

3091 Based on the findings of Chapter 3, it was established that as the available literature
3092 was limited at the time, and for all intents and purpose still is, it was appropriate to
3093 establish whether a high dose of CF had an impact on muscle recovery when
3094 consumed acutely, e.g., immediately post EIMD. It was hypothesised that an acute
3095 dose of CF would attenuate the deleterious symptoms of muscle damage and a higher
3096 (1245 mg vs 830 mg) dose would have a greater effect when compared to a control.
3097 Based on the resultant findings an acute dose had no significant effect on recovery of
3098 muscle function or on measures of perceived soreness. However, large effect sizes
3099 were observed for a number of findings when comparing the highest dose to the
3100 control. When comparing the data as percentage change large effect sizes were
3101 observed between the 1245 mg CF group and the CON for MVIC60% and MVIC30%

3102 at 24 and 48 hr post-EIMD. No significant differences were observed for VAS and
3103 LEFS, however a large effect size was observed between 1245 mg CF and CON at
3104 48 hr for VAS data. Furthermore, moderate effect sizes were observed between 1245
3105 mg CF and 830 mg CF in favour of the higher dose for VAS at 48 hr, and LEFS at 48,
3106 and 72 hr. The findings from Chapter 5a provided valuable information, allowing for an
3107 informed decision to be made on which dose to select for the future study involving
3108 repeated doses of CF (Chapter 6). This study provided evidence that *i*) an acute dose
3109 may be insufficient and *ii*) 1245 mg of CF may be more effective than 830 mg for
3110 assuaging feelings of soreness, albeit not significantly.

3111 For the second experimental study (Chapter 5b) a subgroup analysis was performed
3112 splitting the participants by sex to compare for both inter and intra-sex differences. No
3113 significant differences were observed for any measures for inter- or intra-sex
3114 differences apart from MVIC data analysed as the raw values, when converted to
3115 percentage change no differences were observed. Interestingly however, females
3116 within the CF groups consistently scored lower for levels of perceived soreness than
3117 the CON group, whereas males scored similar values across groups. Specifically for
3118 female VAS scores, when comparing between the treatment groups and control the
3119 data was approaching significance (830 mg vs CON $p = .068$ and 1245 mg vs CON p
3120 $= .059$). Additionally, when comparing soreness between the sexes, females within
3121 the CF groups scored lower than that of the males within the same groups, whereas
3122 in the control group males scored lower than females. For muscle function, MVIC30
3123 percentage change data showed a significant difference between the males and
3124 females in the CF₁₂₄₅ group at 72 hr ($p = .03$), females had returned to baseline values
3125 whereas males only achieved 80%. From this data, it almost appears that the females
3126 gained the greater benefit from CF supplementation when comparing within group
3127 differences for muscle soreness. However, no significant differences were observed
3128 between the groups other than for absolute MVIC data, of which these differences did
3129 not exist when expressed at percentage change. Indeed, this highlights an interesting
3130 area of future research, especially when considering the limited data set and the need
3131 for a fully powered study comparing any sex differences.

3132 For the final experimental study (Chapter 6), the highest dose from the previous
3133 research was selected based off the data that was gathered from Chapter 5. Not only
3134 that, but instead of an acute dose, participants supplemented the CF drink each day

3135 during the experimental protocol and an additional time post-EIMD protocol, of which
3136 there were two separated by 72 hr. This timeline was selected to mimic training
3137 structure for weightlifting sports and somewhat replicate fixture congestion and
3138 tournament settings in team sport, as these times require rapid recovery to ensure
3139 optimal performance and to limit injury risk. For this study it was hypothesised that
3140 based on the data from Chapter 5, indicating that an acute dose was insufficient to
3141 confer a significant benefit for attenuating the symptoms of muscle damage and as
3142 such regular supplementation may be required throughout the recovery period, as
3143 seen with other (poly)phenol research (Bell et al., 2016; Quinlan & Hill, 2020; Tanabe
3144 et al., 2019). Within Chapter 6, no overall significant differences were observed based
3145 on the data, likely due to the limited sample size of the study. However, even though
3146 participant numbers for this study were limited, significant differences and large effect
3147 sizes were observed when analysing the final time point between the CF and CON
3148 group for muscle function as percentage change, as absolute values, and for VAS
3149 data (which was also significantly different). Additionally, immediately post the second
3150 EIMD protocol a large effect size was observed for EMG data expressed as median
3151 frequency percentage change ($75 \pm 3\%$ vs $93 \pm 22\%$, $p = .16$, $d = 1.2$). Again,
3152 immediately post the second protocol only the CON group had a higher EMG
3153 amplitude compared to baseline when expressed as a percentage change (114 ± 39
3154 vs $87 \pm 21\%$, $p = .31$, $d = 0.8$). Therefore, it is possible that following the repeated bout
3155 of strenuous exercise the CON group was in a greater fatigued state than the CF group
3156 due to the possible protective effect of CF allowing for continued recovery as opposed
3157 to impeded recovery. For isokinetic data, the CON group had greater reductions in
3158 isokinetic concentric and eccentric peak torques than the CF group for the non-
3159 dominant limb, the one involved for MVIC. This may imply that the consumption of CF
3160 may aid with the maintenance of maximal performance during a repeated bout of
3161 exercise in a fatigued state.

