UNIVERSITY OF
FORWARD
THINKING
WESTMINSTER

WestminsterResearch
http://www.westminster.ac.uk/westminsterresearch

The Effects of Exercise-Induced Muscle Damage With or Without Omega-3 Supplementation on Inflammation, Muscle Function and Recovery in Healthy Physically Active Younger and Older Adults

Kyriakidou, Yvoni

This is a PhD thesis awarded by the University of Westminster.

© Miss Yvoni Kyriakidou, 2022.

https://doi.org/10.34737/vz800

The WestminsterResearch online digital archive at the University of Westminster aims to make the research output of the University available to a wider audience. Copyright and Moral Rights remain with the authors and/or copyright owners.

The Effects of Exercise-Induced Muscle Damage With or Without Omega-3 Supplementation on Inflammation, Muscle Function and Recovery in Healthy Physically Active Younger and Older Adults

Yvoni Kyriakidou

A thesis submitted to the
University of Westminster
in partial fulfilment of the requirements for the degree of
Doctor of Philosophy

School of Life Sciences
University of Westminster - London
July 2022

Abstract

Unaccustomed eccentric exercise is associated with reductions in muscle force output, muscle pain, avoidance of repeated loading and a transient inflammatory response. Whilst exercise-induced muscle damage (EIMD) is well defined in the literature in healthy younger populations, research into older individuals is lacking. The diversity of exercise protocols used also for younger populations may not be suited to older adults. Omega-3 (n-3) supplementation may minimise EIMD via its anti-inflammatory properties, however, its efficacy remains unclear.
All studies induced mild to moderate EIMD either via downhill running (**Study 1** and **Study 3**) or resistance exercise models (**Study 2** and **3**), and examined the effect of n-3 supplementation (**Study 1**), age (**Study 2**), and EIMD protocols (**Study 3**). Venous plasma was collected for plasma creatine kinase (CK), interleukin (IL)-6, and tumour necrosis factor (TNF)-α, prior, immediately after, and 1-to-72 hours post-EIMD. In addition, at the same time points in Study 2, extracellular vesicles (EVs) were assessed. In all studies, functional measures included maximal voluntary isometric contraction (MVIC), peak power, countermovement jump, range of motion (ROM), delayed onset muscle soreness (DOMS) and thigh circumference.
Key results from **Study 1** included DOMS being significantly lower in omega-3 *vs* placebo group ($p = 0.034$), and peak power significantly suppressed in placebo relative to pre-EIMD but not in omega-3 group, both at 24 hours post-EIMD. IL-6 was increased in placebo ($p = 0.009$) but not in omega-3 ($p = 0.434$) post-EIMD. However, no significant differences in peak power output and IL-6 were observed between groups. In **Study 2**, post EIMD, both CK and TNF-α concentrations were increased in the older group relative to the younger at 72 hours (CK, $p = 0.042$; TNF-α, $p = 0.042$). Both younger and older groups showed a significant reduction in MVIC immediately post-EIMD. EIMD did not substantially alter EV modal size or count in younger or older participants, however, the alteration in EV concentration (ΔCount) and EV modal size (ΔMode) between post-EIMD and pre-EIMD negatively associated with CK activity. In **Study 3**, a significant increase ($p < 0.05$) in CK was recorded in the downhill running compared with two different resistance exercise groups. Downhill running group showed greater decrements on MVIC and jump height than both resistance exercise groups post-EIMD. All groups showed a significant increase in muscle soreness post-EIMD ($p < 0.001$).
This thesis showed that 4 weeks supplementation with 3 g/day of n-3 PUFA offsets the EIMD induced pain response following a single bout of moderate intensity exercise. Older individuals showed a blunted resolution in muscle damage and inflammation relative to their younger counterparts. However, a similar response in muscle function in both groups following EIMD was observed. The combination of the leg press and leg extension protocol with moderate intensity could be used as a muscle-injury inducing model in naive-exercised or older population in future studies.

Acknowledgements

This work would not be possible without the support of many people. Firstly, I would like to thank my Director of Studies, Dr Bradley Elliott. His support, guidance and expertise throughout my Doctorate, and in particular during the COVID-19 pandemic, played a catalytic role to my advancement and progress. I would also like to thank Dr Carly Wood for the advice and feedback provided throughout my PhD project. Furthermore, I would like to thank Professor Jimmy Bell for providing his expertise.

A number of Westminster staff, MSc and BSc students contributed, with particular thanks to Dr Chrystalla Ferrier and Dr Sigrun Lange for their assistance in data curation with the ILab Aries clinical chemistry analyser, and with the nanoparticle tracking analysis and Western blotting. My thanks also to Dr Andrew Dalby for his guidance and invaluable advice on statistical analyses. Thanks are also due to the technical staff, Helen Lloyd, Burak Pirincci and Umar Ahmed for their laboratory support throughout my PhD project.

As a self-funded project, this PhD would not have been possible without external research funding. From the University of Westminster, the 125 Fund Award, the Globally Engaged Research Scholarship and the ‘Stay on Track’ scheme provided financial support for research costs and training, international travel and costs of my studies. The Physiological Society also provided affiliate travel grants and training courses, and opportunities to build collaborative links with Professor Stuart Phillips (McMaster University, Hamilton, Ontario, Canada) for my research development and academic growth.

I would like to acknowledge the efforts of all the volunteers who participated in all the experimental studies of my Doctorate, as well as all my doctoral researcher colleagues within the School of Life Sciences for their support throughout my studies. Special thanks go to my friend and colleague Isabella Cooper for her support both with *ex vivo* data curation in the laboratory and for theoretical discussions throughout my studies.

I would also like to thank my closest friends, specifically Jacqueline for her understanding and for being there for me, and my family for their love and for ongoing support throughout my PhD journey! Very special thanks to my best friend Myrto for her understanding and endless support throughout this challenging academic adventure. We shared many difficult moments as well as moments of joy. Finally, a very big and special thank you to my mum. Mum, I will be eternally grateful to you.

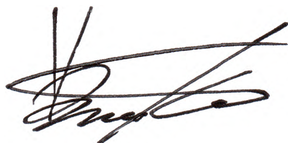
Author's Declaration

I declare that the present work was carried out in accordance with the Guidelines and Regulations of the University of Westminster.

I confirm that this is my own original work and the use of all material from other sources has been properly and fully acknowledged.

Any views expressed in this work are those of the author and in no way represent those of the University of Westminster.

Signed: **Date:** 18/05/2022



Communications Arising

I lead on all aspects of protocol design, data collection, data analyses and preparation of manuscripts for publication of the thesis Chapters that follow.

However, I also gratefully acknowledge input from the co-authors of each publication. In particular, I acknowledge the work of Dr Igor Kraev (**Chapter 5**).

The results presented in this thesis have been published as follows:

Peer Reviewed Publications

1) **Kyriakidou, Y.**, Wood, C., Ferrier, C., Dolci, A. and Elliott, B. (2021). The effect of Omega-3 polyunsaturated fatty acid supplementation on exercise-induced muscle damage. *J Int Soc Sports Nutr*, 18 (1), p.9.

This publication is associated to the present thesis as part of the experimental Study 1 (**Chapter 4**).

2) **Kyriakidou, Y.**, Cooper, I., Kraev, I., Lange, S. and Elliott, B.T. (2021). Preliminary investigations into the effect of exercise-induced muscle damage on systemic extracellular vesicle release in trained younger and older men. *Front Physiol*, 12,723931.

This publication is associated to the present thesis as part of the experimental Study 2 (**Chapter 5**).

Conference Presentations

1) **Kyriakidou, Y**., Wood, C., Cooper, I., Tanner, E. and Elliott, B.T. (2020). *Preliminary investigations into muscle recovery following exercise-induced muscle damage between younger and older males*. American College of Sports Medicine, 17 June 2020, *Medicine & Science in Sports & Exercise,* Vol 52:5 Suppl. San Francisco, California, USA [Poster Communication]

2) **Kyriakidou, Y**., Wood, C., Bell, J. and Elliott, B. (2019). *The effects of ageing process on muscle damage, repair and inflammation*. SLS Doctoral Researcher Conference, University of Westminster, 16 May 2019, London, UK - Best Oral Presentation Award

3) **Kyriakidou**, **Y**., Wood, C., Elliott, B. and Dolci, A. (2018). *The effect of omega-3 supplementation on exercise-induced muscle damage*. Europhysiology, 13-14 September 2018, Proc Physiol Soc 41, PCA184, London, UK [Poster Communication]

4) **Kyriakidou, Y**., Wood, C., Bell, J. and Elliott, B. (2018). *The effect of omega-3 supplementation on exercise-induced muscle damage*. FST Doctoral Conference, University of Westminster, 19 April 2018, London, UK [Oral Communication]

5) **Kyriakidou**, **Y**., Kantorovich, R.C., Bell, J., Monterisi, S. and Dolci, A. (2017). *The potential of omega-3 supplementation to reduce muscle inflammation after muscle-damaging exercise*. American College of Sports Medicine, 1 June 2017, *Medicine & Science in Sports & Exercise,* Vol. 49:5 Suppl. Denver, Colorado, USA [Poster Communication]

6) **Kyriakidou, Y**., Bell, J., Monterisi, S. and Dolci, A. (2017). *PUFAs and physical activity: The potential of omega-3 supplementation to reduce muscle inflammation following a bout of muscle-damaging exercise*. Doctoral Conference, University of Westminster, 27 April 2017, London, UK - Best Poster Presentation Award

Table of Contents

Abstract……………………………………………………………………………....... i
Acknowledgements…………………………………………………………….......... ii
Author's Declaration………………………………………………………….......... iii
Communications Arising……………………………........……………….. iv
Table of Contents……………………………………………………………… vi
List of Figures..……………………... xiv
List of Tables…………... xvii
List of Abbreviations……………………………………………..………. xix
Thesis Format……………………………………………..…................ xxiii

CHAPTER ONE – GENERAL INTRODUCTION…………...……………….…... 1

CHAPTER TWO – REVIEW OF LITERATURE…………..…………………... 9
2.1 Scope of Review………………………………………………………….. 10
2.2 Skeletal Muscle……….……………………………………………………... 11
2.2.1 Overview of Skeletal Muscle Form and Function**………**……….......... 11
2.2.2 Eccentric *vs* Concentric Muscle Actions……………………………........... 15
2.3 Exercise and Muscle Damage………………………..………………….. 18
2.3.1 Mechanism of Action of EIMD………... 20
2.3.2 Time Course of EIMD and the Factors Affecting Recovery from EIMD... 21
2.4 Inflammatory Response after EIMD…………………..………………… 23
2.4.1 Macrophages, Neutrophils and Exercise……………………….……….. 24
2.4.2 Cytokines (IL-6), Signalling Proteins (TNF-a)……………………..…...... 27
2.4.2.1 Interleukin (IL)-6 Response to Exercise……………………………….. 28
2.4.2.2 Tumour Necrosis Factor-α Response to Exercise…………………….. 31
2.4.3 Adaptation…………………..…………………………………………...... 33
2.5 Assessment of Muscle Damage………………………..……………….. 37
2.5.1 Direct Methods of Muscle Damage Assessment……………………..…… 37
2.5.2 Indirect Methods of Muscle Damage Assessment……………………… 39
2.5.2.1 Myocellular Proteins (CK)……………………………………............... 39
2.5.2.2 Delayed Onset Muscle Soreness……...………………….………….. 43
2.5.2.3 Maximal Voluntary Isometric Contraction..……………………….……...... 46

2.5.2.4 Anaerobic Power………..…………………..……………………..…. 49
2.5.2.5 Vertical Jump Performance…..………………………………….....…. 51
2.6 Nutrition and Exercise………………………………………….…………. 55
2.6.1 Dietary Supplements and Ergogenic Aids………………………........ 56
2.7 Omega-3 Polyunsaturated Fatty Acids……………………….............. 58
2.7.1 Biochemistry and Structure of Omega-3 Fatty Acids……….................. 58
2.7.2 Dietary Sources of Omega-3 Fatty Acids……….……………............. 60
2.7.3 Physiological Roles and Health Benefits of Omega-3 Fatty Acids……. 61
2.7.4 Mechanism of Action of Omega-3 Fatty Acids.……............................ 63
2.7.5 Anti-inflammatory Effects of Omega-3 Fatty Acids…………............... 63
2.7.6 Omega-3 Supplementation and Exercise……………………….……. 64
2.7.6.1 Omega-3 Supplementation, Myocellular Proteins and Cytokines…... 65
2.7.6.2 Omega-3 Supplementation, Muscle Function and DOMS…………... 68
2.7.6.3 Omega-3 Supplementation and Lipid Profile…………………..…... 70
2.8. Ageing, Inflammation and Muscle Function…………………………… 74
2.8.1 Ageing and Increased pro-Inflammatory Cytokines.……………..…….. 75
2.8.2 Cellular Senescence and the SASP Theory…………………….…….. 76
2.8.3 Age-Related Changes in Muscle Function………………………………. 77
2.8.4 Ageing and Exercise Recovery…………………………………………… 81
2.9 Summary…...………………………………………………………………… 83

CHAPTER THREE – MATERIALS AND METHODS……………..……..…... 87
3.1 Introduction……………………...………………………………………... 88
3.2 Ethical Approval……….…………..……………………………………... 88
3.3 Data Protection and Confidentiality…….……………………..……... 89
3.4 Human Participants Recruitment……………….……………………..…… 89
3.5 Participants……………………………………………………………….. 89
3.5.1 Inclusion/Exclusion Criteria…...………………..………………………… 90
3.6 Familiarisation……………………..……………………………………….. 90
3.7 Eccentric Muscle-Damaging Exercise Protocol…………………..……... 91
3.8 Anthropometry…………………………………………………………………. 91
3.8.1 Thigh Circumference…………………………….…………………...……. 92
3.9 Maximal Oxygen Uptake ($\dot{V}O_2max$) Test…………………………...……. 93
3.10 Muscle Function………………………………………………...………. 95

3.10.1 Muscle Soreness…… 95
3.10.2 Passive Range of Motion…… 96
3.10.3 Vertical Jump Performance…… 97
3.10.4 Maximal Voluntary Isometric Contraction…… 99
3.10.5 Anaerobic Power…… 100
3.11 Urine Collection, Handling and Analysis…… 101
3.12 Blood Collection, Handling and Analysis…… 102
3.12.1 Creatine Kinase Activity…… 103
3.12.2 IL-6 and TNF-α Analysis…… 103
3.12.3 Triglycerides Analysis…… 104
3.12.4 Blood Lactate Analysis…… 105
3.13 Diet and Activity Control…… 105
3.14 Statistical Analysis…… 107

CHAPTER FOUR – THE EFFECT OF OMEGA-3 POLYUNSATURATED FATTY ACID SUPPLEMENTATION ON EXERCISE-INDUCED MUSCLE DAMAGE…… 109
Abstract…… 110
4.1 Introduction…… 111
4.1.1 Aims and Hypothesis…… 114
4.2 Methods…… 115
4.2.1 Ethical Approval…… 115
4.2.2 Participant Recruitment…… 115
4.2.3 Experimental Design…… 117
4.2.4 Experimental Procedures…… 120
4.2.4.1 Preliminary Testing/Fitness and Familiarisation…… 120
4.2.4.1.1 Determination of 65% of $\dot{V}O_2max$…… 120
4.2.4.2 Experimental Trial…… 121
4.2.4.2.1 Downhill Running Exercise…… 121
4.2.4.3 Diet and Activity Control…… 123
4.2.4.4 Supplementation…… 123
4.2.5 Measurements and Instrumentation…… 125
4.2.6 Statistical analysis…… 125
4.2.6.1 Power Calculation…… 126

4.3 Results.. 127
4.3.1 Participant Characteristics.. 127
4.3.2 Dietary Data.. 128
4.3.3 Laboratory Environmental Conditions... 131
4.3.4 Biomarkers of Muscle Damage and Inflammation.............................. 131
4.3.4.1 Creatine Kinase... 131
4.3.4.2 IL-6... 132
4.3.4.3 TNF-α.. 132
4.3.5 Assessment of Muscle Function.. 134
4.3.5.1 Muscle Soreness.. 134
4.3.5.2 Maximal Voluntary Isometric Strength.. 134
4.3.5.3 Peak Power... 134
4.3.6 Lipid Profile... 137
4.3.6.1 Triglycerides.. 137
4.4 Discussion... 141
Biomarkers of Muscle Damage and Inflammation..................................... 141
Muscle Function.. 143
Triglycerides.. 144
Completed-EIMD *vs* Failed-EIMD Participants....................................... 146
Strengths and Limitations... 147
Recommendations and Future Directions.. 149
4.5 Conclusion... 152

CHAPTER FIVE – THE INFLAMMATORY, FUNCTIONAL AND SYSTEMIC EXTRACELLULAR VESICLE RESPONSES TO EXERCISE-INDUCED-MUSCLE DAMAGE IN PHYSICALLY ACTIVE YOUNGER AND OLDER MALES.. 153
Abstract... 154
5.1 Introduction.. 155
5.1.1 Aims and Hypothesis.. 159
5.2 Methods... 160
5.2.1 Ethical Approval.. 160
5.2.2 Participant Recruitment... 160
5.2.3 Experimental Design... 162

5.2.4 Experimental Procedures…… 165
5.2.4.1 Preliminary Testing and Familiarisation…… 165
5.2.4.1.1 Determination of One Repetition Maximum Load…… 165
5.2.4.2 Experimental Trial…… 166
5.2.4.2.1 Leg Press Exercise…… 166
5.2.4.3 Diet and Activity Control…… 167
5.2.5 Measurements and Instrumentation…… 167
5.2.5.1 EV isolation and characterisation from human plasma…… 168
5.2.5.1.1 Isolation of plasma-EVs…… 168
5.2.5.1.2 Nanoparticle tracking analysis (NTA)…… 169
5.2.5.1.3 Transmission electron microscopy (TEM)…… 169
5.2.5.1.4 Western blot analysis…… 170
5.2.6 Statistical analysis…… 170
5.3 Results…… 171
5.3.1 Participant Characteristics…… 171
5.3.2 Dietary Data…… 172
5.3.3 Biomarkers of Muscle Damage and Inflammation…… 173
5.3.3.1 Creatine Kinase…… 173
5.3.3.2 IL-6…… 176
5.3.3.3 TNF-α…… 178
5.3.4 Assessment of Muscle Function…… 180
5.3.4.1 Muscle Soreness…… 180
5.3.4.2 Maximal Voluntary Isometric Strength…… 182
5.3.4.3 Peak Power…… 184
5.3.4.4 Jump Height…… 186
5.3.4.5 Thigh Circumference (midpoint)…… 188
5.3.5 Extracellular Vesicles…… 190
5.3.6 Blood Lactate…… 194
5.4 Discussion…… 196
Biomarkers of Muscle Damage and Inflammation…… 197
Muscle Function…… 201
Extracellular Vesicles…… 206
Blood Lactate…… 209
Dietary Data…… 211

Strengths and Limitations……………………………………..………………..... 212
Recommendations and Future Directions……………………………...……. 214
5.5 Conclusion………………………………………………………………… 216

<u>**CHAPTER SIX – THE INFLAMMATORY AND MUSCLE FUNCTIONAL RESPONSES TO EXERCISE-INDUCED-MUSCLE DAMAGE AMONGST THREE DIFFERENT EXERCISE PROTOCOLS**</u>……...…...………………….. 217
Abstract…………………………………………………………………….... 218
6.1 Introduction……………………………………………….………... 219
6.1.1 Aims and Hypothesis……………………....................................... 222
6.2 Methods……………………………………………………………......... 224
6.2.1 Ethical Approval……………………………………………………….. 224
6.2.2 Participant Recruitment……………………....................................... 224
6.2.3 Experimental Design…..……………………………………………… 226
6.2.4 Experimental Procedures……………………………………………….. 229
6.2.4.1 Preliminary Testing and Familiarisation…………..……….......... 229
6.2.4.1.1 Determination of One Repetition Maximum Load in Leg Press…..... 229
6.2.4.1.2 Determination of One Repetition Maximum Load in Leg Extension.. 229
6.2.4.1.3 Determination of 65% of $\dot{V}O_2max$ test,,,,,…………………………… 230
6.2.4.2 Experimental Trial……………………………………………...…… 230
6.2.4.2.1 Muscle-Damaging Exercise ………………………………………. 231
6.2.4.3 Diet and Activity Control…………..………………………...…… 232
6.2.5 Measurements and Instrumentation……………………………...…. 232
6.2.6 Statistical analysis……………………………………………………….. 233
6.3 Results……………………………...…………………….......... 234
6.3.1 Participant Characteristics……………………………..……………… 234
6.3.2 Dietary Data……….…………………………………………………… 235
6.3.3 Biomarkers of Muscle Damage and Inflammation…..……………..…… 236
6.3.3.1 Creatine Kinase………………………………………………..…… 236
6.3.3.2 IL-6……………………………………………………………...……… 241
6.3.3.3 TNF-α………………………………………………………………… 243
6.3.4 Assessment of Muscle Function…………………………………………... 245
6.3.4.1 Muscle Soreness……………………...……………………………..... 245
6.3.4.2 Maximal Voluntary Isometric Strength……………………………….…… 248

6.3.4.3 Jump Height……………………………………………………………. 250
6.3.4.4 Peak Power……………………………………………………………. 252
6.3.4.5 Range of Motion (flexion)……..………………………………………. 254
6.3.4.6 Thigh Circumference (midpoint)……………………………………… 256
6.3.5 Blood Lactate……………………………………………………….... 258
6.3.6 Correlations……………………………………………………………. 260
6.4 Discussion……………………………………………………………. 262
Biomarkers of Muscle Damage and Inflammation……………………..……. 262
Muscle Function……………………………………………………………. 266
Blood Lactate……..…………………………………………………………….. 273
Dietary Data…………………………………………………………..……. 274
Strengths and Limitations……………………………..…………………..... 275
Recommendations and Future Directions…………………………...……. 277
6.5 Conclusion………………………………………………………………. 278

<u>**CHAPTER SEVEN – GENERAL DISCUSSION**</u>…………………..……….. 279
7.1 Introduction…..……………………………………………………… 280
7.2 Main Findings in Relation to the Literature…………………..………. 282
7.2.1 Summary of key findings……………..…….. 282
7.2.2 Results with respect to exercise-induced muscle damage and inflammation……………………………………………………………….. 282
7.2.3 Results with respect to muscle function and recovery…..…………………. 287
7.3 Practical Applications……….………………………………………. 289
7.4 Strengths and Limitations……….....……………………………………. 294
7.5 Recommendations for Future Research……….....……………………… 297
7.6 Conclusions……………………………………………………………. 301

References………………………………………………………………….. 302

Appendices………………………………………………………………….. 352
Appendix A: UoW – Risk Assessments……………………….……..…..... 352
Appendix B: UoW – Laboratory Health and Safety 4th Floor………………..... 377
Appendix C: UoW – COSHH Forms – Blood Sampling…..…………………... 386

Appendix D: Participation Information Sheet & Informed Consent Form – Study 1……………………………………….….….……………………………. 390
Appendix E: Participation Information Sheet & Informed Consent Form – Study 2…………………………………………………………...…………… 394
Appendix F: Participation Information Sheet & Informed Consent Form – Study 3……………………………………………………………………….. 398
Appendix G: Medical Questionnaire and WURSS-21 Questionnaire……..... 403
Appendix H: Physical Activity Readiness Questionnaire (PAR-Q)………….. 405
Appendix I: Edinburgh Handedness Inventory Questionnaire..……………. 406
Appendix J: Omega-3 Food List………..……………………………………..… 407
Appendix K: Food Frequency Questionnaire (FFQ)…………...………..…. 408
Appendix L: Representative example of NTA Analysis……………………… 411
Appendix M: Participant's Representative Results – Study 1, Study 2 and Study 3……………………………………………………………………….. 412
Appendix N: Advertisement – Study 1, Study 2 and Study 3…………...….... 415

List of Figures

Figure 2.1 Structure of skeletal muscle……………………………….……..…… 11
Figure 2.2 Skeletal muscle fibre…………….…………………………..……….... 12
Figure 2.3 Schematic representation of the sarcomere ………….…..……..…... 13
Figure 2.4 Types of muscle contraction……………………………….………….... 17
Figure 2.5 Transmission electron microscope image showing a sarcomere of skeletal muscle………………………………………………...…...……..……… 18
Figure 2.6 The ideal length of a sarcomere…………………………………..…. 19
Figure 2.7 Postulated series of events leading to muscle damage from eccentric exercise…………………………………………………………………..... 20
Figure 2.8 Typical magnitude and time course of changes in maximal voluntary contraction (MVC), ROM, DOMS (assessed by visual analogue scale), swelling (measured by circumference) and plasma CK…………….... 23
Figure 2.9 Potential mechanisms for the repeated bout effect…....……...…... 35
Figure 2.10 Representation of a bell-shaped hormesis curve for the EIMD management…………………………………………….…...…………….….. 37
Figure 2.11 The biosynthesis pathway of (n-3) PUFA……….……………… 60
Figure 2.12 Magnetic resonance imaging scans in quadriceps of a 40-year-old triathlete compared with the quadriceps of a 70-year-old triathlete and a 74-year-old sedentary man……………………….…………….…..... 80
Figure 2.13 The multifactorial determinants of elderly skeletal muscle performance…………………………………………………………….………. 81
Figure 3.1 Assessment of body composition using SECA (Seca® mBCA 514 Medical Body Composition Analyzer)……………….………………....... 92
Figure 3.2 Assessment of $\dot{V}O_2$max……………….………………..……… 94
Figure 3.3 Example positioning for the assessment of perceived muscle soreness using a visual analogue scale……………...…………….……....... 96
Figure 3.4 Assessment of jump height using Takei Vertical Jump Meter device…………………………………………………………….…………… 98
Figure 3.5 Assessment of maximal voluntary isometric contraction using the dynamometer KINEO (Globus Kineo 7000)…………………………....... 100
Figure 3.6 Assessment of peak power using Monark cycle ergometer (Monark Ergomedic 894E)…………………………………..……...…... 101

Figure 3.7 Venous blood sampling………………………………………… 103
Figure 4.1 Recruitment pathway and participant withdrawals………..…… 116
Figure 4.2 Schematic of experimental procedures……………..……….. 119
Figure 4.3 Plasma markers of muscle damage and inflammation as a function of time…………………………………………………………...... 133
Figure 4.4 Muscle functional measures prior (pre) and following (post – 72 hours) EIMD…………………………………………………….…..... 136
Figure 4.5 Serum TG by supplementation group on participants who completed the supplementation phase and attempted to complete EIMD……………………………………………………………….….. 138
Figure 4.6 The effect of n-3 supplementation on serum TG in participants who completed EIMD and those who failed EIMD trial…………………..….... 140
Figure 5.1 Recruitment pathway and participant withdrawals……..……… 161
Figure 5.2 Schematic of experimental procedures………………………….. 164
Figure 5.3 Differences in plasma creatine kinase activity between younger and older group…………………………………………….................. 175
Figure 5.4 Differences in plasma IL-6 concentration between younger and older group……………………………………...……..………... 177
Figure 5.5 Differences in plasma TNF-α concentration between younger and older group……………………………...…………………..……... 179
Figure 5.6 Differences in perception of pain between younger and older group………………………………………………….…………..….…..... 181
Figure 5.7 Differences in maximal voluntary isometric contraction between younger and older group……………………………………….……. 183
Figure 5.8 Differences in peak power output between younger and older group…………………………………………………………………..….….... 185
Figure 5.9 Differences in jump height output between younger and older group……………………………………………………………......…...... 187
Figure 5.10 Differences in midpoint thigh circumference between younger and older group…………………………..……………………………… 189
Figure 5.11 Measurement of EV modal size and count in younger and older participants…………………..…………………………………….... 191
Figure 5.12 Alterations in EV modal size and count with exercise, and EV correlations with muscle damage markers…………………………………… 193

Figure 5.13 The effect of EIMD on blood lactate in younger and older participants………..…………………..………………………………..….   195
Figure 6.1 Recruitment pathway and participant withdrawals……………...   225
Figure 6.2 Schematic of experimental procedures……......………………...   228
Figure 6.3 Differences in plasma creatine kinase activity amongst DHR, LGP and LGP+LGE groups…………………………………….………   240
Figure 6.4 Differences in plasma IL-6 concentration amongst DHR, LGP and LGP+LGE groups..…………………………………………………   242
Figure 6.5 Differences in plasma TNF-α concentration amongst DHR, LGP and LGP+LGE groups………………..……………………………...   244
Figure 6.6 Differences in perception of pain amongst DHR, LGP and LGP+LGE groups…………………...……...………………………………...   247
Figure 6.7 Differences in maximal voluntary isometric contraction amongst DHR, LGP and LGP+LGE groups………..……………………………   249
Figure 6.8 Differences in jump height amongst DHR, LGP and LGP+LGE groups……………………………………………..............................   251
Figure 6.9 Differences in peak power output amongst DHR, LGP and LGP+LGE groups……..…………………………………………………...   253
Figure 6.10 Differences in range of motion amongst DHR, LGP and LGP+LGE groups…………………..……………..………………………   255
Figure 6.11 Differences in midpoint thigh circumference amongst DHR, LGP and LGP+LGE groups..…………..………………………………………   257
Figure 6.12 The effect of three EIMD protocols on blood lactate…………   259
Figure 6.13 Correlations between change in CK activity and change in muscle functional markers…………………………………………………...   261

List of Tables

Table 2.1 Summary of studies investigating IL-6 in response to EIMD……. 30

Table 2.2 Summary of studies investigating TNF-α in response to EIMD…. 32

Table 2.3 Summary of studies investigating CK activity in response to EIMD………………………………………………………………………... 42

Table 2.4 Summary of studies investigating DOMS in response to EIMD… 45

Table 2.5 Summary of studies investigating muscle strength in response to EIMD…………………………………………………………………….... 48

Table 2.6 Summary of studies investigating peak power in response to EIMD………………………………………………………………………... 51

Table 2.7 Summary of studies investigating jump height in response to EIMD………………………………………………………………………... 53

Table 2.8 Summary of advantages and disadvantages of direct and indirect methods of muscle damage assessment………..…………….…... 54

Table 2.9 Summary of studies investigating omega-3 supplementation on muscle damage, inflammatory response and muscle function in humans… 71

Table 4.1 Physical characteristics of participants completed EIMD, independent sample t-test comparison between N-3 and PLA group……… 127

Table 4.2 Physical characteristics of participants attempted to complete EIMD……………………………………………………………….……...… 128

Table 4.3 Dietary data at baseline of participants completed EIMD, independent sample t-test comparison between N-3 and placebo group…. 129

Table 4.4 Dietary data post supplementation (+ 3 g of n-3 supplementation) of participants completed EIMD, independent sample t-test comparison between N-3 and placebo group…………………………… 129

Table 4.5 Dietary data at baseline of participants attempted to complete EIMD... 130

Table 4.6 Descriptive characteristics of laboratory environmental conditions during EIMD of participants attempted to complete EIMD…..….. 131

Table 4.7 Mann-Whitney U test in serum TG concentration and percentage change on participants who attempted EIMD by supplementation group……………………………………………………..... 137

Table 4.8 Mann-Whitney U test on TG concentration and percentage change between participants completed the EIMD (only from N-3 group) and those who failed the EIMD……..…………………………………………... 139
Table 5.1 Characteristics and comparisons of younger and older groups at baseline. Independent sample t-test comparison between younger (18-35 years of age) and older ≥60 years of age)…………………….…….…..... 172
Table 5.2 Average dietary intake of younger and older participants. Independent sample t-test comparison between two groups….…………… 172
Table 6.1 Characteristics and comparisons amongst DHR, LGP and LGP & LGE at baseline…………………………....…………………………… 234
Table 6.2 Average daily dietary intake of DHR, LGP and LGE+LGE groups. One-way ANOVA and Kruskal-Wallis tests comparisons amongst the three groups………………………………………………………...…. 236
Table 6.3 Bonferroni *post hoc* tests for CK activity (%) amongst DHR, LGP and LGP+LGE groups at indicated timepoints…………………………….. 238

List of Abbreviations

AA	Arachidonic acid
ACSM	American College of Sports Medicine
ADA	American Dietetic Association
ALA	a-Linoleic acid
ANOVA	Analysis of variance
AP	Antigen presentation
Atm	Atmospheric pressure
ATP	Adenosine triphosphate
%BF	Percentage body fat
BIA	Bioelectrical impedance analysis
BME	Black and minority ethnic
BMI	Body mass index
BSA	Bovine serum albumin
CI	Confidence interval
CK	Creatine kinase
CK-MB	Creatine kinase brain isoform
CK-MM	Creatine kinase muscle isoform
cm	Centimetres
COX-2	Cyclooxygenase-2 inhibitor
CRP	C reactive protein
CV	Coefficient of variation
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
DHR	Downhill running group
DOMS	Delayed-onset muscle soreness
E-C	Excitation-contraction
ECL	Enhanced chemiluminescence
ECM	Extracellular matrix
EER	Estimated energy requirement
EFSA	European Food Safety Authority
EIMD	Exercise-induced muscle damage

ELISA	Enzyme-linked immunosorbent assay
EMG	Electromyography
EPA	Eicosapentaenoic acid
EVs	Extracellular vesicles
FFQ	Food frequency questionnaire
Flot-1	Flotillin-1
GCs	Glucocorticoids
h	Hour
Hb	Haemoglobin
Hct	Haematocrit
HR	Heart rate
HRP	Horseradish peroxidase
IFN-γ	Interferon-gamma
IOC	International Olympic Committee
IL-1β	Interleukin-1 beta
IL-1ra	Interleukin-1 receptor
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-12	Interleukin-12
JNK	c-Jun N-terminal kinase
kcal	Kilocalories
kg	Kilograms
km	Kilometres
LDH	Lactate dehydrogenase
LGP	Leg press group
LGP+LGE	Leg press and leg extension group
LOX	Lipoxygenase
LTs	Leukotrienes
M	Mean
Mb	Myoglobin
Md	Median
mg	Milligrams

MHC	Myosin heavy chain
miRNAs	microRNAs
mL	Millilitres
MLCK	Myosin light chain kinase
mmol/L	Millimoles per litre
MRI	Magnetic resonance imaging
MVIC	Maximal voluntary isometric contraction
N-3	Omega-3 interventional group
n-3 PUFA	Omega-3 polyunsaturated fatty acids
NADPH	Nicotinamide adenine dinucleotide phosphate
NBM	Nude body mass
nm	Nanometres
NTA	Nanoparticle tracking analysis
O	Older group
PAL	Physical activity level
PAR-Q	Physical activity readiness questionnaire
PGE_2	Prostaglandin E_2
PLA	Placebo group
Post-EIMD	After exercise-induced muscle damage
Post-SUP	After supplementation period
Pre-EIMD	Prior to exercise-induced muscle damage
Pre-SUP	Before supplementation period
PUFA	Polyunsaturated fatty acids
RBE	Repeated bout effect
RER	Respiratory exchange ratio
RH	Relative humidity
RM	1 repetition maximum
RMR	Resting metabolic rate
ROM	Range of motion
ROS	Reactive oxygen species
RPE	Rate of perceived exertion
RPM	Revolutions per minute
RT	Room temperature
SASP	Senescence-associated secretory phenotype

SD	Standard deviation
SEM	Standard error mean
T_{amp}	Ambient temperature
TBS	Tris buffered saline
TBW	Total body water
TEM	Transmission electron microscopy
TG	Triglycerides
TLR	Toll-like receptors
TNF–α	Tumour necrosis factor–α
TXs	Thromboxanes
URTI	Upper respiratory tract infection
USDA	U.S Department of Agriculture
USG	Urine specific gravity
VAS	Visual analogue scale
$\dot{V}O_2$	Absolute oxygen uptake per minute
$\dot{V}O_2max$	Maximal oxygen uptake per minute
$\dot{V}CO_2$	Carbon dioxide production per minute
$V_{65\%}$	Treadmill running speed at +1% gradient
V_{test}	Downhill running speed
WADA	World anti-doping agency
WAnT	Wingate anaerobic test
WHR	Waist-hip ratio
WURSS	Wisconsin upper respiratory symptom survey
Y	Younger group
Δ	Difference/Delta
ΔCount	Difference between extracellular vesicles concentration at post-EIMD and pre-EIMD time points
ΔMode	Difference between extracellular vesicles modal size at post-EIMD and pre-EIMD time points
η^2_p	Partial eta squared
μL	Microlitres
μm	Micrometres

Thesis Format

A literature review (**Chapter 2**) provides a brief background, and different theories and mechanisms of the research presented in the thesis. A general methods Chapter follows that outlines the common procedures and analyses performed in the subsequent three independent experimental studies (**Chapter 3**). The first experimental study primarily investigated the effect of the omega-3 polyunsaturated fatty acid supplementation on exercise-induced muscle damage in young population (**Chapter 4**). The second experimental study primarily explored the effect of a resistance muscle-damaging exercise protocol on muscle damage, inflammation and functional changes in recreationally active younger and older adults (**Chapter 5**). Study 3 primarily examined the effect of three different types of eccentric muscle-damaging exercise protocols on muscle damage, inflammation, functional changes and recovery for the optimisation of the EIMD protocol (**Chapter 6**). Throughout the thesis, abbreviations are defined at first use and for clarity a list of abbreviations, figures and tables appear after the contents. **Bold type** is used to refer to sections elsewhere within the thesis.

CHAPTER ONE

General Introduction

Exercise performance concerns many groups of people, such as professional athletes, exercise enthusiasts or people whose profession is physically demanding (e.g., soldiers, miners and builders). It can vary greatly on a daily basis both in very well-trained younger individuals and in older people. This depends on various factors, such as age, weight, training load, overall physical and mental health, nutrition and the environment, all of which influence performance (ACSM, 2009). For example, ageing has been associated with decreased muscle mass and bone density which in turn negatively affect physical performance (Reid et al., 2016). In addition, increased body weight and poor nutrition can both decrease athletic performance, whilst it has been shown that suitable nutritional strategies and supplementation can improve performance and recovery time (ACSM, 2016).

Many of the above factors have been studied individually or in combination to find the best combination to achieve the highest possible performance for a specific task. Some of these factors, such as age or the environment where people must perform in, cannot be changed. However, trying to understand how the human body adapts can help provide interventional techniques or strategies which could decrease the negative effect of these factors on the human body, and that can improve exercise performance or muscle function for different population groups.

An important downside of unaccustomed vigorous-intensity exercise is that it could lead to exercise-induced muscle damage (EIMD; Proske and Morgan, 2001; Clarkson and Hubal, 2002; Paulsen et al., 2012; Peake et al., 2017; Harty et al., 2019; Owens et al., 2019). EIMD includes muscular stiffness, increases in tissue oedema, discomfort and pain on movement, loss of strength and power,

reduced range of motion (ROM), delayed onset muscle soreness (DOMS) and diminished recovery between training sessions (Malm et al., 2000; Jouris et al., 2011; Hyldahl and Hubal, 2014; Ochi et al., 2016; Ives et al., 2017). EIMD is a transient phenomenon, and its symptoms can persist for several days after cessation of exercise. This can affect fit individuals or less well-trained ones, and perhaps even older people; leading to temporarily impeded performance (Wan et al., 2017) or prevention of an individual in participating in normal activity schedules (Peake et al., 2017; Heckel et al., 2019). Therefore, pain after exercise is a factor that may discourage people to engage in physical activities on a daily basis. Enhancing levels of physical activity is a high public health priority, particularly amongst older individuals or those currently inactive. The lack of physical exercise is a great risk factor for developing cardiovascular disease and cancer (Warburton, Nicol and Bredin 2006). In an effort to make exercise more appealing to inactive individuals and recreational athletes, as well as to elderly people, reducing the adverse effects of physical activity is a top priority.

Further, since age-related musculoskeletal decline and impaired muscle regeneration present an important risk for fall in the elderly, it is important to find ways to alleviate the adverse effects of exercise to make physical activity more enjoyable. It is therefore considered essential to apply prevention strategies to reduce EIMD both in younger and older individuals. Indeed, it has been shown that this progressive loss of functional ability is associated with increased risk of falls (Hardy et al., 2007), a high risk of all-cause mortality, cancer and respiratory disease (Celis-Morales et al., 2018). In line with these findings, an increased incidence of type 2 diabetes has been associated with low muscle strength (Celis-Morales et al., 2018). Similarly, it has been observed that the high risk of

cardiovascular disease (CVD) mortality in patients with type 2 diabetes mellitus is decreased in those with greater grip strength (Celis-Morales et al., 2017). These observational studies demonstrated that the maintenance of muscle mass and strength with advanced age is of critical important for the cardio-metabolic health risk management and for the elderly skeletal muscle performance.

One of the aspects of performance that has been of interest to scientists is also the process of recovery which could help individuals maintain their physical performance or improve exercise performance. Since EIMD may involve an inflammatory response and decrements in force-generating capacity, there have been studies focusing on how to decrease symptoms of EIMD, enhance body adaptation and recovery through tolerance to a stressor exercise stimulus (Stupka et al., 1985; Nosaka et al., 2001; Peake et al., 2017; Owens et al., 2019). Strategies to reduce the symptoms of muscle damage and inflammation following EIMD can therefore be useful to athletes and individuals interested in increasing their physical activity (Twist and Eston 2009; Jakeman et al., 2017). Taking that into account, people unaccustomed to exercise, athletes performing at recreational level, elite athletes, military personnel who undergo intense physical stress during their training and usually have restrictions to resting time, and older people may be greatly benefitted from omega-3 (n-3) supplementation. Jouris et al. (2011) have shown that individuals, ranging from athletes at recreational level to sedentary people or patients, who are starting exercise protocols or medical treatments, such as physical therapy, may be greatly benefitted by n-3 supplementation. Indeed, this might improve their recovery after exercise and decrease the amount of typical soreness they experience due to the muscle inflammation (Balnave and Thompson, 1993; Jouris et al., 2011). In that respect,

n-3 supplementation has been associated with the decrease of post-exercise inflammation (Phillips et al., 2003; Jouris et al., 2011; DiLorenzo, Drager and Rankin, 2014). This could promote a healthier lifestyle making exercise and sport more pleasant. In addition, interventional strategies are now developed to promote enhanced muscle and physical function and/or prevent physical limitations, and as such support healthy ageing.

An exploration of the research surrounding EIMD and the physiological processes underlying muscle damage, inflammation and recovery following intense exercise will be conducted and discussed in the literature review that follows. Particular focus will be given to indirect methods of assessment of EIMD via blood biomarkers, such as creatine kinase (CK), interleukin (IL)-6 and tumour necrosis factor (TNF)-α and muscle functional markers, such as maximal voluntary isometric contraction (MVIC), power, DOMS, ROM and jump height. In addition, an examination of the research on n-3 supplementation as a nutritional strategy on EIMD will be presented. Ageing process and interactions with inflammatory responses to EIMD will also be explored. Following this, **Chapter 3** of this work presents the general methods used consistently across all experimental studies.

In **Chapter 4, 5 and 6**, healthy physically active younger and older participants performed different muscle-damaging exercises with or without of omega-3 supplementation in an attempt to examine muscle damage, inflammation and muscle function. More specifically, **Chapter 4** examines the effects of n-3 supplementation on EIMD in healthy recreationally active young adults. **Chapter 5** not only explores the effect of a muscle-damaging exercise in younger and older

adults, but also the effect of EIMD on circulating extracellular vesicles (EVs), as a novel potential biomarker of muscle damage in humans. Whilst exercise is associated with a number of immediate physiological responses, circulating EVs can act as plasma-based biomarkers, reflecting physiological and pathophysiological conditions of the body (Withrow et al., 2016; Zhao et al., 2020). Although associations have previously been observed between EV release profiles in response to inflammatory disorders (Hosseinkhani et al., 2018) and older individuals are noted to have elevated basal systemic inflammatory cytokines concentrations (Franceschi and Campisi, 2014), circulating miRNAs (Jung and Suh, 2014) and increased EV release is seen from senescent cells (Hitomi et al., 2020; Riquelme et al., 2020). **Chapter 6** investigates the effect of three different types of eccentric muscle-damaging exercise protocols on muscle damage, inflammation, functional changes, and recovery in healthy physically active young population to optimise the EIMD protocol used in previous Chapters. The purpose of this is to provide a safer and a more realistic model of EIMD for use in older populations.

Finally, **Chapter 7** of this work draws comparisons of the findings between the experimental Chapters and in relation to the literature. Implications of these results follow, and the subsequent recommendations for future research are presented. Strengths and limitations of this thesis are also discussed along with the final conclusions of this thesis. This work aims to add to the knowledge and evidence-based support for the use of n-3 supplementation as a nutritional strategy on the EIMD, as well as to help better understand the underlying muscle damage, inflammatory and functional responses on the EIMD.

Thus, the primary aim of this Doctorate is to examine the effects of EIMD on muscle damage, inflammation and functional measurements in healthy physically active younger and older adults. Specifically, the objectives of this work are:

1. to assess the effects of n-3 supplementation on muscle damage and inflammation following EIMD in healthy physically active young adults
2. to evaluate muscle function and DOMS between N-3 and placebo group following EIMD
3. to examine muscle damage and inflammation between younger and older participants following EIMD
4. to compare muscle function and DOMS between younger and older participants following EIMD
5. to investigate the effects of EIMD on circulating EV profiles in younger and older participants
6. to examine the differences in muscle damage and inflammatory response, and functional measurements in healthy physically active young male adults following downhill running, leg press and a combination of leg press and leg extension protocol
7. to explore correlations between muscle functional-related changes and biological markers of EIMD, such as CK activity

The primary hypothesis presented here is that EIMD will induce muscle damage and inflammation, attenuate muscle function and recovery which subsequently will decrease exercise performance. Specifically, it is hypothesised that:

1. omega-3 supplementation will decrease muscle inflammation and muscle damage after EIMD

2. omega-3 supplementation will decrease DOMS and improve muscle function measurements after EIMD, and subsequently recovery and exercise performance
3. younger participants will have an improved resolution in muscle damage and inflammatory markers following EIMD compared to older participants
4. muscle functional capacity will be different between younger and older participants following EIMD
5. EIMD will induce plasma EVs in both younger and older participants
6. downhill running protocol will induce greater magnitude of muscle damage and inflammatory markers and larger decrements in muscle function following EIMD compared to both unilateral resistance exercise protocols
7. plasma CK activity will correlate with muscle function markers (e.g., MVIC, DOMS)

CHAPTER TWO

Review of Literature

Description of Chapter

In this chapter, an overview of the literature review of exercise-induced muscle damage (EIMD), omega-3 polyunsaturated fatty acids and age-related changes is given. In addition, different theories and possible mechanisms behind the EIMD will be discussed, as well as different techniques of assessing it. Further, the potential effect of omega-3 supplementation as a nutritional strategy to a) attenuate EIMD and inflammatory response and b) increase muscle function following EIMD will be discussed.

2.1 Scope of Review

In the review of literature that follows, the form and function of skeletal muscle will be examined, which will lead into a discussion of the mechanism and time course of EIMD, the factors affecting recovery from EIMD, and the inflammatory response involved after EIMD. This review, and the thesis as a whole, concentrates on the indirect assessment of muscle damage via blood and functional markers. In addition, the role of nutrition and the dietary supplements in improving exercise performance, and overall health will be discussed, with a particular focus on the n-3 supplementation and its potential beneficial role as a nutritional strategy on EIMD. Finally, some work exploring exercise-induced muscle damage and inflammatory response in older populations, and age-related changes in muscle function will also be investigated. This will help to uncover any potential knowledge gaps within the field.

2.2 SKELETAL MUSCLE

2.2.1 Overview of Skeletal Muscle Form and Function

The human body consists of more than 500 skeletal muscles (Jones et al., 2013) which are controlled by the nervous system, and which connects and supports the skeletal system. Muscle is divided into three tissue types: 1) skeletal muscle, 2) cardiac muscle and 3) smooth muscle. In this work, the term muscle refers to skeletal muscle, unless otherwise specified. Skeletal muscle consists of individual muscle fibres (or myocytes). An individual myofiber is composed of bundles of myofibrils which contain sarcomeres (Figure 2.1).

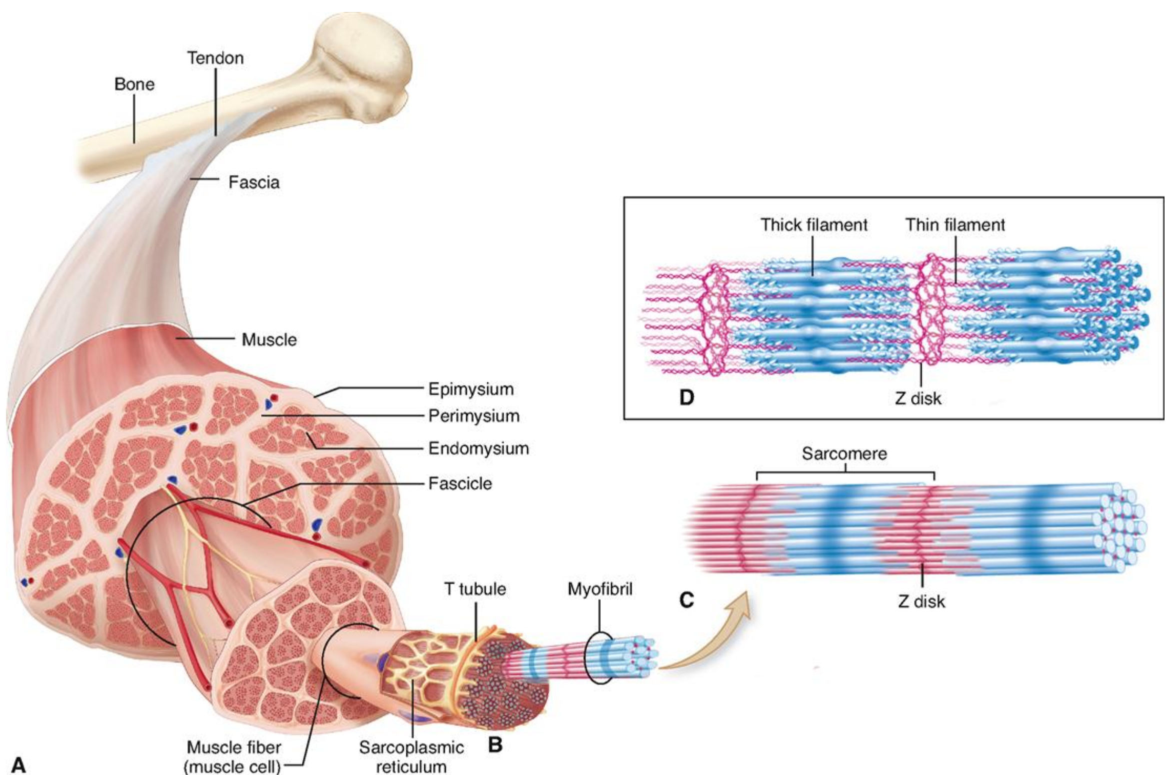


Figure 2.1 Structure of skeletal muscle. **A)** Skeletal muscle composed of bundles of contractile muscle fibres, **B)** Individual fibre showing myofibrils in the sarcoplasm, **C)** Individual myofibril showing a single sarcomere between Z disks, **D)** Molecular structure of myofibril showing thick and thin myofilaments (available from: https://basicmedicalkey.com/physiology-of-the-muscular-system/)

Sarcomeres are responsible for muscle contraction and relaxation which allow the body to perform a variety of different movements, ranging from fast and powerful movements to small and fine motions. The sarcomere is defined as the region between two Z-lines. Each sarcomere comprises a central A-band (thick filaments) and two halves of the I-band (thin filaments). The I-band from the two neighbouring sarcomeres meets at the Z-disk. Each sarcomere is composed of two main protein filaments (Figure 2.2), actin and myosin, which form a protein complex (called actomyosin) by attachment of myosin head on the actin filament. Actin and myosin are the active structures responsible for contracting and shortening the sarcomere; a process called the cross-bridge cycle (Mansfield and Neumann, 2019; Figure 2.3).

Figure 2.2 Skeletal muscle fibre. Coloured scanning electron microscope showing **on the left image:** a cross-sectioned bundle of skeletal muscle fibres (red), called fascicle, together with collagenous connective tissue (white), called perimysium (copyright: Martin Oeggerli), and **on the right image:** a cross-sectioned individual myofiber, red strands represent a bundle of myofibrils which composed of sarcomeres, with exposed intracellular actin and myosin filaments in the sarcoplasm, and white outside layer indicates the cellular sarcolemma. Each fibre is also individually surrounded by a thin layer of connective tissue, called endomysium (copyright: Dennis Kunkel). Available from: https://sciencephoto.com

The most common and widely accepted explanation of the mechanism of muscular contraction is called the 'sliding filament theory' (Wood, 2012; Silverthorn, 2016; Mansfield and Neumann, 2019). In this theory, active force is generated as actin filaments slide past the myosin filaments, resulting in contraction of an individual sarcomere (Figure 2.3). Whilst the sliding filament theory is well supported by experimental evidence *in vitro*, mechanically acto-myosin interactions alone do not seem to explain why eccentric exercise models *in vivo* are capable of such higher force generation than concentric exercise.

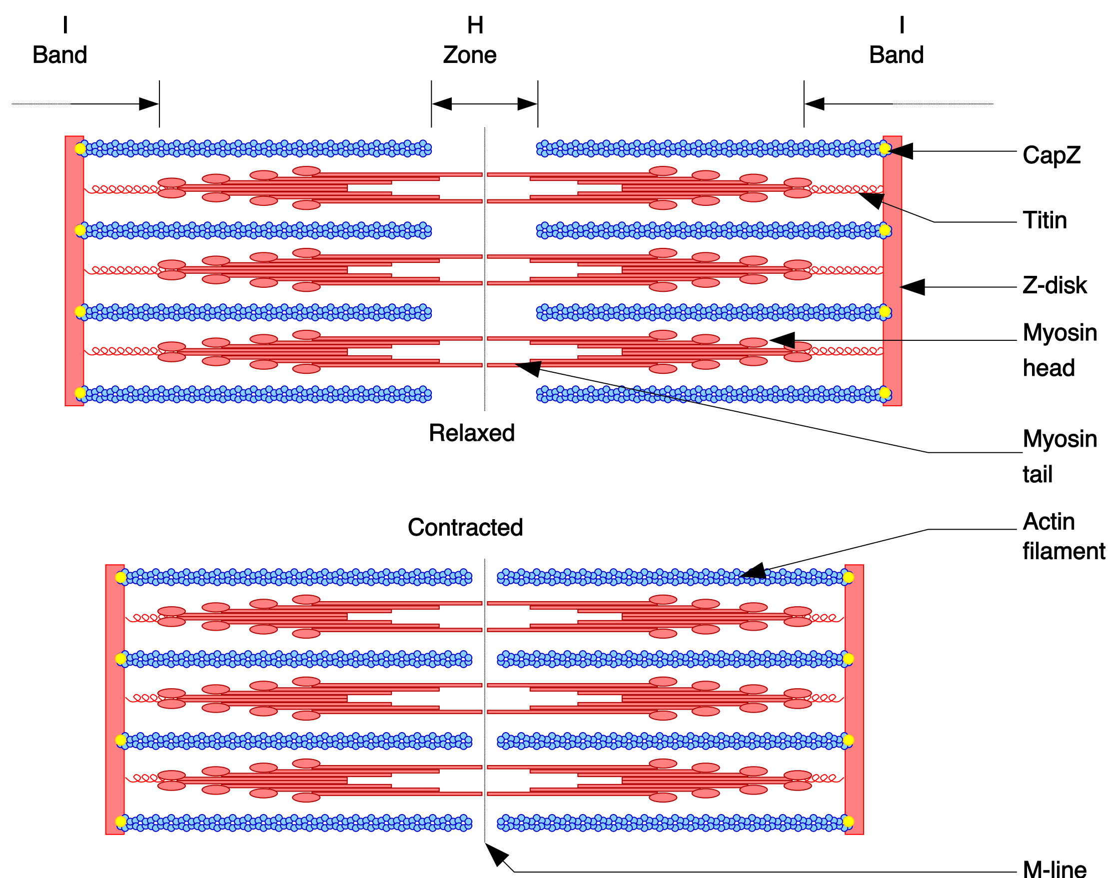


Figure 2.3 Schematic representation of the sarcomere. Muscle fibres in relaxed and contracted positions. The schematic diagram shows the major filament systems that compose the sarcomere: actin (thin), myosin (think) and titin filaments. When a sarcomere contracts, the Z-lines move closer together, and the I band becomes smaller because actin and myosin interaction generates cross-bridges, which slide the myofilaments over each other. At full contraction, the thin and thick filaments overlap completely (Richfield, 2014)

Evidence now suggests that the elastic protein titin plays an important role in active muscle (Nishikawa et al., 2012; Hessel, Lindstedt and Nishikawa, 2017; Montesano et al., 2020). Nishikawa et al. (2012) proposed that titin, previously seen as a passive, spring like role, is actively engaging with actin filaments in a force producing manner. More specifically, in actively contracting muscle fibres, Ca^{2+} influx mechanically activates titin which increases tension and stiffness of the titin spring. Then, the cross-bridges translate and rotate the thin filaments, leading to winding of titin upon them and storage elastic potential energy during force development. Therefore, the 'winding filament theory' provides a simple mechanism by adding the giant titin protein inside active muscle sarcomeres as a ''third filament'' that is regulated by Ca^{2+} influx and cross-bridge cycling, contributing to muscle force enhancement and active shortening during eccentric exercise (Nishikawa et al., 2012; Herzog 2014; Nishikawa, 2016). Thus, the winding filament model builds on the swinging cross-bridge-sliding filament theory and increases its explanatory power for well-established phenomena, including the high force and low cost of eccentric contractions, that remain problematic under the sliding filament theory alone.

Skeletal muscle tissue is important for optimal exercise performance and a significant contributing factor in maintaining optimal health throughout life. As such, muscle tissues are involved in different metabolic pathways. Traditionally, fibres were characterised depending on their colour (red or white), which reflects the myoglobin content.

Type I (or slow twitch) fibres appear red due to the high levels of myoglobin. Red muscles tend to have more mitochondria and use oxidative metabolism to

generate adenosine triphosphate (ATP). These mainly contain the ATPase type I and myosin heavy chain (MHC) type I fibres. They tend to have a low activity level of ATPase and a slower speed of contraction with a more aerobic energy capacity (Radak, 2018).

More glycolytic type II (or fast twitch) fibres, having two subtypes IIA and type IIX, are white because of the relatively low myoglobin content and a reliance on an anaerobic, short term, glycolytic system for energy transfer (Scott et al., 2001). Type II fibres mainly involve the ATPase type II and MHC type II fibres. In addition, fast twitch fibres, especially type IIX fibres, tend to have higher glycogen and creatine phosphate concentrations, and higher enzymes activities associated with glycogenolysis and glycolysis (Valberg, 2008).

Blood lactate concentration is increased during exercise, particularly in type IIX fibres, because of a lack of O_2 to remove the lactate produced by the contracting muscles (Beneke et al., 2011; Dotan, 2012). Therefore, the production of the blood lactate becomes apparent through the anaerobic muscle metabolism and the glycolytic system, as this is the end product of anaerobic glycolysis, the final step of which is the conversion of pyruvate to lactate by the enzyme lactate dehydrogenase (LDH; Rusko et al., 1986; Phypers and Pierce, 2006).

2.2.2 Eccentric *vs* Concentric Muscle Actions

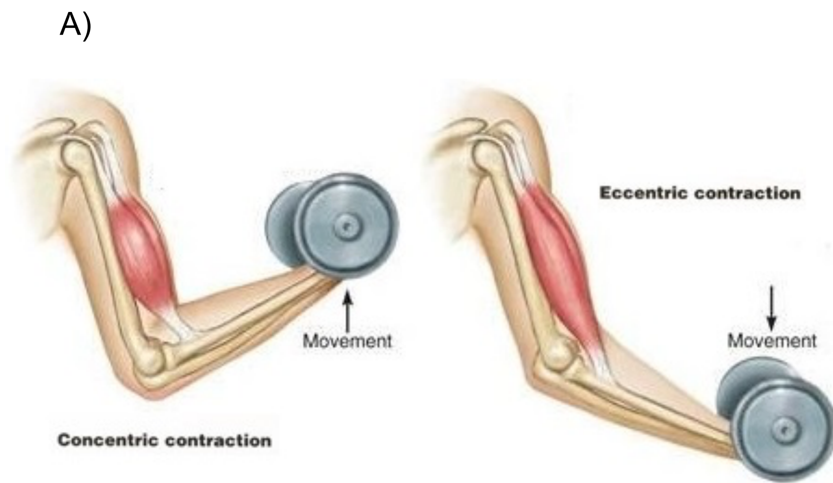
Muscle contractions during exercise can be divided into 3 categories, 1) isotonic contractions (muscle contracts and changes length), 2) isometric contractions (muscle contracts but there is no change in muscle length – muscle force is equal to the resistance) and 3) isokinetic contractions (muscle contracts and changes

length with movements in a constant speed) (Figure 2.4).

There are 2 types of isotonic contraction, the concentric and the eccentric contractions. Concentric muscle contractions may not cause muscle damage; and decrements in force after exercise are restored to baseline in the next few hours (Jones, Newham and Torgan, 1989; Clarkson and Hubal, 2002). On the other hand, eccentric exercise, especially high-force eccentric protocols, produce substantial muscle fibre damage (Newham et al., 1983; Byrne, Twist and Eston, 2004).

A loss of myosin filaments and reduced mitochondria during eccentric contractions, as well as the non-uniform lengthening of sarcomeres can overload the passive cytoskeletal proteins, resulting in greater muscle damage than in concentric exercises (Figure 2.3, Newham et al., 1983; Proske and Morgan, 2001; Herzog, 2014). The recovery time following eccentric actions, such as maximal eccentric exercises and downhill running, can be longer than that associated with concentric ones (Eston et al., 2000; Clarkson and Hubal, 2002). It has been suggested that a mechanism activated during eccentric exercise reduces neural output, and thus muscle activation is limited (Kellis and Baltzopoulos, 1998).

A)



B)

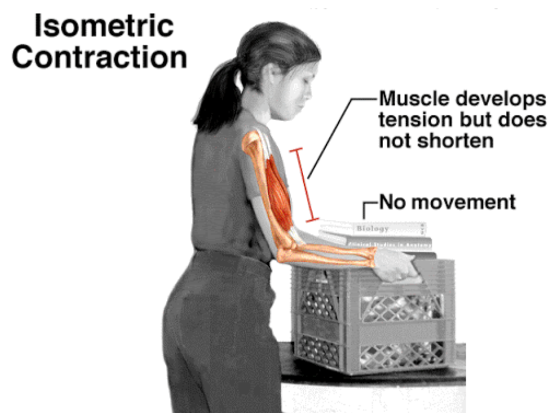


Figure 2.4 Types of muscle contraction. **A) Concentric contraction:** force > load, where biceps muscle shortens during contraction, and **Eccentric contraction:** force < load, where biceps muscle lengthens during contraction. **B) Isometric contraction:** force = load (static contraction), where biceps muscle remains at fixed length during contraction

2.3 EXERCISE AND MUSCLE DAMAGE

EIMD has been an important topic in exercise physiology since 1902, where Hough first suggested that DOMS was a result of micro-lesions in the muscle fibres (Hough, 1902), which was later confirmed by Paulsen et al. (2009) whereby sarcomeres and Z disks are disrupted (Figure 2.5). Since then, however, little work has been done on muscle damage the next 90 years. This started to change over the last two decades, as the interest on EIMD grew in human studies. Most of these studies used eccentric-biased protocols because it is well established that eccentric actions lead to muscle damage (discussed in section 2.2.2; Armstrong et al., 1984; Gibala et al., 1995; Proske and Morgan, 2001; Nosaka et al., 2003; Nederveen et al., 2018).

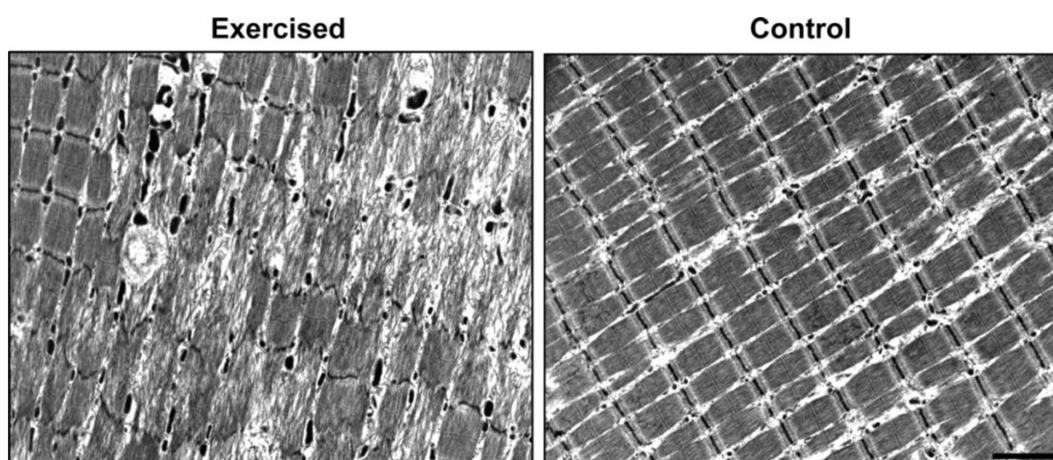


Figure 2.5 Transmission electron microscope image showing a sarcomere of skeletal muscle. Ultrastructural changes (disrupted sarcomeres and Z disks) observed in a myofiber after 1 hour of a bout of eccentric exercise (left) and the undamaged structure of a control (right) (Paulsen et al., 2009)

In addition, it has been demonstrated that there is a significantly larger number of disrupted fibres in eccentric exercise when compared to concentrically biased actions using muscle biopsy (Gibala et al., 1995). An important factor in determining the magnitude of muscle damage is the level of force during muscle

contractions (Clarkson and Sayers 1999; Friden and Lieber 2001). For instance, Nosaka and Sakamoto (2001) examined two eccentric exercises in the elbow flexors with different degrees, one condition was from 100 to 180 degrees and the other was from 50 to 130 degrees. They found a greater muscle damage to the first condition, where elbow flexors are overstretching, compared to the second one. The overstretching was suggested to be a potential reason why eccentric protocols result in greater muscle damage than concentric exercises (Nosaka et al., 2003; Herzog, 2014). The actin and myosin myofilament overlap controls the relationship between sarcomere length and force that is generated in the muscle. In cases when the sarcomere is overstretched the overlap of the myofilaments becomes insufficient resulting in decreased production of force (Figure 2.6).

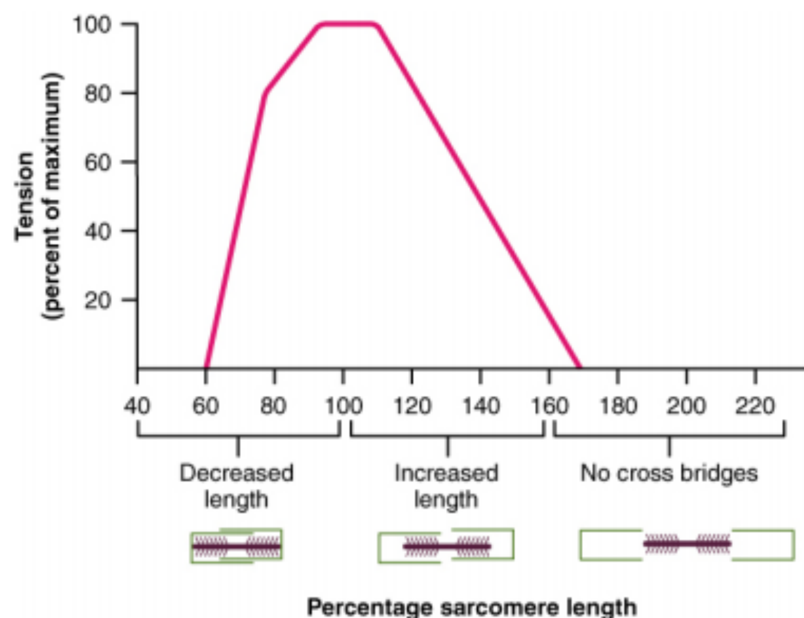


Figure 2.6 The ideal length of a sarcomere. Sarcomeres produce maximal tension when thick and thin filaments overlap between about 80 to 120% approximately 1.6 to 2.6 micrometers (available from: https://openstax.org/details/books/anatomy-and-physiology)

2.3.1 Mechanism of Action of EIMD

Exercise-induced muscle damage is associated with both mechanical and metabolic alterations (Hyldahl and Hubal, 2014). Mechanical factors, such as heavy eccentric exercise, often lead to damage of the excitation-contraction (E-C) coupling system by disturbing intracellular calcium homeostasis and overstretching of sarcomeres in myofibrils resulting in muscular fibre breakdown (Newham et al., 1983; Clarkson and Sayers, 1999; Warren et al., 2001; Lovering and De Deyne, 2004; Herzog, 2014). More specifically, a misalignment of the Z line and the A band with myofibrillar damage occur during eccentric contraction in type II fibres (Newham et al., 1983; Gibala et al., 1995; Byrne, Twist and Eston, 2004). Additionally, metabolic muscle damage can induce EIMD after prolonged exercise regimens due to the production of reactive oxygen species (ROS) generated by neutrophils, enforcing a further damage to the sarcolemma. The initiation of muscle regeneration is then achieved via the inflammatory response that follows the damage to myofibrils (Figure 2.7; Clarkson and Sayers, 1999; Walsh et al., 2011).

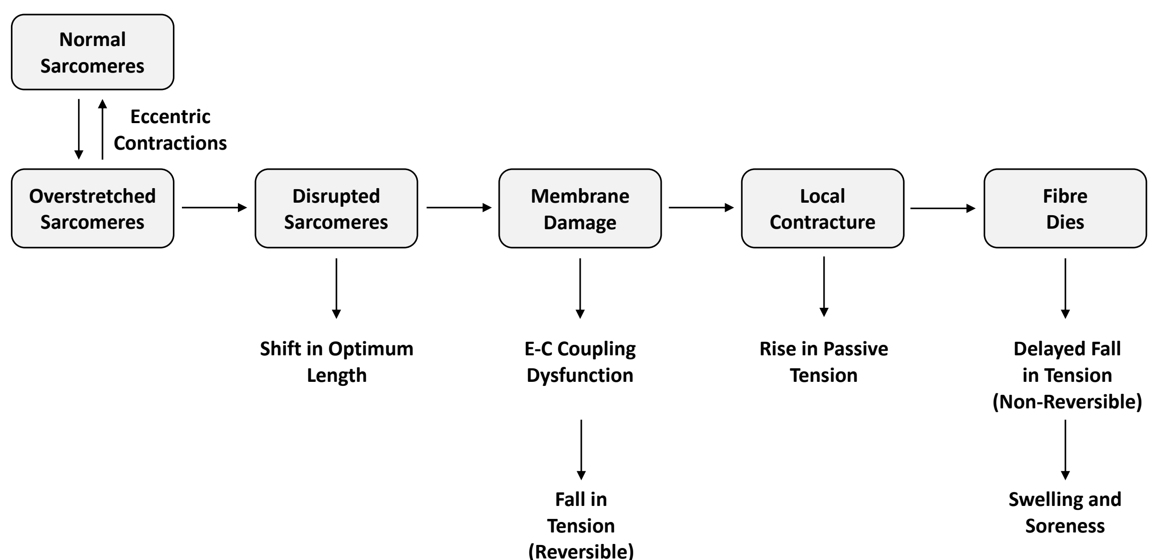


Figure 2.7 Postulated series of events leading to muscle damage from eccentric exercise (Proske and Morgan, 2001)

2.3.2 Time Course of EIMD and the Factors Affecting Recovery from EIMD

The disruption of normal myofibrillar banding patterns is increased immediately after eccentric exercise (Gibala et al., 1995). Disrupted sarcomeres and Z disks in myofibrils peak between 1 and 3 days post-exercise (Newham et al., 1983; Yu et al., 2004; Crameri et al., 2007); and may remain elevated up to 6 to 8 days post-exercise (Jones et al., 1986; Yu et al., 2004). It appears that the magnitude of EIMD, and consequently the muscle strength loss, is related to the recovery time needed to restore muscle strength back to normal. For example, when muscle strength is reduced by ~20% immediately post-exercise it is typically back to baseline within 2 days after exercise (Crameri et al., 2007). Conversely, when it is decreased by ~50%, muscle strength may remain below the pre-exercise values for 7 days post-exercise (Lauritzen et al., 2009; Paulsen et al., 2010).

As can be seen in Figure 2.8, the time course of changes in muscle strength, ROM, DOMS, muscle swelling and plasma CK varies after eccentric exercise. For instance, when recovery time of muscle strength is prolonged, DOMS peaks around day 2 and is not restored by day 4 after the exercise. Whereas, the peak of muscle swelling and plasma markers of muscle damage, such as CK activity, is delayed between 4 and 5 days after maximal eccentric exercise (Damas et al., 2016). Whilst the time course of recovery and the associated negative symptoms may lead to long-term training adaptations, performance may be acutely compromised.

As discussed earlier, greater EIMD and/or prolonged recovery time can arise via mechanical and metabolic mechanisms depending on factors, such as the intensity and the duration of the exercise (Peake et al., 2017). More specifically,

greater mechanical strain is associated with high *vs* low eccentric torque (Nosaka and Newton, 2002; Paschalis et al., 2005) and increasing number of eccentric muscle contractions (Brown et al., 1997) due to the greater damage to contractile proteins and extracellular matrix (ECM; Nosaka, Newton and Sacco, 2002a). In addition, severe EIMD is most likely caused at long *vs* short muscle length, leading to a larger disruption of stretched sarcomeres and a greater degree of non-uniform lengthening of sarcomeres (Child, Saxton and Donnelly, 1998; Nosaka and Sakamoto, 2001).

Moreover, greater degree of muscle damage is caused when the exercise protocol includes a single joint *vs* multiple joints (Soares et al., 2015), where theoretically fewer, smaller and more vulnerable to overstretching muscle groups are recruited. The magnitude of EIMD is also affected by the different muscle groups used (arms *vs* legs; Jamurtas et al., 2005) and knee flexors *vs* extensors (Chen et al., 2011). It is possible due to differences in the level of mechanical loading (Chen et al., 2011). Furthermore, muscle damage is greater following a large number of fast (210 °/s) *vs* slow (30 °/s) velocity eccentric contractions (Chapman et al., 2008). An explanation of this is because faster contractions may cause greater force at longer lengths, which enlarge the degree of damage to contractile proteins as explained earlier (Nosaka, Newton and Sacco, 2002a). Additionally, it is possible that fewer cross bridges are activated by faster contractions, and thus there is a risk of greater mechanical stress produced per active cross bridge (Peake et al., 2017). The recovery time from EIMD is affected by the well-known factor, the repeated bout effect (RBE; Nosaka et al., 2001; Paulsen et al., 2009), where the muscle adapts and is protected. This will be further discussed in section 2.4.3 (adaptation).

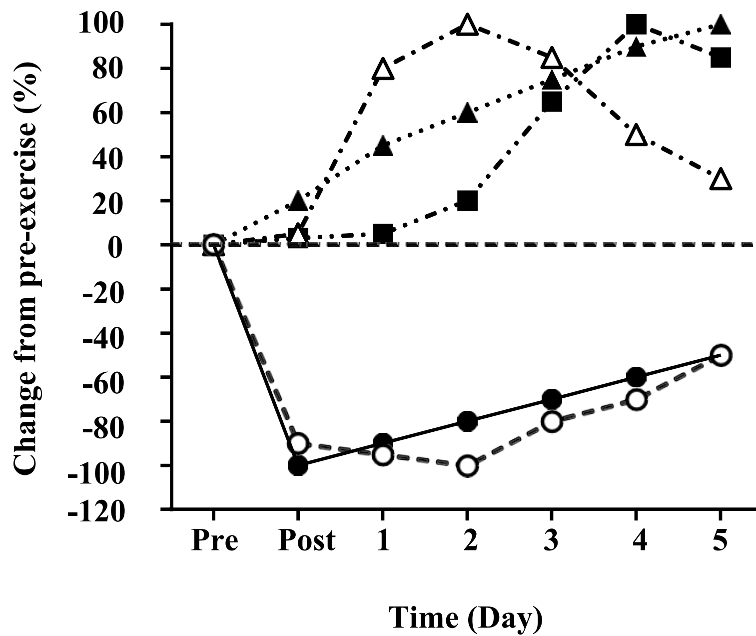


Figure 2.8 Typical magnitude and time course of changes in maximal voluntary contraction (MVC), ROM, DOMS (assessed by visual analogue scale), swelling (measured by circumference) and plasma CK. They were measured before (Pre), immediately after (Post), and 1-5 days after EIMD, and performed by healthy young men who were unaccustomed to the exercise (Data are derived from Damas et al., 2016). ●, strength; ○, range of motion; ▲, swelling; △, soreness; ■, creatine kinase

2.4 INFLAMMATORY RESPONSE AFTER EIMD

Unfamiliar eccentric exercise stimulates an acute inflammatory response (Smith, 1991; Peake, Nosaka and Suzuki, 2005; Peake et al., 2017). The term ''inflammation'' is often used without definition as to whether it is a ''good'' or ''bad'' process. In the context of sports medicine, ''inflammation'' as a response to exercise involves ''clinical, physiological, cellular and molecular'' changes in injured skeletal muscle tissue (Scott et al., 2004). Traditionally, exercise-induced inflammation has been considered as a harmful process related to tissue damage, pain and delayed recovery (Toumi and Best, 2003). However, an updated definition encompassing the regulation and beneficial effects of inflammation after exercise is needed (Peake et al., 2017). In the context of this thesis, inflammation is considered as a detrimental process to participants

undertaking muscle-damaging exercise where recovery is the key to exercise performance.

The notion that inflammation is the main reaction that is taking place to protect, localize and remove injurious agents from the body in order to prepare it for healing and repair is now gaining acceptance (Clarkson and Hubal, 2002; Calder, 2006; Peake et al., 2017). Under non-pathological conditions, such as after EIMD, intramuscular inflammation is a dynamic process that eventually leads to adaptive remodelling and return to homeostasis (Tidball, Dorshkind and Wehling-Henricks, 2014; Chazaud, 2016). More specifically, inflammatory cells, such as macrophages and neutrophils, appear to infiltrate skeletal muscle tissue following primary muscle damage in 55% and 85% of the human and animal studies, respectively (Tidball, 2005; Schneider and Tiidus, 2007), in order to initiate the muscle repair and regeneration process (Peake, Nosaka and Suzuki, 2005; Schoenfeld et al., 2013). The acute phase inflammatory response following exercise involves many mediators such as interleukin -1 receptor antagonist (IL-1ra), IL-6, IL-10 and acute phase proteins (Hirose et al., 2004; Duque and Descoteaux, 2014). The role of all these mediators will be further analysed below.

2.4.1 Macrophages, Neutrophils and Exercise

Regular exercise can stimulate changes in the immune system at a cellular level. It has been observed that immune cells deviate from homeostasis as a response to exercise (Ortega, 1994; Pedersen and Hoffman-Goetz, 2000; Ortega et al., 2005). Phagocytic cells are part of the immune system and play an important role as they are the first line of defence of the organism against pathogens. Macrophages are the main phagocytic cells in the tissues and neutrophils in the

blood (Clarkson and Hubal, 2002; Duque and Descoteaux, 2014). Generally, exercise appears to influence phagocytic processes, which consist of several stages (adherence, chemotaxis, phagocytosis, microbicidal activity). Circulating cells numbers are increased by the exercise, possibly due to increased blood flow and increased catecholamine levels (Bousquet et al., 1996). Macrophages have three main functions in the immune system, 1) phagocytosis, 2) antigen presentation (AP) and 3) cytokine production. Cytokines secreted by T helper cells, such as interferon-gamma (IFN-γ), activate macrophage activity in the inflammatory response (Duque and Descoteaux, 2014). Circulating concentration of cytokines are elevated after exercise (Febbraio and Pedersen, 2002; Gleeson, 2007).

The inflammatory response depends on the type, intensity and duration of exercise (Tidball, 2005; Schneider and Tiidus, 2007). Indeed, chronic moderate exercise stimulates macrophages functions (Okutsu et al., 2008), whereas no effect on macrophages functions has been reported in acute exercise to exhaustion (Ortega, 2003). It also appears that tissue macrophage is decreased by the volume and intensity of exercise in animal studies (Woods et al., 2000), however, in human studies, there is no evidence to date to expand this effect. For example, Woods et al. (1997) showed that MHC type II induction on peritoneal macrophages was suppressed by both moderate and exhaustive exercise in mice that exercised for 4 days during inflammation. More specifically, they found that peritoneal macrophage antigen presentation (AP) was inhibited by exhaustive exercise when measured immediately post-, at 3- and 24-hours post-exercise. However, macrophage AP was suppressed by moderate exercise only at 3 hours post-exercise.

In addition, the time course of inflammatory response after eccentric exercise is variable, depending on the type, intensity and duration of exercise (MacIntyre et al.,1995; Pyne et al., 2000). Indeed, Smith et al. (1989) observed higher neutrophil concentrations after eccentric exercise relative to the concentric protocol using the same participants with similar levels of oxygen consumption. Ortega et al. (1994) also showed that there is an increased neutrophil activity during intense physical activity, leading to the conclusion that neutrophils may contribute to muscle injury. However, there are conflicting conclusions (Nieman et al., 1998a; Novak, Weinheimer-Haus and Koh, 2014); since morphological observations have not only shown neutrophils can damage muscle tissue by releasing high concentrations of cytolytic and cytotoxic molecules (Tiidus, 1998), but they can also release proteases that can help remove cellular debris produced by muscle damage. Chronic moderate exercise decreases pro-inflammatory cytokines and increases anti-inflammatory cytokines (Petersen and Pedersen, 2005). More specifically, it has been demonstrated that acute exercise increases neutrophils apoptosis while chronic exercise delays it (Su et al., 2011; Syu, Chen and Jen, 2012). Therefore, chronic moderate exercise affects neutrophil functions and thus enhances immunity (Woods et al., 1999; Syu, Chen and Jen, 2012).

Finally, it has also been reported that many macrophage functions, such as cytokine production and ROS generation, are dysregulated with ageing (McLachlan et al., 1995). For instance, Woods et al. (2000) examined the effect of exercise on macrophage functions in aged mice and they demonstrated that peritoneal macrophage function in aged mice could be restored by 4 months of training on a treadmill. Given that EIMD stimulates an acute phase inflammatory

response, which involves infiltration of the damaged muscle by macrophages and neutrophils, it has also been reported that sex is another factor that may affect EIMD responses. More specifically, there is a significantly less cellular infiltrate in women than men (Fielding, Manfredi, and Ding, 1993). This finding could be attributed to the female sex hormone 17β-oestradiol, which has antioxidant properties, and it may have a positive effect on the reduction of muscle membrane damage (Tiidus, 1995; Tiidus, 2003). However, the role of oestrogen in the aetiology of EIMD and inflammatory response need to be further examined (Stupka et al., 2000; Clarkson and Hubal, 2002).

Therefore, it appears that macrophages and neutrophils are not only phagocytic, but they also may repair via the release of cytokines as it was mentioned earlier. Thus, the inflammatory response to EIMD remains unclear, because factors, such as exercise protocols (duration and mode of exercise, timing of measurement), age and sex seem to contribute to the effects of acute and chronic exercise (Brickson et al., 2003; Wolach et al., 2005).

2.4.2 Cytokines (IL-6), Signalling Proteins (TNF-α)

Cytokines are glycoproteins, produced primarily by leukocytes, and regulate immune responses (Peake, Nosaka and Suzuki, 2005; Duque and Descoteaux, 2014). Cytokines are produced by different cell types, including immune cells such as macrophages, B and T lymphocytes (Dinarello, 2007). They may include chemokines, interferons, interleukins and tumour necrosis factors and they are important in cell signalling, specifically in host responses to infection, immune responses, inflammation and trauma (Duque and Descoteaux, 2014).

Cytokines can be classified as pro- or anti-inflammatory. Pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, TNF-α, enhance and stimulate the inflammatory response. On the other hand, anti-inflammatory cytokines, such as IL-4, IL-10, IL-1ra and acute phase proteins, such as C-reactive protein (CRP) suppress the inflammatory response. Both pro- and anti-inflammatory cytokines regulate each other to maintain homeostasis and to determine the overall process of the inflammatory response and an adequate repair of the damaged muscle (Pedersen and Toft, 2000; Peake, Nosaka and Suzuki, 2005; Philippou et al., 2018).

2.4.2.1 Interleukin (IL)-6 Response to Exercise

IL-6 is a cytokine which is produced in larger amounts than any other cytokine in association with exercise (Pedersen and Hoffman-Goetz, 2000; Pedersen, Steensberg and Schjerling, 2001; Tarbinian et al., 2011; Brown et al., 2015). Additionally, despite its large variability in response to eccentric exercise, it is widely used as an inflammatory marker and as an indicator of EIMD (Bruunsgaard et al., 1997; Febbraio and Pedersen, 2002; Ormsbee et al., 2015). Most of the studies showed increased IL-6 concentrations after exercise and its relation in response to EIMD, specifically eccentric exercise (Hellsten et al., 1997; Croisier et al., 1999; Smith et al., 2000; Toft et al., 2002; Peak et al., 2005; Smith et al., 2007; Tarbinian et al., 2011; Reihmane and Dela, 2013; Brown et al., 2015). However, IL-6 may be not optimal as an EIMD marker due to the wide variation of exercise protocols, sampling times or participants' training status (Peake, Nosaka and Suzuki, 2005; Paulsen et al., 2012). Bruunsgaard et al. (1997) reported that IL-6 concentrations were higher after eccentric actions compared to those after concentric actions. Others have shown no differences in plasma IL-6

between downhill running and level running, although CK activity was higher after downhill running, a traditional marker of muscle damage (Peake et al., 2005). More details about the function of CK will be discussed in section 2.5.2.1.

Hence, it may be possible that IL-6 might represent an early phase response (Heinrich, Castell, and Andus, 1990; Smith et al., 2000; Liem et al., 2015), suggesting that the exercise-induced increase in IL-6 is a result of an immune response due to local damage in the contracting muscles (Bruunsgaard et al., 1997; Nieman et al., 1998b). Indeed, Febbraio and Pedersen (2002) proposed that the complex signalling cascades that are initiated by intracellular Ca^{2+} ion concentration changes stimulate intramuscular IL-6 release via the upstream activation of the pro-inflammatory transcriptional regulators nuclear factor-κB (NF-κB) and c-Jun N-terminal kinase (JNK), aiding the maintenance of metabolic homeostasis during periods of altered metabolic demands, such as muscular exercise. Therefore, it is plausible that the IL-6 response is to muscle contraction *per se* and may not be related to muscle damage.

Nevertheless, circulating IL-6 depends on several factors, such as mode, intensity and duration of the exercise (Ostrowski, Schjerling and Pedersen, 2000; Febbraio and Pedersen, 2002). For example, Pedersen and Febbraio (2008) suggested eccentric exercise increased plasma IL-6 concentration up to 100-fold. This is supported by another finding which showed that IL-6 was significantly increased after a prolonged endurance activity, such as a marathon race (Bernecker et al., 2013), whereas it peaked immediately after running with a shorter duration (2.5 hours of treadmill running) and was 25-fold above the pre-exercise values (Ostrowski et al., 1998a). Nielsen et al. (1996) also noted only a

2-fold increase in IL-6 after a short duration of activity (6 minutes of maximal rowing exercise). Hence, the type of muscle contraction seems to have a significant effect on the systemic appearance of IL-6. Therefore, regardless of the aforementioned factors in relation to IL-6 response to exercise, IL-6 seems to be a multifunctional cytokine as an ''exercise-factor'' mediating aspects of the exercise-induced acute phase response and the beneficial effects of exercise metabolism. A summary of studies examining plasma IL-6 in response to EIMD are presented in Table 2.1.

Table 2.1 Summary of studies investigating plasma IL-6 in response to EIMD

Authors	Exercise Modality	Experimental Measurements	Peak of IL-6	Recovery to Baseline
Bruunsgaard et al., 1997	Cycling for 20 min at 150% of $\dot{V}O_2max$ & 10 min at 100% of $\dot{V}O_2max$	Pre-exercise, 20 min post-, 30 min post-, 2h post-, 2-, 4- and 7-days post-exercise	2h post-exercise	Yes, 1 day
Ostrowski et al., 1998a	2.5 h of treadmill running, 75% of $\dot{V}O_2max$, 2.5% incline	Pre-exercise, every 30 min during running, and 1, 2, 3, 4, 5, 6h post-exercise	At the end of running 25-fold above the pre-exercise value	Yes, remained until 3h post-running
Croisier et al., 1999	30 maximal eccentric contractions of the knee flexors and extensors	Pre- and immediately post-exercise, and 30-min, 48, 72 and 96h post-exercise	30 min post-exercise	Yes, 48h post-exercise
Ostrowski, Schjerling and Pedersen, 2000	Marathon race	Pre- and immediately post-race, 1.5h, 3h, 1 day & 2 days post-race	100-fold immediately post-running	Yes, 31-fold 1.5h post-exercise
Smith et al., 2000	Bench press eccentric exercise (4 sets, 12 reps)	Pre-exercise and 1.5, 6, 12, 24, 48, 72, 96, 120 and 144h post-exercise	24h post-exercise	Yes, 96h post-exercise
Tarbinian et al., 2011	40 min of bench stepping eccentric exercise	Pre- and immediately post-exercise, and 24 and 48h post-exercise	Immediately post-exercise	Yes, 48h post-exercise

van de Vyver and Myburgh, 2012	Intermittent downhill running (12 x 5 min bouts) for 60 min at 85% of $\dot{V}O_2max$, 10% decline	Pre- and immediately post-exercise, and every day for 9 days post-exercise	Immediately post-exercise	Yes, 1 day
Bernecker et al., 2013	Marathon race	Pre- and immediately post-race	Within 1h post-race	No
Clifford et al., 2016	100-drop jumps	Pre- and immediately post-exercise and at 2, 24, 48, 72h post-exercise	Immediately post- and 2h post-exercise	Yes, 24h post exercise

2.4.2.2 Tumour Necrosis Factor-α Response to Exercise

TNF-α is another inflammatory cytokine that has an important role in regulating inflammatory response (Zhang and An, 2007; Kanda et al., 2013). More specifically, it is one of the first pyrogenic cytokines to be activated during the acute phase of the immune response (Beutler, 1999; Duque and Descoteaux, 2014). TNF-α is secreted by macrophages, neutrophils and T cells, and is regulated by various cytokines, such as IFN-γ and IL-6. IL-6 inhibits the production of TNF-α, while on the other hand, TNF-α can stimulate the expression of IL-6 in muscle cells (Frost and Lang, 2005; Pedersen et al., 2007; Philippou et al., 2018). In a similar action to IL-6, TNF-α promotes the synthesis of CRP from the liver (Duque and Descoteaux, 2014).

Whilst a number of human studies have showed elevated TNF-α concentrations following strenuous exercise (Clarkson, Nosaka and Braun,1992; Brenner et al., 1999; Toft et al., 2000; Tarbinian et al., 2011), others have reported no significant increase in TNF-α after a bout of eccentric exercise (Smith et al., 2000). In particular, Philippou et al. (2018) examined the inflammatory response to EIMD, and they found that TNF-α presented only mild changes over the experimental

period, which did not reach statistical significance. Meanwhile, other studies found minor change in TNF-α concentrations (Ostrowski et al., 1998b; Suzuki et al., 2000), or even 15% lower than pre-exercise values at 6 hours post-exercise (Ostrowski et al., 1998a). In addition, Bruunsgaard et al. (1997) showed that circulating TNF-α was below detection limit (0.1 $pg \cdot m^{-1}$) following eccentric actions. This could reflect a feedback mechanism, as previously mentioned, that IL-6 inhibits TNF-α, and therefore resulting to the inflammation ending (Chrousos, 1995). This could suggest once more that inflammatory response is related to the type and intensity of exercise and/or the muscle groups that are used (Tarbinian et al., 2011; Kanda et al., 2013). It is also possible due to different timing of the blood sampling (MacKinnon, 1999). A summary of studies examining plasma TNF-α in response to EIMD are presented in Table 2.2.

Table 2.2 Summary of studies investigating plasma TNF-α in response to EIMD

Authors	Exercise Modality	Experimental Measurements	Peak of TNF-α	Recovery to Baseline
Bruunsgaard et al., 1997	Cycling for 20 min at 150% of $\dot{V}O_2max$ & 10 min at 100% of $\dot{V}O_2max$	Pre-exercise, 20 min post-, 30 min post-, 2h, 2, 4 and 7 days post-exercise	below the detection limit	N/A
Ostrowski et al., 1998a	2.5 h of treadmill running, 75% of $\dot{V}O_2max$, 2.5% incline	Pre-exercise, every 30 min during running, and 1h, 2h, 3h, 4 h, 5h 6h post-exercise	At the end of running 25% increased	Yes, remained until 3h post-running
Brenner et al., 1999	2h cycling at 60-65% of $\dot{V}O_2max$	Pre- and immediately post-exercise, and 3, 24h and 72h post-exercise	At 72h post-exercise	No
Smith et al., 2000	Bench press eccentric exercise	Pre-exercise and at 1.5, 6, 12, 24, 48, 72, 96, 120 and 144h post-exercise	No significant elevations after exercise	N/A

Toft et al., 2000	Marathon race	A week before race, immediately post-race, and 1.5 and 3h post-race	Immediately after race	Yes, at 3h post-exercise
Suzuki et al., 2000	Marathon race	Pre- and immediately post-race	Below detection limits	N/A
Tarbinian et al., 2011	40 min of bench stepping eccentric exercise	Pre-exercise and immediately post-exercise, and 24 and 48h post-exercise	48h post-exercise	No
van de Vyver and Myburgh, 2012	Intermittent downhill running (12 x 5 min bouts) for 60 min at 85% of $\dot{V}O_2max$, 10% decline	Pre- and immediately post-exercise, and every day for 9 days post-exercise	24h post-exercise	Yes, 1 day
Philippou et al., 2018	50 MVC of knew extensors	Pre-exercise and at 6, 48 and 120h post-exercise	By 50% 6h post-exercise	Yes, at 120h post-exercise

2.4.3 Adaptation

Many studies have found that a single bout of eccentric exercise can produce a protective adaptation making muscle tissue more resistant to damage in following bout of exercise after a week and up to six months (Byrnes et al., 1985; Clarkson, Nosaka and Braun,1992; McHugh, 2003; Chen et al., 2009; Margaritelis et al., 2015). This has been expressed with a faster recovery in strength, less muscle soreness and inflammation after the second exercise bout (Howatson and Someren, 2007; Chen et al., 2016). Although the explanation of the exact mechanism of the RBE is still not fully understood, data suggest it involves neural, mechanical and cellular theories (Figure 2.9; Pizza et al., 1996; Clarkson and Hubal, 2002; McHugh, 2003).

More specifically, the neural component of this involves different explanations. The first suggests that there is a greater recruitment of slow twitch motor units, while the other one suggests there is activation of larger motor unit pool (McHugh, 2003). For each of these two explanations there is conflicting evidence that does not help clarify the neural pathway. In particular, the evidence that supports the explanation of the increased recruitment of slow twitch motor units is based on the fact that there is decreased electromyography (EMG) median frequency for the repeated bout, while on the other hand RBE has been shown with stimulated contractions (Warren et al., 2001). The evidence that supports the second explanation for the neural pathway is that EMG/torque has been shown to increase with eccentric exercise. At the same time though, EMG/torque has not been found to be different between initial and repeated eccentric bouts (Nosaka et al., 2001).

The mechanical theory is based on evidence that dynamic and passive muscle stiffness have been found to increase after eccentric exercise (Reich et al., 2000). However, there is limited information on either passive or dynamic stiffness adaptations to a single bout of eccentric exercise. The cellular theory shows the longitudinal addition of sarcomeres and the adaptation in the inflammatory response that follows a bout of eccentric exercise protocol. This addition of sarcomeres is believed to help reduce the strain on sarcomeres (Morgan, 1990; Brockett et al., 2001). However, submaximal eccentric exercise protocols have not been found to protect against damage from maximum contractions (Nosaka and Newton, 2002). Furthermore, the inflammatory adaptations may limit the extent of damage that usually occurs the days right after the eccentric exercise protocol takes place (Pizza et al., 2002). On the other hand, inflammatory

adaptation cannot explain the decreased mechanical disruption after a repeated bout (McHugh, 2003).

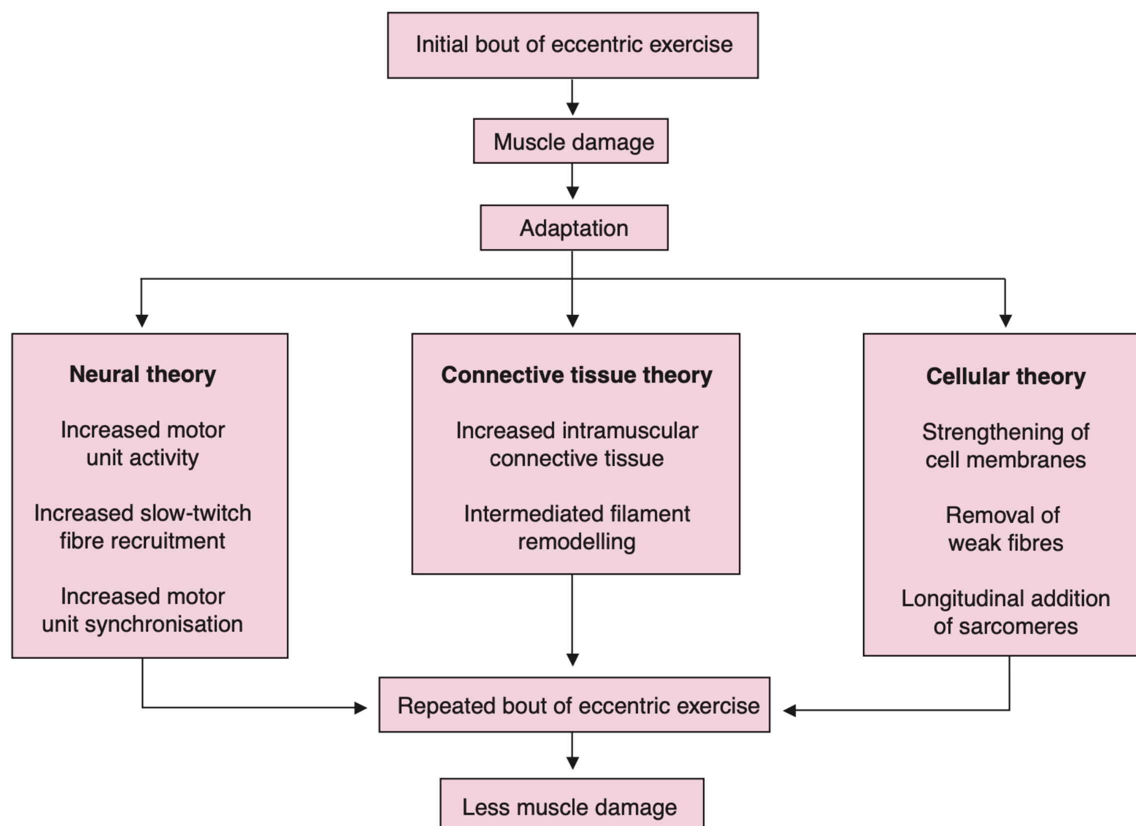


Figure 2.9 Potential mechanisms for the repeated bout effect (taken from McHugh et al., 1999)

Moreover, Nosaka et al. (2001) found that only 2 maximal eccentric contractions have been shown to have a protection against symptoms of EIMD when a repeated bout of eccentric exercise performed after 2 weeks. It does appear that high-force contractions are related with greater mechanical strain when compared with low-force (Nosaka, Newton and Sacco, 2002b). Similarly, another study showed a light eccentrically biased protocol of maximal voluntary contraction (MVC) was effective enough in diminishing muscle damage in a subsequent heavier eccentric protocol (Lavender and Nosaka, 2006). It is plausible that the muscle is less susceptible to overstretching during subsequent

damage (Peake et al., 2017). Therefore, it has been suggested that sarcomeres are increased in muscle fibres during the subsequent adaptation (Morgan, 1990; McHugh et al., 1999).

Furthermore, Pizza et al. (1996) showed both smaller number and lower concentrations of circulating neutrophils in a subsequent exercise after 3 weeks. It has been reported that neutrophils and monocytes produce more damage while entering the fibres and therefore, their reduced activity could affect with less damage in the muscle. Additionally, Smith et al. (2007) concluded that the inflammatory response was blunted following RBE, with plasma IL-6 concentrations reduced following a subsequent muscle damage. The rapid adaptation of the muscle following EIMD increases the possibility to apply a mild eccentric exercise as a strategy to protect a muscle against injury (Clarkson and Tremblay, 1988; Proske and Morgan, 2001). Further, the idea of hormesis applied to exercise models (Radak, Chung and Goto, 2005) for the management of EIMD, suggests that the adaptive response acts in a bell-shaped fashion. Using this theory (see Figure 2.10), Owens et al. (2019) proposed a conceptual region for intervention (yellow text box in Figure 2.10) where the exercise stimulus impairs the recovery time to return to training and competition. This shows that it is important to find a balance between recovery and adaptation.