3162 Collectively, the results provided from the experimental studies conducted for this
3163 thesis add interesting and novel insights to the current body of knowledge and indicate
3164 a pertinent need for further research. Even though there was a lack of statistical
3165 significance within the research the large effect sizes provide some evidence that there
3166 may be a beneficial effect of CF supplementation. These data warrant future studies

3167 including prolonged supplementation of high dose of CF and the potential effects they
3168 may elicit on muscle recovery.

3169 **7.2 Cocoa Flavanols impact on muscle function and perceived soreness**
3170 The growing demands of sport, such as the increase in fixture congestion observed in
3171 soccer, in turn propagates a potential increase in EIMD and for this reason ergogenic
3172 aids to improve recovery are becoming an integral part of many athlete's and general
3173 individual's training regimen. This thesis was to examine the efficacy of such an aid in
3174 a scenario that elicited muscle damage. From the data it was observed that the
3175 consumption of CF whether acute or regular resulted in no significant advantage over
3176 a carbohydrate-protein control recovery beverage for muscle recovery measured *via*
3177 muscle function and muscle soreness.

3178 7.2.1 Muscle function

3179 Specifically for the recovery of muscle function, assessed *via* MVIC and in Chapter 6
3180 EMG, no overall significant effects were observed. This is in line with previous
3181 research that has used MVIC as a recovery marker (de Carvalho et al., 2019; Morgan
3182 et al., 2018; Peschek et al., 2013). A key difference between those studies and the
3183 ones included within the thesis is the selection of muscle group targeted for muscle
3184 damage. The other studies investigated the impact of CF on knee extensor recovery
3185 as opposed to knee flexors. Knee flexors are more susceptible than the knee
3186 extensors to muscle damage (Chen et al., 2011); thus, the knee flexors may be a more
3187 pertinent muscle group to investigate. Even more so when considering the evidence
3188 for knee flexor injuries being amongst the most common in sport (Chumanov,
3189 Schache, Heiderscheit, & Thelen, 2012; Opar et al., 2012; Small, McNaughton, Greig,
3190 Lohkamp, & Lovell, 2009a)

3191 For measures of muscle function other than MVIC, Morgan et al., (2018) found that
3192 supplementation of cacao mucilage provided a significant benefit for the recovery of
3193 countermovement jump height. Indicating there may be some benefit for explosive
3194 power compared to strength. However, as a measure, countermovement jump height
3195 was not included within this thesis, this choice was made due to the difference in
3196 muscle contribution when comparing the role of the knee extensors to the knee flexors.
3197 The biceps femoris has been shown to maintain low level activation during the entire
3198 movement of a countermovement jump, however it only reaches around 40% of
3199 maximal activation, whereas the rectus femoris reaches ~100% of maximal activation

3200 (Mackala, Stodólka, Siemienski, & Coh, 2013). This means that any changes in
3201 muscle fatigue will be more evident in muscles with greater levels of activation during
3202 the exercise.

3203 Within Chapter 5a multiple large effect sizes were observed at 48 hr between the CON
3204 and CF group supplementing 1245 mg acutely for MVIC measures. However, as no
3205 significant difference was observed it is likely that an acute dose was insufficient in
3206 conferring a beneficial effect for the recovery of force generating capability. Peschek
3207 et al., (2013) also found no significant benefit of CF on MVIC following two acute doses
3208 of 350 mg CF, in fact the CF group had a greater loss in MVIC than the control group
3209 (~5% decrease in the control vs 11 and 22% decrease in CF from pre – 48 hr post in
3210 right and left legs, respectively). What is interesting from Peschek et al., (2013) data,
3211 however, is that the CF group MVIC increased from 24 to 48 hr post-EIMD whereas
3212 the control group did not. As the control group did not show signs of impaired muscle
3213 function it is possible that the EIMD protocol (downhill running) was insufficient for the
3214 well-trained endurance athletes used within the study. From a mechanistic perspective
3215 it is essential to elicit a muscle damage response when investigating the effects of an
3216 intervention on muscle damage, something this thesis aimed to do whilst utilising the
3217 laboratory-based protocol. As such, future research is warranted to reach a consensus
3218 about the potential ergogenic effects on recovery an acute dose of CF may have as
3219 there is a dearth of research in the area.