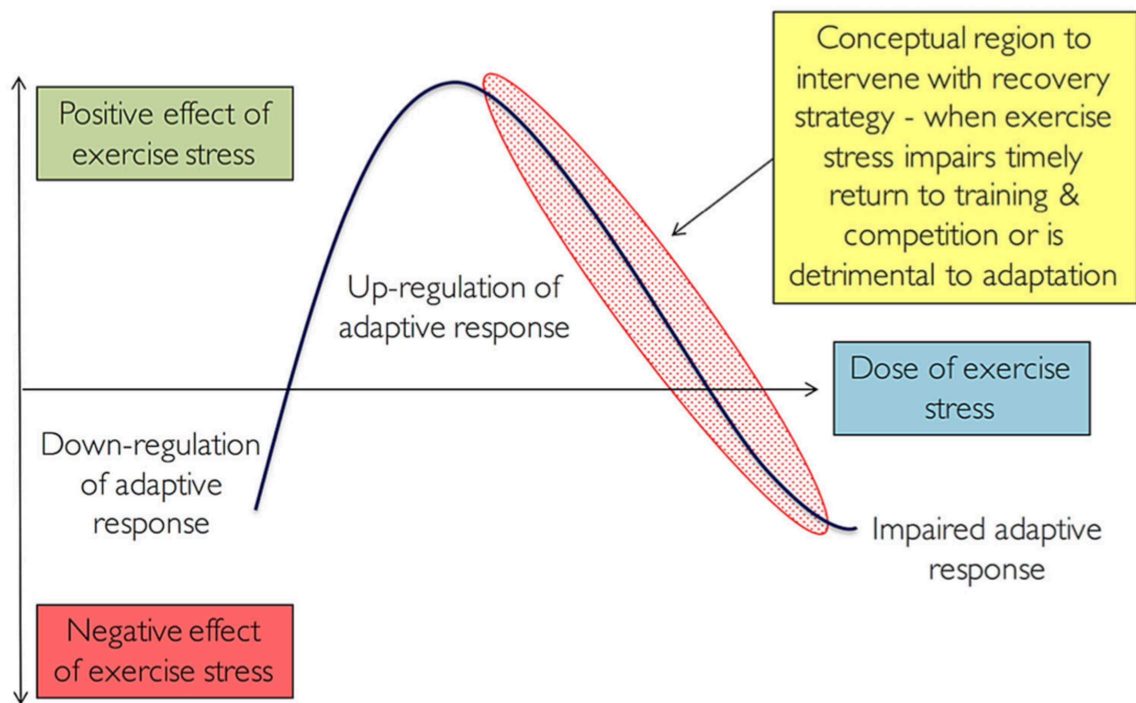


Figure 2.10 Representation of a bell-shaped hormesis curve for the EIMD management. The adaptive response is illustrated as a bell-shaped curve; the beneficial effect of the exercise stress lasts up to the point where the exposure becomes too great. From this point onwards the adaptive response is reduced (taken from Owens et al., 2019)

2.5 ASSESSEMENT OF MUSCLE DAMAGE

2.5.1 Direct Methods of Muscle Damage Assessment

Muscle damage can be measured directly via muscle biopsy or magnetic resonance imaging (MRI). Histological evidence from biopsies shows ultrastructural changes in Z lines, loss in thick myofilaments and disturbances of filaments at the A-Band in type II fibres after a muscle-damaging exercise (Friden, Sjostrom and Ekblom, 1981; Byrne, Twist and Eston, 2004). Findings from human studies revealed that a bout of eccentric exercise create an insult to fibres which leads to damage of the ultrastructural, ECM and to capillaries (Beaton et al., 2002; Paulsen et al., 2012; Nederveen et al., 2018). These disturbances seem to

be the case of the activation of the inflammatory response, and thus the initiation of muscle repair and regeneration process as discussed earlier in the chapter.

However, muscle biopsy is a painful and invasive technique and muscle damage is only investigated from a small sample. Therefore, it may not represent the whole muscle, resulting in overestimating or underestimating the damage (Warren et al., 1999; Clarkson and Hubal, 2002). There is also an argument that the method itself can cause damage and increase inflammation in the muscle, and thus it may affect the results (Roth et al., 2000a; Baird et al., 2012). Indeed, Malm et al. (2000) investigated the effect of multiple muscle biopsies over a period of a week in both control participants and participants who performed eccentric exercise. They showed similar changes in infiltration of leukocytes in both conditions. Therefore, these findings demonstrated that the procedure itself may cause some changes inaccurately attributed to EIMD.

MRI has been a powerful tool and can assess damage in an entire muscle. Mair et al. (1992) investigated the association between MRI and indirect markers of muscle damage, such as muscle strength loss and CK activity after eccentric exercise. They demonstrated that T2 relaxation time showed peak increases 6 days after ECC of knee extensors, which appears to coincide with the delayed peak responses of the CK activity. Findings of Yanagisawa et al. (2015) concurred with those of Mair et al. (1992), who evaluated the effect of EIMD on muscle hardness and assessed the relationship between muscle hardness and indirect markers of muscle damage using MRI. They showed a significant time effect of changes in serum CK activity, in muscle soreness, ROM and elevated magnetic resonance T2 value (an index of oedema) in the medial gastrocnemius

on days 1 to 7 after exercise, suggesting that repeated eccentric muscle contractions damaged muscle fibres. However, muscle damage indicators (e.g., ROM, DOMS) did not correlate with the increased muscle hardness after exercise. Nevertheless, MRI requires a skilled operator, and the cost of the equipment is relatively high. For all these reasons, indirect methods, such as DOMS, MVC, ROM, CK, have been used extensively to assess muscle damage (Clarkson and Hubal, 2002; Paulsen et al., 2012).

2.5.2 Indirect Methods of Muscle Damage Assessment

2.5.2.1 Myocellular Proteins (CK)

Indirect markers of muscle damage have been studied to assess muscle protein efflux in the systemic circulation after exercise. CK activity is one of the several muscle proteins, including LDH, myoglobin (Mb) and troponin, and is the most common plasma marker to assess muscle damage induced by exercise (Croisier et al., 1999; Paulsen et al., 2012). The reason is because the magnitude of increase is significantly greater than other proteins and the cost of the colorimetric assay has been relatively low. Increased CK activity is associated with many diseases, such as myocardial infraction and muscular dystrophy, and thus plasma CK is an important measurement in assessing patients with myopathies or rhabdomyolysis (Moghadam-Kia et al., 2016).

CK catalyses the reversible reaction of creatine and ATP, forming phosphocreatine and ADP. CK is found in 1) skeletal muscle, 2) cardiac muscle and 3) the brain. Eccentric exercise can damage skeletal muscle cell structure at the level of sarcolemma and Z-disks, resulting in an increase in CK activity. There is extensive evidence in the literature regarding the importance of elevated

concentrations of CK related to muscle damage (Byrnes et al., 1985; Clarkson, Nosaka and Braun,1992; Brenner et al., 1999; Clarkson and Hubal, 2002; Jakeman et al., 2009; Baird et al., 2012; van de Vyver and Myburgh, 2012; Hughes et al., 2018). Two types of exercise have been studied related to muscle damage, downhill running and high force eccentric contractions which have shown different responses in plasma CK levels.

Malm et al. (2004) showed a peaked CK activity at 24 hours following a downhill running protocol and remained significantly elevated up to 48 hours following the eccentrically biased exercise. Similar results were reported by Peake and co-workers (2005) that CK activity significantly increased (420% higher than pre-exercise values) at 24 hours after downhill running. In contrast, Clarkson, Nosaka and Braun (1992) suggested increased CK activity about 48 hours after maximal eccentric contractions of the elbow flexors with CK concentrations peaking 2,000 to 10,000 IU between 4 and 6 days after the exercise. Similarly, another study found that CK activity peaked at 48 hours post-eccentric exercise and returned to pre-exercise values on the day 7 post-exercise (Serrao et al., 2007). In addition, it has been demonstrated that the CK release was markedly elevated 96 hours following eccentrically biased exercise (Hyatt and Clarkson, 1998), with a recovery time by the day 10 after exercise (Helers, Ball and Liston, 2002). Therefore, CK concentrations seem to be elevated much earlier after downhill running protocol (Schwane et al., 1987; Eston, Mickleborough, and Baltzopoulos, 1995; Chen, Nosaka and Tu, 2007).

The reason for this variability in CK response to exercise is perhaps the rapid clearance from the blood, the different type of protocols and the degree of muscle

damage is produced. Indeed, one study found that the amount of CK concentration was lower than expected when baseline values were already elevated from a previous exercise suggesting that the increased CK activity from the first bout activated the clearance faster (Nosaka and Clarkson, 1994). Circulating CK is cleared by degradation in the liver and the reticuloendothelial system. The mechanism by which CK is cleared from the circulation is not fully understood. However, it appears that the time course of CK activity in the bloodstream is dependent on the muscle-damaging protocol. CK levels may reflect interactions linked to the scale of muscle disturbance, the magnitude of released CK and its clearance rate from the serum (Thompson, Scordilis and De Souza, 2006; Baird et al., 2012).

Another reason for this variability might be because CK is released from the muscle to the blood stream via the lymphatic system. Sayers, Clarkson and Lee (2000) examined whether the increase of the serum CK after exercise was affected by the changes of the lymph flow. They found a lower increase in CK activity in subjects who immobilized their arm after elbow flexion for 4 days compared to subjects who did not. Similar results were reported by a previous study by Havas, Komulainen and Vihko, (1997) who found significantly lower CK activity in bed-rest group after an 18 km run.

Therefore, CK activity alone might not be an accurate reflection of muscle damage following eccentric exercise (Magal et al., 2010) due to large inter-subject variability in response with a range from 236 to 25,244 IU/L (Clarkson and Hubal, 2002). Generally, individuals with low CK activity in the blood post-exercise are related to less muscle damage, as assessed by MRI (Nosaka and

Clarkson, 1996; Baird et al., 2012). However, Sewright et al. (2008) found that strength loss had a negative relationship with peak CK concentrations after exercise. This is most probably due to temporal muscle disturbance or disruption rather than due to a degree of muscle damage. The variability in circulating CK is still not fully understood (Sewright et al., 2008), since most studies to date have demonstrated equivocal findings; and therefore, this technique has limitations regarding to muscle injury (Warren et al., 1999; Kim and Lee, 2015). A summary of studies examining plasma CK activity in response to EIMD are presented in Table 2.3.

Table 2.3 Summary of studies investigating CK activity in response to EIMD

Authors	Exercise Modality	Experimental Measurements	Peak of CK	Recovery to Baseline
Nosaka and Clarkson, 1994	Maximal eccentric contractions of the forearm flexors	Pre- and post-exercise, and once a day for 10 post-exercise	4 days post-exercise	Yes, 10 days post-exercise
Eston, Mickleborough, and Baltzopoulos, 1995	100 maximal isokinetic eccentric contractions	Baseline, immediately post- and 24, 48, 72, 96h post-exercise	2 days post-exercise	Yes, 4 days post-exercise
Brenner et al., 1999	2h cycling at 60-65% of $\dot{V}O_2max$	Pre- and immediately post-exercise, and 3, 24 and 72h post-exercise	72h post-exercise	No
Chen, Nosaka, and Sacco, 2007	Maximal eccentric contractions of the elbow flexors	Pre-exercise and for 5 consecutive days post-exercise	5 days post-exercise	No
Serrao et al., 2007	60 maximal eccentric contractions of the quadriceps	Pre-exercise, and on day 2, 7 post-exercise and between 21-30 days post-exercise	48h post-exercise	Yes, 7 days post-exercise
Jakeman, et al., 2009	Maximal vertical jumps (10 sets x 10 jumps)	Baseline, and 1, 24, 48, 72 & 96h post-exercise	24h post-exercise	Yes, 48h post-exercise

van de Vyver and Myburgh, 2012	Intermittent downhill running (12 x 5 min bouts) for 60 min at 85% of $\dot{V}O_2$max, 10% decline	Pre- and immediately post-exercise, and every day for 9 days post-exercise	24h post-exercise	Yes, 4-6 days post-exercise
Park and Lee, 2015	40-min downhill running at 70% of $\dot{V}O_2$max at -10% gradient	Pre- and immediately post-exercise, and at 2, 24 and 48h post-exercise	24h post-exercise	Yes, 48h post-exercise
Philippou et al., 2018	50 MVC of knew extensors	Pre-exercise and at 6, 48 and 120h post-exercise	120h post-exercise	No

2.5.2.2 Delayed Onset Muscle Soreness

Delayed onset muscle soreness is an indirect marker to assess EIMD and is the most common symptom of EIMD which affects both elite and exercise enthusiasts. Muscle soreness pain is measured by a visual analogue scale (VAS) from 1 (no soreness) to 10 (very sore) and this correlates with strength loss, reduced ROM, and elevated concentrations of CK in the blood (Baird et al., 2012). Indeed, human studies that assessed muscle fibres from MRI-guided muscle biopsies correlated significantly fibre swelling with increased MRI signal intensity 48 hours after eccentric exercise (Friden, Sjostrom and Ekblom, 1981; Crenshaw et al., 1994; Clarkson and Hubal, 2002).

Muscle soreness is a painful condition that normally manifests in the 24 to 72 hours period following a muscle-damaging exercise (Tsuchiya et al., 2014; Clifford et al., 2016; Jakeman et al., 2017). Although there is no pain or muscular stiffness immediately after the exercise bout, it starts occurring at about 6 to 8 hours later, depending on the individual and the exercise intensity, with a peak soreness between 24 and 48 hours after a muscle-damaging exercise (Newham

et al., 1983; Jones and Round, 1997; Clarkson and Hubal, 2002; Jakeman et al., 2009; Chen et al., 2016); and it generally starts disappearing gradually within 96 hours (Cleak and Eston, 1992; Jakeman et al., 2017).

Similar findings were reported by previous studies which measured muscle soreness at various time points after an exercise bout. For instance, Jones, Newham and Torgan (1989) evaluated muscle soreness at 24, 48, 72 and 96 hours after maximal eccentric, concentric and isometric contractions of the elbow flexors. Soreness scores peaked 48 hours following maximal eccentric actions. Byrnes et al. (1985) also stated that muscle soreness peaked at 42 hours after 30 minutes of downhill running. This was also supported by Chen et al. (2018) who reported significantly increased muscle soreness at 48 hours following MVC of the elbow flexors.

The physiological mechanism of DOMS relates to muscle fibres damage at the level of sarcomere from repeated muscle contractions (Proske and Morgan, 2001). This leads to further damage and the initiation of an inflammatory response followed by fibre swelling, which is a possible cause for continued damage, and may also function in the regeneration process (MacIntyre et al., 1995; Clarkson and Hubal, 2002). It has been suggested that the breakdown products of the damaged tissues may stimulate the nociceptors, and these create a sensation of pain (MacIntyre et al., 1995; Proske and Morgan, 2001; Ren and Dubner, 2010). Prostaglandins are also involved in the production of the soreness (Ricciotti and FitzGerald, 2011). Indeed, they stimulate type III and IV nerve afferents, which send signals of pain to the central nervous system (Buckworth et al., 2013). Although different theories have been proposed (Cleak and Eston,

1992; Gulick and Kimura, 1996; Cheung, Hume and Maxwell, 2003), the exact underlying mechanism of DOMS remains unclear (Proske and Morgan, 2001; Ricciotti and FitzGerald, 2011). Nevertheless, it is generally accepted that there is an association between muscle damage and DOMS and the subsequent inflammatory response (Cheung, Hume and Maxwell, 2003). A summary of studies examining DOMS in response to EIMD are presented in Table 2.4.

Table 2.4 Summary of studies investigating DOMS in response to EIMD

Authors	Exercise Modality	Experimental Measurements	Peak of Soreness	Recovery to Baseline
Newham et al., 1983	20 min stepping eccentric exercise 1 sec/step	Pre-exercise, 2, 10 and 30 min post-, and 1, 5, 24, 48 and 55h post-exercise	Between 24 and 48h post-exercise	4 days after the exercise
Byrnes et al., 1985	30-min of downhill running (-10 degrees slope)	Pre- and at 6, 18 and 42h post-exercise	42h post-exercise	No
Jones, Newham and Torgan, 1989	Maximal voluntary eccentric contractions of elbow flexors	Pre-exercise, 24, 48, 72, 96h post-exercise & after 7 days	48h post-exercise	Yes, 96h post-exercise
Jones and Round, 1997	2h walking backwards downhill on a treadmill (inclined 13°) at 1.3 km/h	On 5-12 days prior to exercise and on 9-14 days post-exercise	2 days post-exercise	Yes, by the 6th day
Jakeman, et al., 2009	Maximal verticals jumps (10 sets x 10 jumps)	Baseline, 1, 24, 48, 72 & 96h post-exercise	24h post-exercise	Yes, 96h post-exercise
van de Vyver and Myburgh, 2012	Intermittent downhill running (12 x 5 min bouts) for 60 min at 85% of $\dot{V}O_2max$, 10% decline	Pre-exercise and on days 1, 2 and 9 post-exercise	24h post-exercise	Yes, 1 day
Clifford et al., 2016	100-drop jumps	Pre- and immediately post- and at 24, 48, 72h post-exercise	48h post-exercise in placebo group	Yes, but not fully recovered by 72h post-exercise in placebo group

Jakeman et al., 2017	100 drop jumps	Pre-exercise, 1, 24, 48, 72 and 96h post-exercise	24h post-exercise	Yes, 96h post-exercise
Chen et al., 2018	30 maximal voluntary eccentric contractions of the elbow flexors at $30^{o}{\cdot}s^{-1}$	2 days prior exercise, immediately post- and 1, 2, 3, 4 and 5 days post-exercise	2 days post-exercise	Yes, not fully recovered by the 5th day post-exercise

2.5.2.3 Maximal Voluntary Isometric Contraction

The prolonged loss of strength with decreases in ROM that arise after eccentric exercise is one of the most commonly used indirect marker of EIMD and is considered to be the best indicator of muscle damage (Proske and Morgan, 2001; Damas et al., 2016). Warren et al. (1999) suggested that MVC presents the most accurate indirect measurement of muscle damage, as a consequence MVC can be included in the assessment of muscle function (Paulsen et al., 2012; Hyldahl et al., 2015; Mickelborough et al., 2015).

Muscle strength loss occurs immediately post-exercise and reveals a linear recovery to baseline measurements (Newham et al., 1983; Clarkson, Nosaka and Braun,1992; Eston, Mickleborough, and Baltzopoulos, 1995; Sayers and Clarkson, 2001; Byrne and Eston, 2002a; Tsuchiya et al., 2015). It has been reported that the greatest and longest duration of strength loss has been related with high-force eccentric exercises (Newham, Jones and Clarkson, 1987; Byrne, Twist and Eston, 2004). These studies have demonstrated over 50% strength loss compared to pre-exercise values with a linear recovery to baseline lasting from 1 to 2 weeks. However, Clarkson et al. (1992) observed that the recovery time might continue up to several weeks. Nosaka and Newton (2002) showed that MVC dropped by 45% immediately after EIMD and it had only recovered by 56% on day 5 post-exercise. On the contrary, downhill running protocol has been

causing a smaller strength loss (about 30% compared to high-force eccentric protocol) with recovery time back to baseline from 4 to 7 days (Eston et al., 2000).

The wide variation in exercise protocols might be an explanation of the diverse findings on EIMD. A large inter-individual variation in response to eccentric exercises is also commonly reported (Clarkson et al., 1992; Sayers and Clarkson, 2001; Sewright et al., 2008; Paulsen et al., 2012). One possibility for this is that individuals may be ‘’low’’, ‘’moderate’’ or ‘’high’’ responders on changes in muscle function following eccentric exercise (Sayers, Knight and Clarkson, 2003; Paulsen et al., 2010). Other contributing factor regarding the inter-individual variation response might be the age (Choi, 2016). Indeed, it was reported that older individuals were more susceptible on a single bout of muscle-damaging exercise than younger participants, showing larger strength decrements (Ploutz-Snyder et al., 2001). It could be also due to the training status (Newton et al., 2008) and genetic factors (Clarkson et al., 2005; Devaney et al., 2007; Baumert et al., 2016). For example, Devaney et al. (2007) examined whether polymorphisms of 2 proteins that code for different components of the sarcomere were related to muscle damage following eccentric exercise. More specifically, in this study they observed a relation between a polymorphism in the sarcomeric protein called myosin light chain kinase (MLCK) and alterations in the serum CK concentration and strength. The SNPs that were tested were the C49T and the C37885A. Findings showed that people that were homozygous for the MLCK 49T allele had a much greater concentration of serum CK and Mb when compared to the heterozygotes (CT). On the other hand, those heterozygous for the MLCK C37885A were found to have increased CK compared to the homozygous wild

type (CC). A summary of studies examining muscle strength in response to EIMD are presented in Table 2.5.

Table 2.5 Summary of studies investigating muscle strength in response to EIMD

Authors	Exercise Modality	Experimental Measurements	Strength Loss	Recovery to Baseline
Clarkson, Nosaka and Braun, 1992	Eccentric contractions of the forearm flexors	Pre-exercise and once a day for 10 days post-exercise	Immediately post-exercise over 50% of pre-exercise values	Yes, 10 days after exercise
Nosaka and Clarkson, 1994	24 maximal eccentric contractions of the forearm flexors	Pre-exercise and once a day for 10 days post-exercise	Immediately post-exercise 55% of pre-exercise values	Yes, 10 days post-exercise
Eston, et al., 1995	100 maximal isokinetic eccentric contractions	Baseline and immediately post- and 24, 48, 72, 96h post-exercise	10 % immediately after exercise	Yes, at day 4
Eston et al., 2000	Downhill running at -12% gradient	Pre-exercise and immediately post- and 24, 72 and 120h post-exercise	Immediately post-exercise	Yes, 72h post-exercise
Sayers and Clarkson, 2001	50 maximal eccentric contractions of the elbow flexors	Baseline and immediately post-and at 36 and 132h post-exercise	On average, 57% reduction immediately post-exercise	Yes, remained 33% lower at 5.5 days post-exercise
Nosaka and Newton, 2002	24 maximal eccentric contractions of the elbow flexors	Pre- and immediately post- and 1, 3, 6, 24, 48, 72, 96 and 120h post-exercise	Immediately post-exercise 45% of pre-exercise values	Yes, recovered 56% by 5 days post-exercise
Byrne and Eston, 2002a	100 barbell squats (10 sets x 10 reps)	Pre-exercise, 1h post- and 1, 2, 3, 4 and 7 days post-exercise	Reduced by 25% at 24h post-exercise	Yes, 4 days post-exercise
Sewright et al., 2008	50 maximal eccentric contractions of the elbow flexors	Baseline and immediately post-and at 0.5 (12-14h), 3, 4, 7 and 10 days post-exercise	Immediately after exercise over 50%	Yes, 10 days after exercise
Mickleborough et al., 2015	20-min downhill running at 70% of $\dot{V}O_2max$ at -16% grade	Baseline, pre- and immediately post- and 2, 24, 48, 72 and 96h post-exercise	24h post-exercise	Yes, 96h post-exercise

Chen et al., 2018	30 maximal voluntary eccentric contractions of the elbow flexors at $30^{o}\cdot s^{-1}$	2 days prior and immediately pre-exercise, immediately post- and 1, 2, 3, 4 and 5 days post-exercise	Immediately after exercise about 45%	Yes, not fully recovered by the 5th day post-exercise

2.5.2.4 Anaerobic Power

Wingate anaerobic test (WAnT) can be useful to measure power output following EIMD (Bar-or, 1987; Comfort and Matthews, 2010); and thus, power output is another indirect measurement of muscle function. Despite the fact that it is reliable and reflects an individual's anaerobic performance, it may be less sensitive than MVIC (Byrne and Eston, 2002a). Byrne and Eston (2002a) found a reduction in peak power during a 30-seconds WAnT cycle test after eccentric exercise. However, it is noteworthy that the decrements and the recovery pattern of the peak power were different of those of the maximal isometric strength, although the two tests had similar time course of recovery. More specifically, there was a linear recovery in MVIC (decreased by 35% 1 hour after the eccentric-biased exercise, 26% at 24 hours and 19% at 48 hours), whereas, the peak power, which was assessed by 30-seconds WAnT test, was decreased by 13% 1 hour post-exercise, reduced further by 18% at 24 hours and 16% at 48 hours before the linear recovery (Byrne and Eston, 2002a). Similarly, Twist and Eston (2005) observed the same pattern in the functional impairment, using a 10 x 6 seconds cycle ergometer sprints. They showed decrements in peak power output by 18% at 48 hours following eccentrically biased exercise. Since WAnT test showed further reductions in peak power at 24 and 48 hours following EIMD, these findings suggest that muscle power may be affected by DOMS (Byrne, Twist and Eston, 2004).

Another study which used a downhill running protocol for 40 minutes at -7% gradient was in agreement with the Byrne and Eston (2002a) study. The authors reported higher decrements in MVC than in peak power, which was assessed by 30-seconds WAnT test, (17% *vs* 5%, respectively) immediately post-EIMD protocol (Nottle and Nosaka, 2007). However, the same study (Nottle and Nosaka, 2007), also reported that the recovery time was fast, and the peak power was 5% higher 5 days later than baseline values. In contrast, Malm et al. (1999), with same test protocol (30-sec WAnT test), have shown no significant change in power output from pre-exercise to 48 hours post-eccentric exercise. Nevertheless, the exercise protocol used may be insufficient to produce changes in muscle function, considering the fact that there was only moderate muscle soreness and no elevation in CK activity.

In addition, a recent study examined the effect of EIMD on performance measurements following a 90-minutes basketball training session (Doma et al., 2018). The training session included multiple sprinting and repeated jumping exercises which caused EIMD, judging by the presence of a significant increase in CK and DOMS in combination with a reduction in jump height. They found significantly lower power output at 24 hours post-training compared with baseline values. Furthermore, Cronin and Keir (2005) examined the relationship between strength and power by assessing different predictors of functional performance, such as maximal strength, sprint and jump squat. They concluded that power output may be a useful indicator to predict exercise performance. A summary of studies examining peak power in response to EIMD are presented in Table 2.6.

Table 2.6 Summary of studies investigating peak power in response to EIMD

Authors	Exercise Modality	Experimental Measurements	Peak Power Loss	Recovery to Baseline
Malm et al., 1999	5 x 30 eccentric-type walking steps (15 steps/legs)	Pre-exercise, 6, 24 and 48h post-exercise	No change before and 48h post-exercise	No
Byrne and Eston, 2002a	100 barbell squats (10 sets x 10 reps at 70% body mass load)	Pre-exercise, 1h post- and 1, 2, 3, 4 and 7 days post-exercise	Reduced 18% from baseline at 48h post-exercise	Yes, on the 7 day post-exercise
Twist and Eston, 2005	100 plyometric jumps (10 set x 10 reps)	Pre-exercise and 30 min, 24, 48, 72h post-exercise	Reduced 18% from baseline at 48h post-exercise	No
Nottle and Nosaka, 2007	40-min downhill running (-7%)	Baseline, 0.5, 48, 72 and 120h post-exercise	Reduced 4.5% from baseline at 30 min post-exercise	Yes, 72h post-exercise
Doma et al., 2018	90-min training session	Pre- and 24h post-training and 1 week post-training	Reduced 3.5% at 24h post-exercise	Yes, but not fully recovered by the 7th day post-training

2.5.2.5 Vertical Jump Performance

Decrements in muscle function following EIMD can also be evidenced by dynamic muscle function, such as jump performance (Chambers et al., 1998; Byrne and Eston, 2002b; Byrne, Twist and Eston, 2004; Twist and Eston 2005; Jakeman, Byrne and Eston, 2010). The vertical jump is a common test of leg extensor muscle power, and it is typically used to monitor both potential athletic performance or an individual's impaired performance and power loss after muscle-damaging exercise (Horita et al., 1999; Avela et al., 1999; Byrne and Eston, 2002b; Kohne, Ormsbee and McKune, 2016). In addition, it has been concluded that decreased performance in maximum vertical jump may be used to indicate fatigue (Chappell et al., 2005; Benjaminse et al., 2008).

The effect of starting position on vertical jump performance has also received significant attention (Selbie and Caldwell, 1996; Bobbert and Casius, 2005; Bobbert and Casius, 2008; Gheller et al., 2015). In most assessments the ‘countermovement jump’ (with arm swing) is performed for maximum height (Bobbert and Casius, 2005; Farrow et al., 2020). In this type of jump, the individual starts from an upright standing position, then performs a preliminary rapid descending movement by flexing the knees and hips and then immediately extends them again to jump vertically up off the ground. Such a movement is an example that uses the ‘stretch-shortening cycle’ model examining neuromuscular function (Komi, 2000), where muscles are ‘pre-stretched’ before shortening in the desired direction. Many human movements, such as running, throwing and jumping, include muscle actions in which a movement in the opposite direction precedes the desired movement. It has been also demonstrated that a pre-stretch motion boosts the power output of the muscles in the subsequent movement (Komi, 1992, pp.169-179).

In addition, a less common type of jump is the ‘squat jump’. In this type, the individual starts from a stationary semi-squatted position where a preliminary downward phase is not employed. Previous work of Chambers et al. (1998) and Byrne and Eston (2002b) have demonstrated that squat jump height is affected to a greater extent than countermovement jump height following EIMD. However, it has been suggested that regardless of the initial jump position, same muscle activation pattern is used to achieve good jump height (Van Soest et al., 1994). Furthermore, it has been proposed that vertical jump height can be enhanced by the use of arm swing (Feltner, Fraschetti and Crisp, 1999; Hara et al., 2006; Hara et al., 2008). However, it has been suggested that ineffective use of arm swing

may mask the effect of any muscular work from the lower extremities (Balster et al., 2016). Thus, as arm swing movements can be key to attained result, proper familiarization is important to avoid training effects from repeated testing. A summary of studies examining jump performance in response to EIMD are presented in Table 2.7.

Table 2.7 Summary of studies investigating jump height in response to EIMD

Authors	Exercise Modality	Experimental Measurements	Jump Performance Loss	Recovery to Baseline
Chambers et al., 1998	90-km ultramarathon race	Pre-exercise, 1, 2, 3, 4, 5, 11, 18, 25 and 32 days post-exercise	Significantly reduced immediately post-race for up to 18 days post-race	Yes
Horita et al., 1999	Exhausting stretch-shortening-cycle-induced muscle damage	Pre-exercise, immediately post- at 2h, and on 2 and 4 days	Initial decline immediately post-exercise, and a delayed decline from 2h to 2 days	Yes
Twist and Eston, 2005	100 plyometric jumps (10 set x 10 reps)	Pre-exercise and 30 min, 24, 48, 72h post-exercise	Decreased and remained below baseline at 24, 48 and 72h post-exercise	No
Vaile et al., 2007	5 sets x 10 eccentric reps at 140% of 1RM in a leg press	Pre-exercise, immediately post-and at 24,48 and 72h post-exercise	Reduced immediately post-exercise	Yes, by 72h post-exercise
Vaile et al., 2008	5 sets x 10 reps at 120% and 2 sets x 10 reps at 100% of 1RM in a leg press	Pre-exercise, immediately post-and at 24, 48 and 72h post-exercise	Reduced immediately post-exercise	Yes, by 72h post-exercise
Jakeman et al., 2010	10 x 10 reps of plyometric drop jumps	Pre-exercise, at 1, 24, 48, 72 and 96h post-exercise	Significant decreased at 24-48h post exercise	Yes, but not fully recovered by 96h post-exercise
Kohne, Ormsbee and McKune, 2016	Downhill running 60 min at 75% of $\dot{V}O_2max$ - 10% gradient	Pre-exercise, immediately post and at 24, 48 and 72h post-exercise	Decrements at 48h post-exercise	Returned to near baseline levels at 72h but not fully recovered

Overall, measurements of muscle function, such as MVC, peak power, jump height, DOMS or ROM, should be used in combination with indirect plasma markers, such as CK and cytokines, to provide more reliable evidence in assessing the magnitude and time-course of EIMD (Warren et al., 1999), as well as to increase the practicability of comparison amongst studies (Powers and Hawley, 2015). A summary of advantages and disadvantages of direct and indirect methods of EIMD assessment are presented in Table 2.8.

Table 2.8 Summary of advantages and disadvantages of direct and indirect methods of muscle damage assessment

Assessment of Muscle Damage	Advantages	Disadvantages
Direct Methods		
Muscle biopsy	90-95% success rate, quick	Painful, invasive, leaves a scar, soreness during recovery, small sample, overestimate/underestimate damage, can cause muscle damage, small risk of infection, skilled staff
MRI	Non-invasive, diagnostic accuracy shows both three-dimensional and cross-sectional images of whole muscle, no radiation	a skilled operator, cost of equipment
Indirect Methods		
CK	Sensitivity, ease and quick, low cost	Venepuncture, may not be an accurate reflection of muscle damage, large variability
DOMS	Non-invasive, minimal equipment required (visual analogue scale) easy to use, low cost	Soreness, pain, self-assessment technique
MVC	Non-invasive, accurate, valid and reliable predictor, quantitative indicator	Specialist equipment (ergometer), laboratory, reduced muscle function, soreness, requires maximal effort
Anaerobic Power	Non-invasive, minimal equipment required, valid and reliable predictor	Specialist equipment (cycle ergometer and computer software), laboratory, assistant required, requires maximal effort

Jump Height	Non-invasive, minimal equipment required, valid and reliable predictor	Specialist equipment (jump meter device with/w computer software), assistant required, requires maximal effort
ROM	Non-invasive, low cost, reliable predictor	Specialist equipment (goniometer), external force (equipment, operator), understand how far to go

MRI, magnetic resonance imaging; CK, creatine kinase; DOMS, delayed onset muscle soreness; MVC, maximal voluntary contraction; ROM, range of motion

2.6 NUTRITION AND EXERCISE

The importance of nutrition in sports is widely recognized, and its role in improving both health and sports performance has widespread acceptance. The American Dietetic Association (ADA) (2009) also stated that optimal nutrition could enhance athletic performance. There are now formal guidelines stated by the International Olympic Committee (IOC) suggesting that diet considerably influences sports performance. All athletes should follow specific nutritional strategies before, during and after exercise and competition to maximise their mental and physical performance (Sousa, Teixeira and Soares, 2014). Evidence-based guidelines from the 2010 IOC consensus statement on the amount, composition and timing of food intake have been demonstrated to help athletes perform more successfully, with less risk of illness and injury (IOC, 2011; Walsh and Oliver, 2016).

In addition to the above, an appropriate diet will help athletes reach an ideal body size and body composition to acquire greater results in their sport. Moreover, athletes can train more and harder when their recovery rate is faster, resulting in improved performance (Sousa, Teixeira and Soares, 2014). On the other hand, many factors inhibit the achievement of optimal nutrition practices by an athlete, including a poor understanding of sports nutrition principles, a failure to recognize

their individual nutrition requirements (based on sport, training load, age, sex, and body type), inappropriate nutrition or dietary deficiencies in macronutrients and poor hydration strategies to maintain physiological processes, lack of nutrition knowledge, limited meal-planning and busy travel schedules (Burke, 1995; Palumbo, 2000; Malinauskas et al., 2007).

2.6.1 Dietary Supplements and Ergogenic Aids

People who train with high frequency and fail to have a balanced diet may find difficulties maintaining their athletic performance at very high level consistently (ADA, 2009). This happens as the body consumes all necessary nutrients to respond to intense training stimulus. When nutrients are limited a negative effect onto performance is commonly observed.

Approximately half of all athletes use supplements, with the most common reasons for their usage are health and performance related (Bean, 2009). UK Sport, amongst other sporting authorities including the IOC and the world anti-doping agency (WADA), advise athletes not to take supplements with no diagnosed health reasons due to the associated risks (Bean, 2009). Although these two large athletic organisations recommend optimal nutrition to replenish lost nutrients to maintain and improve performance, it may be the case that some athletes, or even people from the general population, can be benefitted by specific types of supplementations if they cannot maintain a balanced diet (ADA, 2009); or if their nutritional needs cannot be met by a normal diet.

Dietary supplements in sports are widespread around the world. Supplements classified as ‘Acceptable’ include sports foods, such as drinks, powders, bars and

gels, which provide a useful and practical source of energy and nutrients; and therefore, play an important role in supplementing the diet during both training and competition. A few nutritional ergogenic aids, such as caffeine, creatine, are also ranked in the top 'Acceptable' category as substances with considerable belief for enhanced performance for some athletes on some occasions (Lanham-New et al., 2011). However, the U.S. Food and Drug Administration regularly reports on products found to contain effective amounts of prescriptive drugs, which could lead to harmful side effects (Lanham-New et al., 2011). Hence, athletes should think carefully the issues of efficacy, safety and legality associated with supplements products prior to making the decision to use any of them.

Further, there has been considerable attention to fish oil for their therapeutic potential in many inflammatory disorders, such as rheumatoid arthritis and inflammatory bowel diseases (Endres et al., 1989; Rees et al., 2006; Calder, 2009; Mickleborough, 2013). Since EIMD may involve an inflammatory response fish oil could reduce muscle inflammation. However, only a few nutritional interventions for the muscle damage and the inflammatory response have been conducted in humans to establish whether fish oil supplementation could limit the degree of injury and inflammation associated with EIMD (Tartibian et al., 2009; Tartibian et al., 2011; Santos, et al., 2012; Sousa, Teixeira and Soares, 2014). Fish oils are rich in n-3 fatty acids and may have anti-inflammatory and immunomodulatory effects (Galli and Calder, 2009; Calder 2013; Jeromson et al., 2015). As such, a nutritional strategy that includes fish oil could reduce muscle inflammation arising in response to exercise and immune dysfunction in athletes (Mickleborough, 2013; Calder, 2015). However, the evidence from human studies

on beneficial effect of fish oil in response to exercise is inconclusive as optimal dosage, duration and detailed mechanism are still unclear.

2.7 OMEGA-3 POLYUNSATURATED FATTY ACIDS

2.7.1 Biochemistry and Structure of Omega-3 Fatty Acids

Omega-3 fatty acids are also called polyunsaturated fatty acids (PUFA). There are different types of n-3 PUFA. These are the a-linoleic acid (ALA), the eicosapentaenoic acid (EPA) and the docosahexaenoic acid (DHA) (Calder, 2012). These polyunsaturated fatty acids have all their double bonds in a cis-configuration, which means that the two hydrogen atoms are on the same side of the double bond.

The term n-3 is used to describe the position of the double bond that is closest to the methyl terminus ($-CH_3$) of the acyl chain. Every n-3 fatty acid has this double bond on carbon 3 (Calder and Yaqoob, 2009). There are common and systematic names assigned to n-3 fatty acids. They can also be described based on the number of carbon atoms in the acyl chain, the number of double bonds and the position of the first double bond in relation to the methyl group (Calder, 2012). The shortest and simplest fatty acid is α-linolenic acid [18:3(n-3)]. α-Linolenic acid is synthesized from linoleic acid [18:2(n-6)] by desaturation catalysed by Δ15-desaturase. Plants can synthesise α-linolenic acid because they possess the enzyme Δ15-desaturase. Mammals on the other hand, do not possess the Δ15-desaturase enzyme and are thus, unable to synthesize α-linolenic acid. This is the reason why α-linolenic acid and linoleic acid are considered essential fatty acids. However, once consumed α-linolenic acid can be used to synthesize longer chain fatty acids. This process is mainly taking place in the liver and is led

by a series of linked desaturation and elongation reactions (Calder, 2012). More specifically, Δ6-desaturase converts α-linolenic acid to stearidonic acid [18:4(n-3)] which is then elongated to eicosatetraenoic acid [20:4(n-3)]. Δ5-desaturase further desaturates eicosatetraenoic acid yielding EPA [20:5(n-3)].

Interestingly, converting α-linolenic acid to EPA competes with the process of converting linoleic acid to arachidonic acid (AA) [20:4(n-6)], as the same enzymes are used in both processes. The actions of Δ6- and Δ5-desaturases are regulated by nutrition, hormones and feedback inhibition by end products (Calder, 2012). DHA 22:6(n-3)] is synthesised by the conversion of EPA via docosapentaenoic acid (DPA) [22:5(n-3)] (Figure 2.11). The conversion of EPA to DHA involves limited peroxisomal β-oxidation and the participation of Δ6-Desaturase. It is important to note at this point that the process of converting α-linolenic acid to EPA, DPA and DHA is thought to be generally poor, since the conversion to the end-product which is the DHA is limited (Burge and Calder, 2006; Arterburn et al., 2006). On the other hand, the conversion of stearidonic acid to EPA is thought to be superior, possibly because this process does not involve the rate-limiting Δ6-desaturase enzyme (Figure 2.12; James, Ursin and Cleland, 2003).

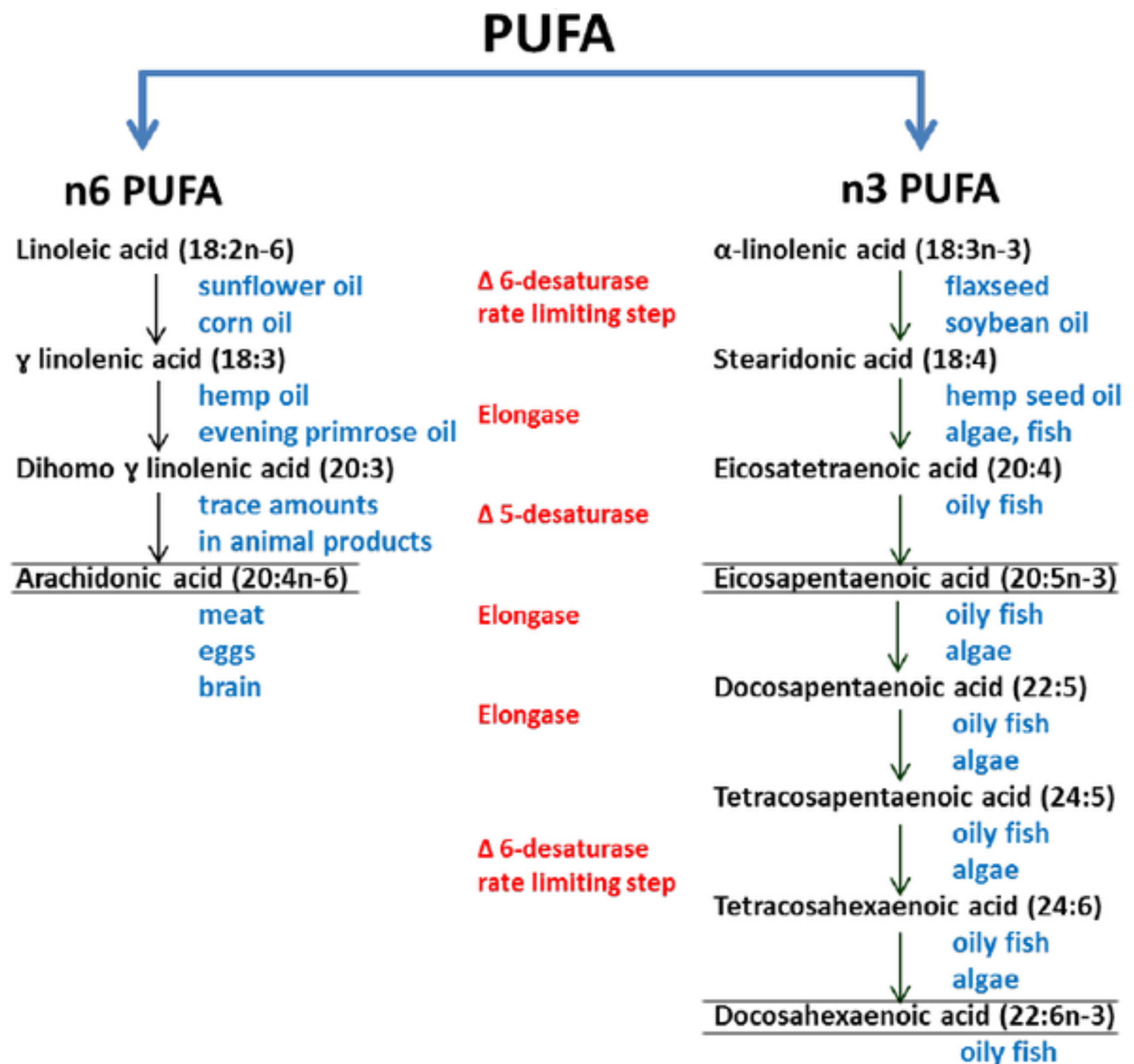


Figure 2.11 The biosynthesis pathway of n-3 PUFA (White et al., 2013)

2.7.2 Dietary Sources of Omega-3 Fatty Acids

There are considerable amounts of α-linolenic acid in a range of different seeds and their oil. Specifically, linseeds (flaxseeds) and their oil contain approximately 45-55% of fatty acids in the form of α-linolenic acid. Walnuts, soybean oil and rapeseed oil typically contain around 10% of fatty acids in the form of α-linolenic acid (Calder, 2012). Dietary intake of α-linolenic acid in Western cultures ranges between 0.5 and 2 g/day (British Nutrition Foundation, 1999). The main PUFA, however, is linoleic acid which is consumed in 5- to 20-fold greater amounts than α-linolenic acid (British Nutrition Foundation, 1999).

Fish is considered one of the major sources of n-3 PUFA. Lean fish such as cod, store lipids in their liver, whereas oily fish such as salmon, tuna, sardines, mackerel store lipids in their flesh (British Nutrition Foundation, 1999). As such, different types of fish contain varied amounts of n-3 and have different ratios EPA:DHA. More specifically, a single lean fish portion can provide approximately 0.2 to 0.3 g of long chain n-3 fatty acids, whereas a single oily fish portion can provide 1.5 to 3.5 g of n-3 fatty acids (Calder, 2012). However, the consumption of oily fish is found to be very low in Western populations (Europe, Australasia and North America), with quantities rarely more than 0.15 to 0.25 g/d (SACN, 2004).

Fish oil is rendered from oily fish flesh or the livers of lean fish. Fish oil is extremely high in n-3 fatty acids, with the EPA and DHA comprising approximately 30% of the fatty acids (Calder, 2012). Depending on the kind of fish that the fish oil was made from, there can be variations in the proportions of EPA, DPA and DHA or in the content of n-3 fatty acids. For instance, cod liver oil has more EPA than DHA, while tuna oil has more DHA than EPA (Calder, 2012).

2.7.3 Physiological Roles and Health Benefits of Omega-3 Fatty Acids

There have been several physiological roles attributed to n-3 PUFA that show potential health or clinical benefits. More specifically, risk factors related to CVD, such as blood pressure, platelet reactivity and thrombosis, vascular function, cardiac arrhythmias, heart rate variability and inflammation have been improved with the consumption of very long-chain n-3 PUFA (Geleijnse et al., 2002; Calder, 2006; von Schacky, 2008).

On the other hand, a systematic review of 79 clinical trials (evidence mainly from supplement trials) involving over 112,000 participants reported that supplementation with n-3 PUFA does not lower the risk of CVD and the risk of all-cause deaths (high-certainty evidence). Even when considering the risk for bias, there was little or no effect of n-3 supplementation (Abdelhamid et al., 2018). Nevertheless, the authors in their recent updated systematic review (Abdelhamid et al., 2020) involving over 160,000 participants, found low-certainty evidence that increasing intake of long chain n-3 PUFA may be slightly protective of coronary death and events. Furthermore, a very recent systematic review and meta-analysis (Khan et al., 2021) stated moderate-certainty of evidence that n-3 PUFA were significantly associated with a decrease in CVD mortality, but not all-cause deaths (with low certainty). In particular, EPA monotherapy showed higher relative improvement in cardiovascular outcomes compared to the combination of EPA and DHA.

Apart from CVD, other diseases can benefit from n-3 PUFA, especially those that have inflammatory elements tight to their development (Calder, 2008). Moreover, some of n-3 PUFA’s physiological actions are their ability to lower fasting serum triglycerides (TG) concentrations (Abdelhamid et al., 2020) and increase insulin sensitivity (Calder, 2012). It has also been reported that supplementation with n-3 PUFA protects muscle cell membrane (Tsuchiya et al., 2016). Omega-3 PUFA are incorporated into phospholipids, resulting in inhibition of ROS and inflammatory markers (Ling, Boyce and Bistrian, 1998). The health benefits that have been related to the consumption of n-3 PUFA are likely to be dose dependant, although this has not been clear in dose-response data (Calder, 2012).

2.7.4 Mechanism of Action of Omega-3 Fatty Acids

Omega-3 PUFA act via multiple mechanisms by which they could regulate metabolism and tissue responses, such as inflammation. Key mediators of inflammation are the eicosanoids, which are produced by 20 carbon PUFA and released from cell membrane phospholipids (Calder, 2006; Calder, 2009). Subfamilies of eicosanoids, such as PGs, thromboxanes (TXs), and leukotrienes (LTs), which are released from leukocytes at the site of inflammation, regulate pain and inflammatory responses (Tilley et al., 2001; Brune, 2004). The n-6 fatty acid AA, the main substrate of eicosanoids synthesis, can be inhibited in the inflammatory cells (Calder, 2009; Tarbinian et al., 2011) by the presence of EPA and DHA. In addition, EPA acts as a substrate for cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, which produce eicosanoids, however, these mediators have different structure of that of AA-derived mediators and influence their effect. Consuming n-3 PUFA leads to increased proportions of EPA and DHA within the cell membranes, consequently the 3-series PGs and TXs, and decreased AA concentrations (Lee et al., 1985; Harris and von Schacky, 2004; Calder, 2010). Therefore, since there is less available substrate for eicosanoids synthesis from AA-derived mediators, n-3 supplementation in the diet has been demonstrated to reduce the production of PGE_2, (Endres et al., 1989; Rodenburg, Bar and De Boer, 1993; Trebble et al., 2003), TXB_2 (Caughey et al., 1996), 4 series-LTE_4 (Von Schacky et al., 1993) and inflammatory cytokines, amongst them the TNF-α, IL-6 and IL-1.

2.7.5 Anti-inflammatory Effects of Omega-3 Fatty Acids

Anti-inflammatory mediators derived from n-3 PUFA and its main bioactive fatty acids, such as EPA and DHA, have been recognised along with their mechanism

of their action (Arita et al., 2005; Calder, 2010). Marine n-3 PUFA seem to have beneficial anti-inflammatory properties, mainly through their effects on the neutrophils and macrophages of the inflammatory response, as mentioned before, by reducing AA concentrations in neutrophils (Lee et al., 1985). It has been suggested that n-3 PUFA alter the activity of the transcription factors, NF-κB and/or peroxisome proliferator-activated receptor (PPAR)-γ (Calder, 2009).

Furthermore, it has been shown that a new group of EPA-derived mediators, the E-series resolvins (Serhan et al., 2000; Calder, 2010) and DHA-derived mediators, the D-series, produced by COX-2/LOX appear to exert anti-inflammatory actions (Hong et al., 2003; Mukherjee et al., 2004). Therefore, it has been suggested that n-3 PUFA can attenuate muscle inflammation and improve the fatigue recovery (Zebrowska et al., 2015; Jakeman et al., 2017). One of the connections between n-3 and muscle inflammation is that these fatty acids induce an anti-inflammatory response via down-regulation of pro-inflammatory cytokines, such as TNF-α and IL-6, reduced production of AA and ROS, consequently, resulting in a decrease in the inflammatory response (Calder, 2006; Tartibian et al., 2009).

2.7.6 Omega-3 Supplementation and Exercise

Over the last few years n-3 supplementation has been of particular interest in sports and exercise performance. Increasing evidence through the mechanisms explained earlier suggests that n-3 supplementation impairs pro-inflammatory cytokines and ROS production, and hence, may show a direct relationship between intense exercise and markers of inflammation (Colbert et al., 2004; Shei, Lindley and Mickleborough, 2014). The effect of n-3 supplementation on EIMD

and its symptoms will be discussed in the following sections whether n-3 supplementation has any beneficial effect in inhibiting EIMD.

2.7.6.1 Omega-3 Supplementation, Myocellular Proteins and Cytokines

It is now known that high levels of inflammation can lower exercise performance (Howell, Chleboun and Conatser, 1993; Sousa, Teixeira and Soares, 2014) and for that reason, n-3 supplementation is being studied as a group of substances that can potentially boost performance and decrease recovery time between training sessions (Jouris et al., 2011). This hypothesis was first examined by Ernst and co-workers (1991), who showed that 3 weeks of 2.8 g per day of EPA and DHA inhibit the increase of the acute-phase proteins after exercise. Tartibian et al. (2011) recruited 45 men to measure inflammatory markers, such as IL-6, TNF-α, PGE_2, during an eccentric exercise protocol. The results of their study indicated that the consumption of n-3 supplementation, with a dosage (324 mg EPA:216 mg DHA), can decrease the impact of exercise-induced inflammatory markers after 40 minutes eccentric exercise.

It has also been reported that 6 weeks of 1.8 g per day of n-3 supplementation (950 mg EPA and 500 mg DHA) could reduce TNF-α, IFN-γ and PGE_2 in elite swimmers after an intense period of annual competitions (Andrade et al., 2007). However, Toft et al. (2000) showed no effect on exercise-induced inflammatory markers (TNF-α, IL-6, IL-1ra) after a marathon, despite providing a higher dose of fish-oil supplementation, 3.6 g/day for 6 weeks. One explanation as to why Toft et al. did not observe a reduction in exercise-induced inflammatory markers is that they did not use eccentric protocol to promote a significant amount of muscle damage. Indeed, as discussed earlier, concentric muscle contractions (even with

a long duration) may not cause muscle damage (Jones, Newham and Torgan, 1989; Clarkson and Hubal, 2002), while eccentric exercise, especially high-force eccentric protocols, leads to substantial muscle fibre damage (Newham et al., 1983; Armstrong et al., 1984; Gibala et al., 1995; Proske and Morgan, 2001; Clarkson and Hubal, 2002; Nosaka et al., 2003; Byrne, Twist and Eston, 2004; Nederveen et al., 2018). Additionally, the blood samples were taken in the acute-phase response, (< 3 hours post-exercise), and delayed effects were not evaluated. By assessing exercise-induced inflammatory response at later time points (e.g., at 24, 48, 72 or 96 hours post-exercise) they could have unveiled a different inflammatory response, as it has been observed elsewhere (Phillips et al., 2003; Tarbinian et al., 2011; DiLorenzo, Drager and Rankin, 2014).

Moreover, Nieman et al. (2009) observed no reduction in IL-6, IL-8, CK and CRP while examining the effect of 2.4 g/day of fish oil for 6 weeks. Their protocol involved participants cycling for 3 hours with average 57% Wmax and a further 10 km vigorous cycling trial that was completed in 13.7 minutes from the placebo group and in 12.7 minutes from the n-3 PUFA group. This was repeated for three consecutive days. However, it is possible that this study did not provide sufficient dosage compared with others (Huffman et al., 2004; Jouris et al., 2011) or did not utilise a muscle-damaging exercise (cycling). Currently there are not optimal supplementation strategies for the efficacy of n-3 supplementation on EIMD. Nevertheless, it has been suggested that an ingestion of a minimum dosage of 3 g daily of n-3 PUFA for at least 3 weeks would be beneficial on EIMD (Simopoulos, 2007; Mickleborough, 2013; McGlory et al., 2014). Other studies, however, have demonstrated that n-3 supplementation has a positive effect on eccentric exercise protocols by reducing the concentrations of IL-6 and TNF-α

(Phillips et al., 2003; Bloomer et al., 2009; DiLorenzo, Drager and Rankin, 2014).

With regards to muscle damage markers, Tarbinian et al. (2011) found that 1.8 g of n-3 supplementation for 1 month reduced serum CK and Mb after bench stepping protocol. Atashak et al. (2013) also reported substantial reduction in CK and CRP increases after lower body resistance training following 1 week of 540 mg EPA and 360 mg DHA, whereas Gray et al. (2014) showed no effect in CK after ingesting 3 g per day of n-3 PUFA (1.3 g EPA/0.3 g DHA) for 6 weeks following knee bending protocol. This is supported by Tsuchiya et al. (2016) who examined the efficacy of 600 mg/day EPA and 260 mg/day DHA and found no significant differences in CK and Mb after eccentric protocol following 8 weeks of supplementation.

Meanwhile, Corder et al. (2016) showed no changes in CRP following resistance training providing 3 g/day DHA for 9 days. However, one possible explanation of the latter finding may be due to the fact that they provided n-3 for too short period to change muscle phospholipids (Metcalf et al., 2007; Smith et al., 2011a). Indeed, McGlory et al. (2014) examined the time course of n-3 PUFA composition in healthy young recreationally active males and they found that is required a more prolonged period (> 4 weeks) of n-3 supplementation to reach a saturation of n-3 PUFA composition in human skeletal muscle. Another reason could be due to the differences in the n-3 PUFA content (EPA:DHA) with other studies (McGlory et al., 2014). It seems that n-3 supplementation is dose and duration dependant in relation to muscle damage and exercise performance. It may be the case to provide a minimum dosage of 1-2 g per day at a leisure level or ingestion of EPA/DHA of 2:1 ratio may have a positive effect to prevent EIMD (Simopoulos,

2007; Mickleborough, 2013). An alternative rationale could also be due to individual's genotype (Baumert et al., 2016) in determining the muscle damage, as well as to ''good'' or ''bad'' response to n-3 supplementation in relation to muscle damage. Nevertheless, the effect of n-3 supplementation to alter exercise-induced muscle inflammation remains unclear.

2.7.6.2 Omega-3 Supplementation, Muscle Function and DOMS

Studies explored how the symptoms of EIMD can be attenuated with the implementation of n-3 supplementation. Tarbinian et al. (2009) provided 324 mg of EPA and 216 mg of DHA per day for 1 month and showed in their study no significant differences in perceived pain and ROM of the lower limbs before, immediately post- and at 24 hours after 40 minutes bench stepping between the groups. However, they demonstrated a significant decrease in DOMS and ROM at 48 hours after the eccentric exercise in the omega-3 group compared with the placebo. In addition, Jouris et al. (2011) demonstrated that 2 g EPA and 1 g DHA per day for just 2 weeks decreased DOMS in the fish oil group. Lembke et al. (2014) also showed that participants with high omega-3 index (the sum of EPA and DHA in erythrocyte membranes) of 2.7 g n-3 per day for 1 month experienced less DOMS at 72- and 96-hours following arm eccentric contractions, however, they found no effect on MVC torque.

In contrast, Lenn et al. (2002) found that 1.8 g per day of EPA and DHA for 30 days do not have any effect on DOMS after 50 eccentric contractions on elbow flexors. A more recent study concluded that MVC and DOMS of knee extensors did not change by 1.6 g daily of n-3 supplementation for 6 weeks (Gray et al., 2014). This is supported by other studies that showed no effect of n-3

supplementation on DOMS (Phillips et al., 2003; DiLorenzo, Drager and Rankin, 2014). However, recent research has suggested that an acute dose of high EPA dose immediately after an intense eccentric exercise may attenuate the symptoms of EIMD, such as muscle soreness and swelling (Jakeman et al., 2017). The lack of consistency between the studies it may be due to the different eccentric protocols or the muscle groups that they examined.

A limited number of human studies examined the efficacy of n-3 supplementation on muscle strength deficit after a muscle-damaging exercise (Shei, Lindley and Mickleborough, 2014). For instance, Houghton and Onambele (2012) observed no differences in muscle strength increments between the groups after resistance exercise for the lower limbs. However, Tsuchiya et al. (2016) showed a suppression in loss of MVC torque in long-term ingestion of n-3 supplementation. More specifically, they found that 600 mg EPA and 260 mg DHA per day for 8 weeks can inhibit the torque increments at about 17% in the omega-3 group after 30 eccentric contractions in elbow flexors. Similarly, their subsequent study has demonstrated reduced muscle strength loss and positive effect on ROM and DOMS following eccentrically biased exercise by providing the same dietary protocol (Ochi et al., 2017). Thus, the effect of n-3 supplementation on muscle strength reduction after EIMD remains questionable. Possible explanations for the disparity of findings on the efficacy of the dietary supplementation with n-3 PUFA on muscle function and DOMS might be due to the diversity of the exercise protocols (i.e., arm flexors *vs* knee extensors), different intensity and duration of the exercise. In addition, it is possible due to the subject population, various dosage, duration of n-3 supplementation and the composition of EPA and DHA (Shei et al., 2014).

2.7.6.3 Omega-3 Supplementation and Lipid Profile

Fish oil can alter blood fatty acids profiles with increased amounts of EPA and DHA in plasma lipids following n-3 supplementation (Rees et al., 2006; Calder, 2013; Lembke et al., 2014; Corder, 2016; Abdelhamid et al., 2018). This hypothesis has been of interest since Bang, Dyerberg and Sinclair (1980) suggested that n-3 PUFA may have potential antiatherogenic properties. More specifically, it has been reported decreased serum concentrations of cholesterol, TG and low-density-lipoprotein (LDL) in Inuit populations, who consume diets rich in n-3 PUFA (Bang, Dyerberg and Sinclair, 1980; Harris, 1997). As mentioned previously, marine n-3 PUFA can reduce serum TG concentrations and generally have positive changes in blood lipids in humans (Harris, 1997; Calabresi et al., 2004; Skulas-Ray et al., 2011).

Smith et al. (2011a; 2011b) examined the n-3 PUFA changes in muscle phospholipids and they showed that muscle lipid composition was altered (~2-fold increase from baseline) by providing 3 g per day of n-3 PUFA for 8 weeks. McGlory et al. (2014) also showed an increase about 2-fold in whole muscle n-3 PUFA composition during 4 weeks by providing 4.5 g per day of n-3 supplementation. However, differences between these two studies might be due to different content of n-3 supplementation or to n-3 PUFA content between membrane *vs* whole muscle, respectively. These data suggest that muscle lipids can be altered by n-3 PUFA. Thus, n-3 supplementation changes muscle phospholipids, which may affect the skeletal muscle metabolic and physical function by enhancing the 3-series PGs and TXs and consequently suppressing AA concentrations (Jeromson et al., 2015). Hence, n-3 supplementation could modulate physiological processes, such as inflammatory response, and changes

in cell membrane actions that influence cell signalling and gene expression (Simopoulos, 2002; Oh et al., 2010). Omega-3 PUFA, therefore, has the potential to improve exercise performance and enhance adaptation (Walser et al., 2006; Gravina et al., 2017). A summary of studies examining the effects of n-3 supplementation on EIMD are presented in Table 2.9.

Table 2.9 Summary of studies investigating omega-3 supplementation on muscle damage, inflammatory response, and muscle function in humans

Authors	Population	Intervention	Exercise Modality	Outcome
Positive Effects				
Ernst, Saradeth and Achhammer, 1991	13 healthy males	1.75 g EPA, 1.05 g DHA/day for 3 weeks	Cycling until exhaustion (Bruce protocol)	Attenuate the rise in acute-phase proteins after exercise
Phillips et al., 2003	40 healthy, untrained males	0.80 g DHA for 2 weeks	Elbow flexor of eccentric contractions (3 sets of 10 reps)	Reduced IL-6
Andrade et al., 2007	20 elite male swimmers	1.8 g fish oil/d containing 950 mg EPA, 500 mg DHA for 6 weeks	6-week period of intense training and competitions	Significantly reduced plasma levels of TNF-α and PGE_2
Bloomer et al., 2009	14 recreational males	2.224 g EPA & 2.208 g DHA for 6 weeks	60-min treadmill climb using a weighted pack	Significantly reduced resting levels of inflammatory markers (TNF-α, C-reactive protein)
Tarbinian et al., 2009	27 healthy untrained males	324 mg EPA, 216 mg DHA/day for 30 days	40-min bench stepping	Significant decrease in DOMS 48h post exercise n-3 (*vs* placebo *vs* control)
Jouris et al., 2011	11 healthy (3 males/8 females)	2 g EPA, 1 g DHA/day for 1 week	Elbow flexor of eccentric contractions (2 sets to failure at 120% 1RM)	Significantly reduced exercise-induced muscle soreness
Tarbinian et al., 2011	45 young healthy males untrained	1.8 g/d n-3 containing 324 mg EPA, 216 mg DHA/day for 30 days	40-min bench stepping	Attenuate IL-6 & TNF-α and CK & Mb following eccentric exercise 24h and 48h

Rodacki et al. 2012	45 healthy elderly women	2 g/day fish oil, 0.4 g EPA & 0.3 DHA for 12 weeks	Lower limb resistance training (3 sets of 8 reps at 50% of 1RM	Improved MIVC, rate of torque development, greater improvements in muscle strength & functional capacity
Atashak et al., 2013	20 young healthy handball players	3 g n-3 containing 540 mg EPA, 360 mg DHA/day for 1 week	lower body resistance exercise (4 sets x 10 reps at 120% 1RM for each three-leg exercise)	Attenuate the increase of CK and CRP values
DiLorenzo, Drager and Rankin, 2014	41 healthy untrained males	2 g DHA for 4 weeks	Elbow flexor of eccentric contractions (6 sets of 10 reps at 140% 1RM)	CK & IL-6 effective
Lembke et al., 2014	64 healthy untrained males & females	2.7 g EPA & DHA for 30 days	Elbow flexor of eccentric contractions (2 sets of 30 maximal efforts)	Decreased DOMS
Tsuchiya et al., 2016	24 healthy, untrained males	0.60 g EPA & 0.26 g DHA for 8 weeks	Elbow flexor of eccentric contractions	Effective in IL-6 and MVC torque
Ochi et al., 2017	21 healthy, untrained males	0.60 g EPA & 0.26 g DHA for 8 weeks	Elbow flexor of eccentric contractions (6 sets of 10 reps at 40% 1RM, 30 ^{o}/s)	Inhibited the muscle strength loss, limitation of ROM and rises in DOMS
Jakeman et al., 2017	27 physically active males	High EPA group; EPA 0.75 g & DHA 0.05 g Low EPA group; EPA 0.15 g & DHA 0.10 g; One dose upon completion of the protocol	Squat jump protocol	Attenuate the symptoms of EIMD, such as muscle soreness and swelling
Kyriakidou et al., 2021a	14 healthy young males	3 g/day n-3 (2145 mg EPA, 858 mg DHA) for 4 weeks	Downhill running (60 min, 65% $\dot{V}O_2max$, -10% gradient)	Significantly decreased DOMS at 24h post-exercise, and attenuated the rise in CK. Peak power and IL-6 did not change in N-3 group but did change in placebo

No Effects				
Raastad et al., 1997	28 male soccer players	5.2 g/day fish oil, 1.6 g EPA & 1.04 g DHA for 10 weeks	Running on the treadmill until exhaustion	No change in anaerobic power or running performance
Toft et al., 2000	20 endurance-trained males	6 g fish oil/day (3.6 g n-3 – 53% EPA, 31% DHA) for 6 weeks	Marathon performance	No difference in cytokine levels (IL-6, IL1-ra, TNF-α) between the 2 groups No influence on exercise-induced increases in leucocytes and CK
Lenn et al., 2002	22 healthy (13 males/9 females)	1.8 g fish oil per day for 30 days	Elbow flexor of eccentric contractions (50 maximal efforts at a 90 °/s)	No significant effects on perceived pain
Peoples et al., 2008	16 male cyclists	8 g/day fish oil for 8 weeks	Submaximal exercise at 55% of peak workload	No change in performance but heart rate, VO_2 lower during steady state endurance exercise
Tarbinian et al., 2009	27 healthy untrained males	324 mg EPA, 216 mg DHA/day for 30 days	40-min bench stepping	No change in pain level between groups before, immediately and 24h post.
Bloomer et al., 2009	14 trained males	2.224 g EPA, 2.208 g DHA/day for 6 weeks	60-min treadmill climb using a weighted pack	No effect on CK & DOMS
Nieman et al., 2009	23 trained cyclists	2.4 g/day fish oil, 3g EPA and 0.4 g DHA for 6 weeks	3 hours of cycling at 57% of maximum power output	No change in 10 km time trial performance (no reduction in IL-6, IL-8 & IL-1ra and CK & CRP)
Houghton and Onambele, 2012	17 healthy females	0.36 g EPA for 3 weeks	Resistance exercise (leg flexions, leg extensions, straight leg dead lifts)	No differences in MVC increments, no effect on DOMS
Gray et al., 2014	20 healthy untrained males	3 g/day fish oil, 1.3 g EPA & 0.3 g DHA for 6 weeks	Knee extensor of eccentric contractions	No change in MVC, DOMS and CK
DiLorenzo, Drager and Rankin, 2014	41 healthy untrained males	2 g DHA for 4 weeks	Elbow flexor of eccentric contractions (6 sets of 10 reps at 140% 1RM)	No effect on DOMS

Lembke et al., 2014	64 healthy untrained males & females	2.7 g EPA & DHA for 30 days	Elbow flexor of eccentric contractions (2 sets of 30 maximal efforts)	No effect on MVC torque
Tsuchiya et al., 2016	24 healthy, untrained males	0.60 g EPA & 0.26 g DHA for 8 weeks	Elbow flexor of eccentric contractions	No effect on CK, Mb and TNF-α
Gravina et al., 2017	26 young healthy soccer players (19 males, 7 females)	0.1 g/kg n-3/d, 1000 mg n-3 (70% EPA, 20% DHA) for 4 weeks	Regular soccer training period	No improvement of strength and power of physical function
Jakeman et al., 2017	27 physically active males	High EPA group; EPA 0.75 g & DHA 0.05 g Low EPA group; EPA 0.15 g & DHA 0.10 g; One dose upon completion of the protocol	Squat jump protocol	No change in CK and IL-6
Kyriakidou et al., 2021a	14 healthy young males	3 g/day n-3 (2145 mg EPA, 858 mg DHA) for 4 weeks	Downhill running (60 min, 65% $\dot{V}O_2max$, -10% gradient)	No improvement of strength and no change in TNF-α

2.8 AGEING, INFLAMMATION, MUSCLE FUNCTION AND EXERCISE

Due to the ageing population, prevention and improvement of age-related diseases have received considerable attention. As people age, they are often challenged with disability which is manifested by various chronic diseases, increasing frailty, and loss of independence. New findings indicate that ageing is a modifiable risk factor, and age-related disorders, including heart diseases, dementia, atherosclerosis, diabetes and most cancers, can be delayed by regulating fundamental ageing mechanisms to increase health span (Tchkonia et al., 2013). One theory suggests the primary cause of these age-related diseases is the chronic, nonmicrobial inflammation. Inflammatory mediators, including increased IL-6, TNF-α circulating concentrations, are linked to depression

(Howren, Lamkin and Suls, 2009), atherosclerosis (Pai et al., 2004; Tuomisto et al., 2006), cancers (O'Connor et al., 2010; Schetter et a., 2010), diabetes (Spranger et al., 2003; Hu et al., 2004) and mortality (Harris et al., 1999; Bruunsgaard et al., 2003). It appears that inflammation is the most important physiological response linked to age-related diseases (Kanapuru and Ershler, 2009) and could lead to sarcopenia (loss of skeletal muscle mass and function) and cachexia (Ferrucci et al., 1999), commonly seen in elderly population (Roubenoff, 2003; Leng et al., 2007; Bandeen-Roche et al., 2009; Lucicesare et al., 2010; Rockwood and Mitnitski, 2011).

2.8.1 Ageing and Increased pro-Inflammatory Cytokines

It is widely accepted that ageing is characterised by elevations of circulating pro-inflammatory cytokines contributing to a chronic inflammatory state, which has been termed inflammageing (Ostan et al., 2008; Franceschi and Campisi, 2014; Kennedy et al., 2014). Whilst having a beneficial role at low doses (physiological inflammation) in tissue repair at young adults, it seems that, with ageing, inflammation gradually increases, leading to consistently elevated concentrations of pro-inflammatory mediators. This may contribute to the pathogenesis of many age-associated diseases and to the progression of the ageing process (Franceschi and Campisi, 2014; Kennedy et al., 2014). Inflammageing is caused by the systemic imbalance between pro- and anti-inflammatory molecules. The imbalance is triggered as a response to an individual's constant exposure to inflammatory stimuli and in particular the continuous initiation of anti-inflammatory processes (Spazzafumo et al., 2013).

2.8.2 Cellular Senescence and the SASP Theory

The termination of cell division is called cellular senescence and it is a process that may contribute to age-related disability and chronic inflammation. Cellular senescence varies according to different environmental factors, including age. In young organisms, senescent cells are advantageous, minimising tumorigenesis and promoting immune responses. Whereas, with chronological ageing, the abundance of senescent cells increases, causing ageing phenotype and age-related pathology (Burton, 2009; Ohtani et al, 2010; Campisi et al., 2011; Waaijer et al., 2012). In addition, it has been found that telomere erosion or other types of DNA damage, such as ROS, mitochondrial damage and protein aggregation, cause cellular senescence (Jeyapalan and Sedivy, 2008; Tchkonia et al., 2010; Weyemi et al, 2012). These activate the tumour suppressor protein p53 and p16INK4a pathways that trigger a senescence response. This process, when fully established becomes irreversible and an intracellular signalling loop, including ROS linked to DNA damage responses (DDRs), NF-κB, IL-6, IL-1α and transforming growth factor-β, enhance this process (Kuilman et al., 2010; Freund et al., 2010).

Such low-grade chronic inflammation is a common feature of ageing and associates with age-related diseases (Franceschi et al., 2007; Vasto et al., 2007; Chung et al., 2009). This systemic inflammation may be due to either an age-related decline in homeostasis of the immune function or may be in part from senescent cells which secrete pro-inflammatory cytokines, chemokines and proteases, termed the senescence-associated secretory phenotype (SASP) (Coppe et al., 2008; Coppe et al., 2010). The SASP is mainly a DDR (Rodier et al., 2009). It produces inflammatory, growth-promoting and remodelling factors

which can potentially explain how cellular senescence changes local tissue microenvironments and spreads malignant phenotypes to neighbouring healthy cells. The increasing burden of senescent cells may contribute to the aetiology of age-related diseases and accelerate progression of these diseases in a positive feedback cycle following their initiation. Proteins, such as IL-6, TNF-α and IGF binding proteins (IGFBPs) that are linked to SASP, increase in multiple tissues with chronological ageing (Freund et al., 2010) and appear in combination with inflammageing. In addition, it has been found that components of the SASP, such as IL-6, IGFBP-2 and plasminogen activator inhibitor-1, are expressed greater in p16INK4a positive senescent cells isolated from fat tissue of older progeroid mice compared with non-senescent cells from the same tissue (Baker et al., 2011). This suggests that the SASP may be a primary driver of age-related systemic inflammation, at least in fat tissue. Therefore, selective elimination of senescent cells or their effects may be a potential strategy to reduce inflammageing, enhance health span and interfere with the link between ageing and chronic disease, as we are seeing in animal studies (Baker et al., 2004; Baker et al., 2008; Childs et al., 2015; Xu et al., 2018), and emerging human studies (Hickson et al., 2019).

2.8.3 Age-Related Changes in Muscle Function

Given that ageing involves a reduction of function of multiple physiological systems, as discussed earlier, there is an essential relationship between inflammation and immunity. Chronic high levels of inflammation are also associated with strength and muscle mass loss, and reduced muscle protein balance; consequently, leading to decrements of mobility, neuromuscular

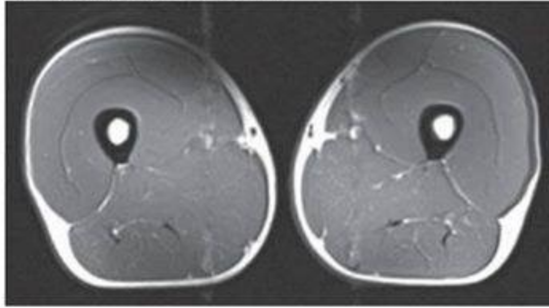
activation, lower-extremity performance, and overall physical activity in older adults (Visser et al., 2002; Cesari et al., 2004; Santos-Eggimann et al., 2009).

In addition, physiological changes, such as changes in fibre type and muscle fibre atrophy, may affect the velocity and force of movements, resulting in reduced performance and potentially in functional disability (Reid and Fielding, 2012). Indeed, skeletal muscle atrophy arises with advancing ageing. Mitchell et al. (2012) in their review showed that the median decline in muscle mass is 0.37% per year for women and 0.47% per year for men throughout the lifespan. These skeletal mass losses are accompanied by a significant decline in strength ranging from 0.3% to 4.2% per day (Wall et al., 2013). Further, at the myocellular level, it is well documented (Verdijk et al., 2007; Snijders et al., 2009; Nilwik et al., 2013) that there is an ageing-induced shift in muscle fibre type, with older individuals showing a larger ratio of type I fibres compared with the younger ones having larger ratio of type II fibres which are also more susceptible to injury. The reduction of the size in type II muscle fibre is also accompanied by an age-related decline in type II muscle fibre satellite cell content and function (Verdijk et al., 2014). This decline may be a main cause for the type II muscle fibre atrophy associated with ageing (Renault et al., 2002; Kadi et al., 2004).

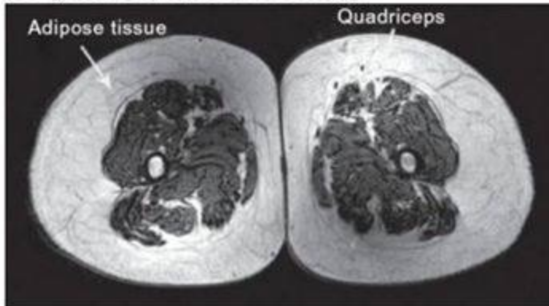
Moreover, the disruption in the regulation of skeletal muscle protein turnover is the key factor of the muscle mass loss, resulting in a negative balance between muscle protein turnover synthesis and muscle protein breakdown (Koopman and van Loon, 1985; Koopman et al., 2007). In addition to the muscle atrophy, it has been demonstrated that ageing is inversely associated with mitochondrial DNA of the vastus lateralis and with mRNA transcription (Short et al., 2005). This

reduction may lead to lower rates of mitochondrial muscle protein synthesis in elderly (Chen et al., 2015). The mitochondrial function (i.e., the ability to produce ATP) is also important for the aged skeletal muscle performance. It has been documented that a number of pathways for producing ATP, including anaerobic glycolysis and oxidative phosphorylation, may be deteriorated in older muscle (Reaburn and Dascombe, 2009; Russ and Lanza, 2011). Previous research has shown an ageing-related decline in anaerobic capacity, due to a decline in enzyme activity, such as LDH and hexokinase, and thus a reduced production of blood lactate (Lanza, Befroy and Kent-Braun, 1985; Pastoris et al., 2000; Kaczor et al., 2006). However, others did not conclude similar results, and showed homogenous enzyme activity between younger and older adults (Essen-Gustavsson and Borges, 1986). Discrepancies may be attributed to the different methodologies, population (i.e., healthy *vs* frail elderly or trained *vs* untrained) or body composition, e.g., muscle mass and muscle architecture and strength (Reaburn and Dascombe, 2009). These losses reduce functional capacity whilst increasing the risk of falls, however, they can be offset by maintenance of physical activity in older age (Figure 2.12; Ganse et al., 2018).

40 year old triathlete



74 year old and sedentary



74 year old triathlete:

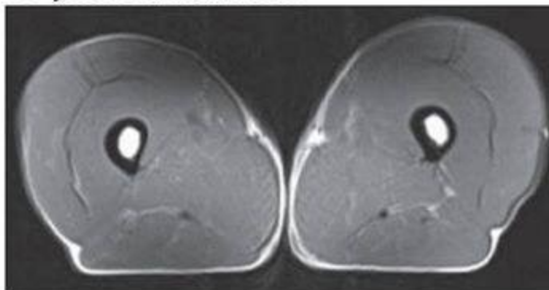


Figure 2.12 Magnetic resonance imaging scans in quadriceps of a 40-year-old triathlete compared with the quadriceps of a 70-year-old triathlete and a 74-year-old sedentary man. Note the significant visual difference between the subcutaneous adipose tissue and intramuscular adipose tissue of the sedentary man *vs* master athletes (Wroblewski et al., 2011)

The relationship between human ageing and physiological function is influenced from a variety of confounding genetic, nutritional and lifestyle factors as illustrated in Figure 2.13. For this reason, many studies have investigated different training protocols, such as resistance-type exercise training, focusing on the maintenance and/or the improvement of maximum strength and power, muscle gains (hypertrophy), and overall functional capacity in older populations (Cadore and

Izquierdo, 2013; Ramirez-Campillo et al., 2014; Holviala et al., 2014; Walker, Peltonen and Häkkineet, 2015).

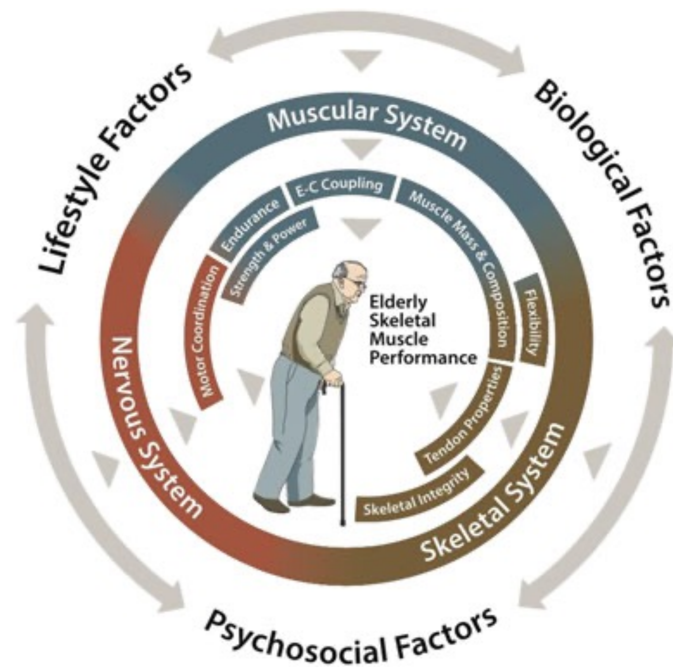


Figure 2.13 The multifactorial determinants of elderly skeletal muscle performance. Diagram by Tim Goheen (taken from Tieland et al., 2018)

2.8.4 Ageing and Exercise Recovery

Both aerobic and resistance exercise, as well as nutritional supplementation of amino acids or n-3 PUFA, have been associated with protection against age-related sarcopenia (Dalle, Rossmeislova and Koppo, 2017) or physical performance (Beaudart et al., 2017), possibly due to their anti-inflammatory and antioxidative properties (Dalle et al., 2017). However, unaccustomed exercise, which may lead to EIMD, may have adverse outcomes in activities of daily living in older adults, resulting in impaired functional reserves with increasing the risk of falls.

In mouse and rat experimental models, ageing-associated differences in muscle cell function and physiology are noted. Following maximal eccentric exercise contraction in rats, the muscle protein synthesis response was lesser in older *vs* younger mice at 48 hours (West et al., 2018). Additionally, in a rat model of DOMS, younger rats showed a quicker recovery from eccentric contraction-induced pain than older rats (Taguchi et al., 2007). *Ex vivo*, extensor digitorum longus (EDL) but not soleus muscle twitch force was reduced, and a significant difference in rate of fatigue onset was noted with repeated contraction, with older EDL muscle showing fatigue resistance to repeated eccentric contractions (Hill et al., 2018). Following two bouts of eccentric exercise separated by 2 days, muscle from older rats showed a reduced rate of force production, whilst younger rats appear fully recovered (McBride et al., 1995). Functional contraction differences were maintained between younger and older muscle at the cellular level; following eccentric contraction in younger (3 months) and older (32 months) rats sarcomeric resting membrane potential was elevated in both age groups but slower to return to baseline in older rats' muscle (McBride, 2000).

However, in human data sets, differences in younger *vs* older muscle recovery are less clear. In middle-aged males (40-60 years of age), Arroyo et al. (2017) did not report differences in the TNF-α or CK response between middle aged and a younger (18-30 years of age) group at 30 minutes to 48 hours post a muscle-damaging protocol. Similarly, no differences were observed in functional strength measures (Arroyo et al., 2017). In addition, Yasar et al. (2019) reported complete recovery in peak power output three days post a sprint interval training protocol (3 x 20 seconds maximal bicycle efforts) in both younger and older participants, however, it is unclear if the sprint protocol utilised was sufficient to induce

decreases in performance, as repeat measurements were not taken immediately post or at days 1 and 2. Further, following a single high intensity interval training (HIIT) cycle training session in younger *vs* older trained cyclists, no effect of age was noted on measures of physical recovery, nor was an effect of age noted for circulating CK concentrations. Nevertheless, it is of interest to note that the perception of fatigue and soreness was elevated in master cyclists 48 hours post HIIT cycle training, relative to trained younger cyclists (Borges et al., 2018). The similarity of functional decreases between ages appeared to be maintained in hyper-acute timeframes, no difference in knee extension torque recovery rate between 7 and 30 minutes was seen in younger (25 ± 3 years) *vs* older (71 ± 4 years) individuals following a knee extension fatigue protocol (Thompson, Conchola and Stock, 2015). Thus, whilst functional differences in muscle recovery from eccentric exercise can been seen in small animal models, caution must be taken in moving these findings into human models.

2.9 SUMMARY

The above evidence exploring n-3 supplementation and exercise-induced muscle damage suggests that it is a subject of interest with many different aspects. More specifically, it is widely established that unaccustomed exercise causes muscle damage and systemic inflammation. EIMD and inflammation are characterised by a milieu of symptoms resulting from myofiber disruption, which could impair recovery and subsequent performance in elite athletes and recreational individuals or prevent an individual in participating in daily activity schedules. Symptoms include increases in tissue oedema and localised swelling, muscular stiffness, muscle soreness, impaired functional capacity (e.g., loss of strength and power, and reduced of ROM) and diminished recovery.

There is some evidence to suggest n-3 PUFA may reduce exercise-induced muscle inflammation. However, the limited number of human studies and the inconsistent results within the current literature make it difficult to draw conclusions. In addition, variations in the duration, the dosage and composition of n-3 supplementation, as well as the usage of different types of exercise protocols makes it difficult to compare findings amongst different studies. Currently, there is no consensus on the optimal duration and dosage for EPA and DHA. Based on the literature, an ingestion of a minimum dosage of 3 g daily of n-3 PUFA or ingestion of EPA/DHA of 2:1 ratio for at least 3 weeks would be beneficial to detect any effect on EIMD (Simopoulos, 2007; Mickleborough, 2013; McGlory et al., 2014). It has also been reported that a mixed EPA and DHA dosage might have a synergic effect, since the ingestion of single EPA or DHA did not cause reduction in the effects of EIMD (Ochi and Tsuchiya, 2018). Omega-3 supplementation has yet to show that mitigates EIMD and improves exercise performance. For that reason, further investigation is required to identify the true relationship between n-3 supplementation and the effects of EIMD.

Maintenance or even improvement of quality of life is a major factor of overall health at any age but becomes a point of emphasis as people age. It is a common belief that with ageing comes an inevitable decline from vitality to frailty and increased susceptibility to multiple diseases and conditions (Hayflick, 2007). Along with the sarcopenia, quality and function of ageing skeletal muscle are directly attributed to modifications in its size, contractile properties and composition (i.e., fibre-type distribution).

Exercise regulates the morbidities of muscle ageing, by increasing muscle strength, improving balance and mobility, and decreasing the likelihood of falling. Habitual exercise or lifelong training has a positive effect on ageing (Zampieri et al., 2015; Elliott et al., 2017) and is prophylactic against age-related functional declines, as exercise at any age stimulates protein synthesis and increased lean muscle mass and strength (Newman et al., 2006; Faulkner et al., 2008; McKendry et al., 2021).

Literature suggests that resistance training increases muscular strength and physical performance of elderly people (Trappe et al., 2003; McCrory et al., 2009; McLeod et al., 2019). Additionally, in older adults, eccentric resistance exercise interventions have been suggested due to high load and potentially greater anabolic response at a low energy cost (Gault and Willems, 2013; Lim, 2016; Franchi, Reeves and Narici, 2017). Aerobic exercise is also important in maintaining optimal skeletal muscle performance (Landi et al., 2014). The age-related decline of aerobic capacity leads to the performance deterioration of physical activities, such as walking or cycling (Lima et al., 2011). Thus, the retention of skeletal muscle mass may contribute to the maintenance of performance with ageing, improve overall quality of life and independence, and promote longevity.

Nevertheless, in the context of exercise performance, if resistance exercise is to be recommended as an effective intervention to delay sarcopenia and/or to initiate muscle hypertrophy (Tieland et al., 2018), eccentric EIMD can be considered to be the basis for hypertrophy. This proposes that micro-trauma in the muscle fibres induced by mechanical and metabolic stress of acute exercise

is a prerequisite for its subsequent growth during the adaptation phase (Clarkson and Tremblay, 1988; Givli, 2015). Further, it has also been suggested that eccentric EIMD may be used to develop safer and more effective personalised training and recovery protocols (Givli, 2015). However, the effects of EIMD must be better understood in older populations. More specifically, improved understanding of any mechanistic ageing-associated differences in muscle damage, inflammation and pain responses may aid in both our understanding of physiological differences in older individuals, and ultimately facilitate personalised exercise prescription in this population.

Whilst significant work has been conducted in younger participants exploring both the effect of n-3 supplementation, and muscle function and exercise recovery, little work has been examined in older populations. Given that n-3 PUFA have potent anti-inflammatory actions and exert anabolic properties, the role of n-3 supplementation in battling sarcopenia could be a novel nutritional strategy. In addition, the diversity of challenging exercise protocols used in the literature in younger populations may not be suited to older adults. Thus, it is warranted to optimise exercise models on responses to muscle damage in naive-exercised or older individuals that would enhance ecological validity in people's everyday lives. Hence, it is of critical importance to optimise diet and lifestyle strategies for maintaining muscle health in both younger and sarcopenic elderly population. Therefore, the following experimental Chapters will focus on muscle damage, inflammation and functionality in both younger and older adults. This Doctorate also aims to establish the technique of EIMD here at the University of Westminster.

CHAPTER THREE

Materials and Methods

3.1 INTRODUCTION

All fitness testing and assessment measures which comprise this thesis were performed in the Human Performance and Sport and Exercise Laboratories of the School of Life Sciences at Cavendish campus, University of Westminster. A maximal and/or exhaustive effort was required on most visits to the laboratory, and thus, safe and appropriate working practices were conducted (**Appendix A**). All plasma, serum and urine samples were handled in accordance with the University's laboratory standard operational procedures and risk assessments (**Appendix B**, **C**). All *ex vivo* samples were assayed in the laboratories of the School of Life Sciences at Cavendish campus, University of Westminster (London, UK). The PhD researcher of this Doctorate complied, throughout the conduct of all studies, with good research practice standards.

3.2 ETHICAL APPROVAL

Prior to the collection of any data, ethical approval for all studies was obtained from the College of Liberal of Arts and Sciences Research Ethics Committee at the University of Westminster, School of Life Sciences (London, UK). All work herein conforms to the standards set by the Declaration of Helsinki of 1975. The nature and purpose of each experimental study was fully explained verbally and in writing to each volunteer (participant information sheet, **Appendix D**, **E**, **F**). Each participant was made fully aware that they were free to withdraw from the study at any time and completed an informed consent form (**Appendix D**, **E**, **F**). The PhD researcher of this Doctorate complied, throughout the conduct of all studies, with University's Code of Practice Governing the Ethical Conduct of Research.

3.3 DATA PROTECTION AND CONFIDENTIALITY

All data was collected and stored securely throughout the duration of this Doctorate in line with the requirements of the Human Tissue Act 2004, the Data Protection Act 2018, the General Data Protection Regulation (GDPR) 2018 and the Freedom of Information Act 2000.

3.4 HUMAN PARTICIPANTS RECRUITMENT

A total of 59 healthy males volunteered to participate in all experimental studies in this Doctorate. Participants were recruited via social media, word of mouth and poster advertisements (**Appendix N**) in the University of Westminster campuses, gyms and sports clubs to ensure recruitment of a range of participants.

3.5 PARTICIPANTS

During visit 1 of each study, participants further to completing the informed consent form, they also completed the medical history questionnaire (**Appendix G**). They were also required to complete an illness-specific health-related questionnaire (WURSS-21; Barrett et al., 2009) to further confirm that they were free from upper-respiratory tract infections (URTI) and to monitor any impairments associated with common cold preceding all trials (**Appendix G**). URTI is extremely common, where approximately 70% of the population experiences a cold in a given year (Barrett et al., 2009). A physical activity readiness questionnaire (PAR-Q) pre-exercise participation screening (**Appendix H**) was alco completed by each participant prior to every visit to confirm they were free from any pain or injury.

3.5.1 Inclusion/Exclusion Criteria

Participants were Caucasian younger males between 18 and 35 years of age (**Chapter 4**, **5** and **Chapter 6**) and older males above 60 years old (**Chapter 5**). Caucasian ethnicity was used as an inclusion criterion throughout all studies as many of the biomarkers used in this thesis have been reported to differ between ethnic groups, including CK (Brewster et al., 2007; Brewster et al., 2012), IL-6 (Chapman et al., 2009; Paalani et al., 2011) and TNF-α (Kalra et al., 2005). Therefore, to maintain consistency and avoid having to account for an additional co-factor only Caucasian men were included in this thesis.

Additionally, participants were non-smokers, free from injuries, any known immune, cardiovascular, or metabolic diseases and not taking any current medication (e.g., non-steroidal anti-inflammatory drugs) or consuming fish oil supplements < 6 months prior to commencing each experimental study. In addition, all participants were unaccustomed to downhill running or to regular eccentric resistance exercise using the legs (e.g., weightlifting, lunges, squats) to minimize the impact of RBE from prior adaptation to eccentric contractions (Brown et al., 1997; Stupka et al., 2001; Thiebaud, 2012; Hyldahl, Chen and Nosaka, 2017).

3.6 FAMILIARISATION

The baseline visits for each study ensured full familiarisation of protocol and testing equipment with safe and correct practice. In addition, during this visit the $\dot{V}O_2max$ (**Chapter 4** and **6**), the 5 repetitions maximum (5RM) of single-leg press (**Chapter 5** and **6**) and 5RM single-leg extension (**Chapter 6**) were determined. During baseline, participants in all studies were also fully informed of the muscle-

damaging protocol and all assessment measures which would be performed in the following visits.

3.7 ECCENTRIC MUSCLE-DAMAGING EXERCISE PROTOCOL

Participants in all experimental studies performed well-established and validated eccentric lower body muscle damaging exercise protocols which will be described in detail to each experimental study (**Chapter 4**, **5** and **6**). Vigorous verbal encouragement was given to participants throughout all maximal and/or exhaustive tests to ensure a maximal effort was produced.

3.8 ANTHROPOMETRY

Prior to commencement of each experimental study (**Chapter 4**, **5** and **6**), height (to nearest 0.1 cm) was measured at baseline using a wall-mounted Holtain Harpenden Stadiometer (Holtain Ltd, Crymych, Wales, UK) fitted with a high speed Veeder-Root counter (Veeder-Root, Elizabethtown, NC, USA) with participants stood in bare feet, heels together with their shoulders and buttocks in contact with the stadiometer. Body weight (to nearest 0.1 kg), BMI and %BF (to nearest 0.1%) were measured at baseline, pre-EIMD and immediately post-EIMD using BIA (Seca® mBCA 514 Medical Body Composition Analyzer, Gmbh&Co. KG, Hamburg, Germany) and with the participant being fasted and with an empty bladder. Watches were removed prior to stepping on the scale and weight offset was set to 0.5 kg to account for minimal exercise clothing (Figure 3.1).

Figure 3.1 Assessment of body composition using SECA (Seca® mBCA 514 Medical Body Composition Analyzer). A participant standing in bare feet on the body composition analyser during assessment

3.8.1 Thigh Circumference

In the experimental trials (**Chapter 5** and **6**), thigh circumference measures were added in the anthropometric measurements. They were obtained in three standardised points on the upper leg (sub gluteal line, midpoint of a line drawn between the proximal pole of the patella and the greater trochanter of the femur, and 5 cm above the superior pole of the patella) of the dominant exercised leg. A non-stretch anthropometric circumference measuring tape (Seca® 201) was used while participants stood on both feet. All diameter measurements were taken prior to each trial, immediately post-, 24, 48 and 72 hours post-EIMD. Circumference measurements were taken as an indicator of acute changes in thigh volume following EIMD (Chen and Hsieh, 2001), which may indicate temporary muscle inflammation and local swelling, likely to occur from osmotic fluid shifts or inflammation which has been related to eccentric muscle damaging exercise (Fielding et al., 2000; Ploutz-Snyder et al., 2001; Vaile et al., 2008). The average

value (cm) of two measurements from each point was used for analysis. However, midpoint thigh girth was chosen for representation of all upper leg measurements, as it was more responsive to change throughout all the measurement points. Measurement sites were marked during the experimental period using a semi-permanent ink marker as a means of standardization between visits and to maintain consistency during follow-up measurements.

3.9 MAXIMAL OXYGEN UPTAKE ($\dot{V}O_2max$) Test

Participants performed a continuous incremental test to volitional exhaustion on a motorised treadmill to determine the $\dot{V}O_2max$ (**Chapter 4** and **6**) (HP Cosmos Mercury 4.0, Nussdorf-Traunstein, Germany), with expired gases analysed by an on-line breath-by breath system (Cortex Metalyser 3B, Biophysik, Leipzig, Germany).

Cortex analyser was turned on to warm up for at least 30 minutes prior to using, and then calibrated for both the gas and flow sensors prior to every test according to the manufacturer’s instructions [a 2-point gas calibration using ambient air and a certified calibration gas (~17% O_2; ~5% CO_2, Specialised Gases, Birmingham: UK) and using a 3 L calibration syringe (Hans Rudolph, Kansas: USA) to calibrate the flow sensor. Participants performed a 3-minute warm-up at a speed at 6 km/h. Following warm-up, the speed was adjusted to 8 km/h at +1% gradient. Every three minutes the speed was increased by 2 km/h until it reached 16 km/h. After, whilst the speed was constant, the gradient was increased by 2.5% every 3 minutes until fatigue. Participants were verbally encouraged to elicit a maximal effort. Throughout the test, rating of perceived exertion [RPE, Borg 6-20 scale (Borg, 1982)], heart rate (HR) using Polar H7 monitor, absolute oxygen

consumption per minute ($\dot{V}O_2$), carbon dioxide production per minute ($\dot{V}CO_2$) and respiratory exchange ratio (RER) were monitored continuously and recorded at the end of every 3 minutes stage. A maximal test was considered when at least one of the following criteria was attained: the participant reached volitional exhaustion, HR was within 10 beats/min of the age-predicted HR maximal, RER was equal or greater than 1.15 (Howley, Bassett and Welch, 1995; Bird and Davison, 1997) and a plateau of $\dot{V}O_2$ was reached despite an increase in exercise intensity. Then, $\dot{V}O_2$max was determined as the highest average $\dot{V}O_2$ obtained during the last 30 seconds sampling period of the test (Figure 3.2).

Figure 3.2 Assessment of $\dot{V}O_2$max. A participant performing the $\dot{V}O_2$max test on a motorized treadmill (HP Cosmos Mercury 4.0)

3.10 MUSCLE FUNCTION

Indirect measurement tools related to strength, power and exercise performance, such as MVIC, peak power, jump height, ROM and muscle soreness were used to assess EIMD in order to provide a wider physiological perspective of the damage response. Indicators of EIMD were collected for all studies in the same standardised manner as listed below; before and after completing the eccentric muscle-damaging exercise to measure the effectiveness of the protocol and to evaluate the recovery on each experimental study.

3.10.1 Muscle Soreness

During experimental trials (**Chapter 4**, **5** and **6**), magnitude of DOMS was quantified using VAS at baseline, pre-, immediately post-, 24, 48 and 72 hours post-EIMD. Muscle soreness was self-rated by participants on a ten-point-validated VAS indicating on a horizontal line with anchor points from 0 (no pain) to 10 (extreme pain) (Carlsson, 1983; McCormack, Horne and Sheather, 1988). Participants were seated with both legs in passive 90° of flexion during a wall squat (thighs parallel to the floor at 90^{0} degrees of the knee joints). Participants then placed a mark at the point on the VAS corresponding to their perception of soreness on the quadriceps muscle. Participants were blinded to the scores they had previously reported (Figure 3.3).

Figure 3.3 Example positioning for the assessment of perceived muscle soreness using a visual analogue scale. Participants performing a muscle soreness test with thighs parallel to the floor at 90^0 degrees of the knee joints

3.10.2 Passive Range of Motion

During **Chapter 6**, knee joint ROM was assessed using a double-armed goniometer (Prestige Medical Protractor Goniometer) at baseline, pre-, immediately post-, 24, 48 and 72 hours post-EIMD. The ROM was measured as passive joint range, created by an external force moving the limb around the joint (e.g., the researcher). The knee joint ROM was measured by the number of degrees from the starting position of a segment to its position at the end of its full range of the movement. A stationary arm holding a protractor was placed parallel with a stationary body segment and a movable arm moved along a moveable body segment. The axis of goniometer was placed over the joint. During the knee joint flexion, the participant was lying supine on flat surface (bed) to stabilise the stationary portion of the body. The opposite leg was held in anatomical position and the dominant knee flexed maximally while performing the movement. The knee joint extension was also recorded with the participant being in the same

supine position. A small towel roll was placed under participant’s ankle and the researcher pushed the knee down to full extension. In both measurements, it was important that the participant did not move his body while moving the joint (this step isolates the joint movement for a more accurate measurement). Thus, when measuring, the same goniometer was used, and same landmarks were kept ensuring consistency. ROM was calculated by the average of two attempts for both joint flexion and extension.

3.10.3 Vertical Jump Performance

In the experimental trial (**Chapter 5** and **6**), vertical jump height was added to assess the effect of muscle-damaging exercise on dynamic muscle function and power performance (Figure 3.4). All vertical jumps were performed at baseline, pre-, immediately post-, 24, 48 and 72 hours post-EIMD. The jump method was determined using the Takei Vertical Jump Meter device (Takei Scientific Instruments Co., Ltd.) which consists of a rubber mat that the participant stands on, with a strap attached to its centre. The strap was connected to a belt that fastened around the participant’s waist, as well as it was connected to the mat with a measurement cord used to measure the vertical jump height where it was set to 0 cm before the jump. The measurement cord slides through the feeder until the participant reaches the maximum height of the jump. After the jump, height was recorded from the length of the measurement cord that has been pulled through the feeder.

Participants were instructed to stand still in an upright manner on the plate before each jump with their shoulders back, arms relaxed by their sides and their knees fully extended to standardise the initial posture. During countermovement phase,

they performed a rapid preparatory downward movement to a self-selected depth and jumped vertically for maximal height with arm swing. The arm movement was not limited to simulate a more realistic setting like in sports. However, the inconsistency of the arm swing patterns of the participants may have been eliminated by keeping the hands on the hips, and thus have a clearer picture of the lower extremity mechanics amongst the participants' countermovement. Nevertheless, this method is not practical as people would normally swing their arms during jumping. Participants performed two maximal jumps, separated by a 1-minute rest. If a participant jumped forward out of the landing area, the jump was repeated. Participants were instructed to provide maximal effort for each repetition to the highest point they could reach. The maximum jump height was recorded from the average value (cm) of two corrected jump attempts (Figure 3.4).