3220 In Chapter 6 large effect sizes were observed 48 hr post the second protocol when
3221 comparing regular supplementation of 1245 mg CF to a CON. These data were
3222 accompanied this time by a statistically significant difference at the final time point for
3223 MVIC60 percentage change ($p = .004$, $d = 3.1$) and MVIC30 percentage change ($p =$
3224 $.005$, $d = 3.2$) with large effect sizes alone noted for the absolute values at the same
3225 time point (MVIC60 $d = 0.8$ and MVIC30 $d = 0.9$). Other studies that utilised repeated
3226 dosing of CF have previously found no benefit for MVIC recovery (de Carvalho et al.,
3227 2019; Morgan et al., 2018). Both of the previous studies incorporated MVIC of the
3228 knee extensors as a measure, however both only included a single angle of
3229 measurement. It is possible that following EIMD with a high amount of eccentric
3230 muscle contractions the optimum angle changes to longer muscle lengths, evidenced
3231 in the knee extensors (Bowers, Morgan, & Proske, 2004) and knee flexors (Brockett,
3232 Morgan, & Proske, 2001). As such, a single angle may over or underestimate changes

3233 in contractile capability following EIMD (Paulsen et al., 2012). Participants within the
3234 study by de Carvalho and colleagues (2019) appear to not have suffered from the
3235 deleterious effects associated with muscle damaging exercise as at 48 hr post-EIMD
3236 both the control and CF group had MVIC scores $\geq 103\%$ of baseline. Conversely, P.
3237 Morgan et al., (2018) found significant reductions in MVIC following the EIMD protocol,
3238 interestingly they found that at the final time point (48 hr post-EIMD) the treatment
3239 group had recovered to $90.8 \pm 14\%$ of baseline, whereas the control group only 85.1
3240 $\pm 15.6\%$ however this difference was non-significant. Similar to Chapter 6, Morgan and
3241 colleagues (2018) supplemented each day of recovery (as well as seven days before
3242 the protocol). In Chapter 6 participants supplemented every day of the testing period,
3243 twice on protocol days, totalling six days of supplementation. Although both studies
3244 saw that the CF group had a greater MVIC percentage on the final day of testing, only
3245 the data within Chapter 6 observed a large effect size between the groups. It is
3246 possible that the reason for this is the large difference between CF dose used in the
3247 two studies, Morgan and colleagues (2018) supplemented with a cacao juice drink
3248 containing 74 mg CF whereas Chapter 6 utilised a dose of 1245 mg. Indicating that
3249 perhaps regular supplementation of a high dose of CF is necessary to gain an
3250 additional benefit for MVIC recovery compared to a carbohydrate protein control.

3251 Regarding the EMG data gathered in Chapter 6 for median frequency, it was found
3252 that the CON group had a similar reduction in median frequency as what has been
3253 observed in other research investigating EMG activity of muscle groups following two
3254 EIMD protocols (Chen, 2003; Starbuck & Eston, 2012; Warren et al., 2000). However,
3255 the CF group within the Chapter did not follow the same pattern, instead the reductions
3256 observed were smaller, albeit not significantly ($75 \pm 3\%$ vs $93 \pm 22\%$, $d = 1.3$). Other
3257 research has observed that supplementation using green tea extract prevented a
3258 decrease in median frequency of the left vastus lateralis during a period of cumulative
3259 fatigue (Machado, da Silva, Souza, & Carpes, 2018). Furthermore, the EMG amplitude
3260 post second EIMD protocol was far greater in the CON group than the CF ($114 \pm 39\%$
3261 vs $87 \pm 21\%$, $d = 0.8$) indicating that although the frequency is reduced the biceps
3262 femoris is still at a greater level of motor unit recruitment than baseline in the CON
3263 group. This may suggest that regular CF, or other (poly)phenol supplementation
3264 provides a protective effect against muscle related fatigue and its impact on task
3265 completion. Therefore, to garner a greater understanding of this proposed mechanism

3266 future research should gather data from other synergist muscles during the exercise
3267 task to assess change in involvement.

3268 7.2.2 Perceived soreness

3269 For measures of perceived soreness, the experimental investigations conducted for
3270 this thesis did not observe any significant differences between CON or CF groups for
3271 either VAS or LEFS. However, similar to muscle function, multiple large effect sizes
3272 were observed in Chapter 5a for VAS at 48 hr post-EIMD ($d = 0.9$) and in Chapter 5b,
3273 48-hr post second EIMD protocol for VAS ($d = 1.9$). For LEFS, large effect sizes were
3274 observed in Chapter 6 at 24 hr post-EIMD ($d = 1.1$), then at 24 hr ($d = 0.8$) and 48 hr
3275 ($d = 1.3$) post second EIMD protocol. This indicates that CF may provide some
3276 analgesic benefit over carbohydrates and protein alone, assuaging feelings of
3277 perceived muscle soreness following a second bout of strenuous exercise. Although
3278 these large effect sizes were observed within this thesis, no significant differences
3279 were found. This is in line with other data from previous CF EIMD research (de
3280 Carvalho et al., 2019; Morgan et al., 2018; Peschek et al., 2013). It is possible that as
3281 the data in this thesis highlights large effect sizes between the groups it may be that
3282 the previous studies did not provide sufficient amounts of CF to induce any analgesic
3283 benefit. Indeed, this thesis included the largest CF dose seen within muscle damage
3284 research at 1245 mg, providing novel insights into CF dosing strategies. Furthermore,
3285 as De Carvalho and colleagues (2019) and Pescheck and colleagues (2013) did not
3286 appear to induce notable muscle damage based on the data, it is possible that the
3287 addition of CF would not significantly influence soreness due to the protocols only
3288 causing moderate increases in VAS scores.