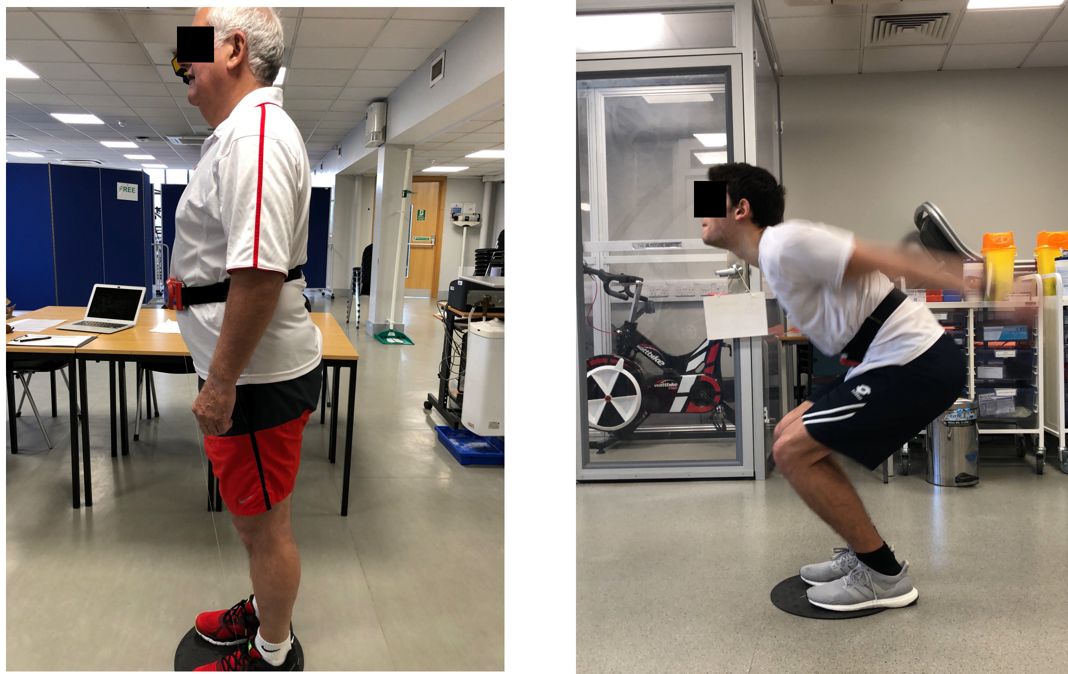


Figure 3.4 Assessment of jump height using Takei Vertical Jump Meter device. Left image: a participant on a standardised initial position. **Right image:** a participant during countermovement phase

3.10.4 Maximal Voluntary Isometric Contraction

During experimental trials (**Chapter 4**, **5** and **6**), MVIC leg strength of the quadriceps was measured at baseline, pre-, immediately post-, 24, 48 and 72 hours post-EIMD to assess the degree of muscle damage. MVIC strength was assessed on KINEO dynamometer (Globus Kineo 7000, Italy). Participants were seated upright and strapped into the dynamometer to limit excess motion. The chair was adjusted so that the leg pad was placed on the lower part of the tibialis anterior, and the pivot was located on the lateral epicondyle of the dominant leg. Maximal force was measured at an angle of 60° leg extension. The protocol consisted of three maximal isometric contractions with 2 minutes recovery between each repetition. Following a 2-minute rest period, participants employed maximal isometric force against the leg pad. Peak force was determined by the average of three maximal isometric contractions lasting 3-5 seconds. The contraction time was recorded by an experimenter. From a pilot study conducted within our laboratory (n = 6 healthy younger participants) the within-day coefficient of variation (CV) for leg extension MVIC was calculated as 6.2% and the day-to-day CV was calculated as 8.7%. Verbal encouragement was given throughout each repetition (Figure 3.5).

Figure 3.5 Assessment of maximal voluntary isometric contraction using the dynamometer KINEO (Globus Kineo 7000). Participants performing a maximal voluntary isometric contraction with their dominant leg

3.10.5 Anaerobic Peak Power

During **Chapter 4**, **5** and **6**, anaerobic peak power was measured at baseline, pre-, immediately post-, 24, 48 and 72 hours post-EIMD using the 10 second WAnT test. Participants performed the test on a cycle ergometer (Monark Ergomedic 894E, Vansbro, Sweden) fixed with an optical sensor (OptoSensor 2000™, Sport Medicine Industries, St. Cloud, USA) with the data obtained by the Monark Anaerobic Test Software.

Participants cycled seated on the cycle ergometer for the sprint protocol with a resistance equal to 7.5% of their body weight (Bar-or, 1987). The seat was adjusted to allow the participant to fully extend the knee with 90º ankle flexion. Participants performed a standardised 5-minute warm-up at 69 revolutions per minute (RPM) which included two 5-second sprints against an unloaded ergometer to familiarise themselves with the test protocol. Participants were then informed with countdown, and they accelerated to maximum RPM 5 seconds

prior to the test, after which the attached weight released at the same time automatically to provide the required resistance. Participants were verbally encouraged throughout the test to maintain their maximal power. At the end of the test a 2-minute cooldown was completed at 69 RPM (Figure 3.6). Seat and handlebar position were also recorded and standardised on follow-up visits.

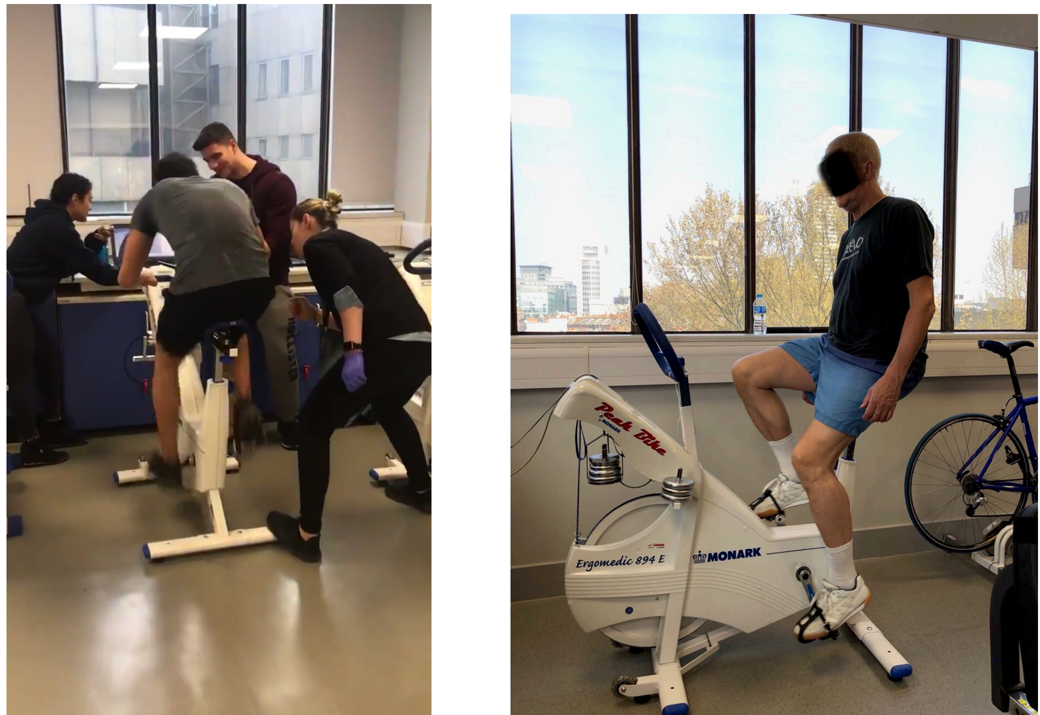


Figure 3.6 Assessment of peak power using Monark cycle ergometer (Monark Ergomedic 894E). Left image: a participant performing the WAnT test. **Right image:** a participant before the initiation of the test

3.11 URINE COLLECTION, HANDLING AND ANALYSIS

During experimental trials (**Chapter 4**, **5** and **6**) all urine samples were collected into 100 mL sterilised plastic containers. Urine sample was collected at baseline and pre-EIMD to assess the hydration status. Hydration level was verified by checking the urine specific gravity (USG) (Atago MASTER-SUR/Na refractometer, Atago Co., Ltd. Tokyo, Japan) upon arrival, which was between

1.001 and 1.029 (Armstrong et al. 2010). Urine colour was also evaluated using the validated urine colour chart (1-8 scale; Armstrong et al., 1998).

3.12 BLOOD COLLECTION, HANDLING AND ANALYSIS

In **Chapter 4**, **5** and **6**, venous blood was drawn by venepuncture using a 21-gauge x 1-¼ inch Vacutainer® Eclipse™ blood collection safety needle from the antecubital fossa vein (Figure 3.7). Whole blood samples were collected at baseline, pre-EIMD, immediately post-EIMD, at 1 and 2 hours post-EIMD (**Chapter 5** and **6**) and during the recovery phase (24, 48 and 72 hours post-EIMD), after the participant seated at rest for 5 minutes. Blood collected immediately post-EIMD was taken 3 minutes after completion of the EIMD bout. A total of 18 mL of blood was collected at each time point in three 6 mL Vacutainer® tubes: one containing K_2 EDTA, one lithium-heparin and one serum SST™ II Advance with separating gel and clot activator (BD, Oxford, UK). The serum vacutainer tube was left for 30 to 40 minutes to clot at room temperature before centrifuging. Whole blood was spun in a refrigerated centrifuge (Hettich Zentrifugen, Universal 320 R, Tuttlingen, Germany) at 5000 revolutions per minute (rpm) for 10 minutes at 4 °C, with the plasma/serum aspirated, aliquoted into Eppendorf tubes and frozen at -80 °C for further analysis. All samples for each assay were defrosted once.

Figure 3.7 Venous blood sampling. It was drawn by venepuncture using a 21-gauge x 1-¼ inch Vacutainer® Eclipse™

3.12.1 Creatine Kinase Activity

Circulating CK activity was measured in heparinised plasma using a clinical chemistry analyser (Werfen ILab Aries, Milano, Italy) (**Chapter 4**, **5** and **6**). CK activity is determined by a coupled enzyme reaction resulting in the production of NADPH proportionate to the CK activity. NADPH concentration was measured using kinetic spectrophotometry at 340 nm with a minimum detection limit of 3 U/L, an undiluted linearity up to 900 U/L. CV was within run <1.2%, total < 2.5%; per the manufacturer's protocol. All samples and standards were analysed in duplicate (**Chapter 4**) and in triplicate (**Chapter 5** and **6**).

3.12.2 IL-6 and TNF-α Analysis

Aliquots of plasma were analysed for IL-6 and TNF-α concentration using Enzyme-Linked Immunosorbent Assay (ELISA) DuoSet kit (R&D Systems,

Minneapolis, Minnesota, USA) according to manufacturer's instruction (**Chapter 4**, **5** and **6**). Briefly, polystyrene microplates were coated with anti-IL-6 or TNF-α capture antibody overnight at room temperature. All samples were dispended in the wells, along with the standards, analysed in duplicate (**Chapter 4**) and in triplicate (**Chapter 5** and **6**) and incubated for 2 hours at room temperature. The detection antibody consists of the biotinylated anti-human IL-6 or TNF-α which was coupled to the HRP with Streptavidin conjugated to horseradish-peroxidase (Streptavidin-HRP), which helped reveal the formation of the complexes through colorimetric reaction. The reading was carried out on SPECTROstar Nano (BMG Labtech, Aylesbury, England) and data analysis was performed with MARS software. Plates were read at 450 nm and blanked to 590 nm. Across all the studies, the mean intra-assay CV for both IL-6 and TNF-α was calculated as 6.6%. For all biochemical analyses, the participant's samples were always assayed within the same plate.

3.12.3 Triglycerides Analysis

Serum TG concentration was measured using a clinical chemistry analyser (Werfen ILab Aries, Milano, Italy) (**Chapter 4**). TG are determined enzymatically by a series of coupled reactions resulting in the production of glycerol. Glycerol is oxidized using glycerol oxidase and H_2O_2 (one of the reaction products) which is measured quantitatively in a peroxidase catalysed reaction that produces colour proportionate to TG concentration. Absorbance was measured at 500 nm. CV was within run <1.7%, total < 2.9%, per the manufacturer's protocol. All samples and standards were analysed in duplicates.

3.12.4 Blood Lactate Analysis

Whole blood lactate was measured in EDTA anticoagulant Vacutainer collection tube immediately after blood sampling using the YSI 2300 glucose and lactate clinical automatic analyser from Yellow Springs Instruments (YSI 2300 STAT Plus, Ohio, USA) (**Chapter 5** and **6**). The YSI employs membrane-bound enzyme electrochemical technology according to the lactate oxidase-base system (www.ysilifesciences.com). The instrument utilises the L-lactate oxidation reaction which catalyses the conversation of L-lactate into pyruvate and hydrogen peroxide in the presence of nicotinamide adenine dinucleotide (NAD^+). The lactate concentration in the YSI 2300 analyser is detected by measuring an electric current. The analyser was calibrated routinely according to the manufacturer's recommendations and met all quality assurance performance standards during the study period. The acceptable upper linear limits of lactate were 30.00 mmol/L, per the manufacturer's protocol. Following calibration, whole blood sample were injected into the sample chamber using a calibrated syringe pipette, and the blood lactate concentration of the sample was determined within one minute. All samples were analysed in duplicate or in triplicate in case of detection error (e.g., air bubbles or froth in the sample drawn into the sipper).

3.13 DIET AND ACTIVITY CONTROL

Participants were requested to maintain their usual diet and exercise routine throughout all experimental studies (**Chapter 4**, **5** and **6**). Participants were also asked to refrain from any exercise for 24 hours prior to baseline visit and 48 hours prior to EIMD visit, and from alcohol and caffeine (coffee, tea, fizzy drinks) 24 hours before all exercise bouts. Further, they were asked to refrain from exercise during the recovery phase (for the subsequent 72 hours following test). Prior to

each trial, participants were provided fluid to consume (5 mL/kg) in the prior 24-hour period to ensure they began exercise testing bouts euhydrated. Participants were also requested to be fasted overnight before the exercise trial, at least 10 hours before their attendance in the laboratory.

Food diaries were provided to record all foods and drinks consumed (including days of the week and of the weekend) prior to baseline (familiarization visit) and the experimental trial (EIMD bout) for all studies. It was important to assess participants' diets whether it was a co-factor to affect both exercise-induced inflammation and function, and the overall performance. Food diaries were analysed using the nutrition analysis software Nutritics® (Research Edition, version 5.092. Nutritics Ltd., Co. Dublin, Ireland) to quantify total energy intake, macro-nutrients (carbohydrates, protein, fatty acids) and n-3 and n-6 fatty acids (**Chapter 4**, **5** and **6**). Average of the two food diaries was taken and compared to the estimated energy requirement (EER) for each participant and recommended intake for recreational athletic populations. Miffin St. Joer's predictive equation (Frankenfield, Roth-Yousey and Compher, 2005) was used to calculate participants' resting metabolic rate (RMR). A physical activity level (PAL) was applied to each participant's RMR to determine EER. PAL factor was calculated using energy expenditure values derived from double-labelled water measures (Black et al., 1996; Food and Nutrition Board, IOM, 2002; German Nutrition Society, 2002). A different PAL was performed according to participants' lifestyle and occupation. A PAL of 1.6 was applied to the recreational active participants with sedentary occupations, 1.7 to those with lightly active occupations or 1.8 to those with moderately active lifestyle occupations.

3.14 STATISTICAL ANALYSIS

The normal distribution and homogeneity of variance of all the parameters were checked with Shapiro-Wilk, Levene's and box's tests, respectively. Frequencies and descriptive statistics were computed for the measured and calculated variables. In normally distributed parameters, differences between two groups were analysed using two-tailed independent samples *t*-tests. When analysing more than two groups a one-way between-groups analysis of variance (ANOVA) was conducted. Where the result of the ANOVA was statistically significant, Tukey's Honestly Significant Difference (HSD) *post hoc* analysis was conducted. The EIMD effects within-group on two different occasions (e.g., pre- *vs* post-EIMD) were determined using paired-samples *t*-test. Exercise-induced changes on outcome measures were analysed using a mixed model (group x time) between-within ANOVA with repeated measures. Bonferroni-adjust pairwise comparisons *post hoc* analysis was used where needed to examine within subject differences. In non-normally distributed parameters, differences between the groups were compared by the Mann-Whitney U test. The Freidman ANOVA was used to determine the main effect of time within-group, and the Wilcoxon signed rank test (using a Bonferroni adjusted alpha value) was performed for *post hoc* analysis to test differences in these variables. The relationships between normally distributed data were investigated using a Pearson correlation coefficient. Between non-normally distributed data a Spearman rank order correlation was performed. For parametric tests, partial eta-squared (η^2_p) values were calculated as measures of effect size when necessary, and were considered small (0.01), medium (0.06) or large (> 0.14). For non-parametric tests, for the Mann-Whitney U test, effect size (r) was calculated by the formula $r = z / \sqrt{n}$; where n = total number of cases; and for the Wilcoxon signed-rank test, effect

size was calculated by the formula $r = z / \sqrt{n}$; where n = the number of observations over the two time points (Pallant, 2016), with effect size being considered small (0.10), medium (0.30) or large (0.50); all were calculated using methods proposed by Cohen (1988). The strength for both Pearson (r) and the non-parametric Spearman (rho) correlations was considered small (0.10 – 0.29), medium (0.30 – 0.49) or large (0.50 – 1.00) (Pallant, 2016). Both linear and non-linear tests statistical significances were expressed as $p < 0.05$. Statistical analyses were performed using SPSS software (IBM SPSS, NY, USA). All figures were generated in GraphPad Prism.

CHAPTER FOUR

The Effect of Omega-3 Polyunsaturated Fatty Acid Supplementation on Exercise-Induced Muscle Damage

(Study one)

The contents of this chapter form the basis of the following peer reviewed publication / conference poster presentation:

Publication

Kyriakidou, Y., Wood, C., Ferrier, C., Dolci, A. and Elliott, B. The effect of omega-3 polyunsaturated fatty acid supplementation on exercise-induced muscle damage. *Journal of the International Society of Sports Nutrition*, 2021; 18 (1):9.

Presentation

Kyriakidou, Y., Wood, C., Elliott, B. and Dolci, A. (2018). The effect of omega-3 supplementation on exercise-induced muscle damage. Europhysiology, 13-14 September 2018, Proc Physiol Soc 41, PCA184, London, UK.

ABSTRACT

Exercise-induced muscle damage (EIMD) results in transient muscle inflammation, strength loss, muscle soreness and may cause subsequent exercise avoidance. Omega-3 supplementation may minimise EIMD via its anti-inflammatory properties, however, its efficacy remains unclear.

PURPOSE: Examine the effects of n-3 supplementation on exercise-induced inflammatory response and muscle function following an unaccustomed muscle-damaging exercise

METHODS: Physically active, healthy males (n = 14, 25.07 ± 4.05 years) provided written informed consent form, then were randomised to 3 g/day n-3 supplementation (N-3, n = 7) or placebo (PLA, n = 7). Following 4 weeks supplementation, a downhill running protocol (60 minutes, 65% $\dot{V}O_2max$, -10% gradient) was performed. CK, IL-6 and TNF-α, perceived muscle soreness, MVIC and peak power were quantified pre, post, and 24, 48 and 72 hours post-EIMD.

RESULTS: Muscle soreness was significantly lower in N-3 *vs* PLA group at 24 hours post-EIMD ($p = 0.034$). IL-6 was increased in PLA ($p = 0.009$) but not in N-3 ($p = 0.434$) following EIMD, however, no significant differences were noted between groups. Peak power was significantly suppressed in PLA relative to pre-EIMD but not in N-3 group at 24 hours post-EIMD. However, no significant difference in peak power output was observed between groups. MVIC, CK and TNF-α were altered by EIMD but did not differ between groups.

CONCLUSION: Omega-3 supplementation for 4 weeks may successfully attenuate minor aspects of EIMD. Whilst not improving performance, these findings may have relevance to soreness-associated exercise avoidance.

Description of Chapter

This chapter focuses on muscle damage, inflammation, muscle function, DOMS and recovery following muscle-damaging exercise in young physically active males. The efficacy of the n-3 supplementation as a nutritional strategy, via its anti-inflammatory properties, remains unclear. Thus, the present study explores the differences on key elements of EIMD between N-3 and PLA group following a downhill running exercise protocol. Secondary objectives of this study are to explore post-supplementation serum triglycerides concentration, and any putative effect of participants' diet.

4.1 INTRODUCTION

The recovery from vigorous athletic performance concerns many groups of people, from high performance athletes to recreationally active individuals. Eccentric exercise, especially novel or high-force eccentric protocols, can produce substantial muscle fibre damage (Clarkson and Hubal, 2002; Owens et al., 2019). Such vigorous-intensity exercise may lead to EIMD (Paulsen et al., 2012). Symptoms of EIMD, such as pain, muscle strength and power loss (Malm et al., 2000; Jouris et al., 2011; Hyldahl and Hubal, 2014; Ochi et al., 2016; Ives et al., 2017); resulting in impairment of exercise performance (Wan et al., 2017). Functionally, muscle strength is reduced by ~20 to ~50% immediately after exercise, and it can take between 2 to 7 days to fully recover (Peak et al., 2017). Systemically, EIMD is paralleled by an inflammatory response involving many mediators, such as IL-1ra, IL-6, IL-10 and acute phase proteins (Duque and Descoteaux, 2014), and a release of muscle specific creatine kinase (Clarkson and Hubal, 2002; Baird et al., 2012). Strategies to reduce muscle damage and inflammation following EIMD can therefore be of use to individuals interested in increasing their rate of recovery and maintaining performance.

Nutrition can affect the biological mechanisms by which the body adapts in exercise and can help improve exercise performance by inducing ergogenic effects (ACSM 2016; Harty et al., 2019). It has been suggested that n-3 PUFA may prove a viable nutritional strategy to attenuate muscle inflammation and improve functional recovery following high-intensity exercise (Philpott et al., 2019). Fish oil is rich in n-3 PUFA that may have anti-inflammatory and immunomodulatory effects (Calder, 2013; Jeromson et al., 2015).

Although animal studies have shown mixed results when evaluating the efficacy of n-3 supplementation on muscle damage, exercise metabolism and exercise performance; human studies have demonstrated that physiological parameters that are linked to improved physical performance and oxygen utilisation, such as blood flow during exercise, can be augmented by dietary n-3 PUFA (Walser et al., 2006; Philpott et al., 2019). Tarbinian et al. (2009), Jouris et al. (2011) and Jakeman et al. (2017) have shown a pain reduction following EIMD with n-3 supplementation. A recent meta-analysis (Lv et al., 2020) also concluded that n-3 supplementation could alleviate DOMS after eccentric exercise. Additionally, Atashak et al. (2013) reported substantial reduction in CK and in C-reactive protein after lower body resistance exercise following 1 week of 540 mg EPA and 360 mg DHA. Further, other studies (Bloomer et al., 2009; DiLorenzo, Drager and Rankin, 2014) have demonstrated that n-3 supplementation has a positive effect on eccentric exercise protocols by reducing the concentrations of IL-6 and TNF-α. However, mixed results have been reported to date, with others (Gray et al., 2014; Tsuchiya et al., 2016) observing no effect of n-3 supplementation on exercise-induced inflammatory and muscle damage markers, and functional markers, such as MVC and DOMS.

It remains unclear whether n-3 supplementation has any beneficial effect in blunting the effects of EIMD, either by increasing the rate of recovery of functional performance, by reducing circulating pro-inflammatory cytokines, or both. Due to this lack of clarity in the literature, the aim of the current study was to add evidence by assessing the effect of n-3 supplementation on EIMD following a downhill running bout. It was hypothesised that 3 g of n-3 supplementation for 4 weeks would attenuate muscle inflammation following EIMD which subsequently would decrease the recovery time, and thus improve exercise performance.

4.1.1 Aims & Hypothesis

The primary aim of this Chapter was to assess the effect of 3 g/day of n-3 supplementation for 4 weeks on EIMD in healthy, young and physically active males. Secondary aim was to compare triglycerides between groups. Specific research objectives were to:

1. assess muscle damage and muscle inflammation following muscle-damaging exercise
2. assess muscle function and DOMS following muscle-damaging exercise
3. examine triglycerides concentration after 4 weeks of 3 g/day n-3 supplementation

The primary hypothesis of this Chapter was that 3 g/day of n-3 supplementation would attenuate muscle damage and inflammation following EIMD which subsequently would decrease the recovery time, and thus improve exercise performance. Specifically, it was hypothesized that:

1. omega-3 supplementation would decrease muscle damage and inflammation following muscle-damaging exercise
2. omega-3 supplementation would mitigate muscle function impairment and DOMS
3. omega-3 supplementation for 4 weeks would alter triglycerides concentration

4.2 METHODS

4.2.1 Ethical Approval

The research protocol of this study received ethical approval from the College of Liberal of Arts and Sciences Research Ethics Committee at the University of Westminster, School of Life Sciences (ETH1617-0182) prior to participant enrolment. Written informed consent was obtained from all participants prior to their participation in the study (**Appendix D**). Participants were recruited between February 2017 and January 2018 from the University of Westminster and from London health clubs.

4.2.2 Participant recruitment

A total of 29 healthy, physically active (self-reported: 4 to 5 times per week structured exercise), Caucasian males aged 18-35 years of age were recruited to participate in this experimental study. Five participants withdrew from the study following acceptance due to the inability to attend testing commitments. One participant reported current consumption of performance enhancing supplements during the first visit, and thus was excluded. Therefore, 23 participants volunteered to participate in the study. Of the 23 participants, a participant discontinued testing during baseline measurements due to inability of blood sampling. Of the remaining 22 participants, 8 participants were removed from the investigation prior to analysis. More precisely, 3 participants discontinued testing following baseline measurements due to inability to attend following visits or due to injury prior to the experimental trial. The remaining 5 participants were unable to complete 60 minutes of downhill running due to pain in calves, hips, upper back or chest. Withdrawals and exclusions from the study are presented in Figure 4.1. Therefore, 14 participants (25.07 ± 4.05 years of age) were included in the final

analysis. Inclusion/exclusion criteria of the participants are described in detail in **Chapter 3** (p. 90).

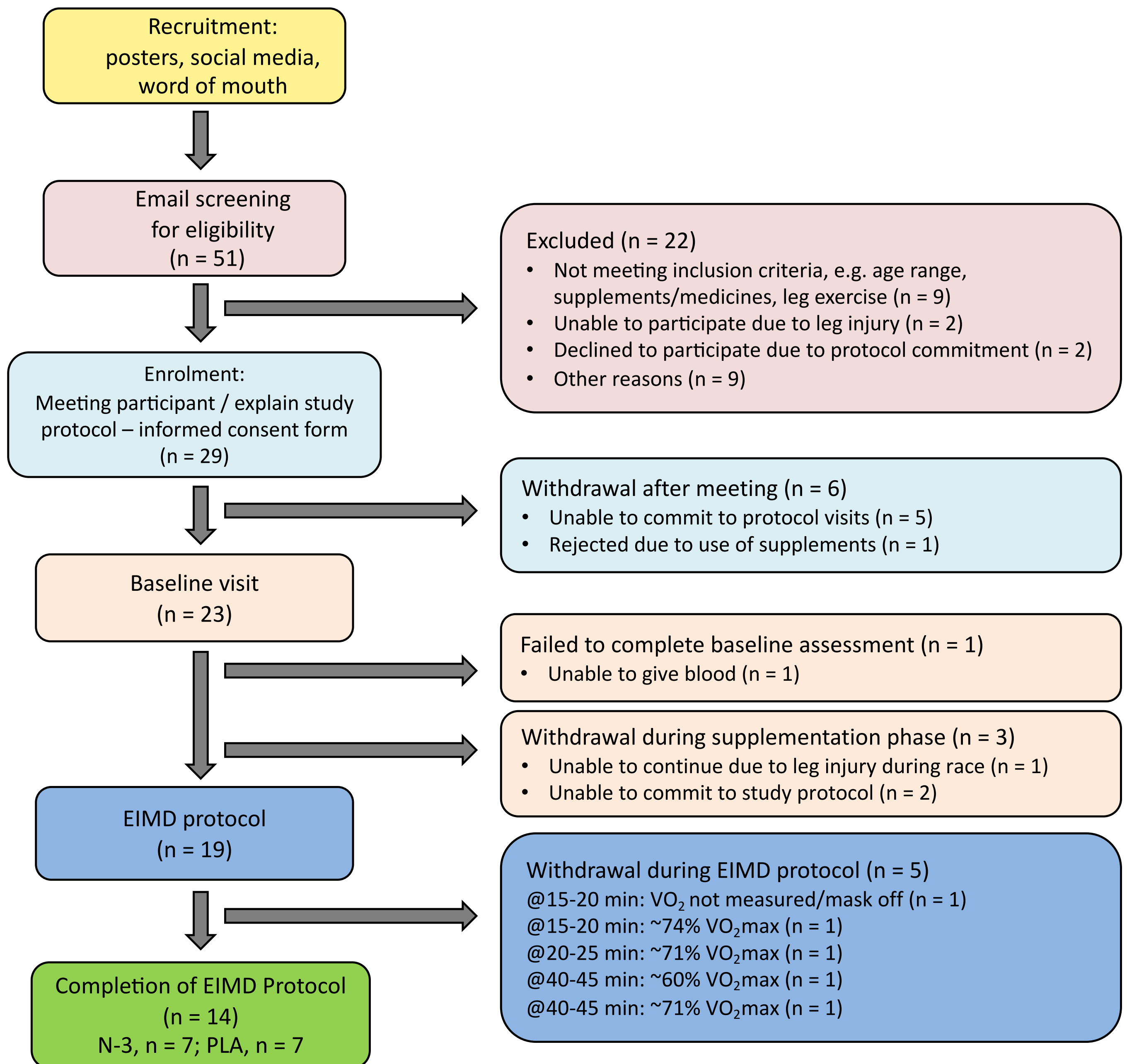


Figure 4.1 Recruitment pathway and participant withdrawals. $\dot{V}O_2$max, maximal oxygen uptake; EIMD, exercise-induced muscle damage

4.2.3 Experimental Design

Participants were single blind randomised to one of two groups, N-3 group (3 g/day of omega-3) or PLA group (taking placebo supplements). After 4 weeks of supplementation, all participants performed a bout of EIMD protocol to examine whether omega-3 supplementation attenuates the muscle inflammation, muscle soreness and force loss following EIMD. All participants were required to attend the Human Performance Laboratory at the same time of day (± 1 hour) in the morning at Cavendish campus, University of Westminster on 6 occasions (included initial visit for study information/informed consent form) over a 6-week period.

During visit 1, participants were informed about the study protocol by providing them the participant information sheet (**Appendix D**) and personally explaining of all procedures, risks and benefits, and given a written informed consent form to sign.

On visit 2 (baseline), in an overnight fasted-state, participants performed baseline measurements to ensure familiarisation of testing equipment. The baseline visit included anthropometric measurements, a urine sample and a venous blood sample. Additionally, perceived muscle soreness, MVIC on the leg and anaerobic peak power were determined as indirect markers of muscle damage, described fully in **Chapter 3**. Following baseline measurements, participants performed a treadmill $\dot{V}O_2max$ test to assess their fitness level before participating in the study and to determine the 65% of their $\dot{V}O_2max$. After this verification was completed, participants were randomly allocated into to one of two groups, N-3 or PLA group, by a computer-generated block randomization in advance

(http://www.randomization.com). Two weeks before beginning EIMD trial, participants completed the WURSS-21 questionnaire on each of the 14 days preceding trial to ensure that they were free from common cold symptoms before testing.

On visit 3 (4 weeks later), participants reported to the laboratory at 07:00 am having fasted overnight and performed the EIMD protocol (downhill running; 60 minutes at 65% $\dot{V}O_2max$ with a -10% gradient). All above measurements were repeated prior to- and immediately-post the EIMD trial. One day before the visit participants were asked to consume water based on their body mass (5 mL/kg) (Dolci et al., 2015) before they reported to the laboratory to ensure adequate hydration before exercise. Identical follow up assessments, except urine sample, were repeated at visits 4, 5 and 6 (24, 48 and 72 hours post-EIMD), during which participants were in a non-fasted state. An overview of the study design is presented in in Figure 4.2.

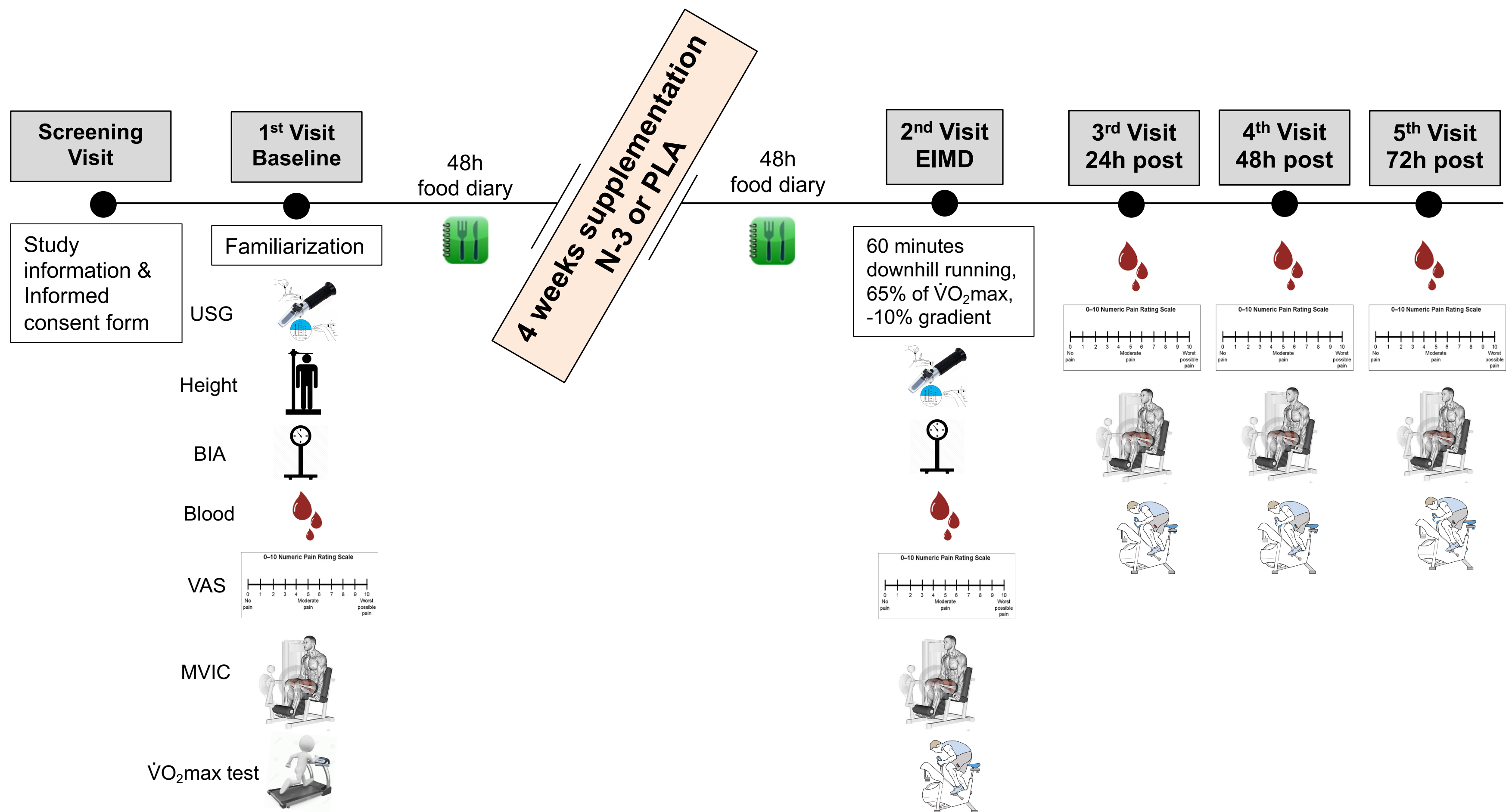


Figure 4.2 Schematic of experimental procedures. USG, urine specific gravity; BIA, bioelectrical impedance analysis; VAS, visual analogue scale for delayed-onset muscle soreness; MVIC, maximal voluntary isometric contraction; $\dot{V}O_2$max, maximal oxygen consumption; N-3, omega-3 supplementation group; PLA, placebo group; EIMD, exercise-induced muscle damage. 2nd visit combines both pre and post measurements, immediately prior and following EIMD stimulus, respectively. A further Wingate test was added on 2nd visit, and all follow up visits

4.2.4 Experimental Procedures

4.2.4.1 Preliminary Testing/Fitness and Familiarisation

On arrival to the Human Performance Laboratory, participants' height, weight, BMI and %BF were measured (detailed description see **Chapter 3**). A urine sample was collected to assess hydration level and venous blood was collected by venepuncture, centrifuged with the plasma aspirated, aliquoted into Eppendorf tubes and frozen at -80 ^{o}C for further analysis. Perceived leg muscle soreness using VAS, maximal isometric leg strength using isokinetic dynamometer and anaerobic power using a 10-second WAnT test were measured to assess participants' baseline muscle function before the EIMD trial. Following baseline measurements, participants performed a treadmill $\dot{V}O_2max$ test to determine the 65%. Instrumentation and procedures used in the experimental trial were presented to participants at this time.

4.2.4.1.1 Determination of 65% of $\dot{V}O_2max$

On the baseline visit, participants performed the $\dot{V}O_2max$ test after anthropometric measurements, blood sampling and functional assessments. Following completion of the $\dot{V}O_2max$ test (detailed description see **Chapter 3**), running speed- $\dot{V}O_2$ relationship was used to obtain the treadmill running speed ($V_{65\%}$) which elicited 65% $\dot{V}O_2max$ at +1% gradient. Then the $V_{65\%}$ was used to estimate the speed for the downhill running (V_{test} = 1.5 x $V_{65\%}$). This estimation was performed because for a given speed, $\dot{V}O_2$ is decreased during downhill running compared to level running (Robergs, Wagner and Skemp, 1997). Following a 10-minute recovery, running speed verification was performed on a downhill run at -10% gradient. Expired gases were analysed on a continuous basis and were averaged every 10 seconds over a period of 2 minutes during this

process with the V_{test} adjusted until 65% $\dot{V}O_2max$ was achieved. This verification lasted no more than 5-6 minutes and the verified running speed was used during the EIMD trial (Fortes et al., 2013, Dolci et al., 2015).

4.2.4.2 Experimental Trial

At the end of the fourth week of supplementation, all participants underwent an EIMD protocol. On the day of the experimental trial, participants reported to the laboratory [ambient temperature (T_{amp}) 21.6 ^{o}C and relative humidity (RH) 29.7%] at 08:00 am having fasted overnight. Participants were asked to consume water based on their body mass (5 mL/kg) before they reported to the laboratory to ensure they began exercise euhydrated. Hydration was verified by checking that the urine specific gravity (USG; Atago MASTER-SUR/Na refractometer, Atago Co., Ltd. Tokyo, Japan) upon arrival was less than 1.028. At 08:15 am, baseline anthropometric measurements and body composition using BIA device were measured to assess whether there were any changes in the body composition after four weeks of supplementation. At 08:30 am a resting blood sample for assessing inflammatory markers were taken before the participant was fitted with a HR monitor (Polar Electro, Kempele, Finland). The participants also rated their perceived muscle soreness at this stage. Then, maximal voluntary isometric contraction on the right leg and anaerobic power test were performed to assess participants' pre-EIMD quadriceps leg strength and power, respectively.

4.2.4.2.1 Downhill Running Exercise

The exercise trial started at 9:00 am with standardised clothes worn by participants (e.g., t-shirt, shorts, socks and running shoes). Following a 3-minute warm up, participants ran for 60 minutes at the individualised predetermined V_{test}

at -10% gradient. During the trial, a harness was provided to secure the participant while using the treadmill. HR and RPE (Borg, 1982) were recorded throughout trial every 10 minutes. A 60-second sample expired gases was collected at 20 and 40 minutes of trial and analysed by an on-line breath-by breath system (Cortex Metalyser 3B, Biophysik, Leipzig, Germany) for $\dot{V}O_2$ to ensure participants were running at 65% $\dot{V}O_2max$. A mouthpiece with a nose clip, to avoid potential gas leakages, were used to adjusted cortex full-face mask. The mouthpiece was placed on to the turbine tube and the cortex was adjusted for the dead space volume difference before the trial. The above procedure has been studied for validity, reliability and stability (Brehm, Harlaar and Groepenhof, 2004; Vogler, Rice and Gore, 2010; Macfarlane and Wong, 2012). Two minutes prior to each sample collection both nose clip and mouthpiece were fitted to allow participant for familiarization and for breathing rates to stabilise. Water was provided every 15 minutes during the downhill running.

Immediately after the muscle-damaging bout participants sat and a blood sample was collected after a standardised period of 3 minutes (post-EIMD). A post-EIMD urine sample was collected, and a body weight (following towelled drying) was also measured at this stage. Participants then rated their perceived muscle soreness, and a maximal isometric leg extension and an anaerobic power test were performed to assess participants' post-EIMD strength and power, respectively. Participants were provided with a standardised amount of water (5 mL/kg) to replace sweat losses and a cereal bar. Participants were asked to refrain from any exercise in this 72-h follow-up period.

4.2.4.3 Diet and Activity Control

Participants were requested to maintain their usual diet and exercise routine throughout the study. Diet and exercise instructions were described in detail in **Chapter 3** (p. 105). In addition, at the start of the supplementation period all participants were provided with a food list (**Appendix J**) with foods low (< 250 mg per serving), moderate (~250 mg per serving) and high (> 500 mg per serving) in omega-3 fatty acids to prevent increasing their omega-3 intake through diet. Cut-off points were used by USDA SR-21 (2008).

Food diaries were provided after the $\dot{V}O_2max$ test to record all foods and drinks consumed one day of the week and one day from the weekend, during 48 hours before the supplementation started as well as during 48 hours prior to the EIMD trial. Written and oral reminders were also provided on a regular basis to ensure diet and exercise practices were maintained consistent throughout the study. It was important to assess the n-3/n-6 ratio in their diets prior to supplementation and to examine how supplementation group affected both exercise-induced inflammation and the overall omega-3 intake.

4.2.4.4 Supplementation

Omega-3 supplementation consisted of 3 gelatine-coated capsules per day (1 consumed in the morning, 1 at lunch and 1 in the evening), each containing 1040 mg of n-3 PUFA (715 mg of EPA and 286 mg DHA) per capsule (Maximum Strength Pure Fish Oil, Nature's Best, UK), for a total of 3900 mg of fish oil daily, containing 3 g of n-3 PUFA (2145 mg of EPA and 858 mg DHA) per day for a period of 4 weeks. Whilst commonly reported side effects of n-3 supplementation, such as unpleasant taste, heartburn, gastrointestinal discomfort and headache

are usually mild (Mazereeuw et al., 2012), the amount of n-3 provided is in line with the nutritional recommendations as part of a normal diet and does not cause any harm or side effects. Daily dosage supplementation of up to about 5 g/day of n-3 PUFA in a long-term consumption is considered safe by the European Food Safety Authority (EFSA, 2012). Longer duration or higher doses may affect immune function due to suppression of inflammatory response (Institute of Medicine, 2005). High doses also might increase bleeding time by reducing platelet aggregation (Institute of Medicine, 2005).

The placebo group received 3 x 600 mg capsules per day of collagen (Troo Healthcare, Colchester, UK), consuming in a matching pattern. Collagen was chosen as the placebo as there is no evidence in the literature that has a pro or anti-inflammatory effect and therefore it will not oppose the action of n-3 supplementation. In addition, in an attempt to isolate the effect of n-3 supplementation collagen was chosen to avoid manipulation of n-6/n-3 ratio.

Participants were only given 1 week of capsules at a time to aid participant retention. Initially written reminders were sent on a daily basis to ensure supplementation practices were maintained consistent throughout the day. Participants' compliance also verified by weekly written and oral reminders, counting remaining capsules at the end of each week and issuing of future weeks capsules took place. Further, participants were asked to guess what group they were at the conclusion of testing with 2 of 7 in placebo and 5 of 7 in N-3 group correctly guessing the supplementation group. Both groups were asked to take the supplements along with their regular meals.

4.2.5 Measurements and Instrumentation

Anthropometric measurements, such as weight, BMI, muscle mass and body fat were recorded at baseline, pre- and post-EIMD. Urine sample was collected prior to each trial (baseline and pre-EIMD) to assess hydration and ensure adequate hydration before exercise.

Blood sampling, for muscle damage (CK activity) and inflammation (IL-6, TNF-α) markers, was obtained at baseline, pre-, post-, 24, 48 and 72 hours post-EIMD. Haematocrit (Hct) with capillary method using a micro-haematocrit centrifuge (Hawksley & Sons Ltd., Lancing, UK) and haemoglobin (Hb) concentration using a photometer (Haemocue, Sheffield, UK) were both analysed immediately on heparinised whole blood in triplicate. Subsequently, concentration of plasma markers (CK, IL-6 and TNF-α) was adjusted for plasma volume changes with the method of (Dill and Costill, 1974; Matomaki et al., 2018).

Following a software malfunction of the dynamometer, the WAnT test was added to the study protocol for the remaining participants (n = 7) to measure anaerobic power and to provide an additional performance assessment. Assessments of muscle function, perceived leg muscle soreness, MVIC and peak power were recorded at baseline, pre-, post-, 24, 48 and 72 hours post-EIMD. All measurements, blood, urine and diet analysis, and instrumentation were described in detail in **Chapter 3**.

4.2.6 Statistical Analysis

Normal distribution of all data was performed by the Shapiro-Wilk test. Baseline characteristics, dietary and hydration data were compared between N-3 and PLA

group using a two-tailed independent samples *t*-test. MVIC and peak power data met all assumptions required for normality and were analysed using a two-way mixed between-within participant repeated measures ANOVA to assess the impact of two different interventions (N-3, PLA) on participants' MVIC and peak power across 5 time points (pre-EIMD, post-EIMD, 24h 48 and 72 hours post-EIMD). Bonferroni-adjust pairwise comparisons *post hoc* analysis was used where needed to examine within subject differences. The examination of the effect of the n-3 supplementation on plasma CK activity, IL-6, TNF-α, TG and DOMS between N-3 and PLA group was performed by the Mann-Whitney U test. Freidman test was used to determine the main effect of time within-group, and *post hoc* with Wilcoxon-signed rank tests (using a Bonferroni adjusted alpha value) were conducted where a significant time was identified. The Wilcoxon signed-rank test was also performed to examine within-group differences in triglycerides between pre- and post-supplementation. Values were expressed as mean ± SD for data from parametric tests and as median and interquartile range for data from non-parametric tests, as recommended by Weissgerber et al. (2017). Statistical significance was accepted as $p < 0.05$. Effect size was calculated using methods proposed by Cohen (1988), with effect sizes considered small (0.20), medium (0.50) or large (0.80). Statistical analyses were performed using SPSS version 26 software (IBM SPSS, NY, USA). All figures were generated in GraphPad Prism (Version 8, GraphPad).

4.2.6.1 Power Calculation

The sample size was estimated from a sample calculation (G*Power 3.1) with an alpha level of 0.05, a power (1-β) of 0.80 and a medium effect size of 0.50 and suggests n = 12 in total would be sufficient.

4.3 RESULTS

4.3.1 Participant Characteristics

The physical characteristics of participants who completed the EIMD are presented in Table 4.1. It can be seen that there was homogeneity in all characteristics of the participants between groups.

Table 4.1 Physical characteristics of participants completed EIMD, independent sample t-test comparison between N-3 and PLA group

	Total (n=14)	N-3 (n=7)	PLA (n=7)	P value
Age (years)	25.07 (± 4.05)	25.57 (± 4.11)	24.57 (± 4.23)	0.662
Weight (kg)	73.04 (± 9.82)	69.34 (± 10.93)	76.74 (± 7.59)	0.167
Height (cm)	179.59 (± 10.23)	174.60 (± 11.64)	184.58 (± 5.77)	0.065
BMI (kg/m^2)	22.60 (± 1.79)	22.62 (± 1.41)	22.58 (± 2.23)	0.967
Body fat (%)	10.75 (± 4.06)	10.40 (± 4.28)	11.10 (± 4.14)	0.762
Muscle mass (kg)	61.00 (± 6.68)	58.76 (± 7.45)	63.62 (± 5.03)	0.204
$\dot{V}O_2max$ (ml/kg/min)	62.42 (± 11.76)	65.11 (± 11.08)	59.74 (± 12.66)	0.415

Values are expressed as mean ± SD. N-3, omega-3 group; PLA, placebo group; BMI, body mass index; $\dot{V}O_2max$, maximal oxygen consumption

The physical characteristics of participants who attempted to complete the EIMD are presented in Table 4.2. Participants who failed to complete 60 minutes of downhill running, managed to complete the trial between 20 and 45 minutes. Follow-up assessments were not conducted in these participants. An independent-samples t-test was conducted to compare physical characteristics between participants who completed and failed the EIMD. There was no significant difference in any characteristic between groups. Mann-Whitney U test was performed to compare percentage of $\dot{V}O_2max$ during EIMD between groups, since this variable did not follow the normal distribution. The results showed, again, no significant difference between participants who completed the EIMD

(Md = 66.22%) and those who failed (Md = 70.12%), U = 23.00, Z = -0.531, p = 0.595 (Table 4.2).

Table 4.2 Physical characteristics of participants attempted to complete EIMD

	Total (n = 19)	Completed EIMD n = 14 (N-3 = 7, PLA = 7)	Failed EIMD n = 5 (N-3 = 2, PLA = 3)	P-value
Age (years)	24.42 (± 4.11)	25.07 (± 4.05)	22.60 (± 4.16)	0.260
Weight (kg)	73.15 (± 10.74)	73.04 (± 9.82)	73.46 (± 14.53)	0.943
Height (cm)	179.44 (± 9.46)	179.59 (± 10.23)	179.00 (± 7.87)	0.908
BMI (kg/m^2)	22.68 (± 2.30)	22.60 (± 1.79)	22.90 (± 3.66)	0.828
Body fat (%)	11.86 (± 5.55)	10.75 (± 4.06)	14.98 (± 8.27)	0.148
Muscle mass (kg)	61.03 (± 6.60)	61.00 (± 6.68)	61.12 (± 7.33)	0.975
$\dot{V}O_2max$ (ml/kg/min)	59.87 (± 11.49)	62.42 (± 11.76)	52.74 (± 7.61)	0.107
RER during EIMD	0.84 (± 0.04)	0.84 (± 0.04)	0.85 (± 0.05)	0.483

Values are expressed as mean ± SD. BMI, body mass index; $\dot{V}O_2max$, peak oxygen consumption; RER, respiratory exchanged ratio; N-3, omega-3 group; PLA, placebo group

4.3.2 Dietary Data

Descriptive characteristics in dietary data at baseline (before supplementation) of participants from both groups (N-3 *vs* PLA) are presented in Table 4.3. An independent-samples t-test was conducted to compare energy, macronutrients and n-6/n-3 ratio between groups, and Mann-Whitney U test was performed to compare n-3 intake [N-3, Md = 7.57 (53.00); PLA, Md = 7.43 (52.00), U = 24.00, Z = -0.064] and n-6 intake [N-3, Md = 8.21 (57.50); PLA, Md = 6.79 (47.50), U = 19.50, Z = -0.640] between groups. There was no significant difference in food intake ($p > 0.05$) at baseline between N-3 and PLA group (Table 4.3).

Table 4.3 Dietary data at baseline of participants completed EIMD, independent sample t-test comparison between N-3 and placebo group

	Total (n = 14)	N-3 (n = 7)	PLA (n = 7)	P-value
Energy (Kcals)	2580.64 (± 789.39)	2664.71 (± 414.35)	2496.57 (± 1077.93)	0.707
CHO (g)	296.64 (± 83.62)	319.71 (± 59.15)	273.57 (± 102.02)	0.321
Protein (g)	109.11 (± 30.21)	108.64 (± 23.20)	109.57 (± 37.94)	0.957
Fat (g)	94.99 (± 46.12)	89.48 (± 28.06)	100.49 (± 61.23)	0.673
n-3 (g)**	1.56 (± 1.43)	1.41 (± 1.20)	1.70 (± 1.72)	0.949
n-6 (g)**	9.47 (± 7.42)	9.85 (± 6.59)	9.09 (± 8.69)	0.522
n-6/n-3 ratio	8.20 (± 4.91)	9.18 (± 4.81)	7.21 (± 5.18)	0.476

*Values are expressed as mean ± SD. N-3, omega-3 group; PLA, placebo; CHO, carbohydrates; n-3, omega-3 fatty acids; n-6, omega-6 fatty acids. ** indicate Mann-Whitney U test*

Descriptive characteristics in dietary data after the supplementation period of participants from both groups (N-3 *vs* PLA) are presented in Table 4.4. An independent-samples t-test was conducted to compare energy, macronutrients, n-3 and n-6 intake between groups. There was a significant difference in n-3 intake with N-3 group showing a higher n-3 intake post supplementation. However, there was no significant difference in any other food intake ($p > 0.05$) between groups (Table 4.4).

Table 4.4 Dietary data post supplementation (+ 3 g of n-3 supplementation) of participants completed EIMD, independent sample t-test comparison between N-3 and placebo group

	Total (n = 14)	N-3 (n = 7)	PLA (n = 7)	P-value
Energy (Kcals)	2574.57 (± 928.64)	2653.43 (± 794.99)	2495.71 (± 1105.43)	0.765
CHO (g)	305.64 (± 134.93)	293.50 (± 68.18)	317.78 (± 185.62)	0.751
Protein (g)	109.79 (± 32.36)	104.06 (± 26.08)	115.52 (± 38.89)	0.529
Fat (g)	92.84 (± 37.37)	104.33 (± 38.87)	81.35 (± 34.74)	0.266
n-3 (g)	2.82 (± 1.92)	3.87 (± 1.90)	1.78 (± 1.34)	0.036*
n-6 (g)	8.47 (± 5.41)	8.65 (± 5.83)	8.29 (± 5.42)	0.906

n-6/n-3 ratio	4.65 (± 3.80)	3.19 (± 3.18)	6.12 (± 4.03)	0.157

**Significant level, $p < .05$; Values are expressed as mean ± SD. N-3, omega-3 group; PLA, placebo; CHO, carbohydrates; n-3, omega-3 fatty acids; n-6, omega-6 fatty acids*

In addition, an independent-samples t-test was conducted to compare participants' hydration status before EIMD between N-3 and PLA group. There was no significant difference in hydration status [N-3, n = 7, (M = 1.015, SD = 0.007) and PLA, n = 7, (M = 1.012, SD = 0.007); $t\ (12) = 0.72$, $p > 0.05$, two-tailed] between groups.

Descriptive characteristics in dietary data at baseline of participants who attempted to complete EIMD are presented in Table 4.5. Whilst the number of participants who failed the EIMD trial were five, however, one participant excluded from the data analysis since he did not return the food diary. An independent-samples t-test was conducted to compare energy and macronutrients, and n-3 and n-6 intake between participants who completed the EIMD and those who failed. There was no significant difference in food intake ($p > 0.05$) between groups (Table 4.5).

Table 4.5 Dietary data at baseline of participants attempted to complete EIMD

	Total (n = 18)	**Completed EIMD n=14 (N-3 = 7, PLA = 7)**	**Failed EIMD n=4 (N-3 = 2, PLA = 3)**	**P-value**
Energy (Kcals)	2739 (± 913.49)	2580 (± 789.38)	3295 (± 1224.44)	0.175
CHO (g)	120.69 (± 47.26)	296.64 (± 83.61)	308.5 (± 135.16)	0.829
Protein (g)	120.69 (± 47.26)	109.1 (± 30.21)	161.25 (± 76.71)	0.269
Fat (g)	105.98 (± 50.73)	94.98 (± 46.11)	144.5 (± 53.19)	0.160
n-3 (g)	1.51 (± 1.40)	1.55 (± 1.43)	1.35 (± 1.50)	0.803
n-6 (g)	8.49 (± 6.77)	9.47 (± 7.42)	5.07 (± 1.16)	0.265
n-6/n-3 ratio	8.49 (± 5.68)	8.19 (± 4.91)	9.54 (± 8.77)	0.689

Values are expressed as mean ± SD. N-3, omega-3 group; PLA, placebo; CHO, carbohydrates; n-3, omega-3 fatty acids; n-6, omega-6 fatty acids

4.3.3 Laboratory Environmental Conditions

Descriptive characteristics in laboratory environmental conditions during EIMD of participants who attempted to complete the EIMD are shown in Table 4.6. An independent-samples t-test was conducted to compare environmental conditions between participants who completed the EIMD and those who failed. There was no significant difference in temperature, relative humidity and atmospheric pressure ($p > 0.05$) during EIMD between groups (Table 4.6).

Table 4.6 Descriptive characteristics of laboratory environmental conditions during EIMD of participants attempted to complete EIMD

	Total (n=19)	**Completed EIMD n=14 (N-3=7, PLA=7)**	**Failed EIMD n=5 (N-3=2, PLA=3)**	**P-value**
T (°C)	21.59 (± 2.64)	21.5 (± 3.00)	21.6 (± 1.49)	0.981
RH (%)	29.0 (± 9.71)	29.7 (± 8.57)	27.0 (± 13.38)	0.606
Atm (mb)	1011 (± 5.47)	1010 (± 5.81)	1014 (± 3.70)	0.231

Values are expressed as mean ± SD. N-3, omega-3 group; PLA, placebo; T, laboratory temperature; RH, relative humidity; Atm, atmospheric pressure

4.3.4 Biomarkers of Muscle Damage and Inflammation

4.3.4.1 Creatine Kinase

Friedman's ANOVA suggested an effect of time on CK activity for both PLA and N-3 groups (both $p < 0.001$), with *post hoc* testing suggesting both groups were increased at 24, 48 and 72 hours relative to baseline ($p < 0.05$, $r = 0.63$ indicating a medium effect size for all three timepoints; Figure 4.3A). However, there was no significant difference between PLA and N-3 at any timepoint.

4.3.4.2 IL-6

Mann-Whitney U test was used to compare plasma IL-6 concentration between N-3 and PLA group. There was no significant difference between groups at any time point. Nevertheless, Friedman's ANOVA revealed plasma IL-6 did not change over time in N-3 group ($p = 0.434$) but did change in PLA group ($p = 0.009$). *Post hoc* testing suggested IL-6 was elevated in the PLA group at immediately post-EIMD relative to baseline ($p < 0.05$, $r = 0.61$ indicating a medium effect size) but no other time points (Figure 4.3B).

4.3.4.3 TNF-α

Mann-Whitney U test was performed to compare plasma TNF-α concentration between groups. Plasma TNF-α did not differ with time in either PLA ($p = 0.274$) or N-3 group ($p = 0.345$; Figure 4.3C).

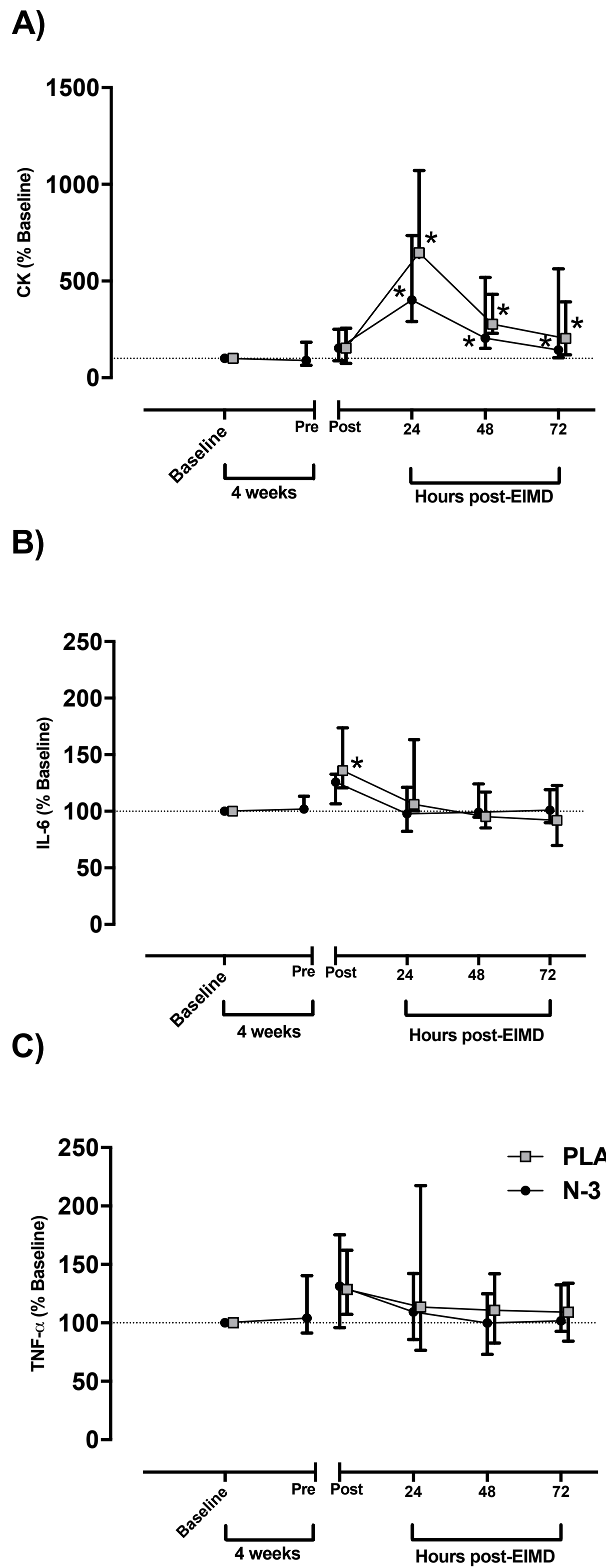


Figure 4.3 Plasma markers of muscle damage and inflammation as a function of time. A) CK (% change from baseline), **B)** IL-6 (% change from baseline) and **C)** TNF-α (% change from baseline). Data shown as medians, error bars indicate interquartile range. Dashed horizontal line indicates 100 % (baseline). * indicates significant difference from Baseline. *Time matched data points offset horizontally to enhance clarity*

4.3.5 Assessment of Muscle Function

4.3.5.1 Muscle Soreness

Mann-Whitney U test was run to compare perceived muscle soreness between N-3 and PLA group at each time point. There was a statistically significant difference in DOMS between groups at 24 hours post-EIMD, with PLA showing a higher muscle soreness compared to N-3 group ($p = 0.034$) with a medium effect size ($r = 0.56$). Friedman's test suggested DOMS significantly differed both within the N-3 and PLA group ($p < 0.001$). Pairwise comparisons suggested that N-3 group had elevated DOMS immediately post ($r = 0.57$) and at 24 hours post-EIMD ($r = 0.59$) relative to pre (all $p < 0.05$), whilst the PLA group maintained DOMS for longer, being elevated immediately post ($r = 0.64$), and at both 24 and 48 hours post-EIMD ($r = 0.60$) relative to pre (all $p < 0.05$; Figure 4.4A).

4.3.5.2 Maximal Voluntary Isometric Strength

No group x time interaction was noted for MVIC (repeated measures ANOVA, $p = 0.813$) or a main effect of group ($p = 0.338$). However, a significant main effect for time was observed for MVIC leg strength ($p = 0.011$, $\eta^2_p = 0.813$). This result suggests a large effect size, with MVIC suppressed relative to pre in both N-3 and PLA groups (N-3 = 29.66 (± 8.78) kg *vs* 20.75 (± 10.55) kg, PLA = 36.50 (± 10.16) kg *vs* 27.13 (± 7.40) kg; both $p < 0.05$) immediately post-EIMD, but no other time points (Figure 4.4B).

4.3.5.3 Peak Power

No group x time interaction was noted for peak power (repeated measures ANOVA, $p = 0.514$) or a main effect of group ($p = 0.310$). However, a significant main effect for time was observed for peak power ($p = 0.014$, $\eta^2_p = 0.841$). This

result suggests a large effect size, with *post hoc* testing suggesting no change in peak power following EIMD in N-3 group, but a suppression in peak power in PLA group at 24 hours relative to pre [pre-EIMD = 825.58 (± 90.67), 24 hours = 763.04 (± 103.09) W, $p < 0.05$] (Figure 4.4C).

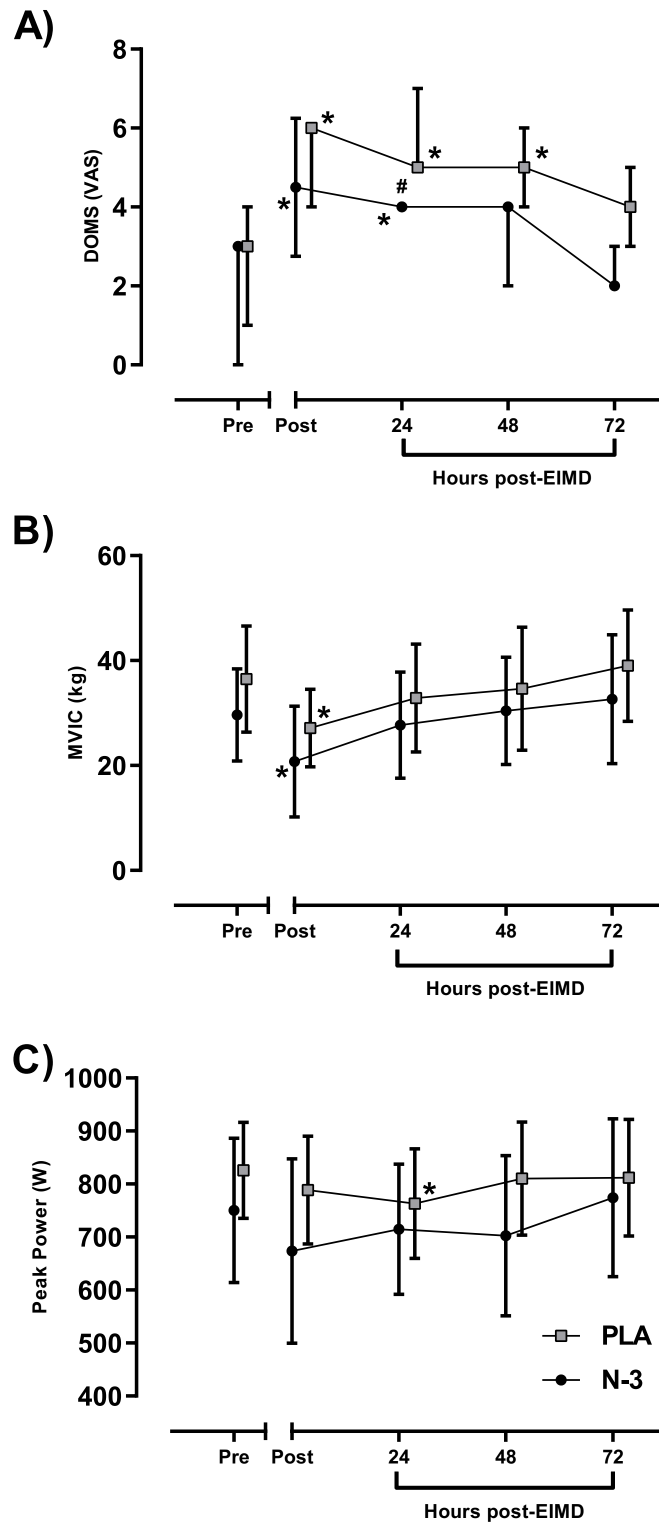


Figure 4.4 Muscle functional measures prior (pre) and following (post – 72 hours) EIMD. A) DOMS, data indicates median, error bars interquartile range. Both **B)** MVIC (kg), and **C)** peak power (W) data indicate means, error bars standard deviation. * indicates significant difference from Pre timepoint, # indicates difference between groups at timepoint indicated. *Time matched data points offset horizontally to enhance clarity*

4.3.6 Lipid Profile

4.3.6.1 Triglycerides

Mann-Whitney U test was performed to compare TG concentration before and after supplementation between N-3 and PLA group from participants who completed the supplementation phase and attempted to complete EIMD (n = 19). No significant difference was observed in serum TG concentration between groups before (pre-SUP, p = 0.630) and after supplementation (post-SUP, p = 0.806; Table 4.7). When TG normalised to pre-supplementation values, again, there was no significant difference in TG percentage change between groups after supplementation (p = 0.210; Table 3.7, visualised in Figure 4.5B).

Table 4.7 Mann-Whitney U test in serum TG concentration and percentage change on participants who attempted EIMD by supplementation group

	N-3 (n = 9)	**PLA (n = 10)**	**U test**	**z standardized test statistic**	**P-value**
TG (mmol/L)					
Pre-SUP	0.67 (0.85)†	0.90 (0.45)§	31.00	-0.482	0.630
Post-SUP	0.65 (0.32)	0.55 (0.44)	42.00	-0.246	0.806
TG (%)					
Pre-SUP	100.00	100.00	45.00	0.000	1.000
Post-SUP	96.43 (37.60)†	83.33 (33.20)§	23.00	-1.254	0.210

Values are expressed as median and interquartile range. Pre-SUP, before supplementation period; Post-SUP, after supplementation period; N-3, omega-3 group; PLA, placebo; TG, triglycerides

†N-3, n = 8

§PLA, n = 9

Further, Wilcoxon signed-rank test suggested there was no significant difference in TG concentration between pre- and post-supplementation in the N-3 group ($z = -0.736$, $p = 0.462$). There was also no significant difference in TG concentration between pre- and post-supplementation for the PLA group ($z = -1.832$, $p = 0.067$; Figure 4.5A).

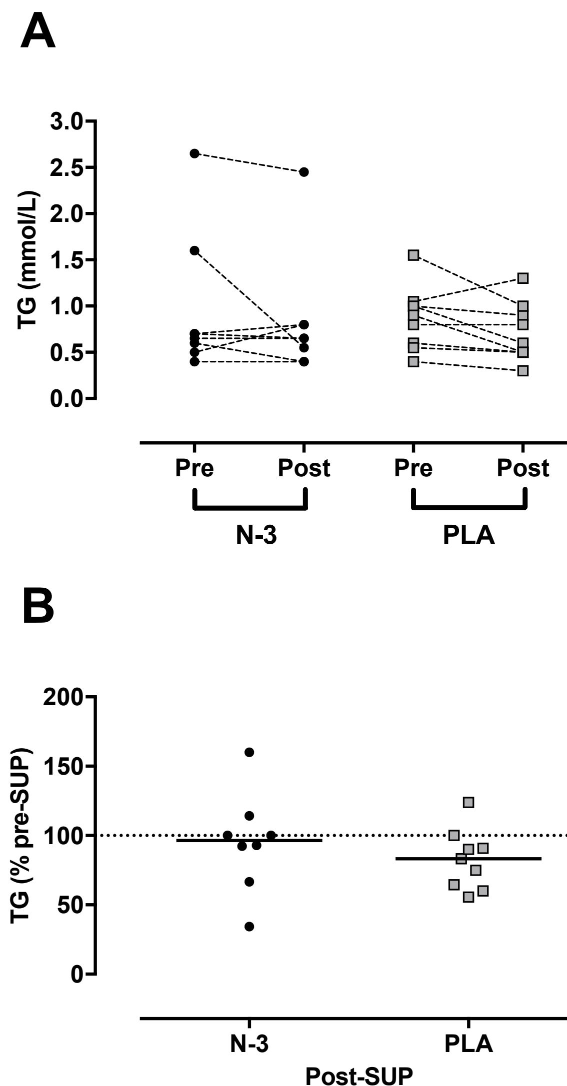


Figure 4.5 Serum TG by supplementation group on participants who completed the supplementation phase and attempted to complete EIMD. **A)** TG (mmol/L) between pre- and post-supplementation in N-3 group and in PLA group, and **B)** TG in percentage change (% pre-SUP) between N-3 and PLA group at post-supplementation period. *Data are presented as individual values. Black circles indicate omega-3 group, grey squares indicate placebo group.*

In addition, TG concentration was analysed between participants who completed EIMD (only from N-3 group) and those who failed to complete EIMD trial to understand whether n-3 supplementation had any effect on EIMD completion. Mann-Whitney U test showed no significant difference in TG concentration between groups before ($p = 0.668$) and after supplementation ($p = 0.463$; Table 4.8). When TG normalised to pre-supplementation values, again, there was no significant difference in TG percentage change between groups after supplementation ($p = 1.000$; Table 4.8, visualised in Figure 4.6B).

Table 4.8 Mann-Whitney U test on TG concentration and percentage change between participants completed the EIMD (only from N-3 group) and those who failed the EIMD

	Completed EIMD (N-3, n = 7)	Failed EIMD (n = 5)	U test	z standardized test statistic	P-value
TG (mmol/L)					
Pre-SUP	0.67 (1.39)†	0.65 (0.51)§	10.00	-0.429	0.668
Post-SUP	0.65 (0.25)	0.45 (0.70)	13.00	-0.735	0.463
TG (%)					
Pre-SUP	100.00	100.00	17.50	0.000	1.000
Post-SUP	96.43 (37.07)†	94.64 (52.68)§	12.00	0.000	1.000

Values are expressed as median and interquartile range. Pre-SUP, before supplementation period; Post-SUP, after supplementation period; N-3, omega-3 group; TG, triglycerides
†Completed EIMD, n = 6
§Failed EIMD, n = 4

Wilcoxon signed-rank test showed that there was no significant difference in TG concentration between pre- and post-supplementation for completed-EIMD group ($z = -0.730$, $p = 0.465$). Similar results were also for TG concentration between pre- to post-supplementation in the failed-EIMD group ($z = -0.184$, $p = 0.854$; Figure 4.6A).

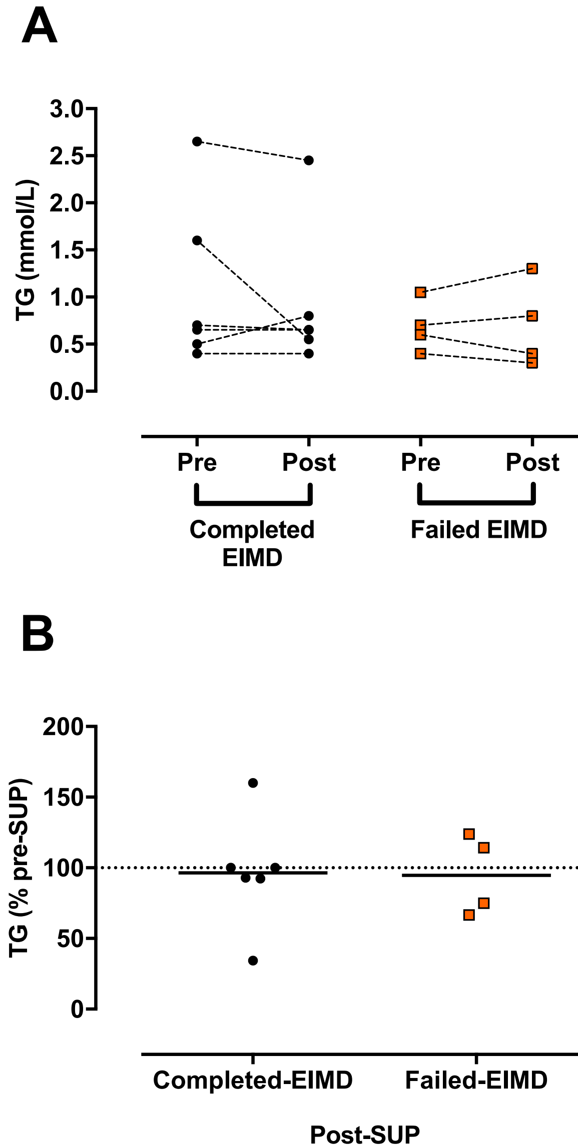


Figure 4.6 The effect of n-3 supplementation on serum TG in participants who completed EIMD and those who failed EIMD trial. **A)** TG (mmol/L) between pre- and post-supplementation in participants who completed EIMD (only from N-3 group) and in those who failed EIMD trial, and **B)** TG in percentage change (% pre-SUP) between completed-EIMD and failed-EIMD group at post-supplementation period. *Data are presented as individual values. Black circles indicate completed-EIMD group, orange squares indicate failed-EIMD group.*

4.4 DISCUSSION

Results presented here suggest that 4 weeks supplementation with 3 g/day of n-3 PUFA offsets the EIMD induced pain response following a single bout of high intensity exercise. Whilst a decreased peak power output in PLA group at 24 hours following EIMD and a blunted time response in pro-inflammatory marker IL-6 was witnessed in the N-3 group immediately post-EIMD, no between group differences were found. Although findings suggest decreased MVIC and increased plasma CK following EIMD, no difference was observed between groups, overall suggesting minimal positive gain in exercise performance with n-3 supplementation.

Biomarkers of Muscle Damage and Inflammation

The data of the current study show a significant increase of CK activity following EIMD before returning to baseline in both groups, mirroring those previously reported (Baird et al., 2012; Hughes et al., 2018). Conversely, Bloomer et al. (2009) reported no significantly increased CK activity following eccentric exercise. This finding is in agreement with the findings by Atashak et al. (2013), another study (Park and Lee, 2015) with a similar exercise protocol, following a 40-minutes downhill running, and Tsuchiya et al. (2016) after an eccentric protocol with a similar dose but longer supplementation period (8 weeks).

The data presented here showed a large degree of variability in circulating CK (from 65 to 4,939 U/L) which is in accordance with previous research (Kim and Lee, 2015). Hence, CK alone might not be an accurate reflection of the degree of muscle damage following eccentric exercise (Magal et al., 2010) due to large inter-individual variability in response with a range from 236 to 25,244 IU/L

(Clarkson and Hubal, 2002). Therefore, the results of the efficacy of the n-3 supplementation on indirect muscle damage biomarkers, such as CK, following maximal exercise performance may be inconsistent due to variability alone, and such markers should not be considered in isolation.

Plasma IL-6 concentration peaked immediately post-EIMD for the PLA group. This peak of plasma IL-6 after exercise is well documented in the literature (Bruunsgaard et al., 1997; Pedersen and Hoffman-Goetz, 2000; Toft et al., 2002; Peak et al., 2005; Smith et al., 2007; Tarbinian et al., 2011; Brown et al., 2015). However, there was no significant difference in plasma IL-6 concentration between N-3 and PLA group. This finding is in accordance with the findings by Tarbinian et al. (2011), who found no significant difference between groups in plasma IL-6 concentration immediately post-exercise.

In a manner similar to plasma IL-6, there were no differences in post-EIMD plasma TNF-α concentration between N-3 and PLA groups. There is conflicting evidence about the behaviour of TNF-α response after muscle-damaging exercise. Toft et al. (2000) have shown that plasma TNF-α was elevated after prolonged exercise, others did not observe any change (Philippou et al., 2018) and others recorded a decrease in the TNF-α (Hirose et al., 2004). In the study by Lenn et al. (2002), TNF-α was not significantly increased, which is a similar result with the present study, where no significant increase was demonstrated in plasma TNF-α concentration. This could be due to a feedback mechanism, that IL-6 inhibits TNF-α (Philippou et al., 2018). Thus, it may be that plasma TNF-α is not an optimal marker to quantify EIMD-induced inflammation.

Muscle Function

A significant change in VAS pain score was reported following EIMD in both groups, further evidence that the exercise protocol used caused significant muscle damage. More specifically, participants' pain perception peaked immediately post-EIMD and remained elevated at 24-48 hours post-EIMD, which is consistent with other findings (Clarkson and Hubal, 2002; Chen et al., 2018). The data presented here demonstrated a significant difference in perceived muscle soreness between groups at 24 hours post-EIMD, suggesting N-3 may have experienced less pain compared to PLA group at this point. Previous studies (Jakeman et al., 2017; Ochi et al., 2017; Lv et al., 2020) also found significant differences in DOMS between groups following EIMD, with the fish oil group having reduced muscle soreness. On the contrary, Jakeman et al. (2017) an acute dose of n-3 PUFA immediately after a muscle-damaging exercise, demonstrated similar muscle soreness between groups. The absence of effect on DOMS might be due to the acute supplementation dose following exercise and is insufficient to change muscle phospholipid content (McGlory et al., 2014; Gerling et al., 2019) relative to the 4 weeks of supplementation used here.

Subsequent exercise performance is significantly affected by EIMD and its symptoms (Owens et al., 2019). The loss of muscle force is considered the most valid indirect measurement of muscle damage (Damas et al., 2016). As expected, and when observing a large effect size, the leg strength significantly decreased immediately post-EIMD in both groups compared with pre-EIMD values. However, there were no significant differences in MVIC between groups nor was any interaction effect observed, suggesting that levels of muscle damage were unchanged by n-3 consumption. These findings match both those of Gravina et

al. (2017), who reported no impact of 4 weeks of n-3 supplementation on leg strength, despite a higher dose (equivalent of 0.1 g/kg/day of supplement in capsules, 1000 mg n-3 PUFA per capsule, with a mean intake of 7 ± 2 capsules per day), and of Gray et al. (2014) who showed no group effect, with a longer duration (6 weeks) of n-3 supplementation. In addition, a recent study by Ramos-Campo et al. (2020), examining muscle damage after eccentric exercise, found no significant difference in strength deficit between the supplementation and placebo group following a 10-week n-3 supplementation. Therefore, the implications of the findings from the previous studies and ours are that n-3 supplementation does not have significant positive effects on muscle strength recovery.

As a secondary measure of muscle function, cycling peak power was examined, with no significant difference between groups. However, the PLA group demonstrated a significant suppression in peak power at 24 hours following EIMD relative to pre, while there was no change in N-3 group relative to pre-EIMD. Decreased cycling peak power output 24 hours following EIMD is in line with previous research (Twist and Eston, 2005). The potential for preservation of voluntary peak power output will be of interest to athletes where repeated maximal powerful performance is required, which is reinforced by differences in perceived pain at this timepoint.

Triglycerides

It has been documented that fish oil can alter blood fatty acids profiles with increased amounts of EPA and DHA in plasma lipids following n-3 supplementation (Rees et al., 2006; Calder, 2013; Lembke et al., 2014; Corder,

2016). Marine n-3 PUFA has been reported that can reduce plasma TG concentrations and generally have positive changes in blood lipids in humans (Harris, 1997; Calabresi et al., 2004; Skulas-Ray et al., 2011). This work, here, demonstrates that participants' TG concentration was not significantly changed following 4 weeks of n-3 supplementation. It seems that 3 g/day of n-3 supplementation for 4 weeks had no impact on lipid profile. One possible explanation of the latter finding may be due to the fact that n-3 PUFA have been provided for too short period to change muscle phospholipids (Metcalf et al., 2007; Smith et al., 2011b). Indeed, McGlory et al. (2014) examined the time course of n-3 PUFA composition in healthy young recreationally active males and they found that is required a more prolonged period (> 4 weeks) of n-3 supplementation to reach a saturation of n-3 PUFA composition in human skeletal muscle. One more reason could be due to the differences in the n-3 PUFA content with previous studies, as it appears that n-3 supplementation is dose and duration dependant in relation to exercise performance and its symptoms.

In addition, participants' hydration status was examined before EIMD to ensure they began exercise euhydrated, to prevent hydration being a cofounding factor for exercise performance. Omega-3 intake was also assessed using a 48-hour diary at pre- and post-supplementation period. No difference in n-3 intake was noted between groups prior to supplementation. As it would be expected, there was an increase in n-3 PUFA intake in the N-3 group relative to the PLA group after supplementation.

Completed-EIMD vs Failed-EIMD Participants

TG concentration was also examined on participants who completed EIMD (only from N-3 group) and those who failed to complete it to understand whether participants who completed EIMD may be protected from muscle damage by prolonged high intake of n-3 PUFA. No significant differences were observed in TG concentration between groups.

In addition, n-3 PUFA intake was examined between participants who completed and those who failed EIMD, and it was not significant different. Whilst the n-3 intake was increased (for the N-3 group) after the supplementation period, the lipid profile between participants who completed and those who failed EIMD was not significantly different. Hence, 4 weeks of n-3 supplementation did not alter phospholipid profiles and therefore, it is not possible to say whether the participants who completed EIMD benefitted from n-3 supplementation as proposed by Lewis et al. (2015) and Tsuchiya et al. (2016). This can also explain why there were not significant differences observed between the two groups.

Moreover, exercise intensity during EIMD and RER were in line with expectations and there was no significant difference between participants who completed and those who failed EIMD. Additionally, the laboratory environmental conditions during EIMD were similar for both groups with no significant differences. There was also no significant difference in dietary data between these two groups, which suggests that the participants who could not completed EIMD it was not due to substrate deficiency. In combination with reported leg and back pains from the participants during EIMD trial, this suggests that these participants withdrew due to non-metabolic peripheral fatigue, which is likely caused by myofibrillar

disruption, and thus reducing contractile function (Green, 1997). Data from participants who withdrew were not collected, and thus it cannot be confirmed whether these participants experienced significant muscle damage.

Strengths and Limitations

Downhill running protocol is a validated commonly used protocol to induce muscle damage (Schwane et al., 1983; Hickner et al., 2001; van de Vyver and Myburgh, 2012; Fortes et al., 2013; Park and Lee, 2015). However, there is a large subject variability due to the mechanical stress occurs when muscles lengthen during downhill running (Eston et al., 1995; Mizrahi et al., 2000). It has been reported in biomechanical studies (Mizrahi et al., 2001; Yokozawa et al., 2005) that the vertical impact loading may result to a greater energy of the body's mass centre by decline grade, resulting in changes to the hip, knee and ankle joints (e.g., foot contact to the ground; Walmsley et al., 1978) during downhill running (Collins and Whittle, 1989). The current study included participants from different sports, such as runners, cyclists, and rowers, and thus they may have different stepping technique.

In an attempt to isolate the effect of n-3 supplementation, collagen was chosen as the placebo in this study to avoid manipulation of n-6/n-3 ratio. There is no evidence in the literature that collagen has a pro or anti-inflammatory effect, and therefore, it would not oppose the action of n-3 supplementation. Whereas other reports have utilized corn oil as a placebo control which is high in n-6, and thus may not represent a true placebo (Jeromson et al., 2015; Philpott et al., 2019). In addition, the use of standardised procedures, such as time visits in the laboratory,

blood sample timing and testing measurements, was another strength of this study.

Some potential limitations of the present study should also be acknowledged. Whilst participants were blinded to the treatment provided, it was not feasible to blind the PhD researcher due to the insufficient support. Although this is a limitation inherent to the study design (i.e., single *vs* double-blinded study), the PhD researcher of this Doctorate fully complied with good research practice standards (Hecksteden et al., 2018). In addition, low statistical power due to the modest sample size played a role in limiting the significance of the statistical comparisons conducted. The strict inclusion criteria, as well as the downhill running task performance made recruitment for participants difficult.

Whilst participants were provided with a food list containing foods with high or low in n-3 fatty acids, they were required not to change their habitual food intake. However, participants' diets were not explicitly controlled during the 4-week loading period. Nevertheless, a 48-hour food diary was recorded immediately before supplementation period and repeated 48 hours before supplementation finished (immediately before EIMD); results of which suggested participants did not change their habitual macronutrient or total caloric intake. Underreporting or overreporting is a well-documented issue with dietary assessment instruments (Black et al., 1991; Gibson, 2005; NIMH, 2017). There is a potential loss of dietary information from mixed dishes, as food ingredients were sometimes counted from mixed dishes. Thus, it is important the accuracy of the food diary. An additional measurement of the habitual omega-3 PUFA intake was recorded by providing a validated food frequency questionnaire (FFQ; Sublette et al., 2011, **Appendix K**).

Participants completed the FFQ in the middle of the supplementation period (end of the second week), and this was repeated at the end of the experimental period. The FFQ was used for validation of food intake from the self-recorded food diary. Another limitation of the current study is that the follow up data from participants who were unable to complete the muscle-damaging exercise were not collected. This is the reason why it was not possible to assess the reasons why certain participants failed to complete the trial. Kohne, Ormsbee and McKune (2016) also used an hour-long downhill running protocol. However, they used an additional physical measurement (squat jump) immediately before and after the hour-long downhill running protocol. Participants who were able to complete at least 30 minutes of downhill and showed decrease in force of about 40%, were retained in the study. Therefore, using an additional test in our study protocol, in a similar manner to of Kohne and co-workers (2016), might have eliminated the dropouts and would increase the sample size.

Recommendations and Future Directions

Future studies should develop a standardized validated muscle-damaging protocol to improve the comparability between studies. In addition, the exercise protocol here utilised a single bout of eccentric exercise. Further investigation is needed to study athletes with multiple training sessions.

Additional blood biomarkers, such as myoglobin and C-reactive protein, may also provide further information in future studies on muscle damage. A clearer picture on change in muscle function could involve examining n-3 supplementation and muscle damage considering multiple functional measurements, such as MVC torque at multiple joint angles, ROM, limb swelling and/or jump height.

Measurements of muscle function should be used in combination with indirect plasma markers to provide more reliable evidence in assessing the magnitude of muscle damage. Ideally, directly measuring muscle damage from muscle biopsies would be optimal, albeit highly invasive. Future studies may also consider taking blood samples at additional acute time points, such as 1, 3, 6 or 12 hours after the muscle-damaging exercise. By doing so, we might have observed an acute inflammatory response difference between groups, as has been observed elsewhere (Jakeman et al., 2017).

The findings of the current study on lipid profile highlight the need for further work in this area. Future studies investigating the effect of n-3 supplementation on lipid profile may also consider using n-3 supplementation for longer duration (> 4 weeks) in order to reach a saturation of n-3 PUFA composition in human skeletal muscle and alter phospholipid profiles (McGlory et al., 2014). Future research may also consider a method to control participants' food intake, such as providing pre-packaged meals or recording complete food diaries (Bingham, 2006) throughout both supplementation period and recovery phase. However, this would incur both significant cost and require participants to have a greater commitment to these methods.

Further research is required to determine optimal supplementation dosage, duration and content of n-3 PUFA as a nutritional strategy to improve exercise performance. Muscle repair is a major component of exercise performance, and it is of interest to explore how it is impacted by a variety of elements. One important such element is the exercise-induced inflammatory response that is known to affect the rate of muscle repair. Exercise-induced inflammatory

response is affected by many factors, as mentioned in the previous Chapter, including ageing. It would therefore be beneficial to investigate the effect of n-3 supplementation during age-associated muscle function loss. Aged populations beyond young male athletes could potentially show increased response to such regimens. It is well established that older individuals can suffer from chronic inflammation which could lead to muscle wasting (Roubenoff, 2003; Rockwood and Mitnitski, 2011; Baker, 2017), and as consequence, resulting in the impairment of physical performance (Dodds et al., 2015). Whilst age-related decline in strength and power have been shown to begin in 40s (Volpi, Nazemi and Fujita, 2004; Faulkner et al., 2007; Baker, 2017) and continue throughout one's lifespan (Metter et al., 1997), it is still unclear whether older people are more susceptible to muscle damage than younger adults due to muscle mass loss and chronic inflammation (Baker, 2017). **Chapter 5** will therefore focus on muscle damage, inflammation and functionality in older adults. More specifically, this work will contribute to our understanding of how different aged populations respond to muscle-damaging exercise without the ergogenic effect of supplementation. This is an important step to understand baseline mechanisms, and which will allow us to distinguish the effects of supplementation when introduced at a later stage.

4.5 CONCLUSION

In summary, an unaccustomed bout of downhill running at moderate intensity was effective to induce muscle damage, since the large effect sizes observed suggest that the muscle function measurements were positively influenced by the muscle-damaging exercise. The findings of the present study support the hypothesis that 4 weeks of 3 g/day n-3 supplementation may attenuate minor aspects of EIMD, as observed in DOMS and peak power. Typically, no significant differences were noted between groups, however, it was observed a blunted inflammatory response immediately after eccentric exercise and a decreased CK activity at 24 hours following muscle-damaging exercise in N-3 group. There were also no significant differences in leg strength between groups indicating that n-3 supplementation will have limited impact on muscle function and subsequent performance. Secondary results also showed that 4 weeks of 3 g/day n-3 supplementation did not affect triglycerides, suggesting that a longer duration of n-3 supplementation might be required. Whilst not improving performance, these findings may have relevance to soreness-associated exercise avoidance.

CHAPTER FIVE

The Inflammatory, Functional and Systemic Extracellular Vesicle Responses to Exercise-Induced Muscle Damage in Physically Active Younger and Older Males

(Study two)

The contents of this chapter form the basis of the following conference presentation:

Publication

Kyriakidou, Y., Cooper, I., Kraev, I., Lange, S. and Elliott, B.T. Preliminary investigations into the effect of exercise-induced muscle damage on systemic extracellular vesicle release in trained younger and older men. *Frontiers in Physiology*, 2021; 12:723931.

Presentation

Kyriakidou, Y., Wood, C., Cooper, I., Tanner, E. and Elliott, B.T. (2020). Preliminary investigations into muscle recovery following exercise-induced muscle damage between younger and older males. American College of Sports Medicine, 17 June 2020, *Medicine & Science in Sports & Exercise,* Vol 52:5 Suppl.

ABSTRACT

Unaccustomed resistance exercise is associated with reductions in muscle force output, avoidance of repeated loading, pain and an inflammatory response. Whilst EIMD is well defined in the literature in healthy younger populations, research into older individuals is lacking. Research has recently suggested that skeletal muscle can also release extracellular vesicles (EVs) into the circulation following a bout of exercise. However, EV's potential role, including as a biomarker, in the response to eccentric resistance exercise remains unclear.

PURPOSE: To examine muscle function, recovery, and inflammatory and EVs response following an unaccustomed EIMD protocol in younger and older males.

METHODS: Twelve (younger, n = 7, 27.0 ± 1.5 years and older, n = 5, 63.0 ± 1.0 years) healthy, physically active males performed a unilateral high intensity eccentric leg press exercise. Venous plasma was collected for assessment of CK, TNF-α, IL-6 and EVs prior to EIMD, immediately after EIMD, and 1-to-72 hours post-EIMD. MVIC, peak power, jump height and DOMS were assessed at all time points except 1 and 2 hours post-EIMD.

RESULTS: Post EIMD, both CK and TNF-α concentrations were increased in the older group relative to the younger at 72 hours (CK, $p = 0.042$; TNF-α, $p = 0.042$). IL-6 did not differ between younger and older groups at any time point. A significant main effect for time was observed for MVIC, with both groups showing a reduction in leg strength immediately post-EIMD. The main effect of group was significant ($p = 0.005$). The younger group produced significantly more power output and jump height than the older group ($p = 0.001$; $p = 0.016$, respectively). No difference in DOMS was observed between groups at any time point. EIMD did not substantially alter EV modal size or EV count in younger or older participants, however, the alteration in EV concentration (ΔCount) and EV modal size (ΔMode) between post-EIMD and pre-EIMD negatively associated with CK activity.

CONCLUSION: The older group had a greater change in muscle damage and inflammatory response by the end of the experimental period, suggesting a blunted resolution relative to the younger group. However, a similar response in muscle function in both groups following EIMD was observed. Findings from this study also suggest that profile of EV release, immediately following exercise, may predict later CK release and play a role in the EIMD response.

Description of Chapter

This chapter focuses on muscle damage, inflammation, muscle function and recovery following muscle-damaging exercise. EIMD is not well studied in older populations, and thus the present study explores the differences on key elements of EIMD between younger and older recreationally active males following a lower body resistance exercise protocol. It also examines the effect of EIMD on plasma extracellular vesicles in younger and older participants. Secondary objectives of this study are to explore post-exercised blood lactate concentration and also any putative effect of participants' diet.

5.1 INTRODUCTION

Human ageing involves a reduction of function of multiple physiological systems, e.g., cardiovascular system, respiratory system, skeletomuscular system, and immuno-senescence (Rebelo-Marques et al. 2018); including muscle mass. Ageing has been also associated with decreased bone density, which in turn negatively affects physical performance (Reid et al., 2016). These losses can be offset by maintenance of physical activity in older age (Holviala et al., 2014; Walker, Peltonen and Häkkineet, 2015).

It is widely accepted that ageing is also characterised by elevations of circulating pro-inflammatory cytokines contributing to chronic inflammatory state, which has been termed ''inflammageing'' (Ostan et al., 2008; Franceschi and Campisi, 2014; Kennedy et al., 2014). In addition, an increased cardiometabolic health risk is associated with the age-related decline in muscle mass and strength (Tyrovolas et al., 2020; An and Kim, 2020). This gradual and progressive decline of skeletal muscle mass and strength with increasing age is collectively referred to as sarcopenia, and is prognostic for mobility disability (Visser et al., 2005) and chronic disease risk (Pedersen and Saltin, 2015). Indeed, it has been shown that

this progressive loss of functional ability is associated with postural instability (Hill et al., 2020), increased risk of falls (Hardy et al., 2007) and a high risk of all-cause mortality, cancer and respiratory disease (Celis-Morales et al., 2018).

Regular exercise and increased physical activity have a myriad of beneficial effects that offset many of the negative physiological effects of human ageing, including minimising loss of muscle function (Pollock et al., 2015; Ganse et al., 2018; Sakugawa et al., 2019), to improve quality of life (Little and Phillips, 2009, Bei et al., 2017; Bagheri et al., 2019), and ultimately to promote health and longevity (Samitz et al., 2011; Holme and Anderssen, 2015). In older adults, eccentric resistance exercise interventions have been suggested due to high load and potentially greater anabolic response at a low energy cost (Gault and Willems, 2013; Lim, 2016; Franchi et al., 2017). However, unaccustomed exercise, and particularly high load eccentric muscle contractions, is associated with temporary muscle damage, muscle pain, reductions in muscle force and power output, an avoidance of repeated loading, localised swelling and transient muscle inflammation (O'Reilly et al., 1987; Eston, Byrne and Twist, 2003; Paulsen et al., 2010; Hyldahl and Hubal, 2014; Owens et al., 2019). Combined, this is often termed EIMD, as discussed in previous Chapters.

These characteristics of EIMD have been well defined in healthy young participants, (Croisier et al., 1999; Nosaka, Newton and Sacco, 2002b; Damas et al., 2016; Kyriakidou et al., 2021a), whilst relatively less work has been conducted in older adults. Although the responsiveness to a single bout of eccentric exercise has been extensively examined in younger population exploring both the effect of EIMD and muscle recovery, there is still some uncertainty about the exact

mechanisms that could potentially make older individuals more susceptible on muscle injury caused by unaccustomed exercise (Baker, 2017). It is also not clear how the ageing process may influence the rate of muscle recovery after vigorous exercise.

It is biologically plausible to hypothesize that EIMD may be worse in older populations, as recovery has been shown to be delayed after a single bout of eccentric-biased exercise when muscle damage and inflammatory markers are measured (Peake et al., 2010; Conceicao et al., 2012). In animal model of DOMS, younger rats show a quicker recovery from eccentric contraction-induced pain than older rats (Taguchi et al., 2007). However, in human data sets, differences in younger *vs* older muscle recovery are less clear. Previous studies using indirect EIMD markers of muscle function, such as strength and muscle soreness, have shown greater muscle damage in younger individuals rather than in older ones (Lavender and Nosaka, 2006; Gorianovas et al., 2013). Thus, it is proposed that there is a pressing need to investigate the effects of EIMD on muscle function and the early phase (i.e., 24, 48, 72 hours post-EIMD) of exercise recovery in older adults.

Further, research recently suggested that skeletal muscle can also release extracellular vesicles (EVs) into the circulation following a bout of exercise. Whilst research into EV profiles has largely focussed on human pathologies (Withrow et al., 2016; Lange et al., 2017; Garcia-Contreras et al., 2017; Dolcetti et al., 2020; Zhao et al., 2020; Urabe et al., 2020) that linked to crucial roles in the pathophysiology of inflammation-associated disorders, explorations of the roles for EVs in normal physiology are fewer, with one plausible mechanism of action

being adaptation and recovery from exercise stimuli. For instance, following treadmill running an increase in circulating EVs was seen in mice (Bei et al., 2017), whilst EV-associated proteins were elevated in humans following 90 minutes exhaustive aerobic exercise (Fruhbeis et al., 2015). Different intensities of aerobic treadmill exercise equally increased circulating EV concentrations, while increases in modal size were only seen with moderate intensity, not low or high intensity exercise (Oliveira et al., 2018). However, exercise modalities outside of endurance exercise have hitherto not been examined. Importantly, EVs have been shown to be involved in acute responses to injury and inflammatory stimuli in non-exercise models (Middel et al., 2016; Slomka et al., 2018). Whilst it is known that eccentric exercise induces the greatest magnitude of EIMD (Clarkson and Hubal, 2002; Herzog, 2014; Owens et al., 2019), it is likely that EVs will be involved in acute aspects of this response. No studies to date have examined the effect of eccentric exercise or the effect of age on circulating EV profiles following exercise.

Therefore, the present study explores muscle damage, inflammation, function and recovery between younger and older male adults following a lower body muscle-damaging resistance exercise. Additionally, this study aimed at isolating, quantifying and size profiling EVs from the plasma of exercised human participants, to investigate whether there is any interplay between acute EIMD-induced changes in EV release profiles in younger and older participants, and whether such EV-related changes would correlate with other biological and muscle functional markers of EIMD, such as CK activity, strength and muscle soreness.

5.1.1 Aims & Hypothesis

The primary aim of this Chapter was to investigate the effect of a resistance muscle-damaging exercise protocol on muscle damage, inflammation, functional changes, and recovery in healthy, physically active younger and older males. Secondary aims were to compare blood lactate response, and the dietary intake between groups. Specific research objectives were to:

1. assess the differences in muscle damage and inflammation following EIMD between younger and older participants
2. compare muscle function and DOMS following EIMD between younger and older participants
3. investigate the effect of EIMD on plasma EV profiles between younger and older participants
4. examine the differences in blood lactate expression, and dietary intake between groups

The hypothesis of this Chapter was that EIMD would induce muscle inflammation and attenuate muscle function and recovery; and subsequently would decrease the exercise performance. Specifically, this study hypothesized that:

1. younger participants would have an improved resolution in muscle damage and inflammatory markers following EIMD compared to older participants
2. muscle functional capacity would be different between younger and older participants following EIMD
3. Extracellular vesicles-related changes would correlate with CK activity, strength and muscle soreness

4. the younger group would differ from the older group in blood lactate following EIMD

5.2 METHODS

5.2.1 Ethical Approval

Ethical approval for this study was provided by the College of Liberal of Arts and Sciences Research Ethics Committee, University of Westminster (ETH1819-0328). Written informed consent was obtained from all participants prior to their participation in the study (**Appendix E**).

5.2.2 Participant Recruitment

A cohort of healthy, physically active, undertaking moderate, regular physical activity (3-5 times per week) younger males (Y) aged 18-35 years and older males (O) aged ≥ 60 years were recruited to participate in this experimental study. Participants were recruited between February and June 2019 from the University of Westminster and from London health clubs.

A total of 15 participants volunteered to participate in this study. Two participants were rejected from the study following acceptance due to not meeting the inclusion criteria (e.g., too strong for the study). Of the 13 participants, a participant discontinued testing during EIMD due to inability of completing the exercise trial. Withdrawals and exclusions from the study are presented in Figure 4.1. Therefore, 12 participants, [Y, n = 7 (27.0 ± 3.5 years) and O, n = 5 (63.0 ± 2.1 years)], were included in the analysis. Physical characteristics of participants who completed the EIMD are presented in Table 4.1. Inclusion/exclusion criteria of the participants are described in detail in **Chapter 3** (p. 90).

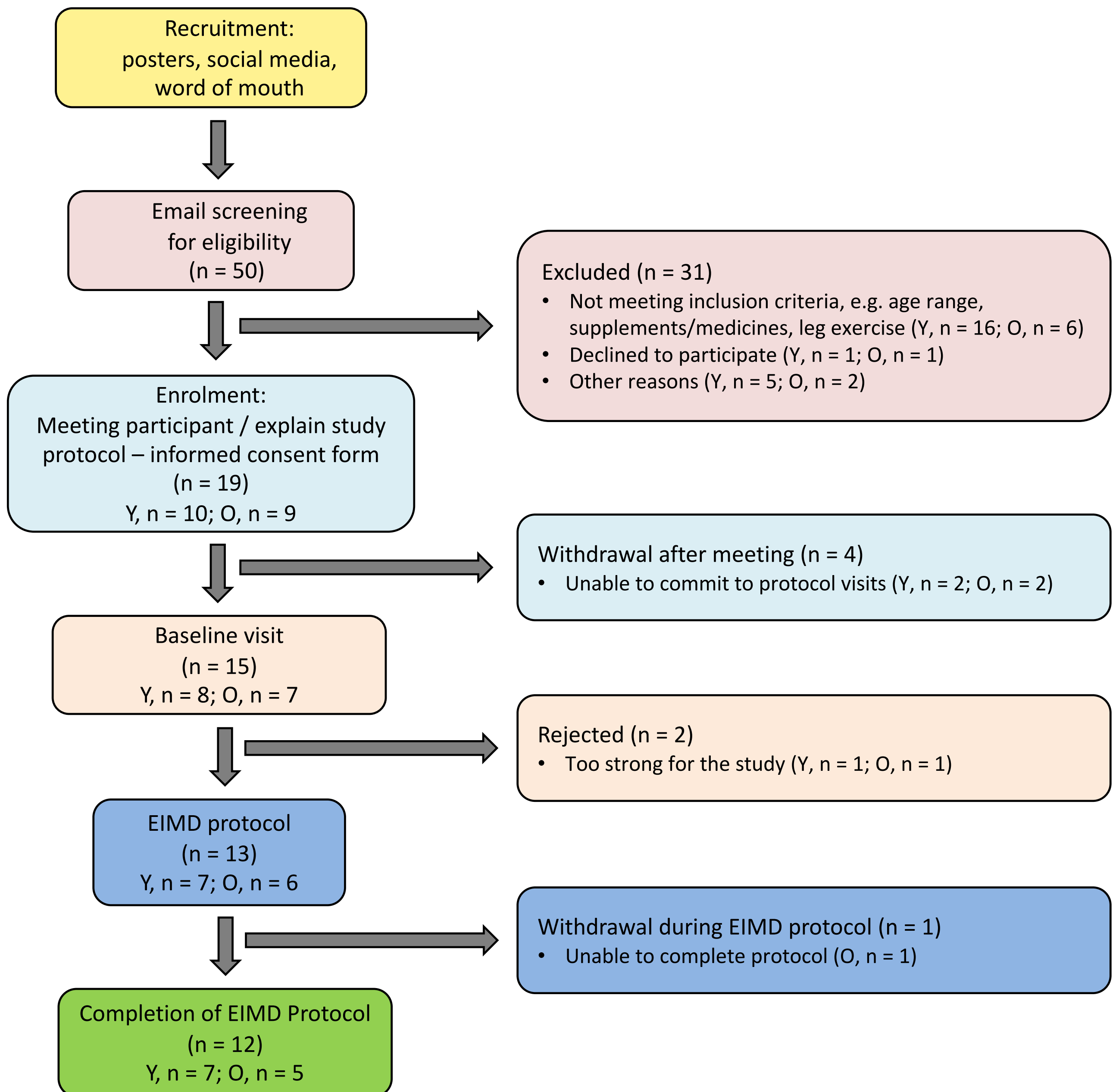


Figure 5.1 Recruitment pathway and participant withdrawals. Y, younger; O, older; EIMD, exercise-induced muscle damage

5.2.3 Experimental Design

Participants were divided into two groups based on age; Y, aged 18-35 years and O, aged ≥ 60 years, which examined the differences in muscle damage and inflammation, muscle function changes and recovery following an EIMD protocol known to successfully induce DOMS in younger individuals (Vaile et al., 2008). All participants were required to attend the Human Performance Laboratory at the same time of day (± 1 hour) in the morning at Cavendish campus, University of Westminster on 6 occasions (included initial visit for study information/informed consent form) over a 2-week period.

During visit 1, participants were informed about the study by providing them with the participant information sheet (**Appendix E**) and personally explaining all procedures, risks and benefits, and given a written informed consent form to sign. They also completed the WURSS-21 questionnaire to ensure that they were free from common cold symptoms before starting baseline measurements, and a questionnaire (Edinburgh Handedness Inventory, **Appendix I**) to confirm leg dominance of their daily activities, accompanied with the question ''if you would shoot a ball on a target, which leg would you use to shoot the ball?'' (Veale, 2014).

On visit 2 (baseline), in an overnight fasted-state, participants performed baseline measurements to ensure familiarisation of testing equipment and to determine 5 repetitions maximum (RM). The baseline visit included anthropometric measurements, a urine and a venous blood sample, and muscle functions of maximal isometric leg strength of the quadriceps, jump height, anaerobic peak power and perceived muscle soreness (described fully in **Chapter 3**). Following baseline measurements, participants' 5RM was also determined.

On visit 3 (seven days later), participants performed the EIMD protocol, a unilateral muscle-damaging exercise protocol, which will be described in detail later. All above measurements were repeated prior to (pre-EIMD) and immediately post (post-EIMD) the EIMD trial, and an additional blood sample taken at 1 and 2 hours post-EIMD. Identical follow-up assessments were repeated at visits 4, 5 and 6 (24, 48 and 72 hours post-EIMD), except the urine sample. An overview of the study design is presented in Figure 5.2.

In addition, a 3-day food diary was provided to record all foods and drinks consumed daily during the 72 hours prior to both baseline and EIMD visit. Participants were also asked to record the WURSS-21 health questionnaire daily in the 1-week preceding the EIMD trial to ensure that they were free from common cold symptoms before EIMD trial.

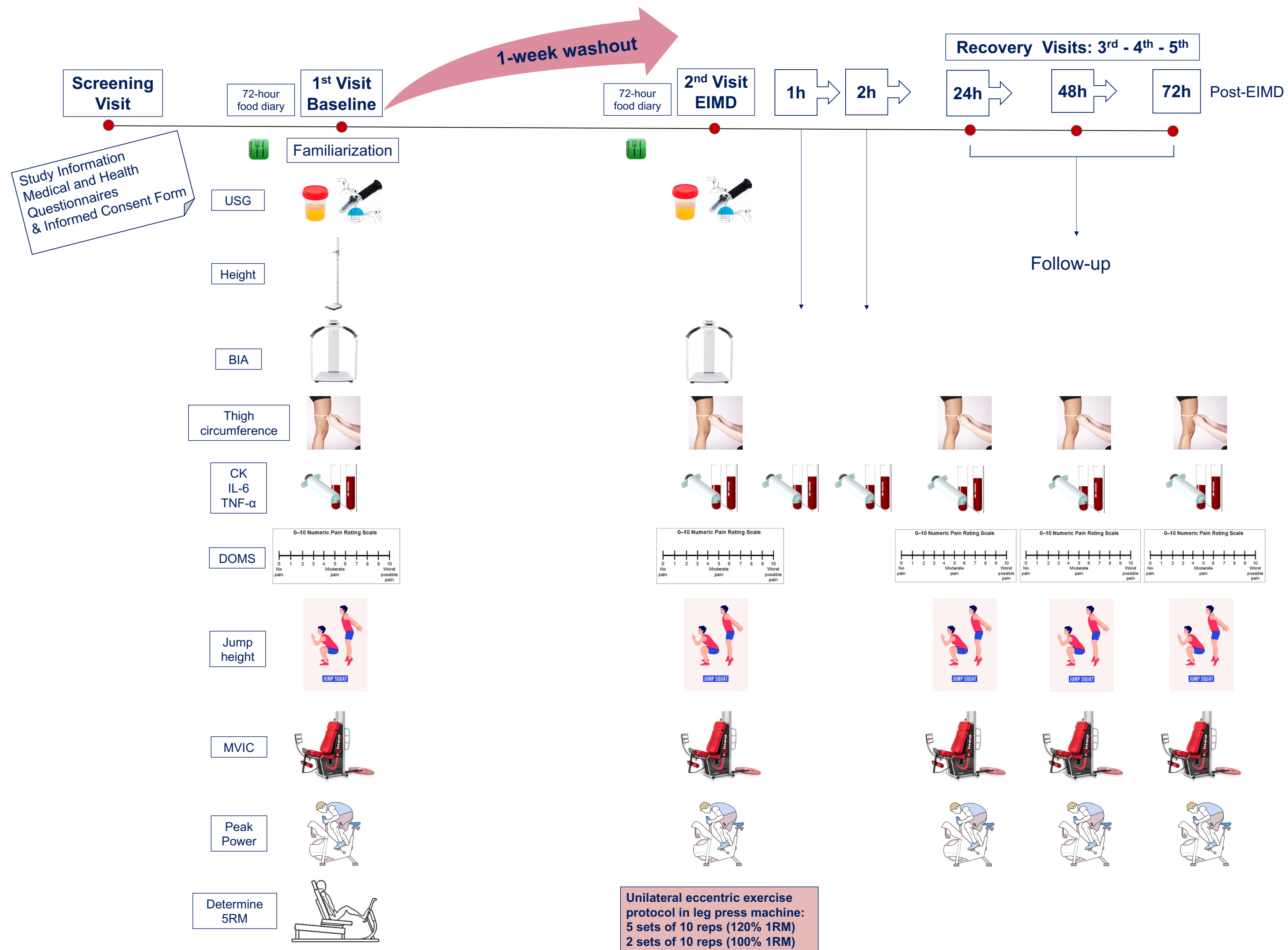


Figure 5.2 Schematic of experimental procedures. USG, urine specific gravity; BIA, bioelectrical impedance analysis; DOMS, delayed-onset muscle soreness via visual analogue scale; MVIC, maximal voluntary isometric contraction; EIMD, exercise-Induced muscle damage. *2nd visit combines both pre and post measurements, immediately prior and following EIMD stimulus, respectively*

5.2.4 Experimental Procedures

5.2.4.1 Preliminary Testing and Familiarisation

On arrival to the Human Performance Laboratory, participants' height, weight, thigh measurements, BMI, muscle mass and body fat were measured. Participants' baseline urine and venous blood sample was also obtained (detailed description see **Chapter 3**). Perceived leg muscle soreness using VAS, jump performance using a jump meter device, MVIC using isokinetic dynamometer and peak power using the 10-sec WAnT test were measured to assess participants' baseline muscle function before the EIMD trial. Following baseline measurements, participants performed a single-leg 5RM test (Body-Solid G9S Multi Station Gym, Taiwan) to determine 5RM. Instrumentation and procedures used in the experimental trial were presented to participants at this time.

5.2.4.1.1 Determination of One Repetition Maximum Load

On the baseline visit, participants performed the 5RM protocol after anthropometric measurements, blood sampling and functional assessments to avoid residual EIMD from baseline affecting experimental measures. Each participant performed 6 concentric repetitions of incremental weight until failure, with 3 minutes rest between sets. 5RM leg press predictive equation: (1.09703 X [5RM, kg]) + 14.2546; Reynolds et al., 2006) was then applied to determine 1RM for each participant. The predicted 1RM weight lifted concentrically at baseline was then utilised to calculate 120% of the weight to be performed eccentrically at EIMD visit. A 5RM protocol for the prediction of 1RM was performed to minimise myofibrillar damage to the contractile proteins of the knee extensors, as well as to avoid adaptations to muscle damage and a potential RBE for the EIMD trial.

5.2.4.2 Experimental Trial

Seven days following baseline visit, all participants underwent an EIMD protocol. On the day of the experimental trial, participants reported to the laboratory [ambient temperature (T_{amp}) 20.3 °C and relative humidity (RH) 27.2%] at 08:00 am having fasted overnight. Participants were asked to consume water based on their body mass (5 mL/kg) before they reported to the laboratory to ensure they began exercise euhydrated. Hydration was verified by checking that the urine specific gravity (USG) (Atago MASTER-SUR/Na refractometer, Atago Co., Ltd. Tokyo, Japan) upon arrival was less than 1.028. A resting blood sample was taken before baseline anthropometric measurements and body composition. Then, muscle functional assessments were performed to assess participants' pre-EIMD soreness, quadriceps leg strength and power.

5.2.4.2.1 Leg Press Exercise

The exercise trial started at 9:00 am with standardised clothes worn by participants (e.g., t-shirt, shorts, socks and training shoes). The protocol comprised of 7 sets of 10 eccentric single-leg press repetitions on a leg press machine (Body-Solid G9S Multi-Station Home Gym, Taiwan), with the first 5 sets of 10 repetitions at 120% of 1RM and final 2 sets of 10 repetitions at 100% of 1RM. A timed rest period of 3 minutes took place between each set. The protocol was performed unilaterally on each participant's dominant leg. Before performing each eccentric contraction, participants raised the weight using both legs, concentrically. Each eccentric contraction lasted 3-5 seconds, during which participants resisted the load with the dominant leg from full knee extension to 90% angle of knee flexion (Vaile et al., 2008). All participants completed all 7 sets. Water was provided ad libitum every 15 minutes.

Immediately after the EIMD bout participants sat and a blood sample was collected after a standardised period of 3 minutes (post-EIMD). Post-EIMD thigh circumferences were assessed before the participants rated their perceived muscle soreness. Then a jump height, maximal isometric leg extension and an anaerobic power test were performed to assess participants' post-EIMD strength and power, respectively. Participants were provided with a standardised amount of water (5 mL/kg) to replace sweat losses and a cereal bar. Participants were asked to refrain from any exercise in this 72-h follow-up period.

5.2.4.3 Diet and Activity Control

Participants were requested to maintain their habitual diet and exercise routine throughout the study. Written and oral reminders were also provided on a regular basis to ensure diet and exercise practices were maintained consistent throughout the study. Diet and exercise instructions were described in detail in **Chapter 3** (p. 105).

5.2.5 Measurements and Instrumentation

Anthropometric measurements, such as weight, BMI, muscle mass and body fat were recorded at baseline, pre- and post-EIMD. Thigh circumferences were taken prior at baseline, pre-, post-, 24, 48 and 72 hours post-EIMD. Urine sample was collected prior to each trial (baseline and pre-EIMD) to assess hydration and ensure adequate hydration before exercise. Blood sampling, for muscle damage (CK activity) and inflammation (IL-6, TNF-α) markers, plasma EVs and blood lactate, was obtained at baseline, pre-, post-, 1, 2, 24, 48 and 72 hours post-EIMD. All assessments of muscle function were recorded at baseline, pre-, post-, 24, 48 and 72 hours post-EIMD. In addition, diet diaries were provided to record

all foods and drinks consumed (including days of the week and of the weekend) 72 hours both prior to baseline (familiarization visit) and prior the experimental trial (EIMD bout). Measurements, blood, urine and diet analysis, and instrumentation were described in detail in **Chapter 3**.

5.2.5.1 EV isolation and characterisation from human plasma

5.2.5.1.1 Isolation of plasma-EVs

Plasma EVs were prepared from the individual plasma (thawed on ice) aliquots (100 µL per individual) from each participant, under the different conditions, using sequential centrifugation and ultracentrifugation according to previously standardised and described protocols and procedures (Kosgodage et al., 2018; Criscitiello et al., 2019; Pamenter et al., 2019), also following the recommendations of The International Society for Extracellular Vesicles (MISEV2018; Théry et al., 2018). For each individual plasma-EV preparation, 100 µL of plasma was diluted 1:5 in Dulbecco's PBS (DPBS, ultrafiltered using a 0.22 µm filter, before use). This was then centrifuged for 20 minutes at 3,000 g at 4 °C, to remove apoptotic bodies and aggregates. Supernatants were then collected and ultra-centrifuged at 100,000 g at 4 °C for 1 hour. This resulted in EV-enriched pellets, which were resuspended each in 500 µL DPBS and thereafter ultra-centrifuged again for 1 hour at 100,000 g, at 4 °C. The final resulting EV pellets were resuspended each in 100 µL of DPBS. The EV pellets were kept frozen at -80 °C until used for nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM) in the procedures described below (all assessments were performed with EV preparations that had not been frozen for longer than 1 week).

5.2.5.1.2 Nanoparticle tracking analysis (NTA)

Plasma-EV quantification and size distribution profiles were established by NTA, based on Brownian motion of particles in suspension, using the NanoSight NS300 system (Malvern, U.K.). For NTA, the EV samples were diluted 1/100 in DPBS (10 µL of EV preparation diluted in 990 µL of DPBS). The diluted EV samples were applied to the NanoSight NS300 (Malvern Panalytical, UK), recording five repetitive reads, 60 seconds each. Particle numbers per frame were 40 to 60, camera settings were at level 10 for recording and for post-analysis the detection threshold was set at 5. Replicate histograms were generated from these videos using the NanoSight software 3.0 (Malvern), representing mean and standard error of the mean (± SEM) of the 5 recordings for each sample.

5.2.5.1.3 Transmission electron microscopy (TEM)

Plasma EVs were further assessed by morphological analysis using TEM. EVs were resuspended in 100 mM sodium cacodylate buffer (pH 7.4). One drop (~3-5 µL) of the EV suspension was placed onto a grid which held a carbon support film which had been previously glow discharged. Following partial drying of the EV suspension, the sample was fixed for 1 minute at room temperature (RT) by placing the grid onto a drop of a fixative solution (2.5% glutaraldehyde) in 100 mM sodium cacodylate buffer (pH 7.0). The grid was applied to the surface of three drops of distilled water for washing of the EV sample, removing excess water using a filter paper. The EVs were then stained for 1 minute with 2% aqueous uranyl acetate (Sigma-Aldrich), removing excess stain with a filter paper and air drying the grid. TEM imaging of EVs was carried out with a JEOL JEM 1400 transmission electron microscope (JEOL, Tokyo, Japan), which was operated at 80 kV, using a magnification of 30,000x to 60,000x. Recording of

digital images was performed with an AMT XR60 CCD camera (Deben, UK).

5.2.5.1.4 Western blot analysis

EVs were assessed for the EV-specific markers CD63 and Flotillin-1 (Flot-1), using western blotting. EV samples were diluted 1:1 in denaturing 2 x Laemmli sample buffer (containing 5% beta-mercaptoethanol, BioRad, U.K.) and heated for 5 minutes at 100 °C. Protein separation was carried out at 165V using 4-20% gradient TGX gels (BioRad U.K.), followed by western blotting at 15V for 1 hour using a Trans-Blot® SD semi-dry transfer cell (BioRad, U.K.). Membranes were blocked with 5% bovine serum albumin (BSA, Sigma, U.K.) in Tris buffered saline (TBS) containing 0.1% Tween20 (BioRad, U.K.; TBS-T) for 1 hour at RT and primary antibody incubation was carried out overnight at 4 °C using the EV-marker CD63 (ab216130, Abcam, U.K.) and Flot-1 (ab41927, Abcam); diluted 1/1000 in TBS-T. The membranes were then washed at RT in TBS-T for 3 x 10 minutes and thereafter incubated with HRP-conjugated anti-rabbit IgG secondary antibodies (BioRad), diluted 1/3000 in TBS-T, for 1 hour at RT. The membranes were then washed for 4 x 10 minutes TBS-T, and visualised, using enhanced chemiluminescence (ECL, Amersham, UK) in conjunction with the UVP BioDoc-ITTM System (Thermo Fisher Scientific, U.K.).

5.2.6 Statistical Analysis

Data were checked for assumptions of normality by the Shapiro-Wilk test. Following Levene's test of equality of variance, group differences between younger and older were determined using two-tailed independent samples *t*-tests (baseline characteristics, dietary data and blood lactate) or Mann-Whitney U tests (CK, IL-6, TNF-α and DOMS) as appropriate. Exercise-induced changes in EV

profiles, functional measurements (MVIC, peak power, jump height) and thigh circumference were analysed using a two-way ANOVA with repeated measures on both factors [group (younger, older) x time (pre-, post-, at 24, 48, 72 hours post-EIMD)]. Bonferroni adjustment was used for *post hoc* analysis to perform pairwise comparisons. The EIMD effects within-group were determined across time in dependent variables CK, IL-6, TNF-α and DOMS using the non-parametric Freidman ANOVA, and the Wilcoxon matched pairs signed ranks test was performed for *post hoc* analysis to test differences in these variables. Values were expressed as mean ± SEM for data from parametric tests, and as median and interquartile range for data from non-parametric tests. Statistical significance was accepted as $p < 0.05$. Effect size was calculated using methods proposed by Cohen (1988). Statistical analyses were performed using SPSS 26 software (IBM SPSS, NY, USA). All figures were generated in GraphPad Prism (Version 8, GraphPad), except generation of NTA curves which was carried out using the Nanosight 3.0 software (Malvern, U.K.). Subsequent power calculations on data presented within was calculated using G*Power (3.1.9.7).

5.3 RESULTS

5.3.1 Participant Characteristics

Participants' characteristics are presented in Table 5.1. Besides age, participants were reasonably homogenous. An independent-samples t-test was conducted to compare physical characteristics between groups. No significant difference was noted in any characteristic between groups (Table 5.1).

Table 5.1. Characteristics and comparisons of younger and older groups at baseline. Independent sample t-test comparison between younger (18-35 years of age) and older ≥60 years of age)

	Younger (18-35) n = 7	Older (≥60) n = 5	P-value
Age (years)	27.00 (± 1.34)	63.00 (± 0.93)	0.001*
Weight (kg)	73.83 (± 3.16)	74.30 (± 5.46)	0.939
Height (cm)	181.14 (± 2.06)	179.60 (± 2.16)	0.624
BMI (kg/m^2)	22.56 (± 0.98)	22.98 (± 1.35)	0.800
Body fat (%)	16.90 (± 2.60)	22.10 (± 2.83)	0.212
Fat mass (kg)	12.87 (± 2.29)	17.03 (± 3.54)	0.326
Fat free mass	60.97 (± 1.64)	57.27 (± 2.10)	0.189
Muscle mass (kg)	29.53 (± 1.04)	27.54 (± 1.22)	0.243
Dominant leg muscle mass (kg)	6.27 (± 0.23)	5.83 (± 0.23)	0.219
Thigh circumference (cm)	52.36 (± 1.55)	49.91 (± 1.24)	0.275
1RM leg press (kg)	152.16 (± 7.66)	135.67 (±11.52)	0.241

**Significant level, $p < 0.05$; Values are expressed as mean ± SEM. BMI, body mass index*

5.3.2 Dietary Data

Descriptive characteristics of the average nutrient intake for both groups are presented in Table 5.2. An independent-samples t-test was conducted to compare energy, macronutrients, and n-3 and n-6 intake between younger and older group. There was no significant difference in dietary data ($p > 0.05$) between groups (Table 5.2).

Table 5.2. Average dietary intake of younger and older participants. Independent sample t-test comparison between two groups

	Younger (18-35) N = 7	Older (≥60) N = 5	P-value
Energy (kcal)	2153.71 (± 126.56)	2367.50 (± 201.26)	0.365
CHO (g)	220.68 (± 20.32)	273.74 (± 28.33)	0.148
Protein (g)	98.48 (± 9.87)	99.20 (± 8.33)	0.959
Fat (g)	84.74 (± 5.68)	94.94 (± 8.95)	0.335
n-3 (g)	1.23 (± 0.34)	1.23 (± 0.28)	0.997

n-6 (g)	5.99 (± 0.94)	6.58 (± 0.72)	0.655
n-6/n-3 ratio	6.52 (± 1.39)	6.81 (± 1.78)	0.897

CHO, Carbohydrates; n-3, omega-3 fatty acids; n-6, omega-6 fatty acids; Values are expressed as mean ± SEM

5.3.3 Biomarkers of Muscle Damage and Inflammation

All markers were analysed as both absolute values and percentage change from pre-EIMD to normalise individual values. The eccentric exercise was effective in provoking significant changes in plasma CK activity but not in plasma IL-6 and TNF-α and blood lactate.

5.3.3.1 Creatine Kinase

Mann-Whitney U test showed significant differences in plasma CK activity between groups at pre-EIMD ($p = 0.028$, $r = 0.63$), immediately post-EIMD ($p = 0.019$, $r = 0.68$), at 1 hour ($p = 0.028$, $r = 0.63$), 2 hours ($p = 0.042$, $r = 0.59$) and 24 hours ($p = 0.004$, $r = 0.82$) post-EIMD indicating a large effect size (Figure 5.3A). Freidman's ANOVA suggested that there were statistically significant differences in CK activity for the younger ($p = 0.020$) and the older ($p < 0.001$) group across time. Pairwise comparisons were performed with a Bonferroni adjustment to alpha values for multiple comparisons.

In the younger group, *post hoc* analysis showed a significant increase in plasma CK activity in the younger group immediately post-EIMD *[Md = 216.00 (245.67) U/L]*, at 1 hour *[Md = 226.33 (221.00) U/L]* and 2 hours *[Md = 228.33 (250.67) U/L]* post-EIMD (all, $p = 0.018$, $r = 0.63$) relative to pre-EIMD *[Md = 211.33 (191.50) U/L]*, indicating a large effect size for all time points. In the older group, *post hoc* analysis revealed a significant increase in plasma CK activity immediately post-EIMD *[Md = 108.50 (81.25) U/L],* at 1 hour *[Md = 108.00 (85.75)*

U/L], 2 hours *[Md = 115.50 (86.00) U/L]*, 24 hours *[Md = 171.50 (56.25) U/L]*, 48 hours *[Md = 168.50 (98.75) U/L]* and 72 hours *[Md = 178.00 (120.83) U/L]* post-EIMD (all, p = 0.043, r = 0.64) relative to pre-EIMD *[Md = 98.50 (66.75) U/L]*, indicating a large effect size for all time points.

Further, a significant between-group difference was found in plasma CK activity percentage change only at 72 hours post-EIMD (p = 0.042, r = 0.59) indicating a large effect size, with older participants showing a greater increase in plasma CK (pre- *vs* 72 hours post-EIMD) compared to the younger counterparts (165.74% *vs* 107.39%, respectively) but no other time points (Figure 5.3B).

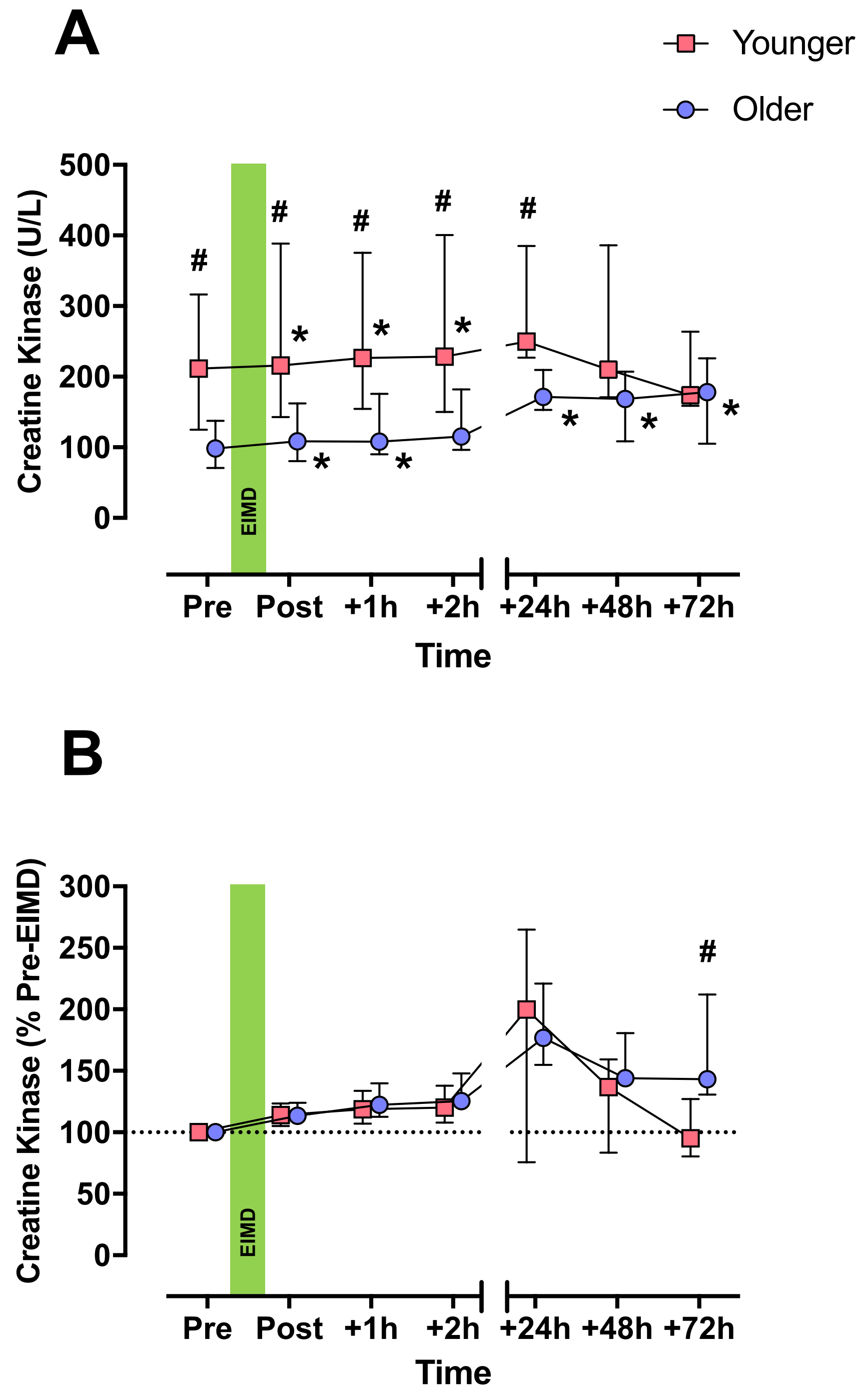


Figure 5.3 Differences in plasma creatine kinase activity between younger and older group. A) CK (U/L) and **B)** CK (% change from pre-EIMD). *Data shown as medians, error bars indicate interquartile range. # indicates significant difference between groups at timepoint indicated, * indicates significant difference from Pre timepoint. Dashed horizontal line indicates 100% (pre-EIMD). The scale brake indicates from hourly testing to 24-hour intervals. Time matched data points offset horizontally to enhance clarity.*

5.3.3.2 IL-6

Mann-Whitney U test revealed that there was no significant difference in either plasma IL-6 or percentage change between groups at any time point ($p > 0.05$; Figure 5.4A-B). Freidman's ANOVA test showed plasma IL-6 did not significantly differ with time in either the younger ($p = 0.270$) or older group ($p = 0.334$; Figure 5.4A). However, the older group showed a less pronounced change than the younger group [Figure 5.4A(ii) for clarity purposes]. When plasma IL-6 was normalized to pre-EIMD values, the older group presented a minor increase at 72 hours post-EIMD relative to pre-EIMD (Figure 5.4B).

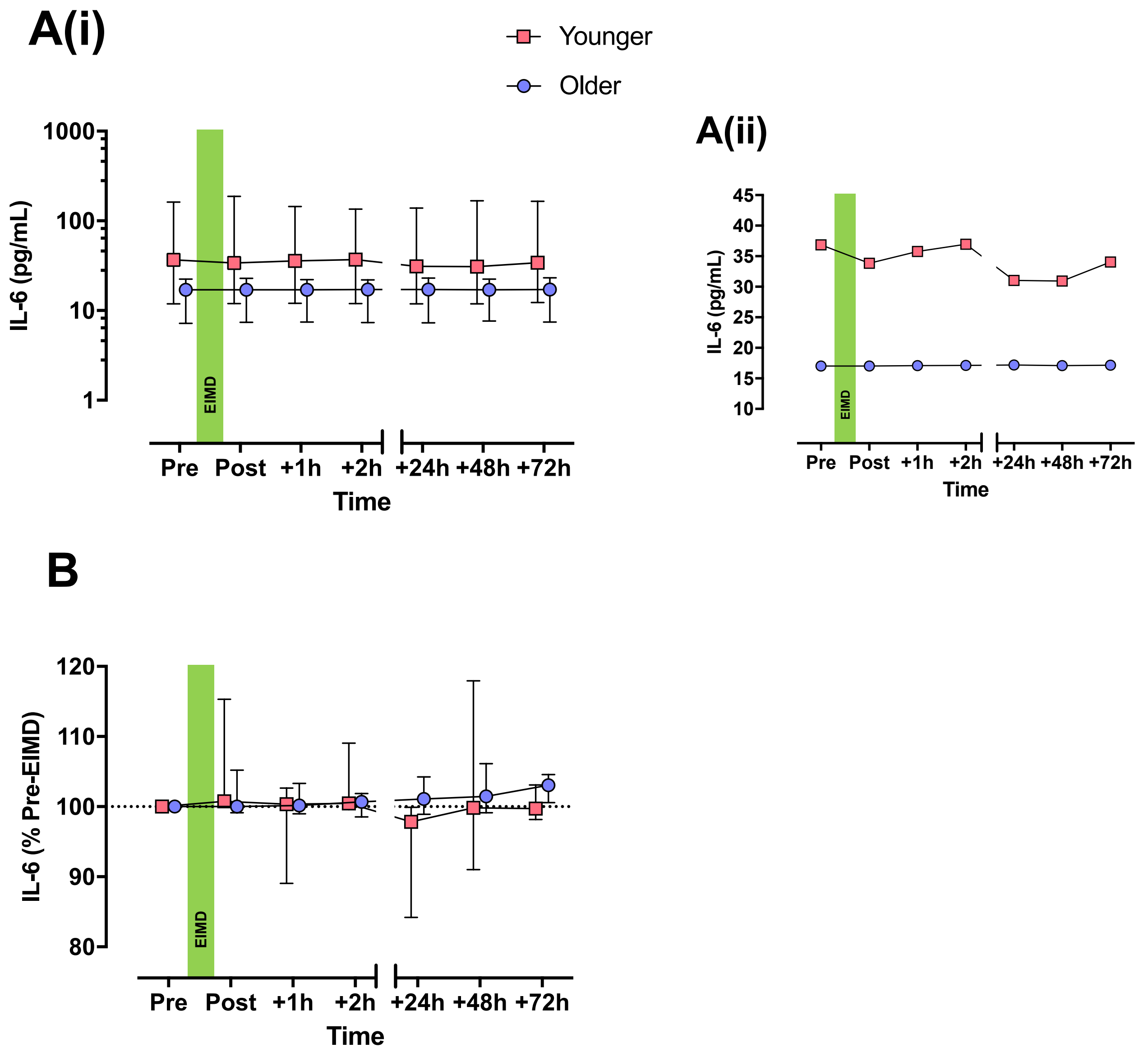


Figure 5.4 Differences in plasma IL-6 concentration between younger and older group. A(i) IL-6 (pg/mL) in logarithmic (log 10) scale for better visualization and **A(ii)** (pg/mL), error bar free to enhance clarity. **B)** IL-6 (% change from pre-EIMD). *Data shown as medians, error bars indicate interquartile range. Dashed horizontal line indicates 100% (pre-EIMD). The scale brake indicates from hourly testing to 24-hour intervals. Time matched data points offset horizontally to enhance clarity.*

5.3.3.3 TNF-α

Mann-Whitney U test showed that there were significant differences in plasma TNF-α concentration between groups at pre-EIMD, post-EIMD, at 2, 24, 48 and 72 hours post-EIMD ($p < 0.042$, $r = 0.59$) and at 1 hour post-EIMD, $p < 0.028$, $r = 0.63$, indicating a large effect size for all time points [Figure 5.5A(i)]. Freidman's ANOVA revealed plasma TNF-α did not change over time in older group ($p = 0.321$) but did change in younger group ($p = 0.043$). Bonferroni *post hoc* testing suggested a significant effect of time at 2 hours *[Md = 21.55 (104.03) pg/mL]* and at 72 hours *[Md = 21.88 (54.30) pg/mL]* post-EIMD (both $p = 0.028$, $r = 0.59$) relative to immediately post-EIMD *[Md = 21.72 (261.48) pg/mL]*, indicating a large effect size for both time points [Figure 5.5A(i) - for visualization clarity refer to Figure 5.5A(ii)].

Further, when TNF-α normalised to pre-EIMD values, a significant between-group difference was found at 72 hours post-EIMD ($p = 0.042$, $r = 0.59$), with older group showing a larger increase in plasma TNF-α (pre *vs* 72 hours post-EIMD) compared to the younger group (104.40% *vs* 89.41%, respectively) but not between any other time points (Figure 5.5B).

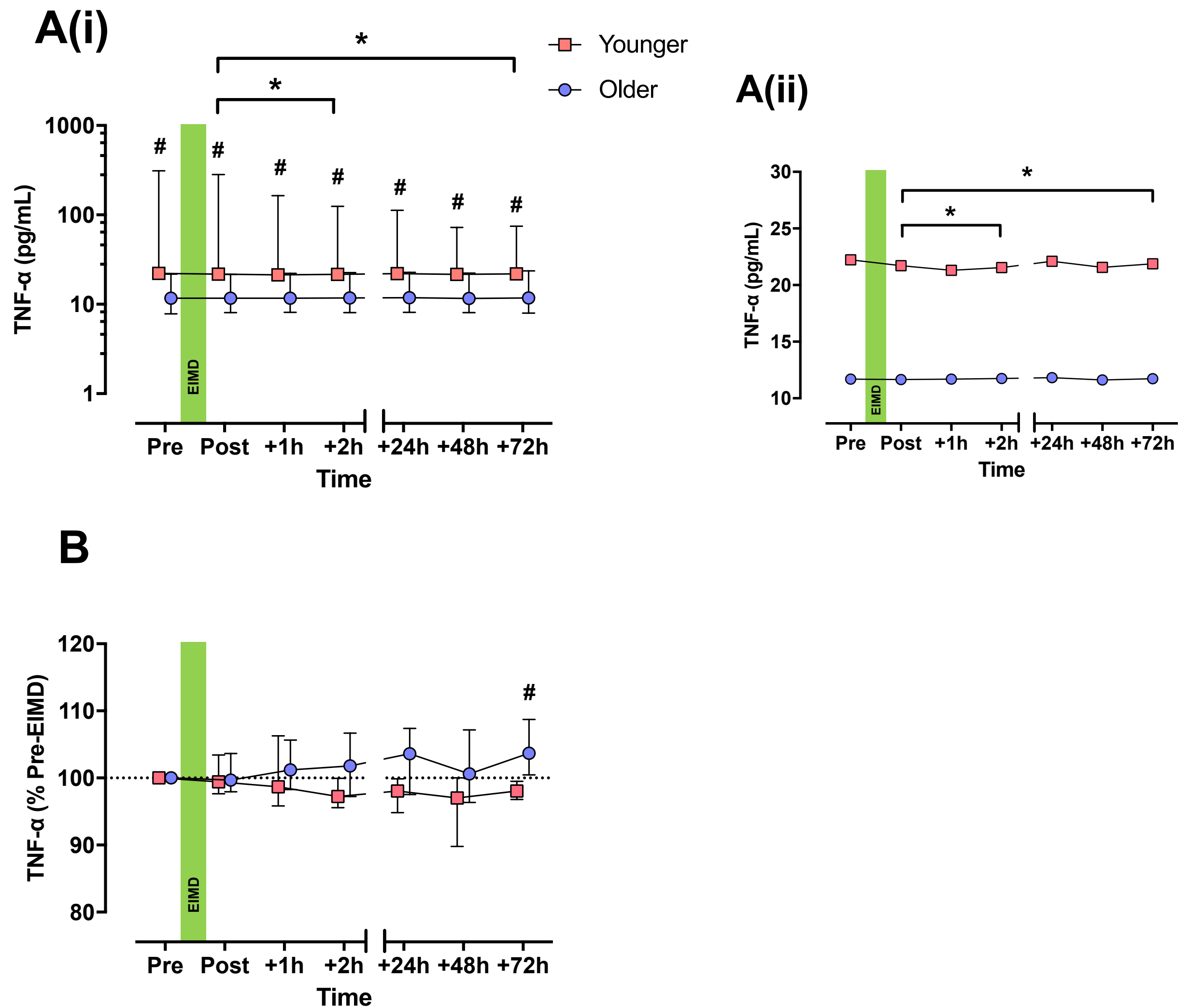


Figure 5.5 Differences in plasma TNF-α concentration between younger and older group. A(i) TNF-α (pg/mL) in logarithmic (log 10) scale for better visualization and **A(ii)** (pg/mL), error bar free to enhance clarity. **B)** TNF-α (% change from pre-EIMD). *Data shown as medians, error bars indicate interquartile range. # indicates significant difference between groups at timepoint indicated, * indicates significant difference from Post timepoint. Dashed horizontal line indicates 100% (pre-EIMD). The scale brake indicates from hourly testing to 24-hour intervals. Time matched data points offset horizontally to enhance clarity.*

5.3.4 Assessment of Muscle Function

All markers of muscle function were analysed as both absolute values and percentage change from pre-EIMD to normalise individual values. The eccentric exercise was effective in provoking significant changes in DOMS and in MVIC but not in jump height, peak power or thigh circumference.

5.3.4.1 Muscle Soreness

Mann-Whitney U test showed no significant difference in either VAS score or percentage change between groups at any timepoint (Figure 5.6A-B). However, following the EIMD protocol, Freidman test suggests a significant increase in DOMS across time in the younger group ($p = 0.014$) and in the older group ($p = 0.034$). Nevertheless, DOMS returned to pre-EIMD values by 72 hours post-EIMD in the younger group, but not in the older group (Figure 5.6A). *Post hoc* pairwise comparisons showed that both younger and older group had significantly elevated DOMS immediately post- *[Y, Md = 4.00 (3.00), p = 0.042, r = 0.54; O, Md = 2.00 (3.75), p = 0.039, r = 0.65]* and at 24 hours post-EIMD *[Y, Md = 5.00 (2.00), p = 0.034, r = 0.57; O, Md = 2.00 (3.25), p = 0.042, r = 0.64]* relative to pre-EIMD *[Y, Md = 1.50 (3.00) and O, Md = 0.00 (2.75)]*, indicating a large effect size for both time points.

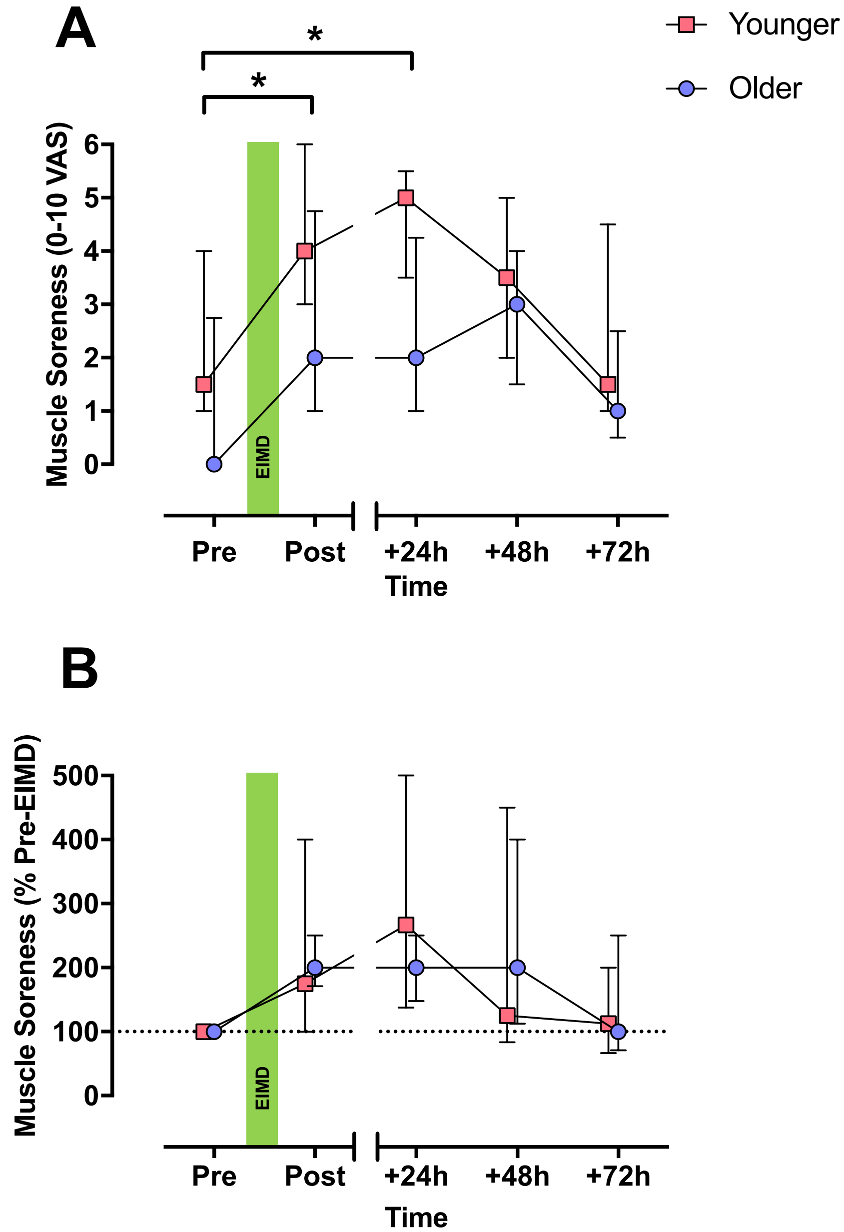


Figure 5.6 Differences in perception of pain between younger and older group. A) VAS score and **B)** DOMS (% change from pre-EIMD). *Data shown as medians, error bars indicate interquartile range. * indicates significant difference from Pre timepoint. Dashed horizontal line indicates 100% (pre-EIMD). The scale brake indicates from hourly testing to 24-hour intervals. Time matched data points offset horizontally to enhance clarity.*

5.3.4.2 Maximal Voluntary Isometric Strength

Mixed ANOVA showed that EIMD had no effect on group by time interaction on leg MVIC ($p = 0.129$), suggesting that MVIC decreased uniformly in the two groups. However, following the EIMD protocol, a main effect of both time ($p = 0.005$, $\eta^2_p = 0.852$) and age group ($p = 0.005$, $\eta^2_p = 0.564$) was noted for MVIC with a large effect size (Figure 5.7A), suggesting that younger participants' MVIC was higher throughout, and the EIMD protocol successfully reduced muscle force in both groups. *Post hoc* testing indicates force significantly decreased immediately post-EIMD *[pooled pre-MVIC, 16.58 (± 0.87) kg to pooled post-MVIC, 13.78 (± 0.79) kg, p = 0.003]*, and then started to return in a linear recovery at 24 hours post-EIMD *[pooled post-MVIC, 13.78 (± 0.79) kg to pooled 24 hours MVIC, 15.57 (± 0.87) kg, p = 0.001]* and at 48 hours post-EIMD *[pooled 48 hours MVIC, 16.07 (± 1.09) kg, p = 0.038]* (Figure 5.7A).

Similarly, no significant interaction between group and time ($p = 0.505$) nor a main effect of age ($p = 0.189$) was found. However, a significant main effect of time was observed for MVIC percentage change ($p = 0.008$, $\eta^2_p = 0.828$) following EIMD, showing a large effect size. Both groups showed a comparable MVIC change, with *post hoc* testing suggesting force significantly decreased immediately post-EIMD in both groups *[pooled pre-MVIC, 100.00% (0.00) to pooled post-MVIC, 84.72% (± 2.85), p = 0.003]*, and then started to return in a linear recovery at 24 hours post-EIMD *[pooled post-MVIC, 84.72% (± 2.85) to pooled 24 hours MVIC, 94.87% (± 2.77), p = 0.002]* and at 48 hours post-EIMD *[pooled 48 hours MVIC, 97.48% (± 3.66), p = 0.022]* (Figure 5.7B).

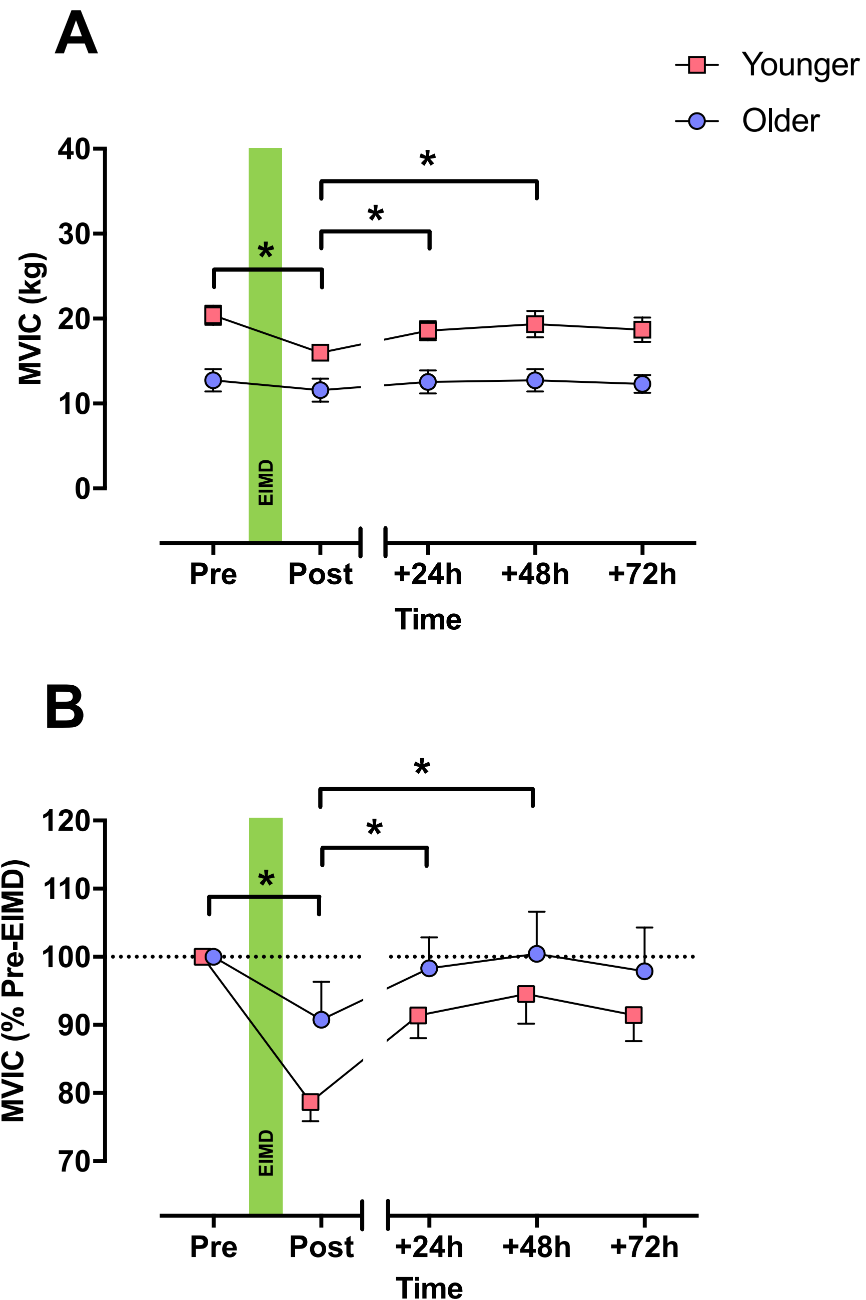


Figure 5.7 Differences in maximal voluntary isometric contraction between younger and older group. A) MVIC (kg) and **B)** MVIC (% change from pre-EIMD). *Data shown as mean (±SEM). * indicates significant difference from Pre or Post timepoint. Dashed horizontal line indicates 100% (pre-EIMD). The scale brake indicates from hourly testing to 24-hour intervals. Time matched data points offset horizontally to enhance clarity.*

5.3.4.3 Peak Power

Mixed ANOVA revealed no significant interaction between group and time ($p = 0.280$) or a main effect of time ($p = 0.077$) on peak power (Figure 5.8A). However, a main effect of age group ($p = 0.001$, $\eta^2_p = 0.690$) was noted; suggesting a large effect size, with the younger group showing a significantly higher peak power output than the older group *[Y, M = 763.60 (± 28.46) W; O, M = 555.38 (± 33.68) W]*.

Similarly, there was no effect on group by time interaction ($p = 0.351$) and no significant time effect ($p = 0.104$) for peak power percentage change. Peak power output was comparable between groups following EIMD (age group, $p = 0.209$), however, it was restored to pre-EIMD values by 72 hours following EIMD in the elderly but not in their younger counterparts (Figure 5.8B).

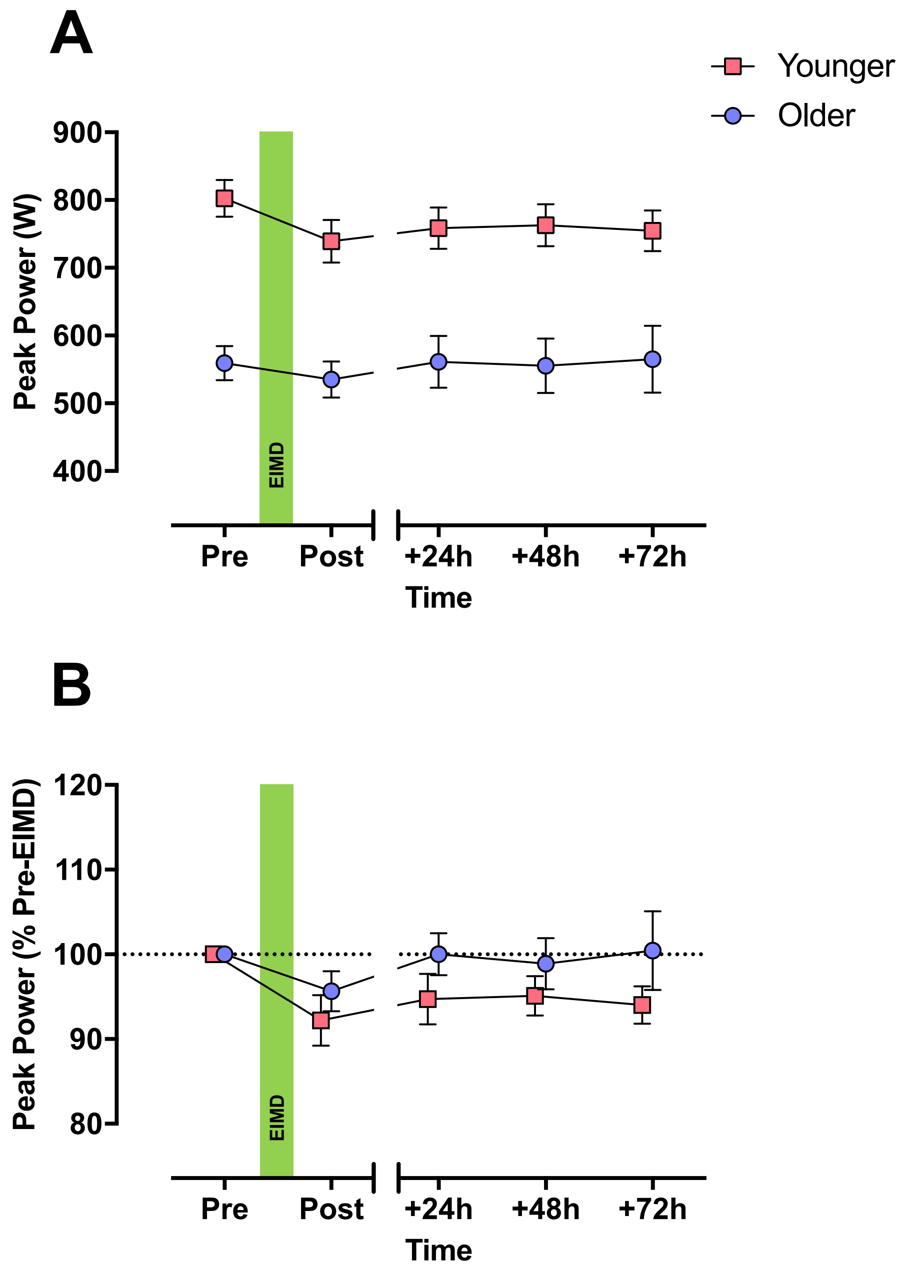


Figure 5.8 Differences in peak power output between younger and older group. A) peak power (W) and **B)** peak power (% change from pre-EIMD). *Data shown as mean (±SEM). Dashed horizontal line indicates 100% (pre-EIMD). The scale brake indicates from hourly testing to 24-hour intervals. Time matched data points offset horizontally to enhance clarity.*

5.3.4.4 Jump Height

EIMD had no significant effect on group by time interaction ($p = 0.089$), or a main effect of time ($p = 0.118$; Figure 5.9A). However, mixed ANOVA suggested a main effect of age group ($p = 0.016$, $\eta^2_p = 0.588$); suggesting a large effect size, with the younger group showing a significantly higher jump performance than the older group *[Y, M = 49.02 (± 2.47) cm; O, M = 38.54 (± 2.21) cm]*.

Whilst the EIMD protocol visually appeared to induce more variability in jump height percentage change, repeated measures ANOVA indicated that the EIMD protocol had no effect on group by time interaction ($p = 0.151$), nor a main effect of either time ($p = 0.200$) or group ($p = 0.668$) on jump height, with both groups showing a reduction immediately post-EIMD (Figure 5.9B).

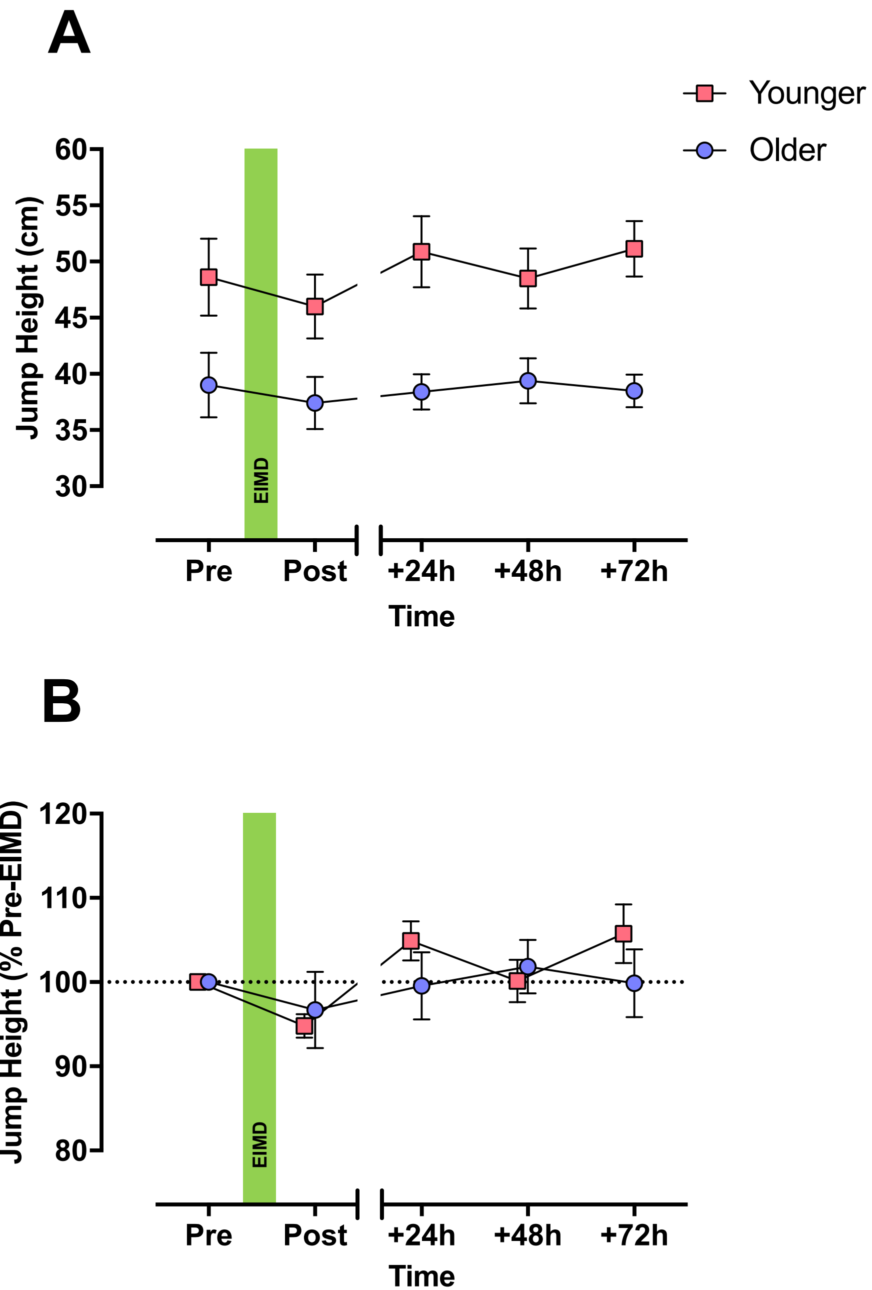


Figure 5.9 Differences in jump height output between younger and older group. A) jump height (cm) and **B)** jump height (% change from pre-EIMD). *Data shown as mean (±SEM). Dashed horizontal line indicates 100% (pre-EIMD). The scale brake indicates from hourly testing to 24-hour intervals. Time matched data points offset horizontally to enhance clarity.*

5.3.4.5 Thigh Circumference (midpoint)

Mixed ANOVA showed no significant interaction between group and time ($p = 0.851$) or a main effect for time ($p = 0.099$) on thigh circumference, with both groups showing a higher thigh circumference immediately post-EIMD (Figure 5.10A). There was also no effect of age group ($p = 0.265$), however, as expected the younger group had greater thigh circumference than the older group *[Y, M = 53.11 (± 1.48) cm; O, M = 50.40 (± 1.75) cm]*.

In a similar manner, when thigh circumference was normalised to pre-EIMD values, EIMD had no significant effect on group by time interaction ($p = 0.840$) or a main effect of time ($p = 0.090$) on thigh circumference. Despite no significant differences between groups ($p = 0.390$), the older group demonstrated a greater thigh circumference change immediately post-EIMD than the younger group *[O, M = 101.13% (± 0.53); Y, M = 100.37% (± 0.31)]* without fully recovered by 72 hours post-EIMD (Figure 5.10B).

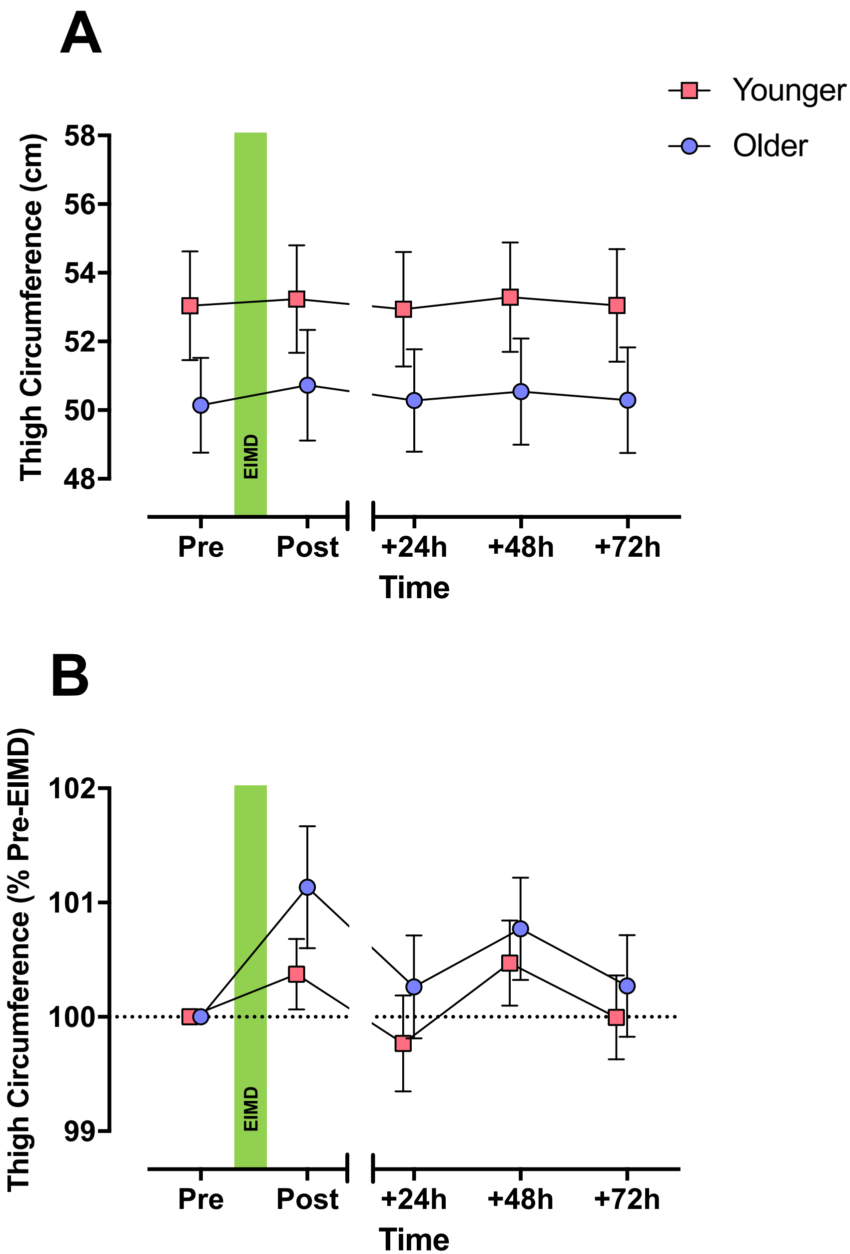


Figure 5.10 Differences in midpoint thigh circumference between younger and older group. A) thigh circumference (cm) and **B)** thigh circumference (% change from pre-EIMD). *Data shown as mean (±SEM). Dashed horizontal line indicates 100% (pre-EIMD). The scale brake indicates from hourly testing to 24-hour intervals. Time matched data points offset horizontally to enhance clarity.*

5.3.5 Extracellular Vesicles

Extracellular vesicle profile (both modal size and particle concentration) was quantified by NTA (representative sample shown Figure 5.11A) and were characterised by Western blotting for EV surface markers (CD63 and Flot-1) (Figure 5.11B) and TEM for morphology (Figure 5.11C). Pre-exercise circulating blood samples suggested that EV modal size did not differ between younger and older participants [younger 109.33 (± 7.64) *vs* older 115.68 (± 6.37) nm, $p = 0.538$, Figure 5.11D], whilst EV count showed a trend of being lower in older participants at rest, relative to younger participants [younger 1.15×10^{10} (± 3.72×10^{9}) *vs* older 2.75×10^{10} (± 3.08×10^{9}), $p = 0.056$, Figure 5.11E].

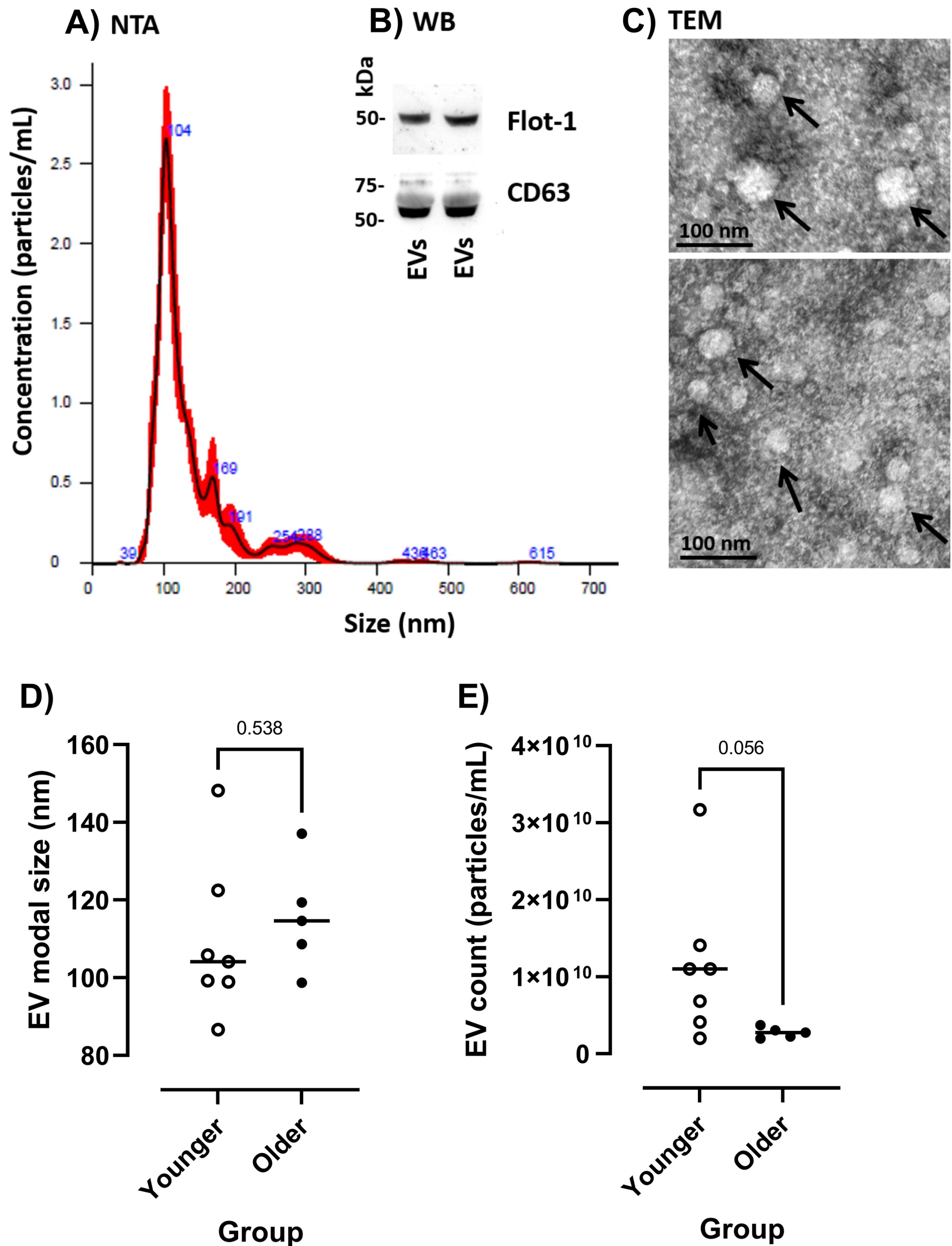
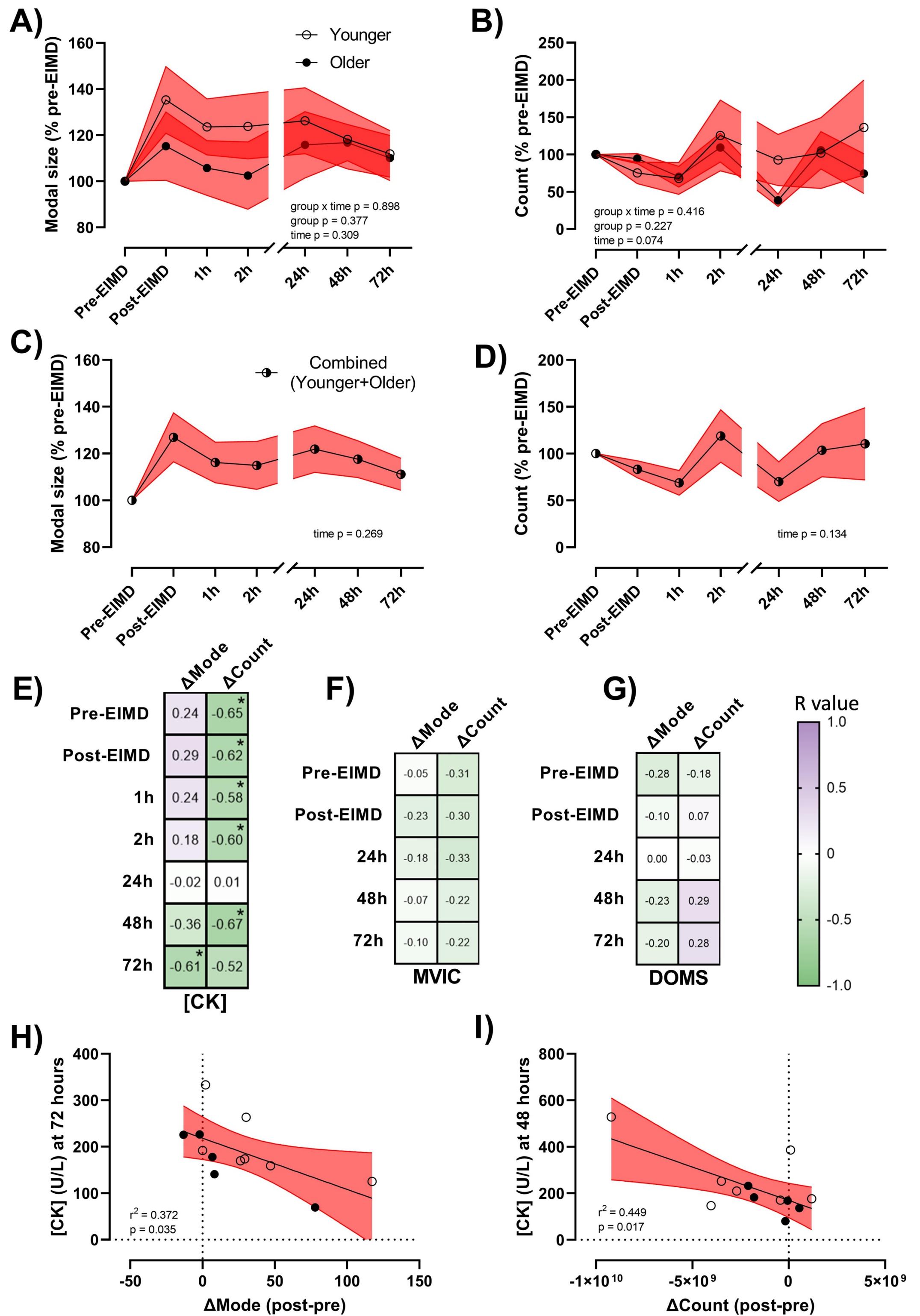


Figure 5.11 Measurement of EV modal size and count in younger and older participants. **A)** Representative example of NTA analysis, SEM shown in red and mean in black line. **B)** Western blotting of human plasma EVs showing positive for Flot-1 and CD63. **C)** Transmission electron microscopy images for human plasma-EVs, showing EV morphology; *scale bar indicates 100 nm.* ***D)*** EV modal size (nm) and **E)** EV count (particles/mL) at pre-EIMD in younger (open circles) and older (closed circles) participants. *Horizontal line indicates group means.*

Whilst the EIMD protocol visually appeared to induce increased expression and greater variability in circulating plasma-EV modal size in the younger group (Figure 5.12A), repeated measures ANOVA suggested EIMD had no significant effect on group by time interaction ($p = 0.898$), nor a main effect of either group (younger or older, $p = 0.377$), or time ($p = 0.309$; Figure 5.12A). In a similar manner, the EIMD protocol did not substantially alter plasma-EV count, with no group by time interaction ($p = 0.416$), nor a main effect of group (younger or older, $p = 0.227$) or time ($p = 0.074$; Figure 5.12B). These results are maintained if participants are examined independent of age ($n = 12$), with one-way ANOVA suggesting no effect of time on EV modal size ($p = 0.269$; Figure 5.12C) or count ($p = 0.134$; Figure 5.12D). As a preliminary study into changes in EV profile with EIMD in younger and older participants, required sample size for future studies using a condition x time model with 7 time points (as presented in Figure 5.12C) was calculated as $n = 398$ per group for EV modal size, and $n = 57$ per group for EV count ($\alpha = 0.05$, power ($1 - \beta = 0.8$), effect size of 0.035 for EV modal and 0.093 for EV count).

To explore a correlation between EV release profiles as a putative biomarker of muscle damage, the numerical difference in EV modal size (ΔMode) and EV count (ΔCount) between post-EIMD and pre-EIMD was examined relative to CK (U/L), MVIC (kg) or DOMS at each time point measured. A significant association between ΔMode and circulating CK was seen at 72 hours only, post-EIMD (Figure 5.12E; $r^2 = 0.372$, $p = 0.035$ visualised in Figure 5.12H). Circulating CK was shown to significantly associate negatively with ΔCount at every time point measured, except 24 hours post-EIMD (Figure 5.12E; largest $r^2 = 0.449$ at 48 hours visualised in Figure 5.12I, $p = 0.017$). No significant associations were

noted between MVIC and either ΔMode or ΔCount (Figure 5.12F), or DOMS and either ΔMode or ΔCount at any time point measured (Figure 5.12G).



E) [CK]	ΔMode	ΔCount
Pre-EIMD	0.24	-0.65*
Post-EIMD	0.29	-0.62*
1h	0.24	-0.58*
2h	0.18	-0.60*
24h	-0.02	0.01
48h	-0.36	-0.67*
72h	-0.61*	-0.52

F) MVIC	ΔMode	ΔCount
Pre-EIMD	-0.05	-0.31
Post-EIMD	-0.23	-0.30
24h	-0.18	-0.33
48h	-0.07	-0.22
72h	-0.10	-0.22

G) DOMS	ΔMode	ΔCount
Pre-EIMD	-0.28	-0.18
Post-EIMD	-0.10	0.07
24h	0.00	-0.03
48h	-0.23	0.29
72h	-0.20	0.28

Figure 5.12 Alterations in EV modal size and count with exercise, and EV correlations with muscle damage markers. A) EV modal size (% pre-EIMD) and **B)** EV count (% pre-EIMD) as a function of timepoint between younger and older groups,

C) EV modal size (% pre-EIMD) and **D)** EV count (% pre-EIMD) as a function of timepoint combined younger and older participants (n = 12). *Red shaded zones indicate SEM and black connected line indicates group means. The scale brake indicates from hourly testing to 24-hour intervals.* **E)** Correlation matrix between change in EV modal size (ΔMode) or in EV count (ΔCount) as a function of CK (U/L), **F)** as a function of MVIC (kg) and **G)** as a function of DOMS (0-10 VAS) at each timepoint measured, with r values as shown. ** indicates significant association between variables (each $p < 0.05$). Colour intensity for r values (purple indicates positive r value, green negative, white = 0) as indicated.* **H)** CK (U/L) at 72 hours as a function of ΔMode (post-EIMD – pre-EIMD and **I)** CK (U/L) at 48 hours as a function of ΔCount (post-EIMD – pre-EIMD). *Red shaded zone indicates 95% confidence intervals. Open circles indicate younger, closed indicate older.*

5.3.6 Blood Lactate

Basal levels of blood lactate did not differ between younger and older participants ($p = 0.748$; Figure 5.13A). Both groups showed a similar pattern response of blood lactate change following EIMD, with blood lactate returning to normal values at 2 hours post-EIMD and remained constant until the end of the recovery period (Figure 5.13B).

Following Levene's test of equality of variance, a paired samples *t*-test was conducted to evaluate the impact of the EIMD in the blood lactate on both groups. There was a statistically significant increase in blood lactate change from pre- *[M = 100.00% (0.00)]* to 1 hour post-EIMD *[M = 498.13% (± 52.40)]* in the younger group [t (4) = -7.598, $p = 0.002$ (two-tailed)]. The eta-squared statistic (η^2_p = 0.935) indicated a large effect size. There was also a statistically significant increase in blood lactate concentration from pre- *[M = 100.00% (0.00)]* to 1 hour post-EIMD *[M = 236.97% (± 43.62)]* for the older group [t (4) = -3.140, $p = 0.035$ (two-tailed), with a large effect size, $\eta^2_p = 0.711$; Figure 5.13C].

Further, an independent samples *t*-test was conducted to compare the blood lactate relative change at 1 hour post-EIMD between younger and older

participants. Blood lactate was significantly different between groups [*t* (8) = 3. 830, p = 0.005, two-tailed], with the younger group showing a greater blood lactate change *[M = 498.13% (± 52.40)]* than the older group *[M = 236.97% (± 43.62)]*. The magnitude of the differences in the means (mean difference = 261.16%, 95% CI: 103.93 to 418.39) was large (η^2_p = 0.647); Figure 5.13D].

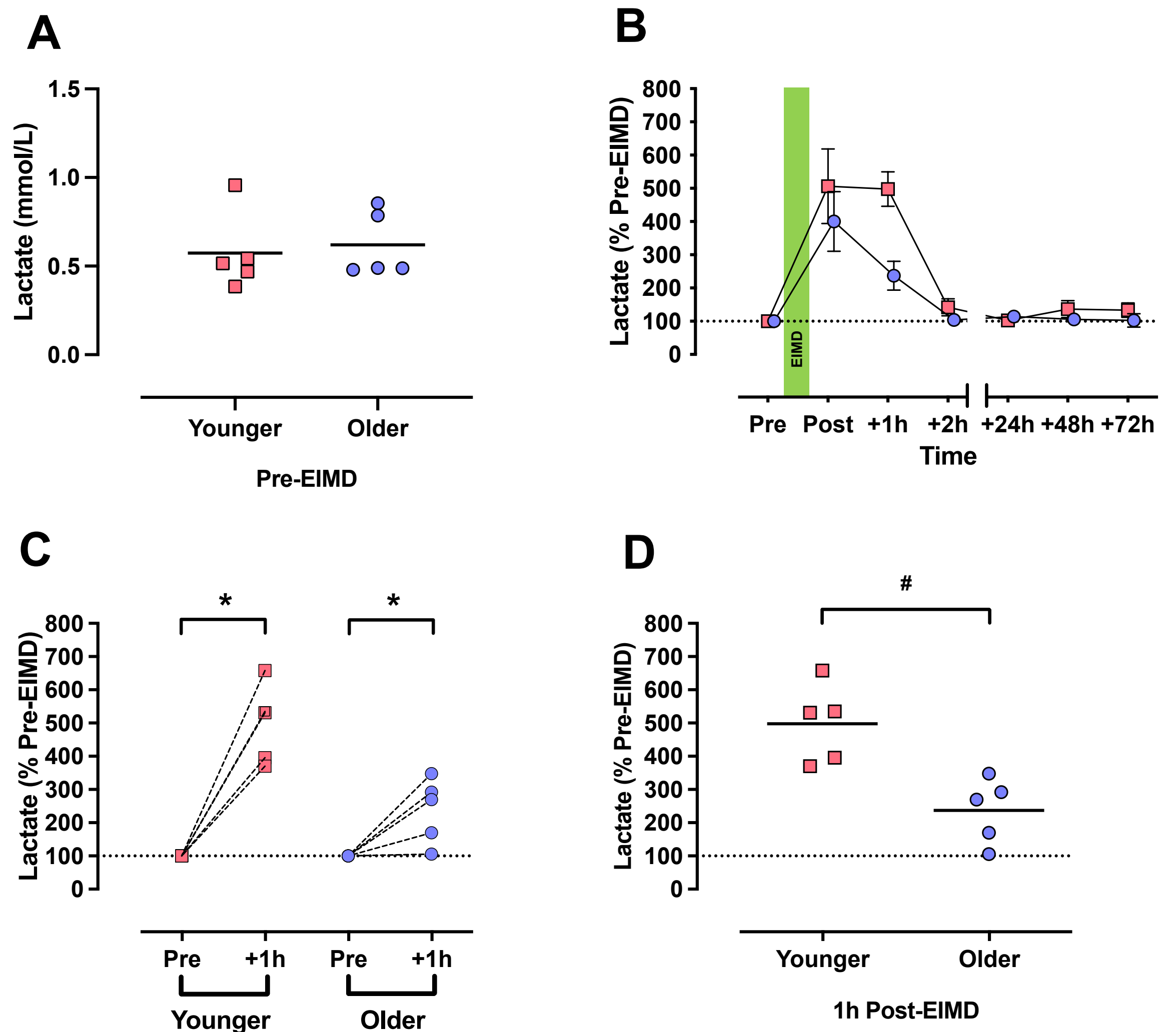


Figure 5.13 The effect of EIMD on blood lactate in younger and older participants. **A)** Basal blood lactate (mmol/L) at pre-EIMD in younger and older participants. *Horizontal line indicates group means*, **B)** Blood lactate (% change from pre-EIMD) of younger and older participants throughout the experimental period, **C)** Blood lactate (% change from pre-EIMD) between pre- and at 1 hour post-EIMD in younger and older group, and **D)** Blood lactate (% change from pre-EIMD) between younger and older group at 1 hour post-EIMD. *Data shown as individual values in graphs **A**, **C** and **D**, and as mean ±SEM in graph **B**. * indicates significant difference from Pre timepoint, # indicates significant difference between groups at timepoint indicated. The scale brake indicates from hourly testing to 24-hour intervals. Dashed horizontal line indicates 100% (pre-EIMD)*

5.4 DISCUSSION

This study examined the effect of an eccentric resistance exercise protocol on muscle damage, inflammation, function and recovery in younger and older healthy, recreationally participants. The leg press exercise induced muscle damage and elicited DOMS, as evidenced by the increased plasma CK, subjective rating of muscle soreness and thigh circumference, in both younger and older participants. The main findings of the present study show that younger group significantly differed from the older group in plasma CK activity and plasma TNF-α, suggesting a blunted resolution in older participants relative to the younger group. Whilst results demonstrate impaired MVIC, peak power and jump performance following EIMD in both groups, with older participants showing a significantly lower output, a relatively homogenous recovery was observed in both groups. In addition, the responses of plasma IL-6, blood lactate and thigh circumference were comparable, since both groups did not differ following the muscle-damaging exercise.

Further, the present study analysed EVs in blood plasma isolated during the acute phase of EIMD and during a recovery period of 72 hours. In this study, a single bout of EIMD was seen to trigger apparent changes to EV concentration and size distribution profiles, but in trained older men there is no clear differences in this EV signature from that of younger men. However, unlike prior studies on the effects of acute endurance exercise on EV release profiles, acute eccentric resistance exercise does not appear to predictably alter EV modal size or EV concentration. Moreover, immediate changes in EV profiles as observed here may associate with later changes in biological markers of muscle damage, such as CK, as found in the current study.

Biomarkers of Muscle Damage and Inflammation

No significant effect on EV profiles was observed in relation to age at pre-exercise values, with younger and older participants showing relatively homogeneous EV profile responses. Nonetheless, older participants had lesser magnitude of CK response than their younger counterparts. However, plasma CK activity was significantly elevated following EIMD in both groups. Intriguingly, older participants demonstrated less increase in plasma CK activity than their younger counterparts, which is similar to other previously reported data for young and older participants (Heckel et al., 2019). This might be due to an ageing-induced shift in muscle fibre type. Indeed, many studies have previously stated that older individuals show larger ratio of type I (or slow twitch) fibres compared with younger ones, and type II (or fast twitch) fibres which are also more susceptible to injury (Verdijk et al., 2007; Snijders et al., 2009; Nilwik et al., 2013). As a result, during microdamage, CK activity tend to be greater in younger adults. However, muscle biopsies would be required to confirm the fibre type shift.

Whilst the younger group showed a greater signal in CK response that gradually returned to pre-exercise values by 72 hours post-EIMD, the CK activity in the older group did not return to pre-exercise absolute values by the end of the recovery period. In addition, a statistically significant difference was found in CK activity percentage change between groups at 72 hours post-EIMD, with the older group showing a larger increase in CK activity (165.74%) compared to the younger group (107.39%). The response to exercise may be attenuated in older muscle rendering it less adaptive to exercise stress, suggesting a better resolution in recovery for the younger participants. Indeed, a review on ageing

human skeletal muscle in athletes suggests that ageing leads to a slower repair and adaptation to response (Fell and Williams, 2008).

Chronological ageing is associated with an increased inflammatory profile (Hager et al., 1994; Bruunsgaard et al., 1999; Toft et al., 2002; Singh and Newman, 2011), surprisingly, such findings were not evident in the present study. For example, resting levels of IL-6 were lower in elderly than younger men. In addition, previous work of Toft et. al (2002) and Brown et al. (2015) showed plasma IL-6 increased after resistance exercise, whereas herein no noticeable changes over the time were observed, with only marginal elevations of IL-6 in both groups following EIMD. This could be due to the exercise intensity and the muscle contractile activity (Ostrowski, Schjerling and Pedersen, 2000). The unilateral leg press protocol, which involved limited muscle mass, may have been insufficient to induce any significant elevations of plasma IL-6, despite large increases in CK activity; suggesting that IL-6 response to muscle damage does not make an important contribution or may not be related to muscle damage. Indeed, IL-6 may not be optimal as an EIMD marker due to the wide variation of exercise protocols, sampling times, age and participant's training status (Peake, Nosaka and Suzuki, 2005; Paulsen et al., 2012).

Hamada et al. (2005) also found a smaller accumulation of cytokines transcripts in the elderly than in the younger men after a bout of eccentric exercise. Particularly, they showed that accumulation of IL-6 transcript was not stimulated in the older participants. It could be hypothesized that the impairment of cytokine responses to EIMD are related to the blunted muscle damage reported previously

in the older group, and such attenuated inflammatory responses in seniors may lead to smaller changes in markers of muscle damage, such as CK responses.

In addition, the plasma IL-6 response was comparable in both groups, suggesting no major differences in inflammatory responses in younger and older individuals following a single bout of EIMD. A plausible reason could be that the participants of this study were recreationally active and habitually engaged in structured exercise (3-5 times per week). Therefore, the training status (trained *vs* untrained) may play an important role in acute inflammatory responses. In fact, evidence suggests that regular exercise and lifelong training improve anti-inflammatory environment via the reduction in visceral fat mass with a subsequent decreased release of adipokines (Suzuki et al., 2006; Gleeson et al., 2011; Minuzzi et al., 2017). Such findings were evident in the present study, although anti-inflammatory markers were not measured to confirm the anti-inflammatory response. Therefore, anti-inflammatory cytokines may be upregulated to local production of pro-inflammatory cytokines and limit the magnitude and duration of the systemic inflammatory response after eccentric exercise (Ostrowski et al., 1999; Peake, Nosaka and Suzuki, 2005).

With regard to plasma TNF-α, Bruunsgaard et al. (1999) demonstrated higher plasma TNF-α response in older individuals. In a similar manner as plasma IL-6 such findings were not observed in the present study, as the younger group showed higher values than the older group. Younger participants also significantly differed in plasma TNF-α over time from their older counterparts. Further, the younger group showed significant changes following EIMD. More specifically, the latter showed both significant reduction and elevation in plasma

TNF-α concentrations at 2 hours and at 72 hours respectively, relative to immediately post-EIMD. A possible reason for this may be due to a feedback mechanism as IL-6 inhibits the production of TNF-α (Frost and Lang, 2005; Philippou et al., 2018). For instance, a transient insignificant increase in the systemic levels of IL-6 at 2 hours post-EIMD was observed, which coincided with the significant down-regulation of TNF-α at 2 hours post-EIMD [Figures 5.5A(ii) and 5.6A(ii), respectively].

Nevertheless, the older group showed, again, a less pronounced change in TNF-α over the time, which is in agreement with the results of Toft et al. (2002). These findings could be related to less muscle damage or less muscular injuries, as discussed earlier, due to an ageing-related shift in muscle fibre type. Furthermore, the older group did not attain absolute values (104.40%) compared to the younger group (89.41%) by the end of the recovery period (at 72 hours post-EIMD), suggesting a blunted resolution relative to the younger group. One possible reason for these findings is that older people lack the optimal functioning of the cells to respond to additional inflammatory stimuli or a dysregulation of the immune function (Ostan et al., 2008; Franceschi and Campisi, 2014; Tieland et al., 2018).

Taken together, whilst an increased expression pattern of inflammatory mediators for the younger men was noted, the latter showed an enhanced resolution, as seen in TNF-α by the end of the experimental period compared to their older counterparts. It seems that the unilateral leg press exercise did not alter plasma concentration of IL-6 and TNF-α, however, plasma TNF-α was significantly greater after exercise (at 72 hours post-EIMD) in older adults.

Indeed, during the initial pro-inflammatory phase, a number of anti-inflammatory cytokines are secreted (Petersen and Pedersen, 2005; Fatouros and Jamurtas, 2016) and systemic levels of cytokines, such as IL-6 and TNF-α, appear to remain unaffected by EIMD. Additionally, an increased CK activity, as it was observed in the younger rather than to the older group, could be related to optimal functioning of the cell, which may decline with age, and may be not simply be a marker of less muscle damage.

Muscle Function

The lower-body resistance exercise bout used in this study induced muscle damage, evidenced by the increased perceived rating of muscle soreness following EIMD in both groups. Previous investigations have demonstrated equivocal findings on age-related differences in muscle soreness following muscle-damaging resistance exercise (Lavender and Nosaka, 2006; Chapman et al., 2008; Heckel et al., 2019). In the current study, the younger group consistently scored higher on perception of pain than the older group during the experimental period. This might have been attributed to a higher muscle damage, as indicated by the increased CK activity for the younger men. Likewise, Lavender and Nosaka, 2006 reported older males experienced lower muscle soreness than younger males following EIMD. A review by Gibson and Helme (2001) also suggested that pain perception is decreased with ageing. This could justify the lower DOMS score of the older group compared with the younger group in the present study.

Whilst significant changes were found in VAS score immediately post and at 24 hours post-EIMD within both groups, which is consistent with other findings

(Jones and Round, 1997; Jakeman, Macrae and Eston, 2009; Heckel et al., 2019), no significant difference was observed between groups during the recovery period. Similarly, Nikolaidis (2017) and Heckel et al. (2019) demonstrated no differences between age groups after lower-body resistance exercise. However, Lavender and Nosaka, (2008) found opposite findings after eccentric exercise. The contrast in research findings was attributed to the magnitude of muscle damage induced by the exercise protocol used (bilateral *vs* unilateral) or due to the different muscle group (arm *vs* leg) involved in the studies. It seems that the effect of ageing on DOMS remains ambiguous with previous studies debating on the findings due to both small sample size or the large variability (Clarkson and Hubal, 2002; Fatouros and Jamurtas, 2016; Peake et al., 2017).

It is of interest to note that although the younger group showed a larger stimulation in muscle soreness at 24 hours post EIMD, it started returning gradually to pre-EIMD values at 48 hours post-EIMD, whereas muscle soreness in the older group was still elevated at 48 hours post-EIMD and was not fully recovered by the end of the experimental period. In accordance with these findings, previous work indicated an impaired and longer recovery period post-exercise and DOMS could negatively affect exercise adherence in the elderly (Raeburn, 2004). A plausible explanation maybe that there is a general deterioration in physiological resilience in older individuals, such as the elasticity of the fibre tissue, which is highly correlated with muscle compliance (Gleim and McHugh, 1997), is diminished by advancing age (Eston, Byrne and Twist, 2003; Sherratt, 2009; Tieland et al., 2018).

Thigh circumference measurements were taken as an indicator of acute changes in thigh volume following EIMD. Thigh volume increased immediately post exercise and at 48 hours post-EIMD in both groups. This result coincided with the peaked DOMS immediately post and at 24-48 hours post-EIMD. It could be speculated that acute changes in both thigh circumference and DOMS may occur from osmotic fluid shifts or local inflammation associated with muscle-damaging exercise (Fielding et al., 2000; Eston, Byrne and Twist, 2003; Paulsen et al., 2010). As expected, lower thigh circumference was noted in older group both in pre-EIMD values and following EIMD, compared to the younger group. However, when normalized to pre-EIMD values, the older group showed a greater change in thigh volume (swelling) immediately post-EIMD (101.13%) compared to the younger group (100.37%); suggesting the older group may have been affected more than the younger group. Nevertheless, no statistically significant differences were witnessed between groups. The absence of significant differences between groups could be due to the fact that the older group was not exercise naïve. Similarly, Lavender and Nosaka (2006) found no significant differences in muscle swelling between younger and older participants.

With regard to the muscular strength, both groups demonstrated a significant suppression in leg strength immediately post-EIMD relative to pre-EIMD, suggesting the EIMD protocol successfully reduced muscle force. However, isometric force uniformly restored in both groups with a linear recovery by the end of the experimental period. A similar pattern was observed with previous studies (Ploutz-Snyder et al., 2001; Byrne and Eston, 2002a). It is reasonable to believe that knee extensors had already obtained a level of adaptation effect through daily activities (e.g., going downstairs, walking), which contain sub-maximal

eccentric muscle actions, although participants had not experienced the pure eccentric load used in the exercise. Additionally, leg muscles may be less susceptible to EIMD than arm muscles since arm musculature are less frequently exposed to eccentric load through daily activities. Hence, it may be that stimuli from daily physical activities prevented a decrease in a longer recovery in MVIC of the legs in this particular group. Therefore, the present data presented here suggest that when younger and older participants are matched for strength and activity status, ageing does not appear to impair recovery from voluntary eccentric exercise.

Nevertheless, the younger group significantly differed on MVIC over time from the older group, with the latter showing a lower strength than their younger counterparts. It has been documented that a reduction in force is observed at the single fibre and whole muscle level in older people, in addition to the pronounced muscle atrophy (Narici and Maganaris, 2006; Russ et al., 2012). Muscle mass also gradually declines in relation to muscle function in elderly (Baumgartner et al., 1998; Volpi, Nazemi and Fujita, 2004; Tyrovolas et al., 2020). Unsurprisingly, lower leg muscle mass in the older group was noted to be reduced in pre-EIMD values compared to the younger group. However, no difference in whole body muscle mass or fat mass was seen in the two populations, unlike that witnessed in wider society (Volpi et al., 2004; Barrios-Silva et al., 2018), which may be due to the physically active older population used here (Harridge and Lazarus, 2017).

In a manner similar to MVIC, pre-EIMD values of peak power were significantly lower for the older group (30.3%) compared to the younger group. These findings were mirrored by previous work of Balmer et al. (2005) who found lower power

output in older healthy trained participants compared to younger ones. Whilst younger participants were stronger, leg press exercise failed to elicit a significant change in peak power, as would have been expected, in the older group; suggesting power performance was not significantly impaired, with both groups showing a similar pattern of recovery following EIMD. A plausible reason could be that participants in this study were habitually active, and lifelong activity has been shown to slow process of sarcopenia (Marzetti et al., 2008) and associated with regulation of function (Elliott et al., 2017). Thus, any potential differences suggested here between populations may be enhanced in exercise naive younger and older individuals. In contrast, Herbert et al. (2015) reported that trained participants aged 63 years old took longer to fully recover after a single bout of intensive exercise compared to their younger counterparts aged 21 years old. The inconsistency in the outcomes may be attributed to several factors, such as the exercise protocol, biochemical adaptations in the pedal stroke Lucia et al. (1998) or glycogen depletion (Heigenhauser, Sutton and Jones, 1983).

Dynamic performance task was also examined and measured by vertical jump height. Not surprisingly, average jump performance was significantly greater in younger group than in older group throughout the experimental period. Likewise, Argaud et al. (2019) and Farrow et al. (2020) exhibited a higher jump height in younger participants compared to their counterparts. The lower jump height in elderly adults could be attributed to an explosive force reduction and to the shortening range of extensor muscles due to age-related declines (Haguenauer, Legreneur and Monteil, 2005), and to a reduction in muscle fibre size, specifically to smaller type II fibres as compared with younger adults (Tieland et al., 2018).

However, in line with muscle strength and peak power, jump performance was comparable between younger and older men following EIMD.

Taken collectively, it seems that the eccentric leg press protocol used in the present study did alter muscle function following EIMD in both groups. Nevertheless, data did not show major differences in muscle function measurements following EIMD between groups. This proposes a similar course in both younger and older groups, with age neither impairing or dampening exercise performance following EIMD. The variation between the results of the present investigation and those of previous studies with respect to recovery after eccentric exercise could be due to the diversity of the exercise protocols (i.e., arm flexors *vs* knee extensors), different intensity and duration of the exercise, different age groups or fitness/activity status and muscular adaptation to regular exercise. Tentatively, these data might suggest that regular physical activity (i.e., repeated bout effect) attenuates changes in indirect markers of muscle damage. It might have been seen a greater deficit in muscle function in naive-exercised individuals, and future work may need to examine differences in responses between naïve- *vs* highly trained-older individuals.

Extracellular Vesicles

Endurance exercise has been shown to alter EV profiles (Oliveira et al., 2020; Soares et al., 2021). Chronic exercise in murine models (3 weeks swim training) was, for example, shown to significantly increase serum EV count (Bei et al., 2017), while the modal size of EVs was unchanged. Both EV count and modal EV size were elevated in race horses following a single bout of sustained (160 km) endurance exercise (Oliveira et al., 2020), which may correlate with previous

observations of larger EVs being associated with inflammation (Slomka et al., 2018). Alternatively, in humans Fruhbeis et al. (2015) reported a significant increase in EV concentration immediately after an incremental cycling exercise to failure (typically 12-20 minutes), but EVs were found to be cleared from the circulation during the early recovery period (90 minutes after exercise). However, the concentration of plasma-EVs remained elevated after exhaustive running. In murine models, Oliveira et al. (2018) showed that 40 minutes of moderate intensity endurance exercise immediately increased EV modal size, but neither low, nor high intensity exercise had any effect on modal size. It is therefore of interest that in the current study it was observed a shift in EV modal size towards larger EVs, at 48 hours post-EIMD (supplementary Figure in **Appendix L**), albeit this trend was not statistically significant. The findings of this study are also in line with previous work of Lovett et al. (2018) who reported no significant change in EVs size or number over time after an acute muscle-damaging exercise (combination of plyometric jumping and downhill running). Thus, it may be that exercise duration, intensity and modality, in addition to differential species responses may yield variable results, and this warrants further exploration to fully understand effects of an acute exercise bout on circulating EVs. Indeed, as already noted, great individual variability is observed in human responses to various exercise modalities, and thus differing EV profile response may in part underlie differing adaptation to these modalities (Trovato et al., 2019). Alternatively, in lieu of changes to the number and morphology of circulating EVs, their transported cargo may be more relevant to the exercise response, and thus future studies may choose to examine this variable.