3289 As muscle tissue samples and markers of inflammation were not measured in this
3290 thesis, the underlying mechanisms by which CF may impact feelings of soreness
3291 remains elusive. Speculatively, it is likely due to the anti-inflammatory and antioxidant
3292 properties of CF. Inflammatory molecules are known to stimulate nociceptors which
3293 are responsible for 'pain signals' indicating that an upregulated anti-inflammatory
3294 response at the site of muscle damage may in turn reduce overall feelings of soreness,
3295 evidenced by the benefits observed following ibuprofen (Tokmakidis, Kokkinidis,
3296 Smilios, & Douda, 2003) and various (poly)phenol compounds (Herrlinger, Chirouzes,
3297 & Ceddia, 2015; Zhang & Tsao, 2016).

3298 Furthermore, the role that CF may have on inflammation and oxidative stress may be
3299 the driving mechanism by which it aids both muscle function and soreness. The
3300 ingestion of CF immediately post-EIMD whether just acutely or as part of a
3301 supplementation period may reduce the acute rise in cytokines and inflammatory
3302 mediators post-EIMD (Paulsen et al., 2012; Pizza et al., 2005). These molecules can
3303 further damage the response by propagating an increase in the accumulation of pro-
3304 inflammatory molecules, e.g., TNF- α and ROS, which can damage healthy bystander
3305 tissues (Paulsen et al., 2012). Reducing or limiting this increase may enhance
3306 recovery by expediting the recovery of muscle function, which may be influenced by
3307 the redox state of the muscle and level of inflammation present (Powers, Ji, Kavazis,
3308 & Jackson, 2011), and reduce soreness, which can increase perceived effort during
3309 exercise and potentially – depending on severity, inhibit performance to avoid pain
3310 (Staiano, Bosio, de Morree, Rampinini, & Marcora, 2018).

3311 Therefore, when combining the evidence from Chapters 5 and 6, it is likely that regular
3312 supplementation is required to confer the benefits that have been observed with other
3313 (poly)phenol nutritional aids like beetroot juice (Clifford, Bell, et al., 2016; Clifford et
3314 al., 2017), curcumin (Ms et al., 2020; Tanabe et al., 2019), and Montmorency tart
3315 cherries (Connolly et al., 2006; Kuehl, Perrier, Elliot, & Chesnutt, 2010). However, for
3316 each article evidencing the benefits of these functional foods there is another that does
3317 not observe any protective effect or instead showcases limited efficacy compared to a
3318 placebo (Abbott, Brashill, Brett, & Clifford, 2020; Costello et al., 2020; Lamb et al.,
3319 2019).

3320 7.2.3 Sex Differences and Cocoa Flavanols

3321 Another novel aspect of this thesis is the intra- and inter-sex comparisons of Chapter
3322 5b. There is potential for sex differences to exist regarding reductions in oxidative
3323 stress following the consumption of different (poly)phenol blends (Burton-Freeman et
3324 al., 2010), and oxidative stress is a key contributor to muscle damage. However, to
3325 date, no research has looked to compare sex differences for the muscle damage
3326 response following CF consumption. Based on the data in Chapter 5b there are no
3327 significant differences, although further research utilising oxidative stress markers is
3328 warranted. However, when comparing female VAS scores from the CON group to the
3329 two CF groups the data was approaching significance (CON vs CF₈₃₀ p = .068 and
3330 CON vs CF₁₂₄₅ p = .059) with both scoring large effect sizes at 24 and 48 hr post EIMD

3331 ($d \geq 1.2$). Specifically, the CF₁₂₄₅ females had significantly lower VAS scores than the
3332 CON females at 24 hr ($p = .03$ $d = 1.6$) and 48 hr ($p = .03$ $d = 1.6$). It is possible that
3333 there is a compounding element to the antioxidant and anti-inflammatory effects of CF
3334 and oestrogen which may in turn reduce feelings of soreness. This is perhaps further
3335 evidenced as females scored lower VAS scores than males did apart from in the CON
3336 group.

3337 As all females were tested within the luteal phase of their menstrual cycle it is likely
3338 that oestrogen will play a role. Evidence has recently shown that females suffer higher
3339 levels of soreness following EIMD when performing in the follicular phase,
3340 characterised by low levels of circulating oestrogen (Romero-Parra, Alfaro-
3341 Magallanes, et al., 2020; Romero-Parra, Barba-Moreno, et al., 2020). Therefore, a
3342 reduction in pro-inflammatory molecules such as TNF- α and IL-1 β would likely
3343 coincide with reduced feelings of soreness as these molecules are known to sensitise
3344 nociceptors within the muscle (Pinho-Ribeiro et al., 2017). Not only that but the
3345 females within CF₁₂₄₅ returned to baseline MVIC levels for MVIC30 at 72 hr whereas
3346 the males within the same group remained at $80 \pm 1\%$ of baseline ($p = .03$ $d = 2.3$). It
3347 is possible that when combining the findings for soreness and muscle function it seems
3348 more evident that females supplementing the highest dose of CF may have had the
3349 greatest benefit. However, more research is needed to elucidate differences in
3350 menstrual cycle phase and (poly)phenol metabolism as well as the inclusion of a
3351 battery of tests to measure inflammation to better understand the mechanisms
3352 involved in recovery. Furthermore, a larger cohort of participants should be included
3353 to reduce the chance of individual variation impacting results.