Unlike research on endurance models, the current literature is lacking in resistance training investigations, and specifically eccentric muscle-damaging protocols, such as those used in the current study. Whilst Cui et al. (2017) assessed three different types of resistance exercise, they reported only changes in circulating microRNAs (miRNAs), not in EV profile states. The present study provides evidence that early changes in EV profile following EIMD significantly correlate with subsequent changes in CK, and thus acute changes in EV profile post-exercise may indicate subsequent magnitude of muscle damage. Both processes may result from the mechanical EIMD stimulus (e.g., the mechanical 'stretching' of the muscle cell membrane may promote both CK and EV release). Alternatively, it is tempting to speculate that the EV response may be causative of subsequent changes in muscle damage markers, such as CK, however, such causality is not possible to ascribe with the data collected here. Outside of EIMD, other types of muscle damage, such as laser membrane ablation and cellular hypoxia, have been reported to induce rapid increases in EV release, however, these have hitherto been performed on either zebrafish (Middel et al., 2016) or mouse models (Scheffer et al., 2015), muscle tissue *ex vivo*, and thus the results presented here are the first to extend these findings into human models of muscle damage.

Furthermore, the current study sought putative age differences in EV release profiles between older and younger individuals following a bout of EIMD. Whilst the results presented here suggest no major differences in EV modal size or EV plasma concentration in younger *vs* older individuals, following a single bout of EIMD, some caution should be taken in the interpretation of these results due to the small sample size assessed and volunteer selection. The findings presented

here are with recreationally active younger and older participants, and thus are not representative of wider physically inactive Western populations (Farrell et al., 2013; Lindsay et al., 2019). Importantly also, no difference in muscle mass or fat mass was seen in this study population, unlike that witnessed in wider society (Volpi et al., 2004; Barrios-Silva et al., 2018). By studying highly active ageing cohorts we can separate physiological differences of ageing from inactivity induced changes (Harridge and Lazarus, 2017). These results, therefore, should be interpreted in light of the relatively physical trained cohort presented here. Any potential differences suggested by the results presented here between age groups may be enhanced when expanding this study to exercise naive younger and older individuals, however, this may reflect effects of long-term inactivity, not ageing per se.

Blood Lactate

EIMD or DOMS has been associated not only with mechanical, but also with alteration of metabolic factors (Tee et al., 2007; Assumpção et al., 2013; Manojlović and Erculj, 2019). A secondary aim of this work was to assess the extent to which muscle damage could affect blood lactate responses and compare blood lactate concentration between younger and older group following EIMD. Basal (pre-exercise) values of blood lactate were found to be lower in the younger group (M = 0.58 mmol/L) than the older group (M = 0.62 mmol/L), although not significantly, so confirms the literature evidencing that a lower blood lactate concentration associates with increased aerobic energy metabolism. Indeed, there is evidence that aerobic capacity declines with advancing ageing (Hollenberg et al., 2006; Tanaka and Seals, 2008; Tieland et al., 2018). Aerobic capacity suggests not only cardiovascular adaptation to oxygen transportation

but also adaptations within the muscle for oxygen usage to meet the energy demands during muscle contraction (Hollenberg et al., 2006).

Both groups presented a similar pattern response in blood lactate during the experimental period, and as expected, a peaked blood lactate concentration was seen in both groups following EIMD; suggesting an indicator related with short-term high-intensity performance (Lacour, Bouvat and Barthélémy, 1990). However, peak blood lactate was lower in older men (2.47 mmol/L) compared to their younger counterparts (2.59 mmol/L) immediately post-EIMD. Indeed, it seems that there is an age-related decrease in peak blood lactate following maximal exercise in master athletes (Benelli et al., 2007; Tanaka and Seals, 2008). In addition, the findings of the present study align with those of Manojlović and Erculj (2019), that higher blood lactate has been previously associated with higher muscle damage after EIMD. Nonetheless, blood lactate concentration declined thereafter for the older group but remained elevated for the younger group until 1-hour post-EIMD.

Furthermore, when blood lactate was normalised to pre-exercise values, the younger group exhibited a statistically greater change of blood lactate than the older group at 1 h post-EIMD, with the younger showing a larger increase in blood lactate (pre *vs* 1 hour post-EIMD) compared to the older group (>193.30% *vs* older). This could be due to an ageing-induced shift in muscle fibre type I, as stated previously. Indeed, it has been suggested that higher blood lactate concentration might be affected by an increased need on type II fibres, and consequently a corresponding shift to increased glycolytic energy production following EIMD (Gleeson et al., 1995; Braun and Dutto, 2003; Chen, Nosaka, and

Sacco, 2007). Another reason for the higher blood lactate production, as seen in the younger group, may be due to a higher muscle mass compared to the older ones, as well as to an augmented rate of lactate efflux into surrounding tissues from damaged muscles because of increased membrane permeability, as suggested by Schneider et al. (2007). This may coincide with the increased DOMS, CK efflux post-EIMD and a larger ratio of type II fibres in the younger group which are also more susceptible to injury, as discussed earlier. Additionally, as a signalling molecule, lactate may be involved in the initiation of the signalling cascade of myocyte (muscle fibres) growth process and mediate enhanced adaptations in younger than in older people (Nalbandian and Takeda, 2016).

Dietary Data

Participants' dietary intake was assessed, since diet plays an important role and may affect EIMD and recovery, and overall performance. Despite seniors having a tendency to consume slightly higher dietary intake than their younger counterparts, the main findings showed no significant differences in all macronutrients and omega-3 or omega-6 fatty acids intake between groups, suggesting both groups followed similar diet pattern and diet was not a confounding factor.

Research suggests that EIMD and inadequate energy intake have been indicated to attenuate re-synthesis of muscle glycogen following exercise (Burke, 2001; Ivy, 2004). In the present study, both groups demonstrated lower energy intakes than the average calculated estimated energy requirements (Y, M = 2780 kcal; O, M = 2486 kcal). Thus, it would be hypothesised that participants' muscle function and glycogen recovery would be impeded. However, participants' performance

or recovery was not affected by their diet or substrate deficiency as younger and older participants presented homogenous muscle function recovery or overall performance.

With regards to n-3 consumption, whilst both groups consumed lower intake than the adequate intake for male adults at 1.6 g/day (Food and Nutrition Board, IOM, 2005) younger and older participants showed comparable consumption. Thus, muscle function recovery followed similar pattern in both groups. It has been reported that n-3 intake may blunt muscle inflammation and pain following EIMD (Tarbinian et al., 2009; Jouris et al., 2011; Lembke et al., 2014; Kyriakidou et al., 2021a) and muscle mass loss (McGlory et al., 2019). However, findings on n-3 consumption and exercise-induced muscle inflammation and function remain unclear, and future studies may consider investigating different dosages and duration of n-3 supplementation to clarify the optimal amount in elderly population.

Strengths and Limitations

In the present study a validated eccentric leg press protocol was employed to elicit muscle damage and DOMS. Whilst DOMS was increased following EIMD, inflammatory markers were not found to be elevated as expected. A plausible reason could be due to the unilateral protocol, and thus a limited muscle mass used (Krzysztofik et al., 2019). Whilst this thesis has previously used a 1-hour downhill running task (**Chapter 4**), this protocol was not considered appropriate here for the older population. In combination with reported leg and back pains from participants during EIMD trial and the large subject variability due to the mechanical stress during decline grade (e.g., foot contact to the ground), a leg

press protocol was chosen with a standardised eccentric contraction. In addition, the use of standardised blood sampling time and various functional measurements in multiple time points was a strength of this study.

Nevertheless, variations of the findings between exercise studies can occur due to small sample size, different study population (healthy *vs* frail elderly or males *vs* females), training status (trained *vs* untrained) or age groups (Clarkson and Hubal, 2002; Toft et al., 2002; Peake et al., 2010; Owens et al., 2019). Thus, the small sample size of the current study and the physically trained cohort may limit true physiological patterns in both groups. However, the strict inclusion criteria made recruitment for participants difficult.

Another limitation of the present study was that no direct markers of muscle damage, for example like morphological changes in muscle cell size, were included. CK activity used as a blood muscle damage marker, which may not be an accurate reflection of a degree of muscle damage when used alone. For that reason, additional indirect biological and muscle function markers are needed for a better estimation of muscle status or the magnitude of muscle damage.

Blood lactate measurements are typically recorded during exercise and in early recovery following exercise (i.e., 3, 6, 10, 20 and 30 minutes) until 1 hour post-exercise to monitor patterns of blood lactate clearance of the accumulated blood lactate (Baron et al., 2005; Goodwin et al., 2007; Menzies et al., 2010; Wirtz et al., 2014). However, blood lactate was not part of the main objectives of this study, and thus immediate post-EIMD measurements such as the ones mentioned above were not taken. Nevertheless, it was interesting to note that

blood lactate concentration returned to the basic level within the next 2 hours post-EIMD. Regarding dietary data, participants' diets were not explicitly controlled during the 2-week experimental period, however, two 72-hour food diaries were recorded immediately before baseline visit and before pre-EIMD trial; results of which suggested participants did not change their habitual diet.

Recommendations and Future Directions

There is a large subject variability in biomarkers due to different exercise protocols (Clarkson and Hubal, 2002), such as unilateral *vs* bilateral. Therefore, a standardized validated resistance muscle-damaging protocol should be developed to improve the comparability between studies. Moreover, the findings of the current study on blood lactate highlight the need for further work on muscle damage and recovery between different age groups in larger cohorts to increase the power of the study. In addition, future studies may investigate the possible physiological reasons of blood lactate analysis during muscle-damaging exercise. This could extend the knowledge about lactate distribution and metabolism during strength training and prediction of muscle damage.

Whilst elderly people may be more susceptible to muscle damage than younger adults due to sarcopenia and chronic inflammation, and thus there is impaired muscle regeneration (Peake et al., 2010; Barberi et al., 2013); adequate energy and n-3 intake, and mode of exercise protocols may play an important role in the maintenance of muscle function capacity with ageing. Experimental work is needed to examine the effect of EIMD on hypertrophy during ageing-associated muscle mass loss to aid muscle function, leading to increase physical activity levels and perhaps even slow muscle loss with ageing. Older participants of this

study reported lower back pains by performing this specific exercise protocol used here. Future work of this Doctorate will focus on the optimisation of the muscle-damaging exercise by comparing three different EIMD protocols, aiming to provide a safer and a more realistic model that would enhance ecological validity in older people's everyday lives.

Finally, while this pilot study on EIMD has presented some interesting results in relation to EVs as putative biomarkers for muscle damage, these findings will need further validation in larger cohorts that can be guided in sample size collection by the results presented here. Future investigations should also conduct in depth analysis of EV cargo composition as this may be of considerable interest for the identification of EV-related biomarkers in EIMD. Therefore, it will be of great interest to perform full EV profiling analysis using RNA sequencing, proteomics and metabolomics to reveal the EV cargo profiles in response to EIMD, also in different age populations. Whilst EV cargo biomarkers have been implicated in the pathophysiology of inflammation-associated disorders, research regarding their role in EIMD and ageing remains limited. This study therefore provides the first insights into the potential of EV-profiling in association with muscle-damaging exercise and ageing and paves the way for future studies, aiming to extend current knowledge on their roles as mediators of health-promoting effects, and as biomarkers, associated with physical activity.

5.5 CONCLUSION

In summary, a unilateral eccentric leg press protocol induced muscle damage in both younger and older groups, as witnessed by significant changes in plasma CK, perceived muscle soreness and MVIC, with large effect sizes. Despite older people showed a blunted resolution in muscle damage and inflammation, as seen in CK and TNF-α, respectively, relative to their younger counterparts, no major differences were noted in exercise-induced plasma IL-6 between groups. Whilst declines in muscle function are commonly reported due to ageing, a similar response in MVIC, peak power, jump height and thigh circumference in both groups following EIMD was observed. Given that exercise performance was not changed in both groups this suggests that the exercise protocol of the current study may have been insufficient to induce a great magnitude of muscle damage in this recreationally trained cohort. Physical responses to eccentric exercise also induce plasma-EV changes that correlate with CK release post exercise. EV profiles did not appear to change significantly in relation to age groups assessed (active younger *vs* older), which importantly may make them a reliable biomarker to assess effects of exercise interventions across age groups. With regards to secondary aims, younger group exhibited significantly higher blood lactate concentration than their older counterparts following EIMD. EIMD seems that was not affected by the participants' diet. Even though the sample size was small, these findings may suggest that regular physical activity or lifelong training may have a beneficial effect on responses to muscle damage throughout the ageing process to support optimal health across the lifespan. As EV release has previously been associated with small animal models of muscle damage, the current study further supports that EV release profiles immediately following exercise may also play a role in the EIMD response in humans.

CHAPTER SIX

The Inflammatory and Muscle Functional Responses to Exercise-Induced Muscle Damage Amongst Three Different Exercise Protocols

(Study three)

ABSTRACT

Unaccustomed eccentric exercise is associated with a decline in force-generating capacity, muscle pain, a transient inflammatory response and may cause subsequent exercise avoidance. Whilst EIMD is well defined in the literature, the diversity of exercise protocols used for younger athletic populations may not be suited to older adults.

PURPOSE: To examine muscle damage, function and inflammatory response following three different unaccustomed EIMD protocols on lower extremity muscles.

METHODS: Twenty healthy recreationally active males (age, 27.35 ± 4.73 years) were randomised to a downhill running (DHR, n = 8), a unilateral eccentric high-intensity leg press (LGP, n = 7) or a unilateral eccentric moderate-intensity leg press and leg extension (LGP+LGE, n = 5) group. Venous plasma was collected for plasma CK, IL-6 and TNF-α immediately before and after EIMD, and 1-to-72 hours post-EIMD. MVIC, DOMS, ROM, peak power, countermovement jump, and thigh circumference were assessed at all time points except 1 and 2 hours post-EIMD.

RESULTS: A significant increase ($p < 0.05$) in CK activity was recorded in the DHR compared to the resistance groups. A significant main effect for time was observed for DOMS ($p < 0.001$), with all groups showing an increase post-EIMD. A significant interaction between group and time ($p = 0.041$) and a main effect for time ($p < 0.001$) on MVIC was noted, with DHR showing greater decrements than both resistance groups immediately post-EIMD. Similarly, a significant group by time interaction ($p = 0.037$) was seen in jump performance, with the DHR revealing greater decrements than both resistance groups post-EIMD. No differences in ROM, peak power and thigh circumference were observed amongst groups at any time point. Plasma IL-6 and TNF-α did not also differ amongst the groups at any time point (each $p < 0.05$). Immediate changes in MVIC negatively associated with later changes in CK activity ($p = 0.004$). Immediate changes in DOMS and muscle mass positively correlated to later changes in CK activity ($p = 0.035$; $p = 0.036$, respectively).

CONCLUSION: These findings suggest that the moderate-intensity LGP+LGE protocol may be a more realistic EIMD model and with a safer use to induce muscle damage with beneficial effects on EIMD responses.

Description of Chapter

This Chapter focuses on muscle damage, inflammation, muscle function, DOMS and recovery following three different lower body muscle-damaging exercises in young recreationally active males. Due to various EIMD protocols, there are equivocal findings on the effect of EIMD and its symptoms, and thus the present study explores the differences on key elements of EIMD amongst downhill running, leg press and a combination of leg press and leg extension protocols. The ultimate goal is to optimise the EIMD protocol from the previous experimental Chapters 4 and 5 in order to be utilised safely to an older population. Secondary objectives of this experimental study are to explore post-exercised blood lactate concentration, and any putative effect of participants' diet amongst these exercise protocols.

6.1 INTRODUCTION

As already discussed in previous Chapters, transient symptoms of EIMD proceeding from unaccustomed eccentric exercise can negatively affect muscle function, reduce training quality, decrease adherence to training, and potentially influence human performance or result in a higher injury prevalence. Strategies to lessen its symptoms (e.g., muscle inflammation, pain, decrements in force and power) may be useful to individuals interested in improving their exercise performance or maintaining their overall daily physical activities (Peake et al., 2017; Heckel et al., 2019). Since the 1980's, the effects of EIMD have been extensively investigated with various methods (e.g., downhill running, stair-stepping and resistance exercises on elbow and knee flexors/extensors) in both animals (McBride et al., 1995; Komulainen et al., 1998; Lovering and Deyne, 2004; Hill et al., 2018) and humans (O'Reilly et al., 1987; Nosaka et al., 2006; Yu et al., 2013; Damas et al., 2016; Owens et al., 2019); however, these investigations have produced equivocal findings. The disparities in findings may be attributed due to differences in participants' training status, age or the modality

of exercise used to induce muscle damage. Indeed, it has been found that physiological responses (i.e., inflammation, muscle function, DOMS oxygen utilisation) are influenced by the exercise modality (Peake, Nosaka and Suzuki, 2005; Fatouros and Jamurtas, 2016).

Whilst it is known that eccentric exercise induces the greatest magnitude of EIMD, its symptoms can be present immediately after EIMD and for up to several days after cessation of exercise, depending on the mode of the muscle-damaging exercise and the individual's susceptibility to the damaging stimulus (Peake et al., 2017; Owens et al., 2019). For instance, various forms of EIMD (e.g., either in their isolated form on an eccentrically biased resistance exercise or as part of the stretch-shortening cycle during downhill running) have been shown to produce multiple physiological responses with a different time course of the symptoms. With regards to muscle function, for example, Nosaka and Newton (2002) demonstrated that MVC dropped by 50% immediately after an eccentric bout of the elbow flexors and it was recovered to 75% at day 2 post-exercise. However, it was not fully recovered by the day 7. By contrast, Eston et al. (2000) showed that downhill running caused smaller force decrements (about 30% compared to high-force eccentric protocol) with a recovery time back to pre-exercise values from 4 to 7 days.

In addition, Damas et al. (2016) reported that muscle soreness seemed to increase from 8 to 24 hours following a combination of leg press and leg extension exercises with a peak between 24 and 48 hours. Previous work of Kyriakidou et al. (2021b) also showed peaks in DOMS at 24 hours in younger adults following a leg press protocol. On the contrary, Byrnes et al. (1985) showed

that muscle soreness peaked at 42 hours after 30 minutes of downhill running (-10 degrees slope). This was also supported by Chen et al. (2018) who reported peak of muscle soreness at 48 hours after a muscle-damaging protocol of the elbow flexors.

The time course of the rise in circulating CK activity seems to be highly variable and is affected by the type of the exercise. Malm et al. (2004) reported that CK activity peaked at 24 hours after downhill running and remained significantly high up to 48 hours post-exercise. Another study, however, found that CK activity peaked at 48 hours post-eccentric exercise of quadriceps femoris muscle and returned to pre-exercise values on the day 7 post-exercise (Serrao et al., 2007). Regarding inflammatory markers, previous studies (Pedersen and Febbraio, 2008; Bernecker et al., 2013) showed that plasma IL-6 was significantly increased following a marathon (e.g., up to ~100-fold), whereas Ostrowski et al. (1998a) showed only up to 25-fold increases after prolonged running (with slower duration and lower intensity) relative to pre-exercise values. Thus, it may be that exercise duration, intensity and modality, in addition to individual variability (e.g., training status, age) may underlie the variability in results seen within the literature to date. This therefore warrants further exploration on the optimisation of muscle-damaging exercise to fully understand the effects of an acute exercise bout on physiological responses and adaptation to these modalities.

In **Chapter 5**, older participants reported leg and lower back pains during the high intensity leg press exercise comprised of 70 repetitions (50 repetitions at 120% of 1RM and 20 repetitions at 100% of 1RM), and thus a new muscle-damaging exercise will be explored here for the optimisation of the EIMD protocol. The

purpose of this is to provide a safer and a more realistic model to induce muscle damage that would enhance ecological validity in older people's everyday lives.

Therefore, this study explores three different types of eccentric exercise which are susceptible to potential muscle damage. Specifically, a protocol of a downhill running, a resistance leg press protocol and a combination of leg press and leg extension exercise will be examined on human physiological responses (e.g., perceived muscle soreness, leg strength and power loss, transient muscle damage and inflammation).

6.1.1 Aims & Hypothesis

The primary aim of this Chapter was to investigate the effect of three different muscle-damaging exercise protocols on muscle damage, inflammation, functional changes, and recovery in healthy, physically active young males. Secondary aims were to compare blood lactate response, and the dietary intake amongst the three groups. Specific research objectives were to:

1. assess the differences in muscle damage and inflammation following EIMD amongst the groups
2. compare muscle function and DOMS following EIMD amongst the groups
3. investigate whether muscle functional-related changes would correlate with biological markers of EIMD, such as CK activity
4. examine the differences in blood lactate expression, and dietary intake amongst groups following EIMD

The hypothesis of this Chapter was that all EIMD protocols would induce DOMS, muscle damage and inflammation, and subsequently would attenuate muscle function. Specifically, this study hypothesized that:

1. downhill running protocol would induce greater magnitude of muscle damage and inflammatory markers following EIMD compared to both unilateral resistance protocols
2. muscle functional capacity would be different amongst groups following EIMD
3. plasma CK activity would correlate with muscle function markers (e.g., MVIC, DOMS)
4. independently of the EIMD group, all groups would have similar blood lactate response following EIMD

6.2 METHODS

6.2.1 Ethical Approval

Ethical approval for this study was provided by the College of Liberal of Arts and Sciences Research Ethics Committee, University of Westminster (ETH1819-0328). Written informed consent was obtained from all participants prior to their participation in the study (**Appendix F**).

6.2.2 Participant Recruitment

A total of 31 healthy, physically active males undertaking moderate, regular physical activity (self-reported: 3-5 times per week) aged 18-35 years volunteered to participate in this experimental study. Participants were recruited between February 2019 and March 2020 from the University of Westminster and from London health clubs.

Ten participants withdrew from the study following acceptance due to inability to attend testing commitments, injuries or cessation of data due to the COVID-19 pandemic. Hence, 21 participants volunteered to participate in the study. Of the 21 participants, one participant discontinued testing following baseline measurements due to the COVID-19 pandemic. Withdrawals and exclusions from the study are presented in Figure 6.1. Therefore, 20 participants [age 27.35 (± 4.73 years), height 179.35 (± 5.77 cm), weight 72.89 (± 7.51 kg)] were included in the analysis. Physical characteristics of participants who completed the EIMD are presented in Table 6.1. Inclusion/exclusion criteria of the participants are described in detail in **Chapter 3** (p. 90).

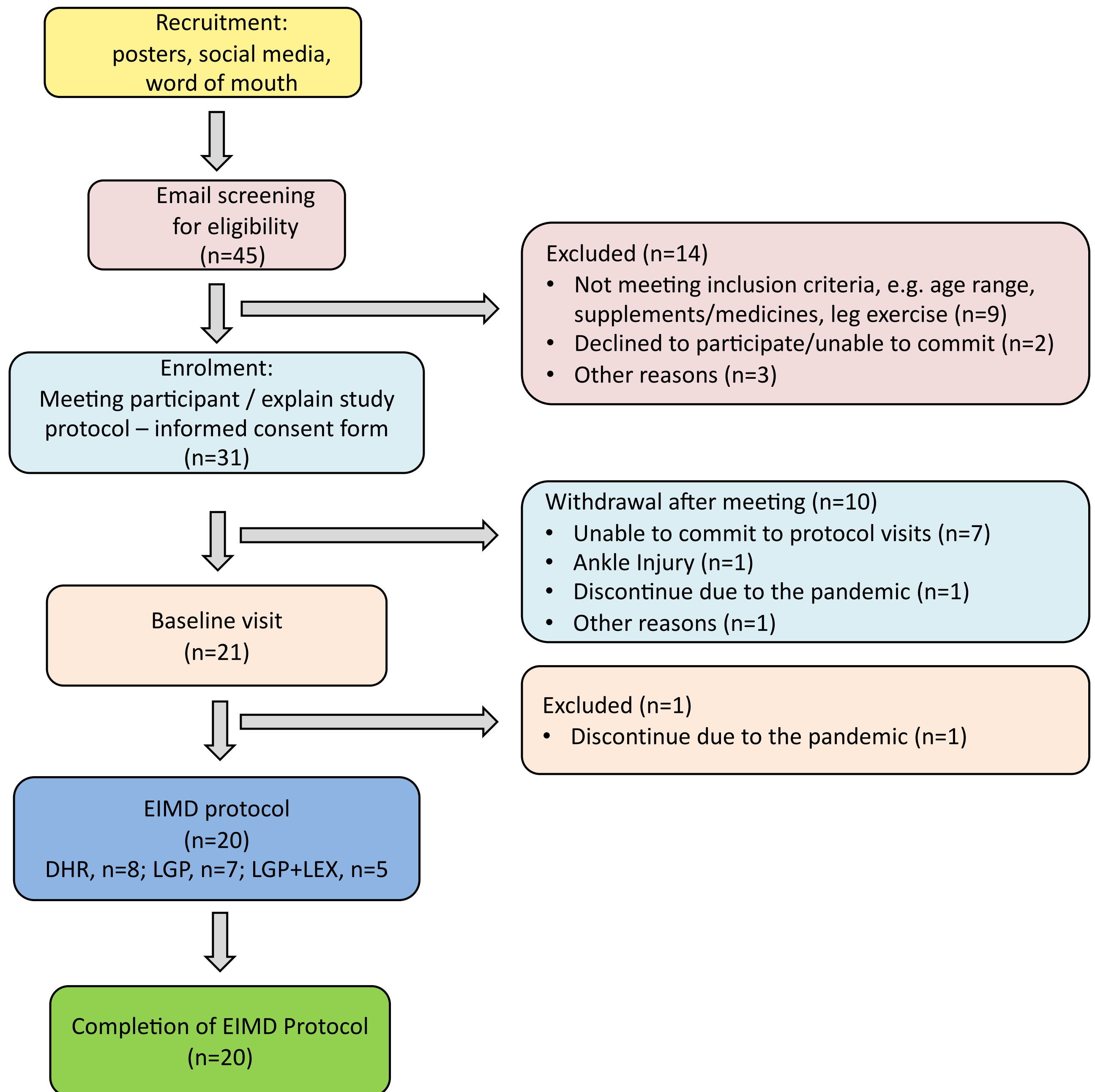


Figure 6.1 Recruitment pathway and participant withdrawals. EIMD, exercise-induced muscle damage

6.2.3 Experimental Design

Participants were randomly assigned to one of the three groups; downhill running (DHR), leg press (LGP) or leg press and leg extension (LGP+LGE) group, which examined the differences in muscle damage and inflammation, muscle function changes and recovery following EIMD. All three exercise protocols were previously designed to elicit muscle damage and DOMS on lower limb muscles (Hickner et al., 2001; van de Vyver and Myburgh, 2012; Fortes et al., 2013; Park and Lee, 2015; Vaile et al., 2008; McKay et al., 2012). All participants were required to attend the Human Performance Laboratory at the same time of day (±1 hour) in the morning at Cavendish campus, University of Westminster on 6 occasions (included initial visit for study information/informed consent form) over a 2-week period.

During visit 1, participants were informed about the study by providing them with the participant information sheet (**Appendix F**) and personally explaining all procedures, risks and benefits, and given a written informed consent form to sign. They also completed the WURSS-21 questionnaire to ensure that they were free from common cold symptoms before starting baseline measurements, and the Edinburgh Handedness Inventory questionnaire to confirm leg dominance of their daily activities, accompanied with the question ‘’if you would shoot a ball on a target, which leg would you use to shoot the ball?’’ (Veale, 2014).

On visit 2 (baseline), in an overnight fasted-state, participants performed baseline measurements to ensure familiarisation of testing equipment, and to determine 5RM on a leg press and leg extension machines and the 65% of their $\dot{V}O_2max$. The baseline visit also included a urine sample, anthropometric measurements

and a venous blood sample, ROM, perceived muscle soreness, countermovement jump, maximal isometric leg strength of the quadriceps and anaerobic peak power (all methods are fully described in **Chapter 3**).

On visit 3 (seven days later), participants performed one of the three EIMD protocols (a downhill running bout, a unilateral eccentric leg press or a unilateral eccentric leg press and leg extension protocol). All above measurements were repeated prior to (pre-EIMD) and immediately post (post-EIMD) the EIMD trial, and an additional blood sample was collected at 1 and 2 hours post-EIMD. Identical follow-up assessments were repeated at visits 4, 5 and 6 (24, 48 and 72 hours post-EIMD), except the urine sample. An overview of the study design is presented in Figure 6.2.

In addition, a 3-day food diary was provided to record all foods and drinks consumed daily during the 72 hours prior to both baseline and EIMD visits. Participants were also asked to record the WURSS-21 health questionnaire daily in the 1-week preceding the EIMD trial to ensure that they were free from common cold symptoms before the EIMD trial.

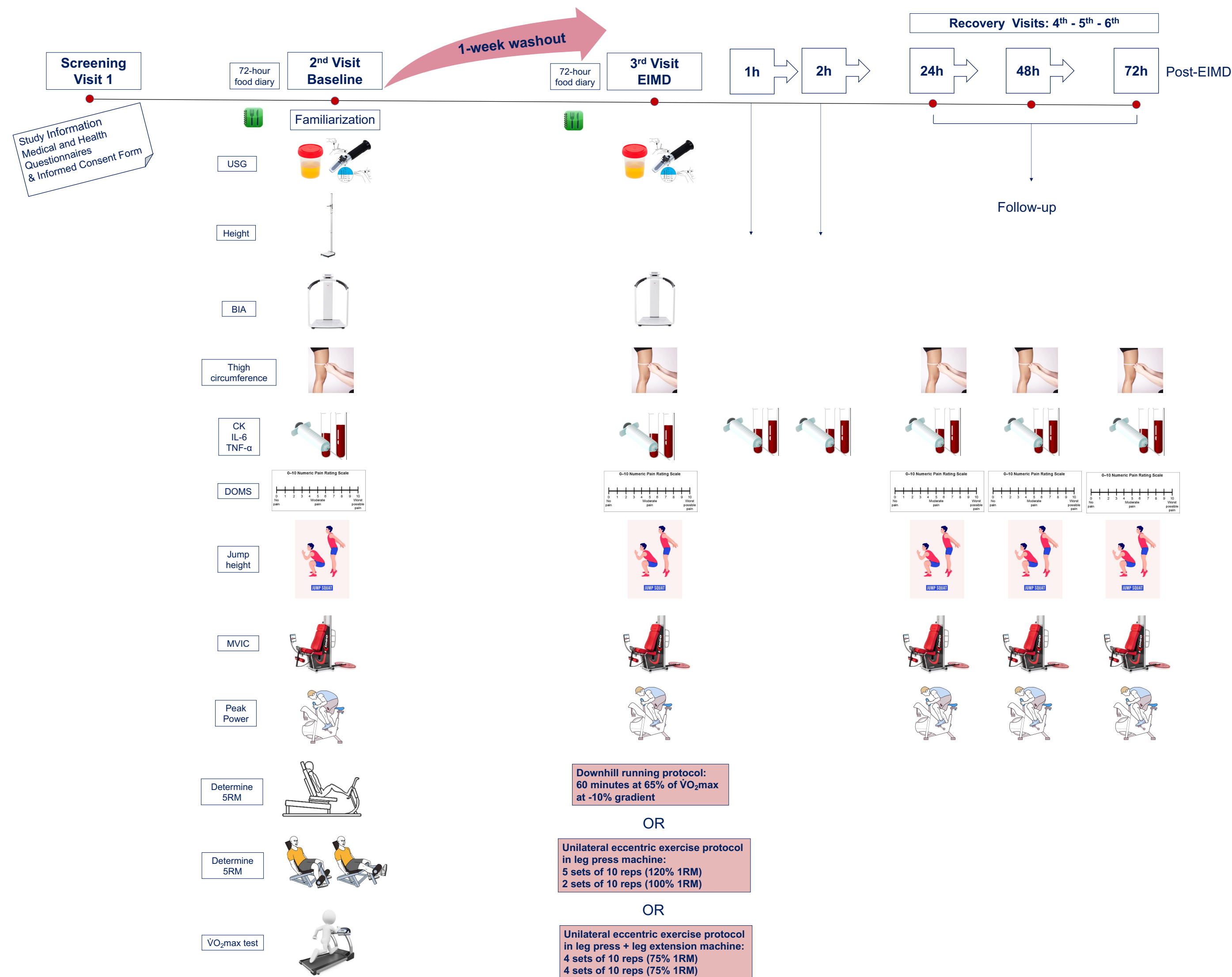


Figure 6.2 Schematic of experimental procedures. USG, urine specific gravity; BIA, bioelectrical impedance analysis; DOMS, delayed-onset muscle soreness via visual analogue scale; MVIC, maximal voluntary isometric contraction; 5RM, 5 repetitions maximum; $\dot{V}O_2max$, maximal oxygen uptake; EIMD, exercise-Induced muscle damage. *3rd visit combines both pre and post measurements, immediately prior and following EIMD stimulus, respectively.*

6.2.4 Experimental Procedures

6.2.4.1 Preliminary Testing and Familiarisation

On arrival to the Human Performance Laboratory, participants' baseline urine and venous blood sample was obtained, and anthropometric and functional measurements, such as ROM, perceived leg muscle soreness, jump height, maximal isometric leg strength and WAnT anaerobic peak power were measured in the same manner as in the **Chapter 4** and **Chapter 5** to standardise methods across all experimental studies. After the preliminary baseline measurements, participants performed a single-leg 5RM leg press and a single-leg 5RM leg extension test (Body-Solid G9S Multi Station Gym, Taiwan) on their dominant leg, and a $\dot{V}O_2max$ test. Instrumentation and procedures used in the experimental trial were presented to participants at this time.

6.2.4.1.1 Determination of One Repetition Maximum Load in Leg Press

A 5RM leg press protocol was performed at baseline visit after all baseline measurements in the same manner as in the **Chapter 5**.

6.2.4.1.2 Determination of One Repetition Maximum Load in Leg Extension

Following completion of 5RM leg press testing, a 5RM leg extension protocol was also employed to determine 1RM of the leg extension. Each participant performed 6 unilateral concentric repetitions of incremental weight until failure with their dominant leg, with 3 minutes rest between sets. Participants grasped the handles, palms facing in with hands positioned slightly wider than shoulder width. They were seated in an upright position with the lower back and hips in contact with the back rest. The height of the seat allowed for a 90-degree angle at the knees, where participants were required to keep their feet in contact with the floor.

Participants placed their ankles behind the roller pad and lifted it upward with their dominant leg until their knee was near full extension. Then, for the eccentric phase of the movement, they returned the roller pad to the starting position with both legs. 5RM leg extension predictive equation: (4.67 + [1.14 x 5RM kg]) (Abadie and Wentworth, 2000) was then applied to determine 1RM for each participant. The predicted 1RM weight lifted concentrically at baseline was then utilised to calculate 75% of the weight to be performed eccentrically at EIMD visit.

6.2.4.1.3 Determination of 65% of $\dot{V}O_2$max test

Following completion of both 5RM leg press and leg extension testing, a $\dot{V}O_2$max test was also performed. The same protocol of $\dot{V}O_2$max test and the determination of 65% of $\dot{V}O_2$max were utilised here as in the **Chapter 4** (detailed description see **Chapter 3**).

6.2.4.2 Experimental Trial

Seven days following baseline visit, all participants underwent an EIMD protocol. On the day of the experimental trial, participants reported to the laboratory [ambient temperature (T_{amp}) 20.1 ^{o}C and relative humidity (RH) 26.5%] at 08:00 am having fasted overnight. Participants were asked to consume water based on their body mass (5 mL/kg) before they reported to the laboratory to ensure they began exercise euhydrated. Hydration was verified by checking that the USG upon arrival was less than 1.028. A resting blood sample was taken before baseline anthropometric measurements and body composition. Then, muscle functional assessments, as described earlier, were performed to assess participants' pre-EIMD soreness, leg strength and peak power.

6.2.4.2.1 Muscle-Damaging Exercise

The exercise trial started at 9:00 am with standardised clothes worn by participants (e.g., t-shirt, shorts, socks and training shoes). Participants performed one of the three EIMD protocols, i) a-60-minutes downhill running at the individualised predetermined 65% $\dot{V}O_2max$ at -10% gradient (detailed description see **Chapter 4**), ii) a leg press exercise comprised of 7 sets of 10 eccentric single-leg press repetitions on a standard leg press machine, with the first 5 sets of 10 repetitions at 120% of 1RM and final 2 sets of 10 repetitions at 100% of 1RM (detailed description see **Chapter 5**) or iii) a series of leg press and leg extension exercises (McKay et al., 2012) which comprised of 4 sets of 10 eccentric single-leg press repetitions on a standard leg press machine (practises remained the same as in the **Chapter 5**) at 75% of 1RM and 4 sets of 10 eccentric single-leg extension repetitions on a standard leg extension machine at 75% of 1RM using the same leg. A timed rest period of 3 minutes took place between each set.

The leg extension exercise was also performed unilaterally (as in the leg press exercise) on each participant's dominant leg. Before performing each eccentric contraction, the weight was raised for them by the investigator after which participants lowered the weight equivalent to 75% of their predetermined unilateral 1RM. Each eccentric contraction was performed in a controlled manner for standardisation amongst the participants. It lasted 3-5 seconds (verbally given by an investigator throughout both leg press and leg extension testing), during which participants resisted the load from horizontal full knee extension to 90% angle of knee flexion. All participants completed all 80 eccentric muscle actions (8 sets x 10 repetitions per set). Water was provided ad libitum every 15 minutes.

Immediately after the EIMD bout participants sat and a blood sample was collected after a standardised period of 3 minutes (post-EIMD). Post-EIMD thigh circumferences and ROM were assessed before the participants rated their perceived muscle soreness. Then a jump height, maximal isometric leg extension and an anaerobic power test were performed to assess participants' post-EIMD strength and peak power. Participants were provided with a standardised amount of water (5 mL/kg) to replace sweat losses and a cereal bar. Participants were asked to refrain from any exercise in the 72-h follow-up period.

6.2.4.3 Diet and Activity Control

Participants were requested to maintain their habitual diet and exercise routine throughout the study. Written and verbal reminders were also provided on a regular basis to ensure diet and exercise practices were maintained throughout the study. Diet and exercise instructions were described in detail in **Chapter 3** (p. 105).

6.2.5 Measurements and Instrumentation

Anthropometric measurements were recorded at baseline, pre- and post-EIMD. Thigh circumferences were taken prior at baseline, pre-, post-, 24, 48 and 72 hours post-EIMD. A urine sample was collected prior to each trial (baseline and pre-EIMD) to assess hydration and ensure adequate hydration before exercise.

Blood sampling, for muscle damage (plasma CK) and inflammation (plasma IL-6, TNF-α) markers, and blood lactate, was obtained at baseline, pre-, post-, 1, 2, 24, 48 and 72 hours post-EIMD. All assessments of muscle function were recorded at baseline, pre-, post-, 24, 48 and 72 hours post-EIMD.

Diet diaries were provided to record all foods and drinks consumed (including days of the week and of the weekend) 72 hours both prior to baseline (familiarization visit) and the experimental trial (EIMD bout). All measurements, blood and urine sampling, diet analysis and instrumentation were described in detail in **Chapter 3**.

6.2.6 Statistical Analysis

Data were initially checked for normality with the Shapiro-Wilk test and homogeneity of variance with the Levene's test. Demographics, dietary data, blood lactate data were tested using one-way between-groups ANOVA to determine 'between group' differences. Tukey HSD test was used for *post hoc* comparisons. The EIMD effects on blood lactate within-group on two different occasions (e.g., pre- *vs* post-EIMD) were determined using paired-samples *t*-test. Exercise-induced changes in functional measurements which followed the normal distribution (MVIC, jump height, peak power, ROM and thigh circumference) were analysed using a mixed between-within ANOVA design [group (x3; DHR *vs* LGP *vs* LGP+LGE) x time (x5; pre-, post-, 24, 48 and 72 hours post-EIMD)]. A Bonferroni *post hoc* test was used to locate pairwise significant differences, where appropriate. Kruskal-Wallis test was conducted to compare differences amongst the three groups for variables which did not follow the normal distribution (CK, IL-6, TNF-α, and DOMS %change). If there was a statistically significant result, then follow-up Mann-Whitney U tests were performed to determine between which pairs of groups there were differences (e.g., DHR *vs* LGP, DHR *vs* LGP+LGE or LGP *vs* LGP+LGE). A Bonferroni-adjusted α was applied to control for Type 1 errors. The relationship between CK and functional measurements (e.g., MVIC and DOMS), muscle mass and thigh

circumference was investigated using a Pearson correlation coefficient or Spearman rank order correlation as appropriate. Values were expressed as mean ± SD for normally distributed parameters, and as median and interquartile range for non-normally distributed parameters. Statistical significance was accepted as $p < 0.05$ throughout all analyses. Effect size was calculated using methods proposed by Cohen (1988). Statistical analyses were performed using SPSS 28 software (IBM SPSS, NY, USA). All figures were generated in GraphPad Prism (Version 9, GraphPad).

6.3 RESULTS

6.3.1 Participant Characteristics

Participants' characteristics are presented in Table 6.1. Participants were reasonably homogenous. One-way ANOVA was conducted to compare physical characteristics amongst three groups. No significant difference was noted in any characteristic between groups (Table 6.1).

Table 6.1. Characteristics and comparisons amongst DHR, LGP and LGP+LGE at baseline

	DHR N = 8	**LGP N = 7**	**LGP+LGE N = 5**	**P-value**
Age (years)	27.37 (± 5.55)	27.29 (± 3.54)	27.40 (± 5.77)	0.999
Weight (kg)	73.02 (± 6.87)	73.83 (± 8.35)	71.36 (± 8.75)	0.866
Height (cm)	178.87 (± 7.30)	181.14 (± 5.46)	177.60 (± 3.21)	0.577
BMI (kg/m^2)	22.82 (± 1.42)	22.56 (± 2.60)	22.58 (± 2.13)	0.963
Body fat (%)	14.25 (± 1.50)	16.90 (± 6.88)	13.44 (± 3.95)	0.395
Fat mass (kg)	10.45 (± 1.70)	12.87 (± 6.08)	9.47 (± 3.71)	0.385
Muscle mass (kg)	30.26 (± 3.33)	29.53 (± 2.74)	29.82 (± 4.36)	0.917

Dominant leg muscle mass (kg)	6.49 (± 0.68)	6.27 (± 0.62)	6.37 (± 1.09)	0.855
Thigh circumference midpoint (cm)	53.36 (± 3.19)	52.36 (± 4.09)	52.38 (± 3.67)	0.841
$\dot{V}O_2$max (mL/kg/min)	58.75 (± 5.92)	55.19 (± 7.35)	54.49 (± 7.05)	0.464
1RM leg press (kg)	192.15 (± 40.66)	152.17 (± 20.26)	169.50 (± 56.55)	0.179
1RM leg extension (kg)	-	-	40.35 (± 14.09)	-

BMI, body mass index; $\dot{V}O_2$max, maximal oxygen uptake; 1RM, 1 repetition maximum. Values are expressed as mean ± SD

6.3.2 Dietary Data

Descriptive characteristics of the average daily nutrient intake for DHR, LGP and LGP+LGE groups are presented in Table 6.2. One-way ANOVA was conducted to compare energy, macronutrients, and n-3 and n-6 intake amongst three groups. No significant difference in CHO, protein, n-3 and n-6 intake ($p > 0.05$) amongst DHR, LGP and LGP+LGE group was noted.

There was a statistically significant difference in mean energy (kcals), $F (2, 17) = 4.38$, $p = 0.029$, with a large effect size ($\eta^2 = 0.340$), and in mean fat (g), $F (2, 17) = 5.66$, $p = 0.013$, with a large effect size ($\eta^2 = 0.400$) amongst the three intervention groups. Following Levene's test of equality of variance, the data meets critical assumptions to use the Tukey HSD test for *post hoc* comparisons. Although there was a significant difference in mean energy amongst groups in the overall ANOVA, *post hoc* pairwise comparisons suggest that DHR group did not significantly differ from either LGP ($p = 0.065$) or LGP+LGE group ($p = 0.050$). LGP group also did not differ from LGP+LGE group ($p = 0.936$). On the other hand, *post hoc* comparisons indicate that the mean fat intake for the DHR group was significantly different from both LGP and LGP+LGE groups, $p = 0.028$ and $p = 0.030$, respectively.

Further, Kruskal-Wallis test was conducted to compare n-6/n-3 ratio amongst three groups *[DHR, Md = 6.61 (5.19); LGP, Md = 7.17 (5.45); LGP+LGE, Md = 7.32 (14.24),* x^2 *(2, n = 20) = 0.04, p = 0.979]*. No significant difference in n-6/n-3 ratio amongst DHR, LGP and LGP+LGE groups was noted (Table 6.2).

Table 6.2. Average daily dietary intake of DHR, LGP and LGP+LGE groups. One-way ANOVA and Kruskal-Wallis tests comparisons amongst the three groups

	DHR N = 8	LGP N = 7	LGP+LGE N = 5	P-value
Energy (kcal)	2553.31 (± 349.94)	2153.71 (± 334.84)	2089.10 (± 217.31)	0.029*
CHO (g)	266.87 (± 48.03)	220.68 (± 53.76)	204.90 (± 49.18)	0.091
Protein (g)	98.75 (± 17.31)	98.48 (± 26.12)	106.00 (± 19.19)	0.800
Fat (g)	113.31 (± 23.39)	84.74 (± 15.04)	82.20 (± 17.08)	0.013*
n-3 (g)	1.29 (± 0.35)	1.23 (± 0.91)	0.94 (± 0.57)	0.617
n-6 (g)	7.77 (± 2.32)	5.99 (± 2.49)	4.39 (± 2.89)	0.086
n-6/n-3 ratio**	7.14 (± 3.18)	7.57 (± 3.60)	9.49 (± 9.58)	0.979

**Significant level, p < 0.05; Values are expressed as mean ± SD. CHO, Carbohydrates; n-3, omega-3 fatty acids; n-6, omega-6 fatty acids. ** indicate Kruskal-Wallis test*

6.3.3 Biomarkers of Muscle Damage and Inflammation

All markers of muscle damage and inflammation were analysed as both absolute values and percentage change from pre-EIMD to normalise individual values.

6.3.3.1 Creatine Kinase

Kruskal-Wallis test showed significant differences in plasma CK activity across groups at 24 hours post-EIMD *[(DHR, n = 8; LGP, n = 7; LGP+LGE, n = 5),* x^2 *(2, n = 20) = 10.12, p = 0.006]* and at 48 hours post-EIMD *[(DHR, n = 8; LGP, n = 7; LGP+LGE, n = 5),* x^2 *(2, n = 20) = 7.63, p = 0.022]*, with DHR group showing a greater increase in plasma CK compared to both LGP and LGP+LGE groups across time (Figure 6.3A).

More specifically, the DHR group had a greater median score [Md = 1695.75 U/L (1602.33)] than the LGP and the LGP+LGE groups at 24 hours post-EIMD, which recorded median values of 249.67 U/L (158.33) and 246.00 U/L (445.50), respectively. Pairwise comparisons were performed with a Bonferroni adjustment to alpha values for multiple comparisons. Plasma CK activity was significantly increased in DHR group compared to LGP group ($p = 0.026$, $r = 0.68$) and to LGP+LGE group ($p = 0.019$, $r = 0.76$), indicating a large effect size for both comparisons at this time point (Figure 6.3A). With regards to 48 hours post-EIMD, the DHR group also showed a greater median score [Md = 629.00 U/L (1060.08)] than the LGP and the LGP+LGE groups which recorded median values of 210.33 U/L (215.33) and 178.00 U/L (252.25), respectively. *Post hoc* pairwise comparisons indicate plasma CK activity was significantly increased in DHR group compared to LGP+LGE group ($p = 0.036$, $r = 0.70$), with a large effect size, but not to LGP group ($p > 0.050$; Figure 6.3A).

Further, when CK activity was normalised to pre-EIMD values, significant among-groups differences were observed in plasma CK activity percentage change at immediately post-EIMD *[(DHR, n = 8; LGP, n = 7; LGP+LGE, n = 5), x^2 (2, n = 20) = 13.93, p < 0.001]*, at 1 hour post-EIMD *[(DHR, n = 8; LGP, n = 7; LGP+LGE, n = 5), x^2 (2, n = 20) = 13.16, p < 0.001]*, at 2 hours post-EIMD *[(DHR, n = 7; LGP, n = 7; LGP+LGE, n = 5), x^2 (2, n = 19) = 12.02, p = 0.002]*, at 24 hours post-EIMD *[(DHR, n = 8; LGP, n = 7; LGP+LGE, n = 5), x^2 (2, n = 20) = 11.52, p = 0.003]*, at 48 hours post-EIMD *[(DHR, n = 8; LGP, n = 7; LGP+LGE, n = 5), x^2 (2, n = 20) = 10.12, p = 0.006],* and at 72 hours post-EIMD *[(DHR, n = 8; LGP, n = 7; LGP+LGE, n = 5), x^2 (2, n = 20) = 6.17, p = 0.046]*, with DHR group, again,

showing a greater increase in plasma CK compared to both LGP and LGP+LGE groups across time (Figure 6.3B).

Bonferroni *post hoc* testing suggested a significant difference between DHR and LGP group, and between DHR and LGP+LGE group immediately post-EIMD, and at 1, 2, 24 and 48 hours post-EIMD, with a large effect size for both comparisons at all time points. Despite DHR group reaching statistical significance compared to LGP group only (p = 0.021) at 72 hours post-EIMD, Bonferroni *post hoc* testing determined no statistical significance at a revised alpha level. Thus, no statistically significant difference between the groups was noted (DHR *vs* LGP, p = 0.063; Figure 6.3B). Please refer to Table 6.3 for more clarity about the pairwise comparisons that were carried out.

Table 6.3. Bonferroni *post hoc* tests for CK activity (%) amongst DHR, LGP and LGP + LGE groups at indicated timepoints

Timepoint	EIMD Group	EIMD Group	P value	r
Post-EIMD	DHR, 172.90% (80.60)	LGP, 114.13% (18.44)	0.016*	0.72
		LGP+LGE, 107.87% (6.94)	0.002*	0.95
1h post-EIMD	DHR, 211.62% (141.23)	LGP, 118.78% (26.60)	0.003*	0.84
		LGP+LGE, 118.90% (20.84)	0.014*	0.79
2h post-EIMD	DHR, 275.08% (92.15)	LGP, 120.00% (29.99)	0.007*	0.78
		LGP+LGE, 119.92% (12.30)	0.012*	0.80
24h post-EIMD	DHR, 714.30% (879.67)	LGP, 199.73% (189.22)	0.008*	0.77
		LGP+LGE, 164.57% (182.75)	0.019*	0.76
48h post-EIMD	DHR, 257.74% (334.60)	LGP, 136.80% (75.61)	0.026*	0.68
		LGP+LGE, 120.75 (92.15)	0.019*	0.76

72h post-EIMD	DHR, 187.59% (176.19)	LGP, 95.23% (46.92)	0.063	-
		LGP+LGE, 114.96% (47.34)	0.071	-

**Significant level, $p < 0.05$; Values are expressed as median ± interquartile range. DHR, Downhill running group; LGP, Leg press group; LGP+LGE, Leg press and leg extension group*

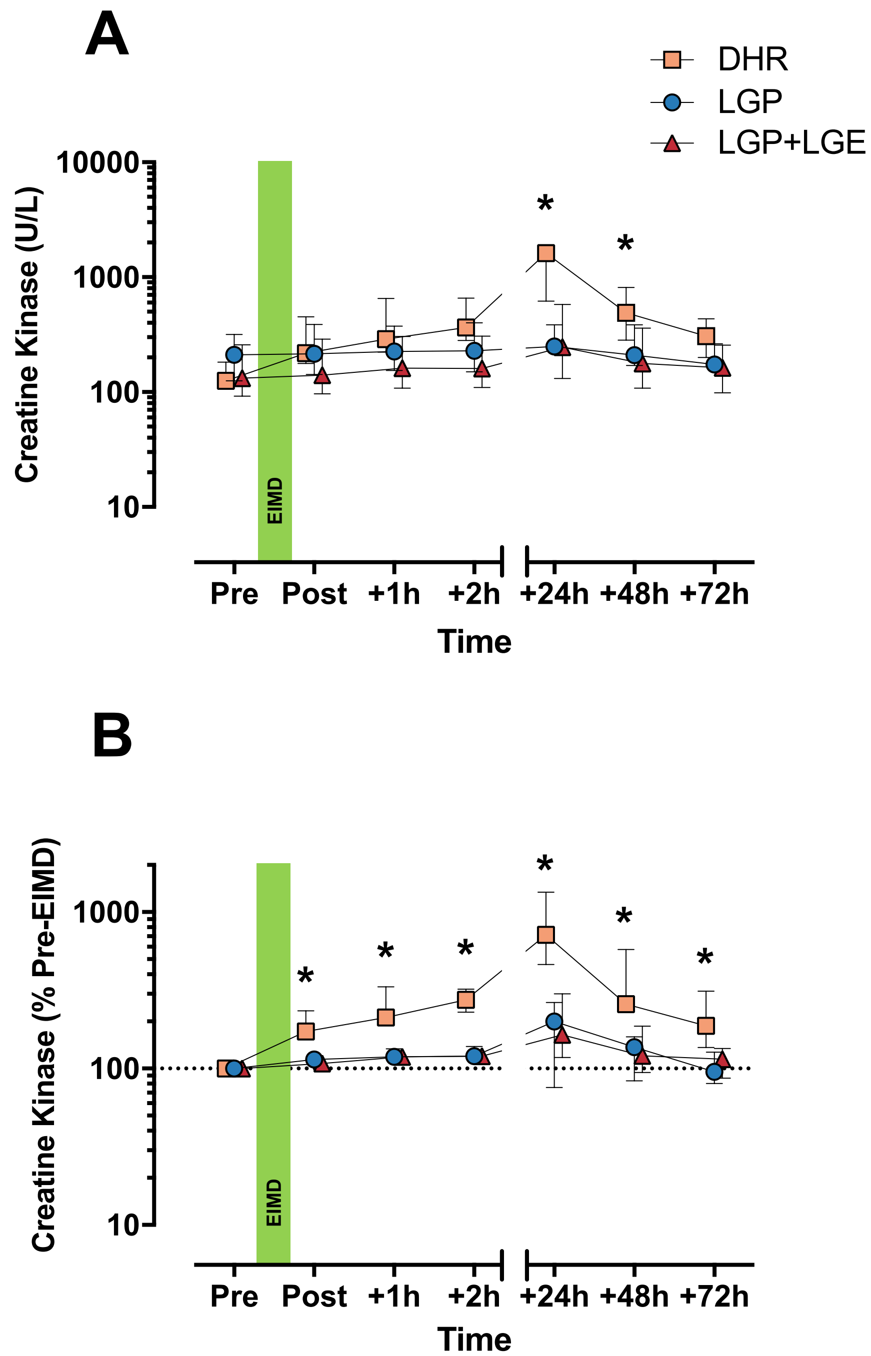


Figure 6.3 Differences in plasma creatine kinase activity amongst DHR, LGP and LGP+LGE groups. A) CK (U/L) and **B)** CK (% change from pre-EIMD) in logarithmic (log10) scale for better visualisation. *Data shown as medians, error bars indicate interquartile range. * indicates significant difference amongst groups at timepoint indicated. Dashed horizontal line indicates 100% (pre-EIMD). The scale brake indicates from hourly testing to 24-hour intervals. Time matched data points offset horizontally to enhance clarity. Y axis shown in log*^{10} *scale.*

6.3.3.2 IL-6

Kruskal-Wallis test suggested that there were no significant differences in either plasma IL-6 or percentage change amongst groups at any time point ($p > 0.05$; Figure 6.4A-B). LGP group showed a greater median than DHR and LGP+LGE groups at all time points [Figure 6.4A(i) - for visualization clarity refer to Figure 6.4A(ii)]. However, when plasma IL-6 was normalized to pre-EIMD values, the DHR group presented a minor increase at 24, 48 and 72 hours post-EIMD compared to LGP and LGP+LGE groups (Figure 6.4B).

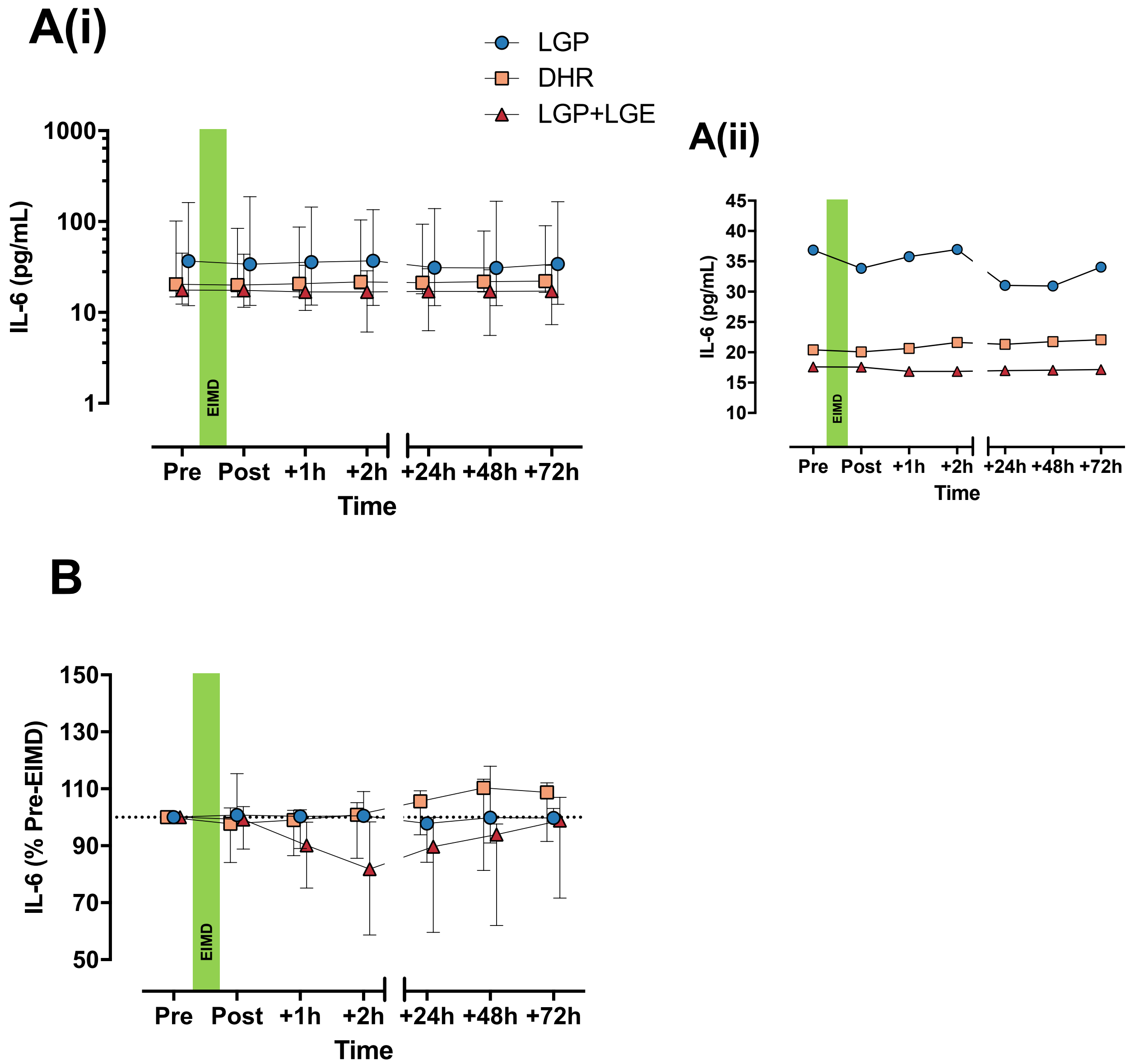


Figure 6.4 Differences in plasma IL-6 concentration amongst DHR, LGP and LGP+LGE groups. A(i) IL-6 (pg/mL) in logarithmic (log10) scale for better visualization and **A(ii)** (pg/mL), error bar free to enhance clarity. **B)** IL-6 (% change from pre-EIMD). *Data shown as medians, error bars indicate interquartile range. Dashed horizontal line indicates 100% (pre-EIMD). The scale brake indicates from hourly testing to 24-hour intervals. Time matched data points offset horizontally to enhance clarity. Y axis shown in log^{10} scale.*

6.3.3.3 TNF-α

Kruskal-Wallis test showed that there were no significant differences in either plasma TNF-α or percentage change across three groups at any time point ($p > 0.05$; Figure 6.5A-B). DHR group showed a greater median than LGP and LGP+LGE groups during the recovery period at 24, 48 and 72 hours post-EIMD [Figure 6.5A(i) - for visualization clarity refer to Figure 6.5A(ii)]. When plasma TNF-α was normalized to pre-EIMD values, the DHR group, again, presented a minor increase at 24, 48 and 72 hours post-EIMD compared to LGP and LGP+LGE groups (Figure 6.5B).

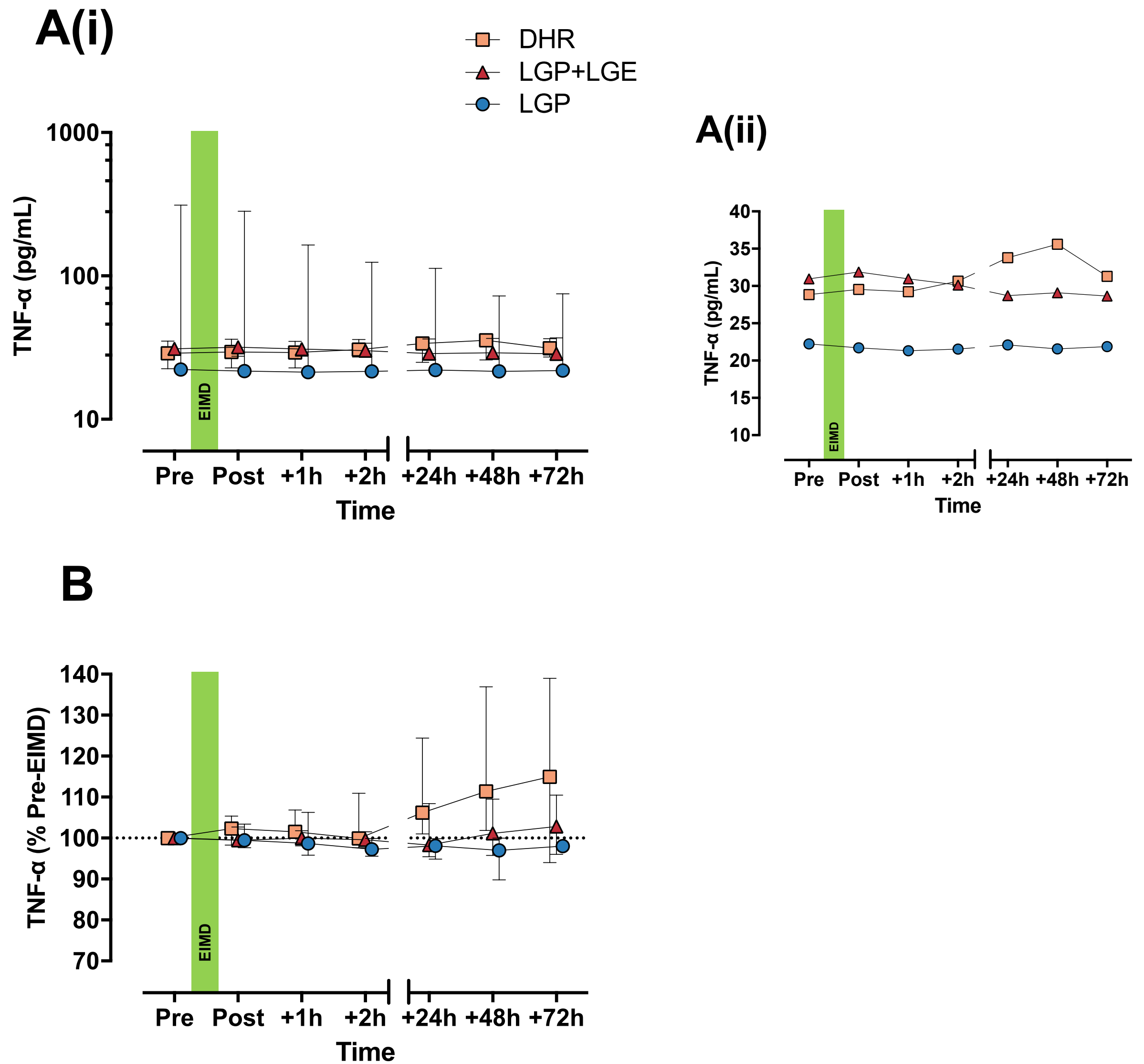


Figure 6.5 Differences in plasma TNF-α concentration amongst DHR, LGP and LGP+LGE groups. A(i) TNF-α (pg/mL) in logarithmic (log 10) scale for better visualization and **A(ii)** (pg/mL), error bar free to enhance clarity. **B)** TNF-α (% change from pre-EIMD). *Data shown as medians, error bars indicate interquartile range. Dashed horizontal line indicates 100% (pre-EIMD). The scale brake indicates from hourly testing to 24-hour intervals. Time matched data points offset horizontally to enhance clarity. Y axis shown in log^{10} scale.*

6.3.4 Assessment of Muscle Function

All markers of muscle function were analysed as both absolute values and percentage change from pre-EIMD to normalise individual values.

6.3.4.1 Muscle Soreness

A two-way mixed model ANOVA was conducted to assess the impact of three different muscle-damaging exercise interventions on participants' perceived muscle soreness across five time points (pre-, post-, 24, 48 and 72 hours post-EIMD). No significant interaction between EIMD group and time ($p = 0.189$) nor a main effect of group ($p = 0.151$) on perceived muscle soreness VAS score was noted, suggesting that all groups showed similar response in DOMS following EIMD. However, a significant main effect of time was observed for DOMS following EIMD ($p < 0.001$, $\eta^2_p = 0.835$), with a large effect size. *Post hoc* pairwise comparisons suggest that all groups had significantly elevated DOMS immediately post-EIMD *[pooled post-DOMS, 5.15 (± 2.03), p < 0.001]*, at 24 hours post-EIMD *[pooled 24 hours post-DOMS, 5.00 (± 1.74), p < 0.001]* and at 48 hours post-EIMD *[pooled 48 hours post-DOMS, 4.50 (± 2.02), p = 0.006]* relative to pre-EIMD *[pooled pre-DOMS, 2.75 (± 1.88)]* (Figure 6.6A).

Further, when DOMS was normalised to pre-EIMD values, Kruskal-Wallis test showed a statistically significant difference in perceived muscle soreness percentage change at 24 hours post-EIMD across groups *[(DHR, n = 8; LGP, n = 7; LGP+LGE, n = 5),* x^2 *(2, n = 20) = 6.45, p = 0.040]* and at 72 hours post-EIMD *[(DHR, n = 8; LGP, n = 7; LGP+LGE, n = 5),* x^2 *(2, n = 20) = 6.74, p = 0.034]* (Figure 6.6B).

More precisely, the DHR group had a greater median score *[(Md = 300.00% (207.08)]* than the LGP and the LGP+LGE groups at 24 hours post-EIMD which recorded median values of 266.67% (362.50) and 125.00% (66.67), respectively. Both DHR and LGP groups reached statistical significance ($p < 0.05$) *vs* the LGP+LGE group at 24 hours post-EIMD, *post hoc* testing determined statistical significance at a revised alpha level $p < 0.025$ ($p = 0.05 / 2$), as this involved two individual comparisons (DHR *vs* LGP+LGE and LGP *vs* LGP+LGE). Thus, no statistically significant difference was observed between groups for both comparisons, with DHR *vs* LGP+LGE, $p = 0.053$ and LGP *vs* LGP+LGE, $p = 0.094$ (Figure 6.6B).

With regards to 72 hours post-EIMD, the DHR group also showed a greater median DOMS score Md = 175.00% (241.67) than the LGP and the LGP+LGE groups, which recorded median values of 112.50% (133.33) and 75.00% (37.50), respectively. Following EIMD protocol, only the DHR group significantly differed from the LGP+LGE group at 72 hours post-EIMD, $p = 0.010$. However, *post hoc* pairwise comparisons (using Bonferroni adjusted alpha value to control for Type 1 error) determined statistical significance at a revised alpha level, $p = 0.029$ (Figure 6.6B).

Figure 6.6 Differences in perception of pain amongst DHR, LGP and LGP+LGE groups. A) VAS score, data shown *as mean, (±SD)* and **B)** DOMS (% change from pre-EIMD), *data shown as median, error bars indicate interquartile range. * indicates significant difference between groups at timepoint indicated. Dashed horizontal line indicates 100% (pre-EIMD). The scale brake indicates from hourly testing to 24-hour intervals. Time matched data points offset horizontally to enhance clarity.*

6.3.4.2 Maximal Voluntary Isometric Strength

A two-factor mixed-design ANOVA was conducted to investigate the impact of three different muscle-damaging exercise interventions (DHR, LGP, LGP+LGE) on participants' leg strength across five time points (pre-, post-, 24, 48 and 72 hours post-EIMD). There was a significant interaction between EIMD group and time on leg MVIC ($p = 0.041$, $\eta^2_p = 0.429$), with a large effect size, suggesting that the three groups showed a different response in MVIC over time. More precisely, DHR group showed a greater strength decrement than LGP and LGP+LGE groups immediately post-EIMD *[DHR pre-EIMD, M = 22.02 (± 4.90) kg; LGP pre-EIMD, M = 20.40 (± 3.00) kg; LGP+LGE pre-EIMD, M = 18.36 (± 3.10) kg; to DHR post-EIMD, M = 15.64 (± 4.73) kg; LGP post-EIMD, M = 15.98 (± 2.42) kg; LGP+LGE post-EIMD, M = 15.20 (± 3.52) kg]*. Leg strength then started to return in a linear recovery at 24 hours post-EIMD for all groups *[DHR 24 hours MVIC, M = 18.81 (± 5.61) kg; LGP 24 hours MVIC, M = 18.59 (± 2.92) kg; LGP+LGE 24 hours MVIC, M = 15.94 (± 4.58) kg]*. However, leg strength dropped again in the LGP and the LGP+LGE groups at 72 hours post-EIMD *[DHR 72 hours MVIC, M = 21.42 (± 6.18) kg; LGP 24 hours MVIC, M = 18.71 (± 3.84) kg; LGP+LGE 24 hours MVIC, M = 17.75 (± 4.06) kg]* (Figure 6.7A).

Similarly, when MVIC was normalised to pre-EIMD values, a significant interaction between EIMD groups and time ($p = 0.040$, $\eta^2_p = 0.430$) was noted following EIMD, with a large effect size. More specifically, DHR group showed a greater strength decrement change than the LGP and LGP+LGE groups immediately post-EIMD *[DHR post-EIMD, M = 70.73% (± 12.33); LGP post-EIMD, M = 78.66% (± 7.42); LGP+LGE post-EIMD, M = 82.34% (± 7.13)]* relative to pre-EIMD. Then, leg strength started to return in a linear recovery, again, at 24 hours

post-EIMD *[DHR 24 hours MVIC, 84.95% (± 12.50); LGP 24 hours MVIC, 91.41% (± 8.90); LGP+LGE 24 hours MVIC, 85.83% (± 17.35)]*, but was not fully restored by 72 hours post-EIMD in all groups (Figure 6.7B).

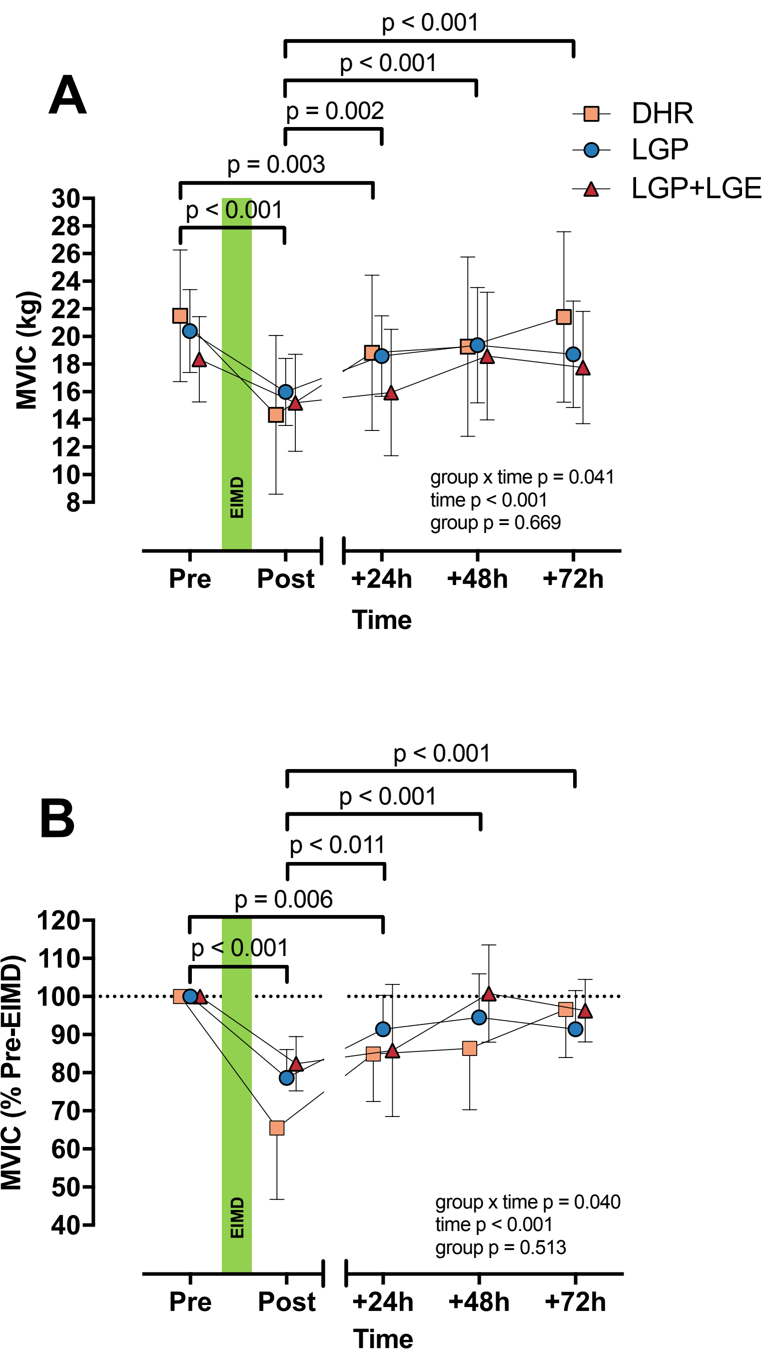


Figure 6.7 Differences in maximal voluntary isometric contraction amongst DHR, LGP and LGP+LGE groups. A) MVIC (kg) and **B)** MVIC (% change from pre-EIMD). *Data shown as mean (±SD). Dashed horizontal line indicates 100% (pre-EIMD). The scale brake indicates from hourly testing to 24-hour intervals. Time matched data points offset horizontally to enhance clarity*

6.3.4.3 Jump Height

A mixed between-within ANOVA was conducted to assess the impact of three different muscle-damaging exercise interventions on participants' jump performance across five time points (pre-, post-, 24, 48 and 72 hours post-EIMD). EIMD had a significant group by time interaction effect ($p = 0.037$, $\eta^2_p = 0.731$), with a large effect size, suggesting that the three interventions groups showed a different response in jump height over time. More specifically, DHR group showed greater mean decrements in jump height than LGP and LGP+LGE groups following EIMD *[DHR, M = 42.40 (± 3.18) vs LGP, M = 49.02 (± 2.44) vs LGP+LGE, M = 48.74 (± 2.19)]* (Figure 6.8A).

In a similar manner, when jump height was normalised to pre-EIMD values, repeated measures mixed ANOVA indicated that the EIMD protocol had a significant group by time interaction effect ($p = 0.034$, $\eta^2_p = 0.737$), again, with a large effect size. DHR group had a greater mean reduction in jump height than LGP and LGP+LGE group following EIMD relative to pre-EIMD *[DHR, M = 90.47% (± 6.56) vs LGP, M = 101.12% (± 5.11) vs LGP+LGE, M = 101.99% (± 4.69)]* (Figure 6.8B).

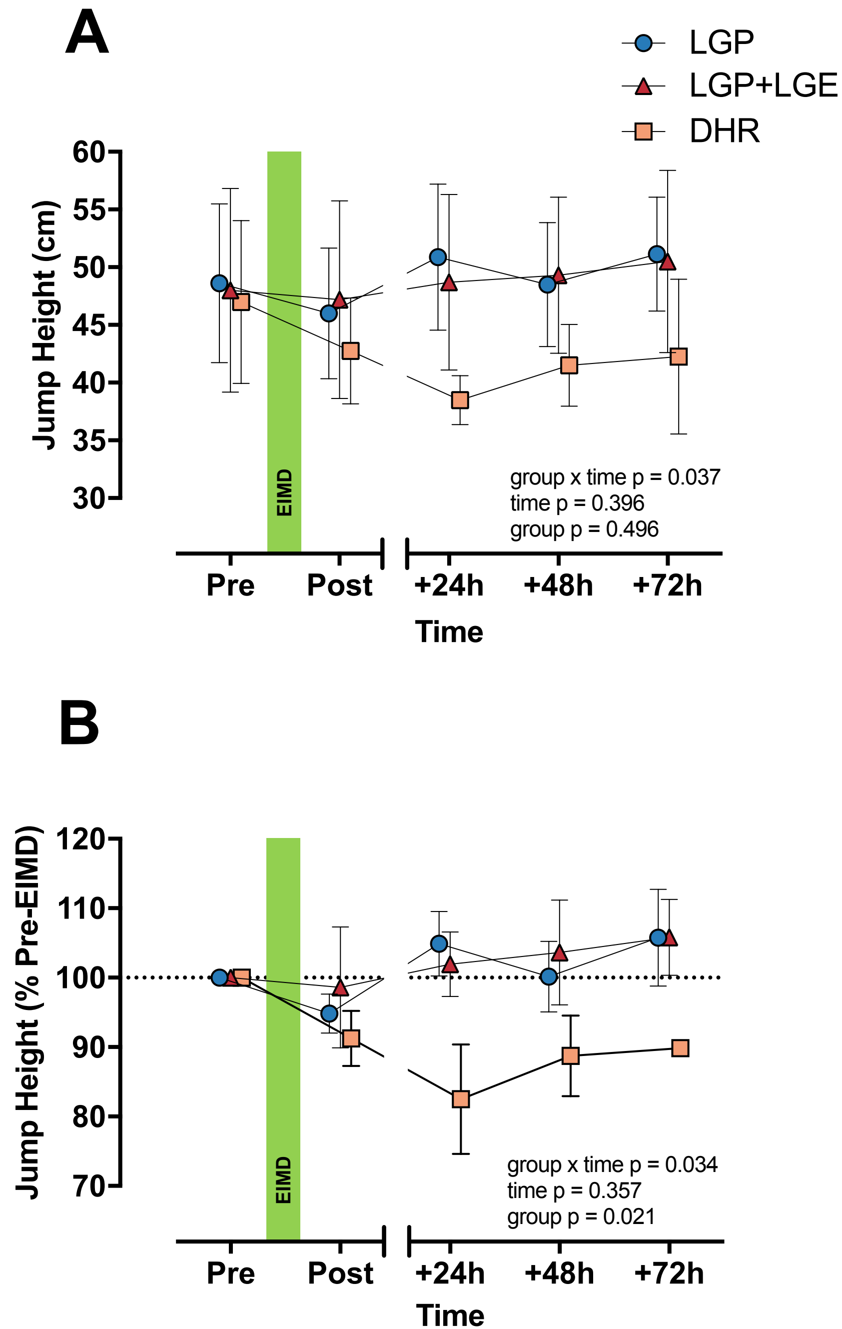


Figure 6.8 Differences in jump height amongst DHR, LGP and LGP+LGE groups. A) jump height (cm) and **B)** jump height (% change from pre-EIMD). *Data shown as mean (±SD). Dashed horizontal line indicates 100% (pre-EIMD). The scale brake indicates from hourly testing to 24-hour intervals. Time matched data points offset horizontally to enhance clarity.*

6.3.4.4 Peak Power

Repeated measures mixed ANOVA revealed no significant interaction between group and time ($p = 0.092$), nor a main effect of either time ($p = 0.193$) or EIMD group ($p = 0.543$) on peak power, suggesting that peak power output was comparable amongst three groups following EIMD (Figure 6.9A).

When peak power was normalized to pre-EIMD values, there was no group by time interaction ($p = 0.097$) or a significant time effect ($p = 0.155$) for peak power percentage change. Peak power output was also comparable amongst groups following EIMD (EIMD group, $p = 0.185$), however, data suggests that peak power was restored to pre-EIMD values by 72 hours following EIMD in the LGP + LGE group but not in the LGP and DHR groups (Figure 6.9B).

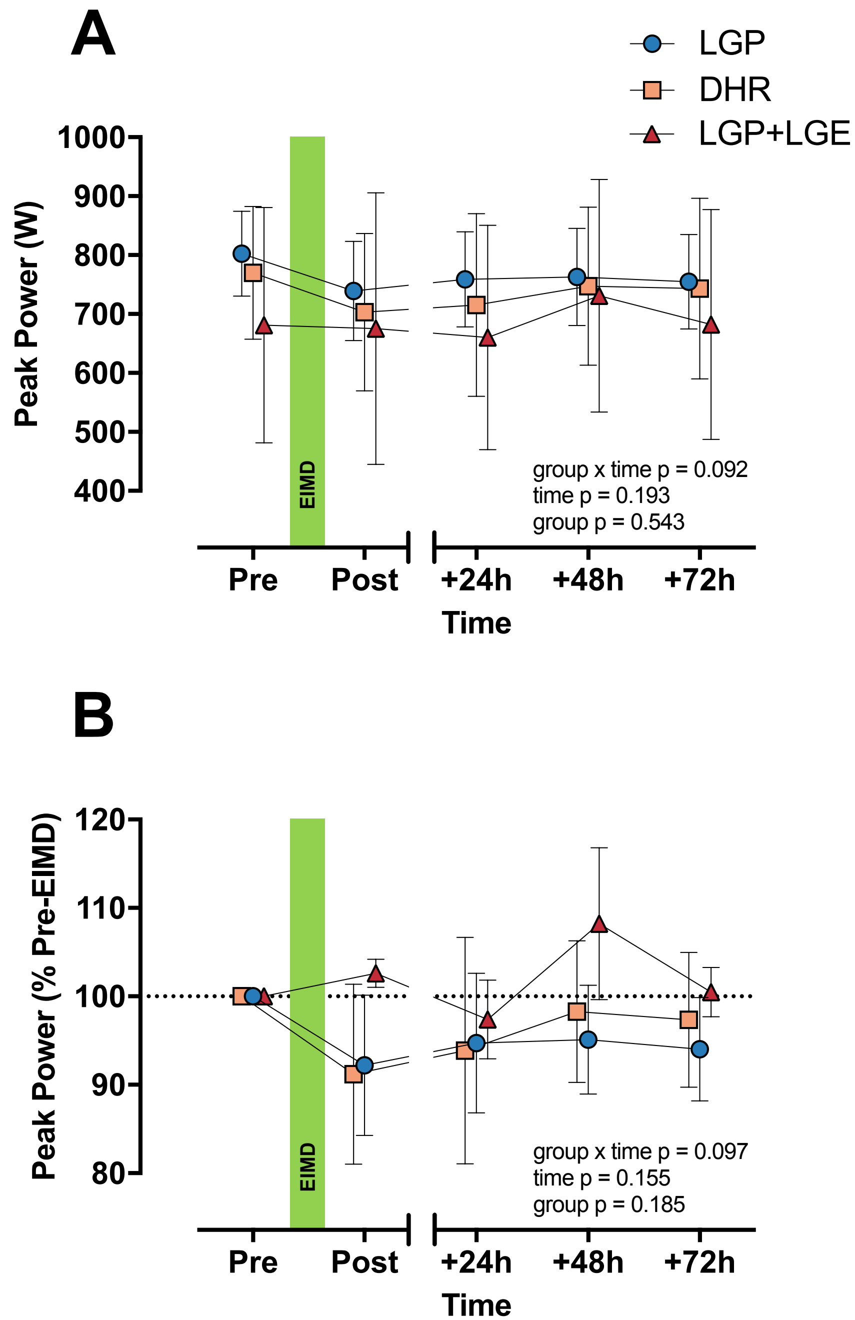


Figure 6.9 Differences in peak power output amongst DHR, LGP and LGP+LGE groups. A) peak power (W) and **B)** peak power (% change from pre-EIMD). *Data shown as mean (±SD). Dashed horizontal line indicates 100% (pre-EIMD). The scale brake indicates from hourly testing to 24-hour intervals. Time matched data points offset horizontally to enhance clarity.*

6.3.4.5 Range of Motion (flexion)

Mixed ANOVA with repeated measures showed that the three different EIMD protocols had no group by time interaction effect ($p = 0.463$), nor a main effect of either time ($p = 0.086$) or group ($p = 0.367$) on ROM, suggesting that participants' ROM was comparable amongst groups. However, LGP + LGE group showed an earlier recovery at 24 hours compared to the DHR and LGP groups which started at 48 hours post-EIMD (Figure 6.10A).

Similarly, when ROM was normalised to pre-EIMD values, no significant interaction between EIMD group and time ($p = 0.455$), nor a main effect of either time ($p = 0.081$) or group ($p = 0.308$) was found for ROM percentage change, with all groups showing a reduction immediately post-EIMD (Figure 6.10B).

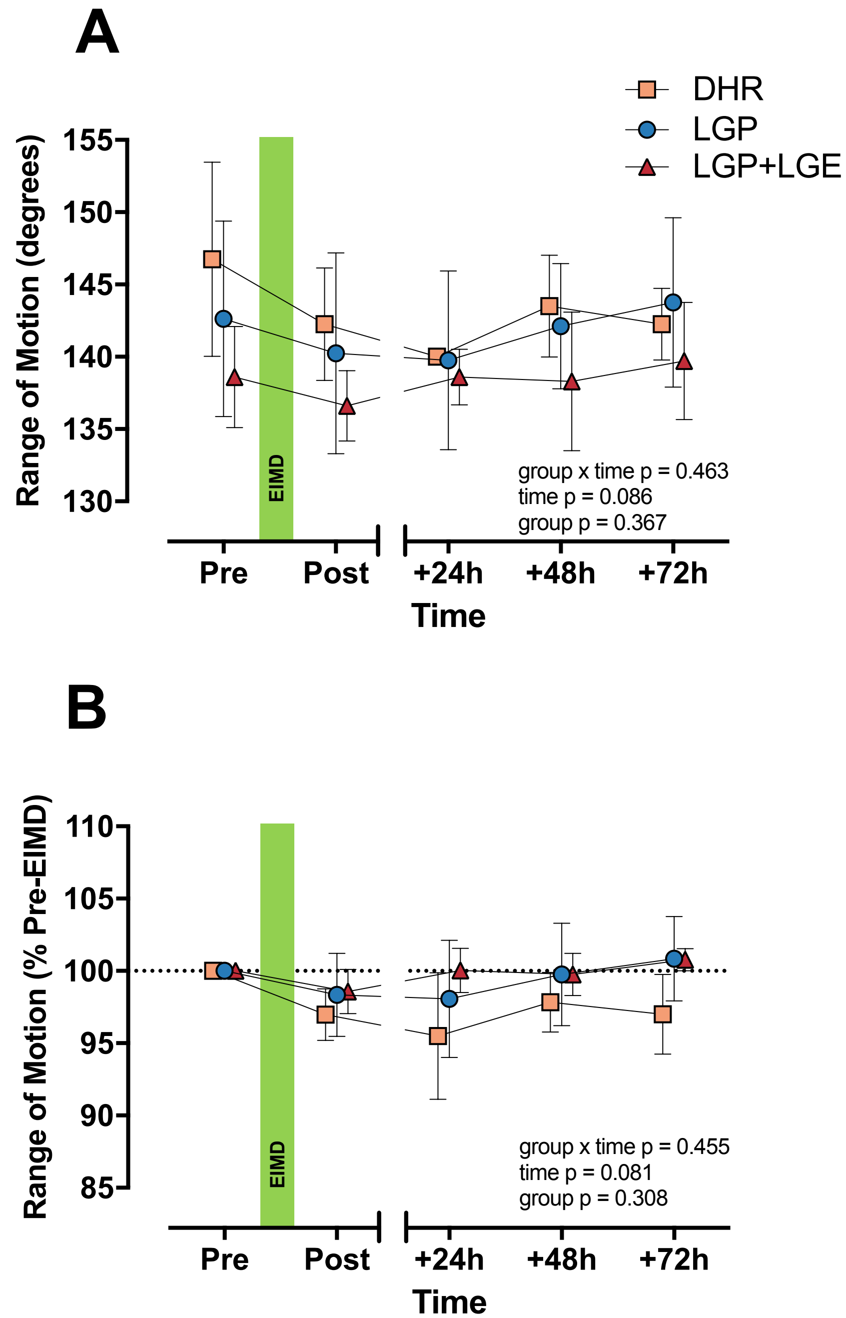


Figure 6.10 Differences in range of motion amongst DHR, LGP and LGP+LGE groups. A) range of motion (degrees) and **B)** range of motion (% change from pre-EIMD). *Data shown as mean (±SD). Dashed horizontal line indicates 100% (pre-EIMD). The scale brake indicates from hourly testing to 24-hour intervals. Time matched data points offset horizontally to enhance clarity.*

6.3.4.6 Thigh Circumference (midpoint)

Mixed ANOVA with repeated measures showed no significant interaction between group and time ($p = 0.076$) or a main effect for time ($p = 0.295$) on thigh circumference (Figure 6.11A). There was also no effect of EIMD group ($p = 0.784$).

Whilst the EIMD protocol visually appeared to induce more variability in thigh circumference percentage change, mixed ANOVA indicated that the EIMD protocol had no effect on group by time interaction ($p = 0.069$), nor a main effect of either time ($p = 0.261$) or group ($p = 0.301$) on thigh circumference following EIMD (Figure 6.11B).

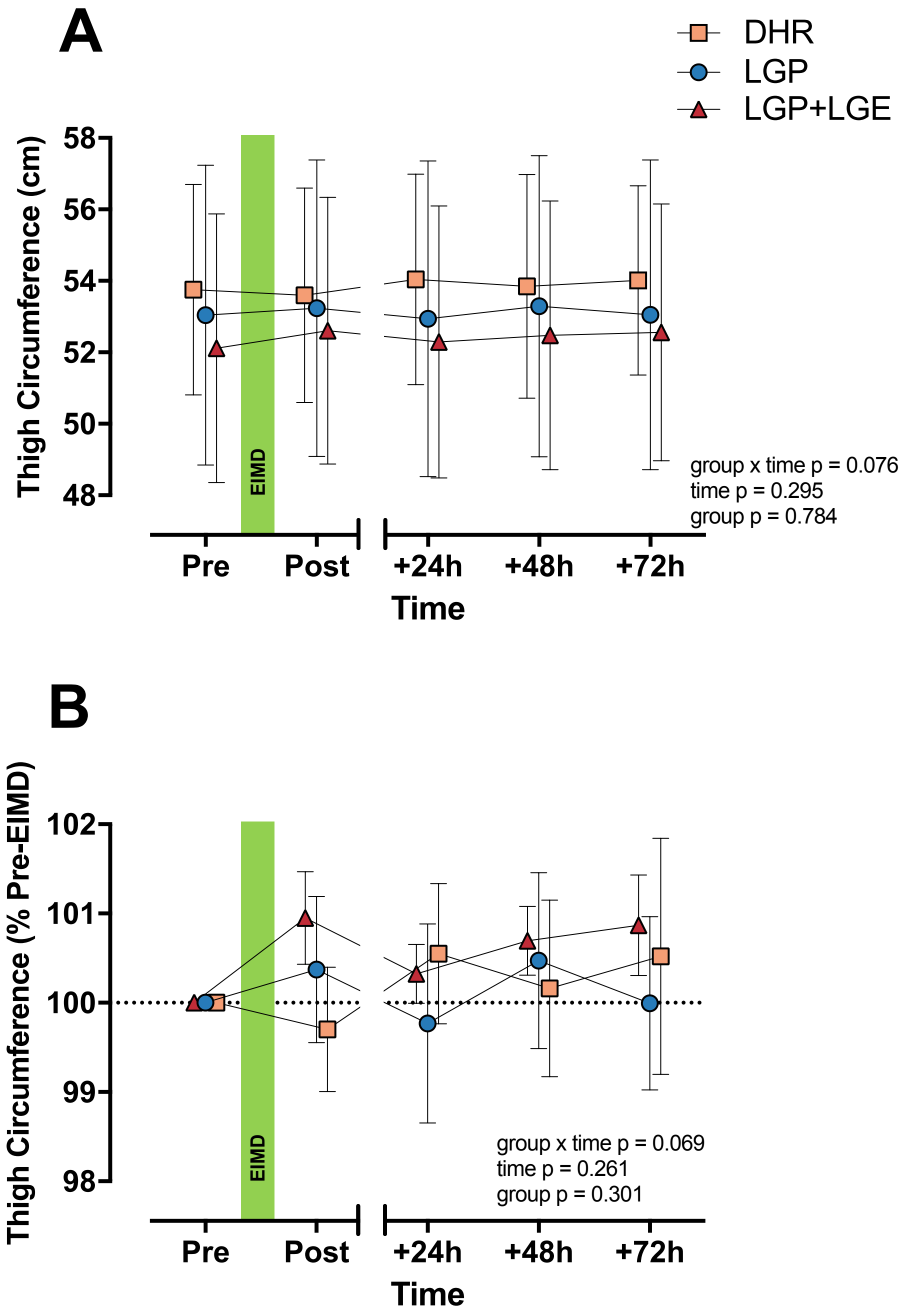


Figure 6.11 Differences in midpoint thigh circumference amongst DHR, LGP and LGP+LGE groups. A) thigh circumference (cm) and **B)** thigh circumference (% change from pre-EIMD). *Data shown as mean (±SD). Dashed horizontal line indicates 100% (pre-EIMD). The scale brake indicates from hourly testing to 24-hour intervals. Time matched data points offset horizontally to enhance clarity.*

6.3.5 Blood Lactate

One-way ANOVA showed that basal levels of blood lactate did not differ amongst DHR, LGP and LGP+LGE groups ($p = 0.799$; Figure 6.12A). Independently of the EIMD protocol, all intervention groups showed a similar pattern response of blood lactate change following EIMD, with blood lactate returning to normal values at 2 hours post-EIMD and remaining constant until the end of the recovery period (Figure 6.12B).

Following Levene's test of equality of variance, a paired samples *t*-test was conducted to evaluate the impact of the EIMD interventions protocols in the blood lactate on all groups. There was a statistically significant increase in blood lactate change from pre-EIMD *[M = 100.00% (0.00)]* to 1 hour post-EIMD *[M = 380.48% (± 110.23)]* in the DHR group [t (5) = -6.233, $p = 0.002$ (two-tailed)]. The mean increase in blood lactate percentage change was 280.48 with 95% CI ranging from -396.16 to -164.80. The eta-squared statistic ($\eta^2_p = 0.886$) indicated a large effect size. There was also a statistically significant increase in blood lactate concentration from pre-EIMD *[M = 100.00% (0.00)]* to 1 hour post-EIMD *[M = 498.13% (± 117.17)]* for the LGP group [t (4) = -7.598, $p = 0.002$ (two-tailed), with a large effect size, $\eta^2_p = 0.935$]. The mean increase in blood lactate percentage change was 398.13 with 95% CI ranging from -543.62 to -252.64, and a significant increase from pre-EIMD *[M = 100.00% (0.00)]* to 1 hour post-EIMD *[M = 403.59% (± 225.80)]* for the LGP-LGE group [t (4) = -3.006, $p = 0.040$ (two-tailed), with a large effect size, $\eta^2_p = 0.693$]. The mean increase in blood lactate percentage change was 303.59 with 95% CI ranging from -583.96 to -23.22 (Figure 6.12C).

Further, one-way ANOVA was conducted to compare blood lactate relative change at 1 hour post-EIMD amongst three groups. No significant difference in mean blood lactate percentage change amongst the three intervention groups was noted, $F(2, 13) = 0.83$, $p = 0.457$ (Figure 6.12D).

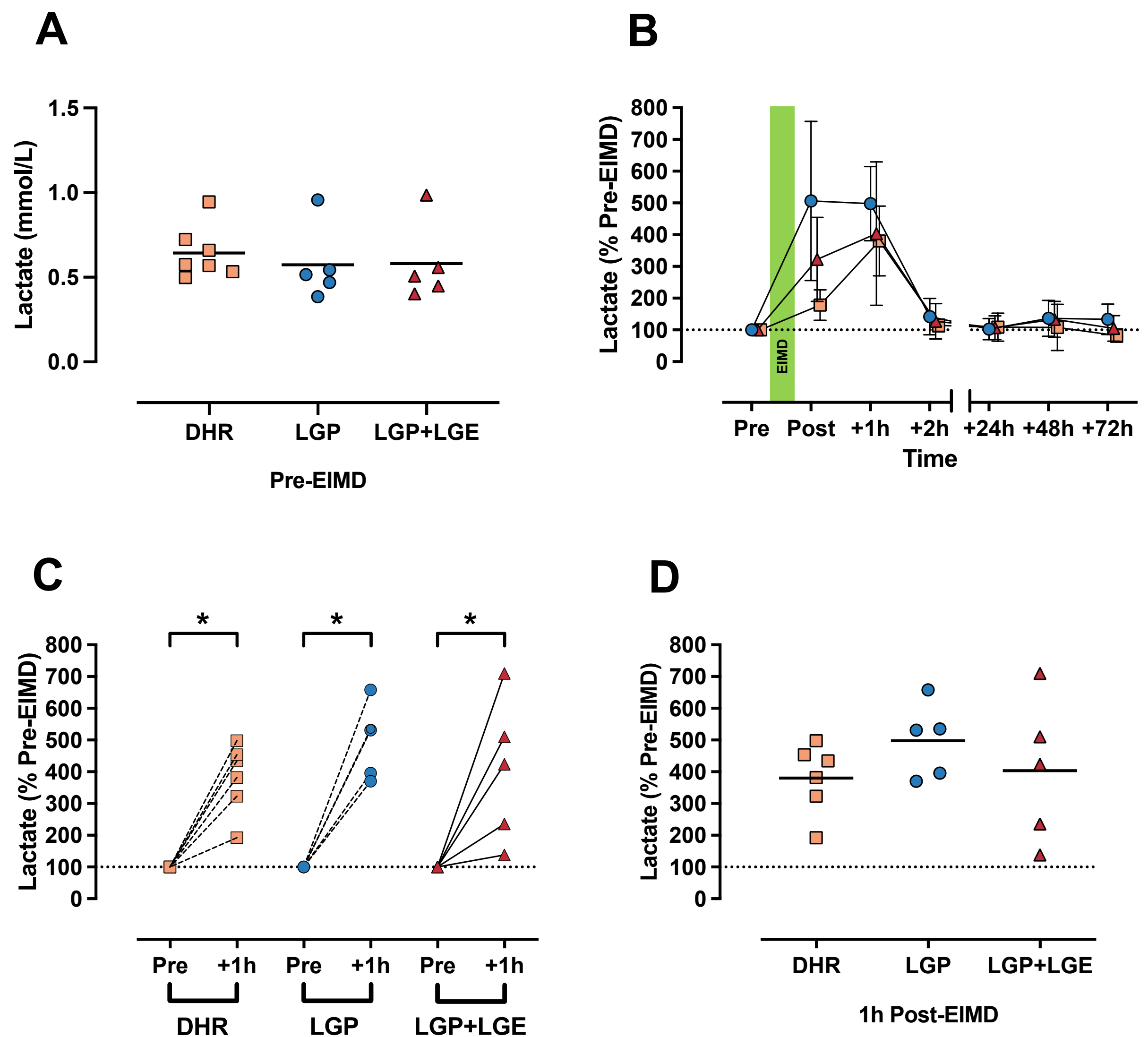


Figure 6.12 The effect of three EIMD protocols on blood lactate. **A)** Basal blood lactate (mmol/L) at pre-EIMD in DHR, LGP and LGP+LGE groups. *Horizontal line indicates group means*, **B)** Blood lactate (% change from pre-EIMD) of DHR, LGP and LGP+LGE groups throughout the experimental period, **C)** Blood lactate (% change from pre-EIMD) between pre- and at 1 hour post-EIMD in DHR, LGP and LGP+LGE groups, and **D)** Blood lactate (% change from pre-EIMD) amongst DHR, LGP and LGP+LGE groups at 1 hour post-EIMD. *Data shown as individual values in graphs **A**, **C** and **D**, and as mean ±SD in graph **B**. * indicates significant difference from Pre timepoint. The scale brake indicates from hourly testing to 24-hour intervals. Dashed horizontal line indicates 100% (pre-EIMD).*

6.3.6 Correlations

To explore a correlation between CK activity, as a biomarker of muscle damage, and functional measurements, the numerical difference in CK activity (ΔCK) between 24 hours post-EIMD and pre-EIMD was examined relative to muscle functional markers' change response (ΔMVIC and Δmuscle mass) between post-EIMD and pre-EIMD. Additionally, ΔCK was examined relative to ΔDOMS and Δthigh circumference between 24 hours post-EIMD and pre-EIMD. The time points were chosen according to the peak timepoint of each marker (Figure 6.13).