3354 7.3 Practical applications

3355 Based on the findings of this thesis, there are some practical applications that could
3356 be considered for the use of CF as an ergogenic aid for muscle recovery. However,
3357 no statistically significant findings were observed overall and only at specific time
3358 points during the experimental studies. Therefore, these should be considered
3359 cautiously and trialled before implementation in a practical setting to judge the
3360 effectiveness.

3361 Firstly, CF consumption at high doses immediately post-EIMD may attenuate the
3362 deleterious effects that muscle damage has on muscle function, specifically with
3363 muscle force production, albeit not significantly compared to a carbohydrate-protein

3364 control. These benefits were noted at the 48-hr post-EIMD in Chapter 5 and then at
3365 the 48-hr point post a second protocol in Chapter 6. Furthermore, the protocol used
3366 within this thesis induced notable muscle damage, characterized *via* strength losses
3367 of $\geq 20\%$ with reductions existing for ≥ 48 -hr (Paulsen et al., 2012). Thus, for athletes
3368 entering a period of performance with multiple strenuous bouts of exercise or periods
3369 that have a need for expedited recovery such as a fixture congested period,
3370 tournament setting in multiple team sports such as hockey, or Olympic athletes that
3371 compete daily or multiple times a day, e.g., judo, may find use of CF or (poly)phenols
3372 to aid recovery.

3373 Secondly, similar results were noted for reductions in perceived pain as CF appeared
3374 to assuage feelings of soreness 48-hr post EIMD protocol in Chapter 5 and 48-hr post
3375 second EIMD protocol in Chapter 6. As muscle soreness can increase the perception
3376 of effort during exercise and reduce exercise performance (Pageaux, 2016) it may be
3377 beneficial during times when soreness is prevalent, e.g., fixture congestion, pre-
3378 season, long lasting competitions such as the tour de France or various tournaments
3379 such as in hockey and tennis. Furthermore, perception of recovery, e.g., reduced
3380 soreness, may be of benefit to athletes in understanding their own recovery and
3381 performance readiness.

3382 From this thesis however, no recommendation can be given with certainty regarding
3383 an effective dose of CF other than the greatest benefit was observed following a dose
3384 of 1245 mg. Pairing this with other research recommendations for (poly)phenol doses,
3385 regular supplementation of ≥ 3 days may be the most efficacious in achieving the
3386 desired effects. Future research should look to further investigate the efficacy of a high
3387 dose of CF for recovery in sporting settings including a wide range of participants
3388 across the athletic pyramid, e.g., elite and/or recreational individuals. For a wide
3389 implementation of CF in athletic settings there is a requirement for more evidence of
3390 their benefits and an increase in the commercial availability of high flavanol cocoa
3391 powder.

3392 Finally, a potentially interesting measure to include in future research regarding
3393 recovery drinks is a palatability scale or short questionnaire regarding the taste,
3394 texture, and enjoyment of the drink. This could allow for increased athlete usage if the
3395 drink is widely considered enjoyable, as one element that detracts from other recovery

3396 aids, such as beetroot juice, is the taste. The development of a comparative index of
3397 nutritional ergogenic aids with reference to the benefits and taste may be worthwhile
3398 for applying their use into a practical setting. Additionally, as taste preferences vary on
3399 individual basis and are influenced at a genetic level (Eriksson, Esberg, Haworth,
3400 Holgerson, & Johansson, 2019), the use of any nutritional aid should be trialled on an
3401 individual basis.

3402 7.4 Future Directions

3403 The series of experimental studies compiled within this thesis have provided some
3404 interesting findings and have also uncovered avenues for future research relating to
3405 CF, muscle recovery and wider nutritional interventions. This section will cover some
3406 of these ideas.

3407 The findings from both Chapter 5 and Chapter 6 have raised a pertinent question, can
3408 a very high dose of CF be efficacious as an ergogenic aid for muscle recovery. Neither
3409 Chapter observed significant differences for treatment however large effect sizes were
3410 found between the groups. As Chapter 6 was underpowered as discussed earlier in
3411 the thesis (see Chapter 6 Section 5) it would be of great interest for future research to
3412 follow a similar loading protocol with a sample size that satisfies a power calculation.
3413 This would allow for a continuation of the research and increase the understanding of
3414 the potential benefits of CF when consumed as a high dose (e.g., >1000 mg).