There was a strong, negative correlation between ΔCK and ΔMVIC, $r = -0.620$, $n = 20$, $p = 0.004$, with immediate changes in MVIC post-EIMD associated with later changes in CK activity at 24 hours post-EIMD (Figure 6.13A). In addition, immediate changes of muscle mass post-EIMD was shown to significantly associate positively with ΔCK at 24 hours post-EIMD, $r = 0.483$, $n = 19$, $p = 0.036$, suggesting a medium correlation between the two variables (Figure 6.13B). A significant positive association between ΔCK and ΔDOMS was also noted, rho = 0.472, $n = 20$, $p = 0.035$, again, proposing a medium correlation between these two variables (Figure 6.13C). No significant association was observed between ΔCK and Δthigh circumference, $r = 0.443$, $n = 20$, $p = 0.050$ (Figure 6.13D).

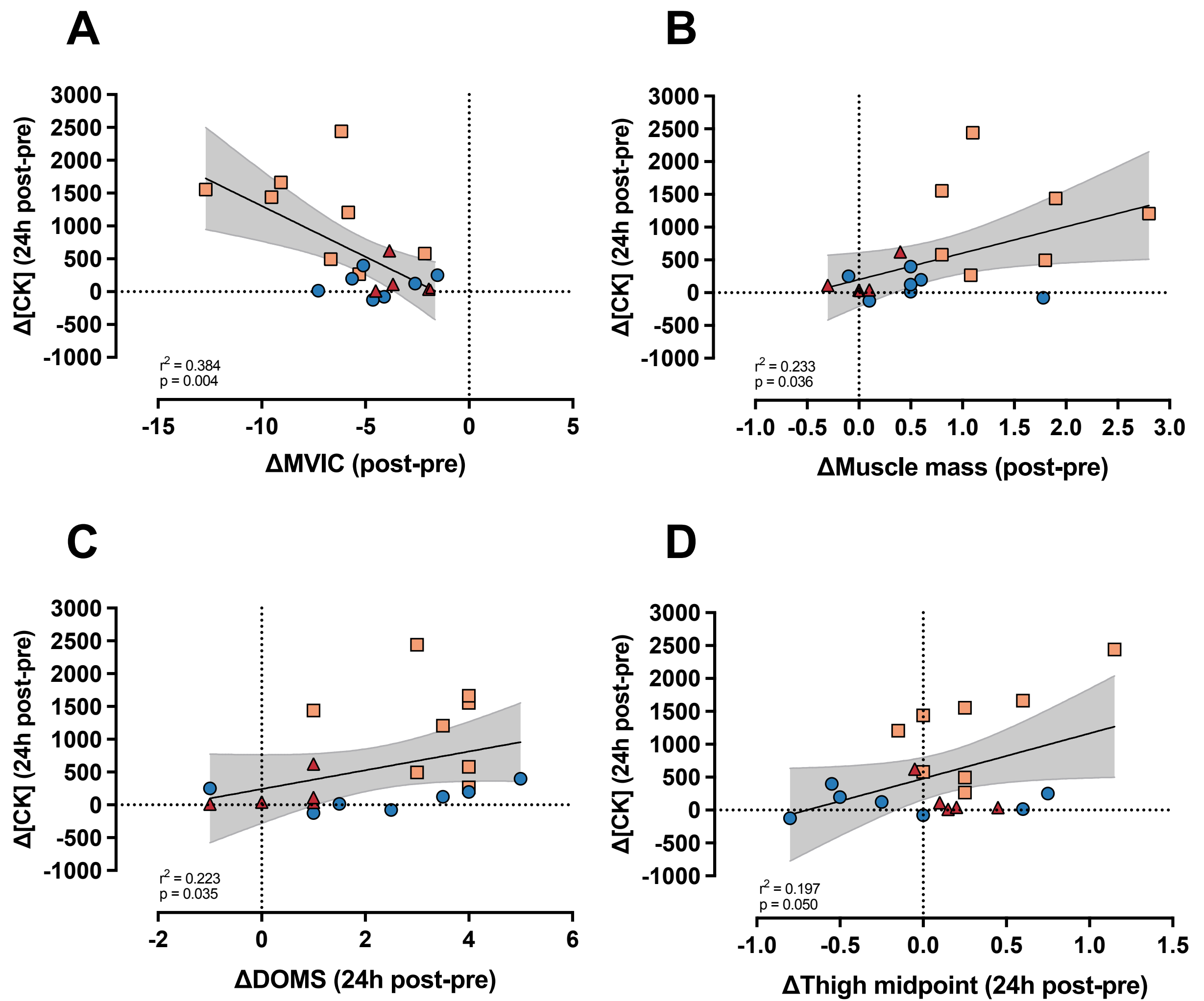


Figure 6.13 Correlations between change in CK activity and change in muscle functional markers. Correlation between change in CK activity (ΔCK, 24 hours post-EIMD – pre-EIMD) and **A)** change in MVIC (ΔMVIC, post-EIMD – pre-EIMD), **B)** change in muscle mass (Δmuscle mass, post-EIMD – pre-EIMD), **C)** change in DOMS (ΔDOMS, 24 hours post-EIMD – pre-EIMD) and **D)** change in thigh circumference (Δthigh midpoint, 24 hours post-EIMD – pre-EIMD). *Pearson correlation coefficient is shown in graphs **A**, **B** and **D,** and Spearman rank order correlation is shown in graph **C**. Grey shaded zone indicates 95% CIs. Orange squares indicate DHR group, blue circles indicate LGP group and red triangles indicate LGP+LGE group.*

6.4 DISCUSSION

The present study investigated the effect of three different eccentric exercise protocols on muscle damage, inflammation, function and recovery in young healthy, recreationally active participants. All EIMD protocols induced different magnitude of muscle damage and elicited DOMS. However, as expected, DHR group demonstrated greater changes in measures of muscle damage than LGP and LGP+LGE groups following EIMD. The main findings of the present study show that the DHR group significantly differed from both resistance groups in indices of muscle damage, such as plasma CK activity, perceived muscle soreness, MVIC and jump height. This may have occurred because downhill running is a whole-body eccentric-based model, whereas both leg press and leg extension resistance exercises are local eccentric-based models (i.e., single muscle group tested). On the other hand, both resistance exercise groups showed homogenous response and recovery in the aforementioned parameters following EIMD. Despite the changes in physical measures, neither the downhill running nor the unilateral resistance protocols altered plasma concentration of IL-6 and TNF-α. Further, immediate changes in muscle function measurements, such as MVIC and DOMS, and skeletal muscle mass, may associate with later changes in biological markers of muscle damage, such as CK, as found in the current study.

Biomarkers of Muscle Damage and Inflammation

The leakage of muscle-specific proteins (e.g., CK, Mb and LDH) into the bloodstream following vigorous exercise are considered common indirect markers of EIMD (Eston et al., 1996; Hickner et al., 2001; Overgaard et al., 2002;

Malm et al., 2004; Byrne, Twist and Eston, 2004; Peake et al., 2005; McKune et al., 2006; van de Vyver and Myburgh, 2012; Park and Lee, 2015). Plasma CK activity was significantly elevated at 24 and 48 hours following EIMD in DHR group compared with the LGP and LGP+LGE groups. These observations may suggest that muscle damage was present to a greater degree following the downhill running protocol performed in the present study. The difference in the magnitude of this elevation may be due to the two legs-running *vs* the unilateral protocols or due to the prolonged downhill running *vs* intermittent resistance exercise with rest intervals. For example, the downhill running task was performed continuously for 60 minutes, whereas the resistance exercise protocols lasted about 45 minutes with 3 minutes rest between each set. Malm et al. (2004) also observed a peaked CK activity at 24 hours after a downhill running protocol which remained significantly elevated up to 48 hours following the eccentrically biased exercise, although there were no significant changes in inflammatory markers, such as IL-6 and IL-1β. In contrast with the findings of the present study, previous studies in EIMD using resistance exercise protocols (specifically, high force eccentrically emphasised exercises) have reported to significantly increase CK activity at 24 hours (Jakeman, Macrae and Eston, 2009) and at 48 hours (Serrao et al., 2007) after EIMD.

In addition, the significantly increased CK activity, as it was observed in the downhill running rather than to the resistance exercises, could be related to the biomechanical and neuromuscular alterations that occur in running (Bontemps et al., 2020). For instance, the ground reaction impact force during downhill movement (Collins and Whittle, 1989; Gottschall and Kram, 2005) may elicit significant damage to the plantar cutaneous and knee flexion receptors because

of the repetitive foot-ground contact (Walmsley, Hodgson and Burke, 1978; Lepers et al., 1997; Hill et al., 2020), and CK may not be simply a marker of muscle damage. Finally, DHR group did not return to pre-EIMD values, with relative values at 307.80% higher compared to the LGP and LGP+LGE groups (107.40%, 111.43%, respectively) by the end of the recovery period (at 72 hours post-EIMD), suggesting a blunted resolution relative to the resistance exercise groups. Generally, individuals with low CK activity in the blood post-exercise are related to less muscle damage, as assessed by MRI (Nosaka and Clarkson, 1996; Baird et al., 2012). However, Sewright et al. (2008) found that strength loss had a negative relationship with peak CK concentrations after exercise. This is most probably due to temporal muscle disturbance or disruption rather than due to a degree of muscle damage. Likewise in the current study, CK activity was significantly negative correlated with strength loss after EIMD.

EIMD is associated with an increased inflammatory response (Bruunsgaard et al., 1997; Pedersen and Hoffman-Goetz, 2000; Toft et al., 2002; Peak et al., 2005; Smith et al., 2007; Brown et al., 2015). Surprisingly, such findings were evident only after the downhill running protocol during the recovery period. More particularly, DHR group showed a minor increase in pro-inflammatory markers (IL-6 and TNF-α) at 24, 48 and 72 hours post-EIMD relative to pre-EIMD values, whereas no noticeable changes in inflammatory markers over the time were observed in both LGP and LGP+LGE groups. This could be related to the exercise intensity (Kraemer et al., 1990; Ostrowski, Schjerling and Pedersen, 2000) or rest intervals (Kraemer et al., 1987; Machado et al., 2011) of the EIMD bout. The unilateral leg press protocol, which involved limited muscle mass compared with the downhill running protocol, may have been insufficient to

induce any significant elevations of plasma IL-6, despite larger increases in plasma CK activity; suggesting that IL-6 response to muscle damage does not make an important contribution or may not be related to muscle damage. In addition, both plasma IL-6 and TNF-α responses were comparable in all EIMD groups, suggesting no major differences in inflammatory responses amongst the three groups following EIMD.

The participants of the current study were recreationally active and habitually engaged in structured exercise (3-5 times per week). Trained people might show lesser EIMD responses (or they are less susceptible to muscle damage) *vs* exercise-naïve people (Newton et al., 2008). Therefore, the training status (trained *vs* untrained) may play an important role in acute inflammatory responses. Previous work of Toft et. al (2002) also showed that plasma IL-6 increased progressively in recreationally active individuals (1-3 times per week) after eccentric exercise, peaked at 4 hours following EIMD and then returned to baseline values at 24 hours post-EIMD. Hence, a plausible reason for the results of the present study could also be due to sampling time. As discussed in previous Chapters, IL-6 may not be optimal as an EIMD marker due to the wide variation of exercise protocols, sampling times, age and participant's training status (Peake, Nosaka and Suzuki, 2005; Paulsen et al., 2012).

Taken together, changes in inflammatory markers evoked by all EIMD bouts were inconsistent, and any putative changes were modest, despite marked increases in CK activity. More specifically, an increased expression pattern of plasma CK activity and inflammatory mediators following EIMD for the DHR group was recorded. However, the resistance exercise groups showed a less pronounced

change over the time and the mediators returned to the pre-EIMD values by the end of the experimental period compared to their DHR counterparts. These findings could be related to less muscle damage or less muscular injuries, as discussed earlier, for the unilateral resistance exercises.

Muscle Function

The degree of muscle damage has been previously investigated by various methods of muscle function. For example, a decline in muscle performance (expressed as MVIC force/torque loss) is commonly used to assess the extent of EIMD. Damas et al. (2016), who examined the response of indirect markers (e.g., ROM, DOMS, CK activity), suggested that loss of MVIC could reflect a sensitive indirect marker for muscle damage. Conversely, Nosaka et al. (2006) showed that loss of MVIC following eccentric exercise is not strongly correlated with changes in markers of muscle damage (e.g., ROM, DOMS).

In the current study, all groups demonstrated a significant suppression in leg strength immediately post-EIMD relative to pre-EIMD, suggesting EIMD was present following all exercise protocols. In line with these observations, it has been suggested that the peak reduction in MVIC generally occurs immediately post-EIMD (Eston et al., 2000; Malm et al., 2004; Nottle and Nosaka, 2007; Baumann et al., 2014; Giandolini et al., 2016; Maeo et al., 2017; Garnier et al., 2018; Lima et al., 2020). Nonetheless, DHR group demonstrated a greater strength decrement than both LGP and LGP+LGE groups following EIMD. A plausible reason may be that downhill running contains a considerable eccentric component that causes substantial muscle damage (Nottle and Nosaka, 2005; Bontemps et al., 2020), and consequently a reduction in muscle strength

(Ahmadi, Sinclair and Davis, 2008; Girard et al., 2018). The latter point is noteworthy, since there are various exercise variables (such as intensity, running speed, slope and alterations in foot strike) to achieve during the downhill running compared with the resistance exercise which has more standardised modalities (Bontemps et al., 2020). Hence, it cannot be claimed that the exact same pool of motor unit was recruited even though all muscle-damaging protocols were in the lower body.

Another reason for this finding is that the structural muscular alterations that occurred during the downhill running may have been attributed to longer muscle length (i.e., overstretched sarcomeres) that led to neuromuscular fatigue (both peripheral/central fatigue; Norbury et al., 2020; Bontemps et al., 2020). Indeed, reviews conducted by Proske and Morgan (2001) and Douglas et al. (2017) concluded that downhill running modality plays an important role in the occurrence of neuromuscular fatigue and muscle damage.

In addition, greater force reductions, occurred in DHR group compared with the LGP and LGP+LGE groups, which may be related to the increased perceived pain that was recorded for the DHR group. A recent study by Smith et al. (2020) demonstrated that maximal isometric strength in the knee extensors was reduced more rapidly when exercising with a greater muscle pain. This is also in agreement with previous experimental work (Graven-Nielsen et al., 2002; Khan et al., 2011). It is plausible that the reduction of the force-generating capacity may be related to the individual's inability to retain sufficient neural drive to maintain target force in line with the theory of the sensory tolerance limit (Hureau, Romer and Amann, 2018).

EIMD is usually accompanied by exercise-induced muscle pain (Clarkson and Hubal, 2002; Jakeman et al., 2017). Indeed, all three muscle-damaging protocols used in this study induced muscle soreness, evidenced by the main effect for time for DOMS. More precisely, DOMS was significantly increased in all groups, with perceived rating of muscle soreness peaking between immediately post-EIMD and at 24 hours after EIMD. However, previous investigations by Chen et al. (2018) have demonstrated a delayed peaked muscle soreness at 48 hours following eccentrically biased protocols.

Whilst there was no significant difference amongst the groups, DHR group consistently scored higher on perception of pain than both LGP and LGP+LGE groups throughout the experimental period. However, when DOMS was normalised to pre-EIMD values, DHR group significantly differed from both resistance exercise groups with a significantly increased perceived soreness at 72 hours following EIMD. This might have been attributed to a higher muscle damage or potential increased membrane permeability (Raja et al., 2020), as indicated by the significant plasma CK activity elevation for the DHR group. Indeed, the leakage of CK into the bloodstream is often related to the appearance of DOMS (Peake et al., 2017) and may also contribute to MVIC force loss (Peake, Nosaka and Suzuki, 2005), in which a greater loss was noted, again, in DHR.

In addition, the significantly increased perceived pain and the delayed muscle soreness recovery in DHR group compared with the two resistance groups may be related to the exercise intensity, modality and duration of the downhill running task. Consequently, it may be plausible that exercised-induced pain contributes to the fatigue process; however, this is not known yet (Norbury et al., 2022).

Further, the experience of muscle pain, by acting as an aversive stimulus, may have relevance to soreness-associated exercise avoidance (Kyriakidou et al., 2021a) or reduction in exercise intensity and performance (Norbury et al., 2022). The contrast in research findings was attributed to the magnitude of muscle damage induced by the exercise protocol used (bilateral *vs* unilateral) or due to the different muscle groups tested (arm flexors *vs* knee extensors) in the studies.

In a manner similar to MVIC, DHR group showed a different response over time, in dynamic muscle power, such as countermovement jump, showing greater and prolonged decrements in jump height than both resistance exercise groups following EIMD. More specifically, the reduction in jump performance was immediate after EIMD in all groups, however, it was further decreased at 24 hours post-EIMD and lasted up to 72 hours post-EIMD for the DHR group. This could be due to non-metabolic fatigue (i.e., muscle damage), resulting in a delayed recovery of jump height and a reduction of physical functional performance. In contrast, jump performance was affected to a lesser extent in LGP and LGP+LGE groups compared to the DHR group, starting to return in pre-exercise values at 24 hours post-EIMD. Greater jump decrements in DHR group could also be due to the different mechanical and neural control contributions, leading to muscle torque being substantially lower compared to the resistance exercise groups. Indeed, Guilhem, Cornu, and Guével (2011) and Guilhem et al. (2013) demonstrated that different modes of eccentric contractions show mode-specific biomechanical and kinematic patterns (e.g., angular velocities) which can consequently lead to different exercise-induced neuromuscular adaptations (Guilhem, Cornu, and Guével, 2011; Guilhem et al., 2013).

With regard to peak power output, all groups indicated comparable peak power performance with a similar pattern of recovery following EIMD. It is possible that this is due to participants' training level or to participants' characteristics (e.g., sex, age). Indeed, such factors play an important role in the extent of EIMD (Bontemps et al., 2020), and in exercise naïve populations or older individuals the outcome may be enhanced. Nevertheless, these data suggest that peak power was restored to pre-EIMD values by 72 hours after EIMD in the LGP+LGE group but not in the LGP and DHR groups. The exercise modality (e.g., intermittent resistance at intensity of 75% of 1RM *vs* 120% of 1RM *vs* 65% of $\dot{V}O_2max$ continuous downhill running, respectively) may have influenced the peak power output.

The time course of changes in ROM varies after EIMD, depending on the mode and intensity of the eccentric exercise (Peak et al., 2017). Indeed, such findings were evident in the present study amongst the three different EIMD protocols. More particularly, the DHR and the high-intensity LGP group showed peak loss of ROM at 24 hours post-EIMD, whereas the moderate-intensity LGP+LGE group demonstrated an early peak loss immediately post-EIMD. Nevertheless, the DHR group showed, once more, greater decrements in ROM than both resistance exercises after EIMD. On the other hand, the findings of this study showed that ROM was not significantly impaired, with all groups showing comparable ROM response following EIMD. The variation between the studies is thought to be related to the modality of the eccentrically eccentric exercise (running *vs* heavy or light resistance exercise), to the velocity of movements (Nogueira et al., 2013) or the numbers of eccentric muscle contractions (Brown et al., 1997).

Due to technique limitations in directly measuring intracellular fibre swelling, many studies (including the present study) measured muscle swelling using circumference of the exercised extremity as an indicator of acute changes in thigh volume following EIMD (Chleboun et al., 1998; Jayaraman et al., 2004; Zainuddin et al., 2005). Whilst no significant differences were witnessed amongst the three groups in thigh circumference following EIMD, interestingly, LGP+LGE group showed the greatest thigh volume amongst groups immediately post-exercise. It is suggested that this is possibly due to the exercised-muscle groups (Chen et al., 2009; Nogueira et al., 2014), which used a combination of the four quadricep muscles and *bicep femoris* muscle groups. The enlarged thigh volume could also be related to temporary oedema. However, muscle biopsies would be required to confirm the fibre swelling.

In addition, the LGP+LGE group along with the DHR group could not recover by the end of the experimental period compared with the LGP group, which was able to return to pre-exercise values. Nonetheless, the absence of any significant muscle damage post-EIMD in all groups could be due to the fact that all participants were physically active. This may also indicate that human muscle sarcolemma was not susceptible to the eccentrically biased exercise in the same way as in animal muscles that are exposed to eccentric exercise (Komulainen et al., 1998, Lovering and Deyne, 2004; Lehti, Kalliokoski and Komulainen, 2007). Indeed, previous work (Crameri et al., 2004; Paulsen et al., 2013; Yu et al., 2013) in voluntary eccentric exercise of human muscles revealed no serious injury as indicated by changes in sarcolemma integrity. Additionally, EIMD-sarcolemma in humans was mostly from mild to moderate, and thus most inflammatory reactions are temporary and reversible. For instance, in an early human study (Crenshaw

et al., 1993), only very few fibres with sarcolemma disruption were induced after ultramarathon running (mainly downhill).

Further, the present study provides evidence that early changes in muscle function following EIMD are significantly associated with subsequent changes in plasma CK, and thus acute changes in MVIC and DOMS post-exercise may indicate subsequent magnitude of muscle damage. In addition, immediate changes of muscle mass post-EIMD were significantly correlated with subsequent changes in plasma CK. Previous research also concluded that eccentric muscle contractions are associated with temporary muscle damage, reductions in muscle force, DOMS and localised swelling (Paulsen et al., 2010; Owens et al., 2019). These processes may result from the mechanical EIMD stimulus (e.g., the mechanical ‘’stretching’’ of the muscle cell membrane may promote both CK release and muscle function changes) or neural control (Franchi, Reeves and Narici, 2017). Alternatively, it is tempting to speculate that muscle function changes may be causative of subsequent changes in muscle damage markers, such as CK; however, such causality is not possible to ascribe with the data collected here. Indeed, human studies that assessed muscle fibres from MRI-guided muscle biopsies correlated significant fibre swelling with increased MRI signal intensity 48 hours after eccentric exercise (Friden, Sjostrom and Ekblom, 1981; Crenshaw Thornell and Friden, 1994; Clarkson and Hubal, 2002).

Collectively, it seems that the downhill running protocol induced temporary muscle function decrements following EIMD, featuring muscle soreness, strength and jump performance loss compared with both unilateral eccentric resistance

protocols. Therefore, it is likely participants from both resistance exercise groups had lesser magnitude of muscle damage than their DHR counterparts. It is possible that this is due to differences in the level of mechanical loading (Chen et al., 2011). Indeed, the disparity in magnitude could be due to the fact that high force eccentrically biased exercises consist of higher forces that are maintained through longer strain range (Eston, Mickleborough and Baltzopoulos, 1995). On the other hand, data did not show major differences in other muscle function measures, such as ROM, peak power and thigh measurements amongst groups. This proposes a similar course in three groups, without impairing or dampening exercise performance following EIMD.

Tentatively, these data might suggest that the combination of leg press and leg extension exercise with moderate intensity may be a relatively more palatable protocol to induce muscle damage to older individuals, however next steps will require testing this method in older individual to assess feasibility and efficacy. It was seen that a faster recovery of muscle function was followed by the latter muscle-damaging protocol compared with the other two eccentric protocols. It might also have been seen a greater deficit in muscle function in naive-exercised individuals, and future work may need to examine differences in responses between naïve- *vs* highly trained-older individuals.

Blood Lactate

Blood lactate measurement has been long used as a marker of exercise intensity and participant's fitness status (Assumpção et al., 2013; Manojlović and Erculj, 2019). The results of the present study suggested that all intervention groups, independently of the type of exercise, showed a similar pattern in blood lactate

response following EIMD, with the blood lactate returning to normal values at 2 hours post-EIMD and remaining constant until the end of the recovery period. In addition, it was observed that blood lactate was significantly increased from pre-EIMD to 1 hour post-EIMD in all groups, which may suggest an indicator related with high-intensity performance (Lacour, Bouvat and Barthélémy, 1990). Further, there was no significant difference in blood lactate change amongst groups following EIMD. Hence, it seems that blood lactate responses were not affected by the mode of EIMD protocols. By contrast, Manojlović and Erculj (2019) showed that higher blood lactate has been associated with greater muscle damage after EIMD. The findings of the current study may be due to the similar muscle mass amongst the groups (DHR, 20.26 kg *vs* LGP, 29.53 kg *vs* LGP+LGE, 29.82 kg), which could indicate similar augmented rate of lactate efflux into surrounding tissues from damaged muscles, as suggested by Schneider et al. (2007). Moreover, this may coincide with the significantly increased DOMS in all groups following EIMD, and the ratio of type II fibres in the younger individuals which are also more susceptible to injury, as discussed in the previous Chapter.

Dietary Data

Participants' dietary intake was assessed, since diet plays an important role and may affect EIMD and recovery, and overall performance. Despite DHR group having a tendency to consume slightly higher dietary intake than the resistance exercise groups, the main findings showed only significant differences in energy and fat intake, suggesting all groups followed similar diet pattern and diet was not a confounding factor. Therefore, participants' performance or recovery was not affected by their diet or substrate deficiency as all groups presented homogenous muscle function recovery or overall performance. With regards to n-3

consumption, whilst all groups consumed lower intake than the adequate intake for male adults at 1.6 g/day (Food and Nutrition Board, IOM, 2005) all participants showed comparable consumption. Thus, muscle function recovery or inflammatory response followed similar pattern in all groups. Evidence suggests that n-3 intake may blunt muscle inflammation and pain following EIMD (Tarbinian et al., 2009; Jouris et al., 2011; Lembke et al., 2014; Kyriakidou et al., 2021a). However, there are equivocal findings on n-3 consumption and exercise-induced muscle inflammation and function, and future investigations should conduct different exercise protocols with or without n-3 supplementation to clarify the optimal amount in younger and older population.

Strengths and Limitations

In the present study three validated EIMD protocols were used to elicit muscle damage and DOMS. Whilst DOMS was significantly increased following EIMD in all groups, inflammatory markers were not found to be elevated as expected. A plausible reason could be that the participants of the present study were habitually active, therefore the training status may play an important role in acute inflammatory responses. Indeed, regular exercise improves anti-inflammatory responses (Gleeson et al., 2011; Minuzzi et al., 2017). Thus, the physically trained cohort in the current study may limit true physiological patterns in all groups. Further, the unilateral eccentric protocols, which involved a limited muscle mass, may have been insufficient to induce any significant elevation of inflammatory markers (Krzysztofik et al., 2019).

Whilst this thesis has previously employed validated and commonly used protocols, such as a 1-hour downhill running task (**Chapter 4**) and a unilateral leg

press exercise (**Chapter 5**), it was warranted to optimise the EIMD protocol. The reasons that led to the protocol optimisation include the large subject variability due to the occurrence of mechanical stress when muscles lengthen during decline grade (Eston, Mickleborough and Baltzopoulos, 1995; Mizrahi, Verbitsky and Isakov, 2000; Hody et al., 2019) and the back pain reports during the leg press protocol from the older participants. Therefore, a combination of leg press and leg extension exercise with a lower intensity was deemed more appropriate, aiming to provide a safer and a more realistic model with standardised eccentric contractions that would enhance ecological validity in older people's everyday lives.

Direct assessment of EIMD (e.g., muscle biopsies) was not included in the present study to further help understand the magnitude and mechanisms of EIMD. Nevertheless, various indirect muscle damage markers of functional measurements, such as MVIC, DOMS, ROM, jump height, peak power, and thigh circumferences, have been used in multiple timepoints, which increased the potential estimation of the EIMD of this study. The findings of this study could provide further knowledge and add information to the literature on EIMD, and it may also help better understand the underlying functional responses on the EIMD.

Variations of the findings between exercise studies can occur due to the sample size, training status or exercise protocols. For instance, the small sample size of the LGP+LGE group (n = 5) compared to the DHR group (n = 8) and the LGP group (n = 7) may have affected the results of the current study. However, the strict inclusion criteria, as well as the inability to collect more experimental data

due to COVID-19 pandemic (Myers et al., 2020) made recruitment for participants difficult.

Recommendations and Future Directions

There is a large participant variability in biomarkers due to different exercise protocols (Clarkson and Hubal, 2002), such as running *vs* resistance or unilateral *vs* bilateral. Therefore, a standardised validated resistance muscle-damaging protocol should be developed to improve the comparability between studies. CK appears to form a more sensitive biomarker of EIMD following exercise, compared to inflammatory biomarkers, such as IL-6 and TNF-α.

Moreover, the findings of the current study on EIMD highlight the need for further work on muscle damage and recovery between different age groups in larger cohorts to increase the power of the study. In addition, more research is needed on EIMD and its impact on hypertrophic responses. Thus, future studies may investigate the possible physiological reasons and molecular mechanisms (i.e., translational enhancement of muscle protein synthesis) of muscle damage to produce muscular hypertrophy and structural remodelling during muscle-damaging exercise, particularly in humans. This could extend our knowledge about muscle repair and regeneration, and morphological adaptations during strength training and the prediction of muscle damage. The mode of exercise protocols may also play an important role in the maintenance of muscle function capacity with ageing. Future work is required to examine the effect of EIMD with or without n-3 supplementation in the maintenance of muscle function capacity and hypertrophy to increase physical activity levels in younger and older population.

6.5 CONCLUSION

In summary, three different eccentric exercise protocols induced mild to moderate muscle damage and DOMS in young healthy, recreationally active participants. More precisely, the downhill running was the protocol that induced more significant changes in plasma CK activity, perceived muscle soreness, MVIC and jump height than the unilateral resistance exercise protocols. No major differences were observed in exercise-induced plasma IL-6 and TNF-α amongst groups. Whilst declines in muscle function were noted in all groups, a similar response in peak power, ROM and thigh circumference following EIMD was observed. Given that exercise performance was not changed in all groups this suggests that the combination of the leg press and leg extension protocol might be sufficient to induce a greater magnitude of muscle damage in older population. With regards to secondary aims, all groups exhibited similar blood lactate concentration following EIMD, suggesting that blood lactate response was not affected by the modality of exercise protocols. All groups followed similar dietary patterns, and therefore differences in participants' performance or recovery were not likely to be affected by their diet. The findings of this study may suggest that the combination of the leg press and leg extension protocol with moderate intensity may have a beneficial effect on responses to muscle damage in naive-exercised or older individuals to support optimal health across the lifespan.

CHAPTER SEVEN

General Discussion

Description of Chapter

This Chapter will discuss the main findings of the three experimental studies, addressing the objectives of each study and results in relation to the literature on muscle damage, inflammation, and muscle function). Comparisons, such as consistent trends between the Chapters, will also be presented. Methodological strengths and limitations will be discussed. Practical applications and the subsequent recommendations will be made for future work, along with the final conclusions of this thesis.

7.1 INTRODUCTION

The overall aim of this Doctorate was to explore muscle damage, inflammation, muscle function, DOMS and recovery following a lower body muscle-damaging exercise in younger or older recreationally active males. Research has predominantly investigated these responses in the younger population, demonstrating negative consequences even amongst well-trained individuals, (i.e., professional athletes, soldiers or miners); such as muscle soreness, reduced strength and power which may compromise recovery and overall performance (Jouris et al., 2011; Paulsen et al., 2012; Hyldahl and Hubal, 2014; Ives et al., 2017; Wan et al., 2017; Heckel et al., 2019; Owens et al., 2019).

Therefore, the aims of this thesis were to (i) examine the efficacy of n-3 supplementation on EIMD in healthy physically active young adults, (ii) investigate muscle damage, inflammation and muscle function measures following EIMD between healthy physically active younger and older adults and (iii) compare three different EIMD exercises on muscle damage, inflammation and muscle function in healthy physically active young adults to optimise the EIMD protocol from the two previous experimental studies in order to ensure that the

protocol is feasible to be used to an older population. Three studies were conducted (**Chapter 4**, **5 and 6**) with biomarkers and muscle function measures as primary dependent variables common to all three studies.

Chapter 4 had 3 main objectives: (i) to assess 4 weeks of 3 g/day n-3 supplementation on muscle damage and inflammation following EIMD in healthy physically active young male adults, (ii) to assess muscle function and DOMS following muscle-damaging exercise and (iii) to examine triglycerides concentration after 4 weeks of 3 g/day n-3 supplementation. The main objectives of **Chapter 5** were (i) to assess muscle damage and inflammation between younger and older participants following EIMD, (ii) to compare muscle function and DOMS following EIMD between younger and older participants and (iii) to investigate the effect of EIMD on circulating EV profiles in younger and older participants. Finally, the objectives of **Chapter 6** were (i) to assess the differences in muscle damage and inflammatory response following downhill running, leg press and a combination of leg press & leg extension, (ii) compare muscle function and DOMS between the three EIMD groups and (iii) investigate correlations between muscle functional-related changes and biological markers of EIMD, such as CK activity. The main findings, implication of key findings, practical applications, strengths and limitations, and future directions for further research are discussed hereafter.

7.2 MAIN FINDINGS IN RELATION TO THE LITERATURE

7.2.1 Summary of key findings

The primary finding from this thesis is the consistent increased response of CK activity to EIMD bouts across all studies independently of n-3 supplementation, exercise protocol or age group. Counter to the above hypotheses, decreased muscle force and increased DOMS immediately post-EIMD were noted in all participants regardless of age or muscle-damaging protocol following EIMD. The muscle functional recovery acted in a similar pattern to the response to prolonged downhill running or intermittent unilateral resistance exercises, although decreases observed in resistance exercise-based protocols were typically smaller than that seen following downhill induced-EIMD. Leg strength and muscle soreness measures regained pre-exercise values within 48 to 72 hours, which mirror EIMD responses from previous research (Eston et al., 2000; Vaile et al., 2008; Clifford et al., 2016).

7.2.2 Results with respect to exercise-induced muscle damage and inflammation

Extensive prior work has documented the adverse effects of EIMD on exercise performance in young adults (Croisier et al., 1999; Nosaka, Newton and Sacco, 2002b; Peake et al., 2017; Damas et al., 2016; Gravina et al., 2017; Ochi et al., 2017; Jakeman et al., 2017; Chen et al., 2018; Heckel et al., 2019). This thesis aimed to examine the related concepts of exercise-induced muscle damage, inflammation and muscle function in both healthy physically active younger and older male adults. It was hypothesised that exercise-induced muscle damage and inflammation would increase, and muscle function would change in response to

acute muscle-damaging protocol. It was also hypothesised that older participants would have a different EIMD responses compared to their younger counterparts.

This Doctorate showed a transient increase in muscle damage (but not excessive muscle damage per se) in both younger and older participants following eccentric based muscle-damaging exercise, evidenced by systemic post-exercise increases in plasma CK at 24 hours following EIMD in all cohorts (**Chapter 4**, **5 and 6**). These findings support previous studies investigating CK responses following a single bout of eccentric exercise, downhill running or maximal eccentric contractions of the lower body (Schwane et al., 1983; Byrnes et al., 1985; Clarkson and Hubal, 2002; Malm et al., 2004; Peake et al., 2005; Serrao et al., 2007; Brancaccio et al., 2008; Jakeman, Macrae and Eston, 2009; Park and Lee, 2015; Kim and Lee, 2015). It is speculated that this primary cellular damage response was caused by mechanical stress associated with the eccentric load of performing downhill running or repeated repetitions in leg press and leg extension. Structural damage to the sarcolemma would result in increased membrane permeability and the witnessed leakage of CK into the cytosol (Baird et al., 2012; Fatouros and Jamurtas, 2016; Owens et al., 2019).

The post-exercise time course of the rise in CK activity and magnitude of muscle damage are highly variable and affected by the individual's factors, such as age (Roth et al., 2000b), sex (Tiidus, 2000; Clarkson and Hubal, 2002), fitness status (Newton et al., 2008), genetic factors (Heled et al., 2007; Yamin et al., 2007; Baumert et al., 2016) and mode of exercise (Jamurtas et al., 2005; Machado et al., 2011; Machado, Pereira and Willardson, 2012; Nieman et al., 2014). CK activity has been shown to increase from 300 - 6000 U/L post-EIMD (Brancaccio

et al., 2008), 365 – 13,498 U/L post-EIMD (Kim and Lee, 2015), 500 – 34,500 U/L post-EIMD (Newham, Jones and Edwards, 1983); with peak concentrations seen between 24 hours to 96 hours post-EIMD after downhill running (Clarkson and Hubal, 2002; Malm et al., 2004; van de Vyver and Myburgh, 2012; Park and Lee, 2015) or a delayed peak between 2 to 6 days after maximal eccentric contractions (Clarkson, Nosaka and Braun, 1992; Stupka et al., 2001; Clarkson and Hubal, 2002; Hyldahl et al., 2011), caused secondary muscle damage due to the elevated production of ROS (Powers et al., 2010a). Conversely, in **Chapters 4**, **5 and 6**, muscle damage was resolved within a 72-hour recovery period (in the younger cohorts), similar to the time course observed by Malm et al. (2004) following downhill running. The similarity in the muscle damage response exhibited in **Chapter 4**, **5 and 6** to that observed by Malm et al. (2004) suggests that the time course and magnitude of muscle damage may be exercise mode dependent. The lack of significant alterations in biomarkers of IL-6 and TNF-α provides further support for no increase in secondary muscle damage.

Muscle damage has been associated with an inflammatory response as part of the repair and regeneration process of damaged tissue (Peake et al., 2017; Owens et al., 2019). Post-exercise increases in biomarkers of inflammation paralleled the muscle damage response, with plasma IL-6 and TNF-α increased immediately post-exercise in response to the single bout of EIMD only in the younger cohorts (**Chapter 4**, **5 and 6**). The observed pro-inflammatory cascade supports the role of inflammation in skeletal muscle remodelling (Suzuki, 2018), as IL-6 and TNF-α are part of the acute phase response that regulate neutrophils which infiltrate the muscle following muscle-damaging exercise (Tidball, 2005; Schneider and Tiidus, 2007; Suzuki, 2018). It also supports the work of previous

studies investigating muscle damage and inflammatory responses on EIMD (Malm et al., 2004; Fatouros et al., 2010; van de Vyver and Myburgh, 2012; Jakeman et al., 2017). Intriguingly, no significant exercise-induced alterations in plasma IL-6 and TNF-α concentrations were observed at the time points considered (except from a significant increase of IL-6 only in the placebo group in **Chapter 4** and TNF-α only in the younger group in **Chapter 5**), suggesting that the use of moderate exercise intensity or short duration of resistance exercise may be insufficient to induce any significant elevations of these markers. These findings are in line with previous reports which showed minor change (Ostrowski et al., 1998a; 1998b; Ostrowski, Schjerling and Pedersen, 2000). Alternatively, IL-6 and TNF-α may not be optimal as an EIMD markers, in part due to the participants who volunteered in this Doctorate being recreationally active and habitually engaged in structured exercise (3-5 times per week). In fact, evidence suggests that regular exercise and lifelong training improves overall anti-inflammatory environment (Gleeson et al., 2011; Minuzzi et al., 2017). However, anti-inflammatory resolution markers were not measured, herein, to confirm the anti-inflammatory response.

In **Chapter 5**, whilst an increased expression pattern of muscle damage and inflammatory mediators for the younger group was noted, the younger group also showed enhanced resolution (as seen in reductions of CK activity and circulating TNF-α) by the end of the experimental period compared to their older counterparts. The response to exercise stress may be attenuated in older muscle that leads to a slower repair and adaptation to response, suggesting a better resolution in recovery for the younger participants (Fell and Williams, 2008). This observation could be related to optimal functioning of the cell, which may decline

with age in line with the inflammageing hypothesis (Ostan et al., 2008; Franceschi and Campisi, 2014; Kennedy et al., 2014), and may not be simply a marker of less muscle damage.

Further, **Chapter 5** presented novel findings by observing that early changes in EV profiles following EIMD significantly correlate with subsequent changes in CK, and thus acute changes in EV profile post-exercise may indicate subsequent magnitude of muscle damage. Whilst the findings of the study suggest no major differences in EV modal size or EV plasma concentration in younger *vs* older individuals following a bout of EIMD, this may make them a reliable biomarker to assess effects of exercise interventions across age groups.

In **Chapter 6**, the main finding was that the DHR group significantly differed in CK activity from both resistance exercise groups at 24 and 48 hours post-EIMD. This suggests that muscle damage may have been present to a greater degree following the downhill running, although exercise performance was not changed in all groups by 72 hours relative to pre-exercise values. The difference in the magnitude of this elevation may be due to the prolonged downhill running (1 hour at 65% of $\dot{V}O_2max$) *vs* intermittent resistance exercise (weight load at 120% of 1RM or at 75% of 1RM) with rest intervals, or due to the two-legs running *vs* the unilateral protocols, and CK may not be simply a marker of muscle damage. By contrast, previous studies in EIMD using high force resistance exercise protocols have reported to significantly increase CK activity at 24 hours (Jakeman, Macrae and Eston, 2009) and at 48 hours (Serrao et al., 2007) after EIMD. With regards to exercise-induced inflammatory response, any putative changes in inflammatory markers evoked by all EIMD bouts were modest, despite marked

increases in CK activity, which are in accordance with a previous study by Malm et al. (2004).

7.2.3 Results with respect to muscle function and recovery

One of the main findings of this thesis support the hypothesis that 4 weeks of 3 g/day of n-3 supplementation may attenuate minor aspects of EIMD, as observed in DOMS and peak power in the N-3 group (**Chapter 4**). In line with previous research (Tarbinian et al., 2009; Jouris et al., 2011; Lembke et al., 2014), **Chapter 4** suggested that n-3 supplementation PUFA offsets the EIMD induced pain response following a single bout of EIMD. There were no significant differences in MVIC between groups, in agreement with previous studies of Houghton and Onambele (2012) and Ramos-Campo et al. (2020), indicating that n-3 supplementation had limited impact on muscle function and subsequent performance. It is likely that the equivocal findings on the efficacy of the dietary supplementation with n-3 PUFA were elicited by a combination of factors, such as the dosage (Mickleborough, 2013), the duration (McGlory et al., 2014) and the composition of EPA:DHA (Shei, Lindley and Mickleborough, 2014) or the diversity of the exercise protocols between studies. An alternative rationale could also be due to individual's genotype (Baumert et al., 2016) in determining the muscle damage, as well as to ''good'' or ''bad'' response to n-3 supplementation in relation to muscle damage. Overall, whilst a minimal positive gain in exercise performance with n-3 supplementation was witnessed in this thesis, these findings may have relevance to soreness-associated exercise avoidance.

Whilst the main findings in **Chapter 5** showed an impaired MVIC in leg strength, peak power and jump performance following EIMD in both younger and older

groups, with older participants showing a significantly lower output, a relatively homogenous recovery was observed in both groups (**Chapter 5**). Nevertheless, previous research (Lavender and Nosaka, 2006; Chapman et al., 2008; Gorianovas et al., 2013), using strength and muscle soreness as indirect EIMD markers of muscle function, have shown greater muscle damage in younger individuals rather than in older ones. A plausible reason could be that the participants of this study were recreationally active and habitually engaged in exercise (3-5 times per week).

Finally, **Chapter 6** showed that a 60-minutes downhill running, a unilateral leg press, and a unilateral leg press and leg extension exercise evoked mild to moderate muscle damage and elicited DOMS in young recreationally active male adults. Indeed, significant decreases in leg strength immediately post-EIMD and increases in muscle soreness following EIMD in all groups were seen. This muscle damage is equivalent to ‘a light pain when walking’ (Vickers, 2001), and thus mirrors that reportedly encountered by soldiers and athletes exposed to unaccustomed exercise with an eccentric component. The extent of this damage (mild to moderate) may stimulate muscle hypertrophy, whilst a more severe muscle damage may interfere with training and daily activities (Kalinski, 2010).

Research has consistently shown that unaccustomed EIMD leads to force and power loss and increased DOMS (Proske and Morgan, 2001; Clarkson and Hubal, 2002; Cheung et al., 2003; Clifford et al., 2016; Jakeman et al., 2017). Nevertheless, unsurprisingly the DHR group significantly differed from both resistance exercise groups in indices of muscle damage post-EIMD (e.g., perceived muscle soreness, MVIC and jump height). Previous research

suggested that it could be related to the biomechanical and neuromuscular alterations that occur in running (Bontemps et al., 2020). This suggests that the moderate intensity resistance exercise of leg press and leg extension might be sufficient to induce muscle damage in naïve-exercised or older individuals. Further, **Chapter 6** provides evidence that early changes in muscle function, following EIMD are significantly associated with subsequent changes in plasma CK, and thus acute changes in MVIC and DOMS post-exercise may indicate subsequent magnitude of muscle damage.

The findings presented here are with physically active participants (younger and older cohorts), all habitually engaged in structured physical activity, and thus are not representative of wider physically inactive Western populations, specifically older populations (Farrell et al., 2013; Lindsay et al., 2019). Importantly also, in the ageing population, reduced physical activity and increases in sedentary time are typically observed (Lindsay et al., 2019). These results, therefore, should be interpreted taking into account that the cohorts presented here consisted of physically active participants.

7.3 PRACTICAL APPLICATIONS

The current research project(s) has generated new information on extracellular vesicles and exercise-induced muscle damage, and has also confirmed that EIMD may have a negative impact on exercise performance (i.e., increases in muscle pain, CK activity and inflammatory markers, decrements in MVIC, jump performance and peak power). There are several valuable findings from the three studies in this thesis that may have potential implications for recreational

individuals and elite athletes or for the military personnel who regularly perform back-to-back training sessions with minimal recovery periods, and even for the older populations.

Post-exercise increases in acute inflammation and muscle damage were observed to peak immediately-post and at 24 hours, respectively, and increases in DOMS and decrements in force were noted immediately following EIMD, returning to pre-exercise values within 48 to 72 hours during the recovery period. Sports scientists/coaches and exercise physiologists should consider the implication of the time course of these exercise-induced responses if athletes are to meet the demands of the next training session with minimal impact from previous sessions. Aiding rapid recovery between high-intensity sessions, where EIMD is a risk, is essential if the next training or competition is within a 48-hour period. Investigating strategies that lessen the magnitude of muscle injury may be needed, such as the n-3 supplementation protocol investigated here.

In **Chapter 4**, **5 and 6**, post exercise responses of CK activity paralleled the responses of subjective measures of perceived muscle soreness. Muscle soreness could impact the quality of subsequent training sessions. Therefore, monitoring of subjective measures of muscle soreness in conjunction with muscle damage and inflammation biomarkers between training could increase understanding of the athlete's perception of pain, and potentially reduce muscular injury risk.

In addition, results in this thesis showed exercise-induced responses of CK activity to be highly individual. Consistent monitoring is recommended to develop

normalised data for individuals allowing prescription of training loads and recovery periods that optimise individual performance (Silva et al., 2018). The practicability of implementing personalised strategies is not without difficulties, however, is now common practice that may result in gains or optimised performance.

Chapter 4 provided insufficient evidence to support the use of prolonged n-3 supplementation (dosing strategy: 2145 mg of EPA and 858 mg DHA, for a total of 3 g of n-3 PUFA per day for a period of 4 weeks loading) to promote recovery and improved exercise performance following an unaccustomed eccentric bout that induced acute muscle damage, inflammation, muscle soreness and functional changes (i.e., strength, peak power) in recreationally active young adults with adequate habitual dietary intakes. From an applied perspective, sports scientists/nutritionists should consider implementation of nutritional interventions that are evidence-based only. Elite athletes or soldiers undertaking vigorous periods of exercise may not benefit from n-3 supplementation to improve performance. However, it is speculated that recreationally active or older individuals with micronutrient deficiencies may respond differently to an n-3 supplementation strategy (McGlory, Calder and Nunes, 2019; Thielecke and Blannin, 2020), and thus, they may benefit from reducing exercise avoidance associated with muscle soreness. Therefore, the potential benefit of n-3 supplementation for enhancing performance requires further investigation.

Given the current debate regarding n-3 anti-inflammatory supplementation blunting exercise-induced muscle inflammation, it is further recommended that sports scientists/nutritionists consider periodised nutritional strategies (Owens et

al., 2019). Current research, however, has suggested that n-3 supplementation has limited effect on EIMD (Toft et al., 2000; Lenn et al., 2002; Houghton and Onambele, 2012; Gravina et al., 2017; Kyriakidou et al., 2021a). The lack of effect could be attributed to the generalised dosing regimen used for all participants instead of a personalised strategy. Research protocols perhaps should implement a personalised strategy to evaluate whether n-3 supplementation can be an efficacious ergogenic aid during exercise performance, based on the individual's genotype to ''good'' or ''bad'' response to n-3 supplementation.

Moreover, the primary finding that n-3 supplementation may help exercise-induced muscle soreness avoidance in addition to the known benefits of maintaining (or even increasing) muscle function capacity or reducing the rate of muscle mass loss, might be influential in providing guidelines to exercise scientists for the most effective strategies to reduce the fall risk in older adults or overall, the improvement of human performance. In the long term, these guidelines may indirectly enhance quality of life and prolong independent living in older adults. Further, the implications of the findings from the previous studies and this thesis are that n-3 supplementation does not have significant positive effects on muscle strength and recovery. Increasing understanding in this area could provide knowledge and evidence-based support for the use of n-3 supplementation as a recovery strategy on the EIMD.

The age-related decline in skeletal muscle mass and strength (referred to as sarcopenia) leads to decrements of mobility and lower-extremity performance in daily tasks, consequently increasing risk of falls (Hardy et al., 2007) and accelerating the progression of independence loss. Whilst regular exercise or

increased physical activity may be prophylactic against age-related functional declines, current practices usually have poor adherence. For instance, those recovering from lower limbs injury or surgery and elderly who are sedentary, typically cannot tolerate lower limb exercise resulting in limited benefit. Due to ageing population, there will likely be an increase in the number of people falling. Thus, there is a need to develop more effective and efficient exercise and nutritional strategies to target these impairments. Better understanding of any mechanistic ageing-associated differences in muscle damage, inflammation, and functional responses may thus help both our understanding of physiological differences in older individuals, and ultimately aid personalised exercise prescription in this population.

Interestingly, this thesis is the first to examine muscle damage and EVs in single bout of resistance exercise in younger and older physically active adults. **Chapter 5** provides evidence that physical responses to eccentric exercise induces plasma EV changes which are significantly associated with changes in CK activity following EIMD. If the post-exercise EV response does indeed reflect physiological injury recovery responses, the magnitude and content of EV profile changes could be of interest for strategies to reduce the impairing effects of EIMD in younger and older populations. This could further aid sports scientists in the prescription of altered training loads or recovery periods to minimise the impact of muscle damage and offsetting the negative effects of muscle damage that could impair performance and increase injury risk.

Finally, eccentric resistance exercise interventions have been proposed in older adults due to high load and potentially greater anabolic response at a low energy

cost (Gault and Willems, 2013; Lim, 2016; Franchi et al., 2017). The findings of **Chapter 6** suggest that the combination of the leg press and leg extension protocol with moderate intensity may be a more realistic EIMD model and may have a beneficial effect (e.g., muscle hypertrophy) on responses to muscle damage in naive-exercised or older individuals to support optimal health. However, this hypothesis should be further tested in these populations. Therefore, EIMD may be used to develop safer and more effective personalised training and recovery protocols (Givli, 2015).

7.4 STRENGTHS AND LIMITATIONS

Strengths and potential limitations are acknowledged in each of the studies in this thesis, as previously addressed in each corresponding Chapter. This section addresses general strengths and limitations affecting the findings of the thesis, and future research should consider these observations.

The use of physically active male participants in all studies was a strength of this thesis, given that trained individuals may respond differently to the exercise bout compared to untrained cohorts. All cohorts of the studies were Caucasian, healthy, recreationally active and unaccustomed to eccentric exercise without taking any medication (e.g., non-steroidal anti-inflammatory drugs) or anti-inflammatory supplements (e.g., fish, cod oil). It has been reported that CK activity in tissues was found to be higher in healthy individuals belonging to Black and minority ethnic (BME) groups with high and fluctuating energy demands compared to Caucasian ones (Brewster et al., 2007; Brewster et al., 2012). Additionally, it has been shown that there is an association between ethnicity and

inflammation with BME individuals having higher IL-6 than Caucasian, and thus IL-6 may act as an important ethnic-specific cytokine (Chapman et al., 2009; Paalani et al., 2011). This may be due to the diverse body composition by ethnicity with higher skeletal muscle in Hispanic and black African males (Silva et al., 2010). Another plausible reason for the ethnic variation in the IL-6 levels may be due to differences in cytokine gene polymorphisms (Delaney et al., 2004; Stowe et al., 2010). Similar findings were also demonstrated in TNF-α, with BME showing higher levels in relation to Caucasian ones in the UK (Kalra et al., 2005). Whilst this criterion decreased biological variability and increased power of this thesis, it does reduce ecological validity.

The use of validated EIMD protocols (downhill running, Fortes et al., 2013; Park and Lee, 2015; leg press protocol, Vaile et al., 2008; and leg press and leg extension protocol, McKay et al., 2012) in all studies to elicit muscle damage and DOMS was also another strength of this thesis. In addition, the use of standardised procedures across all experimental studies, such as collection, storage and analysis procedures, including blood sample timing, urine sampling for assessing hydration status, and functional measurements, was another strength of this thesis. The use also of various indices of muscle function (i.e., MVIC, peak power, DOMS, ROM, jump height and thigh circumferences) in multiple time points helped confirm the presence of exercise-induced muscle damage in each experimental study of this thesis and is recommended for future studies.

Finally, the use of collagen as the placebo (**Chapter 4**) against the N-3 group was another strength. There is no evidence in the literature that has a pro- or anti-

inflammatory effect, and therefore it would not oppose the action of n-3 supplementation. In addition, in an attempt to isolate the effect of n-3 supplementation collagen was chosen to avoid manipulation of n-6/n-3 ratio.

It is acknowledged that findings of the thesis on exercise-induced muscle damage and inflammatory response are limited to the selected biomarkers. Exercise-induced muscle damage and inflammation were assessed indirectly via plasma CK and plasma IL-6 and TNF-α analyses, respectively, across all studies. A limitation of the CK assay is specificity, as this assay quantifies total CK and is not specific for CK isoenzymes present within skeletal muscle (i.e., CK-MM, CK-MB). However, in healthy individuals without certain underlying pathologies (e.g., myocardial infarction, stroke or physical trauma), the total CK tends to be used for muscle activity, as skeletal muscle has high levels of CK and are closely associated with tissue damage or muscle cell disruption (Brancaccio, Maffulli and Limongelli, 2007; Baird et al., 2012). Indeed, the cohorts, in this thesis, were homogenous groups of healthy physically active individuals, thus, observed post-exercise significant elevations in plasma CK could be attributed to muscle damage induced by the EIMD protocols. It should also be considered that the systemic release of plasma CK after exercise indicated an efflux of this intracellular enzyme into the bloodstream due to compromised sarcolemma integrity, but also reflects the clearance rate from the circulation (Warren et al., 1999; Thompson, Scordilis and De Souza, 2006; Baird et al., 2012). Therefore, CK concentrations may not accurately reflect the magnitude of muscle damage following eccentric exercise and level of muscle dysfunction (Warren et al., 1999; Magal et al., 2010) due to large subject variability (Clarkson and Hubal, 2002; Brancaccio, Lippi and Maffulli, 2010).

Furthermore, each study relied on the participants to self-report specific measures (e.g., diet diaries, supplement ingestion, training load); these responses could have been confounded or open to response bias, resulting in underreporting (e.g., potential loss of dietary information from mixed dishes) or overreporting which is a well-documented issue with dietary assessment instruments (Black et al., 1991; Gibson, 2005; NIMH, 2017). Finally, and of importance, the strict inclusion criteria, as well as the inability to collect more experimental data due to the COVID-19 pandemic (Myers et al., 2020) made recruitment for participants difficult.

7.5 RECOMMENDATIONS FOR FUTURE RESEARCH

Suggestions for additional future research have arisen, herein, based on the outcomes of the studies described. Firstly, future research could focus on comparisons of exercise-induced responses in muscle damage and inflammation (pro- and anti-inflammatory biomarkers) associated with other sample types (i.e., blood components and urine), to define whether exercise-induced responses in these sample types precisely reflect skeletal muscle tissue, as this has been questioned (Powers et al., 2010b). Muscle biopsies are often unfeasible in exercise research, given that they are invasive and can cause localised inflammation and muscle damage (Malm, 2001).

Secondly, in this thesis, biomarker responses were compared pre-post EIMD. However, there is a need to classify thresholds of elevated biomarker concentrations that define EIMD and exercise-induced inflammation (Lee et al., 2017) to provide a context for biomarker data and link it to associated measures

of performance and recovery (Buford et al., 2009). This data may need to be established for different activities and on an individual basis due to the high inter-individual variance demonstrated in athletes (Heisterberg et al., 2013; Lee et al., 2017). A standardised validated resistance muscle-damaging protocol should also be developed to improve the comparability between studies. In addition, all the exercise protocols in this thesis, utilised a single bout of eccentric exercise. Further investigation is needed to study individuals with multiple training sessions.

In **Chapter 4**, post-exercise responses of IL-6 and DOMS were visually observed to be lower in the N-3 group compared with the PLA group, and peak power was suppressed in placebo *vs* N-3 group. Therefore, further research into the efficacy of n-3 supplementation to promote recovery and improvement of exercise performance using increased samples sizes and different age groups, is potentially warranted. Supplementing n-3 PUFA immediately post-exercise where inflammation was observed to peak may also be of interest. In addition, future work should explore other underlying mechanisms of EIMD and inflammation as targets to potentially limit EIMD, as links to the NF-κB canonical pathway were unsupported in this thesis.

Further research is also required to address the practical limitations of the findings within this thesis, prior to adopting n-3 PUFA, as a recommendation for exercise performance. Indeed, McGlory et al. (2014) found that is required > 4 weeks of n-3 supplementation to reach a saturation of n-3 PUFA composition in human skeletal muscle. It could, therefore, be speculated that supplementation of n-3 PUFA may be most efficacious if it is given for a more prolonged period.

As such, n-3 PUFA and other ergogenic strategies may be beneficial to mitigate for the decrements in exercise performance that occur during this early recovery period.

Recovery optimisation to aid exercise performance may be achieved through nutritional interventions. However, this thesis showed limited effects of n-3 supplementation in physically active individuals after EIMD. Therefore, sports practitioners should carefully consider the use of n-3 supplementation in this cohort at present. Findings from studies in this thesis can be transferred to professional and recreationally active individuals (younger and older). Further research should also evaluate the implication of n-3 supplementation in older populations.

Further investigation is still needed to clarify the nature and extent of the molecular mechanisms of EIMD involved at intracellular level, and its impact on hypertrophic responses. This would provide us with more compelling evidence on how and for how long EIMD is lessened and the individual can adapt faster through muscle repair and regeneration. Further to this, an *in vitro* investigation, or another study design that could measure the effects of EIMD on innate immunity (e.g., macrophages and neutrophils), should be conducted to confirm the effect of EIMD on exercise performance and recovery in humans.

Finally, expanding on the studies in this thesis, further research should translate the results of **Chapter 4**, **5 and 6** in naïve-exercised younger and older adults and in larger cohorts to confirm the magnitude and time course of the observed exercise-induced responses in muscle damage, inflammation, and functional

measures with or without the ergogenic effect of n-3 supplementation. Additionally, the maintenance of muscle function capacity and hypertrophy with ageing plays an important role to increase physical activity levels in younger and older populations. Therefore, it is recommended that future studies will be performed to identify the changes induced by muscle-damaging exercise on biomarkers of muscle damage and inflammation (including EVs as a putative biomarker for exercise-induced muscle damage and inflammation) and muscle function measures at intracellular level and the subsequent effect on recovery and exercise performance. The novel findings presented in this thesis could inspire the development of new strategies (e.g., the use of extracellular vesicles as a biomarker of muscle damage) to protect those involved in physical activity from the impairing effects of EIMD.

7.6 CONCLUSIONS

In conclusion, this thesis investigated physiological responses to EIMD in healthy physically active younger and older male adults. This thesis used ecologically and controlled approaches to profile exercise-induced muscle damage and associated inflammatory and muscle functional responses. Collectively, this thesis provides an original contribution to the literature through the findings that unaccustomed lower limb eccentric exercise (downhill running/or leg resistance exercises) induced mild to moderate muscle damage paralleled with modest inflammation and changes in muscle function (e.g., DOMS, MVIC, peak power and jump height) with or without n-3 supplementation across all studies. This thesis also showed that extracellular vesicles release immediately following exercise may also play a role in EIMD response in humans.

This thesis showed that (i) 4 weeks supplementation with 3 g/day of n-3 PUFA offsets the EIMD induced pain response following a single bout of moderate intensity exercise, (ii) older people showed a blunted resolution in muscle damage and inflammation relative to their younger counterparts, however, older participants had a comparable muscle functional recovery with their younger counterparts, and (iii) the combination of the leg press and leg extension protocol with moderate intensity could be used as a muscle-injury inducing model in naive-exercised or older populations in future studies. Given that no major differences in functional decrements following EIMD were seen in younger and older physically active cohorts, this suggests that regular or lifelong physical activity may have a beneficial effect on responses to muscle damage throughout the ageing process.

REFERENCES

Abadie, B.R. and Wentworth, M.C. (2000). Prediction of one repetition maximal strength from a 5-10 repetition submaximal strength test in college-aged females. *J Exerc Physiol*, 4 (2), pp.1-6.

Abdelhamid, A.S., Brown, T.J., Brainard, J.S., Biswas, P. Thorpe, G.C., Moore, H.J., Deane, K.H.O., Alabdulghafoor, F.K., Summerbell, C.D., Worthington, H.V., Song, F. and Hooper, L. (2018). Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. *Cochrane Database Syst Rev,* 11 (11), CD003177.

Abdelhamid, A.S., Brown, T.J., Brainard, J.S., Biswas, P., Thorpe, G.C., Moore, H.J., Deane, K.H., Summerbell, C.D., Worthington, H.V., Song, F. and Hooper, L. (2020). Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. *Cochrane Database Syst Rev*, 3 (2), CD003177.

Ahmadi, S., Sinclair, P.J. and Davis, G.M. (2008). Muscle oxygenation after downhill walking-induced muscle damage. *Clin Physiol Funct Imaging*, 28 (1), pp.55-63.

Allen, M.D., Dalton, B.H., Gilmore, K.J., McNeil, C.J., Doherty, T.J., Rice, C.L. and Power, G.A. (2021). Neuroprotective effects of exercise on the aging human neuromuscular system. *Exp Gerontol*, 111465.

An, KO and Kim, J. (2020). Associations between Handgrip Strength with Adverse Cardiometabolic Health among Representative Korean Adults. *Int J Environ Res Public Health*, 17 (1), p.269.

American College of Sports Medicine (ACSM). (2009). Joint Position Statement: Nutrition and athletic performance. *Med & Sci Sports & Exerc*, 41 (3), pp.709-731.

American College of Sports Medicine, Academy of Nutrition and Dietetics, and Dietitians of Canada. (2016). Joint Position Statement: Nutrition and athletic performance. *J Acad Nutr Diet*, 116 (3), pp.501-528.

American Dietetic Association (ADA). (2009). Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance. *J Am Diet Assoc,* 109 (3), pp.509–527.

Andrade, P.M.M., Ribeiro, B.G., Bozza, M.T., Costa Rosa, L.F.B., and do Carmo, M.G.T. (2007). Effects of the fish-oil supplementation on the immune and inflammatory responses in elite swimmers. *Prostaglandins Leuko Essent Fatty Acids*, 77, pp.139–145.

Argaud, S., Pairot de Fontenay, B., Blache, Y. and Monteil, K. (2019). Age-related differences of inter-joint coordination in elderly during squat jumping. *PLoS One*, 14 (9), e0221716.

Arita, M., Bianchini, F., Aliberti, J., Sher, A., Chiang, N., Hong, S., Yang, R., Petasis, N. A. and Serhan, C. N. (2005). Stereochemical assignment, anti-inflammatory properties,

and receptor for the omega-3 lipid mediator resolvin E1. *J Exp Med*, 201 (5), pp.713–722.

Arnett, M.G., Hyslop, R., Dennehy, R.H. and Schneider, C.M. (2000). Age-related variations of serum CK and CK MB response in females. *CJAP*, 25 (6), pp.419-29.

Armstrong, R.B. (1984). Mechanisms of exercise-induced delayed onset muscular soreness: a brief review. *MSSE,* 16 (6), pp.529-538.

Armstrong, L. E., Herrera-Soto, J. A., Hacker, F. T., Casa, D. J., Kavoura, S. A. and Maresh, C. M. (1998). Urinary indices during dehydration, exercise and rehydration. *Int J Sport Nutr*, 8, pp.345-355.

Armstrong, L. E., Pumerantz, A. C., Fiala, K. A., Roti, M. W., Kavouras, S. A., Casa, D. J. and Maresh, C. M. (2010). Human hydration indices: acute and longitudinal reference values. *Int J Sport Nutr Exerc Metab*, 20, pp.145-153.

Arroyo, E., Wells, A.J., Gordon, J.A., 3rd; Varanoske, A.N., Gepner, Y., Coker, N.A., Church, D.D., Fukuda, D.H., Stout, J.R. and Hoffman, J.R. (2017). Tumor necrosis factor-alpha and soluble tnf-alpha receptor responses in young vs. Middle-aged males following eccentric exercise. *Exp Gerontol*, 100, pp.28-35.

Arterburn, L. M., Hall, E. B. and Oken, H. (2006). Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am J Clin Nutr*, 83, pp.1467-1476.

Assumpção, C. de O., Lima, L.C., Oliveira, F.B., Greco, C.C. and Denadai, B.S. (2013). Exercise-induced muscle damage and running economy in humans. *Sci World J*, 2013, p.189149.

Atashak, S., Sharafi, H., Azarbayjani, M.A., Stannard, S.R., Goli, M.A., Haghighi, M.M. (2013). Effect of omega-3 supplementation on the blood levels of oxidative stress, muscle damage and inflammation markers after acute resistance exercise in young athletes. *Kinesiology*, 45 (1), pp.22-29

Avela, J., Kyröläinen, H., Komi, P.V and Rama, D. (1999). Reduced reflex sensitivity persists several days after long-lasting stretch-shortening cycle exercise. *J Appl Physiol*. 86 (4), pp.1292-300.

Baird, M. F., Graham, S. M., Baker, G. J. and Bickerstaff, G. F. (2012). Creatine-Kinase- and Exercise-Related Muscle Damage Implications for Muscle Performance and Recovery. *Journal of Nutrition and Metabolism*, pp.1-13.

Baker, B.A. (2017). An old problem: aging and skeletal-muscle-strain injury. *J Sport Rehabil*. 26 (2), pp.180-188.

Baker, D.J., Jeganathan, K.B., Cameron, J.D., Thompson, M., Juneja, S., Kopecka, A., Kumar, R., Jenkins, R.B., de Groen, P.C., Roche, P. and van Deursen, J.M. (2004).

BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. *Nat Genet*, 36 (7), pp.744-749.

Baker, D.J., Perez-Terzic, C., Jin, F., Pitel, K.S., Niederländer, N.J., Jeganathan, K., Yamada, S., Reyes, S., Rowe, L., Hiddinga, H.J., Eberhardt, N.L., Terzic, A. and van Deursen, J.M. (2008). Opposing roles for p16Ink4a and p19Arf in senescence and ageing caused by BubR1 insufficiency. *Nat Cell Biol*, 10 (7), pp.825-836.

Baker, D.J., Wijshake, T., Tchkonia, T., LeBrasseur, N.K., Childs, B.G., van de Sluis, B., Kirkland, J.L. and van Deursen, J.M. (2011). Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature*, 479 (7372), pp.232-236.

Balmer, J., Potter, C.R., Bird, S.R. and Davison, R.R., (2005). Age-related changes in maximal power and maximal heart rate recorded during a ramped test in 114 cyclists age 15–73 years. *J Aging Phys Act*, 13 (1), pp.75-86.

Balnave, C. D. and Thompson, M.W. (1993). Effect of training on eccentric exercise-induced muscle damage. *J App Physiol*, 75, pp.1545–1551.

Balster, W., Er Lim, C.X., and Wah Kong, p. (2016). Effects of a deeper countermovement on vertical jump biomechanics after three weeks of familiarisation - preliminary findings. *Int J Human Mov Sports Sci*, 4 (4), pp.51-60.

Bandeen-Roche, K., Walston J.D, Huang, Y., Semba, R.D. and Ferrucci, L. (2009). Measuring systemic inflammatory regulation in older adults: evidence and utility. *Rejuvenation Res*, 12 (6), pp.403–410.

Bang, H. O., Dyerberg, J. and Sinclair, H. M. (1980). The composition of the Eskimo food in north western Greenland. *Am J Clin Nutr*, 33, pp.2657-2661.

Barberi, L., Scicchitano, B.M., De Rossi, M., Bigot, A., Duguez, S., Wielgosik, A., Stewart, C., McPhee, J., Conte, M., Narici, M., Franceschi, C., Mouly, V., Butler-Browne, G. and Musaro, A. (2013). Age-dependent alteration in muscle regeneration: critical role of tissue niche. *Biogerontology*, 14, pp.272-292.

Baron, B., Dekerle, J., Depretz, S., Lefevre, T. and Pelayo, P. (2005). Self-selected speed and maximal lactate steady state speed in swimming. *J Sports Med Phys Fitness*, 45 (1), pp.1-6.

Bar-Or, O. (1987). The Wingate anaerobic test an update on methodology, reliability and validity. Sports Medicine, 4 (6), pp.381-394.

Barrett, B., Brown, R.L., Mundt, M.P., Thomas, G.R. Barlow, S.K., Highstrom, A.D. and Bahrainian, M. (2009). Validation of a short form Wisconsin upper respiratory symptom survey (WURSS-21). *Health Qual Life Out,* 7, p.76.

Barrios-Silva, L.V., Parnell, M., Shinwari, Z.B., Chaudhary, G.A., Xenofontos, T., van Bekhoven A. McArthur S. and Elliott, B.T. (2018). Activin subfamily peptides predict chronological age in humans. *Physiol Rep*, 6 (17), e13823.

Baumann, C.W., Green, M.S., Doyle, J.A., Rupp, J.C., Ingalls, C.P. and Corona, B.T. (2014). Muscle injury after low-intensity downhill running reduces running economy. *J Strength Cond Res*, 28 (5), pp.1212–1218.

Baumert, P., Lake, M.J., Stewart, C.E., Drust, B. and Erskine, R.M. (2016). Genetic variation and exercise-induced muscle damage: implications for athletic performance, injury and ageing. *Eur J App Physiol*, 116, pp.1595-1625.

Baumgartner, R.N., Koehler, K.M., Gallagher, D., Romero, L., Heymsfield, S.B., Ross, R.R., Garry, P.J and Lindeman, R.D. (1998). Epidemiology of sarcopenia among the elderly in New Mexico. *Am J Epidemiol*, 147 (8), pp.755-763.

Bean, A. (2009). *Complete Guide to Sports Nutrition*. 6th ed. London: A & C Black.

Beaton, L.J, Tarnopolsky, M.A and Phillips, S.M. (2002). Contraction-induced muscle damage in humans following calcium channel blocker administration. *J Physiol,* 544, pp.849-859.

Beaudart, C., Dawson, A., Shaw, S.C., Harvey, N.C., Kanis, J.A., Binkley, N., Reginster, J.Y., Chapurlat, R., Chan, D.C., Bruyere, O., Rizzoli, R., Cooper, C., Dennison, E.M. and the IOF-ESCEO Sarcopenia Working Group. (2017). Nutrition and physical activity in the prevention and treatment of sarcopenia: systematic review. *Osteoporos Int*, 28, pp.1817-1833.

Beneke, R., Leithäuser, R.M. and Ochentel, O. (2011). Blood lactate diagnostics in exercise testing and training. *Int J Sports Physiol Perform*, 6 (1), pp.8-24.

Benelli, P., Ditroilo, M., Forte, R., De Vito, G. and Stocchi, V. (2007). Assessment of post-competition peak blood lactate in male and female master swimmers aged 40-79 years and its relationship with swimming performance. *Eur J Appl Physiol*, 99 (6), pp.685-93.

Benjaminse, A., Habu, A., Sell, T.C., Abt, J.P., Fu, F.H., Myers, J.B. and Lephart, S.M. (2008). Fatigue alters lower extremity kinematics during a single-leg stop-jump task. *Knee Surg Sports Traumatol Arthrosc*, 16 (4), pp.400-407.

Bernecker, C., Scherr, J., Schinner, S., Braun, S., Scherbaum, W.A. and Halle, M., (2013). Evidence for an exercise induced increase of TNF-α and IL-6 in marathon runners. *Scand J Med & Sci Sports*, 23 (2), pp.207-214.

Beutler, B. (1999). The role of tumor necrosis factor in health and disease. *J Rheumatol*, 57, pp.16-21.

Bingham, S. (2006). Biomarkers in nutritional epidemiology. *Public Health Nutrition,* 5 (6), pp.821-827.

Black, A.E., Goldberg, G.R., Jebb, S.A., Livingstone, M.B., Cole, T.J. and Prentice, A.M. (1991). Critical evaluation of energy intake data using fundamental principles of energy physiology: 2. Evaluating the results of published surveys. *Eur J Clin Nutr,* 45 (12), pp.583-99.

Bloomer, R.J., Larson, D.E., Fisher-Wellman, K.H., Galpin, A.J. and Schilling, B.K. (2009). Effect of eicosapentaenoic and docosahexaenoic acid on resting and exercise-induced inflammatory and oxidative stress biomarkers: A randomized, placebo controlled, cross-over study. *Lipids Health Dis*, 8, p.36.

Bobbert, M.F and Casius, L.J. (2005). Is the effect of a countermovement on jump height due to active state development? *Med Sci Sports Exerc*, 37 (3), pp.440-446.

Bobbert, M.F., Casius, L.J., Sijpkens, I.W. and Jaspers, R.T. (2008). Humans adjust control to initial squat depth in vertical squat jumping. *J Appl Physiol*. 105 (5), pp.1428-1440.

Bontemps, B., Vercruyssen, F., Gruet, M. and Louis, J. (2020). Downhill running: What are the effects and how can we adapt? A narrative review. *Sports Med*, 50, pp.2083-2110.

Borg, G.A. (1982). Psychophysical bases of perceived exertion. *Med Sci Sports Exerc,* 14 (5), pp.377-381.

Borges, N.R., Reaburn, P.R., Doering, T.M., Argus, C.K. and Driller, M.W. (2018). Age-related changes in physical and perceptual markers of recovery following high-intensity interval cycle exercise. *Exp Aging Res*, 44, pp.338-349.

Brancaccio, P., Maffulli, N. and Limongelli, F.M. (2007). Creatine kinase monitoring in sport medicine. *Br Med Bull,* 81 - 82, pp.209-230.

Brancaccio, P., Maffulli, N., Buonauro, R. and Limongelli, F.M. (2008). Serum enzyme monitoring in sports medicine. *Clin Sports Med*, 27 (1), pp.1-18.

Brancaccio, P., Lippi, G. and Maffulli, N. (2010). Biochemical markers of muscular damage. *Clin Chem Lab Med,* 48 (6), pp.757-767.

Braun, W.A. and Dutto, D.J. (2003). The effects of a single bout of downhill running and ensuing delayed onset of muscle soreness on running economy performed 48 h later. *Eur J App Physiol,* 90 (1-2), pp.29-34.

Brehm, M.A., Harlaar, J. and Groepenhof, H. (2004) Validation of the portable VmaxST system for oxygen-uptake measurement. *Gait. Posture*, 20, pp.67–73.

Brenner, M., Natale, V.M., Vasiliou, P., Moldoveanu, A.I., Shek, P.N. and Shepard, R.J. (1999). Impact of three different types of exercise on components of the inflammatory response. *Eur J Appl Physiol Occup Physiol*, 80 (5), pp.452–460.

Brewster, L.M., Mairuhu, G., Sturk, A. and van Montfrans, G.A. (2007). Distribution of creatine kinase in the general population: implications for statin therapy. *Am Heart J*, 154 (4), pp.655-661.

Brewster, L.M., Coronel, C.M., Sluiter, W., Clark, J.F., van Montfrans, G.A. (2012). Ethnic differences in tissue creatine kinase activity: an observational study. *PLoS One*, 7 (3), e32471.

Brickson, S., Ji, L.L., Schell, K., Olabisi, R., St Pierre Schneider, B. and Best, T.M. (2003). M1/70 attenuates blood-borne neutrophil oxidants, activation, and myofiber damage following stretch injury. *J Appl Physiol,* 95, pp.969–976.

British Nutrition Foundation. (1999). Briefing paper: n-3 fatty acids and health. London: British Nutrition Foundation. Available from: http://www.nutrition.org.uk/publications.briefingpapers/n-3-fatty-acids-and-health.

Brockett, C.L., Morgan, D.L., and Proske, U. (2001). Human hamstring muscles adapt to eccentric exercise by changing optimum length. *Med Sci Sports Exerc,* 33 (5), pp.783-790.

Brown, S.J., Child, R.B., Day, S.H. and Donnelly, A.E. (1997). Exercise-induced skeletal muscle damage and adaptation following repeated bouts of eccentric muscle contractions. *J Sports Sci,* 15, pp.215-222.

Brown, W.M.C., Davison, G.W., McClean, C.M. and Murphy, M.H. (2015). A systematic review of the acute effects of exercise on immune and inflammatory indices in untrained adults. *Sports Med Open*, 1 (1), p.35.

Brune, K. (2004). Safety of anti-inflammatory treatment – new ways of thinking. *Rheumatology*, 43 (1), pp.16-20.

Bruunsgaard, H., Galbo, H., Halkjaer-Kristensen, J., Johansen, T.L., MacLean, D.A. and Pedersen, B.K. (1997). Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. *J Physiol,* 499 (3), pp.833-841.

Bruunsgaard, H., Andersen-Ranberg, K., Jeune, B., Pedersen, A.N., Skinhøj, P. and Pedersen, B.K. (1999). A high plasma concentration of TNF-α is associated with dementia in centenarians. *J Gerontol Series A: Biom Sci Med Sci*, 54 (7), pp.357-364.

Bruunsgaard, H., Andersen-Ranberg, K., Hjelmborg, J.B., Pedersen, B.K. and Jeune, B. (2003). Elevated levels of tumor necrosis factor alpha and mortality in centenarians. *Am J Med*, 115 (4), pp.278–283.

Buckworth J., Dishman, R.K., O'Conner, P.J. and Tomporowski, P.D. (2013) *Exercise Psychology* (2nd ed.) USA: Human Kinetics

Buford, T.W., Cooke, H.B., Shelmadine, B.P., Hudson, G.M., Redd, L. and Willoughby, D.S. (2009). Effects of eccentric treadmill exercise on inflammatory expression in human skeletal muscle. *Appl Physiol Nutr Metab,* 34 (4), pp.745-753.

Burke. L. (1995). Practical issues in nutrition for athletes. *J Sports Sci*, 13, pp.583-590.

Burke, L.M., (2001). Energy needs of athletes. *Canad J App Physiol*, 26, pp.202-219.

Burt, D. and Twist, C. (2011). The effects of exercise-induced muscle damage on cycling time-trial performance. *J. Strength. Cond. Res*, 25, pp.2185–2192.

Burton, D.G. (2009). Cellular senescence, ageing and disease. *Age (Dordr)*, 31 (1), pp.1–9.

Byrne, C. and Eston, R. (2002a). Maximal-intensity isometric and dynamic exercise performance after eccentric muscle actions. *J Sports Sci*, 20 (12), pp.951-959.

Byrne, C. and Eston, R. (2002b). The effect of exercise-induced muscle damage on isometric and dynamic knee extensor strength and vertical jump performance. *J Sports Sci*, 20 (5), pp.417-425.

Byrne, C. Twist, C. and Eston, R. (2004). Neuromuscular function after exercise-induced muscle damage: theoretical and applied implications. *Sports Med*, 34, pp.49-69.

Byrnes, W.C., Clarkson, P.M., White, J.S., Hsieh, S.S., Frykman, P.N. and Maughan, R.J. (1985). Delayed onset muscle soreness following repeated bouts of downhill running. *J Appl Physiol*, 59, pp.710-715.

Cadore, E.L. and Izquierdo, M. (2013). New strategies for the concurrent strength-, power-, and endurance- training prescription in elderly individuals. *J Am Med Dir Assoc*, 14 (8), pp.623-624.

Calabresi, L., Villa, B., Canavesi, M., Sirtori, C.R., James, R.W., Bernini, F. and Franceschini, G. (2004). An omega-3 polyunsaturated fatty acid concentrate increases plasma high-density lipoprotein 2 cholesterol and paraoxonase levels in patients with familial combined hyperlipidemia. *Metabolism Clinc and Exp,* 53*,* pp.153–158.

Calder, P.C. (2006). N-3 polyunsaturated fatty acids, inflammation and inflammatory diseases. *Am J Clin Nutr*, 83 (6), pp.1505-1519.

Calder, P.C. (2008). Polyunsaturated fatty acids, inflammatory processes and inflammatory bowel diseases. *Mol Nutr Food Res*, 52, pp.885-897.

Calder, P.C. (2009). Polyunsaturated fatty acids and inflammatory processes: new twists in an old tale. *Biochimie*, 91, pp.791-795.

Calder, P.C. (2010). Omega-3 fatty acids and inflammatory processes. *Nutrients*, 2, pp.355-374.

Calder, P.C. (2012). Mechanisms of action of n-3 fatty acids. *J Nutr*, 142 (3), pp.592-599.

Calder, P.C. (2013). Nutritional benefits of omega-3 fatty acids. In Jacobsen, C., Skall Nielsen, N., Frisenfeldt Horn, A. and Moltke Sorensen, A-D. (eds.). *Food Enrichment with Omega-3 Fatty Acids*. Woodhead Publishing Series in Food Science, Technology and Nutrition, 252, Cambridge, GB: Woodhead Publishing, pp.3-26.

Calder, P.C. (2015). Functional roles of fatty acids and their effects on human health. *J Parenter Enter Nutr*, 39, pp.18–32.

Calder, P.C. and Yaqoob, P. (2009). Understanding omega-3 polyunsaturated fatty acids. *Postgrad Med*, 121, pp. 148–57.

Campisi, J., Andersen, J.K., Kapahi, P. and Melov, S. (2011). Cellular senescence: a link between cancer and age-related degenerative disease? *Semin Cancer Biol*. 21 (6), pp.354–359.

Carlsson, A.M. (1983). Assessment of chronic pain. I. Aspects of the reliability and validity of the visual analogue scale. *Pain,* 16 (1), pp.87-101.

Caughey, G.E., Mantzioris, E., Gibson, R.A., Cleland, L. G. and James, M.J. (1996). The effect on human tumor necrosis factor a and interleukin 1β production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. *Am J Clin Nutr*, 63, pp.116-122.

Ceddia, M.A. and Woods, J.A. (1999). Exercise suppresses macrophage antigen presentation. *J Appl Physiol*, 87, pp.2253-2258.

Celis-Morales, C.A., Petermann, F., Hui, L., Lyall, D.M., Iliodromiti, S., McLaren, J., Anderson, J., Welsh, P., Mackay, D.F., Pell, J.P., Sattar, N., Gill, J.M.R. and Gray S.R. (2017). Associations between diabetes and both cardiovascular disease and all-cause mortality are modified by grip strength: evidence from UK Biobank, a prospective population-based cohort study. *Diabetes Care*, 40 (12), pp.1710-1718.

Celis-Morales, C.A., Welsh, P., Lyall, D.M., Steell, L., Petermann, F., Anderson, J., Iliodromiti, S., Sillars, A., Graham, N. Mackay, D.F., Pell, J.P., Gill, J.M.R., Sattar, N. and Gray, S.R. (2018). Associations of grip strength with cardiovascular, respiratory, and cancer outcomes and all-cause mortality: prospective cohort study of half a million UK Biobank participants. *Br Med J,* 361, k1651.

Cesari, M., Penninx, B.W., Pahor, M., Lauretani, F., Corsi, A.M., Rhys, Williams, G., Guralnik, J.M. and Ferrucci, L. (2004). Inflammatory markers and physical performance in older persons: the InCHIANTI study. *J Gerontol A Biol Sci Med Sci*, 59 (3), pp.242-248.

Chambers, C., Noakes, T.D., Lambert, E.V. and Lambert, M.I. (1998). Time course of recovery of vertical jump height and heart rate versus running speed after a 90-km foot race. *J Sports Sci*, 16 (7), pp. 645-651.

Chapman, D.W., Newton, M., McGuigan, M. and Nosaka, K. (2008). Effect of lengthening contraction velocity on muscle damage of the elbow flexors. *Med Sci Sports Exerc,* 40, pp.926-933.

Chapman, B.P., Khan, A., Harper, M., Stockman, D., Fiscella, K., Walton, J., Duberstein, P., Talbot, N., Lyness, J.M. and Moynihan, J. (2009). Gender, race/ethnicity, personality, and interleukin-6 in urban primary care patients. *Brain Behav Immun*, 23 (5), pp.636-642.

Chappell, J.D., Herman, D.C., Knight, B.S., Kirkendall, D.T., Garrett, W.E. and Yu, B. (2005). Effect of fatigue on knee kinetics and kinematics in stop-jump tasks. *Am J Sports Med*, 33 (7), pp.1022–1029.

Chazaud, B. (2016). Inflammation during skeletal muscle regeneration and tissue remodeling: application to exercise-induced muscle damage management. *Immunol Cell Biol*, 94 (2), pp.140-145.

Chen, T.C., Nosaka, K. and Sacco, P. (2007). Intensity of eccentric exercise, shift of optimum angle, and the magnitude of repeated-bout effect. *J Appl Physiol,* 102 (3), pp.992-999.

Chen, T.C. and Hsieh, S.S. (2001). Effects of a 7-day eccentric training period on muscle damage and inflammation. *Med Sci Sports Exerc*, 33 (10), pp.1732-1738.

Chen, T.C., Nosaka, K. and Tu, J.H. (2007). Changes in running economy following downhill running. *J Sports Sci,* 25 (1), pp.55-63.

Chen, T.C., Nosaka, K., Lin, M.J., Chen, H.L., and Wu, C.J. (2009). Changes in running economy at different intensities following downhill running. *J Sports Sci*, 27 (11), pp.1137–1144.

Chen, T.C., Lin, K.Y., Chen, H.L., Lin, M.J. and Nosaka, K. (2011). Comparison in eccentric exercise-induced muscle damage among four limb muscles. *Eur J Appl Physiol,* 111, pp.211-223.

Chen, T.C., Chen, H.L., Lin, M.J., Wu, C.J. and Nosaka, K. (2009). Muscle damage responses of the elbow flexors to four maximal eccentric exercise bouts performed every 4 weeks. *Eur Appl Physiol*, 106 (2), pp.267-275.

Chen, T.C., Chen, H.L., Lin, M.J., Yu, H.I. and Nosaka, K. (2016). Contralateral repeated bout effect of eccentric exercise of the elbow flexors. *Med Sci Sports Exerc,* 48, pp.2030-2039.

Chen, T.C., Lin, M.J., Chen, H.L., Lai, J.H., Yu, H.I. and Nosaka, K. (2018). Muscle damage protective effect by two maximal isometric contractions on maximal eccentric exercise of the elbow flexors of the contralateral arm. *Scand J Med Sci Sports*, 28 (4), pp.1354-1360.

Chen, W.T., Chou, K.H., Liu, L.K., Lee, P.L., Lee, W.J., Chen, L.K., Wang, P.N. and Lin, C.P. (2015). Reduced cerebellar gray matter is a neural signature of physical frailty. *Hum Brain Mapp*, 36 (9), pp.3666-3676.

Cheung, K., Hume, P.A. and Maxwell, L. (2003). Delayed onset muscle soreness: treatment strategies and performance factors. *Sports Med*, 33 (2), pp.145–164.

Child, R.B., Saxton, J.M. and Donnelly, A.E. (1998). Comparison of eccentric knee extensor muscle actions at two muscle lengths on indices of damage and angle-specific force production in humans. *J Sports Sci,* 16, pp.301-308.

Childs, B.G., Durik, M., Baker, D.J. and van Deursen, J.M. (2015). Cellular senescence in aging and age-related disease: from mechanisms to therapy. *Nat Med*, 21 (12), pp.1424-1435.

Chleboun, G.S., Howell, J.N., Conatser, R.R. and Giesey, J.J. (1998). Relationship between muscle swelling and stiffness after eccentric exercise. *Med Sci Sports Exerc*. 30 (4), pp.529-535.

Choi, S.J. (2016). Age-related functional changes and susceptibility to eccentric contraction-induced damage in skeletal muscle cell. *Int Med Res*, 5, pp.171-175.

Chrousos, G. (1995). The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *N Eng J Med*, 33 (20), pp.1351-1362.

Chung, H.Y., Cesari, M., Anton, S., Marzetti, E., Giovannini, S., Seo, A.Y., Carter, C., Yu, B.P. and Leeuwenburgh, C. (2009). Molecular inflammation: underpinnings of aging and age-related diseases. *Ageing Res Rev*, 8 (1), pp.18-30.

Clarkson, P.M. and Dedrick, M.E. (1988). Exercise-induced muscle damage, repair, and adaptation in old and young subjects. *J Gerontol*, 43 (4), pp.91–96.

Clarkson, P.M., Hoffman, E.P., Zambraski, E., Gordish-Dressman, H., Kearns, A., Hubal, M., Harmon, B., Devaney, J.M. (2005). ACTN3 and MLCK genotype associations with exertional muscle damage. *J Appl Physiol*, 99, pp.564-569.

Clarkson, P.M. and Hubal, M.J. (2002). Exercise-induced muscle damage in humans. *Am J Phys Med Rehabil*, 81, pp.52–69.

Clarkson, P.M., Nosaka, K. and Braun, B. (1992). Muscle function after exercise-induced muscle damage and rapid adaptation. *Med Sci Sports Exerc,* 24, pp.512–520.

Clarkson, P.M. and Sayers, S.P. (1999). Etiology of exercise-induced muscle damage. *Can J App Physiol*, 24, pp.234-248.

Clarkson, P.M. and Tremblay, I. (1988). Exercise-induced muscle damage, repair, and adaptation in humans. *J Appl Physiol*, 65 (1), pp.1-6.

Cleak, M.J. and Eston, R.G. (1992). Delayed onset muscle soreness: mechanisms and management. *J Sports Sci,* 10 (4), pp.325-341.

Clifford, T., Bell, O., West, D.J., Howatson, G. and Stevenson, E.J. (2016). The effects of beetroot juice supplementation on indices of muscle damage following eccentric exercise. *Eur J Appl Physiol*, 116 (2), pp.353-362.

Cohen, J. (1988). *Statistical power analysis for the behavioural sciences*. Hillsdale, NJ: Lawrence Erlbaum Associates.

Colbert, L.H., Visser,M., Simonsick, E.M., Tracy, R.P., Newman, A.B. Kritchevsky, S.B., Pahor, P., Taaffe, D.R., Brach, J., Rubin, S. and Harris, T.B. (2004). Physical Activity, Exercise, and Inflammatory Markers in Older Adults: Findings from The Health, Aging and Body Composition Study. *J Am Ger Soc*, 52 (7), pp.1098-1104.

Collins, J.J. and Whittle, M.W. (1989). Impulsive forces during walking and their clinical implications. *Clin Biom*, 4, pp.179-187.

Comfort, P. and Matthews, M. (2010). Assessment and needs analysis. Chapter 3. In: *Sports Rehabilitation and Injury Prevention*. UK: Wiley-Blackwell.

Conceição M.S., Libardi, C.A., Nogueira, F.R., Bonganha, V., Gáspari, A.F., Chacon-Mikahil, M.P., Cavaglieri, C.R. and Madruga, V.A. (2012). Effects of eccentric exercise on systemic concentrations of pro- and anti-inflammatory cytokines and prostaglandin (E2): comparison between young and postmenopausal women. *Eur J Appl Physiol*, 112 (9), pp.3205-13.

Coppé, J.P., Patil, C.K., Rodier, F., Sun, Y., Muñoz, D.P., Goldstein, J., Nelson, P.S., Desprez, P.Y. and Campisi, J. (2008). Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol*, 6 (12), pp.2853-68.

Coppé, J.P., Patil, C.K., Rodier, F., Krtolica, A., Beauséjour, C.M., Parrinello, S., Hodgson, J.G., Chin, K., Desprez, P.Y. and Campisi, J. (2010). A human-like senescence-associated secretory phenotype is conserved in mouse cells dependent on physiological oxygen. *PLoS One*, 5 (2), p.e9188.

Corder, K.E., Newsham, K.R., McDaniel, J.L., Ezekiel, U.R. and Weiss, E.P. (2016). Effects of short-term docosahexaenoic acid supplementation on markers of inflammation after eccentric strength exercise in women. *J Sports Sci Med,* 15 (1), pp.176-183.

Crameri, R.M., Aagaard, P., Qvortrup, K., Langberg, H., Olesen, J. and Kjaer, M. (2007). Myofibre damage in human skeletal muscle: effects of electrical stimulation versus voluntary contraction. *J Physiol*, 583, pp.365-380.

Crenshaw, A.G, Thornell, L.E. and Friden, J. (1994). Intramuscular pressure, torque and swelling for the exercise-induced sore vastus lateralis muscle. *Acta Physiol Scand*, 152, pp.265-277.

Croisier, J.L., Camus, G., Venneman, I., Deby-Dupont, G., Juchmes-Ferir, A., Lamy, M., Crielaard, J.M., Deby, C. and Duchateau, J. (1999). Effects of training on exercise-induced muscle damage and interleukin 6 production. *Muscle & Nerve,* 22 (2), pp.208-212.

Cronin, J.B. and Keir, T.H. (2005). Strength and power predictors of sports speed. *J Strength Cond Res*, 19 (2), p.349.

Damas, F., Nosaka, K., Libardi, C.A., Chen, T.C. and Ugrinowitsch, C. (2016). Susceptibility to exercise-induced muscle damage: A cluster analysis with a large sample. *Int J Sports Med*, 37 (8), pp.633–640.

Dalle, S., Rossmeislova, L. and Koppo, K. (2017). The role of inflammation in age-related sarcopenia. *Front Physiol*, 8, p.1045.

Devaney, J.M., Hoffman, E.P., Gordish-Dressman, H., Kearns, A., Zambraski, E., Clarkson, P.M. (2007). IGF-II gene region polymorphisms related to exertional muscle damage. *J Appl Physiol*, 102, pp.1815–1823.

Delaney, N.L., Esquenazi, V., Lucas, D.P., Zachary, A.A. and Leffell, M.S. (2004). TNF-alpha, TGF-beta, IL-10, IL-6, and INF-gamma alleles among African Americans and Cuban Americans. Report of the ASHI Minority Workshops: Part IV. *Hum Immunol*, 65 (12), pp.1413-1419.

Dill, D.B. and Costill, D.L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol*, 37, pp.247–248.

DiLorenzo, F.M., Drager, C.J. and Rankin, J.W. (2014). Docosahexaenoic acid affects markers of inflammation and muscle damage after eccentric exercise. *J Strength Cond Res*, 28 (10), pp.2768–2774.

Dinarello, C.A. (2007). Historical insights into cytokines. *Eur J Immunol*, 37 (1), pp.34-45.

Dodds, R.M., Roberts, H.C., Cooper, C. and Sayer, A.A. (2015). The Epidemiology of Sarcopenia. *J Clin Densitom*, 18 (4), pp.461-6.

Dolci, A., Fortes, M.B., Walker, F.S., Haq, A., Riddle, T. and Walsh, N.P. (2015). Repeated muscle damage blunts the increase in heat strain during subsequent exercise heat stress. *Eur J Appl Physiol,* 115 (7), pp.1577-1588.

Doma, K., Leicht, A. S., Sinclair, W. H., Schumann, M., Damas, F., Burt, D. and Woods, C. (2018). The impact of exercise-induced muscle damage on physical fitness qualities in elite female basketball players. *J Strength Cond Res*, 32 (6), pp.1731-1738.

Dotan, R. (2012). Reverse lactate threshold: a novel single-session approach to reliable high-resolution estimation of the anaerobic threshold. *Int J Sports Physiol Perform*, 7 (2), pp.141-51.

Douglas, J., Pearson, S., Ross, A. and McGuigan, M. (2017). Eccentric exercise: physiological characteristics and acute responses. *Sports Med*, 47 (4), pp.663–675.

Duque, G.A. and Descoteaux, A. (2014). Macrophage cytokines: involvement in immunity and infectious diseases. *Front Immunol*, 5 (491), pp.1-12.

Elliott, B.T., Herbert, P., Sculthorpe, N., Grace, F.M., Stratton, D. and Hayes, L.D. (2017). Lifelong exercise, but not short-term high-intensity interval training, increases GDF11, a marker of successful aging: a preliminary investigation. *Physiol Rep*, 5 (13), e13343.

Endres, S., Ghorbani, R., Kelley, V.E., Georgilis, K., Lonnemann, G., van der Meer, J., Cannon, J.G., Rogers, T.S., Klempner, M.S., Weber, P.C., Schaefer, E.J., Wolff, S.M. and Dinarello, C.A. (1989). The effect of dietary supplementation with N-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N Eng J Med*, 320, pp.265-271.

Ernst, E., Saradeth, T. and Achhammer, G. (1991). N-3 fatty acids and acute-phase proteins. *Eur J Clin Invest*, 21 (1), pp.77-82.

Essen-Gustavsson, B. and Borges, O. (1986). Histochemical and metabolic characteristics of human skeletal muscle in relation to age. *Acta Physiol Scand*, 126, pp.107–114.

Eston, R., Byrne, C. and Twist, C. (2003). Muscle function after exercise-induced muscle damage: Considerations for athletic performance in children and adults. *J Exerc Sci Fitness*, 1 (2), pp.85-96.

Eston, R.G., Finney, S., Baker, S. and Baltzopoulos, V. (1996). Muscle tenderness and peak torque changes after downhill running following a prior bout of isokinetic eccentric exercise. *J Sports Sci*, 14 (4), pp.291-299.

Eston, R.G., Lemmey, A.B., McHugh, P. Byrne, C. and Walsh, S.E. (2000). Effect of stride length on symptoms of exercise-induced muscle damage during a repeated bout of downhill running. *Scand J Med Sci Sports,* 10 (4), pp.199-204.

Eston, R.G., Mickleborough, J. and Baltzopoulos, V. (1995). Eccentric activation and muscle damage: biomechanical and physiological considerations during downhill running. *Br J Sp Med*, 29 (2), pp.89-94.

European Food Safety Authority (EFSA). (2012). Scientific Opinion on the Tolerable Upper Intake Level of eicosopentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). *EFSA J*, 10 (7), p.2815.

Farrell, L., Hollingsworth, B., Propper, C., and Shields, M.A. (2013). The socioeconomic gradient in physical inactivity in England. *Soc. Sci. Med*, 123, pp.55-63.

Farrow, A.C., Gonzales, J.U., Agu-Udemba, C.C., Sobolewski, E.J., Thompson, B.J., Palmer, T.B. (2020). Effects of age on vertical jump performance and muscle morphology characteristics in females. *J Sports Med Phys Fitness*, 60 (8) pp.1081-1088.

Fatouros, I.G. and Jamurtas, A.Z. (2016). Insights into the molecular etiology of exercise-induced inflammation: opportunities for optimizing performance. *J inflamm Res*, 9, pp.175–186.

Fatouros, I.G., Chatzinikolaou, A., Douroudos, I.I., Nikolaidis, M.G., Kyparos, A., Margonis, K., Michailidis, Y., Vantarakis, A., Taxildaris, K., Katrabasas, I., Mandalidis, D., Kouretas, D. and Jamurtas, A.Z. (2010). Time-course of changes in oxidative stress and antioxidant status responses following a soccer game. *J Strength Cond Res*, 24 (12), pp.3278-3286.

Faulkner, J.A., Davis, C.S., Mendias, C.L. and Brooks, S.V. (2008). The aging of elite male athletes: age-related changes in performance and skeletal muscle structure and function. *Clin J Sport Med*, 18 (6), pp.501-7.

Faulkner, J.A., Larkin, L.M., Claflin, D.R. and Brooks, S.V. (2007). Age-related changes in the structure and function of skeletal muscles. *Clin Exp Pharmacol Physiol*, 34 (11), pp.1091-1096.

Febbraio, M.A. and Pedersen, B.K. (2002). Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. *FASEB J,* 16 (11), pp.1335-1347.

Fell, J. and Williams, A.D. (2008). The effect of aging on skeletal muscle recovery from exercise: possible implications for aging athletes. *J Aging Phys Act*, 16 (1), pp.97–115.

Feltner, M.E., Fraschetti, D.J. and Crisp, R.J. (1999). Upper extremity augmentation of lower extremity kinetics during countermovement vertical jumps. *J Sports Sci*, 17 (6), pp.449-466.

Ferrucci, L., Harris, T.B., Guralnik, J.M., Tracy, R.P., Corti, M.C., Cohen, H.J., Penninx, B., Pahor, M., Wallace, R. and Havlik, R.J. (1999). Serum IL-6 level and the development of disability in older persons. *J Am Geriatr Soc*, 47 (6), pp.639-646.

Fielding, R.A. Manfredi, T.J. and Ding, W. (1993). Acute phase response in exercise: III. Neutrophil and IL-1 beta accumulation in skeletal muscle. *Am J Physiol*, 265, pp.166-172.

Fielding, R.A., Violan, M.A., Svetkey, L., Abad, L.W., Manfredi, T.J., Cosmas, A. Bean, J. (2000). Effects of prior exercise on eccentric exercise-induced neutrophilia and enzyme release. *Med Sci Sports Exerc*, 32 (2), pp.359–364.

Food and Nutrition Board (I.O.M). (2005). Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients). *A report of the Panel on Macronutrients, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes,*

and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Washington, DC: National Academy Press.

Fortes, M.B., Di, F.U., Dolci, A., Junglee, N.A., Crockford, M.J., West, L., Hillier-Smith, R., Macdonald, J.H. and Walsh, N.P. (2013). Muscle-damaging exercise increases heat strain during subsequent exercise heat stress. *Med Sci Sports Exerc,* 45 (10), pp.1915-1924.

Franceschi, C. and Campisi, J. (2014). Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J Gerontol A Biol Sci Med Sci*, 69 (1), pp.4–9.

Franceschi, C., Capri, M., Monti, D., Giunta, S., Olivieri, F., Sevini, F., Panourgia, M.P., Invidia, L., Celani, L., Scurti, M., Cevenini, E., Castellani, G.C. and Salvioli, S. (2007). Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mech Ageing Dev*, 128 (1), pp.92-105.

Franchi, M.V., Reeves, N.D. and Narici, M.V. (2017). Skeletal muscle remodelling in response to eccentric vs. concentric loading: Morphological, molecular, and metabolic adaptations. *Front Physiol*, 8, p.447.

Freund, A., Orjalo, A.V., Desprez, P.Y. and Campisi, J. (2010). Inflammatory networks during cellular senes- cence: causes and consequences. *Trends Mol Med*, 16 (5), pp.238–246.

Friden, J. and Lieber, R. L. (2001). Serum creatine kinase level is a poor predictor of muscle function after injury. *Scand J Med Sci Sports,* 11 (2), pp.126-127.

Friden, J., Sjostrom, M. and Ekblom, B. (1981). A morphological study of delayed muscle soreness. *Experientia*, 37, pp.506-507.

Frost, R.A and Lang, C.H. (2005). Skeletal muscle cytokines: Regulation by pathogen-associated molecules and catabolic hormones. *Curr Opin Clin Nutr Metab Care*, 8, pp.255-263.

Galli, C. and Calder, P.C. (2009). Effects of fat and fatty acid intake on inflammatory and immune responses: a critical review. *Ann Nutr Metab*, 55, pp.123-129.

Ganse, B., Ganse, U., Dahl, J. Degens, H. (2018). Linear decrease in athletic performance during the human life span. *Front Physiol*, 9, p.1100.

Garnier, Y.M., Lepers, R., Dubau, Q., Pageaux, B. and Paizis, C. (2018). Neuromuscular and perceptual responses to moderate-intensity incline, level and decline treadmill exercise. *Eur J Appl Physiol*, 118 (10), pp.2039–2053.

Gault, M.L. and Willems, M.E. (2013). Aging, functional capacity and eccentric exercise training. *Aging Dis*, 4 (6), pp.351-63.

Geleijnse, J.M., Giltay, E.J., Grobbee, D.E., Donders, A.R.T. and Kok, F.J. (2002). Blood pressure response to fish oil supplementation: meta-regression analysis of randomized trials. *J Hypertens*, 20, pp.1493–1499.

Gheller, R.G., Dal Pupo, J., Ache-Dias, J., Detanico, D., Padulo, J., dos Santos, S.G. (2015). Effect of different knee starting angles on intersegmental coordination and performance in vertical jumps. *Hum Mov Sci*, 42, pp.71-80.

Giandolini, M., Horvais, N., Rossi, J., Millet, G.Y., Morin, J.B. and Samozino, P. (2016). Acute and delayed peripheral and central neuromuscular alterations induced by a short and intense downhill trail run. *Scand J Med Sci Sports*, 26 (11), pp.1321–1333.

Gibala, M.J., MacDougall, J.D., Tarnopolsky, M.A., Stauber, W.T. and Elorriaga, A. (1995). Changes in human skeletal muscle ultrastructure and force production after acute resistance exercise. *J Appl Physiol*, 78, pp.702-708.

Gibson, R.S. (2005). *Principles of Nutritional Assessment.* 2nd ed. Oxford: Oxford University Press.

Gibson, S.J. and Helme, R.D. (2001). Age-related differences in pain perception and report. *Clin Geriatr Med*, 17 (3), pp.433-56.

Girard, O., Banzet, S., Koulmann, N., Chennaoui, M., Drogou, C., Chalabi, H. and Racinais, S. (2018). Larger strength losses and muscle activation deficits in plantar flexors induced by backward downhill in reference to distance-matched forward uphill treadmill walk. *Eur J Sport Sci,* 18 (10), pp.1346-1356.

Givli, S. (2015). Contraction induced muscle injury: towards personalized training and recovery programs. *Ann Biomed Eng*, 43 (2), pp.388-403.

Gleeson, M. (2007). Immune function in sport and exercise. *J Appl Physiol*, 103 (2), pp.693-699.

Gleeson, M., Blannin, A.K., Zhu, B., Brooks, S. and Cave, R. (1995). Cardiorespiratory, hormonal and haematological responses to submaximal cycling performed 2 days after eccentric or concentric exercise bouts. *J Sports Sci,* 13 (6), pp.471- 479.

Gleeson, M., Bishop, N.C., Stensel, D.J., Lindley, M.R., Mastana, S.S. and Nimmo, M.A. (2011). The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nat Rev Immunol*, 11 (9) pp.607-15.

Gleim, G.W. and McHugh, M.P. (1997). Flexibility and its effects on sports injury and performance. *Sports Med,* 24, pp. 289-99.

Goodwin, M.L., Harris, J.E., Hernández, A. and Gladden, L.B. (2007). Blood lactate measurements and analysis during exercise: a guide for clinicians. *J Diabetes Sci Technol*, 1 (4), pp.558-69.

Gorianovas, G., Skurvydas, A., Streckis, V., Brazaitis, M., Kamandulis, S. and McHugh, M.P. (2013). Repeated bout effect was more expressed in young adult males than in elderly males and boys. *BioMed Res Int*, 2013, p.218970.

Gottschall, J.S. and Kram, R. (2005). Ground reaction forces during downhill and uphill running. *J Biomech*, 38 (3), pp.445-452.

GraphPad Prism, GraphPad Software, San Diego, California, USA.

Graven-Nielsen, T., Lund, H., Arendt-Nielsen, L., Danneskiold-Samsøe, B. and Bliddal, H. (2002). Inhibition of maximal voluntary contraction force by experimental muscle pain: a centrally mediated mechanism. *Muscle Nerve*, 26 (5), pp.708-712.

Gravina, L., Brown, F.F., Alexander, L., Dick, J., Bell, G., Witard, O.C and Galloway, S.D.R. (2017). N-3 fatty acid supplementation during 4 weeks of training leads to improved anaerobic endurance capacity, but not maximal strength, speed or power in soccer players. *Int J Sport Nutr Exerc Metabol*, 27, pp.305-313.

Gray, P., Chappell, A., Jenkinson, A.M., Thies, F. and Gray, S.R. (2014). Fish oil supplementation reduces markers of oxidative stress but not muscle soreness after eccentric exercise. *Int J Sport Nutr Exerc Metab*, 24 (2), pp.206-214.

Green, H.J. (1997). Mechanisms of muscle fatigue in intense exercise. *J Sports Sci,* 15 (3), pp.247-256.

Guilhem, G., Cornu, C. and Guével, A. (2011). Muscle architecture and EMG activity changes during isotonic and isokinetic eccentric exercises. *Eur J Appl Physiol*, 111 (11), pp.2723-2733.

Guilhem, G., Cornu, C., Maffiuletti, N.A. and Guével, A. (2013). Neuromuscular adaptations to isoload versus isokinetic eccentric resistance training. *Med Sci Sports Exerc*, 45 (2), pp.326-235.

Gulick, D.T. and Kimura, I.F. (1996). Delayed onset muscle soreness: what is it and how do we treat it? *J Sport Rehab,* 5, pp.234-243.

Hager, K., Machein, U., Krieger, S., Platt, D., Seefried, G. and Bauer, J. (1994). Interleukin-6 and selected plasma proteins in healthy persons of different ages. *Neurobiol Aging*, 15 (6), pp.771-2.

Haguenauer, M., Legreneur, P. and Monteil, K.M. (2005). Vertical jumping reorganization with aging: a kinematic comparison between young and elderly men. *J Appl Biomech*, 21 (3), pp. 236-46.

Hamada, K., Vannier, E., Sacheck, L.M., Witsell, A.L. and Roubenoff, R. (2005). Senescence of human skeletal muscle impairs the local inflammatory cytokine response to acute eccentric exercise. *FASEB J*, 19, pp.264-266.

Hara, M., Shibayama, A., Takeshita, D. and Fukashiro, S. (2006). The effect of arm swing on lower extremities in vertical jumping. *J Biomech*. 39 (13), pp.2503-2511.

Hara, M., Shibayama, A., Takeshita, D., Hay, D.C. and Fukashiro, S. (2008). A comparison of the mechanical effect of arm swing and countermovement on the lower extremities in vertical jumping. *Hum Mov Sci*, 27 (4), pp.636-648.

Hardy, S.E., Perera, S., Roumani, Y.F., Chandler, J.M. and Studenski, S.A. (2007). Improvement in usual gait speed predicts better survival in older adults. *J Am Geriatr Soc*, 55 (11), pp.1727–1734.

Harridge, S.D. and Lazarus, N.R. (2017). Physical activity, aging, and physiological function. *Physiol (Bethesda)*, 32 (2), pp.152-161.

Harris, W.S. (1997). N-3 fatty acids and serum lipoproteins: Human studies. *Am J Clin Nutr*, 65 (5), pp.1645-1654.

Harris, T.B., Ferrucci, L., Tracy, R.P., Corti, M.C., Wacholder, S., Ettinger, W.H.Jr., Heimovitz, H., Cohen, H.J. and Wallace, R. (1999). Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am J Med*, 106 (5), pp.506-512.

Harris, W.S. and von Schacky, C. (2004). The omega-3 index: a new risk factor for death from CHD? *Prevent Med*, 39, pp.212-220.

Harty, P.S., Cottet, M.L., Malloy, J.K. and Kerksick, C.M. (2019). Nutritional and supplementation strategies to prevent and attenuate exercise-induced muscle damage: a brief review. *Sports Med Open*, 5 (1), p.1.

Havas, E., Komulainen, J. and Vihko, V. (1997). Exercise-induced increase in serum creatine kinase is modified by subsequent bed rest. *Int J Sport Med*, 18, pp.578-582.

Hayflick, L. (2007). Biological aging is no longer an unsolved problem. *Ann N Y Acad Sci*, 1100, pp.1-13.

Heckel, Z., Atlasz, T., Tekus, E., Kőszegi, T., Laczko, J. and Vaczi, M. (2019). Monitoring exercise- induced muscle damage indicators and myoelectric activity during two weeks of knee extensor exercise training in young and old men. *PLoS One,* 14 (11), e0224866.

Hecksteden, A., Faude, O., Meyer, T. and Donath, L. (2018). How to construct, conduct and analyze an exercise training study? *Front Physiol*, 9, p.1007.

Heigenhauser, G.J.F., Sutton, J.R. and Jones, N.L. (1983). Effect of glycogen depletion on the ventilatory response to exercise. *J App Physiol, Res, Env Exerc Physiol,* 54, pp.470-474.

Heinrich, P.C., Castell, J.V. and Andus, T. (1990). Interleukin-6 and the acute phase response. *Biochem, J*, 265, pp.621-636.

Heisterberg, M.F., Fahrenkrug, J., Krustrup, P., Storskov, A., Kjær, M. and Andersen, J.L. (2013). Extensive monitoring through multiple blood samples in professional soccer players. *J Strength Cond Res,* 27 (5), pp.1260-1271.

Heled, Y., Bloom, M.S., Wu, T.J., Stephens, Q., and Deuster, P.A. (2007). CK-MM and ACE genotypes and physiological prediction of the creatine kinase response to exercise. *J Appl Physiol*, 103, pp.504-510.

Helers, G.G., Ball, T.E. and Liston, L. (2002). Creatine kinase levels are elevated during 2-A-day practices in collegiate football players. *Athl Train*, 37 (2), pp.151-156.

Hellsten, Y., Frandsen, U., Orthenblad, N., Sjodin, B. and Richter, E.A. (1997). Xanthine oxidase in human skeletal muscle following eccentric exercise: a role in inflammation. *J Physiol,* 498 (1), pp.239-248.

Herbert, P., Grace, F.M. and Sculthorpe, N.F. (2015). Exercising caution: prolonged recovery from a single session of high-intensity interval training in older men. *J Am Ger Soc*, 63 (4), pp.817-818.

Herzog, W. (2014). Mechanisms of enhanced force production in lengthening (eccentric) muscle contractions. *J Appl Physiol*, 116 (11), pp.1407-1417.

Hessel, A.L., Lindstedt, S.L. and Nishikawa, K.C. (2017). Physiological mechanisms of eccentric contraction and its applications: a role for the giant titin protein. *Front Physiol*, 8, p.70.

Hickner, R. C., Mehta, P. M., Dyck, D., Devita, P., Houmard, J. A., Koves, T., and Byrd, P. (2001). Relationship between fat-to-fat-free mass ratio and decrements in leg strength after downhill running. *J Appl Physiol*, 90 (4), pp.1334-1341.

Hickson, L.J., Langhi Prata, L.G.P., Bobart, S.A., Evans, T.K., Giorgadze, N., Hashmi, S.K., Herrmann, S.M., Jensen, M.D., Jia, Q., Jordan, K.L., Kellogg, T.A., Khosla, S., Koerber, D.M., Lagnado, A.B., Lawson, D.K., LeBrasseur, N.K., Lerman, L.O., McDonald, K.M., McKenzie, T.J., Passos, J.F., Pignolo, R.J., Pirtskhalava, T., Saadiq, I.M., Schaefer, K.K., Textor, S.C., Victorelli, S.G., Volkman, T.L., Xue, A., Wentworth, M.A., Wissler Gerdes, E.O., Zhu, Y., Tchkonia, T. and Kirkland, J.L. (2019). Senolytics decrease senescent cells in humans: Preliminary report from a clinical trial of Dasatinib plus Quercetin in individuals with diabetic kidney disease. *EBioMedicine*, 47, pp.446-456.

Hill, C., James, R.S., Cox, V.M. and Tallis, J. (2018). The effect of increasing age on the concentric and eccentric contractile properties of isolated mouse soleus and extensor digitorum longus muscles. *J Gerontol Series A, Biol Sci and Med Sci*, *73*, pp.579-587.

Hill, M.W., Hosseini, E.A., McLellan, A., Price, M.J., Lord, S.R. and Kay, A.D. (2020). Delayed impairment of postural, physical, and muscular functions following downhill compared to level walking in older people. *Front Physiol*, 11, 544559.

Hirose, L., Nosaka, K., Newton, M., Laveder, A., Kano, M., Peake, J. and Suzuki, K. (2004). Changes in inflammatory mediators following eccentric exercise of the elbow flexors. *Exerc Immunol Rev,* 10, pp.75-90.

Hitomi, K., Okada, R., Loo, T.M., Miyata, K., Nakamura, A.J. and Takahashi, A. (2020). DNA damage regulates senescence-associated extracellular vesicle release via the ceramide pathway to prevent excessive inflammatory responses. *Int J Mol Sci*, 21 (10), p.3720.

Hody, S., Croisier, J.L., Bury, T., Rogister, B. and Leprince, P. (2019). Eccentric muscle contractions: risks and benefits. *Front Physiol*, 10, p.536.

Hoffman-Goetz, L. and Pedersen, B.K. (1994). Exercise and the immune system: a model of the stress response? *Immunol Today*, 15, pp.382-387.

Hollenberg, M., Yang, J., Haight, T.J. and Tager, I.B. (2006). Longitudinal changes in aerobic capacity: implications for concepts of aging. *J Gerontol A Biol Sci Med Sci*, 61, pp.851–858.

Holme, I. and Anderssen, S.A. (2015). Increases in physical activity is as important as smoking cessation for reduction in total mortality in elderly men: 12 years of follow-up of the oslo ii study. *Br J Sports Med*, 49, pp.743-748.

Holviala, J., Hakkinen, A., Alen, M., Sallinen, J., Kraemer, W. and Hakkinen, K. (2014). Effects of prolonged and maintance strength training on force production, walking, and balance in aging women and men. *Scand J Med Sci Sports*, 24 (1), pp.224-233.

Hong, S., Gronert, P. Devchand, R.-L., Moussignac, C. N. and Serhan, C. N. (2003). Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood and glial cells: autocoids in anti-inflammation. *J Biol Chem*, 278, pp.14677-14687.

Horita, T., Komi, P.V., Nicol, C. and Kyröläinen, H. (1999). Effect of exhausting stretch-shortening cycle exercise on the time course of mechanical behaviour in the drop jump: possible role of muscle damage. *Eur J Appl Physiol Occup Physiol*, 79 (2), pp.160-167.

Hosseinkhani, B., Kuypers, S., van den Akker, N.M.S., Molin, D.G.M. and Michiels, L. (2018). Extracellular vesicles work as a functional inflammatory mediator between vascular endothelial cells and immune cells. *Front Immunol*, 9, p.1789.

Hough, T. (1902). Ergographic studies in muscular soreness. *Am J Physiol*, 7, pp.76-92.
Houghton, D. and Onambele, G.L. (2012). Can a standard dose of eicosapentaenoic acid (EPA) supplementation reduce the symptoms of delayed onset of muscle soreness? *J Int Soc Sports Nutr,* 9 (1), p.1.

Howell, J.N, Chleboun, G. and Conatser, R. (1993). Muscle stiffness, strength loss, swelling and soreness following exercise-induced injury in humans. *J. Physiol*, 464, pp.183–196.

Howley, E.T., Bassett, D.R. Jr and Welch, H.G. (1995). Criteria for maximal oxygen uptake: review and commentary. *Med Sci Sports Exerc*, 27, pp.1292-1301.

Howren, M.B., Lamkin D.M. and Suls, J. (2009). Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosom Med*, 71 (2), pp.171–186.

Hu, F.B., Meigs, J.B., Li, T.Y., Rifai, N. and Manson, J.E. (2004). Inflammatory markers and risk of developing type 2 diabetes in women. *Diabetes*, 53 (3), pp.693–700.

Huffman, D.M., Altena, T.S., Mawhinney, T.P. and Thomas, T.R. (2004). Effect of n-3 fatty acids on free tryptophan and exercise fatigue. *Eur J Appl Physiol,* 92 (4-5), pp.584-591.

Hughes, J.D., Denton, K., Lloyd, R.S., Oliver, J.L., and Croix, M.D.S. (2018). The impact of soccer match play on the muscle damage response in youth female athletes. *Int J Sports Med*, 39 (5), pp.343-348.

Hureau, T., Romer, L. and Amann, M. (2018). The 'sensory tolerance limit': a hypothetical construct determining exercise performance? *Eur J Sport Sci,* 18, pp.13–24.

Hyatt J.P. and Clarkson, P.M. (1998). Creatine kinase release and clearance using MM variants following repeated bouts of eccentric exercise. *Med Sci Sports Exerc*, 30 (7), pp.1059-1065.

Hyldahl, R.D., Chen, T.C. and Nosaka, K. (2017). Mechanisms and Mediators of the Skeletal Muscle Repeated Bout Effect. *Exerc Sport Sci Rev*, 45 (1), pp.24-33.

Hyldahl, R.D. and Hubal, M.J. (2014). Lengthening our perspective: Morphological, cellular and molecular responses to eccentric exercise. *Muscle & Nerve*, 49 (2), pp.155-170.

Hyldahl, R.D., Nelson, B., Xin, X., Welling, T. Groscost, L., Hubal, M.J., Chipkin, S., Clarkson, P.M. and Parcell, A.C. (2015). Extracellular matrix remodeling and its contribution to protective adaptation following lengthening contractions in human muscle. *FaSEB J*, 29, pp.1-12.

Hyldahl, R.D., Xin, L., Hubal, M.J., Moeckel-Cole, S., Chipkin, S. and Clarkson, P.M. (2011). Activation of nuclear factor-κB following muscle eccentric contractions in humans is localized primarily to skeletal muscle-residing pericytes. *FASEB J*, 25, pp.2956–2966.

IBM SPSS Statistics for Mac, Armonk, NY, U.S.A: IBM Corp.

International Olympic Committee (IOC). (2011). Consensus statement on sports nutrition 2010. *J Sports Sci*, 29 (1), pp.3-4.

Ivy, J.L. (2004). Regulation of muscle glycogen repletion, muscle protein synthesis and repair following exercise. *J Sports Sci Med*, 3 (3), pp.131-8.

Jakeman, J.R., Byrne, C. and Eston, R.G. (2010). Lower limb compression garment improves recovery from exercise-induced muscle damage in young, active females. *Eur J Appl Physiol*, 109 (6), pp.1137-1144.

Jakeman, J.R., Lambrick, D.M., Wooley, B., Babraj, J.A. and Faulkner, J.A. (2017). Effect of an acute dose of omega-3 fish oil following exercise-induced muscle damage. *Eur J Appl Physiol*, 117, pp.575–582.

Jakeman, J., Macrae, R. and Eston, R. (2009). A single 10-min bout of cold-water immersion therapy after strenuous plyometric exercise has no beneficial effect on recovery from the symptoms of exercise-induced muscle damage. *Ergonomics,* 52, pp.456–460.

James, M.J., Ursin, V.M. and Cleland, L.G. (2003). Metabolism of stearidonic acid in human subjects: comparison with the metabolism of other n-3 fatty acids. *Am J Clin Nutr*, 77, pp.1140–1145.

Jamurtas, A.Z., Theocharis, V., Tofas, T., Tsiokanos, A., Yfanti, C., Paschalis, V., Koutedakis, Y. and Nosaka K. (2005). Comparison between leg and arm eccentric exercise of the same relative intensity on indices of muscle damage. *Eur J Appl Physiol,* 95, pp.179-185.

Jayaraman, R.C., Reid, R.W., Foley, J.M., Prior, B.M., Dudley, G.A., Weingand, K.W. and Meyer, R.A. (2004). MRI evaluation of topical heat and static stretching as therapeutic modalities for the treatment of eccentric exercise-induced muscle damage. *Eur J Appl Physiol*, 93 (1-2), pp.30-38.

Jeromson, S., Gallagher, I.J., Galloway, S.D.R. and Hamilton, D.L. (2015). Omega-3 fatty acids and skeletal muscle health. *Marine Drugs*, 13 (11), pp.6977- 7004.

Jones, D.A. and Round, J.M. (1997). *Human muscle damage induced by eccentric exercise or reperfusion injury: A common mechanism*? In ed. Salmon S., 64-75. New York, Oxford Press.

Jeyapalan, J.C. and Sedivy, J.M. (2008). Cellular senescence and organismal aging. *Mech Ageing Dev*, 129 (7-8), pp.467–474.

Jones, H.R., Burns, T., Aminoff, M.J. and Pomeroy, S. (2013). *The Netter Collection of Medical Illustrations: Nervous System—Spinal Cord and Peripheral Motor and Sensory Systems*, 2nd ed. Philadelphia: Elsevier.

Jones, D.A., Newham, D.J., Round, J. M. and Tlfree, S. E. (1986). Experimental human muscle damage: morphological changes in relation to other indices of damage. *J Physiol*, 375, pp.435-448.

Jones, D.A., Newham, D.J. and Torgan, C. (1989). Mechanical influences on long lasting human muscle fatigue and delayed onset pain. *J Physiol*, 412, pp.415-427.

Jouris, K.B., McDaniel J.L. and Weiss, E.P. (2011). The effect of omega-3 fatty acid supplementation on the inflammatory response to eccentric strength exercise. *J Sports Sci Med*, 10, pp.432-438.

Kaczor, J.J., Ziolkowski, W., Antosiewicz, J., Hac, S., Tarnopolsky, M.A. and Popinigis, J. (2006). The effect of aging on anaerobic and aerobic enzyme activities in human skeletal muscle. *J Gerontol A Biol Sci Med Sci*, 61, pp.339–344.

Kadi, F., Charifi, N., Denis, C. and Lexell, J. (2004). Satellite cells and myonuclei in young and elderly women and men. *Muscle Nerve*, 29 (1), pp.120-7.

Kalinski, M. (2010). Cellular mechanisms of muscle injury during exercise. *Br J Sports Med*, 44 (1), pp.1-82.

Kalra, L., Rambaran, C., Chowienczyk, P., Goss, D., Hambleton, I., Ritter, J., Shah, A., Wilks, R. and Forrester, T. (2005). Ethnic differences in arterial responses and inflammatory markers in Afro-Caribbean and Caucasian subjects. *Arterioscler Thromb Vasc Biol*, 25 (11), pp.2362-2367.

Kanapuru, B. and Ershler, W.B. (2009). Inflammation, coagulation, and the pathway to frailty. *Am J Med*, 122 (7), pp.605–613.

Kanda, K. Sugama, K., Hayashida, H., Sakuma, J., Kawakami, Y., Miura, S., Yoshioka, H., Mori, Y. and Suzuki, K. (2013). Eccentric exercise-induced delayed-onset muscle soreness and changes in markers of muscle damage and inflammation. *Exerc Immunol Rev*, 19, pp.72-85.

Kellis, E. and Baltzopoulos, V. (1998). Muscle activation differences between eccentric and concentric isokinetic exercise. *Med Sci Sports Exerc*, 30, pp.1616-1623.

Kennedy, B.K., Berger, S.L., Brunet, A., Campisi, J., Cuervo, A.M., Epel, E.S., Franceschi, C., Lithgow, G.J., Morimoto, R.I., Pessin, J.E., Rando, T.A., Richardson, A., Schadt, E.E., Wyss-Coray, T. and Sierra, F. (2014). Geroscience: linking aging to chronic disease. *Cell*, 159 (4), pp.709-13.

Khan, S.U., Lone, A.N., Khan, M.S., Virani, S.S., Blumenthal, R.S., Nasir, K., Miller, M., Michos, E.D., Ballantyne, C.M., Boden, W.E. and Bhatt, D.L. (2021). Effect of omega-3 fatty acids on cardiovascular outcomes: A systematic review and meta-analysis. *EClinicalMedicine*, 3, p.32.

Khan, S., McNeil, C., Gandevia, S. and Taylor, J. (2011). Effect of experimental muscle pain on maximal voluntary activation of human biceps brachii muscle. *J Appl Physiol*, 111, pp.743–750.

Kim, J. and Lee, J. (2015). The relationship of creatine kinase variability with body composition and muscle damage markers following eccentric muscle contractions. *J Exerc Nutr Bioch,* 19 (2), p.123.

Kohne, J.L., Ormsbee, M.J. and McKune, A.J. (2016). The effects of a multi-ingredient supplement on markers of muscle damage and inflammation following downhill running in females. *J Int Soc Sports Nutr*, 13 (44), pp.1-13.

Komi, P.V. (1992). Stretch-shortening cycle. In: Komi, V. (ed.) *Strength and Power in Sport*. Oxford: Blackwell Science, pp.169–179.

Komi, P.V. (2000). Stretch-shortening cycle: a powerful model to study normal and fatigued muscle. *J Biomech*, 33 (10), pp.1197-206.

Komulainen, J., Takala, T.E., Kuipers, H. and Hesselink, M.K. (1998). The disruption of myofibre structures in rat skeletal muscle after forced lengthening contractions. *Pflugers Arch*, 436 (5), pp.735-741.

Koopman, R. and van Loon, L.J. (1985). Aging, exercise, and muscle protein metabolism. *J Appl Physiol*, 106 (6), pp.2040-8.

Koopman, R., Saris, W.H., Wagenmakers, A.J. and van Loon, L.J. (2007). Nutritional interventions to promote post-exercise muscle protein synthesis. *Sports Med*, 37 (10), pp.895-906.

Kraemer, W.J., Marchitelli, L., Gordon, S.E., Harman, E., Dziados, J.E., Mello, R., Frykman, P., McCurry, D. and Fleck, S.J. (1990). Hormonal and growth factor responses to heavy resistance exercise protocols. *J Appl Physiol,* 69 (4), pp.1442-1450.

Kraemer, W.J., Noble, B.J., Clark, M.J. and Culver, B.W. (1987). Physiologic responses to heavy-resistance exercise with very short rest periods. *Int J Sports Med*, 8 (4), pp.247-252.

Krzysztofik, M., Wilk, M., Wojdała, G. and Gołaś, A. (2019). Maximizing muscle hypertrophy: a systematic review of advanced resistance training techniques and methods. *Int J Environ Res Public Health*, 16 (24), p.4897.

Kuilman, T., Michaloglou, C., Mooi, W.J. and Peeper, D.S. (2010). The essence of senescence. *Genes Dev*, 24 (22), pp.2463–2479.

Kyriakidou, Y., Wood, C., Ferrier, C., Dolci, A. and Elliott, B. (2021a). The effect of Omega-3 polyunsaturated fatty acid supplementation on exercise-induced muscle damage. *J Int Soc Sports Nutr*, 18 (1), p.9.

Kyriakidou, Y, Cooper, I., Kraev, I., Lange, S. and Elliott, B.T. (2021b). Preliminary investigations into the effect of exercise-induced muscle damage on systemic extracellular vesicle release in trained younger and older men. *Front Physiol*, 12, 723931.

Lacour, J.R., Bouvat, E. and Barthélémy, J.C. (1990). Post-competition blood lactate concentrations as indicators of anaerobic energy expenditure during 400-m and 800-m races. *Eur J Appl Physiol Occup Physiol*, 61 (3-4), pp.172-6.

Lancaster, G.I., Khan, Q., Drysdale, P., Wallace, F., Jeukendrup, A.E., Drayson, M.T. and Gleeson, M. (2005). The physiological regulation of toll-like receptor expression and function in humans. *J. Physiol*, 563, pp.945-955.

Landi, F., Marzetti, E., Martone, A.M., Bernabei, R. and Onder, G. (2014). Exercise as a remedy for sarcopenia. *Curr Opin Clin Nutr Metab Care*, 17 (1), pp.25-31.

Lanham-New, S.A., Stear, S.J., Shirreffs, S.M. and Collins, A.L. (2011). *Sport and Exercise Nutrition*. United Kingdom: Wiley-Blackwell.

Lanza, I.R., Befroy, D.E. and Kent-Braun, J.A. (1985). Age-related changes in ATP-producing pathways in human skeletal muscle in vivo. *J Appl Physiol*, 99 (5), pp.1736–1744.

Lauritzen, F., Paulsen, G., Raastad, T., Bergersen, L. H. and Owe, S. G. (2009). Gross ultrastructural changes and necrotic fiber segments in elbow flexor muscles after maximal voluntary eccentric action in humans. *J Appl Physiol*, 107, pp.1923-1934.

Lavender, A.P. and Nosaka, K. (2006a). Changes in fluctuation of isometric force following eccentric and concentric exercise of the elbow flexors. *Eur J Appl Physiol,* 96 (3), pp.235-240.

Lavender, A.P. and Nosaka, K. (2006b). Responses of old men to repeated bout of eccentric exercise of the elbow flexors in comparison with young men. *Eur J Appl Physiol*, 97 (5), pp.619–626.

Lavender, A.P. and Nosaka, K. (2006c). Comparison between old and young men for changes in makers of muscle damage following voluntary eccentric exercise of the elbow flexors. *Appl Physiol Nutr Metab*, 31, pp.218–225.

Lavender, A.P. and Nosaka, K. (2008). Changes in markers of muscle damage of middle-aged and young men following eccentric exercise of the elbow flexors. *J Sci Med Sport*, 11 (2), pp.124–131.

Lee, T.H., Hoover, R.L., Williams, J.D., Sperling, R. I., Ravalese, J., Spur, B.W., Robinson, D.R, Corey, E.J., Lewis, R.A. and Austen, K.F. (1985). Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic Acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. *N Engl J Med*, 312, pp.1217-1224.

Lee, E.C., Fragala, M.S., Kavouras, S.A., & Queen, R.M., Pryor, J.L. and Casa, D.J. (2017). Biomarkers in sports and exercise: Tracking health, performance, and recovery in athletes. *J Strength Cond Res,* 31 (10), pp.2920-2937.

Lehti, T.M., Kalliokoski, R. and Komulainen, J. (2007). Repeated bout effect on the cytoskeletal proteins titin, desmin, and dystrophin in rat skeletal muscle. *J Muscle Res Cell Motil,* 28 (1), pp.39-47.

Lembke, P., Capodice, J., Hebert, K. and Swenson, T. (2014). Influence of omega-3 (n3) index on performance and wellbeing in young adults after heavy eccentric exercise. *J Sports Sci Med*, 13, pp.151–156.

Leng, S.X., Xue, Q.L., Tian, J., Walston, J.D. and Fried, L.P. (2007). Inflammation and frailty in older women. *J Am Geriatr Soc*, 55 (6), pp.864–871.

Lenn, J., Uhl, T., Mattacola, C., Boissonneault, G., Yates, J., Ibrahim, W., Bruckner, G. (2002). The effects of fish oil and isoflavones on delayed onset muscle soreness. *Med Sci Sports Exerc*, 34, pp.1605–1613.

Lepers, R., Bigard, A.X., Diard, J.P., Gouteyron, J.F., Guezennec, C.Y. (1997). Posture control after prolonged exercise. *Eur J Appl Physiol*, 76, pp.55-61.

Lewis, E, Radonic, P., Wolever, T. and Wells, G. (2015). 21 days of mammalian omega-3 fatty acid supplementation improves aspects of neuromuscular function and performance in male athletes compared to olive oil placebo. *J Int Soc Sports Nutr,* 12 (1), p.28.

Liem, R.I., Onyejekwe, K., Olszewski, M. Nchekwube, C., Zaldivar, F.P., Radom-Aizik, S., Rodeghier, M.J. and Thompson, A.A. (2015). The acute phase inflammatory response to maximal exercise testing in children and young adults with sickle cell anaemia. *Br J Haematol,* 171 (5), pp.854-861.

Lim, J.Y. (2016). Therapeutic potential of eccentric exercises for age-related muscle atrophy. Integr *Med Res*, 5 (3), pp.176-181.

Lima, G.A., Vilaça, K.H., Lima, N.K., Moriguti, J.C. and Ferriolli, E. (2011). Balance and aerobic capacity of independent elderly: a longitudinal cohort study. *Rev Bras Fisioter*, 15 (4), pp.272-777.

Lindsay, T., Westgate, K., Wijndaele, K., Hollidge S. Kerrison, N., Forouhi, N., Griffin, S., Wareham, N., and Brage, S. (2019). Descriptive epidemiology of physical activity energy expenditure in UK adults (The Fenland study). *Int J Behav Nutr Phys Act*, 16, p.126.

Ling, P.R., Boyce, P. and Bistrian, B.R. (1998). Role of arachidonic acid in the regulation of the inflammatory response in TNF-alpha-treated rats. *J Parenter Entr Nutr*, 22, pp.228-275.

Lovering, R.M. and De Deyne, P.G. (2004). Contractile function, sarcolemma integrity, and the loss of dystrophin after skeletal muscle eccentric contraction-induced injury. *Am J Physiol Cell Physiol*, 286 (2), pp.230-238.

Lucia, A., Pardo, J., Durántez, A., Hoyos, J. and Chicharro, J.L. (1998). Physiological differences between professional and elite road cyclists. *Int J Sports Med,* 19, pp.342-348.

Lucicesare, A., Hubbard, R.E., Searle, S.D. and Rockwood, K. (2010). An index of self-rated health deficits in relation to frailty and adverse outcomes in older adults. *Aging Clin Exp Res*, 22 (3), pp.255–260.

Macfarlane, D.J. and Wong, P. (2012). Validity, reliability and stability of the portable Cortex Metamax 3B gas analysis system. *Eur J Appl Physiol*, 112, pp.2539-2547.

Machado, M., Koch, A.J., Willardson, J.M., Pereira, L.S., Cardoso, M.I., Motta, M.K., Pereira, R. and Monteiro, A.N. (2011). Effect of varying rest intervals between sets of assistance exercises on creatine kinase and lactate dehydrogenase responses. *J Strength Cond Res*, 25, pp.1339-1345.

Machado, M., Pereira, R. and Willardson, J.M. (2012). Short intervals between sets and individuality of muscle damage response. *J Strength Cond Res*, 26, pp.2946-2952.

MacKinnon, L.T. (1999). *Advances in exercise immunology*. Human Kinetics, Champaign, Ill., pp.201-227.

MacIntyre, D.L., Reid, W.D. and McKenzie, D.C. (1995). Delayed Muscle Soreness. The Inflammatory Response to Muscle Injury and its Clinical Implications. *Sports Med*, 20, pp.24-40.

Maeo, S., Ando, Y., Kanehisa, H. and Kawakami, Y. (2017). Localization of damage in the human leg muscles induced by downhill running. *Sci Rep*, 7 (1), p.5769.

Magal, M., Dumke, C., Urbiztondo, Z., Cavill, M., Triplett, N., Quindry, J., Mcbride, J. and Epstein, Y. (2010). Relationship between serum creatine kinase activity following exercise-induced muscle damage and muscle fibre composition, *J Sports Sci,* 28 (3), pp.257–266.

Malinauskas, B.M., Overton, R.F., Cucchiara, A. J., Carpenter, A.B. and Corbett, A.B. (2007). Summer league college baseball players: Do dietary intake and barriers to eating healthy differ between game and non-game days? *Sport Manag Rel Topics J*, 3 (2), pp.23–34.

Malm, C., Lenkei, R. and Sjodin, B. (1999). Effects of eccentric exercise on the immune system in men. *J Appl Physiol,* 86 (2), pp.461-468.

Malm, C., Nyberg, P., Engstrom, M., Sjodin, B., Lenkei, R., Ekblom, B. and Lundberg, I. (2000). Immunological changes in human skeletal muscle and blood after eccentric exercise and multiple biopsies. *J Physiol,* 529 (1), pp.243-262.

Malm, C. (2001). Exercise-induced muscle damage and inflammation: Fact or fiction? *Acta Physiol Scand*, 171 (3), pp.233-239.

Malm, C., Sjodin, T.L., Sjoberg, B., Lenkei, R., Renstrom, P., Lundberg, I. E. and Ekblom, B. (2004). Leucocytes, cytokines, growth factors and hormones in human skeletal muscle and blood after uphill or downhill running. *J Physiol*, 556 (3), pp.983-1000.

Manojlović, V. and Erčulj, F. (2019). Using blood lactate concentration to predict muscle damage and jump performance response to maximal stretch-shortening cycle exercise. *J Sports Med Phys Fitness*, 59 (4), pp.581-586.

Mansfield, P.J. and Neumann, D.A. (2019). Structure and Function of Skeletal Muscle. In: *Essentials of Kinesiology for the Physical Assistant* (3rd ed). St. Louis, Missouri: Elsevier

Margaritelis, N., Theodorou, A., Baltzopoulos, V., Maganaris, C., Paschalis, V., Kyparos, A. and Nikolaides, M. (2015). Muscle damage and inflammation after eccentric exercise: can the repeated bout effect be removed? *Phys Rep*, 3 (12), e12648.

Marzetti, E., Lawler, J.M., Hiona, A., Manini, T., Seo, A.Y. and Leeuwenburgh, C. (2008). Modulation of age-induced apoptotic signalling and cellular remodelling by exercise and calorie restriction in skeletal muscle. *Free Rad Biol Med*, 44 (2), pp.160–168.

McBride, T.A., Gorin, F.A. and Carlsen, R.C. (1995). Prolonged recovery and reduced adaptation in aged rat muscle following eccentric exercise. *Mechan Ageing Develop*, 83, pp.185-200.

McBride, T. (2000). Increased depolarization, prolonged recovery and reduced adaptation of the resting membrane potential in aged rat skeletal muscles following eccentric contractions. *Mechan Ageing Develop*, 115, pp.127-138.

McCormack, H.M., Horne, D.J. and Sheather, S. (1988). Clinical applications of visual analogue scales: a critical review. *Psychol Med,* 18 (4), pp.1007-1019.

McCrory, J.L., Salacinski, A.J., Hunt, S.E. and Greenspan, S.L. (2009). Thigh muscle strength in senior athletes and healthy controls. *J Strength Cond Res*, 23 (9), pp.2430-2436.

McGlory C., Calder, P.C. and Nunes, E.A. (2019). The influence of omega-3 fatty acids on skeletal muscle protein turnover in health, disuse, and disease. *Front Nutr*, 6, p.144.

McGlory, C., Galloway, S.D.R., Hamilton, D.L., McClintock, C., Breen, L., Dick, J.R., Bell. J. G. and Tipton, K.D. (2014). Temporal changes in human skeletal muscle and blood lipid composition with fish oil supplementation. *Prostagl Leukotr Ess Fat Acids*, 90 (6), 199-206.

McGlory, C., Gorissen, S.H.M., Kamal, M., Bahniwal, R., Hector, A.J., Baker, S.K., Chabowski, A. and Phillips, S.M. (2019). Omega-3 fatty acid supplementation attenuates skeletal muscle disuse atrophy during two weeks of unilateral leg immobilization in healthy young women. *FASEB J*, 33, pp.4586–4597.

McHugh, M.P., Connolly, D.A., Eston, R.G. and Gleim, G.W. (1999). Exercise-induced muscle damage and potential mechanisms for the repeated bout effect. *Sports Med*, 27, pp.157-170.

McHugh, M.P. (2003). Recent advances in the understanding of the repeated bout effect: the protective effect against muscle damage from a single bout of eccentric exercise. *Scand J Med Sci Sports*, 13, pp.88-97.

McKay, B.R., Ogborn, D.I., Bellamy, L.M., Tarnopolsky, M.A. and Parise, G. (2012). Myostatin is associated with age-related human muscle stem cell dysfunction. *FASEB J*, 26 (6), pp.2509-2521.

McKendry, J., Stokes, T., Mcleod, J.C. and Phillips, S.M. (2021). Resistance exercise, aging, disuse, and muscle protein metabolism. *Compr Physio,* 11 (3), pp.2249-2278.

McKune, A.J., Smith, L.L., Semple, S.J., Mokethwa, B. and Wadee, A.A. (2006). Immunoglobulin responses to a repeated bout of downhill running. *Br J Sports Med*, 40 (10), pp.844-849.

Mcleod, J.C., Stokes, T. and Phillips, S.M. (2019). Resistance exercise training as a primary countermeasure to age-related chronic disease. *Front Physiol* 10, p.645.

Meeusen, R., Watson, P., Hasegawa, H., Roelands, B. Piacentini, M.F. (2006). Central fatigue. *Sports Med,* 36 (10), pp.881-909.

Menzies, P., Menzies, C., McIntyre, L., Paterson, P., Wilson, J. and Kemi, O.J. (2010). Blood lactate clearance during active recovery after an intense running bout depends on the intensity of the active recovery. *J Sports Sci*, 28 (9), pp.975-982.

Metcalf, R.G., James, M.J., Gibson, R.A., Edwards, J.R., Stubberfield, J., Stuklis, R., Roberts-Thomson, K., Young, G.D. and Cleland, L. G. (2007). Effects of fish-oil supplementation on myocardial fatty acids in humans. *Am J Clin Nutr*, 85, pp.1222–1228.

Metter, E.J., Conwit, R., Tobin, J. and Fozard, J.L. (1997). Age-associated loss of power and strength in the upper extremities in women and men. *J Gerontol A Biol Sci Med Sci,* 52 (5), pp.267-276.

Mickleborough, T.D. (2013). Omega-3 polyunsaturated fatty acids in physical performance optimization. *Int J Sport Nutr Exerc Metab,* 23 (1), pp.83-96.

Mickleborough, T.D., Sinex, J.A., Platt, D. Chapman, R.F. and Hirt, M. (2015). The effects PCSO-524[(R)], a patented marine oil lipid and omega-3 PUFA blend derived from the New Zealand green lipped mussel *(Perna canaliculus)*, on indirect markers of muscle damage and inflammation after muscle damaging exercise in untrained men: a randomized, placebo controlled trial. *J Int Soc Sports Nutr,* 12 (10), pp.1-17.

Minuzzi, L.G., Rama, L., Bishop, N.C., Rosado, F., Martinho, A., Paiva, A. and Teixeira, A.M. (2017). Lifelong training improves anti-inflammatory environment and maintains the number of regulatory T cells in masters athletes. *Eur J appl Physiol*, 117 (6), pp.1131-1140.

Mitchell, W.K., Williams, J., Atherton, P., Larvin, M., Lund, J. and Narici, M. (2012). Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review. *Front Physiol*. 3, p.260.

Mizrahi, J., Verbitsky, O. and Isakov, E. (2000). Fatigue-related loading imbalance on the shank in running: A possible factor in stress fractures. *Annals Biomed Eng*, 28, pp.463-469.

Mizrahi, J., Verbitsky, O. and Isakov, E. (2001). Fatigue-induced changes in decline running. *Clin Biomech*, 16, pp.207-212.

Moghadam-Kia, S., Oddis, C.V. and Aggarwal, R. (2016). Approach to asymptomatic creatine kinase elevation. *Cleve. Clin. J. Med*, 83 (1), pp.37-42.

Montesano, P., Palermi, S., Massa, B. and Mazzeo, F. (2020). From ''sliding'' to ''winding'' filaments theory: A narrative review of mechanisms behind skeletal muscle contraction. *J Human Sport Exerc*, 15 (3), pp.S806-S814.

Morgan, D.L. (1990). New insights into the behavior of muscle during active lengthening. *Biophys. J*, 57, pp.209-221.

Mukherjee, P.K., Marcheselli, V.L., Serhan, C.N. and Bazan, N.G. (2004). Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. *Proc Natl Acad Sci, USA*, 101, pp.8491-8496.

Myers, K.R., Tham, W.Y., Yin, Y., Cohodes, N., Thursby, J.G., Thursby, M.C., Schiffer, P., Walsh, J.T., Lakhani, K.R. and Wang, D. (2020). Unequal effects of the COVID-19 pandemic on scientists. *Nat Hum Behav*, 4 (9), pp.880-883.

Nalbandian, M. and Takeda, M. (2016). Lactate as a signaling molecule that regulates exercise-induced adaptations. *Biology (Basel)*, 5 (4), pp.38.

Narici, M.V. and Maganaris, C.N. (2006). Adaptability of elderly human muscles and tendons to increased loading. *J Anat,* 208 (4), pp.433–443.

Nederveen, J.P., Joanisse, S., Snijders, T., Thomas, A.C.Q., Kumbhare, D. and Parise, G. (2018). The influence of capillarization on satellite cell pool expansion and activation following exercise-induced muscle damage in healthy young men. *J Physiol*, 596 (6), pp.1063-1078.

Newham, D.J., McPhail, G. Mills, K.R. and Edwards, R.H. (1983). Ultrastructural changes after concentric and eccentric contractions of human muscle. *J Neurol Sci,* 61 (1), pp.109-122.

Newham, D.J., Jones, D.A. and Clarkson, P.M. (1987). Repeated high-force eccentric exercise: effects on muscle pain and damage. *J Appl Physiol,* 63 (4), pp.1381-1386.

Newham, D.J., Jones, D.A. and Edwards, R.H. (1983). Large delayed plasma creatine kinase changes after stepping exercise. *Muscle Nerve*, 6, pp.380-385.

Newman, A.B., Kupelian, V., Visser, M., Simonsick, E.M., Goodpaster, B.H., Kritchevsky, S.B., Tylavsky, F.A., Rubin, S.M. and Harris, T.B. (2006). Strength, but not muscle mass, is associated with mortality in the health, aging and body composition study cohort. *J Gerontol A Biol Sci Med Sci*, 61 (1), pp.72-77.

Newton, M.J., Morgan, G.T., Sacco, P., Chapman, D.W. and Nosaka, K. (2008). Comparison of responses to strenuous eccentric exercise of the elbow flexors between resistance-trained and untrained men. *J Strength Cond Res*, 22, pp.597–607.

Nieman, D.C., Johanssen, L.M., Lee, J.W. and Arabatzis, K. (1990). Infectious episodes in runners before and after the Los Angeles Marathon. *J Sports Med Phys Fitness*, 30, pp.316-328.

Nieman, D.C., Nehlsen-Cannarella, S.L., Fagoaga, O.R., Henson, D.A., Utter, A., Davis, J.M., Williams, F. and Butterworth, D.E. (1998a). Effects of mode and carbohydrate on the granulocyte and monocyte response to intensive, prolonged exercise. *J Appl Physiol*, 84 (4), pp.1252-1259.

Nieman, D.C., Nehlsen-Cannarella, S.L., Fagoaga, O.R., Henson, D.A., Utter, A., Davis, J.M., Williams, F. and Butterworth, D.E. (1998b). Influence of mode and carbohydrate on the cytokine response to heavy exertion. *Med Sci Sports Exerc*, 30 (5), pp.671-678.

Nieman, D.C., Henson, D.A., McAnulty, S.R. Jun, F. and Maxwell, K.R. (2009). N-3 polyunsaturated fatty acids do not alter immune and inflammation measured in endurance athletes. *Int J Sport Nutr Exerc Metabol*, 19, pp.536-546.

Nieman, D.C., Luo, B., Dréau, D., Henson, D.A., Shanley, R. A., Dew, D. and Meaney, M.P. (2014). Immune and inflammation responses to a 3-day period of intensified running versus cycling. *Brain Behav Immun,* 39, pp.0-5.

Nikolaidis, M.G. (2017). The Effects of Resistance Exercise on Muscle Damage, Position Sense, and Blood Redox Status in Young and Elderly Individuals. *Geriatrics (Basel)*, 2 (3), p.20.

Nilwik, R., Snijders, T., Leenders, M., Groen, B.B.L., Kranenburg, J., Verdijk, L.B. and Loon, L.J.C. (2013). The decline in skeletal muscle mass with aging is mainly attributed to a reduction in type II muscle fiber size. *Exp Gerontol*, 48 (5), pp.492–498.

NIMH. (2017). *National Cancer Institute: 24h dietary recall (24h) ta a glance*. United States: NCI’s Division of Cancer Control and Population Sciences.

Nishikawa, K.C., Monroy, J.A., Uyeno, T.E., Yeo, S.H., Pai, D.K. and Lindstedt, S.L. (2012). Is titin a 'winding filament'? A new twist on muscle contraction. *Proc Biol Sci,* 279 (1730), pp.981-990.

Nishikawa, K. (2016). Eccentric contraction: unraveling mechanisms of force enhancement and energy conservation. *J Exp Biol*, 219 (2), pp.189-196.

Nogueira, F.R.D., Conceição, M.S., Vechin, F.C., Mendes, Jr. E.M., Rodrigues, G.F.C., Fazolin, M.A., Chacon-Mikahil, M.P. and Libardi, C.A. (2013). The effect of eccentric contraction velocity on muscle damage: A review. *Isok Exer Sci*, 21, pp.1-9.

Norbury, R., Smith, S.A., Burnley, M., Judge, M. and Mauger, A.R. (2022). The effect of elevated muscle pain on neuromuscular fatigue during exercise. *Eur J Appl Physiol*, 122 (1), pp.113-126.

Nosaka, K. and Clarkson, P.M. (1994). Effect of eccentric exercise on plasma enzyme activities previously elevated by eccentric exercise. *Eur J Appl Physiol Occup Physiol*, 69, pp.492-497.

Nosaka, K. and Clarkson, P.M. (1996). Variability in serum creatine kinase response after eccentric exercise of the elbow flexors. *Int J Sport Med*, 17, pp.120-127.

Nosaka, K., Lavender, A., Newton, M. and Sacco, P. (2003). Muscle damage in resistance training – Is muscle damage necessary for strength gain and muscle hypertrophy? *Int J Sport Health Sci*, 1 (1), pp.1-8.

Nosaka, K. and Newton M. (2002). Difference in the magnitude of muscle damage between maximal and submaximal eccentric loading. *J Strength Cond Res,* 16, pp.202-208.

Nosaka, K., Newton, M. and Sacco, P. (2002a). Delayed-onset muscle soreness does not reflect the magnitude of eccentric exercise-induced muscle damage. *Scand J Med Sci Sports*, 12 (6), pp.337–346.

Nosaka, K., Newton, M. and Sacco, P. (2002b). Muscle damage and soreness after endurance exercise of the elbow flexors. *Med Sci Sports Exerc,* 34, pp.920-927.

Nosaka, K. and Sakamoto, K. (2001). Effect of elbow joint angle on the magnitude of muscle damage to the elbow flexors. *Med Sci Sports Exerc*, 33, pp.22-29.

Nosaka, K., Sakamoto, K., Newton, M. and Sacco, P. (2001). How long does the protective effect on eccentric exercise-induced muscle damage last? *Med Sci Sports Exerc*, 33, pp.1490-1495.

Nottle, C. and Nosaka, K. (2005). The magnitude of muscle damage induced by downhill backward walking. *J Sci Med Sport*, 8 (3), pp.264-273.

Nottle, C. and Nosaka, K. (2007). Changes in power assessed by the Wingate anaerobic test following downhill running. *J Str Cond Res,* 21 (1), p.145.

Novak, M.L., Weinheimer-Haus, E.M. and Koh, T.J. (2014). Macrophage activation and skeletal muscle healing following traumatic injury. *J Pathol*, 232 (3), pp.344-355.

O'Connor, P.M., Lapointe, T.K., Beck, P.L. and Buret, A.G. (2010). Mechanisms by which inflammation may increase intestinal cancer risk in inflammatory bowel dis- ease. *Inflamm Bowel Dis,* 16 (8), pp.1411–1420.

Ochi, E., Tsuchiya, Y. and Nosaka, K. (2016). Differences in post-exercise t2 relaxation time changes between eccentric and concentric contractions of the elbow flexors. *Eur J Appl Physiol*, 116, pp.2145–2154.

Ochi, E. and Tsuchiya, Y. (2018). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in muscle damage and function. *Nutrients*, 10 (552), pp.1-13.

Ochi, E., Tsuchiya, Y. and Yanagimoto, K. (2017). Effect of eicosapentaenoic acids-rich fish oil supplementation on motor nerve function after eccentric contractions. *J Int Soc Sports Nutr*, 14 (23), pp.1–8.

Oh, D.Y., Talukdar, S., Bae, E.J., Imamura, T., Morinaga, H., Fan, W. and Olefsky, J.M. (2010). GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell*, *142*, pp.687–698.

Ohtani, N., Yamakoshi, K., Takahashi, A. and Hara, E. (2010). Real-time in vivo imaging of p16gene expression: a new approach to study senescence stress signaling in living animals. *Cell Div*, 5, p.1.

Okutsu, M., Suzuki, K., Ishijima, T., Peake, J. and Higuchi, M. (2008). The effects of acute exercise-induced cortisol on CCR2 expression on human monocytes. *Brain Behav. Immun*, 22, pp.1066-1071.

O'Reilly, K.P., Warhol, M.J., Fielding, R.A., Frontera, W.R., Meredith, C.N. and Evans, W.J. (1987). Eccentric exercise-induced muscle damage impairs muscle glycogen repletion. *J Appl Physiol*, 63, pp.252-256.

Ormsbee, M., Ward, E., Bach, C., Arciero, P., McKune, A. and Panton, L. (2015). The impact of a pre-loaded multi-ingredient performance supplement on muscle soreness and performance following downhill running. *J Int Soc Sports Nutr*, 12 (1), p.2.

Ortega, R. (1994). Physiology and biochemistry: influence of exercise on phagocytosis. *Int J Sports Med*, 15, pp.172-178.

Ortega, E., Rodriguez, M.J., Barriga, C. and Forner, M.A. (1996). Corticosterone, prolactin, and thyroid hormones as hormonal mediators of the stimulated phagocytic capacity of peritoneal macrophages after high-intensity exercise. *Int J Sports Med*, 17, pp.149-155.

Ortega, E. (2003). Neuroendocrine mediators in the modulation of phagocytosis by exercise: physiological implications. *Exerc Immunol Rev*, 9, pp.70-93.

Ortega, E., Marchena, J.M., Garcia, J.J., Barriga, C. and Rodriguez, A.B. (2005). Norepinephrine as Mediator in the Stimulation of Phagocytosis Induced by Moderate Exercise. *Eur J App Physiol*, 93, pp.714-718.

Ostan R., Bucci, L., Capri, M., Salvioli, S., Scurti, M., Pini, E., Monti, D. and Franceschi, C. (2008). Immunosenescence and immunogenetics of human longevity. *Neuroimmunomodulation*, 15 (4-6), pp.224–240.

Ostrowski, K., Hermann, C., Bangash, A., Schjerling, P., Nielsen, J.N. and Pedersen, B.K. (1998a). A trauma-like elevation of plasma cytokines in humans in response to treadmill running. *J Physiol*, 513 (3), pp.889-894.

Ostrowski, K., Rohde, T., Zacho, M., Asp, S. and Pedersen, B. K. (1998b). Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *J Physiol*, 508, pp.949–953.

Ostrowski, K., Rohde, T., Asp, S., Schjerling, P. and Pedersen, B.K. (1999). Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *J Physiol,* 515, pp.287-291.

Ostrowski, K., Schjerling, P. and Pedersen, B.K. (2000). Physical activity and plasma interleukin-6 in humans - effect of intensity of exercise. *Eur J Appl Physiol*, 83, pp.512-515.

Overgaard, K., Lindstrøm, T., Ingemann-Hansen, T. and Clausen, T. (2002). Membrane leakage and increased content of Na+ -K+ pumps and Ca2+ in human muscle after a 100-km run. *J Appl Physiol*, 92 (5), pp.1891–1898.

Owens, D.J., Twist, C., Cobley, J.N., Howatson, G. and Close, G.L. (2019). Exercise-induced muscle damage: What is it, what causes it and what are the nutritional solutions? *Eur J Sport Sci*, 19 (1), pp.71-85.

Paalani, M., Lee, J.W., Haddad, E., Tonstad, S. (2011). Determinants of inflammatory markers in a bi-ethnic population. *Ethn Dis*, 21 (2), pp.142-149.

Pai, J.K., Pischon, T., Ma, J., Manson, J.E., Hankinson, S.E., Joshipura, K., Curhan, G.C., Rifai, N., Cannuscio, C.C., Stampfer, M.J. and Rimm, E.B. (2004). Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med*, 351 (25), pp.2599-2610.

Pallant, J. (2016). *SPSS Survival Manual*. 6th Ed. England: McGraw Hill Education.

Palumbo, C.M. (2000). Case problem: Nutrition concerns related to the performance of a baseball team. *J Am Diet Ass*, 100, pp.704–705.

Park, K.S. and Lee, M.G. (2015). Effects of unaccustomed downhill running on muscle damage, oxidative stress and leukocyte apoptosis. *J Exerc Nutr Biochem*, 19 (2), pp.55-63.

Paschalis, V., Koutedakis, Y., Jamurtas, A. Z., Mougios, V. and Baltzopoulos, V. (2005). Equal volumes of high and low intensity of eccentric exercise in relation to muscle damage and performance. *J Strength Cond Res,* 19, pp.184-188.

Pastoris, O., Boschi, F., Verri, M., Baiardi, P., Felzani, G., Vecchiet, J., Dossena, M. and Catapano, M. (2000). The effects of aging on enzyme activities and metabolite concentrations in skeletal muscle from sedentary male and female subjects. *Exp Gerontol,* 35 (1), pp.95–104.

Paulsen, G., Egner, I.M., Drange, M., Langberg, H., Benestad, H.B., Fjeld, J.G., Hallen, J. and Raastad, T. (2010). A COX-2 inhibitor reduces muscle soreness, but does not influence recovery and adaptation after eccentric exercise. *Scand J Med Sci Sports*, 20 (1), e195-207.

Paulsen, G., Egner, I., Raastad, T, Reinholt, F., Owe, S., Lauritzen, F., Brorson, S.H. and Koskinen, S. (2013). Inflammatory markers CD11b, CD16, CD66b, CD68, myeloperoxidase and neutrophil elastase in eccentric exercised human skeletal muscles. *Histochem Cell Biol*, 139 (5), pp.691-715.

Paulsen, G., Lauritzen, F., Bayer, M.L., Kalhovde, J.M., Ugelstad, I., Owe, S.G., Hallen, J., Bergersen, L.H. and Raastad, T. (2009). Subcellular movement and expression of HSP27, alphaB-crystallin, and HSP70 after two bout of eccentric exercise in humans. *J Appl Physiol*, 107, pp.570-582.

Paulsen, G., Mikkelsen, U.R., Raastad, T. and Peake, J.M. (2012). Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? *Exerc. Immunol Rev*, 18, pp.42–97.

Peake, J.M., Della-Gatta, P. and Cameron-Smith, D. (2010). Aging and its effects on inflammation in skeletal muscle at rest and following exercise-induced muscle injury. *Am J Physiol Regul Integr Comp Physiol*, 298 (6), pp.1485–1495.

Peake, J.M., Neubauer, O., Della Gatta, P.A. and Nosaka, K. (2017). Muscle damage and inflammation during recovery from exercise. *J Appl Physiol*, 122, pp.559-570.
Peake, J.M, Nosaka, K. Muthaib, M. and Suzuki, K. (2006). Systemic inflammatory responses to maximal versus lengthening contractions of the elbow flexors. *Exerc Immunol Rev*, 12, pp.72-85.

Peake, J.M, Nosaka, K. and Suzuki, K. (2005). Characterization of inflammatory responses to eccentric exercise in humans. *Exerc Immunol Rev*, 11, pp.64–85.

Peake, J.M., Suzuki, K., Wilson, G., Hordern, M., Nosaka, K., Mackinnon, L. and Coombes, J.S. (2005). Exercise-induced muscle damage, plasma cytokines and markers of neutrophil activation. *Med Sci Sports Exerc*, 37 (5), pp.737-745.

Pedersen, B. and Febbraio, M. (2008). Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol Rev,* 88, pp.1379-1406.

Pedersen, B.K. and Hoffman-Goetz, L. (2000). Exercise and the immune system: regulation, integration, and adaptation. *Physiol Rev,* 80 (3), pp.1055-1081.

Pedersen, B.K. and Saltin, B. (2015). Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases. *Scand J Med Sci Sports,* 25 (3), pp.1–72.

Pedersen, B.K, Steensberg, A. and Schjerling, P. (2001). Muscle-derived interleukin-6: possible biological effects. *J Physiol*, 536 (2), pp.329-337.

Pedersen, B.K and Toft, A.D. (2000). Effects of exercise on lymphocytes and cytokines. *Br J Sports Med*, 34, pp.246-251.

Petersen, A.M.W. and Pedersen, B.K. (2005). The anti-inflammatory effect of exercise. *J Appl Physiol*, 98 (4), pp.1154-1162.

Phillips, T., Childs, A.C., Dreon, D.M., Phinney, S. and Leeuwen-burgh, C. (2003). A dietary supplement attenuates IL-6 and CRP after eccentric exercise in untrained males. *Med Sci Sports Exerc*, 35, pp.2032-2037.

Philippou, A., Maridaki, M., Psarros, C. and Koutsilieris, M. (2018). Systemic responses of inflammation-related factors following eccentric exercise in humans. *Am J Sports Sci*, 6 (2), pp.32-37.

Phypers, B. and Pierce, J.M.T. (2006). Lactate physiology in health and disease. *Con Edu Anes Crit Care & Pain*, 6 (3), pp.128-132.

Pizza, F.X., Davis, B.H., Henrickson, S.D., Mitchell, J.B., Pace, J.F., Bigelow, N., DiLauro, P. and Naglieri, T. (1996). Adaptation to eccentric exercise: effect on CD64 and CD11b/CD18 expression. *J Appl Physiol,* 80 (1), pp.47-55.

Pizza, F.X., Koh, T.J., McGregor, S.J. and Brooks, S.V. (2002). Muscle inflammatory cells after passive stretches, isometric contractions, and lengthening contractions. *J Appl Physiol*, 92, pp.1873-1878.

Ploutz-Snyder, L.L., Giamis, E.L., Formikell, M. and Rosenbaum, A.E. (2001). Resistance training reduces susceptibility to eccentric exercise-induced muscle dysfunction in older women. *J Gerontol*, 56, pp.384-390.

Powers, S.K. and Howley, E.T. (2015). *Exercise physiology: Theory and application to fitness and performance.* 9th ed. New York: McGraw-Hill.

Powers, S.K., Duarte, J., Kavazis, A.N. and Talbert, E.E. (2010a). Reactive oxygen species are cell signalling molecules for skeletal muscle adaptation. *Experimental Physiology*, 95 (1), 1-9.

Powers, S.K., Smuder, A.J., Kavazis, A.N., & Hudson, M.B. (2010b) Experimental guidelines for studies designed to investigate the impact of antioxidant supplementation on exercise performance. *Int J Sport Nutr Exerc Metab,* 20 (1), pp.2-14.

Proske, U. and Morgan, D.L. (2001). Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J Physiol*, 537 (2), pp.333-345.

Pyne, D.B, Smith, J.A. Baker, M.S., Telford, R.D. and Weidemann, M.J. (2000). Neutrophil oxidative activity is differentially affected by exercise intensity and type. *J Sci Med Sport*, 3, pp.44-54.

Raastad, T., Høstmark, A.T. and Strømme, S.B. (1997). Omega-3 fatty acid supplementation does not improve maximal aerobic power, anaerobic threshold and running performance in well-trained soccer players. *Scand J Med Sci Sports*, 7, pp.25–31.

Radak, Z. (2018). *Skeletal muscle, function, and muscle fiber types*. In The Physiology of Physical Training. UK: Elsevier.

Radak, Z., Chung, H.Y. and Goto, S. (2005). Exercise and hormesis: oxidative stress-related adaptation for successful aging. *Biogerontology*, 6 (1), pp.71-75.

Raja, S.N., Carr, D.B., Cohen, M., Finnerup, N.B., Flor, H., Gibson, S., Keefe, F.J., Mogil, J.S., Ringkamp, M., Sluka, K.A., Song, X.J., Stevens, B., Sullivan, M.D., Tutelman, P.R., Ushida, T. and Vader, K. (2020). The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. *Pain*, 161 (9), pp.1976-1982.

Ramírez-Campillo, R., Castillo, A., de la Fuente, C.I., Campos-Jara, C., Andrade, D.C., Álvarez, C., Martínez, C., Castro-Sepúlveda, M., Pereira, A., Marques, M.C. and Izquierdo, M. (2014). High-speed resistance training is more effective than low-speed resistance training to increase functional capacity and muscle performance in older women. *Exp Gerontol*, 58, pp.51-77.

Reaburn, P. (2004). Recovery for ageing athletes. *Sports Coach*, 26 (4), pp.12-14.

Reaburn, P. and Dascombe, B. (2009). Anaerobic performance in master athletes. *Eur Rev Aging Phys Act*, 6, pp.39-53.

Rees, D., Miles, E.A., Banerjee, T., Wells, S. J., Roynette, C.E., Wahle, K.W. and Calder, P.C. (2006). Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans: a comparison of young and older men. *Am J Clin Nutr*, 83 (2), pp.331–342.

Reich, T.E., Lindstedt, S.L., LaStayo, P.C. and Pierotti, D.J. (2000). Is the spring quality of muscle plastic? *Am J Physiol Regul Integr Comp Physiol*, 278, pp.1661-1666.

Reid, N., Daly, R.M., Winkler, E.A.H., Gardiner, P.A., Eakin, E.G., Owen, N., Dunstan, D.W. and Healy, G.N. (2016). Associations of monitor-assessed activity with performance-based physical function. *PLoS ONE*, 11 (4), e0153398.

Reid, K.F. and Fielding, R.A. (2012). Skeletal muscle power: a critical determinant of physical functioning in older adults. *Exerc Sport Sci Rev*. 40 (1), pp.4-12.

Reihmane, D. and Dela, F. (2013). Interleukin-6: Possible biological roles during exercise. *Eur J Sport Sci*, 14, pp.242-250.

Ren, K. and Dubner, R. (2010). Interactions between the immune and nervous system in pain. *Nat Med*, 16 (11), pp.1267-1276.

Renault, V., Thornell, L.E., Eriksson, P.O., Butler-Browne, G. and Mouly, V. (2002). Regenerative potential of human skeletal muscle during aging. *Aging Cell*, 1 (2), pp.132-9.

Reynolds, J.M., Gordon, T.J. and Robergs, R.A. (2006). Prediction of one repetition maximum strength from multiple repetition maximum testing and anthropometry. *J Str Cond Res*, 20 (3), pp.584-592.

Rhea, M. (2004). Determining the magnitude of treatment effects in strength training research through the use of the effect size. *J Strength Cond Res*, 18, pp.918–920.

Ricciotti, E. and Fitzgerald, G.A. (2011). Prostaglandins and inflammation. *Arterioscl Thromb Vasc Biol*, 31 (5), pp. 986–1000.

Richfield, D. (2014). Medical gallery of David Richfield. *WikiJournal Med*, 1 (2), DOI:10.15347/wjm/2014.009. ISSN 2002-4436.

Riquelme, J.A., Takov, K., Santiago-Fernández, C., Rossello, X., Lavandero, S., Yellon, D.M. and Davidson, S.M. (2020). Increased production of functional small extracellular vesicles in senescent endothelial cells. *J Cell Mol Med*, 24 (8), pp.4871-4876.

Robergs, R.A., Wagner D.R. and Skemp, K.M., (1997). Oxygen consumption and energy expenditure of level versus downhill running. *J Sports Med Phys Fitness*, 37 (3), pp.168-174.

Rockwood, K. and Mitnitski, A. (2011). Frailty defined by deficit accumulation and geriatric medicine defined by frailty. *Clin Geriatr Med*, 27 (1), pp.17–26.

Rodacki, C.L., Rodacki, A.L.F., Pereira, G., Naliwaiko, K., Coelho, I., Pequito, D. and Fernandes, L.C. (2012). Fish-oil supplementation enhances the effects of strength training in elderly women. *Am J Clin Nutr*, 95 (12), pp.428–36.

Rodenburg, J.B., Bar, P.R., De Boer, R.W. (1993). Relations between muscle soreness and biochemical and functional outcomes of eccentric exercise. *J Appl Physiol*, 74 (6), pp.2976–2983.

Rodier, F., Coppé, J.P., Patil, C.K., Hoeijmakers, W.A., Muñoz, D.P., Raza, S.R., Freund, A., Campeau, E., Davalos, A.R. and Campisi, J. (2009). Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat Cell Biol*, 11 (8), pp.973-979.

Roth, S.M., Martel, G.F. and Rogers, M.A. (2000a). Muscle biopsy and muscle fiber hypercontraction: a brief review: *Eur J Appl Physiol*, 83, pp.239-245.

Roth, S.M., Martel, G.F., Ivey, F.M., Lemmer, J.T, Metter, E.J., Hurley, B.F. and Rogers, M.A. (2000b). High-volume, heavy-resistance strength training and muscle damage in young and older women. *J Appl Physiol*, 88, pp.1112-1118.

Roubenoff, R. (2003). Sarcopenia: Effects on body composition and function. *J Gerontol*, 58 (11), pp.1012-1017.

Rusko, H., Luhtanen, P., Rahkila, P., Viitasalo, J., Rehunen, S. and Härkönen, M. (1986). Muscle metabolism, blood lactate and oxygen uptake in steady state exercise at aerobic and anaerobic thresholds. *Eur J Appl Physiol Occup Physiol*, 55 (2), pp.181-186.

Russ, D.W and Lanza, I.R. (2011). The impact of old age on skeletal muscle energetics: supply and demand. *Curr Aging Sci*, 4, pp.234–247.

Russ, D.W., Gregg-Cornell, K., Conaway, M.J., Clark, B.C. (2012). Evolving concepts on the age-related changes in “muscle quality”. *J Cachexia Sarcopenia Muscle*, 3, pp.95–109.

Salmons, S. (1997) *Muscle damage*. Oxford, UK: Oxford University Press.

Samitz, G., Egger, M. and Zwahlen, M. (2011). Domains of physical activity and all-cause mortality: Systematic review and dose-response meta-analysis of cohort studies. *Int J Epidemiol*, 40, pp.1382-1400.

Santos-Eggimann, B., Cuenoud, P., Spagnoli, J. and Junod, J. (2009). Prevalence of frailty in middle-aged and older community-dwelling Europeans living in 10 countries. *J Gerontol A Biol Sci Med Sci*, 64, pp.675-681.

Santos, E., Silva, A., de, C.C., Moura, J., de, O.Q., Franca, G., Asciutti, L. (2012). Omega-3 supplementation attenuates the production of C-reactive protein in military personnel during 5 days of intense physical stress and nutritional restriction. *Biol Sport*, 29 (2), pp.93-99.

Sayers, S.P. and Clarkson, P.M. (2001). Force recovery after eccentric exercise in males and females. *Eur J Appl Physiol*, 84, pp.122-126.

Sayers, S., Knight, C. and Clarkson, P. (2003). Neuromuscular variables affecting the magnitude of force loss after eccentric exercise. *J Sports Sci*, 21 (5), pp.403-410.

Sayers, S.P., Clarkson, P.M. and Lee, J. (2000). Activity and immobilization after eccentric exercise: I. Recovery of muscle function. *Med Sci Sports Exerc*, 32, pp.1587-1592.

Schindler, R., Mancilla, J., Endres, S., Ghorbani, R., Clark, S. C. and Dinarello, C.A. (1990). Correlations and interactions in the production of interleukin-6 (IL- 6), IL-1, and tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF. *Blood*, 75, pp.40-47.

Schetter, A.J., Heegaard, N.H. and Harris C.C. (2010). Inflammation and cancer: interweaving microRNA, free radical, cytokine and p53 pathways. *Carcinogenesis*, 31 (1), pp.37–49.

Schneider, D.A., Berwick, J.P., Sabapathy, S., and Minahan, C.L. (2007). Delayed onset muscle soreness does not alter O_2 uptake kinetics during heavy-intensity cycling in humans. *Int J Sports Med,* 28 (7), pp.550-556.

Schneider, B.S.P. and Tiidus, P.M. (2007). Neutrophil infiltration in exercise-injured skeletal muscle. *Sports Med*, 37 (10), pp.837-856.

Schoenfeld, B.J. and Contreras, B. (2013). Is Postexercise Muscle Soreness a Valid Indicator of Muscular Adaptations? *Strength Cond J,* 35 (5), pp.16-21.

Schwane, J.A., Johnson, S.R., Vandenakker, C.B. and Armstrong, R.B. (1983). Delayed-onset muscle soreness and plasma CPK and LDH activities after downhill running. *Med Sci Sports Exerc*, 15 (1), pp.51-56.

Schwane, J.A., Williams, J.S. and Sloan, J.H. (1987). Effects of training on delayed muscle soreness and serum creatine kinase activity after running. *Med Sci Sports Exerc*, 19 (6), pp.584-590.

Scott, W., Stevens, J. and Binder-Macleod, S.A (2001). Human skeletal muscle fiber type classifications. *J Physical Therapy Rehab,* 81 (11), pp.1810-1816.

Scott, A, Khan, K.M., Roberts, C.R., Cook, J.L. and Duronio, V. (2004). What do we mean by the term "inflammation"? A contemporary basic science update for sports medicine. *Br J Sports Med*, 38 (3), pp.372-380.

Selbie, W.S. and Caldwell, G.E. (1996). A simulation study of vertical jumping from different starting postures. *J Biomech*, 29 (9), pp.1137-1146.

Serhan, C.N., Clish, C.B., Brannon, J., Colgan, S.P., Gronert, K. and Chiang, N. (2000). Anti-inflammatory lipid signals generated from dietary n-3 fatty acids via cyclooxygenase-2 and transcellular processing: a novel mechanism for NSAID and n-3 PUFA therapeutic actions. *J Physiol Pharmacol*, 4, pp.643-654.

Serrão, F.V., Mendes da Silva Serrão, P.R., Foerster, B., Tannús, A., Pedro, V.M. and Salvini, T.F. (2007). Assessment of the quadriceps femoris muscle in women after injury

induced by maximal eccentric isokinetic exercise with low angular speed. *J Sports Sci Med*, 6, pp.106-116.

Sewright, K.A., Hubal, M.J., Kearns, A., Holbrook, M.T. and Clarkson, P.M. (2008). Sex differences in response to maximal eccentric exercise. *Med Sci Sports Exerc*, 40, pp.242-251.

Shei, R.J., Lindley, M.R. and Mickleborough, T.D. (2014). Omega-3 polyunsaturated fatty acids in the optimization of physical performance. *Mil Med,* 179 (11), pp.144-156.

Sherratt, M.J. (2009). Tissue elasticity and the ageing elastic fibre. *Age (Dordr)*, 31 (4), pp.305–325.

Short, K.R., Bigelow, M.L., Kahl, J., Singh, R., Coenen-Schimke, J., Raghavakaimal, S. and Nair, K.S. (2005). Decline in skeletal muscle mitochondrial function with aging in humans. *Proc Natl Acad Sci U S A*, 102 (15), pp.5618-23.

Silva, A.M., Shen, W., Heo, M., Gallagher, D., Wang, Z., Sardinha, L.B. and Heymsfield, S.B. (2010). Ethnicity-related skeletal muscle differences across the lifespan. *Am J Hum Biol*, 22 (1), pp.76-82.

Silva, J.R., Rumpf, M.C., Hertzog, M., Castagna, C., Farooq, A., Girard, O. and Hader, K. (2018). Acute and residual soccer match-related fatigue: a systematic review and meta-analysis. *Sports Med*, 48 (3), pp.539-583.

Silverthorn, D.U. (2016). Muscles. In Human Physiology: An integrated approach (7th ed.). San Francisco, CA: Pearson. pp.377-416.

Simopoulos, A. (2002). The importance of the ration of mega-6/omega-3 essential fatty acids. *Biomed. Pharmacother,* 56, 365-379.

Simopoulos, A.P. (2007). Omega-3 fatty acids and athletics. *Curr Sports Med Rep*, 6, pp.230–236.

Singh, T. and Newman, A.B. (2011). Inflammatory markers in population studies of aging. *Ageing Res Rev*, 10 (3), pp.319–329.

Skulas-Ray, A.C., Kris-Etherton, P.M., Harris, W.S., Heuvel, J.P.V., Wagner, P.R. and West, S.G. (2011). Dose-response effects of omega-3 fatty acids on triglycerides, inflammation, and endothelial function in healthy persons with moderate hypertriglyceridemia. *Am J Clin Nutr,* 93 (2), pp.243-252.

Smith, G.I., Julliand, S., Reeds, D.N., Sinacore, D.R., Klein, S., Mittendorfer, B. (2015). Fish oil-derived n-3 PUFA therapy increases muscle mass and function in healthy older adults. *Am J Clin Nutr*, 102, pp.115–22.

Smith, G.I., Atherton, P., Reeds, D.N., Mohammed, B.S., Rankin, D., Rennie, M.J. and Mittendorfer. B. (2011a). Omega-3 polyunsaturated fatty acids augment the muscle

protein anabolic response to hyperinsulinaemia-hyperaminoacidaemia in healthy young and middle-aged men and women. *Clin Sci (Lond.),* 121 (6), pp.267-278.

Smith, G.I., Atherton, P., Reeds, D.N., Mohammed, B.S., Rankin, D., Rennie, M. J., and Mittendorfer. B. (2011b). Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: A randomized controlled trial. *Am J Clin Nutr*, 93 (2), pp.402-412.

Smith, L., Anwar, A., Fragen, M., Rananto, C., Johnson, R. and Holbert. D. (2000). Cytokines and cell adhesion molecules associated with high-intensity eccentric exercise. *Eur J Appl Physiol*, 82, pp.61-67.

Smith, L.L. (1991). Acute inflammation: the underlying mechanism in delayed onset muscle soreness? *Med Sci Sports Exerc*, 23, pp.542-551.

Smith, L.L., McCammon, M., Smith, S., Chamness, M., Israel, R.G. and O'Brien, K.F. (1989). White blood cell response to uphill walking and downhill jogging at similar metabolic loads. *Eur J Appl Physiol Occup. Physiol*, 58 (8), pp.833-837.

Smith, L.L., McKune, A. J., Semple, S. J., Sibanda, E., Steel, H. and Anderson, R. (2007). Changes in serum cytokines after repeated bouts of downhill running. *Appl Physiol Nutr Metab*, 32, pp.233-240.

Smith, S.A., Micklewright, D., Winter, S.L. and Mauger, A.R. (2020). Muscle pain induced by hypertonic saline in the knee extensors decreases single-limb isometric time to task failure. *Eur J Appl Physiol*, 120 (9), pp.2047-2058.

Snijders, T., Verdijk, L.B. and van Loon, L.J. (2009). The impact of sarcopenia and exercise training on skeletal muscle satellite cells. *Ageing Res Rev*, 8, pp.328–338.

Soares, S., Ferreira-Junior, J.B., Pereira, M.C., Cleto, V.A., Castanheira, R.P., Cadore, E.L., Brown, L.E., Gentil, P., Bemben, M.G. and Bottaro, M. (2015). Dissociated time course of muscle damage recovery between single- and multi-joint exercises in highly resistance-trained men. *J Strength Cond Res,* 29, pp.2594-2599.

Sousa, M. Teixeira, V.H. and Soares, J. (2014). Dietary strategies to recover from exercise-induced muscle damage. *Int J Food Sci Nutr*, 65 (2), pp.151-163.

Spazzafumo, L., Olivieri, F., Abbatecola, A.M., Castellani, G., Monti, D., Lisa, R., Galeazzi, R., Sirolla, C., Testa, R., Ostan, R., Scurti, M., Caruso, C., Vasto, S., Vescovini, R., Ogliari, G., Mari, D., Lattanzio, F. and Franceschi, C. (2013). Remodelling of biological parameters during human ageing: evidence for complex regulation in longevity and in type 2 diabetes. *Age (Dordr),* 35 (2), pp.419-29.

Spranger, J., Kroke, A., Möhlig, M., Hoffmann, K., Bergmann, M.M., Ristow, M., Boeing, H. and Pfeiffer, A.F. (2003). Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective

Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Diabetes*, 52 (3), pp.812-817.

Stowe, R.P., Peek, M.K., Cutchin, M.P and Goodwin, J.S. (2010). Plasma cytokine levels in a population-based study: relation to age and ethnicity. *J Gerontol A Biol Sci Med Sci*, 65 (4), pp.429-433.

Stupka, N., Lowther, S., Chorneyko, K., Bourgeois, J. M., Hogben, C. and Tarnopolsky, M. A. (2000). Gender differences in muscle inflammation after eccentric exercise. *J Appl Physiol,* 89, pp.2325-2332.

Stupka, N., Tarnopolsky, M.A., Yardley, N.J. and Phillips, S.M. (2001). Cellular adaptation to repeated eccentric exercise-induced muscle damage. *J Appl Physiol*, 91, pp.1669-1678.

Su, S.H., Jen, C.J. and Chen, H.I. (2011). NO signaling in exercise training-induced anti-apoptotic effects in human neutrophils. *Biochem Biophys Res Commun*, 405 (1), pp.58-63.

Sublette, M.E., Ellis, S.P., Geant, A.L. and Mann, J.J. (2011). Meta-analysis: Effects of eicosapentaenoic acid in clinical trials in depression. *J Clin Psychiatry*, 72 (12), pp.1577-1584.

Suzuki, K., Nakaji, S., Yamada, M., Totsuka, M., Sato, K. and Sugawara, K. (2002). Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. *Exerc Immunol, Rev*, 8. pp.6-48.

Suzuki, K., Nakaji, S., Yamada, M., Liu, Q., Kurakake, S., Okamura, N., Kumae, T., Umeda, T. and Sugawara, K. (2003). Impact of a competitive marathon race on systemic cytokine and neutrophil responses. *Med Sci Sports Exerc*, 35, pp.348-355.

Suzuki, K., Peake, J., Nosaka, K., Okutsu, M., Abbiss, C.R., Surriano, R., Bishop, D., Quod, M.J., Lee, H., Martin, D.T. and Laursen, P.B. (2006). Changes in markers of muscle damage inflammation and HSP70 after Ironman triathlon race. *Eur J Appl Physiol*, 98, pp.525–534.

Suzuki, K., Yamada, M., Kurakake, S., Okamura, N., Yamaya, K., Liu, Q., Kudoh, S., Kowatari, K., Nakaji, S. and Sugawara, K. (2000). Circulating cytokines and hormones with immunosuppressive but neutrophil-priming potentials rise after endurance exercise in humans. *Eur J Appl Physiol*, 81, pp.281-287.

Suzuki, K. (2018). Cytokine response to exercise and its modulation. Antioxidants, 7 (1), p.17.

Syu, G.D., Chen, H.I. and Jen, C.J. (2012). Differential effects of acute and chronic exercise on human neutrophil functions. *Med Sci Sports Exerc*, pp.1021-1027.

Taguchi, T., Matsuda, T. and Mizumura, K. (2007). Change with age in muscular mechanical hyperalgesia after lengthening contraction in rats. *Neurosc Res*, 57, pp.331-338.

Tanaka, H. and Seals, D.R. (2008). Endurance exercise performance in Masters athletes: age-associated changes and underlying physiological mechanisms. *J Physiol*, 586 (1), pp.55-63.

Tartibian, B., Maleki, B.H. and Abbasi, A. (2009). The effects of ingestion of omega-3 fatty acids on perceived pain and external symptoms of delayed onset muscle soreness in untrained men. *Clin J Sport Med*, 19 (2), pp.115-119.

Tartibian, B., Maleki, B.H. and Abbasi, A. (2011). Omega-3 fatty acids supplementation attenuates inflammatory markers after eccentric exercise in untrained men. *Clin J Sport Med*, 21 (2), pp.131-137.

Tchkonia, T., Morbeck, D.E., Von Zglinicki, T., Van Deursen, J., Lustgarten, J., Scrable, H., Khosla, S., Jensen, M.D. and Kirkland, J.L. (2010). Fat tissue, aging, and cellular senescence. *Aging Cell*, 9 (5), pp.667-684.

Tchkonia, T., Zhu, Y., van Deursen, J., Campisi, J. and Kirkland, J.L. (2013). Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. *J Clin Invest*, 123, 966-972.

Tee, J.C., Bosch, A.N. and Lambert, M.I. (2007). Metabolic Consequences of Exercise-Induced Muscle Damage. *Sports Med,* 37, pp.827–836.

Thiebaud, R.S. (2012). Exercised-induced muscle damage: is it detrimental or beneficial? *J Trainology*, 1, pp.36-44.

Thielecke, F. and Blannin, A. (2020). Omega-3 fatty acids for sport performance-are they equally beneficial for athletes and amateurs? A narrative review. *Nutrients*, 12 (12), p.3712.

Thompson, H.S., Scordilis, S.P. and De Souza, M.J. (2006). Serum creatine kinase activity varies with ovulatory status in regularly exercising, premenopausal women. *Horm Res*, 65 (3), pp.151-158.

Thompson, B.J., Conchola, E.C. and Stock, M.S. (2015). Effects of age and muscle action type on acute strength and power recovery following fatigue of the leg flexors. *Age*, 37, 111.

Tidball, J.G. (2005). Inflammatory processes in muscle injury and repair. *Am J Physiol Regul Integr Comp Physiol*, 288 (2), pp.345-353.

Tidball, J.G., Dorshkind, K. and Wehling-Henricks, M. (2014). Shared signaling systems in myeloid cell-mediated muscle regeneration. *Development*, 141 (6), pp.1184-1196.

Tieland, M., Trouwborst, I. and Clark, B.C. (2018). Skeletal muscle performance and ageing. *J Cachex Sarcop Mucscle*, 9, pp.3–19.

Tiidus, P.M. (1995). Can estrogens diminish exercise induced muscle damage? *Can J Appl Physiol*, 20 (1), pp.26-38.

Tiidus, P.M. (1998). Radical species in inflammation and overtraining. *Can J Physiol Pharmacol*, 76, pp.533-538.

Tiidus P.M. (2000). Estrogen and gender effects on muscle damage, inflammation, and oxidative stress. *Can J Appl Physiol*, 25, pp.274-287.

Tiidus, P.M. (2003). Influence of estrogen on skeletal muscle damage, inflammation, and repair. *Exerc Sport Sci Rev*, 31 (1), pp.40-44.

Tilley, S.L., Coffman, T.M. and Koller, B.H. (2001). Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *J Clin Invest*, 108, pp.15-23.

Toft, A.D., Jensen, L.B., Bruunsgaard, H., Ibfelt, T., Halkjaer-Kristensen, J., Febbraio, M. and Pedersen, B.K. (2002). Cytokine response to eccentric exercise in young and elderly humans. *Am J Physiol Cell Physiol*, 283 (1), pp.289-295.

Toft, A.D., Thorn, M., Ostrowski, K., Asp, S., Moller, K., Iversen, S., Hermann, C., Sondergaard, S.R. and Pedersen, B.K. (2000). N-3 polyunsaturated fatty acids do not affect cytokine response to strenuous exercise. *J Appl Physiol* (1985), 89 (6), pp.2401-2406.

Toumi, H. and Best, T.M. (2003). The inflammatory response: friend or enemy for muscle injury?. *Br J Sports Med*, 37 (4), pp.284-286.

Trappe, S., Gallagher, P., Harber, M., Carrithers, J., Fluckey, J. and Trappe, T. (2003). Single muscle fibre contractile properties in young and old men and women. *J Physiol*, 552 (Pt 1), pp.47-58.

Trebble, T., Arden, N.K., Stroud, M.A., Wootton, S.A., Burdge, G.C., Miles, E.A., Ballinger, A.B., Thompson, R.L and Calder, P.C. (2003). Inhibition of tumour necrosis factor-a and interleukin-6 production by mononuclear cells following dietary fish-oil supplementation in healthy men and response to antioxidant co-supplementation. *Br J Nutr*, 90, pp.405–412.

Trost, Z., France, C.R. and Thomas, J.S., (2011). Pain-related fear and avoidance of physical exertion following delayed-onset muscle soreness. *Pain*, 152 (7), pp.1540-1547.

Tsuchiya, Y., Kikuchi, N. Shirato, M. and Ochi, E. (2015). Differences of activation pattern and damage in elbow flexor muscle after isokinetic eccentric contractions. *Isokin Exerc Sci*, pp.1-7.

Tsuchiya Y., Sakuraba, K. and Ochi, E. (2014). Effect of eccentric contractions of elbow flexor on bone formation and resorption markers. *J Sports Med Phys Fitness*, 54, pp.651-657.

Tsuchiya, Y., Yanagimoto, K., Nakazato, K., Hayamizu, K. and Ochi, E. (2016). Eicosapentaenoic and docosahexaenoic acids-rich fish oil supplementation attenuates strength loss and limited joint range of motion after eccentric contractions: A randomized, double-blind, placebo-controlled, parallel-group trial. *Eur J Appl Physiol*, 116, pp.1179–1188.

Tuomisto, K., Jousilahti, P., Sundvall, J., Pajunen, P., Salomaa, V. (2006). C-reactive protein, interleukin-6 and tumor necrosis factor alpha as predictors of incident coronary and cardiovascular events and total mortality. A population-based, prospective study. *Thromb Haemost*, 95 (3) pp.511–518.

Twist, C. and Eston, R. (2005). The effects of exercise-induced muscle damage on maximal intensity intermittent exercise performance. *Eur J Appl Physiol,* 94 (5-6), pp.652-658.

Twist, C., and Eston, R.G. (2009). The effect of exercise-induced muscle damage on perceived exertion and cycling endurance performance. *Eur J Appl Physiol,* 105 (4), pp.559-567.

Tyrovolas, S., Panagiotakos, D., Georgousopoulou, E., Chrysohoou, C., Tousoulis, D., Haro, J.M. and Pitsavos, C. (2020). Skeletal muscle mass in relation to 10 year cardiovascular disease incidence among middle aged and older adults: the ATTICA study. *J Epidemiol Community Health,* 74, pp.26-31.

U.S. Department of Agriculture, Agricultural Research Service (2008). USDA National Nutrient Database for Standard Reference, Release 21. Nutrient Data Laboratory. Available from:
https://www.ars.usda.gov/northeast-area/beltsville-md/beltsville-human-nutrition-research-center/nutrient- data-laboratory/docs/sr21-home-page/

Vaile, J.M., Gill, N.D. and Blazevich, A.J. (2007). The effect of contrast water hydrotherapy on symptoms of delayed onset muscle soreness. *J Strength Cond Res*, 21 (3), pp.697-702.

Vaile, J., Halson, S., Gill, N. and Dawson, B., (2008). Effect of hydrotherapy on the signs and symptoms of delayed onset muscle soreness. *Eur J Appl Physiol*, 102 (4), pp.447-455.

Valberg, S.J. (2008). Skeletal muscle function. In: Kaneko, J.J., Harvey, J.W. and Bruss, M.L. (eds.) *Clinical Biochemistry of Domestic Animals* (6th ed). USA: Elsevier, 459-484.

van de Vyver, M. and Myburgh, K.H. (2012). Cytokine and satellite cell responses to muscle damage: interpretation and possible confounding factors in human studies. *J Muscle Res Cell Motil*, 33, pp.177-185.

Van Soest, A.J., Bobbert, M.F. and Van Ingen Schenau, G.J. (1994). A control strategy for the execution of explosive movements from varying starting positions. *J Neurophysiol*, 71 (4), pp.1390-1402.

Vasto, S., Candore, G., Balistreri, C.R., Caruso, M., Colonna-Romano, G., Grimaldi, M.P., Listi, F., Nuzzo, D., Lio, D. and Caruso, C. (2007). Inflammatory networks in ageing, age-related diseases and longevity. *Mech Ageing Dev*, 128 (1), pp.83-91.

Veale, J.F. (2014). Edinburgh Handedness Inventory - Short Form: a revised version based on confirmatory factor analysis. *Laterality*, 19 (2), pp.164-177.

Verdijk, L.B., Koopman, R., Schaart, G., Meijer, K., Savelberg, H.H., van Loon, L.J. (2007). Satellite cell content is specifically reduced in type II skeletal muscle fibers in the elderly. *Am J Physiol Endocrinol Metab*, 292, pp.151–157.

Verdijk, L.B., Snijders, T., Drost, M., Delhaas, T., Kadi, F. and van Loon, L.J. (2014). Satellite cells in human skeletal muscle; from birth to old age. *Age (Dordr)* 36 (2), pp.545-7.

Vickers, A.J. (2001). Time course of muscle soreness following different types of exercise. *BMC Musculoskelet Disord*, 2, p.5.

Visser, M., Pahor, M., Taaffe, D.R., Goodpaster, B.H., Simonsick, E.M., Newman, A.B., Nevitt, M. and Harris, T.B. (2002). Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. *J Gerontol A Biol Sci Med Sci,* 57 (5), pp.326-332.

Visser, M., Goodpaster, B.H., Kritchevsky, S.B., Newman, A.B., Nevitt, M., Rubin, S.M., Simonsick, E.M. and Harris, T.B. (2005). Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident mobility limitations in well-functioning older persons. *J Gerontol A Biol Sci Med Sci,* 60 (3), pp.324–333.

Vogler, A.J., Rice, A.J. and Gore, C.J. (2010) Validity and reliability of the Cortex MetaMax3B portable metabolic system. *J Sports Sci*, 28, pp.733–742.

Volpi, E., Nazemi, R. and Fujita, S. (2004). Muscle tissue changes with aging. *Curr Opin Clin Nutr Metab Care*, 7 (4), pp.405-410.

von Schacky, C. (2008). Omega-3 fatty acids: antiarrhythmic, proarrhythmic or both? *Curr Opin Clin Nutr Metab Care*, 11, pp.94–99.

Waaijer, M.E., Parish, W.E., Strongitharm, B.H., van Heemst, D., Slagboom, P.E., de Craen, A.J., Sedivy, J.M., Westendorp, R.G., Gunn, D.A. and Maier, A.B. (2012). The number of p16INK4a positive cells in human skin reflects biological age. *Aging Cell,* 11 (4), 722-725.

Walker, S., Peltonen, H. and Häkkinen, K. (2015). Medium-intensity, high-volume "hypertrophic" resistance training did not induce improvements in rapid force production in healthy older men. *AGE,* 37, p.41.

Wall, B.T., Dirks, M.L. and van Loon, L.J. (2013). Skeletal muscle atrophy during short-term disuse: implications for age-related sarcopenia. *Ageing Res Rev*, 12 (4), pp.898-906.

Walmsley, B., Hodgson, J.A. and Burke, R.E. (1978). Forces produced by medial gastrocnemius and soleus muscles during locomotion in freely moving cats. *J Neurophysiol*, 41 (5), pp.1203-1216.

Walser, B., Giordano, R.M. and Stebbins, C.L. (2006). Supplementation with omega-3 polyunsaturated fatty acids augments brachial artery dilation and blood flow during forearm contraction. *Eur J Appl Physiol*, 97 (3), pp.347-354.

Walsh, N.P., Gleeson, M., Pyne, D.B., Nieman, D.C., Dhabhar, F.S., Shephard, R.J., Oliver, S.J., Bermon, S. and Kajeniene, A. (2011). Position statement. Part two: maintaining immune health. *Exerc Immunol Rev*, 17, pp.64–103.

Walsh, N.P. and Oliver, S.J. (2016). Exercise, immune function and respiratory infection: An update on the influence of training and environmental stress. *Immunol. Cell Biol*, 94 (2), pp.132-139.

Wan, J.J., Qin, Z., Wang, P.Y., Sun, Y. and Liu, X. (2017). Muscle fatigue: General understanding and treatment. *Exp Mol Med*, 49 (10), e384.

Warburton, D.E.R., Nicol, C.W. and Bredin, S.S.D. (2006). Health benefits of physical activity: the evidence. *Canad Med Assoc J*, 174 (6), pp.801-809.

Warren, G.L., Lowe D.A. and Armstrong, R.B. (1999). Measurement tools used in the study of eccentric contraction-induced injury. *Sports Med*, 27, pp.43–59.

Warren, G.L., Ingalls, C.P., Lowe, D.A. and Armstrong, R.B. (2001). Excitation-contraction uncoupling: major role in contraction-induced muscle injury. *Exerc Sport Sci Rev*, 29, pp.82-87.

Weissgerber, T.L., Savic, M., Winham, S.J. Stanisavljenic, D., Garovic, V.D. and Milic, N.M. (2017). Data visualization, bar naked: A free tool for creating interactive graphics. *J Biol Chem*, 292 (50), pp.20592-20598.

West, D.W.D., Marcotte, G.R., Chason, C.M., Juo, N., Baehr, L.M., Bodine, S.C. and Baar, K. (2018). Normal ribosomal biogenesis but shortened protein synthetic response to acute eccentric resistance exercise in old skeletal muscle. *Front Physiol*, 9, p.1915.

Weyemi, U., Lagente-Chevallier, O., Boufraqech, M., Prenois, F., Courtin, F., Caillou, B., Talbot, M., Dardalhon, M., Al Ghuzlan, A., Bidart, J.M., Schlumberger, M. and Dupuy, C. (2012). ROS-generating NADPH oxidase NOX4 is a critical mediator in oncogenic H-

Ras-induced DNA damage and subsequent senescence. *Oncogene*, 31 (9), pp.1117-1129.

White, H.M., Richert, B.T. and Latour, M.A. (2013). Impacts of nutrition and environmental stressors on lipid metabolism. Chapter 10 In: *Lipid Metabol*, pp.211-232.

Wirtz, N., Wahl, P., Kleinöder, H. and Mester, J. (2014). Lactate Kinetics during Multiple Set Resistance Exercise. *J Sports Sci Med*, 13 (1), pp.73-77.

Wolach B, Gavrieh R, Ben-Dror SG, Zigel L, Eliakim A, Falk B. (2005). Transient decrease of neutrophil chemotaxis following aerobic exercise. *Med Sci Sports Exerc,* 37 (6), pp.949-954.

Wood, A.W. (2012). Skeletal muscle biophysics. In: Wood, A.W. (ed.) *Physiology, Biophysics, and Biomedical Engineering*. FL, USA: Taylor & Francis, 158-162.

Woods, J.A., Ceddia, M.A., Kozak, C and Wolters, B. W. (1997). Effects of exercise on the macrophage MHC II response to inflammation. *Int J Sports Med*, 18, pp.483-488.

Woods, J.A., Davis, J.M., Smith, J.A. and Nieman, D.C. (1999). Exercise and cellular innate immune function. *Med Sci Sports Exerc*, 31 (1), pp.57-66.

Woods, J.A., Lu, Q., Ceddia, M.A. and Lowder, T. (2000). Exercise-induced modulation of macrophage function. *Immunol Cell Biol*, 78, pp.545-553.

Wroblewski, A.P., Amati, F., Smiley, M.A., Goodpaster, B. and Wright, V. (2011). Chronic exercise preserves lean muscle mass in masters athletes. *Phys Sportsmed*, 39 (3), pp.172-8.

Xu, M., Pirtskhalava, T., Farr, J.N., Weigand, B.M., Palmer, A.K., Weivoda, M.M., Inman, C.L., Ogrodnik, M.B., Hachfeld, C.M., Fraser, D.G., Onken, J.L., Johnson, K.O., Verzosa, G.C., Langhi, L.G.P., Weigl, M., Giorgadze, N., LeBrasseur, N.K., Miller, J.D., Jurk, D., Singh, R.J., Allison, D.B., Ejima, K., Hubbard, G.B., Ikeno, Y., Cubro, H., Garovic, V.D., Hou, X., Weroha, S.J., Robbins, P.D., Niedernhofer, L.J., Khosla, S., Tchkonia, T. and Kirkland, J.L. (2018). Senolytics improve physical function and increase lifespan in old age. *Nat Med*, 24 (8), pp.1246-1256.

Yamin, C., Amir, O., Sagiv, M., Attias, E., Meckel, Y., Eynon, N., Sagiv, M. and Amir, R.E. (2007). ACE ID genotype affects blood creatine kinase response to eccentric exercise. *J Appl Physiol*, 103, pp.2057-2061.

Yanagisawa, O., Sakuma, J., Kawakami, Y., Suzuki, K. and Fukubayashi, T. (2015). Effect of exercise-induced muscle damage on muscle hardness evaluated by ultrasound real-time tissue elastography. *SpringerPlus*, 4 (308), pp.1-9.

Yasar, Z., Dewhurst, S. and Hayes, L.D. (2019). Peak power output is similarly recovered after three- and five-days' rest following sprint interval training in young and older adults. *Sports*, 7.

Yokozawa, T., Fujii, N., and Ae, M. (2005). Kinetic characteristics of distance running on downhill slope. *Int J Sport Health Sci*, 3, pp.35-45.

Yu, J.G., Carlsson, L. and Thornell, L.E. (2008). Evidence for myofibril remodeling as opposed to myofibril damage in human muscles with DOMS: an ultrastructural and immunoelectron microscopic study. *Histochem. Cell Biol*, 121, pp.219-227.

Yu, J.G., Liu, J.X., Carlsson, L., Thornell, L.E. and Stål, P.S. (2013). Re-evaluation of sarcolemma injury and muscle swelling in human skeletal muscles after eccentric exercise. *PLoS One*, 8 (4), e62056.

Zainuddin, Z., Newton, M., Sacco, P. and Nosaka, K. (2005). Effects of massage on delayed-onset muscle soreness, swelling, and recovery of muscle function. *J Athl Train*, 40 (3), pp.174-180.

Zampieri, S., Pietrangelo, L., Loefler, S., Fruhmann, H., Vogelauer, M., Burggraf, S., Pond, A., Grim-Stieger, M., Cvecka, J., Sedliak, M., Tirpáková, V., Mayr, W., Sarabon, N., Rossini, K., Barberi, L., De Rossi, M., Romanello, V., Boncompagni, S., Musarò, A., Sandri, M., Protasi, F., Carraro, U. and Kern, H. (2015). Lifelong physical exercise delays age-associated skeletal muscle decline. *J Gerontol A Biol Sci Med Sci*, 70 (2), pp.163-173.