3415 A study investigating the fate of the various flavanols and their metabolites following
3416 the consumption of a high dose would also provide a greater insight into the possible
3417 bioactivity of CF. This would also aid with the creation of a recommended amount if
3418 perhaps there is a ceiling for the absorption of CF, so a comparative study with various
3419 doses may provide insight into that. Furthermore, as it has been recently identified that
3420 the metabolites of CF from the gut microflora may still remain in circulation >24-hr
3421 post-consumption (Gómez-Juaristi et al., 2019; Spencer, Schroeter, Rechner, & Rice-
3422 Evans, 2001), any future study should look to include numerous timepoints that span
3423 over a 24-hr period. Not only that but these studies may benefit from including both
3424 plasma and urinary samples to identify the rate of appearance in circulation but also
3425 the rate of excretion. To allow for possible practical applications of research
3426 investigating the bioavailability of CF, a commercial high flavanol cocoa powder may
3427 be the best option. This way other investigations can take place using the same
3428 product and it would be accessible for the general public to acquire and consume.

3429 Another important question for future studies is whether CF exert any benefit on EIMD
3430 when elicited by an ecologically valid protocol, i.e., repeated sprints, soccer simulation
3431 or real-world sporting scenarios, i.e., match play. These are the scenarios that may
3432 require expedited recovery the most, especially in soccer due to the increased
3433 prevalence of fixture congestion and reduced recovery time (Julian et al., 2020). Even
3434 though female soccer has fewer fixtures than male soccer, there are still times when
3435 recovery may be crucial for team success, specifically during tournament scenarios.
3436 Throughout the thesis the muscle damaging protocol used did not replicate the
3437 demands of any specific exercise other than maximal contractions of the knee flexors.
3438 However, Chapter 6 from within this thesis followed a structure akin to a fixture
3439 congested period albeit with muscle damaging protocols as opposed to soccer
3440 simulation or match play. Based on the findings of that investigation it may be possible
3441 that CF would exert a benefit for recovery following the second bout of soccer exercise
3442 72 hr post the initial. The study uncovered that the individuals within the CF group
3443 were unimpeded by the second bout of exercise for recovery of muscle function and
3444 soreness, whereas the CON had their recovery impeded by the second bout,
3445 evidenced by drop-offs in MVIC and increased soreness. Large effect sizes were
3446 observed for all MVIC measures and VAS at 48 hr post the second protocol between
3447 the groups. This improved recovery could be pertinent in an actual sporting scenario
3448 in which rapid recovery could be a determining factor for success. As such, future
3449 studies should look to supplement CF alongside exercise protocols that mimic the
3450 demands of match play within a fixture congested schedule, possibly including an
3451 extra-time period during the second bout of exercise due to the added physiological
3452 and biomechanical load and exacerbated fatigue (Field, Corr, et al., 2020; Field, Page,
3453 et al., 2020). The inclusion of performance markers to assess recovery such as
3454 countermovement jump height, reactive stress index, muscle force output, and sprint
3455 time will greatly enhance the knowledge base for the impact of CF on muscle recovery
3456 and exercise performance.

3457 One of the interesting findings observed in Chapter 6 related to the EMG data obtained
3458 immediately post the second exercise bout that week was related to the median EMG
3459 frequency. The CON group had a reduced median frequency to $75 \pm 3\%$ of baseline,
3460 indeed this reduction implies that other synergist muscles may have been recruited to
3461 aid with exercise task completion. The CF group however, reduced to $91 \pm 14\%$ of

3462 baseline which indicates a greater usage of the knee flexors during the exercise task.
3463 Perhaps the difference observed came as a result of the supplementation of CF
3464 attenuating muscle damage allowing for a greater level of direct hamstring muscle
3465 usage. To better understand the role of CF on muscle fatigue future studies should
3466 look to collect EMG data from other contributing muscle groups for the chosen
3467 exercise. In the case of this thesis for the chosen exercise (glute-hamstring bridge)
3468 other hip extensors such as the gluteus maximus may have been preferentially
3469 recruited to aid with task completion. It has been previously reported that when in a
3470 fatigued condition the role of the hamstring muscle group appears to be different
3471 compared to non-fatigued (Edouard et al., 2018). Indeed, other muscles perform
3472 compensatory mechanisms to maintain performance and protect the fatigued muscle.
3473 Gathering more EMG data for various contributing muscle groups will allow for greater
3474 insight into the fatigue mechanics elicited from EIMD and whether CF or other
3475 (poly)phenol treatment may reduce muscle fatigue evidenced by the data.

3476 7.5 Limitations of the thesis

3477 The main limitation of this thesis is the lack of direct muscle biopsies, inflammatory
3478 markers, and oxidative stress markers. These markers would have provided a greater
3479 understanding of the mechanisms by which CF may act on a damaged muscle to aid
3480 recovery.

3481 The reason that such markers were not included is due to cost restrictions and the
3482 limited facilities available, instead it was opted to only measure for non-invasive,
3483 accessible, and tangible measures of muscle damage such as changes in muscle
3484 function and perceived soreness. As discussed in Chapter 2, section 5, changes in
3485 muscle function, such as reductions in force generating capabilities, is considered one
3486 of the best measures of muscle damage (Paulsen et al., 2012; Warren et al., 1999),
3487 whilst also providing practical evidence of an individual's capacity to perform exercise.
3488 Importantly, as the choice of measure was maximal voluntary isometric contraction
3489 multiple angles were chosen as to not over or underestimate changes in force
3490 generating capability of the knee flexors. Changes in perceived muscle soreness are
3491 a more subjective marker however and inherently individual as ways of measuring
3492 pain, e.g., *via* a VAS, are reliant on what a participant considers a great deal of pain
3493 as an anchor point. For this reason, two methods of measuring soreness were
3494 included (VAS and LEFS) and recent relevant literature was utilised to best inform the

3495 optimal use of a VAS and its anchor points (Reed & Van Nostran, 2014). Mechanistic
3496 markers would have provided valuable insight and future studies should look to
3497 incorporate inflammatory markers and oxidative stress markers if feasible.

3498 During Chapter 5 there was a split of males and females. Females completed the
3499 experiment during the luteal phase of the menstrual cycle, and to calculate this, a
3500 calendar method was employed alongside a menstrual cycle history questionnaire to
3501 account for >2 previous cycles. Although this method can be relatively accurate and
3502 has been validated in the field, it is not as accurate as hormonal testing (Wideman et
3503 al., 2013). Ideally, regular blood sampling would take place to accurately define the
3504 menstrual cycle phase alongside regular individual tracking of the menstrual cycle,
3505 including >2 previous cycles. Recently, it has been recommended that to reduce
3506 possible variability in the data, as each menstrual cycle is relatively variable within and
3507 between individuals, outcome measures should be repeated in another cycle (Elliott-
3508 Sale et al., 2021). This undertaking would be extremely difficult in EIMD research,
3509 namely due to the repeated bout effect and as such is not something that could be
3510 achieved within the time frame of this PhD. Additionally, it was difficult to balance the
3511 groups for contraceptives used by each female participant, of the 13 females within
3512 the study eight were classed as naturally menstruating, three were on the oral
3513 combined pill and two were on the Depo-Provera injection. For the most accurate
3514 comparisons all participants would have either been naturally menstruating or all on
3515 one specific contraceptive. However, this would reduce the generalisability of the
3516 cohort as not everyone is on one type of contraceptive.

3517 Another limitation of the thesis is the sole use of a laboratory-based exercise protocol
3518 to induce muscle damage. Even though the protocol induced notable EIMD it is not an
3519 ecological valid method of doing so due to the isolation of the knee flexors. Utilising a
3520 more ecologically valid protocol such as a repeated sprint protocol, one that replicates
3521 the demands and movement patterns of real-world exercise, make findings more
3522 generalisable to sporting settings. Additionally, team sports such as soccer commonly
3523 include changes of direction, physical contact with other players, jumping, kicking and
3524 other movement patterns, something most EIMD protocols lack. Therefore, for
3525 research to better translate to the practical setting selecting the protocol is a pertinent
3526 aspect to consider. It is worth noting that laboratory-based protocols are still an

3527 important feature of muscle damage research due to their established reliability in
3528 eliciting the desired muscle damage response.

3529 Another limitation of the thesis is the reduced data set for the final experimental study
3530 (Chapter 6), due to the coronavirus pandemic data collection was hindered drastically.
3531 The repeat national lockdowns that occurred three times from March 2020 to March
3532 2021 meant that further data collection was not possible. From the end of the final
3533 lockdown, it was decided that there would be no return to the laboratory to continue
3534 with the study due to the time intensive nature of the protocol and the high probability
3535 of frequent dropouts due to possibility of a positive covid-19 test result. Not only that
3536 but University policy was reactionary to the ever-changing pandemic environment
3537 making forward planning almost impossible from a research perspective, especially
3538 when having to factor in six consecutive testing days, the University itself put all
3539 research endeavours 'on hold' during each national lockdown and during the local
3540 lockdowns also. Furthermore, it was also considered that many potential participants
3541 would be in a state of detraining and suffer a large muscle damage response, as most
3542 gyms were closed many individuals were unable to perform regular resistance training.
3543 This could result in skewed data from an exaggerated response from 'untrained'
3544 individuals. Not only that but participant injury risk was considered also. As the testing
3545 period incorporated two bouts of muscle damaging protocols it was possible that this
3546 may also increase the withdrawal rate of participants due to fear of injury. Due to the
3547 final study being underpowered the findings should be approached cautiously as
3548 reduced cohort numbers can increase the chance of large effect sizes, which were
3549 noted within the study.