Żebrowska, A., Mizia-Stec, K., Mizia, M., Gąsior, Z. and Poprzęcki, S. (2015). Omega-3 fatty acids supplementation improves endothelial function and maximal oxygen uptake in endurance-trained athletes. *Eur J Sport Sci*, 15 (4), pp.305–314.

Zhang, J.M. and An, J. (2007). Cytokines, inflammation and pain, *Int Anesthesiol Clin*, 45 (2), pp.27–37.

APPENDICES

Appendix A: UoW – Risk Assessments

GENERAL RISK ASSESSMENT

Activity:	Physiology Laboratory	**Brief description of work activity:** C4.04 is a Physiology Laboratory used for teaching and by project students. It housed the BodPod and the METi Man. Practical work may involve collection of human material such as blood, saliva and urine.	**Assessed By:**	Helen Lloyd
Dept./Faculty:	FST - TSD		**Date:**	14.08.18
Location:	C4.04		**Review Date:**	14.08.19

1. What are the hazards?	2. Who might be at harm and how? *E, C, S, Mp, V, Em, Mp, Dp **	3. Current control measures	4. Initial Risk Rating: *H/M/L **	5. Additional control measures (if required)	6. Action by whom?	7. Action by when? (Date)	8. Date done	9. Residual risk rating. *H/M/L*
Gases attached to equipment (if in room) Cortex Gas 5% CO2/20% O2 Servomex calibration Gas 1) 99.99% nitrogen 2) 8% CO2, 20% O2	Employees and laboratory users due to materials present	Securely stored on equipment. Regulator attached to indicate volume of gas in the cylinder. Visual checks when using.	**LOW RISK**					**LOW RISK**
Gas cylinder for Bomb calorimeter (100% O2, 110ml)	Employees and laboratory users due to materials present	Stored in a labelled cupboard. Visual checks before use. Only trained persons to use.	**LOW RISK**					**LOW RISK**

		Regulator attached to indicate volume.						
Exposure to hazardous substances (mainly disinfectants)	Employees and laboratory users due to materials present	Follow GLP and ensure substances present no risk of a hazard (explosion, implosion or release of toxic or flammable gases) or that these have been addressed. Any spillages to be mopped up immediately, including following the spillage procedure for spilt hazardous substances. Use COSHH risk assessments for substances hazardous to health Chemical and biological waste materials are disposed through established procedures to minimise risk Materials, including biological waste for autoclaving have a checklist attached to confirm contents	**LOW RISK**	Review staff exposure on individual basis (sensitivities/ allergies etc.) in light of occupational health advice.	Dinesh	As required		**LOW RISK**
Biological materials, such as human blood and human bodily fluids.	Employees and laboratory users due to materials present	To follow the FST code of practice for work in biology laboratories To follow the FST code of practice/procedure for dropped or split materials. Use COSHH risk assessments for substances hazardous to health Chemical and biological waste materials are disposed through	**LOW RISK**					**LOW RISK**

		established procedures to minimise risk Blood contaminated materials must be immediately disposed of in autoclave bags or sharps bins as appropriate to avoid Blood Borne Viruses Only trained staff should work with biological materials PPE must be used at all times when working with biological materials Hands washed in designated sinks prior to leaving lab						
Electrical hazards	Employees and laboratory users due to materials present	Technicians conduct first-line equipment maintenance in their area(s) of work Any faults are immediately reported and sign posted on equipment indicating 'out of order' Equipment fault communicated to laboratory users Equipment operated only by technicians trained in their use Any irregularities in electrical equipment must be reported to the Micro-science team Leader PAT testing undertaken for all equipment	**LOW RISK**					**LOW RISK**

		Laboratory users should undertake a pre-use visual inspection of electrical equipment						
Personal contamination	Employees exposed to or contaminated with hazardous (biological and chemical) substances	COSHH Assessments are undertaken for all hazardous substances Staff should adhere to good laboratory practices Working benches are to be cleaned/sanitised after use/work with hazardous substances Hands are to be washed in designated sink regularly and prior to leaving Laboratory Desk area should be kept free from biological and chemical hazards by ensuring all materials are kept in the experimental preparation zone	**LOW RISK**					**LOW RISK**
Manual Handling	Employees whilst lifting or moving equipment.	Refer to manufacturers' instructions when moving heavy gym equipment Make use of manual handling aids present (trolleys, benches and push/pull sticks). Plastic trays and metal baskets to be used for transporting materials If necessary, seek support from other staff when lifting/moving heavy items.	**LOW RISK**					**LOW RISK**

		Technicians should attend the university manual handling workshop Use correct method when moving bicycle ergometers and other heavy equipment						
Slips, Trips & Falls	Employees due to spillages or obstructions on floor.	Any spillages to be mopped up immediately, including following the spillage procedure for spilt hazardous substances. Good housekeeping, i.e. equipment stored appropriately and area kept tidy. Floor to be clutter free and cleaned periodically by University cleaning contractors. Working areas and walkways are well lit and clutter free To observe and avoid use of trailing cables across the floor	**LOW RISK**					**LOW RISK**
Glassware and Sharps	Employees and users of the laboratory due to broken glass or needles	First Aid available (ext. 5555) for staff injuries. Sharps boxes available for disposal of broken glass and needles. Needles must not be disposed in biological autoclave waste bags PPE to be worn (Gloves available).	**LOW RISK**					**LOW RISK**

GENERAL RISK ASSESSMENT

Activity:	Human Performance research	**Brief description of work activity:** C4.08 is a research laboratory housing various gym equipment such as exercise bikes and the treadmill. It also houses the Altitude Chamber. Practical work may involve collection of human material such as blood, saliva and urine.	**Assessed By:**	Helen Lloyd
Dept./Faculty:	FST - TSD		**Date:**	15.08.18
Location:	C4.08		**Review Date:**	15.08.19

1. What are the hazards?	2. Who might be at harm and how? *E, C, S, Mp, V, Em, Mp, Dp **	3. Current control measures	4. Initial Risk Rating: *H/M/L **	5. Additional control measures (if required)	6. Action by whom?	7. Action by when? (Date)	8. Date done	9. Residual risk rating. *H/M/L*
Gases attached to equipment. <u>Cortex Gas</u> 1 x 110ml 1) 5% CO_2/20% O_2 <u>Servomex calibration Gas</u> 2 x 110ml 3) 99.99% nitrogen 4) 8% CO_2, 20% O_2	Employees and laboratory users due to materials present	Securely stored on equipment. Regulator attached to indicate volume of gas in the cylinder. Visual checks when using.	**LOW RISK**					**LOW RISK**
Exposure to hazardous substances (mainly disinfectants)	Employees and laboratory users due to materials present	Follow GLP and ensure substances present no risk of a hazard (explosion, implosion or release of toxic or flammable gases) or that these have been addressed. Any spillages to be mopped up immediately, including following the spillage procedure for spilt hazardous substances.	**LOW RISK**	Review staff exposure on individual basis (sensitivities/ allergies etc.) in light of occupational health advice.	Dinesh	As required		**LOW RISK**

		Use COSHH risk assessments for substances hazardous to health Chemical and biological waste materials are disposed through established procedures to minimise risk Materials, including biological waste for autoclaving have a checklist attached to confirm contents						
Biological materials, such as human blood and human bodily fluids.	Employees and laboratory users due to materials present	To follow the FST code of practice for work in biology laboratories To follow the FST code of practice/procedure for dropped or split materials. Use COSHH risk assessments for substances hazardous to health Chemical and biological waste materials are disposed through established procedures to minimise risk Blood contaminated materials must be immediately disposed of in autoclave bags or sharps bins as appropriate to avoid Blood Borne Viruses Only trained staff should work with biological materials PPE must be used at all times when working with biological materials	**LOW RISK**					**LOW RISK**

		Hands washed in designated sinks prior to leaving lab						
Electrical hazards	Employees and laboratory users due to materials present	Technicians conduct first-line equipment maintenance in their area(s) of work Any faults are immediately reported and sign posted on equipment indicating ‘out of order’ Equipment fault communicated to laboratory users Equipment operated only by technicians trained in their use Any irregularities in electrical equipment must be reported to the Micro-science team Leader PAT testing undertaken for all equipment Laboratory users should undertake a pre-use visual inspection of electrical equipment	**LOW RISK**					**LOW RISK**
Risk of injury when using gym equipment	Laboratory users	All users of gym equipment must be supervised and trained in proper use of the equipment. All users of gym equipment must fill in the PAR Q form, and must not use the equipment if they have any medical condition which may increase risk of injury	**LOW RISK**	All users must follow the correct procedure when using the equipment and be trained by their supervisor or a member of technical staff.	All users	As required		**LOW RISK**

Risk of suffocation when using the Altitude Chamber	The subjects in the altitude chamber and all the people in the vicinity if there is leakage of gases from the Altitude Chamber	Subjects in the altitude chamber must be supervised at all times. Unauthorised people must not be allowed in C4.08 when the Altitude chamber is in use. Warning signs to be posted on the door when the altitude chamber is in use. Monitor to show Oxygen levels, inside the altitude chamber. All users to wear an oxygen saturation probe when inside the chamber.	**LOW RISK**	All users must follow the correct procedures when using the Altitude Chamber. (please see the specific Risk Assessment for the Altitude chamber)	All users	As required		**LOW RISK**
Personal contamination	Employees exposed to or contaminated with hazardous (biological and chemical) substances	COSHH Assessments are undertaken for all hazardous substances Staff should adhere to good laboratory practices Working benches are to be cleaned/sanitised after use/work with hazardous substances Hands are to be washed in designated sink regularly and prior to leaving Laboratory Desk area should be kept free from biological and chemical hazards by ensuring all materials are kept in the experimental preparation zone	**LOW RISK**					**LOW RISK**
Manual Handling	Employees whilst lifting or	Refer to manufacturers' instructions when moving heavy gym equipment	**LOW RISK**					**LOW RISK**

	moving equipment.	Make use of manual handling aids present (trolleys, benches and push/pull sticks). Plastic trays and metal baskets to be used for transporting materials If necessary, seek support from other staff when lifting/moving heavy items. Technicians should attend the university manual handling workshop Use correct method when moving bicycle ergometers and other heavy equipment						
Slips, Trips & Falls	Employees due to spillages or obstructions on floor.	Any spillages to be mopped up immediately, including following the spillage procedure for spilt hazardous substances. Good housekeeping, i.e. equipment stored appropriately and area kept tidy. Floor to be clutter free and cleaned periodically by University cleaning contractors. Working areas and walkways are well lit and clutter free To observe and avoid use of trailing cables across the floor	**LOW RISK**					**LOW RISK**
Glassware and Sharps	Employees and users of	First Aid available (ext. 5555) for staff injuries.	**LOW RISK**					**LOW RISK**

	the laboratory due to broken glass or needles	Sharps boxes available for disposal of broken glass and needles. Needles must not be disposed in biological autoclave waste bags PPE to be worn (Gloves available)						

RISK ASSESSMENT – Multi-Use Gym

Activity:	Using the Multi-use Gym	**Brief description of work activity:** Strength training Repetition max assessment	**Assessed By:**	Helen Lloyd
Dept./Faculty:	FST		**Date:**	14/03/2018
Location:	4.04		**Review Date:**	14/03/2018

1. What are the hazards?	2. Who might be at harm and how? *E, C, S, Mp, V, Em, Mp, Dp **	3. Current control measures	4. Initial Risk Rating: *H/M/L **	5. Additional control measures (if required)	6. Action by whom?	7. Action by when? (Date)	8. Date done	9. Residual risk rating. *H/M/L*
Musculoskeletal injury	E, C, V, M, S **Risk of** Musculoskeletal injury. **Caused by** extra demand placed on the musculoskeletal system when performing physical activity.	Pre-screening (PAR-Q) for old/existing injuries. Strict adherence to the agreed protocol which includes a warm up and warm down. The subject is monitored by a person trained in the use of the equipment. Visual communication is maintained between the subject and experimenter throughout the session. . Participants are familiarised with equipment and trained in appropriate techniques. All user made aware of first aid procedures via SOP and to dial 5555/999 in an emergency.	(L2 x S2 = 4) **LOW RISK**	Risk assessments for project students and teaching practicals to include all items here plus any additions resulting from different protocols and or supplementation studies.	Any user of the multi-use gym.	Every time the multi-use gym is used.		(L2 x S2 = 4) **LOW RISK**
Cardiovascular complications	E, C, V, M, S **Risk of** Cardiovascular complications **caused by** Extra strain placed on the cardiovascular system when exercising.	Pre-screening (PAR-Q) to assess the subject's current level of fitness and status of health. All user made aware of first aid procedures via SOP and to dial 5555/999 in an emergency.	(L1 x S5 = 5) **LOW RISK**	Risk assessments for project students and teaching practicals to include all items here plus any additions resulting from different protocols and or supplementation studies.	Any user of the multi-use gym	Every time the multi-use gym is used.		(L1 x S5 = 5) **LOW RISK**

Faint, nausea or vomiting	E, C, V, M, S **Risk of** subject fainting or feeling nauseous **caused by** exertion.	Pre-screening (PAR-Q) and informed consent to make sure participant has eaten and is hydrated (if appropriate, in studies where fasting is required, spate risk assessments must be completed). Strict adherence to the agreed protocol which includes a warm up and warm down. The subject is monitored by a person trained in the use of the equipment. Visual communication is maintained between the subject and experimenter throughout the session. . Participants are familiarised with equipment and trained in appropriate techniques. All user made aware of first aid procedures via SOP and to dial 5555/999 in an emergency.	(L1 x S3=R3) **LOW RISK**	Risk assessments for project students and teaching practicals to include all items here plus any additions resulting from different protocols and or supplementation studies.	Any user of the multi-use gym	Every time the multi-use gym is used.		(L1 x S3=R3) **LOW RISK**
Slips, trips and falls	E, C, V, M, S **Risk of** slips trips or falls **caused by** loose equipment/ wires/water /sweat etc.	All loose wires will be taped down. All equipment will be stored safely. Water/sweat will be moped up as soon as it is spilt. The area around the multi-gym will be kept tidy and free from clutter. Bags and coats will be stored away from the equipment.	(L1 x R4 =R4) **LOW RISK**	Risk assessments for project students and teaching practicals to include all items here plus any additions resulting from different protocols and or supplementation studies.	Any user of the multi-use gym	Every time the multi-use gym is used.		(L1 x R4 =R4) **LOW RISK**
Injury	E, C, V, M, S **Risk** of getting fingers trapped in between weights **caused**	All users will be inducted into correct use of the multi-gym via a familiarisation session. This will include reading the SOP (which will include this risk assessment) and being made award of potential hazards.	(L1 x R4) **LOW RISK**	Risk assessments for project students and teaching practicals to include all items here plus any additions resulting from different protocols and or supplementation studies.	Any user of the multi-use gym	Every time the multi-use gym is used.		(L1 x R4) **LOW RISK**

	by unsafe use of equipment.							

RISK ASSESSMENT – SECA Body Composition

Activity:	Seca	**Brief description of work activity:** Using the Seca	**Assessed By:**	Burak Pirincci and Helen Lloyd
Dept./Faculty:	Health Sciences		**Date:**	19/05/2017
Location:	C4.04		**Review Date:**	01/08/17

1. What are the hazards?	**2. Who might be at harm and how?** ***E, C, S, Mp, V, Em, Mp, Dp ****	**3. Current control measures**	**4. Initial Risk Rating:** ***H/M/L ****	**5. Additional control measures (if required)**	**6. Action by whom?**	**7. Action by when? (Date)**	**8. Date done**	**9. Residual risk rating.** ***H/M/L***
Electric Shock	E, S, V **Risk of** electric shock **caused by** equipment malfunction or water near the SECA.	The equipment is PAT tested annually. The equipment is maintained in line with the user manual. The user must have dry hands and feet before using the equipment. The equipment will be visually checked before every use. Any water spills near the equipment should be cleaned up immediately. If spills occur on the equipment, it should be turned off and it should be fully dried before use.	L1 x S4 = R4 **LOW RISK**					L1 x S4 = R4 **LOW RISK**
Contracting a foot infection	V, MP, S, E **Risk of** infection **caused by** previous participants using SECA with infection.	The foot plate must be wiped with disinfectant wipes before stepping on. Wipes will be placed next to the machine. Anyone knowingly suffering from any foot infection (verruca, athlete's foot etc.) must not act as a participant.	(L1 x S2) = R2 **LOW RISK**					(L1 x S2) = R2 **LOW RISK**

Psychological discomfort	E, S, Mp, V **Risk of** psychological discomfort caused by the results of the assessment	Participants will be asked if they are comfortable with the procedure. The assessor will explain how results are interpreted against norm values. The participant will be reassured if they are upset.	(L1 x S2) = R2 **LOW RISK**					(L1 x S2) = R2 **LOW RISK**
Slips, trips and falls	E, S, Mp, V **Risk of** tripping **caused by** trailing cables between the SECA and printer.	Cables tidied away along the skirting board or behind the equipment out of the way of footfall.	(L1 x S2) = R2 **LOW RISK**					(L1 x S1) = R2 **LOW RISK**

RISK ASSESSMENT - Treadmill

Instrument:	Treadmill	**Brief description of work activity:** Maximal (>85% Max hr) exercise on treadmill: e.g. VO2 Max test	**Assessed by:**	Helen Lloyd
Dept./Faculty:	FST		**Date:**	24.03.17
Location:	C4.08, C4.07		**Review Date:**	24.08.17

1. What are the hazards?	2. Who might be at harm and how? *E, C, S, Mp, V, Em, Mp, Dp **	3. Current control measures	4. Initial Risk Rating: *H/M/L **	5. Additional control measures (if required)	6. Action by whom?	7. Action by when? (Date)	8. Date done	9. Residual risk rating. *H/M/L*
Faint,nausea or vomiting	**E, S, Mp, V** **Risk of** subject fainting or feeling nauseous **caused by** exercising maximally.	In the event of the subject feeling the onset of either nausea or syncope, ensure they are positioned supinely, with their feet raised to aid central venous return. Visual communication is maintained between the participant and investigator. Investigator is aware of first aid procedure. Spill kit available if the participant vomits.	(L1 x S3) = R3 **LOW RISK**					(L1 x S3) = R3 **LOW RISK**
Musculoskeletal injury	E, S, Mp, V **Risk of** musculoskeletal injury **caused by** extra demand placed on the musculoskeletal system when performing physical activity.	All participants will be pre-screened using the PAR-Q. Participants will sign informed consent forms. Individuals with existing lower limb or muscular injuries will not take part. All participants will warm up on the treadmill for at least five minutes.	(L2 x S2) = R4 **LOW RISK**					(L2 x S2) = R4 **LOW RISK**

		All participants will cool down for 5 minutes and be encouraged to stretch.						
Slips, trips and falls	E, S, Mp, V **Risk of** participant falling from the treadmill **caused by** a participant that may be unfamiliar with using a treadmill, or a participant that pushes themself to exhaustion and stumbles.	Participants will be required to do a familiarisation period on the treadmill before the exercise test. Participants will be required to wear a harness.	(L1 x S3) = R3 **LOW RISK**					(L1 x S3) = R3 **LOW RISK**
Cardiovascular Complications	E, S, Mp, V **Risk of** cardiovascular complications **caused by** extra strain placed on the cardiovascular system when exercising	Pre-screening questionnaire to assess the subject's current level of fitness and status of health. Individuals with existing cardiovascular conditions who have been advised not to exercise will not be allowed to be a participant. Participants will be monitored throughout testing and are required to wear a heart rate monitor. Participants will be chaperoned should they wish to leave the room within 20 minutes after testing has finished. The person carrying out the test will know the procedure in the event of a first aid incident (which is to call 5555 to get a first aider).	(L2 x S5) = R10 **MEDIUM RISK**				N/A	(L2 x S5) = R10 **MEDIUM RISK**

Injury from broken Treadmill	E, S, Mp, V **Risk of** flywheel becoming detached from the cycle ergometer **caused by** loose attachments and poor maintenance.	The treadmill will be checked and maintained by technical staff in line with the maintenance in the user manual. It will undergo an annual service. Any faults should be reported to technical staff immediately.	(L1 x S3) = R3 **LOW RISK**					(L1 x S3) = R3 **LOW RISK**
Bacterial infection	E, S, Mp, V **Risk of** bacterial, viral infection from mouthpiece **caused by** contaminated mouthpiece	Breathing apparatus are sterilised immediately after use in Virkon solution. This ensures the breathing apparatus are sterile and safe for the next subject to use. Gloves should be worn when handling contaminated breathing apparatus.	(L1 x S2) = R2 **LOW RISK**					(L1 x S2) = R2 **LOW RISK**

RISK ASSESSMENT – Monark Cycle Ergometer and Computer

Instrument:	Monark Cycle ergometer and computer	**Brief description of work activity:** Performing a wingate test.	**Assessed by:**	Helen Lloyd
Dept./Faculty:	FST		**Date:**	14.08.18
Location:	C4.07, C4.04		**Review Date:**	14.08.19

1. What are the hazards?	2. Who might be at harm and how? *E, C, S, Mp, V, Em, Mp, Dp **	3. Current control measures	4. Initial Risk Rating: *H/M/L **	5. Additional control measures (if required)	6. Action by whom?	7. Action by when? (Date)	8. Date done	9. Residual risk rating. *H/M/L*
Post exercise syncope and nausea and vomiting.	E, S, Mp, V **Risk of** participant fainting or feeling nauseous **caused by** exercising maximally	Ensure participant continues to pedal sub-maximally for a minimum of 5 minutes following the completion of the test in order to prevent lower limb venous blood pooling, and reduce the chance of syncope and other nausea related conditions. In the event of the subject feeling the onset of either nausea or syncope, ensure they are positioned supinely, with their feet raised to aid central venous return. Participants must not be left alone for the 20 minutes post Wingate and must be monitored closely following its completion. Participants must not leave the room within the 20 minutes post Wingate without a chaperone. The person carrying out the test will know the procedure in the event of a first aid	(L2 x S3) = R6 **MEDIUM RISK**				N/A	(L2 x S3) = R6 **MEDIUM RISK**

		incident (which is to call 5555 to get a first aider). A bowl and spillage kit are always present to collect any vomit or clean any spillage.						
Musculoskeletal injury	E, S, Mp, V **Risk of** musculoskeletal injury **caused by** extra demand placed on the musculoskeletal system when performing physical activity.	All participants will be pre-screened using the PAR-Q. Participants will sign informed consent forms. Individuals with existing lower limb or muscular injuries will not take part. All participants will warm up on a cycle ergometer for at least five minutes. During the final minute of this process, participants will perform several short maximal sprints in order to replicate the intensity of the protocol. All participants will cool down on a cycle ergometer for 5 minutes and be encouraged to stretch.	(L2 x S2) = R4 **LOW RISK**				N/A	(L2 x S2) = R4 **LOW RISK**
Slips, trips and falls	E, S, Mp, V **Risk of** participant falling from the cycle ergometer **caused by** a participant that may be unfamiliar with riding a cycle ergometer or the bike tipping from a strong athlete exerting during the protocol.	Allow the participant to become accustomed with riding a cycle ergometer before the exercise test. 4 nominated people to secure the Monark to the floor during testing (ideally 1 on each corner of the bike).	(L1 x S3) = R3 **LOW RISK**					(L1 x S3) = R3 **LOW RISK**
Cardiovascular Complications	E, S, Mp, V **Risk of** cardiovascular	Pre-screening questionnaire to assess the subject's current level of fitness and status of health.	(L2 x S5) = R10 **MEDIUM RISK**				N/A	(L2 x S5) = R10 **MEDIUM RISK**

	complications **caused by** extra strain placed on the cardiovascular system when exercising	Individuals with existing cardiovascular conditions who have been advised not to exercise will not be allowed to be a participant. Participants will be monitored throughout testing. Participants will be chaperoned should they wish to leave the room within 20 minutes after testing has finished. The person carrying out the test will know the procedure in the event of a first aid incident (which is to call 5555 to get a first aider).						
Injury from broken Monark	E, S, Mp, V **Risk of** flywheel becoming detached from the cycle ergometer **caused by** loose attachments and poor maintenance.	The Monark cycle ergometer is positioned so that it is facing a wall and that it is within 1.0m of that wall. Monark cycle ergometers will checked and maintained by technical staff in line with the maintenance in the user manual.	(L1 x S3) = R3 **LOW RISK**					(L1 x S3) = R3 **LOW RISK**
Injury from entanglement	E, S, Mp, V **Risk of** shoelaces becoming caught in the flywheel and clothing caught in the chain **caused by** inappropriate clothing.	Participants must Secure shoelaces and any lose clothing prior to exercise.	(L1 x S3) = R3 **LOW RISK**					(L1 x S3) = R3 **LOW RISK**

RISK ASSESSMENT – Venepuncture

Instrument:	Venepunture	**Brief description of work activity:** The puncture of a vein as part of a medical procedure, typically to withdraw a blood sample or for an intravenous injection.	**Assessed by:**	Helen Lloyd
Dept./Faculty:	FST		**Date:**	24.03.17
Location:	C4.08, C4.04, C4.07		**Review Date:**	24.08.17

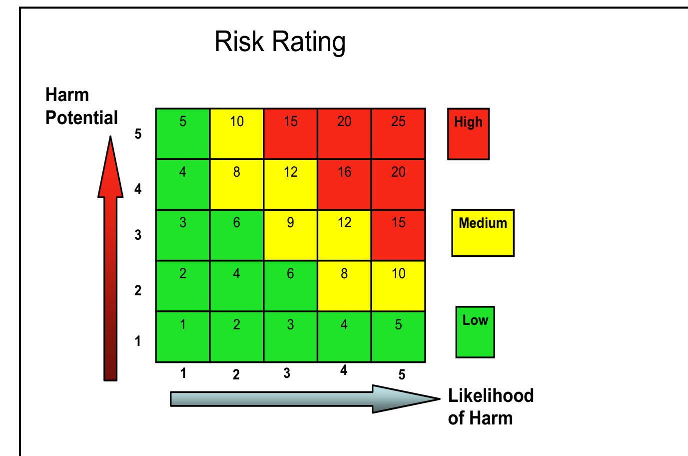
1. What are the hazards?	2. Who might be at harm and how? *E, C, S, Mp, V, Em, Mp, Dp **	3. Current control measures	4. Initial Risk Rating: *H/M/L **	5. Additional control measures (if required)	6. Action by whom?	7. Action by when? (Date)	8. Date done	9. Residual risk rating. *H/M/L*
Infection	**E, S, Mp, V** **Risk of** cross infection **caused by** contaminated equipment and/or participants with infection or needle stick injury.	Correct use of PPE Adherence to the blood and blood products procedure and waste disposal procedures. Pre-screened participants. Adherence to the needle stick policy and first aid procedure in the event of an incident.	(L1 x S5) = R5 **LOW RISK**					(L1 x S5) = R5 **LOW RISK**
Bruising	**E, S, Mp, V** **Risk of** bruising **caused by** insufficient pressure application on site of blood sampling following procedure.	Ensure immediate pressure should be exerted on the site of blood sampling. This should be sustained until bleeding has stopped.	(L2 x S2) = R4 **LOW RISK**					(L2 x S2) = R4 **LOW RISK**

Fainting	**E, S, Mp, V** **Risk of** vasovagal fainting **caused by** adverse reaction/phobia to blood sampling.	Take blood in seated or lying position to reduce the possibility of heavy impact with floor or furniture if subject faints. Ask participant if they are ok with having their blood taken. Person taking blood must be inducted on the first aid policy.	(L1 x S2) = R2 **LOW RISK**					(L1 x S2) = R2 **LOW RISK**
Haemotosis	**E, S, Mp, V** **Risk of** heamatosis **caused by** excessive needle trauma to a vein.	Risk is minimised by appropriately trained staff (must have undertaken training in phlebotomy). Person taking blood must be inducted on the first aid policy. Compression should be applied to help stabilise a hematoma.	(L1 x S5) = R5 **LOW RISK**					(L1 x S5) = R5 **LOW RISK**
Blood spill	**E, S, Mp, V** **Risk of** blood spillage **caused by** incorrect use of tourniquet or insufficient compression following sampling.	Risk is minimised by appropriately trained staff (must have undertaken training in phlebotomy). Correct use of Tourniquet will reduce possibility of blood spurting under pressure. Adherence to venepucture SOP.	(C1 x S2) = R2) **LOW RISK**					(C1 x S2) = R2) **LOW RISK**

*** Please see overleaf for guidance on completion**

Guidance on completing the risk assessment

1. **Description of the work:** A general description of the work e.g. Teaching; Travelling; Workshop activities; Grounds Maintenance; Office Activities; Waste collection;
2. **Task or Process:** A brief description of the specific work being carried out e.g. use of workshop or laboratory equipment; using ladders for maintenance purposes; manual handling of materials; bulk waste collection etc. or the process being assessed.
3. **The Hazard(s):** A brief description of the potential for causing harm or loss e.g. moving parts of machinery; entanglement; contact with electricity, lifting heavy loads etc.
4. **Persons at Risk:** E = Employees; C = Contractors; V = Visitors; Mp = Members of the public; S = Students; EM = Expectant Mothers; DP = Disabled persons. The types and numbers of person at risk may impact the degree and likelihood of the risk.
5. **Existing Controls:** Considerations could include:- guarding; training; safe systems of work; segregation; safety equipment; examination and testing; emergency arrangements.
6. **Risk Classification:** In considering the likelihood of an injury or incident occurring the following potential contributory factors should be considered:-
   - How frequent the work is carried out? A higher frequency may increase the risk.
   - Whether those carrying out the work are more at risk e.g. a disabled person, an expectant mother, or someone with little experience.
   - Are suitable tools and equipment available that are properly maintained?
   - Has a safe system of work been established and implemented?
   - Has suitable information, instruction and training been provided?
   - Is there adequate supervision?
   - Are the controls in place adequate or are additional controls required?



Likelihood of occurrence

1. **Improbable**
2. **Remote**
3. **Possible**
4. **Probable**
5. **Certainty**

Potential Severity of injury of financial loss

1. **None**
2. **Negligible**
3. **Minor**
4. **Major**
5. **Fatal**

Risk = Likelihood X Severity

Using the values above determine the risk classification by multiplying the likelihood and the potential severity you consider appropriate and enter the result in the matrix above to obtain the risk rating: e.g. If you consider the potential harm to be major (4) and the likelihood to be remote (2) the risk classification is 4 x 2 = 8 which is classed as a “Medium” risk using th1

Appendix B: UoW - Laboratory Health and Safety 4th Floor

UNIVERSITY OF WESTMINSTER
SCHOOL OF LIFE SCIENCES

BIOLOGY SAFETY PRECAUTIONS
CODE FOR THE HANDLING OF HUMAN BLOOD AND BLOOD PRODUCTS

1. All human blood and blood products must be treated as potentially hazardous materials and disposable gloves must be worn when handling them (eye protection should also be considered).

   Normal microbiological safe handling procedures must be used (see **Code of Practice for the Use of Microorganisms and Materials likely to contain them**, *Appendix 1*).

   Other human body fluids which must be treated in the same way as blood and blood products include cerebrospinal fluid (CSF) and amniotic fluid.

   There is a separate code for the use of urine and saliva provided in *Appendix 7*.

   If in doubt, please consult the Biological Safety Officer.

2. Care must be taken to avoid contaminating the skin and eyes of those using the blood or blood product. Also contamination of laboratory areas and apparatus must be avoided. Spillages of human blood or a blood product must be cleared up immediately using "*Virkon*", following the instructions in *Appendix 2* (**Procedure for dropped or spilt Cultures**). Phenolic-based disinfectants, such as "*Hycolin*", should not be used on material likely to contain viruses, such as human blood. **NOTE**: due to the risk of explosion and release of toxic gas, hypochlorite-based disinfectants, such as "*Chloros*", are not allowed except by special permission (and only if their use can be justified) from the School Laboratory Manager, Research Director or Biological Safety Officer.

3. A COSHH Assessment must be made of any work involving the use of human blood and blood products although, in normal circumstances, adherence to the relevant Code of Practice and Appendix(ices) should be adequate for the control procedures.

4. The use of scalpels, needles, glass pipettes and other sharps should be avoided.
5. Human blood and blood products for use in University laboratories should be obtained from the safest source that is reasonably practicable. The following guidelines should be used:-

(a) Blood from laboratory-bred animals should be used in preference to human blood.
Human blood should only be used when there is no alternative.

(b) All human blood that is used for class practicals must be screened for hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV). This will be after consultation with the Biological Safety Officer (BSO).

(c) Any staff or students who donate blood for use on University sites must be certified (either by the National Blood Service or similar reputable body) to be HIV negative, HBV negative, HCV negative, and have a haemoglobin level of more than 140 g l^{-1} (for men) or 120 g l^{-1} (for women). Such screening must have taken place not more than 6 months before the date of donation.
Although blood from such individuals may be used in class practicals, there is no guarantee that samples are free from HIV, HBV and HCV at the time of use and appropriate care must always be taken (*i.e.* human blood must always be regarded as a potentially hazardous material).

(d) Only authorised persons can take blood from blood donors listed in 5(c) above. Authorized individuals include appropriately qualified members of the University Medical Service and appropriately qualified members of staff of the School of Biosciences (for whom the Head of School has provided written authorization).

(e) All other use of unscreened blood (*ie.* blood from sources other than that listed in 4(c) above) requires written authorization from the Biological Safety Officer and counter signature from the Head of the School of Biosciences.

6. All surfaces and bench tops must be disinfected after use with an appropriate, freshly prepared, hypochlorite-type disinfectant (*e.g.* 5% AChloros≅, 1% ADomestos≅). All blood or blood product-contaminated material must be autoclaved prior to disposal or washing. All waste for autoclaving must be fully labelled as designated in *Code of Practice, para 3*.

7. It is the responsibility of the academic supervisor to ensure that students or research personnel for whom they are responsible have been adequately trained and briefed in the use and hazards of human blood and blood products.

BIOLOGY SAFETY PRECAUTIONS

CODE FOR THE USE OF URINE AND SALIVA

Human urine and saliva should be treated as potentially infectious materials and normal microbiological safe handling procedures used at all times (see *Appendix 1*, **Code of Practice for the Use of Microorganisms and Materials likely to contain them**).

A COSHH assessment must be made and recorded for any work involving the use of human urine and saliva although, in normal circumstances, adherence to the relevant Code of Practice and Appendix(ices) should be adequate for risk control procedures.

1. Disposable gloves must be worn at all times when handling urine and saliva samples.

2. Any spillages in the laboratory must be mopped up immediately using an appropriate disinfectant (as specified in *Appendix 6*) and using the procedure described in *Appendix 2*.

3. Particular care should be taken with saliva and urine collection.

4. All samples must be fully labelled and stored in screw-top bottles (not overfilled) using only a designated fridge or freezer.

5. The use of glass pipettes and other sharps should be avoided if at all possible.

6. Contaminated glassware and discarded and contaminated items must be placed in the receptacles provided for their disposal. All waste for autoclaving must be fully labelled as designated in *Code of Practice, para 3*.

7. All glassware and other contaminated items must be autoclaved before washing.

8. Spillages of any human body fluids, including blood, urine and saliva, must be cleared up immediately using "*Virkon*", following the instructions in *Appendix 2* (**Procedure for dropped or spilt Cultures**). Phenolic-based disinfectants, such as "*Hycolin*", should not be used on material likely to contain viruses, such as human blood. **NOTE**: due to the risk of explosion and release of toxic gas, hypochlorite-based disinfectants, such as "*Chloros*", are not allowed except by special permission (and only if their use can be justified) from the School Laboratory Manager, Research Director or Biological Safety Officer.

BIOLOGY SAFETY PRECAUTIONS

CODE OF PRACTICE FOR THE USE OF MICROORGANISMS AND MATERIALS LIKELY TO CONTAIN THEM

Whatever your biology specialism, this Code applies to **you**. Students following Biological Sciences, Biomedical Sciences, Physiology, Sports Science, Environmental Science, *etc*. will all come into contact with materials which are potential biohazards. These instructions do **not** just apply to microbiology.

Every microorganism, culture, sample and isolate must be considered as potentially pathogenic and a COSHH risk assessment done by the lecturer\researcher prior to using it (see *Appendix 10*). Direct contact with such must not be made, *e.g*. with skin, nose, respiratory tract, eyes and mouth.

If you have any medical condition, including pregnancy, that could make working with microorganisms (or chemicals) in the laboratory especially hazardous, it is **your** duty to inform the relevant member of staff in confidence. This can be the lecturer in charge, the Module/Course Leader, the School Disability Liaison Officer or your Personal Tutor.

1. At all times you must be properly attired in the laboratory in an appropriate laboratory coat which must be correctly fastened at all times. The School of Biosciences provides laboratory coats for students. These MUST be put on a coat hanger and returned to the rack after use, and NOT thrown on the bench or floor.

   You must wear any other protective clothing (*e.g*. gloves, masks, safety spectacles, visors) as instructed by the member of staff in charge.

2. You must not eat, drink, smoke or apply cosmetics in the laboratory, on any laboratory floors, on stairs, or in lifts.

3. You must not suck or bite pencils or pens. You must not wear sandals, cumbersome jewellery or hats.

4. You must not lick labels prior to sticking them to apparatus (use tap water, self-adhesive labels or marker pen).

5. Avoid touching your face, hair, eyes, mouth, *etc*. whilst in the laboratory. Long hair must be tied back.

6. You must keep your available bench space clear, clean, tidy and free of inessential clutter, e.g. books.

7. You must not remove any materials from the laboratory, e.g. microbial cultures, without the permission of the lecturer in charge.

8. Manipulations by loop or pipette should be performed in a manner to minimise the production of aerosols.

9. Pipetting by mouth of any material is forbidden. You must always use the teats, syringes, and pipette-fillers provided.

10. All manipulations should be performed aseptically, using plugged pipettes, and the contaminated pipettes disposed of in the containers indicated by the lecturer in charge.

11. Contaminated glassware, plasticware, microscope slides and discarded Petri dishes, *etc*. must be placed in the receptacles indicated by the lecturer in charge.

12. It should be recognised that certain procedures or equipment produce aerosols of contaminated material, e.g. the breaking of any fluid film, centrifugation and the agitation of fluids in shaking or orbital incubators.

13. Report all personal accidents, minor cuts and abrasions, breakages and spillages of cultures and reagents to the lecturer in charge. Cuts and abrasions must be protected by an adequate waterproof dressing.

14. If instructed, before leaving your bench, swab the area down with an appropriate disinfectant.

15. Before leaving the laboratory, return personal protective clothing, hang up your lab coat correctly, and wash your hands with soap (preferably germicidal).

BIOLOGY SAFETY PRECAUTIONS

PROCEDURE FOR DROPPED OR SPILT CULTURES

1. Wearing gloves, flood the affected area with the disinfectant provided and allow to act for at least 10 minutes. Note that a wider area than that visible may be contaminated by aerosols.

   NOTE: due to the risk of explosion and release of toxic gas, hypochlorite-based disinfectants, such as "*Chloros*", are not allowed except by special permission (and only if their use can be justified) from the School Laboratory Manager, Research Director or Biological Safety Officer.

2. Mop up the area with absorbent cotton wool, paper towel or tissue and discard it into an autoclavable plastic bag.

3. Broken glassware must be picked up with forceps or dustpan and brush and put into a suitable container for autoclaving. Such containers can be obtained from the technical staff and their attention MUST be drawn to the fact that broken glassware requires sterilization. The dustpan and brush or forceps should also be sterilized.

4. Dispose of the gloves appropriately and wash your hands thoroughly using germicidal soap.

5. Personal items, e.g., books, pencil cases, *etc*. must also be disinfected if they have been contaminated. To prevent this necessity, it is best to keep the benches clear of such items.

BIOLOGY SAFETY PRECAUTIONS

INFECTION CONTROL PROCEDURES

The sterility and safe disposal of needles
The avoidance of injury from needles
The safe handling of blood spills
Procedures in the event of a needle stick injury
Hepatitis B immunisation.

1) The sterility and safe disposal of needles
    a) All needles used for teaching and for practice are disposable needles.
    b) Needles are purchased from a reliable supplier and will carry the CE mark.
    c) Needles may only be used if they are taken from a sealed packet.
    d) Needles must not be put down onto any surface before or after use.
    e) Unused needles that have been removed from a packet must be disposed of in the same way as a used needle.
    f) Needles removed from a patient are placed directly into a sharps box without being put down in any intermediary container.
    g) Sharps boxes are provided in each clinical or teaching room and should be placed on the blood trolley which should be as near as possible to the person taking the sample.
    h) If a sharps box appears to be full, so that the needles will not easily fall into the box or so that the self-closing mechanism will not operate, then a new box should be obtained immediately.

NB. The covers of sharps boxes are never to be removed. If it is necessary to temporarily place infected needles anywhere other than the sharps box, as soon as possible they should be safely disposed of and the surface properly cleaned.

2) The avoidance of injury from needles

Care is to be taken at all times when using needles. Do not rush.

Particular care should be taken after the needles are removed from a patient. Hospital needle-stick injury records show that most occur during the disposal of the needles. Do not turn around quickly when holding used needles. Other persons should keep a safe distance from the person disposing of used needles.

3) The safe handling of blood spills
    a) Wearing gloves, flood the affected area with the disinfectant provided and allow to act for at least 10 minutes.
    b) NOTE: due to the risk of explosion and release of toxic gas, hypochlorite-based disinfectants, such as "Chloros", are not allowed except by special permission (and only if their use can be justified) from the School Laboratory Manager, Research Director or Biological Safety Officer.
    c) Mop up the area with absorbent cotton wool, paper towel or tissue and discard it into an autoclavable plastic bag.
    d) Broken glassware must be picked up with forceps or dustpan and brush and put into a suitable container for autoclaving. Such containers can be obtained from the technical staff and their attention MUST be drawn to the fact that broken glassware requires sterilization. The dustpan and brush or forceps should also be sterilized.
    e) Dispose of the gloves appropriately and wash your hands thoroughly using germicidal soap.
    f) Personal items, e.g. books, pencil cases, etc. must also be disinfected if they have been contaminated. To prevent this necessity, it is best to keep the benches clear of such items.

4) Procedures in the event of a needle stick injury

a) If the injury occurs before the needle has been inserted into the patient, the needle should be discarded into the sharps box in the usual manner.

b) If the injury occurs from a contaminated needle, then immediately:

i) <u>First Aid</u>

Encourage bleeding by squeezing gently for a few seconds.
Wash the wound thoroughly with soap and water.
Wash any blood splashes on broken skin, or to the eyes or mouth, with copious amounts of running water.
Dispose of the needle safely

ii) <u>Reporting procedure</u>

The incident should be then reported to the supervisor and reported online at:

https://myintranet.westminster.ac.uk/my-tools/emergency/report-an-incident.

If the incident involves a student then Student Health should be informed on extension 68005.

iii) Action to be taken

If the source of the blood contamination is known (i.e. the patient), they should be asked about any known infections.

Advice on assessment of Hep B & C and HIV risk can be obtained from the University Health Service on ext 5186.

The injured person should be advised to attend a clinic for blood tests as soon as possible but within 72 hrs. The nearest appropriate clinic is: The Mortimer Market Centre, (which is a sexual health clinic), off Capper Street, off Tottenham Court Road. Tel 0207 530 5111 (Health Advisors). An alternative testing centre is JS Pathology Services, 80 Harley Street. This is a private laboratory, and would cost approximately £50 for the series of tests.

iv) Blood tests and Immunisation

This is for information, as tests and any follow up will be under the direction of the clinic carrying out the testing. They will:

Consider whether appropriate to take samples of the subject's blood for long term storage, Hep B Antigens, AntiHC and HIV, all taken with informed consent.
Consider Hep B immunisation update or Hep B specific immunoglobulin.
Consider antiviral prophylactic drugs if high risk HIV.
Follow up if donor is positive for HIV, Hep B or Hep C: offer blood test for HIV after 3 months; blood test for AntiHC after 6 months; blood test for Anti HBc after 6 weeks.

5) Procedures regarding immunisation against Hepatitis B.

It is the responsibility of all persons taking blood to ensure they have had the hepatitis B vaccination.

Hepatitis B vaccinations can be done at your GP or a travel clinic. A cost may be incurred.

Keith Redway
Biological Safety Office

Appendix C: UoW – COSHH Forms – Blood Sampling

UNIVERSITY OF WESTMINSTER
School of Life Sciences
Control of Substances Hazardous to Health (COSHH) Sheet........ of..........
******APPENDIX 10 - MICROBIAL HAZARDS (ACDP GROUPS 1 & 2 ONLY)******

Title of Experiment **Room/Laboratory**

1. Venous blood sampling | C4.06 / C4.04

Brief description of work

A tourniquet is used to occlude venous return and to aid visualisation of vein to be bleed. Area of skin is cleaned with alcohol swab and allowed to dry for 30 seconds. Single use sterile needle is mated with vacutainer system for collection; needle cap is loosened but not removed. Anchor view with thumb distal to site of sampling, avoiding sterile sample zone. Remove cap with free hand and puncture vein. Once required volume of blood has been collected remove tourniquet then needle (order important) and immediately dispose of needle in sharps bin (do not recap). Place sterile dressing over puncture site and apply firm pressure for 5 minutes. Replace dressing and secure with tape if necessary. Dispose of contaminated swabs and dressings into hazardous waste bin. Post sample any potentially contaminated areas are to be cleaned with Virkon.

2. LIST OF CULTURES USED (continue on a separate sheet if necessary)

	NAME OF CULTURE **(GENERIC AND SPECIFIC NAME)**	**CULTURE TYPE**	MAX. QUANTITY EXPOSED TO	**INSERT ACDP GROUP NUMBER BELOW (1 or 2 ONLY ARE ALLOWED)**	**KNOWN HAZARDS**
	Microorganism (ACDP Category 1 or 2 only). Give full name, strain code(s) and UOW number (if known).	*e.g.* broth culture, Petri dish, fermenter.	*e.g.* 20 ml broth for UB, 15 ml agar for plate.		20 (Biological hazard). ACDP = Advisory Committee on Dangerous Pathogens.
A	n/a	Human blood	12ml	2	20 (Biological hazard/infectious material)

NOTE: 1) EXPLOSIVE 2) OXIDIZING 3) FLAMMABLE 4) TOXIC 5) HARMFUL 6) CORROSIVE 7) IRRITANT 8) CARCINOGEN 9) MUTAGEN 10) TERATOGEN 11) DUST 12) INHALATION 13) INGESTION 14) SKIN ABSORPTION 15) SKIN OR EYE 16) INJECTION 17) MAXIMUM EXPOSURE LIMIT 18) OPERATIONAL EXPOSURE STANDARD 19) RADIATION 20) OTHER (specify)

3. PERSONNEL INVOLVED WITH CULTURES (continue on a separate sheet if necessary)

SURNAME/CLASS	**INITS**	**STATUS**	**INVOLVEMENT** - DATE OF USE or DAILY/WEEKLY/MONTHLY/OCCASIONALLY
Dolci	A	Lecturer	Weekly
Kyriakidou	Y	PhD student	Weekly
Ferraroli	J	Technician	Occasionally

4. OTHER GROUPS/PERSONS WHO MAY HAVE ACCESS TO THE CULTURES
(e.g. students [name class], cleaners, maintenance staff, contractors, visitors, storekeepers, *etc.*)

Level 6 students conducting research on this project, teaching and technical staff present in room where blood is being taken/handled. Technical staff in dealing with hazardous waste.

5. EMERGENCY PROCEDURES
if any of the cultures or procedures identified above is likely to pose a special hazard in an emergency, then identify below action to be taken

SPILLAGE/UNCONTROLLED RELEASE:	FIRE:
Follow instructions in *Appendix 2* of UOW Regulations for the Use of Biological Materials (**Procedure for dropped or spilt cultures**).	**N/A**

If personnel are affected (fume, contamination*, etc.*) treatment to be adopted:
(N.B. Antidotes and special treatment may be obtained through............**N/A**...............)

Wash affected area of skin with germicidal soap. Change contaminated clothes and wash with water then detergent and water.
If eyes affected, wash with plenty of water.

In situations of sharps injury follow UoW procedures in the event of a sharps injury. Briefly, encourage bleeding from injury site by squeezing the injured area gently; wash liberally with soap and water. Seek medical assistance if the risk of transfer of infection is considered plasuable (*does the donating individual have an infective disorder*?).

6. CONTROL MEASURES TO BE ADOPTED

1) personal protective equipment needed (clear eyewear, gloves, appropriate clothing for human sampling)
2) safe disposal of sharps
3) safe disposal of hazardous contaminated waste
4) regular cleaning of the workplace utilizing Virkon
5) adequate washing facilities

STORAGE - SAFETY CONSIDERATIONS	HANDLING PRECAUTIONS
Use secure, fully labelled containers in fridge, freezer or incubator.	Follow instructions in *Appendix 1* of UOW Regulations for the Use of Biological Materials (**Code of Practice for the Use of Microorganisms**). All students must receive adequate practical instruction from the lecturer in charge.

DISPOSAL PROCEDURES DURING AND AT END OF EXPERIMENT

Follow instructions in *Appendix 1*. Technicians to follow instructions in *Appendix 8* (**Instructions for technical staff**).

7. REVIEW AND MONITORING OF CONTROL MEASURES

Control measures to be reviewed every 12 months, or earlier if there is a change of personal in charge or event prompting a review of measures in place.

Medical monitoring required? **N** Occupational health nurse informed? **N**

8. OTHER RELEVANT INFORMATION

All experiments must comply with the UoW regulation of the use of biological materials, the codes of practice and the relevant appendices. Collected whole blood must comply with the Human Tissues Act, unless immediately rendered acellular for plasma or serum.

9. Name of Assessor (BLOCK CAPITALS): Dr ALBERTO DOLCI Signed:

Status of Assessor (Lecturer/Post-doc/Technician): Lecturer Date: 7 – 11 - 2016

Keith Redway
May 2005

UNIVERSITY OF WESTMINSTER
School of Life Sciences
Control of Substances Hazardous to Health (COSHH) Sheet........ of..........
******APPENDIX 10 - MICROBIAL HAZARDS (ACDP GROUPS 1 & 2 ONLY)******

	Title of Experiment	Room/Laboratory
1.	Venous blood sampling	C4.08 / C4.05

Brief description of work

Venepuncture - blood sampling. After verbal screening for known presence of pathogens (such as HIV or Hepatitis B/C), a tourniquet is used to occlude venous return and to visualize vein to be bleed. Area of skin is cleaned with alcohol swab and allowed to airdry for 30 seconds. Single use sterile needle is mated with vacutainer system for collection; needle cap is loosened but not removed. Anchor view with thumb distal to site of sampling, avoiding sterile sample zone. Remove cap with free hand and puncture vein. Once required volume of blood has been collected remove tourniquet then needle (order important) and immediately dispose of needle in sharps bin (do not recap). Place dressing over puncture site and apply firm pressure for 5 minutes. Replace dressing and secure with tape if necessary. Dispose of contaminated swabs and dressings into hazardous waste bin. Equipment surrounding the venous sampling suite (chair, armrest, reusable equipment) should be cleaned with Virkon (2%).

2. LIST OF CULTURES USED (continue on a separate sheet if necessary)

NAME OF CULTURE (GENERIC AND SPECIFIC NAME)		CULTURE TYPE	MAX. QUANTITY EXPOSED TO	INSERT ACDP GROUP NUMBER BELOW (1 or 2 ONLY ARE ALLOWED)	KNOWN HAZARDS
Microorganism (ACDP Category 1 or 2 only). Give full name, strain code(s) and UOW number (if known).		*e.g.* broth culture, Petri dish, fermenter.	*e.g.* 20 ml broth for UB, 15 ml agar for plate.		20 (Biological hazard). ACDP = Advisory Committee on Dangerous Pathogens.
A	n/a	Human blood	12ml	2	20 (Biological hazard/infectious material)

NOTE: 1) EXPLOSIVE 2) OXIDIZING 3) FLAMMABLE 4) TOXIC 5) HARMFUL 6) CORROSIVE 7) IRRITANT 8) CARCINOGEN 9) MUTAGEN 10) TERATOGEN 11) DUST 12) INHALATION 13) INGESTION 14) SKIN ABSORPTION 15) SKIN OR EYE 16) INJECTION 17) MAXIMUM EXPOSURE LIMIT 18) OPERATIONAL EXPOSURE STANDARD 19) RADIATION 20) OTHER (specify)

3. PERSONNEL INVOLVED WITH CULTURES (continue on a separate sheet if necessary)

SURNAME/CLASS	**INITS**	**STATUS**	**INVOLVEMENT** - DATE OF USE or DAILY/WEEKLY/MONTHLY/OCCASIONALLY
Elliott	B	Lecturer	Weekly/Monthly
Kyriakidou	Y	PhD student	Daily/Weekly

4. OTHER GROUPS/PERSONS WHO MAY HAVE ACCESS TO THE CULTURES
(e.g. students [name class], cleaners, maintenance staff, contractors, visitors, storekeepers, *etc.*)

Level 6 students conducting research on this project, teaching and technical staff present in room where blood is being taken/handled. Technical staff in dealing with hazardous waste.

5. EMERGENCY PROCEDURES
if any of the cultures or procedures identified above is likely to pose a special hazard in an emergency, then identify below action to be taken

SPILLAGE/UNCONTROLLED RELEASE:	FIRE:
Follow instructions in *Appendix 2* of UOW Regulations for the Use of Biological Materials (**Procedure for dropped or spilt cultures**).	**N/A**

If personnel are affected (fume, contamination*, etc.*) treatment to be adopted:
(N.B. Antidotes and special treatment may be obtained through............**N/A**...............)

Wash affected area of skin with germicidal soap. Change contaminated clothes and wash with water then detergent and water.
If eyes affected, wash with plenty of water.

In situations of sharps injury follow UoW procedures in the event of a sharps injury. Briefly, encourage bleeding from injury site by squeezing the injured area gently; wash liberally with soap and water. Seek medical assistance if the risk of transfer of infection is considered plasuable (*does the donating individual have an infective disorder*?).

6. CONTROL MEASURES TO BE ADOPTED

1) personal protective equipment needed (clear eyewear, gloves, appropriate clothing for human sampling)
2) safe disposal of sharps
3) safe disposal of hazardous contaminated waste
4) regular cleaning of the workplace utilizing Virkon
5) adequate washing facilities

STORAGE - SAFETY CONSIDERATIONS	HANDLING PRECAUTIONS
Use secure, fully labelled containers in fridge, freezer or incubator.	Follow instructions in *Appendix 1* of UOW Regulations for the Use of Biological Materials (**Code of Practice for the Use of Microorganisms**). All students must receive adequate practical instruction from the lecturer in charge.

DISPOSAL PROCEDURES DURING AND AT END OF EXPERIMENT

Follow instructions in *Appendix 1*. Technicians to follow instructions in *Appendix 8* (**Instructions for technical staff**).

7. REVIEW AND MONITORING OF CONTROL MEASURES

Control measures to be reviewed every 12 months, or earlier if there is a change of personal in charge or event prompting a review of measures in place.

Medical monitoring required? **N** Occupational health nurse informed? **N**

8. OTHER RELEVANT INFORMATION

All experiments must comply with the UoW regulation of the use of biological materials, the codes of practice and the relevant appendices. Collected whole blood must comply with the Human Tissues Act, unless immediately rendered acellular for plasma or serum.

9. Name of Assessor (BLOCK CAPITALS): Dr BRADLEY ELLIOTT Signed:

Status of Assessor (Lecturer/Post-doc/Technician): Lecturer Date: 18 – 03 - 2019

Keith Redway
May 2005

Appendix D: Participation Information Sheet & Informed Consent Form – Study 1

PARTICIPATION INFORMATION SHEET

PUFAs and physical activity: The potential of Omega-3 supplementation to reduce muscle-inflammation after muscle-damaging exercise

Researcher: Yvoni Kyriakidou
Supervisor: Dr Alberto Dolci

There is evidence from many studies that omega-3 fatty acids have anti-inflammatory actions and this suggests a potential role for these fatty acids to reduce the amount of muscle-inflammation.

The aim of the research is to proof that supplementation with omega-3 fatty acids can attenuate the inflammation caused by the performance of exercise and thus reduce pain, discomfort and appearance of upper respiratory tract infections.

You are being invited to take part in a research study on omega-3 supplementation, which involves physically active participants (e.g going to the gym, members of sports clubs) undergoing exercised-induced muscle damage (EIMD) using physical activity at an intensity of 65% $\dot{V}O_2max$ in order to induce muscle-inflammation.

The study will involve you:
During the first visit to the laboratory a $\dot{V}O_2max$ test will be performed in order to assess your fitness level before participating in the study. Additionally, a urine sample, a blood sample, a mucosal sample, maximal isometric right-leg quadriceps strength and a perceived leg muscle soreness test will be taken. Height, weight and body composition with bioelectrical impedance will also be measured.

Then, you will take a supplement (either omega-3 or placebo) for 4 weeks. At the end of the 4th week you will be asked to come to the University of Westminster, New Cavendish Building, Human Performance Laboratory on four occasions to take part in tests as follows:

- You will be asked to perform a running exercise, downhill at 65% $\dot{V}O_2max$ on -10% gradient for 60 minutes. A urine sample, a blood sample, maximal isometric right-leg quadriceps strength and a perceived leg muscle soreness test will be taken before and after trial (running downhill), as well as body composition with bioelectrical impedance will be measured before and after the trial.

You may experience a muscle discomfort temporarily as a result of performing the exercise bout. Your legs may be pain- free for about 8 hours but slight soreness may appear and peak over the next 24 to 48 hours. The muscle uneasiness you may feel will vary from person to person but all discomfort usually subsides within 3 days.

- 24 hours after the exercise bout downhill running, you will be asked to provide a urine sample, a blood sample and assess how sore your legs feel by placing a mark on a visual scale and by performing a strength test for 10 minutes.

- 48 hours and 72 hours after the experimental trial (downhill running), you will be asked to provide all the same measurements taken after 24 hours of your downhill running.

Additionally, you will be asked to record a daily health questionnaire in the 2 weeks preceding trials. Diet diaries will be provided to record all food and drinks consumed during 48 hours before the supplementation starts and during 48 hours prior to the trial. Also, 24 hours before the trial you will be asked to consume water, the amount of which will depend on your body weight, in order to ensure that you are hydrated before the test.

You should not undertake downhill running or eccentric exercise as part of your normal training throughout the study. Additionally, you should be free from medications or supplements. You should, also, refrain from alcohol and caffeine 24 hours before the downhill running and refrain from eating and drinking an hour before the mucosal sample collection.

Please note mucosal samples include human tissue cells. You will be asked to give your consent for research to be undertaken on these samples.

Please note:

- Your participation in this research is entirely voluntary.

- You have the right to withdraw at any time without giving a reason.
- Wherever practicable, withdrawal from the research will not affect any treatment and/or services that you receive.
- You have the right to ask for your data to be withdrawn as long as this is practical, and for personal information to be destroyed.
- You do not have to answer particular questions either on questionnaires or in interviews if you do not wish to do so.
- Your responses will normally be made anonymous, unless indicated above to the contrary, and will be kept confidential unless you provide explicit consent to do otherwise.
- No individuals should be identifiable from any collated data, written report of the research, or any publications arising from it.
- All computer data files will be encrypted and password protected. The researcher will keep files in a secure place and will comply with the requirements of the Data Protection Act.
- All hard copy documents, e.g. consent forms, completed questionnaires, etc. will be kept securely and in a locked cupboard, wherever possible on University premises. Documents may be scanned and stored electronically. This may be done to enable secure transmission of data to the university's secure computer systems.
- As detailed above, this research includes the collection of small amounts of your human tissue, e.g. blood and mucosal samples. If you give your consent for its inclusion in the research the researcher will ensure any remaining or excess tissue is destroyed at the end of the study.
- Please notify the researcher immediately if any adverse symptoms arise during or after the research.
- If you wish you, can receive information on the results of the research. Please indicate if you would like to receive this information, and if you like to be informed of any abnormal results.
- The researcher can be contacted during and after participation by email (w1609303@my.westminster.ac.uk).
- If you have a complaint about this research project you can contact the project supervisor, Dr Alberto Dolci by e-mail (A.Dolci@westminster.ac.uk).

CONSENT FORM

Title of Study: PUFAs and physical activity: The potential of omega-3 supplementation to reduce muscle-inflammation after muscle-damaging exercise

Lead researcher: Alberto Dolci and Yvoni Kyriakidou

I have read the information in the Participation Information Sheet, and I am willing to act as a participant in the above research study.

Name: ________________________________

Signature: ___________________________ Date: _______________

This consent form will be stored separately from any data you provide so that your responses remain anonymous.

I have provided an appropriate explanation of the study to the participant

Researcher Signature_____________________________

Appendix E: Participation Information Sheet & Informed Consent Form – Study 2

PARTICIPATION INFORMATION SHEET

The effects of ageing processes on muscle damage, repair and inflammation

Researcher: Yvoni Kyriakidou

Supervisor: Dr Bradley Elliott

You are being invited to take part in a research study examining changes in muscle function and muscle inflammation following exercise. There is evidence that exercise-induced muscle damage (EIMD) is caused by strenuous and unaccustomed exercise and is related to strength loss, pain, muscle soreness and impaired recovery; and can result to exercise avoidance. Muscle damage can affect fit individuals, less well-trained ones and it perhaps can prevent older people from following their normal activity schedule. Indeed, EIMD has not been well studied in older individuals. This study involves physically active participants (e.g., going to the gym, members of sports clubs) undergoing EIMD using physical activity on a leg press exercise protocol in order to induce muscle-inflammation.

The aim of this research is therefore to examine this, by looking at a group of younger (18-35 years of age) and older (above 60 years of age) people before and after an EIMD protocol. To do this, we will take a small blood sample before and after EIMD protocol and use this sample to measure the concentration of markers of muscle damage and how these change during the recovery phase.

The study will involve you:

Attending the University on 5 occasions, for approximately 2 hours on the 1st visit, 4 hours on the 2nd visit and 30 minutes on the 3rd, 4th and 5th visit.

1) During the first visit to the laboratory, a familiarization session will take place and 1 repetition maximum will be determined on a leg press machine.
   - Urine sample and a small blood sample will be taken.
   - Functional measurements: perceived leg muscle soreness via visual analogue scale, a maximal isometric leg quadriceps strength and peak power test will be measured.

- Height, weight, body composition and thigh circumference will also be measured.

2) Following 1 week, you will be asked to come to the University of Westminster, New Cavendish Building, Human Performance Laboratory on 4 occasions to take part in tests as follows:
   - You will be asked to perform a leg press exercise (7 sets of 10 repetitions).
   - Before and immediately post-exercise you will be asked to provide all the same measurements taken during the first visit. In addition, a small blood sample will be measured again at 1- and 2-hours post-exercise.

You may experience a muscle discomfort temporarily as a result of performing the exercise bout. Your legs may be pain- free for about 8 hours but slight soreness may appear and peak over the next 24 to 48 hours. The muscle uneasiness you may feel will vary from person to person, but all discomfort usually subsides within 3 days.

3) 24 hours after the exercise bout, you will be asked to provide a small blood sample and assess how sore your legs feel by placing a mark on a visual scale and by performing a maximal isometric quadriceps strength and power test.

4) 48 hours and 72 hours after the experimental trial, you will be asked to provide all the same measurements taken after 24 hours after the exercise.

Additionally, you will be asked to record a daily health questionnaire in the 1-week preceding trial. A diet diary will be provided to record all food and drinks consumed during 72 hours prior to baseline visit and prior to the exercise trial. Also, 24 hours before the trial you will be asked to consume water, the amount of which will depend on your body weight, in order to ensure that you are hydrated before the test.

You should not undertake any eccentric exercise (resistance exercise, downhill running) as part of your normal training throughout the study. Additionally, you should be free from medications or supplements. You should, also, refrain from alcohol and caffeine 24 hours before the exercise trial and refrain from eating and drinking in the morning of the exercise bout.

If you wish, we will provide you with a copy of all of your results from this experiment for your information.

Please note:

- Your participation in this research is entirely voluntary.

- You have the right to withdraw at any time without giving a reason.
- Wherever practicable, withdrawal from the research will not affect any treatment and/or services that you receive.
- You have the right to ask for your data to be withdrawn as long as this is practical, and for personal information to be destroyed.
- You do not have to answer particular questions either on questionnaires or in interviews if you do not wish to do so.
- Your responses will normally be made anonymous, unless indicated above to the contrary, and will be kept confidential unless you provide explicit consent to do otherwise, for example, the use of your image from photographs and/or video recordings. [NOTE: it may not be possible to maintain confidentiality in certain circumstances, e.g., where issues of child safety have been identified. You should seek clarification from the researcher and/or their supervisor if you are concerned about this].
- No individuals should be identifiable from any collated data, written report of the research, or any publications arising from it.
- All computer data files will be encrypted and password protected. The researcher will keep files in a secure place and will comply with the requirements of the Data Protection Act.
- All hard copy documents, e.g., consent forms, completed questionnaires, etc. will be kept securely and in a locked cupboard, wherever possible on University premises. Documents may be scanned and stored electronically. This may be done to enable secure transmission of data to the university's secure computer systems.
- As detailed above, this research includes the collection of small amounts of your human tissue, e.g., blood samples. If you give your consent for its inclusion in the research the researcher will ensure any remaining or excess tissue is destroyed at the end of the study.
- Please notify the researcher immediately if any adverse symptoms arise during or after the research.
- If you wish you, can receive information on the results of the research. Please indicate if you would like to receive this information, and if you like to be informed of any abnormal results.
- The researcher can be contacted during and after participation by email (w1609303@my.westminster.ac.uk).
- If you have a complaint about this research project you can contact the project supervisor, Dr Bradley Elliott by e-mail (B.Elliott@westminster.ac.uk) or by telephone (0207 911 5000 ext 64582).

CONSENT FORM

Title of Study: The effects of ageing processes on muscle damage, repair and inflammation

Lead researcher: Bradley Elliott and Yvoni Kyriakidou

I have read the information in the Participation Information Sheet, and I am willing to act as a participant in the above research study.

Name: _________________________________

Signature: ___________________________ Date: ________________

This consent form will be stored separately from any data you provide so that your responses remain anonymous.

I have provided an appropriate explanation of the study to the participant

Researcher Signature_____________________________

Appendix F: Participation Information Sheet & Informed Consent Form – Study 3

PARTICIPATION INFORMATION SHEET

The effects of downhill running, leg press and leg extension protocol on muscle damage and muscle inflammation

Researcher: Yvoni Kyriakidou

Supervisor: Dr Bradley Elliott

You are being invited to take part in a research study examining changes in muscle function and muscle inflammation following exercise. There is evidence from many studies that exercise-induced muscle damage (EIMD) is caused by strenuous and unaccustomed exercise and is related to strength loss, pain, muscle soreness and impaired recovery; and can result to exercise avoidance. This study involves physically active participants (e.g. going to the gym, members of sports clubs, athletes) undergoing EIMD using physical activity in order to induce muscle-inflammation. The aim of this research is therefore to examine this, by looking at a group of young (18-35 years of age) people before and after an EIMD protocol. To do this, we will assess muscle function and take a small blood sample before and after EIMD protocol and use this sample to measure the concentration of markers of muscle damage and how these change during the recovery phase.

The study will involve you:

Attending the University on 5 occasions, for approximately 2 hours on the 1st visit, 4 hours on the 2nd visit and about 30 minutes on the 3rd, 4th and 5th visit.

1) During the first visit to the laboratory, a $\dot{V}O_2max$ test will be performed in order to assess your fitness level before participating in the study and 5 repetitions maximum will be determined on a leg press and leg extension machine.

- Urine sample and a small blood sample will be taken.
- Functional measurements: perceived leg muscle soreness via visual analogue scale, a maximal isometric leg quadriceps strength and peak power will be measured.
- Height, weight, body composition and thigh circumference will also be measured.

2) Following 1 week, you will be asked to come to the University of Westminster, New Cavendish Building, Human Performance Laboratory on 4 occasions to take part in tests as follows:
   - You will be asked to perform an exercise on a leg press and leg extension machine.
   - Before and immediately post-exercise you will be asked to provide all the same measurements taken during the first visit. In addition, a small blood sample will be measured again at 1- and 2-hours post-exercise.

You may experience a muscle discomfort temporarily as a result of performing the exercise bout. Your legs may be pain- free for about 8 hours but slight soreness may appear and peak over the next 24 to 48 hours. The muscle uneasiness you may feel will vary from person to person, but all discomfort usually subsides within 3 days.

3) 24 hours after the exercise bout, you will be asked to provide a small blood sample, and assess how sore your legs feel by placing a mark on a visual scale and by performing a maximal isometric quadriceps strength and peak power tests.

4) 48 hours and 72 hours after the experimental trial, you will be asked to provide all the same measurements taken 24 hours after the exercise.

Additionally, you will be asked to record a daily health questionnaire in the 1-week preceding trial. A diet diary will be provided to record all food and drinks consumed during 72 hours prior to the trial. Also, 24 hours before the trial you will be asked to consume water, the amount of which will depend on your body weight, in order to ensure that you are hydrated before the test.

You should not undertake downhill running or eccentric exercise (resistance/weight lifting) as part of your normal training throughout the study. Additionally, you should be free from medications or supplements. You should, also, refrain from alcohol and caffeine 24 hours before the exercise trial and refrain from eating and drinking in the morning of the exercise bout.

If you wish, we will provide you with a copy of all of your results from this experiment for your information.

Please note:

- Your participation in this research is entirely voluntary.

- You have the right to withdraw at any time without giving a reason.
- Wherever practicable, withdrawal from the research will not affect any treatment and/or services that you receive.
- You have the right to ask for your data to be withdrawn as long as this is practical, and for personal information to be destroyed.
- You do not have to answer particular questions either on questionnaires or in interviews if you do not wish to do so.
- Your responses will normally be made anonymous, unless indicated above to the contrary, and will be kept confidential unless you provide explicit consent to do otherwise, for example, the use of your image from photographs and/or video recordings. [NOTE: it may not be possible to maintain confidentiality in certain circumstances, e.g., where issues of child safety have been identified. You should seek clarification from the researcher and/or their supervisor if you are concerned about this].
- No individuals should be identifiable from any collated data, written report of the research, or any publications arising from it.
- All computer data files will be encrypted and password protected. The researcher will keep files in a secure place and will comply with the requirements of the Data Protection Act.
- All hard copy documents, e.g., consent forms, completed questionnaires, etc. will be kept securely and in a locked cupboard, wherever possible on University premises. Documents may be scanned and stored electronically. This may be done to enable secure transmission of data to the university's secure computer systems.
- As detailed above, this research includes the collection of small amounts of your human tissue, e.g., blood samples. If you give your consent for its inclusion in the research the researcher will ensure any remaining or excess tissue is destroyed at the end of the study.
- Please notify the researcher immediately if any adverse symptoms arise during or after the research.
- If you wish you, can receive information on the results of the research. Please indicate if you would like to receive this information, and if you like to be informed of any abnormal results.
- The researcher can be contacted during and after participation by email (y.kyriakidou@ westminster.ac.uk).
- If you have a complaint about this research project you can contact the project supervisor, Dr Bradley Elliott by e-mail (B.Elliott@westminster.ac.uk) or by telephone (0207 911 5000 ext 64582).

CONSENT FORM

Title of Study: The effects of downhill running, leg press and leg extension protocol on muscle damage and muscle inflammation

Lead researcher: Bradley Elliott and Yvoni Kyriakidou

I have read the information in the Participation Information Sheet, and I am willing to act as a participant in the above research study.

Name: ________________________________

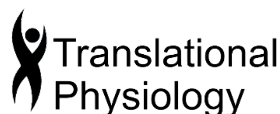
Signature: ___________________________ Date: _______________

This consent form will be stored separately from any data you provide so that your responses remain anonymous.

I have provided an appropriate explanation of the study to the participant

Researcher Signature_____________________________

Appendix G: Medical Questionnaire and WURSS-21



Version 4 - 2019

MEDICAL QUESTIONNAIRE
STRICTLY CONFIDENTIAL

Participant code:________________________________

State if you have suffered from any of the following:

- Severe anxiety or depression
- Hay fever or any allergies
- Liver disorder
- Bladder disorder
- Any recurrent infections
- Rheumatic Fever (Rheumatism)
- Any impairment of immunity
- Stomach or Bowel Complaint
- Diabetes
- Infection of Kidneys
- Tumors or other masses
- Eating or Mental Disorder
- Epilepsy
- Any blood disorder
- Genito-Urinary Complaints
- Asthma
- Alcohol or drug related problems
- Thyroid or gland problems
- High or Low Blood Pressure
- Heart condition/Angina
- Fainting or Migraine

In the last 2 years:
Have you had any specialist or hospital investigation, X-Ray, ECG, MRI, CT scan or other diagnostic imaging?___________

Is any investigation pending?
If so, please specify____

Have you suffered an injury?
If so, state when and how____

Are you at present on any form of treatment, medication or medical advice?
If so please specify____

Are you at present taking any dietary supplements or over the counter pain killers?
If so, please specify____

Do you feel in good health?____

Do you drink alcohol? If so, how many units per week?____________________________

Are you a smoker? If so, please give details__________________________________

Researcher:________________________________ Date__________________________

Wisconsin Upper Respiratory Symptom Survey – 21 --- *Daily Symptom Report*

Day:	*Date:*	*Time:*	*ID*:

Please fill in one circle for each of the following items:

	Not sick	Very mildly		Mildly		Moderately		Severely
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
How sick do you feel **today**?	O	O	O	O	O	O	O	O

Please rate the **average severity of your cold symptoms over the last 24 hours** for each symptom:

	Do not have this symptom	Very mild		Mild		Moderate		Severe
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Runny nose	O	O	O	O	O	O	O	O
Plugged nose	O	O	O	O	O	O	O	O
Sneezing	O	O	O	O	O	O	O	O
Sore throat	O	O	O	O	O	O	O	O
Scratchy throat	O	O	O	O	O	O	O	O
Cough	O	O	O	O	O	O	O	O
Hoarseness	O	O	O	O	O	O	O	O
Head congestion	O	O	O	O	O	O	O	O
Chest congestion	O	O	O	O	O	O	O	O
Feeling tired	O	O	O	O	O	O	O	O

Over the last 24 hours, how much has your cold interfered with your ability to:

	Not at all	Very mildly		Mildly		Moderately		Severely
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Think clearly	O	O	O	O	O	O	O	O
Sleep well	O	O	O	O	O	O	O	O
Breathe easily	O	O	O	O	O	O	O	O
Walk, climb stairs, exercise	O	O	O	O	O	O	O	O
Accomplish daily activities	O	O	O	O	O	O	O	O
Work outside the home	O	O	O	O	O	O	O	O
Work inside the home	O	O	O	O	O	O	O	O
Interact with others	O	O	O	O	O	O	O	O
Live your personal life	O	O	O	O	O	O	O	O

Compared to yesterday, I feel that my cold is…

Very much better	Somewhat better	A little better	The same	A little worse	Somewhat worse	Very much worse
O	O	O	O	O	O	O

WURSS -21© (Wisconsin Upper Respiratory Symptom Survey) 2004
Created by Bruce Barrett MD PhD et al., UW Department of Family Medicine, 777 S. Mills St. Madison, WI 53715, USA

Appendix H: Physical Activity Readiness Questionnaire (PAR-Q)

Physical Activity Readiness
Questionnaire - PAR-Q
(revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
☐	☐	**1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	**2. Do you feel pain in your chest when you do physical activity?**
☐	☐	**3. In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	**4. Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	**5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	**6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	**7. Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME ________________________________

SIGNATURE ________________________________ DATE ________________

SIGNATURE OF PARENT ________________________________ WITNESS ________________
or GUARDIAN (for participants under the age of majority)

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

© Canadian Society for Exercise Physiology

Supported by: Health Canada Santé Canada

continued on other side...

Appendix I: Edinburgh Handedness Inventory Questionnaire

Edinburgh Handedness Inventory - Short Form

Please indicate your preferences in the use of hands in the following activities or objects:

	Always right	Usually right	Both equally	Usually left	Always left
Writing	☐	☐	☐	☐	☐
Throwing	☐	☐	☐	☐	☐
Toothbrush	☐	☐	☐	☐	☐
Spoon	☐	☐	☐	☐	☐

Scoring:

For each item: Always right = 100; Usually right = 50; Both equally = 0; Usually left = -50; Always left = -100

To calculate the Laterality Quotient add the scores for the four items in the scale and divide this by four:

Writing score	
Throwing score	
Toothbrush score	
Spoon score	
Total	
Total ÷ 4 (Laterality Quotient)	

Classification:	Laterality Quotient score:
Left handers	-100 to -61
Mixed handers	-60 to 60
Right handers	61 to 100

Appendix J: Omega-3 Food List

Eat these foods at any time

Meats, Potatoes, Rice, Noodles, Pizza, Pasta, Flour tortillas, Eggs, Margarine, Milk, Yogurt, Bread, Cereals, Oatmeal, Juices, Spinach, Winter squash, Brussels sprouts, Cauliflower, Broccoli, Kale, Brazil nuts, Cashews, Hazelnuts, Peanuts, Pumpkin seeds, Soya oil, Sunflower oil, Peanut butter, **plus anything else not listed below**.

Eat these occasionally (1-2 times per week is fine)

Prawns/Shrimp, Lobsters, Scallops, Fish sticks, Octopus, Tuna, Macadamia nuts

Avoid these during the 4 weeks of supplementation

Salmon, Mackerel, Herring, Sardines, Anchovies, Trout, Halibut, Oysters, Crab, Squid, fish oil, Cod liver oil, Fish Roe (caviar), White fish, Walnuts & Walnut oil, Almonds, Butternuts, Flax seeds & Flaxseed oil, Chia seeds, Hemp seeds, Corn oil, Soy milk

Appendix K: Food Frequency Questionnaire (FFQ)

SELF REPORT (version 9 25 06) ID#
DATE:_____________ RATER____ PT INIT

Omega 3 Fatty Acid Questionnaire

1) Gender: ❒ Male (1) ❒ Female (2)

2) About how long ago was your last meal? ________hours

3) Have you eaten any fish or shellfish--such as shrimp, crab, lobster etc.—in the past 24 hours? (include sushi made with fish or shellfish)
1) ❒ No
2) ❒ Yes

4) How many times have you eaten fish or shellfish in the past week?
1) ❒ 0 times
2) ❒ 1-3 times
3) ❒ More than 3 times

5) Over the past 6 months, about how often have you eaten fish or shellfish in any form?
1) ❒ Never
2) ❒ Less than 1 time each month
3) ❒ 1 time each month
4) ❒ 2-3 times each month
5) ❒ 1 time each week
6) ❒ 2 times each week
7) ❒ 3-4 times each week
8) ❒ 5-6 times each week
9) ❒ 1 time each day
10) ❒ 2 or more times each day

If NEVER, Skip to Question 8.

6) Each time you ate fish or shellfish, how much did you eat?
1) ❒ Less than 2 ounces or less than one fillet or less than 4 pieces of sushi
2) ❒ 2 to 7 ounces or about 1 fillet or 4 -14 pieces of sushi
3) ❒ More than 7 ounces or more than 1 fillet or more than 14 pieces of sushi

7) Please check off the **types of fish or shellfish you eat most frequently** (Check off as many as are appropriate for you) .
1) ❒ Bass
2) ❒ Bluefish
3) ❒ Catfish
4) ❒ Clams
5) ❒ Cod
6) ❒ Crab
7) ❒ Flounder
8) ❒ Haddock
9) ❒ Halibut
10) ❒ Herring
11) ❒ Kingfish
12) ❒ Lobster
13) ❒ Mackerel
14) ❒ Mahi Mahi
15) ❒ Mussels
16) ❒ Oysters
17) ❒ Salmon
18) ❒ Sardines
19) ❒ Scallops
20) ❒ Sea Trout
21) ❒ Shark
22) ❒ Shrimp
23) ❒ Skate
24) ❒ Snapper
25) ❒ Sole
26) ❒ Swordfish
27) ❒ Tilapia
28) ❒ Tilefish
29) ❒ Trout (freshwater)
30) ❒ Tuna
31) ❒ Turbot
32) ❒ Whitefish
33) ❒ Whiting

8) In the past 6 months, about how often did you eat walnuts?

1) ❒ Never
2) ❒ Less than 1 time each month
3) ❒ 1 time each month
4) ❒ 2-3 times each month
5) ❒ 1 time each week
6) ❒ 2 times each week
7) ❒ 3-4 times each week
8) ❒ 5-6 times each week
9) ❒ 1 time each day
10) ❒ 2 or more times each day

If NEVER, Skip to Question 10.

9) Each time you ate walnuts, how much did you eat?

1) ❒ Less than ¼ cup
2) ❒ ¼ to ½ cup
3) ❒ More than ½ cup

10) In the past 6 months, about how often did you use canola cooking oil?

1) ❒ Never	6) ❒ 2 times each week
2) ❒ Less than 1 time each month	7) ❒ 3-4 times each week
3) ❒ 1 time each month	8) ❒ 5-6 times each week
4) ❒ 2-3 times each month	9) ❒ 1 time each day
5) ❒ 1 time each week	10) ❒ 2 or more times each day

If NEVER, Skip to Question 12.

11) Each time you used canola cooking oil, how much did you use?

1) ❒ Less than 1 teaspoon
2) ❒ 1-2 teaspoons
3) ❒ 2 teaspoons
4) ❒ 3 teaspoons (1 tablespoon)
5) ❒ More than 1 tablespoon

12) In the past 6 months, about how often did you eat flaxseed?

1) ❒ Never
2) ❒ less than 1 time each month
3) ❒ 1 time each month
4) ❒ 2-3 times each month
5) ❒ 1 time each week
6) ❒ 2 times each week
7) ❒ 3-4 times each week
8) ❒ 5-6 times each week
9) ❒ 1 time each day
10) ❒ 2 or more times each day

If NEVER, Skip to Question 14.

13) Each time you ate flaxseeds, about how much did you eat?

1) ❒ Less than 1 teaspoon
2) ❒ 1-2 teaspoons
3) ❒ 2 teaspoons
4) ❒ 3 teaspoons (1 tablespoon)
5) ❒ More than 1 tablespoon

SELF REPORT (version 9 25 06) ID#
DATE:____________ RATER___ PT INIT

14) In the past 6 months, about how often did you use flaxseed oil?

1) ❒ Never
2) ❒ Less than 1 time each month
3) ❒ 1 time each month
4) ❒ 2-3 times each month
5) ❒ 1 time each week
6) ❒ 2 times each week
7) ❒ 3-4 times each week
8) ❒ 5-6 times each week
9) ❒ 1 time each day
10) ❒ 2 or more times each day

If NEVER, Skip to Question 16.

15) Each time you used flaxseed oil, how much did you have?

1) ❒ Less than 1 teaspoon
2) ❒ 1-2 teaspoons
3) ❒ 2 teaspoons
4) ❒ 3 teaspoons (1 tablespoon)
5) ❒ More than 1 tablespoon

16) In the past 6 months, about how often did you use cod liver oil?

1) ❒ Never
2) ❒ Less than 1 time each month
3) ❒ 1 time each month
4) ❒ 2-3 times each month
5) ❒ 1 time each week
6) ❒ 2 times each week
7) ❒ 3-4 times each week
8) ❒ 5-6 times each week
9) ❒ 1 time each day
10) ❒ 2 or more times each day

If NEVER, Skip to Question 18.

17) Each time you used cod liver oil, how much did you have?

1) ❒ Less than 1 teaspoon
2) ❒ 1-2 teaspoons
3) ❒ 2 teaspoons
4) ❒ 3 teaspoons (1 tablespoon)
5) ❒ More than 1 tablespoon

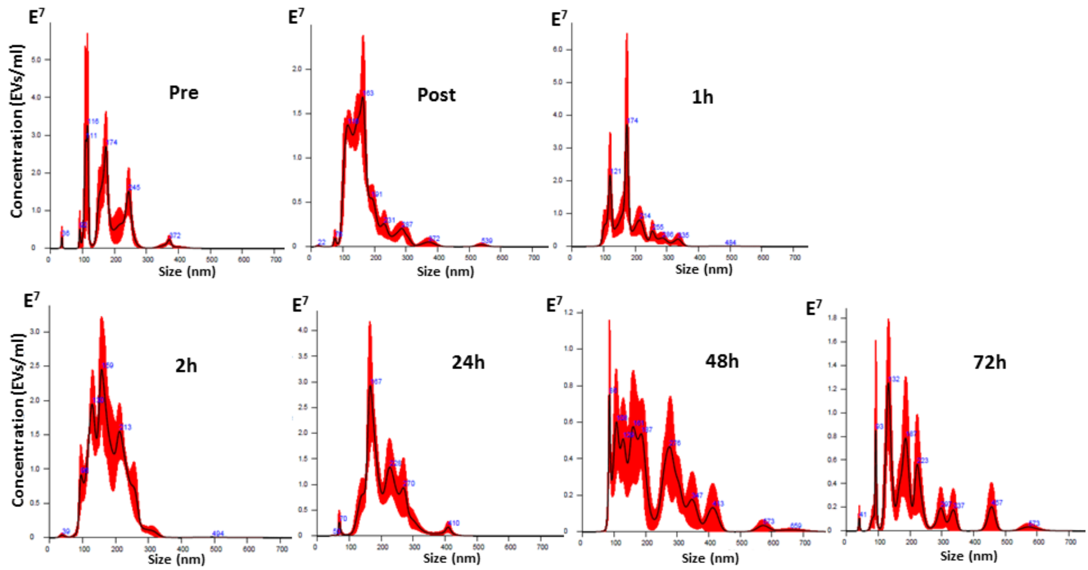
18) In the past 6 months, have you used an Omega 3 fatty acids or fish oil supplement at least once each week?

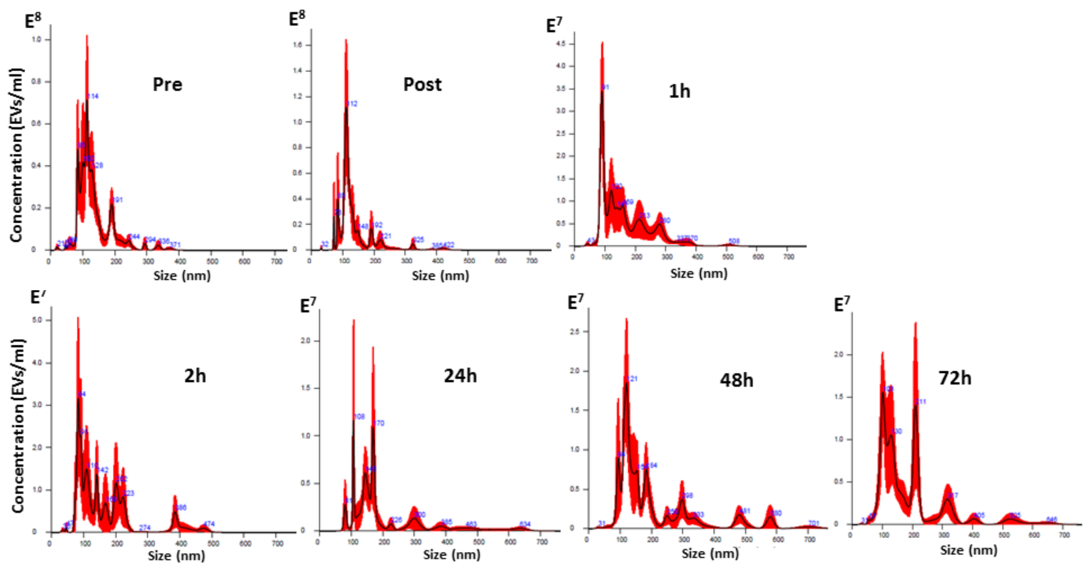
❒ NO (YOU ARE FINISHED WITH THIS QUESTIONNAIRE)
❒ YES – What type of an Omega 3 fatty acids or fish oil supplement do you take?

Please write the name of the supplement below:

____

Appendix L: Representative example of NTA Analysis





Appendix M: Participant's Representative Results – Study 1, Study 2 and Study 3

Study 1

Training Plan

The charts on this page provide summary information on your anthropometric measures on the day of the downhill running test, alongside the results of both the aerobic and anaerobic fitness tests, and with some normative data to compare your results with.

In addition we have created a training program on the following pages based on your results.

You can use the program for running, cycling or rowing. If you follow the running program you will need the following information from this page

- Speed at which you reached VO_2max (called vVO_2max)
- Maximum Heart Rate (called Hrmax)
- Vmax. This is simply your maximum sprinting speed.

- If you choose to use the program for cycling or rowing you will need to complete some additional tests outline on the following page.

Blood Markers

Haematocrit	
0.453 L/L	Normal

Haemoglobin	
149 g/L	Normal

Aerobic Fitness Measured by Oxygen Uptake

VO_2max	4.83 L/min	Weight	75.9 kg

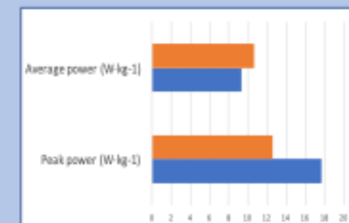
Relative VO_2max

Very Poor	Poor	Fair	Good	Excellent
				63.64 ml/kg/min

Anaerobic Fitness (10s Wingate)

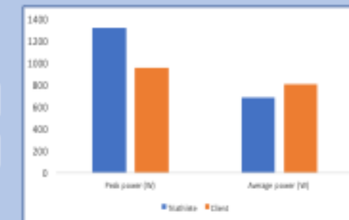
Performance relative to national level triathletes

Peak Power (Pmax)	956.37 W
Average Power	809.6 W
Fatigue Index	25.62 %
Peak Power Percentile	90%



Isometric Leg Extension

Max Recorded	48.6 kg
Average Recorded	40.14 kg



VO_2max/Speed KPH

60% vVO_2max	9.6 Kph
vVO_2max	16 Kph
120% vVO_2max	19.2 Kph
130% vVO_2max	20.8 Kph
140% vVO_2max	22.4 Kph

Summary of Anthropometric Measurements

Age	23 years		
Height	1.87 M		
Weight	75.9 kg		
Muscle Mass	64.3 kg	% BF	10.9 %
Fat Free Mass	67.6 kg	Max Heart Rate	197 BPM

BMI: 21.7 kg/m2 (Under, Normal, Over, Obese)

Body Fat Classification

- [ ] Obese
- [ ] Average
- [ ] Fitness
- [x] Athlete
- [ ] Essential

Study 2 – Example of Older Participant's results

UNIVERSITY OF WESTMINSTER

The charts on this page provide summary information on your anthropometric measures on the day of the baseline fitness assessments, alongside the results of both anaerobic and functional fitness tests along with some normative data to compare your results with.

Information from this page:

- Body Mass Index (called BMI)
- Vertical jump is your highest attempt of the jump performance
- Norm value (of the jump) is the average jump value of the population of the age 65 years old

Anaerobic Power Output (10s Wingate test):

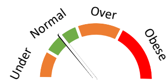
- Peak Power (called Pmax)
- Relative Pmax is the peak power when taking the body weight into consideration

*both graphs are related to peak power output: Figure (a) absolute Pmax in comparison to adults over 60 years old, & Figure (b) relative Pmax (according to your body weight) in comparison to adults over 60 years old with the same weight

- Fatigue Index: shows the % of power lost from the beginning to end of the test (below it is presented the average value of all 6 tests). The % expresses the decline compared to peak power output

Summary of Anthropometric Measurements / Body Composition

Age	65 years	Total Energy Expenditure	2882 kcal/day	Skeletal Muscle Mass	25.8 kg (27.8-36.6)
Height	1.80 m	Resting Energy Expenditure	1601 kcal/day	Fat Free Mass	55.2 kg (normal)
Weight	69.6 kg	Max Heart Rate	155 beats per min	Fat Mass	14.4 kg (normal)
				% Body Fat	20.7%



BMI

21.5 kg/m2

Body Fat Classification

- [ ] Overweight
- [x] Average
- [ ] Fitness
- [ ] Athlete
- [ ] Essential

Jump Performance

Vertical Jump	34.0 cm
Average Norm Value	31.6 cm

Anaerobic Fitness (10s Wingate)

Peak Power (Pmax)	502.9 W
Average Power	418.5 W
Fatigue Index	26.6%
Peak Power Percentile	70%

Isometric Leg Strength

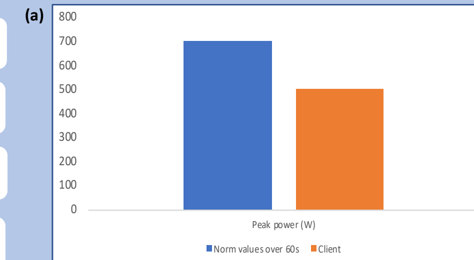
Max Recorded	13.5 kg
Average Recorded	12 kg

Leg Press (one leg)

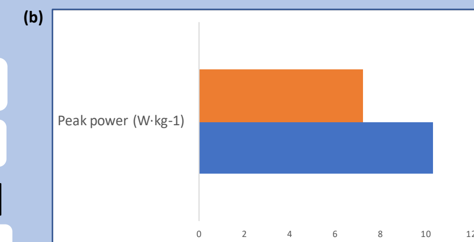
1 Max Repetition (100%)	133.7 kg

Performance relative to adults 60s and over

(a)



(b)



Study 3

The charts on this page provide summary information on your anthropometric measures on the day of the $\dot{V}O_2$max, alongside the results of both the aerobic and anaerobic fitness tests along with some normative data to compare your results with.

<u>Information from this page</u>:

- Speed at which you reached $\dot{V}O_2$max during the $\dot{V}O_2$max test (called v$\dot{V}O_2$max)
- Moderate intensity during flat running (called 65% v$\dot{V}O_2$max)
- Relative $\dot{V}O_2$max is related to your body weight (see example in p.6)
- Vertical jump is your highest attempt of the jump performance
- Norm value (of the jump) is the average jump value of the population of the age 35 years old
- Body Mass Index (called BMI)

Anaerobic Power Output (10s Wingate test):

- Peak Power (called Pmax)

*both graphs are related to power output: Figure a) absolute Pmax & absolute average power in comparison to triathletes and Figure b) relative Pmax & relative average power (according to your body mass) in comparison to triathletes

- Fatigue Index: shows the % of power lost from the beginning to end of the test (below is the average value of all 6 tests). The % expresses the decline compared to peak power output

$\dot{V}O_2$max/Speed KPH

v$\dot{V}O_2$max	16 Kph
65% v$\dot{V}O_2$max	10.4 Kph
120% v$\dot{V}O_2$max	19.2 Kph
130% v$\dot{V}O_2$max	20.8 Kph
140% v$\dot{V}O_2$max	22.4 Kph

Jump Performance

Vertical Jump	57.5 cm
Average Norm Value	52.8 cm

Aerobic Fitness Measured by Oxygen Uptake

$\dot{V}O_2$max: 5.02 L/min

Weight: 81.5 kg

Relative $\dot{V}O_2$max

Poor	Below Average	Fair	Good	Excellent
				62 mL/kg/min

Summary of Anthropometric Measurements / Body Composition

Age	35 years	Total Energy Expenditure	3163 Kcal/day
Height	1.81 m	Resting Energy Expenditure	1757 kcal/day
Weight	81.5 kg	Max Heart Rate	185 BPM

BMI

25 kg/m²

Skeletal Muscle Mass	32.6 kg (28.1-37.0)
Fat Free Mass	68.11 kg (normal)
Fat Mass	13.8 kg (normal)
% Body Fat	16.8 %

Body Fat Classification

- ☐ Obese
- ☐ Average
- ☑ Fitness
- ☐ Athlete
- ☐ Essential

Performance relative to national level triathletes

Anaerobic Fitness (10s Wingate)

Peak Power (Pmax)	840.5 W
Average Power	725.51 W
Fatigue Index	25.63%
Peak Power percentile	97%

Isometric Leg Strength

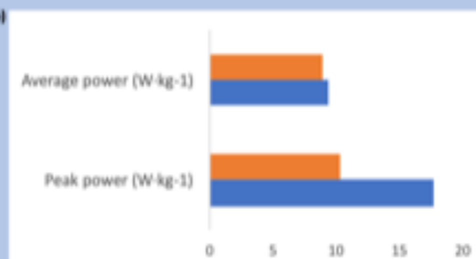
Max Recorded	26.55 kg
Average Recorded	23.55 kg

Leg Press (1 leg)

1 Max Repetition (100%)	153.6 kg

(a)

(b)



Appendix N: Advertisement - Study 1, Study 2 and Study 3

Study 1

Take your Training to the NEXT LEVEL!!

Join our study and learn

- How to train smarter!
- Go further and faster!
- Recover more quickly!
- Nutrition to support your training!

FREE VO_2MAX TEST

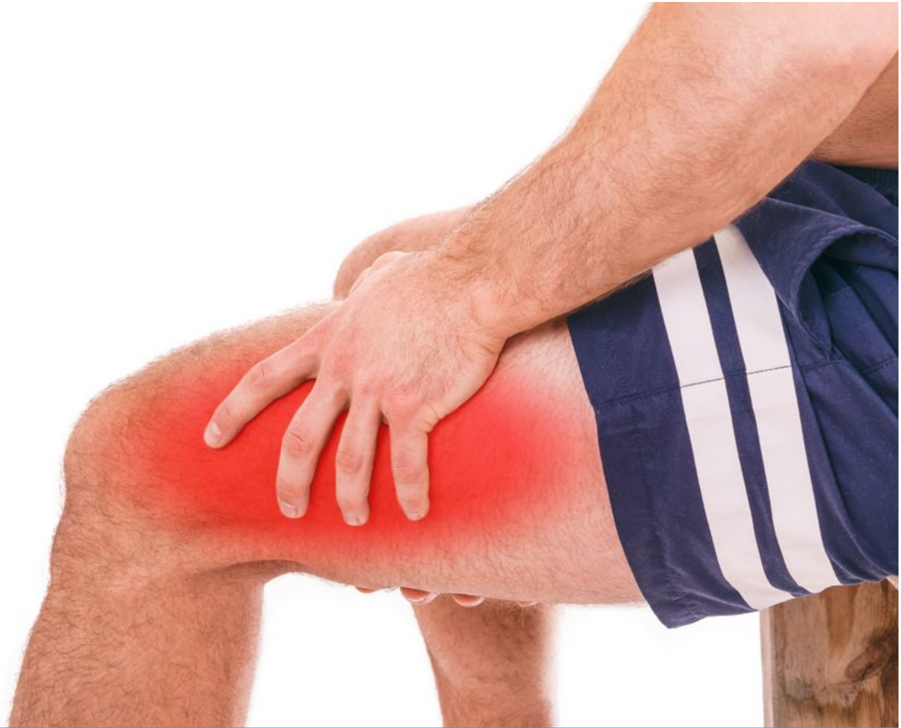
Are You?
Caucasian, male aged 18-35?
physically active?

READY TO REACH YOUR PEAK?

UNIVERSITY OF
LEADING
THE WAY
WESTMINSTER

For more info contact:
Yvoni Kyriakidou
Email: w1609303@my.westminster.ac.uk

Study 2



WHY DOES EXERCISE HURT?

We are researching why muscles sometimes hurt following intense exercise

To do this:
- **we are seeking physically active, Caucasian males between 18-35 and over 60 years of age**

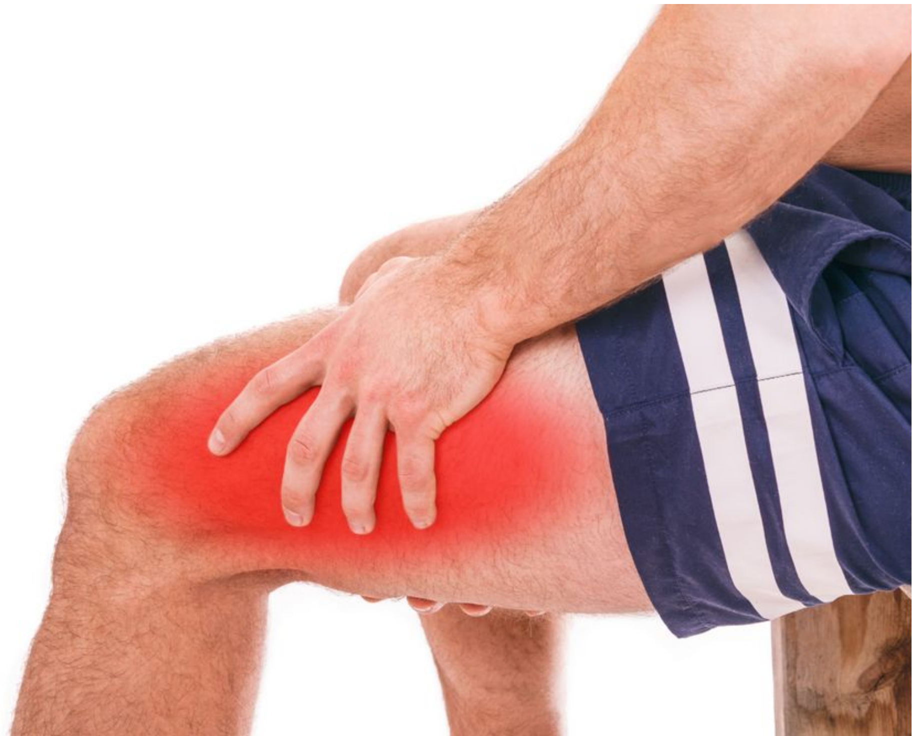
Take part in our research study and receive:
- **FREE body composition, aerobic & anaerobic fitness assessments**

Visits take place in our central London laboratory

Contact Yvoni Kyriakidou for details
E: Y.Kyriakidou@westminster.ac.uk

Ethical approval for this study is provided by the College of Liberal Arts & Sciences Research Ethics Committee

Study 3



WHY DOES EXERCISE HURT?

We are researching why muscles sometimes hurt following intense exercise

To do this:

- **we are seeking physically active, Caucasian males between 18-35 years of age**

Take part in our research study and receive:

- **FREE body composition, aerobic & anaerobic fitness assessments**

Visits take place in our central London laboratory

Contact Yvoni Kyriakidou for details
E:Y.Kyriakidou@westminster.ac.uk

Ethical approval for this study is provided by the College of Liberal Arts & Sciences Research Ethics Committee