3550 As with all research, ethical considerations arose throughout this thesis, especially due
3551 to the nature of the maximal, strenuous exercise that participants volunteered to
3552 perform. Within both Chapter 5 and Chapter 6, a total of three participants withdrew
3553 due to 'injury related reasons', indicating that at times for a few individuals the muscle
3554 damage response experienced was too severe for them to feel comfortable continuing
3555 with the research. Participant health and wellbeing remained the utmost importance
3556 throughout this PhD. Indeed, all participants were reminded to continue to rest
3557 following the completion of the study before recommencing any training and ensure
3558 they were fully recovered following the protocol.

3559 7.6 Reflections During a Global Pandemic

3560 Conducting the final 18 months of this PhD during a global pandemic created
3561 difficulties that could not have been predicted in the months leading up to the
3562 beginning of the covid-19 period. The main issue was the cessation of data collection
3563 due to the closure of the University, this period of 'no data' has continued till now in
3564 the summer of 2021. Measures were put in place to return to data collection in October
3565 – December 2020, however the second national lockdown immediately ended that
3566 endeavour. Thankfully, enough data was collected prior to the University closure to
3567 allow for statistical analysis for a third and final study, albeit an underpowered one.

3568 The pandemic also provided time to reflect on the PhD experience and just how much
3569 it has changed over the previous 18 months. The greatest shift was in the environment
3570 (not just for the obvious reasons), from conducting research, meetings, conferences,
3571 and teaching in person it was now performed remotely. Not only that but the PhD
3572 research community that had been ever present during the first half of the PhD had
3573 now all but vanished along with virtually all in-person social interactions. This along
3574 with living alone magnified the isolated feelings that had begun to brew over the initial
3575 lockdown. As time went on, I adjusted to the 'new world' and the new normal.

3576 As data collection could not be continued, progress had to be made elsewhere. In this
3577 regard, the first publication of the thesis occurred in June/July 2020 (what is now
3578 Chapter 5 in the thesis) and in July 2021 published what is now Chapter 3. Alongside
3579 this I wrote an article for The Conversation in August 2020 (translated into French in
3580 December 2020) about the benefits of CF and a brief history of chocolate. This article
3581 was among the top read from those published by academics at the University of
3582 Huddersfield in 2020. Authoring the article improved my ability to write for a lay
3583 audience whilst still managing to get key information across to the reader. Additionally,
3584 in 2020 and 2021 I was part of the organising committee for the internal Engage
3585 conference at the University of Huddersfield aimed at postgraduate research students
3586 within the School of Human and Health Sciences. Due to the pandemic the conference
3587 was held online on both occasions. In doing so, the committee's main aim was
3588 recreating the supportive, community feeling that was present at previous in-person
3589 versions. To summarise, despite the circumstances I believe I have made progress in
3590 key aspects of being a researcher and academic.

3591 A similarity between the Conquistadors and I is that we both had our own New Worlds
3592 to find our footing in. All in all, although the coronavirus pandemic impacted the final
3593 study, preventing it from reaching statistical power, it does not detract from the
3594 interesting and novel findings of this thesis.

3595

3596 7.7 Conclusion

3597 To summarise, the three experimental studies that complete this thesis indicate that *i*)
3598 an acute dose of CF has no significant impact on muscle damage over a carbohydrate-
3599 protein control, *ii*) there are no significant differences between sex regarding an acute
3600 dose of CF on markers of muscle damage with a small benefit observed for soreness
3601 in females when consuming 1245 mg CF, *iii*) regular supplementation of 1245 mg CF
3602 may reduce accumulated fatigue associated with a repeated bout of strenuous
3603 exercise indicated by the recovery of muscle function, maintenance of knee flexor
3604 performance during a multi-joint exercise task whilst in a fatigued state, and the
3605 reduction of perceived soreness following the second bout.

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4656 **Appendices**

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4658 **Appendix 1 – Example of Consent Form**

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4660 **CONSENT FORM**

4661

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

4662

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

4663

4664 (one copy to be retained by Participant / one copy to be retained by Researcher)

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4668 Appendix 2 – Example of Institutional Ethical Approval
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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)

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4673 Appendix 3 – Menstrual Cycle History Questionnaire

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4675 **MENSTRUAL CYCLE QUESTIONNAIRE**

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4677 **Participant number:** _____

4678 **Please answer the following questions:**

4679 **1. Do you have periods? YES NO**

4680 • **If YES how regular are they? Every month 4-9 times a year**

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4682 **2. How long does your menstrual cycle usually last, from day 1 of bleeding to day 1 of the next**
4683 **bleed? _____ Days.**

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4685 **3. How long were your previous two menstrual cycles?**

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4687 **4. How many days does your menstrual flow usually last? _____ Days.**

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4689 **5. When was the approximate start date of your most recent cycle (i.e first day of bleeding)?**

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4691 **6. What date do you expect your next cycle will begin approximately?**

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4693 **7. Do you use contraceptive pills or any other form of female contraception? YES NO**

4694 • **If YES please answer the following:**

4695 • **Brand:**

4696 • **Duration (years/months):**

4697 • **How often do you take a contraceptive pill? Everyday Every month**

4698 • **Other, please state: _____**

4699 • **Any additional details:**

4700